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Oil source and peroxidation status interactively affect growth performance and oxidative status in broilers from 4 to 25 d of age

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Abstract

The objectives of this experiment were to evaluate the effect of oil source and peroxidation status on broiler performance and oxidative stress. Broilers (initial BW 85.1 ± 7.8 g) were allotted to 40 cages with 5 birds per cage in a completely randomized design. The 4 × 2 factorial arrangement of treatments consisted of oil source (palm oil, soybean oil, flaxseed oil, and fish oil) and peroxidation status (fresh or peroxidized). Broilers were fed experimental diets for 20 d to measure growth performance; on day 21 of the experiment, plasma and liver samples were harvested for analysis of oxidative stress including thiobarbituric acid reactive substances (TBARS), protein carbonyls (PC), 8-hydroxy-2'-deoxyguanosine (8-OH-2dG), glutathione peroxidase activity (GPx) and superoxide dismutase and catalase (CAT). An interaction occurred between oil source and peroxidation status where broilers fed peroxidized oils had reduced ADFI, ADG, G:F, and plasma GPx in all oil sources except for fish oil ($P \leq 0.04$). Plasma 8-OH-2dG was increased by feeding peroxidized oils ($P = 0.01$). An interaction occurred in liver TBARS where broilers fed peroxidized palm oil had greater liver TBARS compared to fresh palm oil ($P = 0.09$). An interaction was noted for liver PC where broilers fed palm, flaxseed, and fish oil had similar liver PC regardless of peroxidation status, while broilers fed peroxidized soybean oil had increased liver PC compared to the fresh soybean oil diet ($P = 0.04$). Oil source affected plasma TBARS and 8-OH-2dG ($P = 0.01$), plasma PC ($P = 0.09$), liver 8-OH-2dG ($P = 0.08$), and liver CAT ($P = 0.02$). Correlations between oil composition with growth performance and oxidative stress markers imply that oil UFA:SFA, p-anisidine value, DDE, total polar compounds, and polymerized triglycerides should be measured as an indicator of oil quality, with growth performance being correlated to plasma TBARS, PC, and GPx. In conclusion, the degree of unsaturation and peroxidation status of dietary oils affected growth performance and markers of oxidative stress in poultry.

Keywords

broilers, growth, oxidative stress, peroxidized oils

Disciplines

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Comments

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Oil source and peroxidation status interactively affect growth performance and oxidative status in broilers from 4 to 25 d of age

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ABSTRACT The objectives of this experiment were to evaluate the effect of oil source and peroxidation status on broiler performance and oxidative stress. Broilers (initial BW 85.1 ± 7.8 g) were allotted to 40 cages with 5 birds per cage in a completely randomized design. The 4 × 2 factorial arrangement of treatments consisted of oil source (palm oil, soybean oil, flaxseed oil, and fish oil) and peroxidation status (fresh or peroxidized). Broilers were fed experimental diets for 20 d to measure growth performance; on day 21 of the experiment, plasma and liver samples were harvested for analysis of oxidative stress including thiobarbituric acid reactive substances (TBARS), protein carbonyls (PC), 8-hydroxy-2'-deoxyguanosine (8- OH-2dG), glutathione peroxidase activity (GPx) and superoxide dismutase and catalase (CAT). An interaction occurred between oil source and peroxidation status where broilers fed peroxidized oils had reduced ADFI, ADG, G:F, and plasma GPx in all oil sources except for fish oil ($P \leq 0.04$). Plasma 8-OH-2dG was increased by feeding peroxidized oils ($P = 0.01$). An interaction occurred in liver TBARS where broilers fed peroxidized palm oil had greater liver TBARS compared to fresh palm oil ($P = 0.09$). An interaction was noted for liver PC where broilers fed palm, flaxseed, and fish oil had similar liver PC regardless of peroxidation status, while broilers fed peroxidized soybean oil had increased liver PC compared to the fresh soybean oil diet ($P = 0.04$). Oil source affected plasma TBARS and 8-OH-2dG ($P = 0.01$), plasma PC ($P = 0.09$), liver 8-OH-2dG ($P = 0.08$), and liver CAT ($P = 0.02$). Correlations between oil composition with growth performance and oxidative stress markers imply that oil UFA:SFA, p-anisidine value, DDE, total polar compounds, and polymerized triglycerides should be measured as an indicator of oil quality, with growth performance being correlated to plasma TBARS, PC, and GPx. In conclusion, the degree of unsaturation and peroxidation status of dietary oils affected growth performance and markers of oxidative stress in poultry.

Key words: broilers, growth, oxidative stress, peroxidized oils

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INTRODUCTION

Poultry diets commonly contain supplemental oils which are high in polyunsaturated fatty acids (**PUFA**) to increase the energy density of the feed. While the fatty acid profile of a dietary oil is known to affect lipid digestibility, and therefore its energy value (Wiseman et al., 1998), it also plays a role in the susceptibility of oils to peroxidize because PUFA are more prone to lipid peroxidation than saturated fatty acids (Holman, 1954). Lipid peroxidation is a chain reaction that produces and consumes compounds such as peroxides, aldehydes, and polar compounds, while diminishing the antioxidant capacity of the oil. The degree of peroxidation differs based on the temperature and duration of thermal processing, as well as oil composition. Because there is no single measurement that describes the overall peroxidation status of oils, it is more biologically descriptive to measure multiple markers of peroxidation.

Feeding peroxidized oils to poultry negatively impacts growth performance (Takahashi and Akiba, 1999; Anjum et al., 2004; Tavárez et al., 2011), potentially through the induction of in vivo oxidative stress (Tavárez et al., 2011; Boler et al., 2012). Oxidative stress occurs in vivo when reactive oxygen species (**ROS**) and free radical products overcome the capacity of antioxidants and antioxidant enzymes to convert these species to less reactive species, resulting in tissue-damaging free radicals binding to lipids, proteins, and DNA (Kalyanaraman, 2013). Typically, thiobarbituric acid reactive substances (**TBARS**) are measured as a marker of lipid damage, protein carbonyl concentration

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(**PC**) is measured for protein damage, and 8-hydroxy-2′-deoxyguanosine (**8-OH-2dG**) is measured to indicate DNA damage. Antioxidants work to combat and detoxify ROS and are commonly measured by superoxide dismutase (**SOD**), catalase (**CAT**), and glutathione peroxidase (**GPx**) activities. Because there are limited data available regarding the effects of feeding thermally processed oils on markers of oxidative stress in poultry, the objectives of this study were to evaluate the effect of oil source and peroxidation status on broiler performance and markers of oxidative stress.

MATERIALS AND METHODS

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

Animals and Housing

Day-old, Ross 308 (Welp Hatchery, Bancroft, IA) chicks were brooded in raised wire battery cages (Petersime Incubator Co., Gettysburg, OH) located within an environmentally controlled room. The chicks received supplemental heat starting at 35°C on 1 d of age, decreasing 2°C every week with light provided at 23L:1D. Chicks were allowed 3 d of acclimation to the batteries and fed a common starter diet during this acclimation period. On 4 d of age (day 0 of the experiment), chicks were individually wing banded, weighed (initial BW 85.1 ± 3.5 g), and randomly assigned to one of 8 dietary treatments. The experimental unit consisted of a battery cage of 5 broilers, with 5 replications for each treatment allotted to 40 battery cages for a total of 200 broilers. Broilers had ad libitum access to their experimental diets and water for the duration of the 20-d experimental period.

Diets

Dietary treatments were arranged in a 4 × 2 factorial within a completely randomized design. Factors consisted of 4 oil sources: a 5% inclusion of palm oil, soybean oil, flaxseed oil, or fish oil; in combination with peroxidation status: fresh or peroxidized oil. Prior to diet manufacturing, lipid peroxidation was achieved by placing 2.5 L of oil into a 5-L round-bottom glass flask and heating with an electric heating mantle with a power controller. Oils were thermally processed at 90°C for 72 h with a continuous infusion of air at a rate of 3 L/min, while the fresh oils had no thermal or air infusion treatment. To characterize the quality of oil used in each treatment, FA profile, oil quality, lipid peroxidation products, and total tocopherols were analyzed (Table 1), using methodologies shown in Table 2. Diets contained 5% of their respective fresh or peroxidized oil and were formulated to meet or exceed NRC (1994) nutrient recommendations (Table 3).

Data Collection

Broiler chicks were monitored daily over the duration of the experiment and on days 0 and 20 of the experiment (4 and 25 d of age); all broilers were individually weighed and ADG was calculated. Feed disappearance was measured on day 20 of the experiment (25 d of age) to calculate ADFI and used to calculate feed efficiency (G:F). During the experiment, non-treatment-related mortality included 1 bird in each of the following treatments: peroxidized soybean oil, fresh flaxseed oil, peroxidized flaxseed oil, fresh fish oil, and peroxidized fish oil diets, and 2 birds fed the peroxidized palm oil diets. Therefore, growth performance data was corrected accordingly for mortality. On day 21, 2 broilers per pen were euthanized via carbon dioxide asphyxiation, and blood was obtained via cardiac venipuncture using a 10-mL vacuum tube containing sodium heparin, which was then centrifuged at 2,500× *g* for 15 min at 4°C and plasma was harvested. Plasma samples were immediately frozen at −80°C and stored until subsequent analysis of Trp concentration and oxidative stress markers. In addition, a section of liver tissue from the right lobe was excised from each bird and snap-frozen in liquid nitrogen, transported on dry ice, and stored at −80°C until analysis for markers of oxidative stress.

Plasma Tryptophan

Plasma-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.

Oxidative Stress Markers

Multiple oxidative stress markers were measured in the plasma and liver obtained from the 2 randomly selected birds/cage using commercially available assay kits, each carried out according to the manufacturer's instructions (Cayman Chemical Company, Ann Arbor, MI) (Table 4). Assays were run in triplicate in 96-well microplates and intra-assay CV of ≤5.0%. Liver tissues were homogenized in their respective buffers and the supernatants were stored at −80°C and assayed within 1 mo of homogenization. Plasma and liver samples were assessed for TBARS, an indicator of lipid damage, where 100 mg of liver tissue was homogenized per mL of RIPA buffer (Cayman Chemical Co., Ann Arbor, MI; #10010263), centrifuged at 1,600× *g* for 10 min at 4°C and then the supernatant was used to run the assay or 100 μL of plasma was used in assays. No dilutions were required for plasma or liver quantification of TBARS, with values reported as μM of MDA. Protein damage was measured by PC concentration

Table 1. Compositional analysis of fresh and thermally processed oils.

	Palm oil		Soybean oil		Flaxseed oil		Fish oil	
Temperature, °C[1]	22.5	90	22.5	90	22.5	90	22.5	90
FA, % of total oil								
C14:0, Myristic	1.34	1.43	0.08	0.11	0.04	0.03	10.00	11.33
C16:0, Palmitic	46.41	49.07	11.09	15.83	5.21	6.55	20.16	22.80
C16:1, Palmitoleic	0.19	0.22	0.00	0.00	0.00	0.00	13.06	14.48
C 18:0, Stearic	4.56	4.80	4.51	6.45	3.84	4.74	3.78	4.28
C18:1, Oleic	38.48	39.17	23.89	30.52	15.70	18.66	5.75	6.36
C18:2, Linoleic	8.73	5.06	52.72	42.14	16.24	16.76	1.79	1.85
C18:3, Linolenic	0.29	0.24	6.86	3.73	58.56	52.89	1.97	1.92
C20:5, EPA[2]	0.00	0.00	0.00	0.00	0.00	0.00	16.91	14.27
C22:6, DHA[2]	0.00	0.00	0.00	0.00	0.00	0.00	16.55	13.18
UFA:SFA[2]	0.91	0.81	5.32	3.41	9.96	7.80	1.65	1.36
Double bonds[3]	57	50	150	126	224	211	212	181
AnV[2,4]	3.9	87.0	0.8	384.1	ND	ND	38.4	436.6
PV, mEq/kg[2]	15.1	607.4	15.0	616.2	11.2	128.2	15.0	20.4
PTAGS, %[2]	0.0	4.9	0.0	31.7	0.0	21.8	0.0	9.9
Total polar compounds, %[2]	13.2	29.6	4.7	61.4	10.7	50.2	5.3	30.0
Aldehydes, mg/kg	59.1	1327.2	14.4	1553.0	44.8	540.3	176.7	278.3
2,4-decadienal	1.2	84.5	2.0	555.2	0.5	89.7	1.1	4.5
4-hydroxynonenal	45.1	231.0	1.2	384.6	0.6	58.0	6.6	8.5
Acrolein	5.8	10.4	5.5	26.6	28.9	118.2	86.1	231.3
Decenal	0.2	197.0	0.2	88.6	1.8	11.7	0.3	0.8
Heptadienal	0.1	5.1	1.6	56.7	6.6	227.5	44.1	22.2
Heptanal	1.4	60.5	0.6	94.9	3.8	9.8	4.9	2.8
Hexanal	3.9	242.8	1.7	59.4	1.5	4.2	9.0	1.9
Octenal	0.6	277.2	0.4	134.8	0.5	5.6	2.7	1.8
Pentanal	0.5	23.0	0.9	15.7	0.5	1.2	21.8	3.2
Undecadienal	0.1	2.1	0.1	31.1	0.1	1.7	0.1	0.3
Undecenal	0.3	193.5	0.2	105.4	0.2	12.7	0.1	0.9
Ratio[5]	6.23	8.20	0.22	3.42	0.07	0.24	0.05	0.04
Total tocopherols, mg/kg	58	<10	802	37	267	<10	119	125
Alpha	58	<10	71	37	21	<10	119	125
Beta	<10	<10	<10	<10	<10	<10	<10	<10
Delta	<10	<10	196	<10	<10	<10	<10	<10
Gamma	<10	<10	535	<10	246	<10	<10	<10

[1]The temperature of 22.5°C was fresh oil and not subjected to heat, whereas 90°C oils were heated for 72 h with constant air flow (3 L/min).

[2]Abbreviations: EPA, eicosapentaneoic acid; DHA, docosahexaenoic acid; UFA:SFA, unsaturated fatty acid:saturated fatty acid ratio; PV, peroxide value; AnV, p-anisidine value; DDE, 2,4-decadienal; PTAGS, polymerized triglycerides; LOQ, limit of quantification; ND, p-anisidine value could not be detected in flaxseed oil because of color interference.

[3]The double bonds present in the experimental oils were calculated taking the number of double bonds in a FA multiplied by the analyzed FA composition.

[4]There is no unit for p-anisidine value.

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

Table 2. Method of oil analysis.

Analyte	Method
Aldehydes[1]	Wang et al. (2016)
p-Anisidine value[2]	AOCS Cd 18-90
Fatty acids[2]	AOCS Ce 1a-13
Peroxide value[2]	AOCS Cd 8b-90
Polymerized triacylglycerides[3]	AOAC 993.25
Tocopherols[3]	AOCS Ce 8-89
Total polar compounds[3]	AOCS Cd 20-91

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

in both plasma and liver samples. Briefly, 200 mg of liver tissue was homogenized per mL in 50 mM phosphate buffer containing 1 mM EDTA and centrifuged at 10,000× *g* for 15 min at 4°C, and then supernatant was assayed to determine PC concentration. No dilutions were required for plasma and liver quantification of PC with results expressed in nmol/mL.

Guanine is the nucleic acid base that is most prone to oxidative damage, with 8-OH-2dG from DNA being the form of oxidized guanine that is most commonly studied (Wu et al., 2004; Mateos and Bravo, 2007). Therefore, in the current experiment 8-OH-2dG in the plasma and liver was measured where 25 mg of liver was homogenized after which DNA was extracted using ZR Genomic DNA-Tissue MiniPrep (Zymo Research, Irvine, CA). Following DNA extraction, DNA yields were determined using Gen5 software on Cytation 5 Imaging Reader (BioTek, Winooski, VT) and DNA yields ranged from 20 to 40 μg DNA. DNA was then digested by nuclease P1 (Sigma-Aldrich, St. Louis, MO) to convert double-stranded DNA to single-stranded DNA, and then 1 unit of alkaline phosphatase (Roche Diagnostics, Mannheim, Germany) was added per 100 μg of DNA to convert nucleotides to nucleosides

Table 3. Ingredient and calculated composition of treatment diets, as-fed basis.[1]

Item, %	Percent
Corn	47.06
Soybean meal	43.41
Oil[1]	5.00
Dicalcium phosphate	1.83
Limestone	1.10
Vitamin mineral premix[2]	0.63
Sodium chloride	0.50
Methionine hydroxy analog	0.34
Choline chloride 60	0.10
L-threonine	0.03
TOTAL	100.00
Calculated composition	
ME, kcal/kg	3135
CP, %	25.1
Lys, %	1.42
Met + Cys, %	1.06
Thr, %	1.00
Ca, %	1.00
Available phosphorus, %	0.45

[1]Oil treatments included fresh and peroxidized palm oil, soybean oil, flaxseed oil, and fish oil. The temperature of 22.5°C was fresh oil and not subjected to heat, whereas 90°C oils were heated for 72 h with constant air flow (3 L/min). Analyzed crude fat for diets containing the 22.5 and 90°C for palm oil, soybean oil, flaxseed oil, and fish oil were 6.6 and 6.8%, 6.4 and 6.3%, 6.4 and 6.0%, and 4.8 and 5.1%, respectively.

[2]Provided the following per kilogram of diet: selenium, 250 μg; vitamin A, 8250 IU; vitamin D_3, 2750 IU; vitamin E, 17.9 IU; menadione, 1.1 mg; vitamin B_{12}, 12 μg; biotin, 41 μg; choline, 447 mg; folic acid, 1.4 mg; niacin, 41.3 mg; pantothenic acid, 11 mg; pyridoxine, 1.1 mg; riboflavin, 5.5 mg; thiamine, 1.4 mg; iron, 282 mg; magnesium, 125 mg; manganese, 275 mg; zinc, 275 mg; copper, 27.5 mg; iodine, 844 μg.

Table 4. Assay kits and dilution factors used to determine oxidative status in plasma and liver.[1]

Assay kit	Catalog Number[2]	Plasma Dilution factor	Liver Dilution factor
TBARS	7,00,870	ND	ND
PC	1,00,05,020	ND	ND
8-OH-2dG	5,89,320	1:75	ND
SOD	7,06,002	NA	1:10
GPx	7,03,102	1:20	1:20
CAT	7,07,002	NA	1:10,000

[1]Cayman Chemical Company (Ann Arbor, MI).

[2]TBARS, thiobarbituric acid reactive substances; 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.

and was stored at 4°C until use. The supernatant was assayed to determine the 8-OH-2dG of liver and no dilution was required; however, plasma was diluted 1:75 in sample buffer prior to assessment and both plasma and liver 8-OH-2dG is expressed in pg/mL.

To assess antioxidant machinery, SOD, GPx, and CAT activities were evaluated. Superoxide dismutase and CAT were measured in liver homogenates while GPx was measured in plasma 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, and centrifuged at 1,500× 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× g for 15 min at 4°C, and the supernatant was diluted 1:10,000 in sample buffer and assayed to determine CAT, which was expressed as nmol $min^{-1}mL^{-1}$. Glutathione peroxidase activity was measured in liver and plasma where 100 mg of liver tissue was homogenized in 1 mL of 50 mM Tris-HCl containing 5 mM EDTA and 1 mM DTT, and centrifuged at 10,000× g for 15 min at 4°C. Plasma and liver homogenates were diluted 1:20 in sample buffer prior to being assayed and were expressed as nmol min^{-1} mL^{-1}. Lastly, total antioxidant capacity was measured in plasma via ferric reducing antioxidant power (**FRAP**). 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 ascorbic acid standards (Benzie and Strain, 1996; Gabler et al., 2005). No sample dilution was necessary for this assay with results expressed as μM (FRAP value).

Statistical Analysis

One battery cage containing 5 birds was considered the experimental unit for broiler performance. For the analysis of plasma Trp and oxidative status in the plasma and liver, the 2 birds selected were averaged within pen, and considered as the experimental unit. As a consequence, there were 5 replicates per dietary treatment for all data. Data were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS, 2009) with means reported and separated using LSMEANS. The main effect of oil source and peroxidation status and their interaction were fixed effects, with cage as a random effect. If the overall model was significant at $P \leq 0.10$, comparisons were made using an unadjusted t-test, and discussed accordingly. In addition, the relationship between oil composition with growth performance and oxidative stress markers and the relationship between growth performance and oxidative stress markers were evaluated by simple linear correlation (Pearson correlation coefficients).

RESULTS AND DISCUSSION

Peroxidation Status and Diet Composition

The poultry industry utilizes a variety of oil sources such as palm, soybean, flaxseed, and fish oil as energy sources in diet formulation; however, oil quality can be compromised through lipid peroxidation. Lipid peroxidation can be achieved through thermal processing of oils rich in PUFA, resulting in a decrease in unsaturated fatty acids (**FA**), an increase in saturated FA,

and increased generation of lipid peroxidation products (Shurson et al., 2015). Lipid peroxidation occurs in 3 consecutive phases: initiation, propagation, and termination (Gutteridge, 1995) which produce and consume a variety of lipid peroxidation products, some of which may cause pathophysiological effects in cells and tissues (Esterbauer et al., 1991). Therefore, the current study was designed to evaluate oils with widely differentiating fatty acid compositions and differing in susceptibility to peroxidation based on the ability to uptake oxygen (Holman, 1954) on broiler performance and oxidative status.

As expected, FA analysis of the 4 oil sources (Table 1) shows that palm oil was high in palmitic acid (C16:0), soybean oil was high in linoleic acid (C18:2n6), flaxseed oil was rich in linolenic acid (C18:3n3), and fish oil was containing high amounts of the omega-3 long-chain FA eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**) (C20:5n3 and C22:6n3, respectively). Palm oil contained about 50% palmitic acid (C16:0); however, fish oil also contained a moderate amount of palmitic acid (20%). Although PUFA are susceptible to lipid peroxidation, the omega-3 fatty acids, linolenic acid, EPA, and DHA have been shown to mitigate oxidative stress and have beneficial effects on inflammation (Song and Horrobin, 2004; Gomaa and Abd El-Aziz, 2016). Because of this, how palm, soybean, flaxseed, and fish oils, fresh or thermally peroxidized could modulate in vivo markers of oxidative stress was of interest. To thermally peroxidize the oils, temperature and duration of thermal processing was based on past experiments including Liu et al. (2014b), who thermally processed 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 peroxide values (**PV**) and aldehydes such as 4-hydroxynonenal (**HNE**) and 2,4-decadienal (**DDE**).

As shown in Table 1, compared to their peroxidized counterpart, each fresh oil (i.e., 22.5°C) had a higher unsaturated to saturated fatty acid ratio (**UFA:SFA**) where the UFA:SFA ranged from 0.91 to 0.81 for palm oil, 5.32 to 3.41 for soybean oil, 9.96 to 7.80 for flaxseed oil, and 1.65 to 1.36 for fish oil, respectively. Although the UFA:SFA ratio is a fairly crude measure related to the degree of unsaturation and thus susceptibility to peroxidation, the degree of FA unsaturation in each oil (total number of double bonds) was also determined, and similar to the change in UFA:SFA, there was a consistent decrease in the total number of double bonds due to thermally processing each oil (Table 1). This was expected because the progression of lipid peroxidation involves the hydrogenation of FA, thereby decreasing the number of double bonds available for peroxidation (Holman, 1954; Yin et al., 2011).

Prior to diet formulation, the peroxidation status of the oils by measuring common lipid peroxidation products (Table 1) was also determined. As expected, PV, which measures hydroperoxides formed in the initiation phase of lipid peroxidation, increased for all thermally processed oil treatments compared to their fresh oil counterparts where PV increased by 592.3, 601.2, 117.0, and 5.4 mEq/kg for palm, soybean, flaxseed, and fish oil, respectively. The same trend was noted for p-anisidine value (**AnV**) which measures the molecular weight of saturated and unsaturated aldehydes. In addition, individual aldehydes including acrolein, hexanal, DDE, and HNE were all increased due to thermal processing. Total polar compounds (**TPC**), which measure monoglycerides, diglycerides, and free fatty acids, were analyzed, and polymerized triglycerides (**PTAGS**), which measure the molecular size of polymeric compounds formed in the tertiary phase of lipid peroxidation, were measured and both increased due to thermal processing, supporting the statement that the oils evaluated were peroxidized. These analyses of these peroxidized oils were similar to the changes in fatty acid composition and lipid peroxidation products reported previously (Engberg et al., 1996; Kerr et al., 2015) due to thermal processing.

Total tocopherols were measured, because depending upon the degree of processing vegetable oils contain natural antioxidants including tocopherols which protect the oil from autoxidation (Seppanen and Csallany, 2002). In the current experiment (Table 1), fresh soybean oil had the highest concentration of total tocopherols (802 mg/kg) followed by flaxseed oil (267 mg/kg), fish oil (119 mg/kg), and palm oil (58 mg/kg). Thermally processing the oils virtually depleted total tocopherol concentrations aside from fish oil, where the total tocopherol concentration of fresh fish oil was 119 and 125 mg/kg in the thermally processed oil. Miyagawa et al. (1991) reported that thermally processing a blend of soybean oil and rapeseed oil at frying temperature (185°C) resulted in a rapid degradation rate for gamma tocopherol followed by delta- and alpha-tocopherol, respectively. Based on these data, alpha-tocopherol has the greatest retention rate when thermally processed. This could explain why a drop in alpha-tocopherol level in processed fish oil and a less dramatic drop in the other oils was not observed.

Broiler Performance

The effects of oil source and peroxidation status on broiler performance over the 20-d performance period are reported in Table 5. An interaction ($P = 0.01$) between oil source and peroxidation status was noted for ADG where broilers fed peroxidized palm oil, soybean oil, and flaxseed oil had a 13.08%, 23.45%, and 22.95% reduction, respectively, in ADG compared to their fresh oil counterparts. However, peroxidation status did not affect ADG in broilers fed fish oil, thus driving the interaction. The lower ADG in broilers fed fish oil compared to the other oils tested in the current experiment is in contrast to Scaife et al. (1994), who reported similar weight gain in 56-day-old broilers fed fresh (e.g., unperoxidized) marine oil in comparison to broilers fed

Table 5. Effect of oil source and peroxidation status on broiler growth performance.[1]

Oil	Peroxidation[2]	ADFI, g	ADG, g	G:F, g:g
Palm	22.5	56.5^{a,b}	46.8^{a}	0.829^{a}
Palm	90	52.8^{b}	40.7^{b}	0.769^{b}
Soybean	22.5	57.4^{a}	47.0^{a}	0.819^{a}
Soybean	90	48.1^{c}	36.0^{c}	0.749^{b,c}
Flax	22.5	56.1^{a,b}	47.3^{a}	0.845^{a}
Flax	90	49.0^{c}	36.5^{c}	0.744^{b,c}
Fish	22.5	46.9^{c}	34.0^{c}	0.726^{c}
Fish	90	46.4^{c}	34.5^{c}	0.746^{b,c}
Statistics				
	SEM	1.26	1.20	0.015
Oil × Peroxidation, P value		0.01	0.01	0.01
Oil, P value		0.01	0.01	0.01
Peroxidation, P value		0.01	0.01	0.01
Main effect				
Oil	Peroxidation			
Palm		54.6^{a}	43.7^{a}	0.799^{a}
Soybean		52.7^{a}	41.5^{a}	0.784^{a}
Flax		52.6^{a}	41.9^{a}	0.794^{a}
Fish		46.6^{b}	34.3^{b}	0.736^{b}
	Fresh	54.2	43.8	0.805
	Peroxidized	49.1	36.9	0.752

[1]Data are means of 5 replications per treatments with 5 broilers/cage except for palm oil—22.5°C—which represents 4 replications. Average initial BW, 85.1 ± 7.8 g. The trial lasted 20 d (4 to 25 d of age). Superscripts reflect dietary treatment differences (abc, $P \leq 0.05$).

[2]The temperature of 22.5°C was fresh oil and not subjected to heat, whereas the 90°C oils were heated for 72 h with constant air flow (3 L/min).

tallow, rapeseed oil, and soybean oil. The reduction in ADG for broilers fed peroxidized oils in the current experiment is in agreement with Anjum et al. (2004) who reported a decrease in ADG of 4.2% in broilers fed peroxidized soybean oil in comparison to those fed fresh soybean oil. The current data are also in agreement with a review of 26 broiler experiments reviewed by Hung et al. (2017) who reported that broilers fed peroxidized oil had reduced ADG of 11.1% compared to birds fed their respective control (unperoxidized) oil.

A similar interaction ($P = 0.01$) between oil source and peroxidation status was noted for ADFI (Table 5), in which birds fed peroxidized soybean oil and flaxseed oil exhibited reduced ADFI (16.3 and 12.5% respectively, $P \leq 0.01$) compared to broilers fed the fresh oil counterparts, while birds fed peroxidized palm oil had only a 6.6% reduction in ADFI compared to broilers fed fresh palm oil ($P = 0.06$), but peroxidation status did not affect ADFI in broilers fed palm oil or fish oil. Consequently, broilers fed fresh fish oil appear to be the main factor driving the interaction that was observed, similar as that for ADG. The reduction in feed intake, especially in the fish oil treatment, could be a result in an increase in the dietary UFA:SFA. Crespo and Esteve-Garcia (2001) determined that increasing UFA:SFA in the dietary oils resulted in increased feed intake. Furthermore, broilers fed diets containing 4.2% redfish oil had reduced ADFI compared to a control group and groups fed redfish meal (Hulan et al., 1988). However, these results are in contrast to others (Scaife et al., 1994; Korver and Klasing, 1997; Farhoomand and Checaniazer, 2009; Lelis et al., 2009) who did not report differences in ADFI in broilers fed lipids which differed in fatty acid profiles, including fish oil. Peroxidation status also contributed to this interaction where broilers fed peroxidized oils had decreased ADFI, aside from broilers fed fish oil. This is in agreement with other studies that have shown decreased feed intake in birds fed peroxidized oils (Wang et al., 1997; Anjum et al., 2004; Tavárez et al., 2011). In the Hung et al. (2017) review, ADFI of broilers fed peroxidized oils was reduced by 6.6% in comparison to broilers fed fresh oil groups. While Hung et al. (2017) concluded that the reason ADFI decreased in broilers fed peroxidized oils was due to the PV of the oil, other lipid peroxidation products such as aldehydes, acids, ketones, and PTAGS could also contribute to the decreased ADFI as suggested by Shermer and Calabotta (1985).

An interaction ($P = 0.01$) was observed for G:F where broilers fed peroxidized palm, soybean, and flaxseed oils all had reduced G:F in comparison to their fresh oil counterparts, while broilers fed fish oil had no change in G:F, regardless of peroxidation status (Table 5). It would have expected broilers fed fish oil to respond similarly to the other treatment groups, but because this was not the case the interactions observed are apparently driven by broilers fed fresh fish oil. It is possible that the inclusion rate of 5% fish oil had a negative effect on diet palatability accounting for the reduction in growth performance in broilers fed fish oil. The oil source effect on G:F is supported by others (Pumchasov and Nir, 1992; Scaife et al., 1994; Crespo and Esteve-Garcia, 2001), who reported increased G:F when dietary PUFA intake increased in broiler diets. In contrast, Hulan et al. (1988) reported reduced feed efficiency in broilers fed 4.2% redfish oil. Furthermore, the effect of peroxidation on G:F is in agreement with others (Wang et al., 1997; Anjum et al., 2004; Tavárez et al., 2011) where broilers fed peroxidized oil sources had reduced G:F. This is supported in the review by Hung et al. (2017) who reported a 4.6% reduction in G:F in broilers fed peroxidized oil 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) which could account for the reduction in growth performance observed in broilers fed peroxidized oil sources.

Because growth performance parameters were affected by oil source and peroxidation status, a correlation analysis among multiple measures of lipid peroxidation products and performance parameters was conducted (Table 6). The UFA:SFA was positively correlated with ADFI, ADG, and G:F ($P \leq 0.10$), which was expected because as the unsaturation of fatty acids increases, they are more digestible because of their ability to form a micelle compared to saturated fatty acids (Wiseman and Salvador, 1991). This was also

Table 6. Pearson correlation coefficients among oil composition and lipid peroxidation products with growth performance.[1]

	Oil composition and lipid peroxidation products[2]											
Criterion	UFA:SFA	PV	AnV	TALD	ACR	HEX	DDE	HNE	Ratio	TPC	PTAGS	TOC
ADG	0.32	–	−0.59	−0.37	−0.61	–	−0.31	–	–	−0.49	−0.50	0.52
	(0.05)		(0.01)	(0.02)	(0.01)		(0.05)			(0.01)	(0.01)	(0.01)
ADFI	0.27	–	−0.60	−0.30	−0.62	–	−0.29	–	–	−0.45	−0.47	0.50
	(0.10)		(0.01)	(0.06)	(0.01)		(0.07)			(0.01)	(0.01)	(0.01)
G:F	0.33	–	−0.42	−0.36	−0.47	–	−0.27	–	–	−0.42	−0.43	0.42
	(0.04)		(0.01)	(0.02)	(0.01)		(0.10)			(0.01)	(0.01)	(0.01)

[1]Top value represents correlation (r value) and bottom value in parenthesis represents significance (P value). If no value is given, it was not found to be significant (–) at $P \leq 0.10$.

[2]UFA:SFA, unsaturated:saturated fatty acid ratio; PV, peroxide value; AnV, p-anisidine value; TALD, sum of 11 aldehydes; ACR, acrolein; HEX, hexanal; DDE, 2,4-decadienal; HNE, 4-hydroxynonenal; Ratio, ratio of aldehydes as described by Wang et al. (2016); TPC, total polar compounds; PTAGS, polymerized triglycerides; TOC, total tocopherols.

expected because as each oil was thermally processed, the UFA:SFA ratio decreased across all oils evaluated. In contrast to Hung et al. (2017), PV was not found to be correlated to any growth performance parameters, while AnV, total aldehydes, acrolein, DDE, TPC, and PTAGS were all negatively correlated to these growth parameters ($P \leq 0.10$). This was expected because these lipid peroxidation products were all increased due to thermal processing of the oils. Lastly, total tocopherols were positively correlated to ADG, ADFI, and G:F ($P \leq 0.01$), which was expected because thermal processing reduced tocopherol content in all oils evaluated, except for fish oil.

Relationship between Oil Source and Peroxidation on Plasma Trp

Plasma Trp is 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 (Baranyiova, 1991; Seve, 1999; Kerr et al., 2005). In growing broilers, it has been clearly shown that feeding a Trp-deficient diet can have profound effects on feed intake (Rosa et al., 2001; Corzo et al., 2005). 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 (Wang et al., 2018), it may be important to determine if there is a relationship between the consumption of peroxided SO and plasma Trp in young birds. As shown in Figure 1, there was an interaction between oil source and peroxidation status ($P = 0.01$), where birds fed peroxidized flaxseed oil had a reduced plasma Trp compared to birds fed fresh flaxseed oil, while there was no impact of feeding peroxidized palm oil, soybean oil, or fish oil on plasma Trp compared to birds fed these same oils that were not thermally stressed. This is in contrast to that reported in growing and finishing pigs (Lindblom et al., 2018; Overholt et al., 2018) where feeding peroxidized soybean oil resulted in reduced plasma Trp. No explanation for these differences could be put forth and no data to compare with in poultry could be found, suggesting additional research is needed in this area.

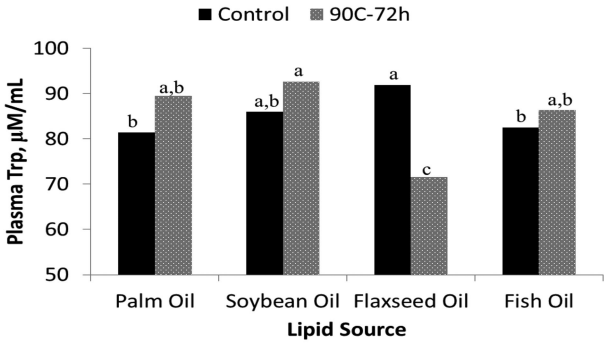

Figure 1. Plasma TRP as affected by oil source and lipid peroxidation status. Data represent the mean of 2 birds per cage and 5 cages per oil × peroxidation combination, with blood obtained on day 21 of the experiment (26 d of age) with birds having full access to feed and water. Pooled SEM = 3.05, oil × peroxidation, $P = 0.01$; oil, $P = 0.10$; peroxidation status, $P = 0.86$. Superscripts reflect dietary treatment differences (abc, $P \leq 0.05$).

Markers of Oxidative Stress

Markers of oxidative status as affected by oil source and peroxidation status were also evaluated. Increased oxidative stress has been shown to reduce animal growth, feed intake, and feed efficiency (Takahashi and Akiba, 1999; Tavárez et al., 2011; Boler et al., 2012). Oxidative stress occurs when free radical production overwhelms antioxidant production causing damage to lipids, proteins, and DNA. Because fatty acid profile (Liu et al., 2014b) and peroxidation status (Takahashi and Akiba, 1999) have been shown to affect oxidative status, measuring indices of lipid, protein, and DNA oxidative damage, as well as multiple enzymatic antioxidants in plasma (Table 7) were also conducted. Consumption of lipid peroxidation products results in the accumulation of aldehydes in the gastric lumen which are then absorbed through the small intestine where they are concentrated and metabolized in the liver (Kanazawa and Ashida, 1998). For this reason, and

Table 7. Effect of oil source and peroxidation status on broiler oxidative stress in plasma.[1]

Oil	Peroxidation[2]	TBARS[3] μM/mL	PC nmol/mL	8-OH-2dG pg/mL	GPx nmolmin^{-1}mL^{-1}	FRAP μM
Palm	22.5	12.0	10.1	10,161	2,733^{a,b,c}	257.2
Palm	90	11.9	12.0	12,981	2,074^{b,c}	192.8
Soybean	22.5	13.3	10.8	11,063	2,555^{a,b}	169.2
Soybean	90	13.5	17.0	19,034	1,734^{c}	162.5
Flax	22.5	15.3	11.3	17,265	3,149^{a}	195.4
Flax	90	16.1	12.9	17,059	1,798^{c}	165.8
Fish	22.5	16.4	16.5	10,165	2,067^{b,c}	229.7
Fish	90	18.6	14.6	14,199	2,119^{b,c}	162.9
Statistics						
SEM		1.27	1.86	1768	247	36.8
Oil × Peroxidation, *P* value		0.80	0.19	0.15	0.04	0.81
Oil, *P* value		0.01	0.09	0.01	0.45	0.44
Peroxidation, *P* value		0.39	0.15	0.01	0.01	0.12
Main effect						
Oil	Peroxidation					
Palm		11.9^{c}	11.1^{b}	11,571^{b}	2228	225.0
Soybean		13.4^{b,c}	13.9^{a,b}	15,048^{a,b}	2144	165.8
Flax		15.7^{a,b}	12.1^{a,b}	17,162^{a}	2473	180.6
Fish		17.5^{a}	15.6^{a}	12,182^{b}	2093	196.3
	Fresh	14.2	12.2	12,163	2538	212.9
	Peroxidized	15.0	14.1	15,818	1931	171.0

[1]Data are means of 5 replications per treatments with 2 broilers/cage except for fresh palm oil which represents 4 replications. Superscripts reflect dietary treatment differences (abc, $P \leq 0.05$).

[2]The temperature of 22.5°C was fresh oil and not subjected to heat, whereas 90°C oils were heated for 72 h with constant air flow (3 L/min).

[3]TBARS, thiobarbituric acid reactive substances; PC, protein carbonyls; 8-OH-2dG, 8-hydroxy-2′deoxyguanosine; GPx, glutathione peroxidase activity.

because a major function of the liver is detoxification of pro-oxidants, the measure of oxidative balance in the liver was also conducted (Table 8). Similar to the performance data, a correlation analysis was performed between oil composition and lipid peroxidation measures with oxidative stress markers in the plasma and liver (Table 9).

Thiobarbituric Acid Reactive Substances No significant interaction ($P = 0.80$) was observed between dietary oil source and peroxidation status on plasma TBARS (Table 7). Plasma TBARS were, however, affected by oil source ($P = 0.01$), where broilers fed fish oil had the greatest plasma TBARS concentration followed by flaxseed oil, soybean oil, and palm oil (17.5, 15.7, 13.4, and 11.9 μM/mL, respectively). It was hypothesized that plasma TBARS increased with increasing FA unsaturation of the oils of the treatment diets because the more polyunsaturated an oil is, the more susceptible it is to peroxidation (Holman, 1954; Yin et al., 2011). This was confirmed by a correlation analysis between plasma TBARS and double bonds in the dietary oil (r = 0.55, $P = 0.01$, data not shown). In comparison, Lin et al. (2006) induced oxidative stress in broilers via heat stress and observed increases in plasma TBARS. While it would have been expected that broilers fed peroxidized oils have increased plasma TBARS, the numerical increase observed in the current trial was not found to be significant ($P = 0.39$, Table 7). The current results are similar to Tavárez et al. (2011) who reported no differences in plasma TBARS concentration between broilers fed fresh and peroxidized soybean oil. Furthermore, Liu et al. (2014c) reported that oil source influenced serum TBARS in pigs, where pigs fed corn oil and canola oil had increased serum TBARS in comparison to pigs fed poultry fat and tallow. However, this is in contrast to other studies which have reported increases in plasma TBARS in broilers (Engberg et al., 1996; Takahashi and Akiba, 1999) and pigs (Liu et al., 2014c) fed peroxidized oils compared fresh oil treatments. Despite the lack of effect of peroxidation status on plasma TBARS, lipid peroxidation compounds in the dietary oil including PV, AnV, acrolein, hexanal, and HNE were correlated ($P \leq 0.05$) to plasma TBARS (Table 9).

There tended ($P = 0.09$) to be an interaction between oil source and peroxidation status for liver TBARS (Table 8). In general, broilers fed peroxidized palm oil had increased liver TBARS in comparison to broilers fed fresh palm oil (9.6 and 13.0 μM/mL, respectively), while the opposite effect was observed in broilers fed soybean oil (15.4 and 11.2 μM/mL for broilers fed fresh and peroxidized soybean oil, respectively), with no difference in liver TBARS noted between broilers fed flaxseed oil and fish oil due to thermal processing. There was a tendency ($P = 0.10$) for broilers fed flaxseed oil to have increased liver TBARS in comparison to broilers fed palm oil and fish oil with broilers fed soybean oil as intermediates (Table 8). This observation is supported by a positive correlation between UFA:SFA and liver TBARS (r = 0.39, $P = 0.02$). This agrees with

Table 8. Effect of oil source and peroxidation status on broiler oxidative stress in liver.[1]

Oil	Peroxidation	TBARS[2] μM/mL	PC nmol/mL	8-OH-2dG pg/mL	SOD U/mL	GPx nmolmin^{-1} mL^{-1}	CAT nmolmin^{-1} mL^{-1}
Palm	Fresh	9.6^{c}	96.5^{a,b,c}	163.7	165.9	2,592	26,248
Palm	Peroxidized	13.0^{a,b,c}	93.6^{a,b,c}	165.2	207.2	2,364	32,670
Soybean	Fresh	15.4^{a}	87.2^{b,c}	138.4	140.5	2,175	43,653
Soybean	Peroxidized	11.2^{b,c}	117.4^{a}	152.2	167.6	2,160	43,341
Flax	Fresh	14.0^{a,b}	112.0^{a,b}	192.0	151.0	2,134	51,372
Flax	Peroxidized	14.7^{a,b}	93.9^{a,b,c}	194.9	197.8	2,499	61,454
Fish	Fresh	11.2^{b,c}	79.6^{c}	175.21	152.4	2,081	25,907
Fish	Peroxidized	11.2^{b,c}	97.3^{a,b,c}	221.24	187.5	2,292	31,802
Statistics							
	SEM	1.44	8.87	22.79	24.06	302.96	9232
Oil × Peroxidation, P value		0.09	0.04	0.74	0.98	0.78	0.95
Oil, P value		0.10	0.32	0.08	0.61	0.73	0.02
Peroxidation, P value		0.98	0.29	0.33	0.03	0.70	0.40
Main effect							
Oil	Peroxidation						
Palm		11.3^{a,b}	95.1	164.5^{a,b}	186.6	2,478	29,459^{b}
Soybean		13.3^{a,b}	102.3	145.3^{b}	154.0	2,168	43,497^{a}
Flax		14.4^{a}	103.0	193.4^{a}	174.4	2,316	56,413^{a}
Fish		11.2^{b}	88.5	198.2^{a}	170.0	2,187	28,854^{b}
	Fresh	12.6	93.8	167.3	152.5	2,245	36,795
	Peroxidized	12.5	100.57	183.4	190.0	2,329	42,317

[1]Data are means of 5 replications per treatments with 2 broilers/cage except for fresh palm oil which represents 4 replications. Superscripts reflect dietary treatment differences (abc, $P \leq 0.05$).

[2]TBARS, thiobarbituric acid reactive substances; PC, protein carbonyls, 8-OH-2dG, 8-hydroxy-2-deoxy-guanosine; SOD, superoxide dismutase; GPx, glutathione peroxidase activity; CAT, catalase activity.

Table 9. Pearson correlation coefficients among oil composition and peroxidation measures with oxidative stress markers.[1]

	Oil composition and lipid peroxidation products[2,3]											
Criterion[2]	UFA:SFA	PV	AnV	TALD	ACR	HEX	DDE	HNE	Ratio	TPC	PTAGS	TOC
Plasma												
TBARS	–	−0.34 (0.04)	0.36 (0.05)	–	0.61 (0.01)	0.36 (0.02)	–	0.32 (0.05)	−0.52 (0.01)	–	–	–
PC	–	–	0.35 (0.07)	–	–	–	0.32 (0.05)	–	–	–	0.31 (0.06)	–
8-OH-2dG	0.37 (0.02)	0.33 (0.05)	0.58 (0.01)	0.38 (0.02)	–	–	0.46 (0.01)	0.37 (0.02)	–	0.55 (0.01)	0.56 (0.01)	–
GPx	0.28 (0.09)	−0.34 (0.04)	−0.34 (0.07)	−0.41 (0.01)	–	–	−0.36 (0.03)	−0.36 (0.03)	–	−0.47 (0.01)	−0.46 (0.01)	0.36 (0.03)
Liver												
TBARS	0.39 (0.02)	–	-	–	–	–	–	–	–	–	–	0.33 (0.04)
PC	–	–	0.40 (0.03)	–	–	–	0.36 (0.04)	0.30 (0.06)	–	0.30 (0.06)	0.30 (0.07)	–
8-OH-2dG	–	–	-	–	0.41 (0.01)	–	–	–	–	–	–	–

[1]Top value represents correlation (r value) and bottom value in parenthesis represents significance (P value). If no value is given, it was not found to be significant (–) at $P \leq 0.10$.

[2]UFA:SFA, unsaturated:saturated fatty acid ratio; PV, peroxide value; AnV, p-anisidine value; TALD, sum of 11 aldehydes; ACR, acrolein; HEX, hexanal; DDE, 2,4-decadienal; HNE, 4-hydroxynonenal; Ratio, ratio of aldehydes as described by Wang et al. (2016); TPC, total polar compounds; PTAGS, polymerized triglycerides; TOC, total tocopherols; TBARS, thiobarbituric acid reactive species; PC, protein carbonyls; 8-OH-2dG, 8-hydroxy-2′deoxy-guanosine; GPx, glutathione peroxidase activity.

[3]No correlations for plasma ferric reducing antioxidant power or liver GPx were observed. The only correlation for liver superoxide dismutase was with TOC (r = −0.30, $P = 0.07$), the only correlation for liver catalase was with UFA:SFA (r = 0.48, $P = 0.01$), and were not included in the table.

the plasma TBARS data where it was shown in Table 9, and is also supported by the plasma observed that an increase in TBARS with increasing number of double bonds present in the dietary oil. The main effect of peroxidation status did not affect liver TBARS ($P = 0.98$). These data agree with others (Takahashi and Akiba, 1999; Tavárez et al., 2011) who also observed similar liver TBARS concentrations in broilers fed peroxidized and fresh soybean oil in broilers.

Protein Carbonyls Increased ROS and PC concentration in mitochondria of breast muscle has been linked to low feed efficiency in broilers (Bottje et al., 2002;

Iqbal et al., 2004). In the current study, there was no interaction between oil source and peroxidation status on plasma PC ($P = 0.19$). However, plasma concentration of PC (Table 7) tended to differ among the oil sources ($P = 0.09$), with broilers fed fish oil having the greatest plasma PC concentration (15.6 nmol/mL) and broilers fed palm oil having the lowest plasma PC concentration (11.1 nmol/mL), with flaxseed oil and soybean oil having intermediate concentrations (12.1 and 13.9 nmol/mL, respectively). This result was similar to that of plasma TBARS leading us to speculate that the number of double bonds in the dietary oil played a role in the increase in plasma PC because lipid peroxidation can lead to protein oxidation (Fellenberg and Speisky, 2006). However, no correlation between plasma PC and UFA:SFA or with the number of double bonds was noted (Table 9). While peroxidation status numerically increased plasma PC, this increase was not found to be significant ($P = 0.15$). This result is in contrast to experiments in swine where consumption of peroxidized soybean oil (PV = 180 mEq/kg oil) increased plasma PC in comparison to pigs fed fresh soybean oil (Lu et al., 2014). Despite the fact that plasma PC concentration was not significantly increased in birds fed the peroxidized oils, plasma PC was positively correlated to lipid peroxidation products including AnV, DDE, and PTAGS (Table 9).

There was an interaction between oil source and peroxidation status observed in liver PC ($P = 0.04$; Table 8), where PC concentrations were significantly increased by 25.7% in broilers fed peroxidized soybean oil; however, no changes were observed in broilers fed peroxidized palm oil, flaxseed oil, or fish oil. Interestingly, the main effects of oil source and peroxidation status on liver PC were not found to be significant ($P = 0.32$ and $P = 0.29$, respectively). Lu et al. (2014) determined that feeding pigs peroxidized soybean oil increased in liver PC so it would have been expected a similar outcome. No explanation for these responses could be put forth and no published data are available to compare with results from the current experiment with. Similar to plasma PC, liver PC were correlated with peroxidation compounds including AnV, DDE, HNE, TPC, and PTAGS analyzed in the dietary oils.

8-hydroxy-2′-deoxyguanosine All cellular DNA components are susceptible to base pair damage due to ROS; however, out of the nucleic acids, guanine is the most readily oxidized (Wu et al., 2004; Mateos and Bravo, 2007), making 8-OH-2dG the most prevalent measurement for oxidative damage of DNA. No interactions between oil source and peroxidation status were observed for plasma 8-OH-2dG ($P = 0.15$) (Table 7). Plasma 8-OH-2dG was affected by both oil source ($P = 0.01$) and peroxidation status ($P = 0.01$). Broilers fed flaxseed oil had the greatest plasma 8-OH-2dG concentration (17,162 pg/mL) in comparison to broilers fed palm oil and fish oil (11,571 and 12,182 pg/mL, respectively), with broilers fed soybean oil being intermediate (15,048 pg/mL; Table 7). It was hypothesized that the UFA:SFA of the dietary oil may have played a role in the increase in plasma 8-OH-2dG which is supported by a positive correlation between plasma 8-OH-2dG and UFA:SFA (r = 0.37, $P = 0.02$). Furthermore, broilers fed peroxidized oil diets had greater plasma 8-OH-2dG concentration (15,818 pg/mL) compared to broilers fed fresh oils (12,163 pg/mL). This was also expected because DNA is sensitive to oxidative damage and is supported by the observation of multiple correlations among lipid peroxidation products and plasma 8-OH-2dG as shown in Table 9. Unfortunately, there are no published data in poultry was found regarding plasma 8-OH-2dG from which to compare this data with.

There was no interaction observed between oil source and peroxidation status for liver 8-OH-2dG ($P = 0.74$) (Table 8); however, there was a tendency ($P = 0.08$) for oil source to increase 8-OH-2dG. Broilers fed fish oil and flaxseed oil had the greatest liver 8-OH-2dG (198.2 and 193.4 pg/mL, respectively) in comparison to soybean oil (145.3 pg/mL), with palm oil being intermediate (164.5 pg/mL). It was hypothesized that the degree of unsaturation of the dietary oil influenced liver 8-OH-2dG. In contrast to plasma samples, peroxidation status did not affect liver 8-OH-2dG ($P = 0.33$). Interestingly, among all composition and lipid peroxidation products, acrolein was the only lipid peroxidation product positively correlated with liver 8-OH-2dG (Table 9). These data suggest that the antioxidant defense system was apparently functioning properly because there was minimal oxidative damage of lipids, proteins, and DNA in the liver.

Superoxide Dismutase, Glutathione Peroxidase, Catalase, and FRAP It was also of interest to examine SOD, GPx, and CAT activities to provide a better understanding of total antioxidant balance. There was no interaction noted between oil source and peroxidation status ($P = 0.98$) and no oil source effect ($P = 0.61$) on liver SOD. There was a peroxidation effect ($P = 0.03$) on liver SOD where broilers fed peroxidized oils had a 19.7% increase in SOD activity in comparison to broilers fed fresh oils. This is in agreement with Altan et al. (2003) who reported a 47.4% increase in plasma SOD in broilers that were exposed to heat as an inducer of oxidative stress in comparison to broilers under thermal neutral conditions. This is in contrast to Lin et al. (2006) who induced oxidative stress via heat exposure to broilers and reported no differences in liver SOD. Surprisingly, the only correlation for liver SOD was with total tocopherols (r = −0.30, $P = 0.07$; data not shown). It would have been expected that correlations between liver SOD and lipid peroxidation measures would have been expected because feeding peroxidized lipids increased liver SOD, but this was not the case and is a reminder that correlations are not a cause-and-effect relationship.

Glutathione peroxidase is a common enzymatic antioxidant studied in livestock (Altan et al., 2003; Boler et al., 2012). In the current study, there was an

interaction between oil source and peroxidation status ($P = 0.04$) on plasma GPx activity, where plasma GPx activity was decreased in broilers fed peroxidized palm oil, soybean oil, and flaxseed oil in comparison to their fresh oil counterparts, while peroxidation status had no effect on plasma GPx in broilers fed fish oil. Interestingly, this mimics the changes noted in total tocopherol concentrations of each oil and is supported by the correlation between total tocopherols and plasma GPx (r = 0.36, $P = 0.03$). Overall, oil source did not affect plasma GPx activity ($P = 0.44$), while feeding peroxidized oils reduced ($P = 0.01$) plasma GPx activity. This was expected because consuming lipid peroxidation products activates the antioxidant systems in vivo until the antioxidant system becomes overwhelmed, which explains why GPx activity was decreased in broilers fed peroxidized oils. This is in agreement with an experiment conducted in swine where pigs fed peroxidized oils had reduced plasma GPx activity (Boler et al., 2012); however, the opposite effect occurred in the plasma GPx activity of heat stressed broilers (Altan et al., 2003).

In the current experiment, there was no interaction between oil source and peroxidation status ($P = 0.78$) on liver GPx activity. Similarly, neither oil source nor peroxidation status had an effect on liver GPx activity ($P \geq 0.70$). Due to this lack of effect, it was not surprising that no correlations were noted between liver GPx activity and oil composition or lipid peroxidation products (data not shown). Because of the differential effects of peroxidation status on markers of oxidative stress in the liver, it may be postulated that the antioxidant defense system was not affected to any measureable degree.

Coordination between GPx and CAT is required for adequate clearing of H_2O_2 (Baud et al., 2004). In the current experiment, there was no interaction observed between oil source and peroxidation status for liver CAT ($P = 0.95$). Likewise, there was no effect of peroxidation status on liver CAT ($P = 0.40$); however, liver CAT was affected by oil source ($P = 0.02$). This is in contrast to Altan et al. (2003) who reported a 36.8% increase in plasma CAT activity in heat stressed broilers compared to birds at a thermal neutral temperature. The only correlation noted for liver CAT was with UFA:SFA (r = 0.48, $P = 0.01$) seems reasonable as oil source affected liver CAT activity. Because liver GPx was not affected by peroxidation status, it makes sense that liver CAT was also not affected. Catalase is similar to GPx in that it detoxifies H_2O_2 to produce H_2O; however, it is more efficient in comparison to GPx when H_2O_2 concentration is high (Finaud et al., 2006), suggesting that the antioxidant system was functioning properly. No data regarding the effect of oil source on catalase activity from which to compare the current data with could be found.

Lastly, FRAP in the plasma as a general indicator of total antioxidant capacity was also measured. There was no interaction noted between oil source and peroxidation status ($P = 0.81$), and plasma FRAP was not affected by either oil source or peroxidation status ($P \geq 0.12$). This is in contrast to Lin et al. (2006) who observed a decrease in plasma FRAP when broilers were under oxidative stress conditions due to exposure to heat. It would have been expected that FRAP would decrease in broilers fed peroxidized oils because of the observed increase in 8-OH-2dG and decrease in GPx activity in plasma.

Table 10. Pearson correlation coefficients of broiler performance with measures of oxidative stress in plasma.[1]

	TBARS[2]	PC[2]	8-OH-2dG[2]	GPx[2]
ADG	−0.35	−0.48	–	0.44
	(0.03)	(0.01)		(0.01)
ADFI	−0.42	−0.43	–	0.45
	(0.01)	(0.01)		(0.01)
G:F	–	−0.45	–	0.33
		(0.01)		(0.04)

[1]Top value represents correlation (*r* value) and bottom value in parenthesis represents significance (*P* value). If no value is given, it was not found to be significant (–) at $P \leq 0.10$.

[2]Plasma TBARS, thiobarbituric acid reactive species; PC, protein carbonyls; 8-OH-2dG, 8-hydroxy-2′deoxy-guanosine; GPx, glutathione peroxidase activity.

[3]The only correlation noted in the liver oxidative stress markers was for liver TBARS was with ADFI (r = 0.28, $P = 0.09$) so all liver oxidative stress markers were removed from the table.

Relationship Between Growth Performance and Markers of Oxidative Stress

To relate the effects of dietary oil quality to oxidative stress and broiler performance, an additional correlation analysis between broiler growth performance and markers of oxidative stress (Table 10) was conducted. Interestingly, there were no correlations between growth performance and markers of oxidative stress in the liver apart from liver TBARS which tended to be correlated to ADFI (r = 0.28, $P = 0.09$). Therefore, markers of oxidative stress in liver were not reported in Table 10. Correlations were observed between growth performance and oxidative stress markers in plasma suggesting that feeding peroxidized oils elicits a response on oxidative stress in plasma which thereby affects animal performance. Plasma PC were negatively correlated and GPx was positively correlated with all measurements of growth performance, while plasma TBARS was negatively correlated to ADG and ADFI (Table 10). In contrast, plasma 8-OH-2dG was not correlated with any growth performance measures ($P \geq 0.11$).

CONCLUSIONS

Results in the present study reveal that feeding 5% palm, soybean, flaxseed, and fish oils in either a fresh or peroxidized state differentially affected broiler growth performance and markers of oxidative stress in the plasma and liver. The observed correlations between oil composition with growth performance and oxidative stress markers imply that it is worthwhile to measure multiple lipid peroxidation products, namely UFA:SFA,

AnV, DDE, TPC, and PTAGS as observed in the current experiment, because they were most consistently correlated to measures of both growth performance and oxidative stress. The data also suggest that growth performance is affected by oxidative stress as measured by plasma measures of TBARS, PC, and GPx. Collectively, these data provide insight on the implications of feeding multiple oil sources and peroxidation state on successfully inducing metabolic oxidative stress, and consequently negatively affecting growth performance.

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