Effects of thermally oxidized canola oil and tannic acid supplementation on nutrient digestibility and microbial metabolites in finishing pigs¹

Bonjin Koo and Charles Martin Nyachoti²

Department of Animal Science, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

ABSTRACT: The present study investigated the effects of oxidized canola oil and tannic acid (TA) supplementation on nutrient digestibility and microbial metabolites in finishing pigs. Four experimental diets were formulated to include 5% of either fresh canola oil (0 mEq/kg of peroxide value) or oxidized canola oil (180 mEq/kg of peroxide value). An equal portion of each diet was supplemented with 0.1% TA. Eight pigs (initial body weight of 82.89 ± 2.26 kg) were surgically fitted with a T-cannula at the distal ileum. At the beginning of the experiments, all pigs were fed a nitrogen-free diet for 7 d. Then, they were assigned to 4 dietary treatments according to an incomplete replicated 4×3 Latin square design with 3 periods to give 6 replicates per treatment. Each experimental period lasted for 9 d, starting with a 5-d adaptation period followed by 2 consecutive 2-d periods for fecal (day 6 and day 7) and ileal digesta (day 8 and day 9) collection. The inclusion of oxidized oil reduced ($P < 0.05$) the standardized ileal digestibility (SID) of Pro and the apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of NDF. Also, pigs fed the oxidized oil tended to have lower ($P \leq 0.10$)

lactate and ammonia-nitrogen concentrations in the ileum and lower ($P \leq 0.10$) acetate and propionate concentrations in the feces than those fed the fresh oil. The supplementation of 0.1% TA reduced ($P < 0.05$) the AID of NDF and the ATTD of gross energy, crude protein, and NDF. Additionally, the TA-supplemented diet tended to have a lower ($P \leq 0.10$) digestible energy content than the nonsupplemented diet. Pigs fed the TA-supplemented diet showed reduced ($P < 0.05$) AID and SID of His, Cys, and Pro compared with those fed the nonsupplemented diet. There were interactive effects ($P < 0.05$) of oxidized oil and TA supplementation on acetate, isovalerate, total short chain fatty acids, and pH level in the ileum. In conclusion, the results indicated that the effects of oxidized oil are greater on microbial activities rather than nutrient digestibility. Although negative effects of dietary TA supplementation were observed in relation to nutrient digestibility, TA supplementation was found to ameliorate these negative effects of oxidized oil on microbial metabolites. Therefore, further studies are required to investigate the interaction between dietary oxidized oil and TA in relation with the gut microbiota in pigs.

Key words: microbial metabolites, nutrient digestibility, oxidized oil, pigs, standardized ileal digestibility, tannic acid

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²Corresponding author: Martin.Nyachoti@umanitoba.ca

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INTRODUCTION

Vegetable oils are widely used as a supplemental lipid in swine diets. Also, the soaring prices of conventional ingredients have encouraged the use of alternative feed ingredients, such as flaxseed meal and distiller's dried grains with

solubles (Woyengo et al., 2014), resulting in high unsaturated fatty acid content in diets (Eastwood et al., 2009; Stein and Shurson, 2009). Because unsaturated fatty acids are prone to oxidation (Benzie, 1996), it is highly probable that the fatty acids in the ingredients or diet are oxidized during the fermentation, drying, and pelleting processes or during storage (Shurson et al., 2015). Thus, pigs are more likely to consume oxidized oils that generate an assortment of peroxidation products (Liu et al., 2014b). Peroxidation products such as peroxides, aldehydes, polymers, and polar compounds increase epithelial cell death, damage intestinal structures (Rosero et al., 2015), and modify amino acid (AA) transporters (Lv et al., 2012), possibly reducing nutrient digestibility. The chemical reaction of lipid peroxidation products and dietary nutrients may reduce nutrient utilization in pigs (Nielsen et al., 1985). Furthermore, modified nutrient residues and dietary lipid peroxidation products may negatively affect microbial fermentation and microbial metabolites in the gut (Bomba et al., 2002). However, to our knowledge, no previous studies have examined the effects of oxidized oil on nutrient digestibility at the ileal level or on microbial metabolites.

Gallotannins, commonly called tannic acids (TA), are a type of polyphenols showing strong antioxidative capacity against lipid peroxidation (Nakamura et al., 2003). Also, due to their ability to bind protein, TA can directly interact with bacterial cell membranes and exhibit antimicrobial properties, reducing the production of toxic metabolites such as ammonia and biogenic amines in the gut (Huang et al., 2017). However, there are very limited studies on the effects of dietary TA supplementation in pigs.

Therefore, it was hypothesized that oxidized oil in a diet would have negative effects on nutrient digestibility and supplemental TA would ameliorate the negative effects in finishing pigs. The objective of this study was to investigate the effects of oxidized oil and TA supplementation in a swine diet on nutrient digestibility and microbial metabolites.

MATERIALS AND METHODS

All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Committee (AC11013), and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

Lipid Preparation and Experimental Diets

Food-grade canola oil (Loblaw Companies Limited, Brampton, ON, Canada) was obtained from a local store. To oxidize fresh canola oil, a stainless steel laboratory pot containing the required amount of canola oil was placed on a heating block along with an immersed oil thermometer. Fresh canola oil (0 mEq/kg of oil) was heated at 95 °C with a constant air flow of 60 L/min for 27 h until reaching the targeted peroxide value (180 mEq/kg of oil). Fresh oil and processed oils were stored at 4 °C to minimize unexpected peroxidation before feed mixing. Two experimental diets were formulated to include either 5% oxidized canola oil or fresh canola oil (Table 1). An equal portion of each diet was supplemented with 0.1% TA (gallotannins extracted from gall nuts; Sigma-Aldrich, St. Louis, MO) to give a total of 4 experimental diets. Also, a cornstarch-sucrose-based nitrogen (N)-free diet was formulated.

Animals, Housing, and Experimental Design

Eight pigs (Terminal offspring of Landrace × Large White sows sired by Large White × Pietrain; Topigs Norsvin, Winnipeg, MB, Canada) with an initial body weight of 82.89 ± 2.26 kg were individually housed in plastic-covered woven metal floor pens (1.47×1.14 m) in a temperature-controlled room (23–24 °C). Following the 5-d presurgical period, pigs were fitted with a T-cannula at the distal ileum. After surgery, pigs were cared for as described by Koo et al. (2018) and were allowed a 7-d recovery period before starting the experiment. Each pen was equipped with a stainless steel feeder and a nipple drinker that allowed ad libitum access to water. At the beginning of the experiment, all pigs were fed a N-free diet for 7 d to determine basal endogenous losses of AA and calculate standardized ileal digestibility (SID) of AA in experimental diets. Then, they were allocated to 4 dietary treatments according to an incomplete replicated 4×3 Latin square design with 3 periods to give 6 replicates per treatment. Each experimental period lasted for 9 d, starting with a 5-d adaptation period followed by 2 consecutive 2-d periods for the fecal (day 6 and day 7) and ileal digesta (day 8 and day 9) collection. Daily feed allowance was set at 3.5 times the energy requirement for maintenance ($106 \text{ kcal ME/kg BW}^{0.75}$) per day, which was close to the voluntary feed intake. All pigs were weighed at the beginning of each period. Two equal feed portions were provided in the form of a dry mash at 0800 and 1600 h.

Sample Preparation and Experimental Procedure

Freshly voided fecal samples were collected twice daily using the grab method from the cleaned pen floor on day 6 and day 7 in each period, transferred to plastic bags, and immediately frozen at -20°C . Rectal massage was performed to collect fresh fecal samples for analysis of short chain fatty acids (SCFA). These subsamples were transferred to sterile bags, immediately snap frozen in liquid N, and transferred to a -80°C freezer where they were stored until required. Fecal pH was determined immediately after defecation. Ileal digesta to be used for the digestibility assessment were collected from 0800 to 2000 h in plastic bags containing 10 mL of 10% (v/v) formic acid to minimize bacterial fermentation. Two bags of ileal digesta were collected at 1100 h and 1400 h without formic acid; these subsamples were used for pH determination, and the remaining samples were snap frozen in liquid N and transferred to a -80°C freezer where they were stored until required for SCFA analysis. Samples for digestibility assessment were removed when the bags (dimension; 3×7 inches) were filled with approximately 75% ileal digesta and immediately frozen at -20°C . The pH of the ileal digesta was measured by directly submerging the pH probe, whereas the pH of the fecal samples was measured by thoroughly mixing equal amounts of feces with deionized water in 50-mL tubes and submerging the pH probe (AB15 plus, Fisher Scientific, Toronto, ON, Canada). The ileal digesta and fecal samples were thawed and independently pooled for each pig per period. Samples were homogenized, and subsamples were taken for further processing. The ileal digesta samples were lyophilized. The fecal samples were dried in a forced air oven at 60°C for 3 d. Diet, ileal digesta, and fecal samples were ground to pass through a 1-mm sieve before chemical analysis.

Chemical Analyses

All samples were analyzed for DM, OM, GE, ether extracts (EE), NDF, CP, and titanium dioxide. Diet and ileal samples were further analyzed for AA. Diet and fecal samples were also analyzed for Ca and P contents. Dry matter was determined according to official method 934.01 of the Association of Official Analytical Chemists (AOAC, 2006). Nitrogen content was determined using a combustion analyzer (model CNC-2000; Leco Co., St. Joseph, MI; method 984.13A-D) and was then used to calculate the CP concentration ($\text{N} \times 6.25$). The GE was determined using an isoperibol bomb

calorimeter (Parr Instrument Co., Moline, IL) that was previously calibrated using benzoic acid as the standard. Samples for titanium analysis were ashed and digested according to the method proposed by Lomer et al. (2000) and read on an inductively coupled plasma spectrometer (Vista-MPX, Varian Canada Inc., Mississauga, ON, Canada). The content of the EE was determined using the hexane extraction method 920.39A of the AOAC (2006). The Ca (method 968.08) and P (method 946.06) concentrations were analyzed according to the AOAC (2006) and read on a Varian inductively coupled plasma mass spectrometer (Vista-MPX). The NDF contents were analyzed according to the method of Goering and van Soest (1970) using α -amylase (Sigma number A3306, Sigma Chemical Co., St. Louis, MO) and sodium sulfite and were corrected for ash using an adapted Ankom 200 Fiber analyzer (Ankom Technology, Fairport, NY). Ash content was determined according to official method 942.05 of the AOAC (2006). Organic matter was calculated as DM minus ash. The AA composition was determined after acid hydrolysis according to method 994.12 of the AOAC (2006). Total sulfur AA content was determined after performic acid oxidation followed by acid hydrolysis (AOAC, 2006; method 985.18). Tryptophan was not determined. The AA in samples was analyzed using an AA analyzer (SYKAM, Eresing, Germany). The SCFA concentrations were determined using gas chromatography (Varian Chromatography System, model Star 3400; Varian Medical Systems, Palo Alto, CA) with a capillary column (30×0.5 mm; Restek Corp., Bellefonte, PA) according to the method described by Erwin et al. (1961). Briefly, 1 mL of 25% metaphosphoric acid was mixed with 5 mL of digesta fluid in a 15-mL centrifuge tube, and the mixture was frozen overnight. Samples were then thawed, neutralized with 0.4 mL of 25% NaOH, and vortexed. Thereafter, 0.64 mL of 0.3 M oxalic acid was added to the samples, which were vortexed again. The samples were then centrifuged for 20 min at $3,000 \times g$ at 4°C , and 2 mL of the supernatant was transferred into a gas chromatography vial for SCFA analysis. Ammonia-N concentration in feces and digesta samples was determined using the method described by Novozamsky et al. (1974). Briefly, 1.5 mL of a reagent containing 200 mL of 0.05% sodium nitroprusside and 10 mL of 4% ethylenediaminetetraacetic acid was added to 50 μL of sample in a 10-mL test tube. A solution containing 10% sodium hypochlorite (2.5 mL) was then added to the mixture. Test tubes containing the resulting mixture were placed in a test tube rack

Table 1. Ingredients and nutrient composition of experimental diets¹, % (as-fed basis)

Ingredient	Fresh	Oxidized	Nitrogen free
Corn	71.6	71.6	-
Soybean meal	20.0	20.0	-
Corn starch	-	-	66.6
Sucrose	-	-	20.0
Cellulose	-	-	5.0
Fresh canola oil	5.0	-	5.0
Oxidized canola oil	-	5.0	-
Limestone	1.0	1.0	1.0
Monophosphate	0.8	0.8	0.8
Salt	0.3	0.3	0.3
Vitamin-mineral premix ²	1.0	1.0	1.0
Titanium dioxide	0.3	0.3	0.3
Total	100	100	100
Nutrient composition ³			
Net energy, Mcal/kg	2.66	2.66	-
Gross energy, Mcal/kg	4.11	4.12	4.01
Crude protein	14.47	14.50	0.07
Ether extracts	6.35	6.36	4.51
Indispensable AA			
Arg	0.30	0.30	-
His	0.79	0.80	-
Ile	0.59	0.54	-
Leu	0.33	0.35	-
Lys	1.20	1.25	-
Met	0.70	0.73	-
Phe	0.26	0.27	-
Thr	0.65	0.64	-
Val	0.44	0.47	-
Dispensable AA			
Ala	0.93	0.93	-
Asp	1.54	1.58	-
Cys	0.26	0.26	-
Glu	2.72	2.79	-
Gly	0.53	0.53	-
Pro	0.93	0.95	-
Ser	0.88	0.89	-
Tyr	0.43	0.43	-

¹Fresh canola oil (0 mEq/kg of peroxide value) was heated to 95°C and was oxidized by continuously bubbling air into the oil at a rate of 80 L/min to reach 180 mEq/kg of peroxide value (9 mEq/kg in a diet). Peroxide values were determined according to AOCS (2007) methods. Each diet was supplemented with either 0 or 0.1% of tannic acid (gallotannin extracted from gall nuts; Sigma-Aldrich, St. Louis, MO).

²Supplied the following per kilogram of diet: 2,000 IU vitamin A, 300 IU vitamin D, 11 IU vitamin E, 4 mg vitamin K, 1 mg biotin, 500 mg choline chloride, 1 mg folic acid, 22.5 mg niacin, 10 mg pantothenic acid, 4 mg riboflavin, 1.5 mg thiamin, 4.5 mg vitamin B6, 20 µg vitamin B12, 10 mg copper as copper sulphate, 0.4 mg iodine (as potassium iodine), 100 mg iron as ferrous sulfate, 10 mg of manganese (as manganese oxide), 0.3 mg selenium as sodium selenite; 100 mg of zinc as zinc oxide.

³Analyzed values except for net energy.

and incubated in complete darkness for 30 min, and then the mixture was immediately read at 630 nm. Peroxide values were determined according to the

acetic acid chloroform method of the American Oil Chemists' Society (AOCS, 2007).

Calculation and Statistical Analyses

Apparent total tract digestibility (ATTD), apparent ileal digestibility (AID), endogenous loss of AA at the distal ileum, and SID were calculated using the equations provided by Koo et al. (2018). Hindgut disappearance was calculated as ATTD minus AID. Ammonia-N concentration was determined by calculating the concentration from a regression equation of the standard curve ($R^2 = 0.99$) of ammonia sulfate (range: 0–30 mg/L). All data were analyzed using the MIXED procedure (version 9.4; SAS Inst. Inc., Cary, NC), with each animal serving as the experimental unit. The model included oxidized oil, TA supplementation, and their interaction as the fixed variables and period as the random variable. The LSMeans statement along with the Tukey-adjusted PDIF option was used to calculate and separate the mean values of each dietary treatment. Differences were considered significant at $P < 0.05$, and trends ($0.05 \leq P \leq 0.10$) were also presented.

RESULTS

All pigs were healthy and readily consumed their daily feed allowance throughout the experiment.

Apparent Ileal Digestibility, Apparent Total Tract Digestibility, Hindgut Disappearance, and Digestible Energy

Dietary inclusion of oxidized oil versus fresh oil did not affect the ATTD or AID of the DM, OM, GE, CP, and EE (Table 2). Also, no difference in the digestible energy (DE) content was found between the oxidized oil and fresh oil diets. However, less AID and ATTD of NDF were observed ($P < 0.05$) in pigs fed the oxidized oil diet compared with those fed the fresh oil diet. Dietary TA supplementation did not affect the AID of DM, OM, GE, CP, or EE, but did reduce ($P < 0.01$) the AID of NDF (Table 2). Dietary TA supplementation lowered ($P < 0.05$) the ATTD of GE, CP, and NDF by 1.5, 3.5, and 8.7%, respectively. Similarly, pigs fed the diet supplemented with TA tended to show reduced ($P = 0.06$) ATTD of DM and OM compared with those fed the diet without TA. A trend towards lower ($P = 0.08$) hindgut disappearance of CP was observed in pigs fed the TA supplemented diet than those fed the nonsupplemented diet. The diet supplemented with TA tended to have lower ($P = 0.09$)

Table 2. Effects of oxidized oil and tannic acid on apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) and hindgut disappearance of nutrient in pigs

	Experimental diets ¹				SEM	<i>P</i> -value		
	Fresh		Oxidized			Oil	TA	Oil × TA
	(−)	(+)	(−)	(+)				
AID, %								
DM	74.6	74.9	76.3	73.6	1.16	0.87	0.32	0.21
OM	76.8	77.3	78.5	75.9	1.06	0.88	0.32	0.17
GE	76.5	77.1	78.2	75.8	1.09	0.86	0.42	0.19
CP	75.9	76.6	77.9	74.6	1.14	0.97	0.25	0.10
Ether extracts	86.5	86.0	84.6	85.8	0.75	0.18	0.69	0.28
NDF	36.2	27.5	30.2	13.7	4.05	<0.05	<0.01	0.27
ATTD, %								
DM	88.5	87.8	88.3	86.2	0.68	0.22	0.06	0.35
OM	90.2	89.6	90.0	88.1	0.62	0.19	0.06	0.35
GE	88.5	87.7	88.3	86.1	0.69	0.20	0.04	0.34
CP	87.6	85.0	88.1	83.7	1.00	0.67	<0.01	0.38
Ether extracts	73.8	73.9	73.1	72.7	1.38	0.52	0.91	0.87
NDF	63.5	57.4	55.6	44.2	3.13	<0.01	<0.05	0.42
Calcium	47.3	43.5	46.5	40.2	3.91	0.60	0.21	0.76
Phosphorus	37.2	36.8	40.9	32.3	3.13	0.90	0.17	0.20
Hindgut disappearance, %								
DM	13.9	12.8	12.0	12.6	0.93	0.27	0.83	0.36
OM	13.4	12.3	11.5	12.2	0.85	0.25	0.83	0.30
GE	12.0	10.6	10.1	10.3	0.84	0.20	0.47	0.35
CP	11.7	8.4	10.2	9.1	1.18	0.74	0.08	0.37
Ether extracts	−11.6	−12.2	−11.5	−12.0	1.40	0.94	0.72	0.96
NDF	27.4	29.9	30.3	34.1	4.13	0.40	0.45	0.88
Digestible energy, Mcal/kg								
Ileal	3.14	3.17	3.21	3.13	0.045	0.81	0.56	0.22
Total tract	3.64	3.61	3.63	3.55	0.028	0.24	0.09	0.42

¹Fresh = 5 % of fresh canola oil; oxidized = 5 % of oxidized canola oil (180 mEq/kg of peroxide value); (+) = with 0.1% of tannic acids (gallotannin extracted from gull nuts); (-) = without tannic acids.

total tract DE than the nonsupplemented diet. No interactions between the oil source and the TA supplementation were observed in relation with nutrient digestibility, hindgut disappearance, or DE content (Table 2).

Apparent Ileal Digestibility and Standardized Ileal Digestibility of Amino Acids

Endogenous losses of AA (mg/kg of DM intake) were as follows: 18,008 for CP, 478 for Arg, 1,433 for His, 185 for Ile, 414 for Leu, 364 for Lys, 107 for Met, 224 for Phe, 457 for Thr, 332 for Val, 669 for Ala, 676 for Asp, 234 for Cys, 834 for Glu, 1,504 for Gly, 4453 for Pro, 594 for Ser, 176 for Tyr. Inclusion of 5% oxidized oil in place of fresh oil did not affect the AID (Table 3) nor the SID (Table 4) of AA with the exception of Pro, which was reduced ($P < 0.05$). Dietary TA supplementation decreased ($P < 0.05$) the AID and SID of His, Cys,

and Pro. No interactive effects of oxidized oil and TA supplementation were found on the AID or SID of the AA.

Short Chain Fatty Acid and Ammonia-Nitrogen Concentrations and pH Values in Ileal Digesta and Feces

Interactive effects of oil type and TA supplementation were observed ($P < 0.05$) on the concentrations of acetate, isovalerate, total volatile fatty acids, total SCFA, and pH in ileal digesta (Table 5). Pigs fed the oxidized oil diet tended to have lower ($P \leq 0.10$) acetate and propionate concentrations in feces in addition to lower ($P \leq 0.10$) lactate and ammonia-N concentrations in ileal digesta than those fed the fresh oil diet. Dietary TA supplementation tended to decrease ($P \leq 0.10$) the acetate and total volatile fatty acids concentrations and to increase the lactate concentration in ileal digesta.

Table 3. Apparent ileal digestibility (AID) of amino acids (AA) in pigs fed oxidized oil diet with or without tannic acid

	Experimental diets ¹				SEM	<i>P</i> -value		
	Fresh		Oxidized			Oil	TA	Oil × TA
	(−)	(+)	(−)	(+)				
Indispensable AA								
Arg	87.6	87.6	89.0	87.6	0.90	0.45	0.44	0.47
His	64.7	60.9	63.2	55.4	2.52	0.18	<0.05	0.44
Ile	76.5	78.5	76.9	81.2	2.09	0.46	0.15	0.59
Leu	84.0	84.0	85.5	84.6	0.94	0.27	0.66	0.63
Lys	81.2	82.0	82.1	83.2	1.54	0.51	0.54	0.94
Met	85.1	85.0	87.5	85.2	0.80	0.12	0.14	0.17
Phe	83.8	85.1	85.2	85.6	1.14	0.44	0.48	0.72
Thr	72.1	73.4	74.4	75.9	1.78	0.19	0.43	0.97
Val	76.9	73.1	78.1	78.0	2.32	0.21	0.42	0.44
Dispensable AA								
Ala	80.5	80.8	81.9	80.9	1.33	0.57	0.77	0.63
Asp	81.8	82.3	83.1	82.8	1.14	0.44	0.91	0.73
Cys	75.2	71.3	75.7	72.9	1.39	0.47	<0.05	0.71
Glu	86.6	86.0	87.1	86.3	0.80	0.63	0.40	0.92
Gly	73.4	71.0	74.6	72.9	2.13	0.48	0.34	0.85
Pro	83.5	79.9	83.0	75.4	1.74	0.15	<0.01	0.23
Ser	81.1	80.8	81.9	81.3	1.16	0.60	0.71	0.89
Tyr	82.2	84.8	84.9	84.3	1.12	0.32	0.36	0.18

¹Fresh = 5 % of fresh canola oil; oxidized = 5 % of oxidized canola oil (180 mEq/kg of peroxide value); (+) = with 0.1% of tannic acids (gallotannin extracted from gull nuts); (-) = without tannic acids.

Table 4. Standardized ileal digestibility (SID) of amino acids (AA) in pigs fed oxidized oil diet with or without tannic acid¹

	Experimental diets ²				SEM	<i>P</i> -value		
	Fresh		Oxidized			Oil	TA	Oil × TA
	(−)	(+)	(−)	(+)				
Indispensable AA								
Arg	93.4	92.7	94.6	92.8	0.90	0.48	0.17	0.51
His	87.1	82.8	86.8	79.5	2.52	0.47	<0.05	0.55
Ile	81.9	83.3	82.4	85.4	2.09	0.55	0.30	0.72
Leu	87.2	87.1	88.6	87.5	0.94	0.32	0.52	0.58
Lys	86.0	86.6	86.9	87.4	1.54	0.58	0.72	0.97
Met	88.8	88.9	91.1	88.9	0.80	0.17	0.22	0.17
Phe	87.2	88.0	88.5	88.5	1.14	0.43	0.75	0.75
Thr	81.9	82.4	83.7	84.3	1.78	0.31	0.76	0.99
Val	83.4	79.9	84.4	84.0	2.32	0.30	0.41	0.51
Dispensable AA								
Ala	87.1	87.2	88.7	87.2	1.33	0.57	0.62	0.56
Asp	85.9	86.2	87.2	86.5	1.14	0.49	0.88	0.68
Cys	83.1	79.9	84.1	80.9	1.39	0.46	<0.05	1.00
Glu	89.5	88.7	89.9	88.9	0.80	0.70	0.29	0.87
Gly	99.5	96.4	101.2	97.5	2.13	0.53	0.12	0.88
Pro	127.3	122.8	126.3	116.8	1.74	0.04	<0.01	0.14
Ser	87.4	86.8	88.2	87.1	1.16	0.67	0.50	0.82
Tyr	86.2	88.2	88.7	88.0	1.12	0.33	0.57	0.24

¹Values for SID were calculated by correcting apparent ileal digestibility values for basal endogenous losses (mg/kg of DMI), which were determined by feeding a 5%-casein diet used to quantify endogenous CP and AA losses: CP, 18,008; Arg, 478; His, 1,433; Ile, 185; Leu, 414; Lys, 364; Met, 107; Phe, 224; Thr, 457; Val, 332; Ala, 669; Asp, 676; Cys, 234; Glu, 834; Gly, 1,504; Pro, 4453; Ser, 594; Tyr, 176.

²Fresh = 5 % of fresh canola oil; oxidized = 5 % of oxidized canola oil (180 mEq/kg of peroxide value); (+) = with 0.1% of tannic acids (gallotannin extracted from gull nuts); (-) = without tannic acids.

Table 5. Short chain fatty acids (SCFA), and ammonia-nitrogen concentration, and pH in fresh feces and ileal digesta of pigs fed oxidized oil diet with or without tannic acid

	Experimental diets ¹				SEM	<i>P</i> -value		
	Fresh		Oxidized			Oil	TA	Oil × TA
	(−)	(+)	(−)	(+)				
Ileal SCFA ² , mmol/L								
Acetic acid	28.54 ^x	17.13 ^y	20.44 ^{xy}	22.44 ^{xy}	2.463	0.58	0.07	< 0.05
Propionic acid	1.89	0.33	0.36	0.81	0.791	0.46	0.43	0.21
Butyric acid	0.79	0.55	0.70	0.94	0.133	0.25	0.98	0.07
Isovaleric acid	0.23 ^x	0.11 ^y	0.16 ^{xy}	0.21 ^x	0.023	0.53	0.21	< 0.01
Valeric acid	0.12	0.07	0.01	0.01	0.059	0.17	0.65	0.64
Lactate	23.75	26.50	16.47	23.64	2.731	0.07	0.08	0.41
Total VFA ³	31.57 ^x	18.12 ^y	21.63 ^{xy}	24.09 ^{xy}	2.661	0.47	0.05	< 0.05
Total SCFA	55.32 ^x	40.82 ^{xy}	35.63 ^y	47.73 ^{xy}	4.350	0.16	0.79	< 0.05
Fecal SCFA, mmol/L								
Acetic acid	41.70	40.07	31.80	28.87	5.663	0.08	0.69	0.91
Propionic acid	16.37	15.10	12.93	12.20	1.770	0.09	0.58	0.88
Isobutyric acid	3.64	3.80	3.56	3.16	0.423	0.41	0.79	0.52
Butyric acid	7.99	9.78	7.87	7.88	1.280	0.41	0.46	0.46
Isovaleric acid	5.21	5.59	5.32	4.79	0.605	0.58	0.90	0.46
Valeric acid	1.61	1.37	1.46	1.30	0.184	0.50	0.22	0.80
Lactate	1.69	0.44	1.28	1.00	0.498	0.87	0.14	0.35
Total VFA ³	74.91	75.24	62.93	58.20	9.004	0.12	0.81	0.78
Total SCFA	76.59	75.68	64.22	59.21	9.183	0.13	0.75	0.83
Ammonia-nitrogen, mg/L								
Ileal	96	98	88	82	6.5	0.08	0.79	0.58
Fecal	456	520	535	474	54.7	0.75	0.97	0.24
pH								
Ileal	7.11 ^x	7.38 ^{xy}	7.38 ^y	7.19 ^{xy}	0.103	0.70	0.72	< 0.05
Fecal	6.13	6.31	6.36	6.28	0.101	0.29	0.62	0.21

¹Fresh = 5 % of fresh canola oil; oxidized = 5 % of oxidized canola oil (180 mEq/kg of peroxide value); (+) = with 0.1% of tannic acids (gallotannin extracted from gull nuts); (-) = without tannic acids.

²Iso-butyric acid was not detected.

³VFA, volatile fatty acids.

^{xy}Means in the same row with different superscripts differ ($P < 0.05$).

DISCUSSION

Rising feed costs have forced producers to find alternative feed ingredients, such as distiller's dried grains with solubles (Woyengo et al., 2014); these substitutes possibly increase dietary unsaturated fatty acids, thereby elevating the incidence of dietary lipid peroxidation (Stein and Shurson, 2009). In swine production, alternative feed ingredients are also more likely to be fed to finishing pigs because of their greater utilization of fibrous ingredients, and because of the more significant feed consumption requirements of older animals as compared with younger pigs (Shi and Noblet, 1993; NRC, 2012). Therefore, it is of interest to further investigate the effects of dietary lipid peroxidation in finishing pigs in particular.

Thermally oxidized oil has been used to investigate the effects of dietary lipid peroxidation (or fat quality in feed ingredients) on growth performance and the antioxidative defense system in pigs (Liu et al., 2014b; Lu et al., 2014a; Lindblom et al., 2018). Peroxide values have been generally used as an indicator of peroxidation or fat quality during the initial stage of lipid peroxidation (Shurson et al., 2015). It was found that pigs fed a diet containing 5% oxidized oil with a peroxide value of 180 mEq/kg underwent oxidative stress, showing increased concentrations of thiobarbituric acid reactive substances in serum and of stress-related enzymes in plasma (Lu et al., 2014b). Thus, in the present study, to investigate the effects of dietary lipid peroxidation, which can induce oxidative stress, fresh canola oil, a common oil source in

North America, was thermally oxidized to a peroxide value of 180 mEq/kg and included in a conventional swine diet. The complete diet contained 9 mEq/kg of peroxide value.

Several studies have focused on nutrient digestibility to explain reduced growth performance in animals fed an oxidized oil-containing diet, but the results were controversial. Engberg et al. (1996) reported that broilers fed a diet containing oxidized oil (17 mEq/kg of diet) showed significantly lower GE and EE retention compared with those fed a diet containing fresh oil diet. Also, in weanling pigs fed a diet containing oxidized fish oil (39 mEq/kg of diet), the ATTD of CP was reduced by 10% compared with pigs fed a fresh oil diet (Yuan et al., 2007). Similarly, Nielsen et al. (1985) found that AA utilization was inhibited when dietary proteins reacted with oxidized lipids (methyl linolenate) in rats. The authors postulated that reduced nutrient digestibility may be attributable to the chemical reaction of lipid peroxidation products with dietary nutrients, impaired gut function caused by oxidative stress, polymers produced during lipid peroxidation, and modification of the fatty acid profile of oil. In contrast, other studies with nursery pigs (DeRouchey et al., 2004; Liu et al., 2014a) showed that nutrient and energy digestibility (ATTD of DM, GE, or EE) were not affected by a diet containing oxidized oil (4–24 mEq/kg of diet). The latter findings are in agreement with our results: No differences were observed in pigs fed a fresh oil diet of an oxidized oil diet (9 mEq/kg of diet). It appears that pigs tend to respond to dietary oxidized oil in a dose-dependent manner. This is supported by the study of Lindblom et al. (2018), wherein a diet containing 10% oxidized soybean oil (10 mEq/kg of diet) did not affect nutrient and energy digestibility in growing pigs, but 15 mEq/kg in the diet significantly decreased the ATTD of EE, N retention, the DE/GE, and the metabolizable energy/DE compared with a fresh oil diet. Apart from the degree of lipid peroxidation, animals may also differentially respond to dietary oxidized oil in relation to age (body weight), physical condition, weather (heat or cold stress), dietary antioxidant levels, and oil source (fatty acid profile). It should be noted that the SID of Pro was reduced by the inclusion of oxidized oil, suggesting that oxidized oil may have increased the secretion of mucin, which is rich in Pro, thereby reducing Pro digestibility. This is supported by Jüngst et al. (2007), who found that biliary mucin hypersecretion was promoted by systemic lipid peroxidation in humans. The close linkage between lipid peroxidation and biliary

mucin has been extensively researched in humans (Sreejayan and von Ritter, 1999). However, further research is needed that focuses on the endogenous AA originated from mucin in association with oxidized oil consumption in pigs.

Interestingly, the results for NDF digestibility were similar to those for the concentration of microbial metabolites (lactate, acetate, propionate, and ammonia-N) in the gut, as both were lower in the oxidized oil diet. Greater SCFA production is generally considered beneficial because SCFA play important trophic roles in the proliferation of epithelial cells, antibacterial roles in the gut, and serve as an additional energy source for intestinal epithelial cells and peripheral tissue (Montagne et al., 2003). To our knowledge, there are no previous studies on the interaction between dietary oxidized oil and fiber digestibility or gut microbiota. Thus, a clear explanation for our results is not available. However, considering that intestinal microbes preferably ferment carbohydrates that produce SCFA in the gut or alternatively ferment proteinaceous compounds releasing ammonia-N in the gut (Wenk, 2001), our results may be strongly correlated with microbial alteration or digesta transit time in the gut. Previous studies (DeRouchey et al., 2004; Liu et al., 2014b) discuss the increasing ratio of unsaturated and saturated fatty acids, and the shorter carbon chain of corn oil or canola oil, during lipid peroxidation. This may affect gastric mobility and the intestinal passage ratio, possibly changing the rate of NDF fermentation in the gut. This is supported by the slowing gastric emptying rate for the digestion of lipids with longer carbon chains (Hunt and Knox, 1968) and the stimulation of cholecystokinin, a key hormone that reduces intestinal mobility (Maher and Clegg, 2018). Furthermore, oxidized oil is associated with toxic products (e.g., acrolein, 4-hydroxynonenal, and malondialdehyde) resulting from the degradation of lipid peroxides (Shurson et al., 2015). These products may serve as oxidants, inducing oxidative stress in gut microbiota and inhibiting their activities in the gut, as reported previously by Perez et al. (2008) and Joshi et al. (2011). However, a microbial assay was not carried out in the present study and therefore further studies will be required to establish the interaction between oxidants and gut microbiota in pigs.

Dietary tannins, mostly originating from tannin-rich ingredients (e.g., sorghum and pulses), are regarded as an antinutritional factor, mostly because of their ability to precipitate dietary proteins in the gut, thus reducing CP digestibility in pigs and poultry (Nyachoti et al., 1997; Woyengo and

Nyachoti, 2012; Pan et al., 2016). However, there is a growing interest in plant secondary metabolites (Nyachoti et al., 2018) such as tannins because of their antibacterial, antidiarrheal, antioxidant, and anti-inflammatory effects (Huang et al., 2017). Other studies (Lu et al., 2014a, 2014b) reported that dietary supplementation with propyl gallate successfully quenched the free radicals of oxidized oils. The supplementation also ameliorated the negative effects of oxidized oil on growth performance and the antioxidant defense system in pigs. In the present study, TA, hydrolysable polymers of gallates, were used as an antioxidant source (Arapitsas, 2012). However, because of the lack of information on the antioxidant effects of TA in pigs, the dose of TA administered in the current study was determined through antimicrobial studies, finding that 0.1% of hydrolysable tannins could beneficially modify gut microbiota or microbial metabolites in pigs (Biagi et al., 2010; Lee et al., 2010) and chicken (Jamroz et al., 2009).

In contrast to our expectation, no substantial detrimental effects of oxidized oil on nutrient digestibility were observed. Rather, TA supplementation reduced digestibility of energy and selected AA (His, Cys, and Pro). It has been well documented that animals, particularly herbivores, release tannin-binding salivary proteins (e.g., Pro-rich proteins and histatins) to save dietary protein as a first line of defense against dietary tannins (Mueller-Harvey, 2006). Because the phenolic groups of tannins bind very tightly to proteins by forming hydrogen bonds with the NH groups of peptides, they have high affinities for Pro and His (Chung et al., 1998). Therefore, it appears that TA supplementation stimulated the release of salivary proteins, resulting in their interaction and an elevation in endogenous losses rather than direct inhibition of dietary AA digestion. Considering that cystatins also account for salivary proteins along with α -amylase, proline-rich proteins, histatins, and immunoglobulin A (Fábán et al., 2012), cystatins may have been stimulated with other salivary proteins and reduced the degradation of cysteine-containing proteins due to their strong inhibitory action on cysteine proteases (Fábán et al., 2012). It should be noted that tannins directly form complexes with cell wall components (e.g., pectin, cellulose, and lignin; Chung et al., 1998). Specifically, hydrogen bonds are formed between the hydroxyl groups of tannins and the oxygen atoms of the glycosidic linkages of polysaccharides (Jakobek, 2015). The formation of these complexes may reduce the number of binding sites

upon which microbial extracellular enzymes can act, thereby reducing the digestibility of cell wall components. Aside from the direct interaction between NDF and TA, TA possibly reduce microbial activities in the gut. Indeed, TA are known to possess antimicrobial properties, inhibiting extracellular microbial enzymes, metabolic oxidative phosphorylation, and directly binding to microbial cell membranes, thereby modifying cell wall morphology and increasing membrane permeability (Huang et al., 2017). This may be one contributing factor to our findings of the reductions in NDF digestibility as well as microbial metabolite production (SCFA and ammonia-N) in the gut. This is partially supported by Beauchemin et al. (2007), who found that ruminal acetate and ammonia-N production was linearly reduced by increasing levels of tannin extract (0, 1, and 2% of quebracho tannin extract). However, it seems that TA selectively bind or inhibit microbes because the production of ileal lactate, mostly produced by lactic acid bacteria, was enhanced by TA supplementation, as Pietro et al. (2008) and Jamroz et al. (2009) confirmed their selective antimicrobial property. Additional studies should be performed to elucidate the effect of dietary TAs on gut microbiota and their activities.

Interactive effects of oxidized oil and TA supplementation were found for SCFA concentration and pH levels in the ileum. It appears that dietary TA counteract the effects of oxidized oil on SCFA concentration in the ileum. The underlying reasons for these apparent interactions are not clear. One possible reason is that dietary TA donates hydrogen atoms to free radicals rather than binding with microbial membranes, thereby promoting microbial fermentation and SCFA production. This is partly supported by Nakamura et al. (2003), who found that TA exhibited strong antioxidative effects against lipid peroxidation and the free radicals generated as a result. Ileal pH values were reduced inversely with SCFA concentration in ileum, suggesting that SCFA production leads to an acidic gut environment.

In conclusion, our results showed that the inclusion of oxidized canola oil in place of fresh canola oil in a swine diet (9 mEq/kg of diet) has a negligible effect on nutrient and energy digestibility. Microbial fermentation seemed to be negatively affected by oxidized oil in the diet. Although TA supplementation decreased the ATTD of nutrients and energy, it may be beneficial in terms of the microbial metabolites modified by oxidized oil in the gut, promoting SCFA production and an acidic

environment in the ileum. However, further studies are needed to investigate the effects of dietary oxidized oil on the composition of gut microbiota and the interaction between lipid peroxidation products and dietary antioxidants such as TA.

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