ABSTRACT

CHANG, PETRA L.Y.C. Strategic use of Lipids to Improve Health and Productivity in Pigs and Sows. (Under the direction of Dr. E. van Heugten).

The use of lipids in swine diets has many facets yet to be explored. Lipid quality is often disregarded and its effects on pig health are not fully understood. Moreover, the role of dietary essential fatty acids in sow reproduction and piglet health is an intricate topic that needs to be further investigated. To address these current issues, four experiments were conducted.

The objective of experiment 1 was to determine the effects of peroxidized oil and antioxidant supplementation on growth performance, oxidative status and response to vaccination of nursery pigs. One hundred and seventy-six nursery pigs were randomly assigned to a 2 x 2 factorial arrangement; factors consisted of peroxidation status of corn oil and antioxidant supplementation. Pig performance, serum malondialdehyde concentration and response to vaccine were not affected by peroxidized corn oil or supplementation of antioxidant during the 31 d feeding period. Serum vitamin E concentrations decreased (interaction, $P < 0.001$) progressively in pigs fed peroxidized oil and increased ($P < 0.001$) with antioxidant supplementation.

In experiment 2, a large field study was conducted to determine the impact of lipid peroxidation on performance, health, medical treatment, viability and oxidative status of nursery pigs in a dose-dependent manner. A total of 2,200 nursery pigs were fed dietary treatments with 5 degrees of lipid peroxidation: 0, (diet with 5% control oil), 25, 50, 75, and 100% (diet with peroxidized corn oil) peroxidation during 43 d. Pig feed efficiency and final pen gain at the end of the study decreased linearly ($P < 0.05$) with increasing dietary peroxidation level. Antibody titers to vaccination and oxidative stress markers in serum did not change due to peroxidation of the diet. Vitamin E concentrations and total antioxidant capacity in serum decreased ($P = 0.05$) linearly with increasing level of peroxidation. Percentage of pigs removed due to sickness or size, pigs treated and culled increased linearly ($P < 0.05$) with increasing level of peroxidation, while percentage of full-value pigs decreased linearly ($P < 0.05$) with increasing dietary peroxidation level.

Experiment 3 aimed to determine and verify the impact of linoleic acid supplementation on reproductive performance of sows under practical field conditions and evaluate the impact of commercial antioxidants on oxidative stress markers and sow

performance. Six hundred and five sows were randomly allotted to a 2 x 2 factorial design; factors consisted of levels of linoleic acid (LA; 1.4 or 3.3%) and antioxidant supplementation (0 or 0.1%). Sows were fed dietary treatments from farrowing until weaning; sows were tracked during lactation and until their subsequent cycle. Feed efficiency was improved (P = 0.03) in the 1.4% LA treatment, but daily gain and feed intake were not different during lactation. Litter performance during lactation was not impacted by sow dietary treatment, and serum total antioxidant capacity was higher (P = 0.02) in sows fed 1.4% LA compared to sows fed 3.3% LA. Vitamin E concentrations in serum were highest in mature sows fed 1.4% LA compared to other treatments (interaction, $P < 0.01$). Wean-to-estrus interval, wean-to-farrow interval, percentage of sows bred and sows returning to estrus were not affected by dietary treatment. Culling rate increased (interaction, $P < 0.02$) in young sows fed 3.3% LA with antioxidant. Total pigs born, number of pigs born alive and stillborn pigs in the subsequent cycle of sows were not affected by dietary treatment.

In experiment 4, sixty lactating sows were fed supplemental arachidonic acid enriched oil (ARA; 1 vs 2%) or eicosapentaenoic acid rich oil (EPA; 1 vs 2%) compared to a control palm kernel oil treatment for two weeks prior to weaning. The objectives of the study were to determine the dose and duration of ARA and EPA supplementation required to efficiently enrich sow milk and intestinal tissue of suckling piglets and to determine its effects on pre- and post-weaning performance of pigs in a commercial production environment. Milk ARA concentrations increased progressively by day and with increasing levels of ARA (interaction, $P < 0.04$). Milk EPA and docosahexaenoate (DHA) increased ($P < 0.001$) with increasing levels of dietary EPA treatment. Fatty acid profile in the ileum of piglets was measured weekly while sows were receiving dietary treatments and one week after supplementation had ended. Concentrations of EPA and DHA in intestinal mucosa of pigs from sows fed EPA increased in the first two weeks of supplementation and declined post-weaning (interaction, $P < 0.001$). Concentrations of ARA in intestinal mucosa of piglets increased ($P < 0.001$) with sow ARA supplementation. Sows fed control palm kernel oil and 1% EPA treatments weaned heavier piglets and these pigs had improved ADG at weaning compared to pigs from sows fed 2% ARA ($P < 0.05$). Pig body weight and daily gain post-weaning were not affected by dietary supplementation of the sow. Mortality, total pig gain and weight produced at the end of the study were not affected by fatty acid supplementation.

In conclusion, these studies demonstrated that peroxidation of lipids decreased pig health and productivity under field conditions; sow subsequent performance was not improved by increasing linoleic acid intake; and, lastly, sow supplementation of long chain polyunsaturated fatty acids influenced piglet intestinal fatty acid composition, but these did not impact pig performance. Further research with dietary supplementation of essential fatty acids is encouraged to determine levels and duration of feeding necessary to improve sow and litter performance.

Strategic use of Lipids to Improve Health and Productivity in Pigs and Sows

by

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BIOGRAPHY

Petra Lee Yu Caetano Chang was born on March 3rd, 1986 in Campinas, Brazil. She received her B.Sc. degree in Agriculture Engineering in 2010 by the Luiz de Queiroz College of Agriculture at the University of Sao Paulo, Brazil. In 2009 she spent 8 months doing an internship with Murphy-Brown LLC in a sow farm in North Carolina. In the Spring of 2011 she was admitted at North Carolina State University to pursue her studies as a MS student in the Department of Animal Science under the direction of Dr. Eric van Heugten and Dr. Chad Stahl. She graduated in January of 2013 and continued her Ph.D. under the direction of Dr. Eric van Heugten. Her research explored various uses of lipids in swine nutrition.

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CHAPTER I: LITERATURE REVIEW

INTRODUCTION

Lipids are commonly added to swine diets as a means to increase energy density and improve feed efficiency. Lipid supplementation to diets improves pellet quality, reduces dust and improves palatability, while also being a source of essential fatty acids and improving absorption of fat-soluble vitamins. Supplementation of up to 6% of the diet is commonly acceptable and does not interfere with feed handling characteristics (NRC, 2012). Under heat stress, addition of lipids to diets improves growth performance of pigs (Stahly, 1984; Spencer et al., 2005), a feat which can be attributed to the low heat increment of lipids.

Nonetheless, energy is the most expensive component of swine diets, therefore the inclusion of lipids depends on its cost per energy content and its impact on growth performance of pigs. Because corn is the main source of energy in swine diets, economic evaluations should consider the price of lipid relative to the price of corn (van Heugten et al., 2015).

Commercial lipid sources available to swine vary greatly in quality and composition (Shurson et al., 2015) and these can negatively impact animals. Special consideration will be given to peroxidation of lipids and its detrimental effects in pigs.

Concurrently, there has been increasing interest in specific lipids, such as fish oil, for their potentially therapeutic effects. These effects pertain to their fatty acid composition, more specifically omega-3 fatty acids, and their derivatives as eicosanoids. These compounds have shown to exert anti-inflammatory effects and have been studied extensively (Simopoulos, 1991). Additionally, essential fatty acids found in vegetable oils are precursors to omega-6 eicosanoids, and these signal a wide variety of biological functions in animals. This review will highlight research conducted in swine fed these essential fatty acids and their longer chain derivatives.

LIPIDS

Lipids comprise a diverse class of molecules which are often insoluble in water, but highly soluble in nonpolar organic solvents. Lipids have many biological functions, including formation of membrane bilayers, energy storage, as hormone molecules, vitamins and eicosanoids. Fatty acids (FA) are simple lipids consisting of a polar head and a hydrocarbon tail, while the main components of lipid bilayers have a phosphoric acid residue attached to the carbon tail and are referred to as phospholipids (Horton et al., 2006).

Fatty acid nomenclature is defined by the length of the hydrocarbon chain, degree of unsaturation (number and position) and stereochemistry of double bonds (most naturally occurring FAs are in the *cis* configuration) with reference to its carboxylic end (Δ designation). Furthermore, an n- or ω- (omega) designation indicates the position of the first double bond relative to the methyl end (Das, 2006). For example, arachidonic acid (ARA) can be identified as 20:4n-6 Δ5, 8, 11, 14, as depicted below:

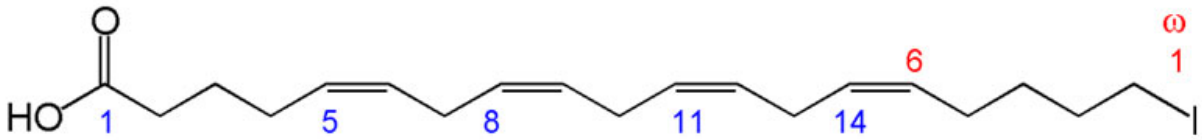

Figure 1. Arachidonic acid (ARA) structure.

Fatty acid synthesis

In mammals, synthesis of FA occurs in the cytosol and involves 2 main enzymes: acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) complex. The biotin-dependent ACC enzyme catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, a reaction requiring ATP. The FAS complex contains a transacylase, which produces malonyl-ACP (acyl carrier protein, ACP). Next, a synthase (3-ketoacyl ACP synthase, KAS) condensates acetyl-ACP and malonyl-ACP to form acetoacetyl-ACP. Subsequent reduction followed by a dehydration and another reduction takes place, and the newly formed acyl-ACP molecule is then condensed again with another malonyl-ACP molecule, and the sequence of reactions is repeated until 16 carbon palmitoyl-ACP is assembled. A thioesterase cleaves off the fatty acid, which needs to be activated to acyl-CoA in an ATP-dependent reaction catalyzed by acyl-CoA synthetase to be further metabolized (Horton et al., 2006).

To produce longer and unsaturated FAs, elongases and desaturases are employed mainly in the endoplasmic reticulum of animals. Mammals cannot insert double bonds past the Δ9 carbon, but plants have Δ12 and Δ15 desaturases that give rise to linoleic acid (LA, 18:2n-6 Δ9, 12) and α-linolenic acid (ALA, 18:3n-3 Δ9, 12, 15), respectively. Thus, these are referred to as essential fatty acids.

Fatty acid β-oxidation

Activated fatty acyl-CoA can be degraded in the mitochondria and peroxisomes two carbons at a time, in a process called β-oxidation. This process initiates with the oxidation of carbons 2 (α) and 3 (β) by acyl-CoA dehydrogenase, forming Δ^2- trans-enoyl-CoA. Hydration by Δ^2- enoyl-CoA hydratase produces 3-hydroxyacyl-CoA, which is further oxidized by L-3-hydroxyacyl-CoA dehydrogenase to form 3-ketoacyl-CoA. Finally, the latter is cleaved to yield acetyl-CoA and a fatty acyl-CoA two carbons shorter than the original. The newly formed acyl-CoA goes on to another cycle of β-oxidation until the entire molecule has been converted to acetyl-CoA (Botham and Mayes, 2009b). Acetyl-CoA is oxidized in the citric acid cycle, releasing two molecules of CO_2.

Oxidation of unsaturated fatty acids requires two additional enzymes, an isomerase and a reductase. These enzymes are necessary to convert the *cis* configuration to *trans,* so it can be used by the hydratase.

Odd-chain fatty acids are oxidized to acetyl-CoA molecules until propionyl-CoA is left, which can be converted to succinyl-CoA and enter the citric acid cycle. β-oxidation of fatty acids produces great amounts of energy yielding $FADH_2$ and NADH during the oxidation steps, and these can be further oxidized through the electron-transport chain to yield ATP.

Additionally, translocation of fatty acyl-CoA into the mitochondrial matrix is carnitine dependent. Carnitine-palmitoyltransferases (CPT) catalyze the transfer of acyl residues to carnitine and back to acyl-CoA, so acylcarnitine is carried into the matrix while carnitine is carried out (Botham and Mayes, 2009b).

Lipid absorption and transport

Dietary lipids are often in the form of triacylglycerols, more than 95% of which are absorbed by combined action of the stomach, intestines, liver and pancreas. Upon ingestion, gastric lipases can hydrolyze up to 25% of triacylglycerols in the stomach (Dupuis et al., 2001). In the intestines, pancreatic lipase hydrolyzes triacylglycerols at the C1 and C3 positions, generating free fatty acids and a monoacylglycerol. Phospholipases are also secreted by the pancreas and hydrolyze glycerophospholipids into free fatty acid and lysophospholipid. Bile acids are secreted into the lumen of the small intestine to coat fat particles and form micelles. These micelles are transported to the intestinal wall where they are absorbed by enterocytes.

Bile acids are synthesized in the liver, stored in the gallbladder and released into the small intestine by induction of cholecystokinin (Agellon, 2008).

Micelle components dissociate into an aqueous phase or into the brush border membrane and diffuse across the unstirred water layer of the small intestine and through the brush border membrane by passive diffusion or though protein carriers. Facilitated transport of free cholesterol and medium and long chain fatty acids into enterocytes is achieved by fatty acid binding proteins. Moreover, Na^+/H^+ exchangers present in the brush border membrane contribute to long chain FA uptake by enterocytes (Thomson and Wild, 2001). Once in the cytosol, monoglycerides and FAs are re-esterified into triacylglycerols by the phosphatidic acid pathway and monoacylglycerol pathway (Kuksis and Lehner, 2001). Some long chain FAs will pass directly into circulation, and the rest will form lipoproteins or chylomicrons and leave the basolateral membrane to circulation through the lymphatic system (Thomson and Wild, 2001). Medium and short chain free fatty acids are mostly absorbed into the hepatic portal vein (Bender, 2009).

In circulation, chylomicrons deliver triacylglycerol mainly to adipose tissue, heart and muscle, and about 20% goes to the liver. In the endothelium, lipoprotein lipases hydrolyze chylomicrons to free fatty acids and glycerol. Free fatty acids are transported into tissues and chylomicron remnants rich in cholesterol and cholesteryl esters are taken up and metabolized by the liver (Botham and Mayes, 2009c).

Lipid metabolism is chiefly regulated by the hormones insulin and glucagon, which have opposing effects (insulin is anabolic, while glucagon is catabolic). In general, after a meal, insulin is secreted and increases fatty acid synthesis in liver, while decreasing rate of lipolysis in adipose tissue, reducing circulation of triacylglycerol and fatty acids. Insulin supports lipogenesis by several mechanisms, including stimulation of acetyl-CoA carboxylase (ACC) and lipoprotein lipase in adipocytes (Wang and Eckel, 2009), as well as increasing transport of glucose into cells and activating pyruvate dehydrogenase (branch point of carbon partitioning to FA synthesis) (Botham and Mayes, 2009c).

Glucagon is secreted in the fasted state, decreasing FA synthesis in liver (by inactivating ACC), increasing lipolysis in adipose tissue (activation of hormone-sensitive lipase), thus increasing circulation of FAs and increasing FA oxidation and ketogenesis in liver.

Moreover, catecholamines (epinephrine, norepinephrine) induce lipolysis in adipose tissue through activation of hormone-sensitive lipase via protein kinase A (Horton et al., 2006).

Digestibility of lipids

Digestibility of lipids is typically high, with animal fats being less digestible than vegetable oils and increasing in digestibility as animals get older (Cera et al., 1990). Other factors may contribute to digestibility, including free fatty acid (FFA) content, carbon chain length and degree of saturation (NRC, 2012). Increasing free fatty acid content is known to decrease digestibility of lipids (Jorgensen and Fernandez, 2000; Powles et al., 1995). However, growth performance of pigs has shown controversial results. DeRouchey et al. (2004) did not observe negative impacts on performance using increasing concentrations of FFA in nursery pigs. Similarly, Mendoza and van Heugten (2014) fed choice white grease and acidulated soybean oil to nursery pigs and concluded that increasing levels of FFA did not affect pig performance.

Moreover, unsaturated: saturated FA (U:S) ratio affects digestibility of dietary lipids (Stahly, 1984; Powles et al., 1995), with unsaturated lipids being more digestible than saturated lipids (Wiseman et al., 1990; Powles et al., 1994), even though this has been disputed by some (Jorgensen and Fernandez 2000; Kil et al., 2010).

The digestible energy (DE) values for various lipids are presented by the NRC (2012), which were estimated using the following equation:

$$DE = \{36.898 - [0.005 \times FFA] - [7.33 \times e^{(0.906 \times U:S)}]\} / 4.184$$

where DE = digestible energy (kcal/kg); FFA = free fatty acid (g/kg) and U:S = unsaturated: saturated fatty acid ratio.

Metabolizable energy (ME) is calculated as 98% of DE; however, this does not take into account post absorptive utilization of lipids. Therefore, net energy (NE) of lipids calculated as 98% of ME may often be underestimated (NRC, 2012; Kerr et al., 2015).

LIPID PEROXIDATION

Digestibility can also be affected by lipid moisture content, insolubles and unsaponifiables (MIU), and peroxidation (Kerr, et al. 2015). Lipid peroxidation initiates with formation of free lipid radicals and hydroperoxides, which are then decomposed to secondary

compounds (propagation step), finishing with formation of tertiary oxidation products (Figure 2) (AOCS, 2005). Most decomposed products of hydroperoxides, such as alcohols, aldehydes, furans, hydrocarbons, ketones and acids, are responsible for undesirable odors and flavors in oxidized oils (Kim and Min, 2008). Saturated lipids are less prone to peroxidation, and peroxidation rates increase with increasing degree of unsaturation of lipids. Additionally, increasing temperature, irradiation and oxygen pressure boosts the rate of peroxidation.

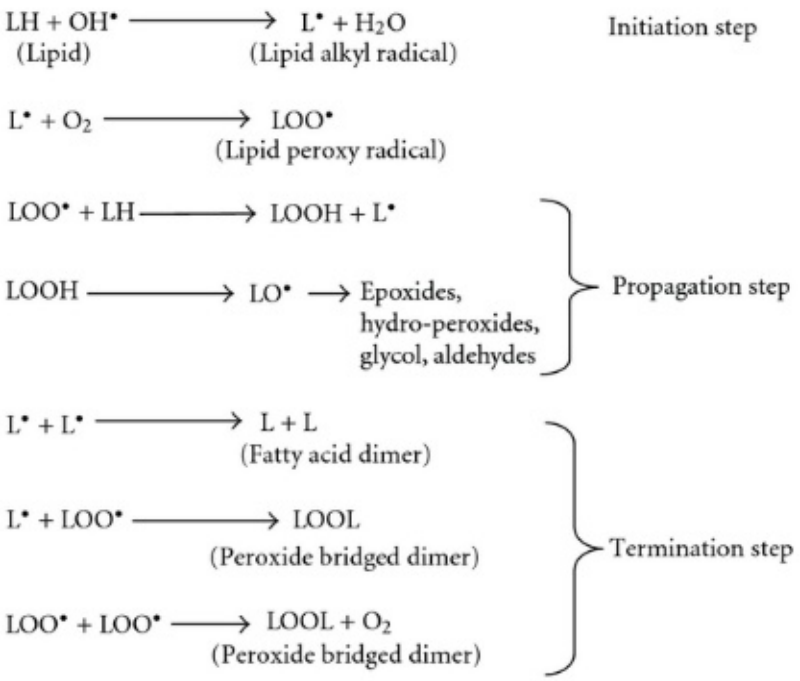

Figure 2. Free radical induced lipid peroxidation process.

Because several compounds are produced during peroxidation (Figure 3), different analytical tests can be used to measure peroxidation. Preferentially, these should be combined to better determine the peroxidation status of the lipid (NRC, 2012).

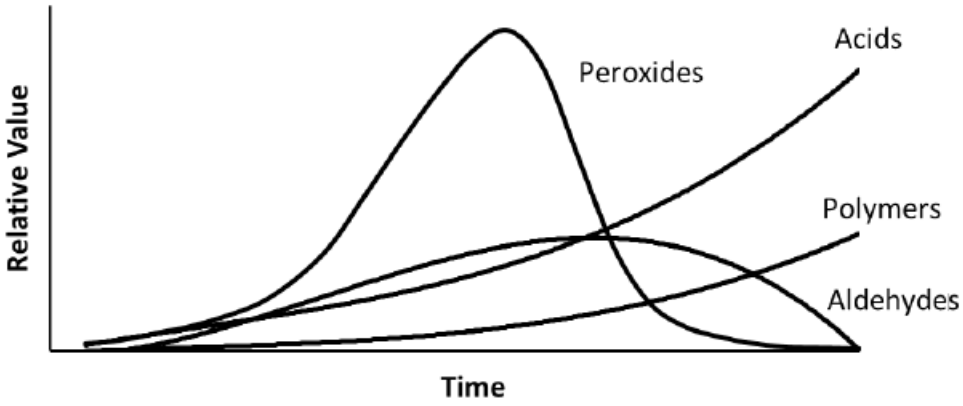

Figure 3. Progression of lipid peroxidation over time on production and degradation of peroxidation products (Fitch Haumann, 1993).

The most common indicative assays of peroxidation include peroxide value (PV), anisidine value (AnV) and thiobarbituric acid reactive substances (TBARS) (NRC, 2012; Shurson et al., 2015; Kerr et al., 2015). Peroxide value measures primary oxidation products (hydroperoxides and peroxides), while AnV and TBARS measure formation of aldehydes in the propagation step. The reaction of TBARS with malondialdehyde (MDA) produces a conjugated-double-bond compound which is measured to indicate oxidation in the TBARS assay. Specific aldehydes (4-hydroxynonenal and 2, 4-decadienal) produced in the termination step can also be determined (Kerr et al., 2015).

Furthermore, predictive tests such as the active oxygen method (AOM) and oxidative stability index (OSI) expose samples to increased temperatures and oxygen pressure, accelerating the peroxidation process. The AOM measures peroxide values over time, while OSI measures conductivity changes due to formation of volatile organic acids, such as formic acid. Nonetheless, all of these and other assays have limitations (NRC, 2012; Liu et al., 2014a; Shurson et al., 2015), which further complicates the estimation of peroxidation and predicates the need for novel approaches.

Absorption and metabolism of peroxides in the intestine is related to the redox status of the gut. Generally, the proportion of reducible glutathione (GSH) to its oxidized product, glutathione disulfide (GSSG), is a useful indicator of redox status in enterocytes (Circu and Aw, 2011). Glutathione concentration in the intestine determines peroxide accumulation in the

lumen, in which increased glutathione availability decreases concentration of peroxides in the lumen (LeGrand and Aw, 2001), as supported by Aw et al. (1992a,b). As peroxides enter the cell, reduction is achieved by glutathione peroxidase, producing GSSG and hydroxide. GSSG can be reduced back to GSH by GSSG reductase, in a NADPH dependent reaction (LeGrand and Aw, 2001). Moreover, the cysteine/cystine (Cys/CySS) concentration contributes to the GSH/GSSG redox couple, and it is important in maintaining extracellular and luminal redox state (Circu and Aw, 2011).

Kanazawa and Ashida (1998) proposed that dietary oxidized lipids decompose to aldehydes and ketones in the gastric lumen, where most of them are absorbed and incorporated in gastric tissue, whereas remaining aldehydes pass to the small intestine where they are absorbed and finally accumulate in the liver.

Toxicity of dietary lipid peroxides has been reported in rats. Kanazawa et al. (1985) found secondary products of oxidation to cause enlarged livers and increased serum transaminase activity. Tsunada et al. (2003) found increased peroxide content in tissues, along with a decreased GSH and increased GSSG concentration at 2 weeks of feeding peroxidized fish oil, after which redox status was comparable to controls, suggesting an adaptive response to prolonged feeding. Moreover, GSH peroxidase and GSSG reductase activities were increased in the jejunum after 8 weeks of feeding. Peroxidized oil also depressed enterocyte proliferation and apoptosis in the small intestine, but not in the colon.

Lipid sources and quality

A variety of feed-grade lipids are currently used in swine diets, such as rendered fats, restaurant grease, DDGS derived oils and vegetable oils. As illustrated by van Kempen and McComas (2002) and more recently by Shurson et al. (2015), there is great variation in the quality and composition of lipid sources available to the industry.

To investigate potential effects of different commercial lipid sources, growing pigs were fed 8% of either soybean oil, tallow, poultry fat, pet food-grade poultry fat, yellow grease, brown grease or restaurant grease, plus a basal control diet with no added lipids (Chang et al., 2015). As expected, soybean oil had the greatest concentration of unsaturated fatty acids, whereas tallow was most saturated. Brown grease had the greatest concentration of free fatty acids (23.2%) and MDA (74.2 mmol/L lipid), while restaurant grease had the greatest anisidine

value (36), followed by yellow grease (19) and brown grease (12), indicating significant peroxidation of these lipids. Unexpectedly, pigs fed yellow grease had the lowest feed intake and lost weight during the 10-d trial. Malondialdehyde concentrations in serum were highest in these pigs (11.25 mmol/L), but did not differ among other lipid sources. Supplementation of lipids to the basal diet increased apparent total tract digestibility of fat from 40.0% to 81.3% and improved digestibility of gross energy, but there were no differences in the digestibility of fat amongst lipid sources. The general lack of differences between the lipid sources indicates that the composition and the quality characteristics of the supplemental lipids used in this experiment had little impact on apparent total tract digestibility of fat and gross energy in growing pigs. These observations are in agreement with results reported by DeRouchey et al. (2004), in which peroxidized choice white grease fed to nursery pigs for 35 d did not affect digestibility of lipids or fatty acids. Similar results were seen by Liu et al. (2014a) when feeding peroxidized lipids to nursery pigs.

In contrast, Harrell et al. (2010) found that inclusion of 20 to30% DDGS in nursery pig diets compromised growth performance to the same extent as feeding oxidized (PV= 7.5 mEq/kg of diet) corn oil. In like manner, Song et al. (2014) fed oxidized DDGS to weaned pigs and found decreased growth performance and poorer carcass characteristics (hot carcass weight, dressing percentage, loin eye area), but no impact on muscle lipid oxidation. On the other hand, Hanson et al. (2015) included oxidized DDGS to sow and nursery diets and found no detrimental effects on post weaning oxidative status of pigs, attributing these effects mainly to the high content of sulfur amino acids present in DDGS source.

Furthermore, controlled forced peroxidation of lipids has been implemented to test its adverse effects on pigs. Liu et al. (2014b) tested slow (95°C for 72 h) and rapid (185°C for 7 h) heating of vegetable oils for peroxidation indicators and found that PV, TBARS, hexanal and 2, 4-decadienal (DDE) were positively correlated and were higher in slow heating vs. rapid heating; while AnV positively correlated with HNE and AOM, which increased in rapid heating, and negatively with OSI. Subsequently, these oils were fed to weaned pigs at 10% of the diet during 39 d (Liu et al., 2014c). Pigs had decreased gain when fed the rapidly heated oils and the highest TBARS value in heated canola oil correlated with the poorest growth performance. Liver weight of pigs was increased when rapidly heated oils were fed, which was

attributed by the authors to hepatic oxidative stress caused by secondary oxidation products, demonstrated by high levels of TBARS and HNE in this group of pigs.

Rosero et al. (2015a) obtained increasing levels of AnV and MDA by heating soybean oil with a constant oxygen flow for 12 d, and reported increasing peroxide values that seemed to plateau at similar concentrations at day 9 and d 12. Nonetheless, FFA and MIU values were not altered at any time point. Nursery pig performance was abated by increasing peroxidation, as was apparent total tract digestibility of gross energy and fat. Jejunum mucosa had increased concentration of MDA and decreased antioxidant capacity as peroxidation increased, and absorption of mannitol and D-xylose tended to decrease with peroxidation, indicating a reduced absorptive capacity in the small intestine due to consumption of peroxidized lipids.

Dietary peroxidized lipids have been shown to affect antioxidant systems in swine. Growing pigs receiving oxidized sunflower oil at 9% of the diet had increased TBARS concentrations in intestinal epithelial cells, together with decreased α-tocopherol and reduced enzymatic activities of catalase, glutathione peroxidase and superoxide dismutase (Ringseis et al., 2007). Plasma cholesterol was reduced in pigs fed oxidized oil, and there was a tendency for DNA-binding activity of PPARγ to be lower in those pigs.

Shi-bin et al. (2007) fed 5% oxidized (PV= 786 mEq/kg) fish oil to weaned pigs and observed depressed growth performance and feed efficiency, accompanied by a reduction in activities of superoxide dismutase and glutathione peroxidase and increased TBARS concentrations in serum and liver. Correspondingly, feeding 5% oxidized corn oil (PV= 150 mEq/kg) decreased glutathione peroxidase activity in plasma, tended to increase plasma TBARS and reduced plasma and liver vitamin E in growing pigs (Boler et al., 2012). Oxidation also led to poorer performance and inferior carcass characteristics.

On the other hand, Luci et al. (2007) found increased superoxide dismutase mRNA and activity, as well as increased catalase activity in the liver of pigs fed 9% oxidized sunflower oil. PPARα, SREBP-1 and SREBP-2 target genes were also up-regulated when pigs were fed oxidized oil.

Comparably, rat and mice studies with oxidized lipids demonstrated reductions in liver and plasma triacylglycerol and cholesterol, as well as increased activation of PPARα, the latter which was more pronounced under moderate heating of lipids (for review, see Ringseis and Eder, 2011).

The effects of dietary peroxidized lipids in poultry were recently reviewed by Kerr at al. (2015) and include overall depressed feed efficiency and energy digestibility, debilitated immune function, increased mortality and reduced meat quality. In accordance, Liang et al. (2015) recently demonstrated reduced gain, increased MDA in jejunum and plasma, decreased catalase and glutathione S-transferase activities in plasma and altered immune function in broilers fed oxidized soybean oil at different PV levels for 22 d.

Taken together, these results clearly indicate the detrimental effects of peroxidized lipids, and caution should be used when feeding these to animals. Antioxidants have shown to counteract some of these effects, as described next.

Antioxidants

To prevent peroxidation of lipids and vitamins, antioxidants can be added to diets. Various natural and synthetic compounds display antioxidant properties, including natural carotenoids, flavonoids, tocopherols and citric acid, and synthetic ethoxyquin, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and propyl gallate (Wanasundara and Shahidi, 2005; Jacela et al., 2010).

Antioxidants can be further classified by their mode of action into primary and secondary antioxidants. Primary antioxidants (carotenoids, flavonoids, tocopherols, BHT, BHA, ethoxyquin, propyl gallate, among others) delay or inhibit the initiation or impede the propagation step of lipid peroxidation by reacting with lipid and peroxide radicals, becoming more stable, non-radical products. These are most effective when added during the initiation step and before propagation of peroxidation ensues. Primary antioxidants are mono or polyhydroxy phenols with numerous ring substitutions. After hydrogen donation, the antioxidant radical formed is stabilized by delocalization of the unpaired electron around the phenolic ring (Reische et al., 2008). Antioxidant radicals can then bind each other and form deactivated antioxidant dimers or regenerate by reducing reactions with other antioxidants (Wanasundara and Shahidi, 2005).

Secondary antioxidants slow the rate of peroxidation by many possible actions, including chelating pro-oxidant metals and deactivating them, chemically reducing primary antioxidants, decomposing hydroperoxides to non-radical species, deactivating singlet oxygen, absorbing UV radiation or acting as oxygen scavengers. Citric acid, phosphoric acid and

ethylenediaminetetraacetic acid (EDTA) chelate metals to produce a more stable oxidized product. Moreover, vitamin C and sulfites scavenge oxygen and chemically reduce compounds (Reische et al., 2008).

Among the synthetic antioxidants, the most commonly used in swine diets include a mixture of ethoxyquin, BHA, BHT and propyl gallate. Ethoxyquin (6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline) is a yellow liquid that when added to oils acts as a free radical terminator. BHA and BHT are most effective in animal fats and are susceptible to volatilization during frying compounds (Reische et al., 2008). BHA and BHT can work synergistically: BHA reaction with a peroxide radical forms a BHA phenoxy radical, which then abstracts a hydrogen from the hydroxyl group of BHT. BHA is regenerated and the newly formed BHT phenoxy radical can react with a peroxide radical and act as a chain terminator (Belitz et al., 2009). Propyl gallate is more effective in vegetable oils than BHA and BHT, but also degrades at high temperatures and should be used together with metal chelators to avoid formation of dark-colored complexes (Reische et al., 2008).

Tocopherols and tocotrienols are homologs with vitamin E activity in the diet. Vegetable oils are the most concentrated source of vitamin E, with soybean oil being the main commercial source of natural vitamin E (Reische et al., 2008). α-tocopherol has the highest antioxidant activity compared to its β, γ and δ isomers (Ullrey, 1981) and it is the most abundant in pigs (Lauridsen et al., 2002). α-tocopherol donates hydrogen to a peroxide radical to form a α-tocopheryl semiquinone radical, which in turn can donate another hydrogen molecule to a radical and produce methyltocopherylquinone or dimerize with another semiquinone radical. Methyltocopherylquinone is unstable and converts to α-tocopherylquinone, while the α-tocopheryl dimer remains active (Reische et al., 2008).

Ascorbic acid, or vitamin C, acts as a primary antioxidant in vivo by donating hydrogen molecules. It can also be a scavenger, reductant and regenerate primary antioxidants in feed. It further works synergistically with vitamin E, donating hydrogen atoms to tocopheryl radicals, forming tocopherol and dehydroascorbic acid (Reische et al., 2008).

Furthermore, enzymatic antioxidants are crucial during oxidative imbalance and are frequently quantified in oxidative stress studies. Catalase is a peroxidase containing four heme groups found mainly in peroxisomes. Selenium-containing glutathione peroxidase catalyzes removal of H_2O_2 by oxidizing glutathione to glutathione disulfide (GSSG) (Botham and

Mayes, 2009a). Superoxide dismutase (SOD) catalyzes rapid removal of superoxide anion $\cdot O_2^-$ by reducing its metal ion component (Cu, Zn) and then oxidizing it again, producing O_2 and H_2O_2, which can further be metabolized by peroxidases. It is found in mitochondria and cytosol in aerobic tissue (Horton et al., 2006).

Supplementation of commercial antioxidant blends has been researched in pigs. Positive effects of antioxidant in nursery pig performance were seen by Harrell et al. (2010), but antioxidant supplementation was not able to correct reduced growth performance in pigs fed oxidized corn oil or DDGS source. In contrast, Lu et al. (2014a) fed weaned pigs 5% oxidized soybean oil combined with antioxidant blend and vitamin E, reporting similar growth rates for pigs fed antioxidant and antioxidant plus vitamin E to pigs receiving a control diet. Increase in liver weight, plasma aspartate transaminase and bilirubin indicated liver damage in pigs fed oxidized oil. Moreover, addition of antioxidant blend, but not vitamin E alone, protected against oxidative stress indicated by elevated TBARS and protein carbonyl in plasma and liver in animals fed oxidized oil. In the same study, inferior carcass characteristics (weight, backfat thickness, lean mass and loin eye area) induced by dietary oxidized oil were prevented by addition of antioxidant blend (Lu et al., 2014b). Similarly, Boler et al. (2012) fed growing pigs 5% of oxidized corn oil and a commercial antioxidant blend (tert-butylhydroquinone and ethoxyquin) and found that inclusion of antioxidant decreased protein carbonyl in plasma and improved shelf life of pork.

Vitamin supplementation and its effects on pork quality have shown controversial results. Vitamin E generally impacts meat quality by reducing lipid oxidation in swine and poultry, as reviewed by Jensen et al. (1998). Lauridsen et al. (1999) found improved water holding capacity and reduced lipid oxidation in *longissimus dorsi* of animals fed synthetic vitamin E as dl-α-tocopheryl acetate. Conversely, vitamin E supplementation in combination with oxidized DDGS feeding did not affect TBARS concentrations in *longissimus dorsi* in finishing pigs (Song et al., 2014).

Furthermore, Pion et al. (2004) did not find significant improvement in meat quality when feeding vitamin C to finishing pigs, while Peeters et al. (2006) found improved meat color with supplementation of vitamin C and vitamin E to finishing pigs.

The effect of antioxidants and oxidized oils has been investigated in broilers. Dibner et al. (1996) fed oxidized (PV= 212 mEq/kg) poultry fat to broilers in combination with

ethoxyquin (125 ppm) and observed abated feed conversion and hematocrit concentration due to oxidation. Non-oxidized poultry fat in combination with antioxidant displayed highest hematocrit concentration and feed conversion. Oxidized fat reduced epithelial half-life in small intestine and increased proliferation of hepatocytes, which was not ameliorated by antioxidant addition. Moreover, oxidized fat reduced IgA concentration in intestinal tissue. In contrast, antioxidant supplementation (125 ppm of ethoxyquin) was able to correct the negative effects caused by oxidized fats (PV= 75 or 150 mEq/kg) on feed conversion in broilers (McGill et al. 2011a, b).

Additionally, antioxidants have shown positive effects on packaged broiler meat. Tavárez et al. (2011) fed oxidized (PV= 180 mEq/kg) soybean oil and antioxidant blend to broilers and found diminished growth performance of birds consuming oxidized oil, and improved weight gain and feed intake, but not G:F, in animals fed antioxidant blend. Packaged breast meat had highest TBARS concentrations at d 7 for animals fed the peroxidized diet without antioxidant supplementation, suggesting that antioxidant protected against further oxidation when supplemented to other treatments. Liver TBARS concentrations were highest in birds fed oxidized oil with antioxidant and lowest concentrations were found in fresh oil plus antioxidant treatment. Oxidized oil decreased vitamin E in serum, while antioxidant supplementation increased vitamin E concentration in serum and liver. Serum and liver vitamin A was lowest in broilers fed oxidized oil without antioxidant.

Comparably, Delles et al. (2014) fed broilers low (PV= 23 mEq/kg) and highly (PV=121 mEq/kg) oxidized soybean oil with or without supplementation of a mineral antioxidant blend for 42 d. Oxidation increased TBARS and protein carbonyl and decreased protein sulfhydryl retention in packaged meat, while antioxidant supplementation reduced both TBARS and protein carbonyl and increased protein sulfhydryl retention in meat. Moreover, superoxide dismutase, catalase and glutathione peroxidase activity in breast muscle was reduced by high peroxidation and increased with antioxidant supplementation.

Altogether, there is still controversy remaining on the efficacy of antioxidants on abating unfavorable effects of peroxidation. Further research is necessary to address these concerns.

ESSENTIAL FATTY ACIDS AND EICOSANOIDS

Linoleic (LA; 18:2n-6) and α-linolenic (ALA; 18:3n-3) acids are nutritionally essential to many animals, for the reason that most cannot insert a double bond past the Δ9 position of fatty acids. Mammals lack the Δ12 desaturase enzyme necessary to catalyze the formation of linoleic or linolenic acid, rendering them indispensable in these animals' diets (Rooke et al., 2003). Nonetheless, vegetable oils are a rich source of essential fatty acids; oils derived from corn, soy and sunflower seeds all contain high concentrations of linoleic acid, while flaxseed oil remains the most notable source of α-linolenic acid (Kostik et al., 2013). Moreover, fish oils are the most concentrated source of longer chain fatty acids, eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3).

The essentiality of linoleic and linolenic acids derives from their functions as eicosanoid percursors. Eicosanoids, which refer to a group of 20-carbon polyunsaturated fatty acids (PUFAs), include prostaglandins (PG), thromboxanes (TX), leukotrienes (LT) and lipoxins (LX). Arachidonic acid (ARA; 20:4n-6) is the major precursor of these molecules, and, if not supplied in the diet, it is synthesized from linoleic acid (Palmquist, 2009).

The conversion of LA to longer chain PUFAs requires a Δ6-desaturase in its first step, which is also the first enzyme used in the conversion of ALA to its longer chain derivatives. The enzyme has greater affinity for ALA, however, LA is usually found in greater amounts in cells, hence more n-6 long chain PUFAs are produced normally (Sprecher, 2000). Also, the conversion efficiency of ALA to long chain n-3 PUFAs is low in pigs, and feeding these fatty acids directly to the animals increases tissue accumulation compared to feeding its precursor alone (Brenna et al., 2009).

Eicosapentaenoic acid (EPA; 20:5n-3) is also a substrate that leads to formation of prostanoids (such as TXA_3 and PGI_3) and leukotrienes, which are converted through the same enzyme systems as ARA. Furthermore, EPA has been described to compete with ARA, reducing production of ARA metabolites (Higgs et al., 1986).

Prostanoids (PGs and TXs) are products of the reactions catalyzed by PGH synthase (PGHS) with ARA, which entails cyclooxygenase (COX) and peroxidase activities. The COX enzyme catalyzes the formation of one hydroperoxide PG2 from ARA and two molecules of O2. PG_2 is then reduced to PGH_2 in the peroxidase active site. PGH_2 is later converted to

several different short-lived regulatory molecules, which are commonly cell specific and act not far from their site of production (Smith and Murphy, 2008).

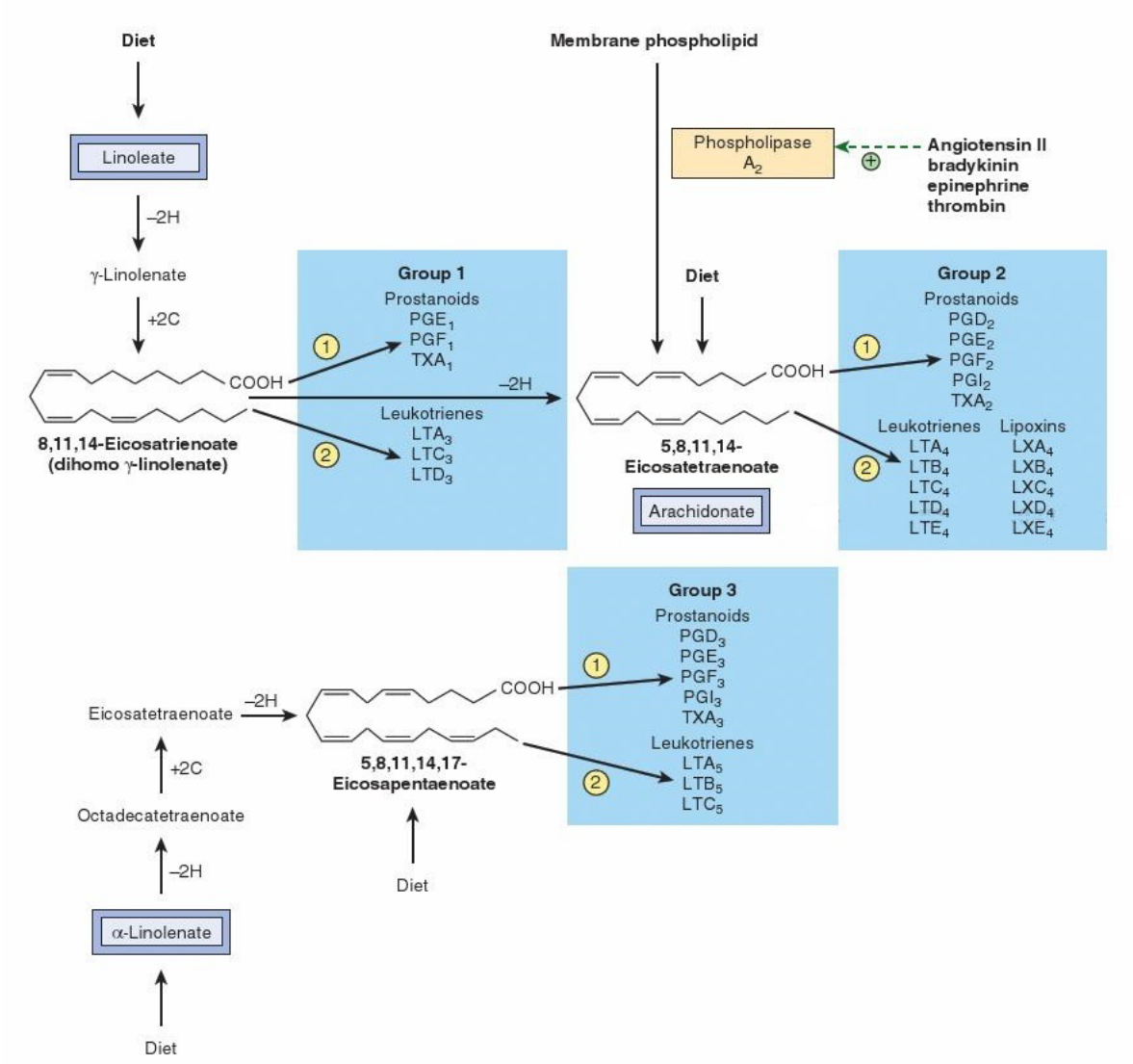

Figure 4. Eicosanoid biosynthesis pathways. ①, cyclooxygenase pathway; ②, lipoxygenase pathway. Source: Botham and Mayes, 2009b.

There are two COX isoenzymes, COX-1 and COX-2. The former seems to prefer FAs for substrate (such as ARA) and is responsible for regulating mucin secretion in the stomach; while COX-2 uses both FAs and 2-arachidonoylglycerol (Rouzer and Marnett, 2003), and it is

pro-inflammatory. Aspirins (NSAIDS) inhibit both isoenzymes, while corticosteroids are used to inhibit transcription of COX-2.

Perhaps the most described effect of prostanoids is platelet aggregation induced by TXA_2, which are synthesized in platelets in response to injury, also causing vasoconstriction in smooth muscles. Oppositely, PGI2 synthesized in blood vessels inhibits platelet aggregation (Smith and Murphy, 2008).

PGE_2 is a well-known pro-inflammatory molecule that provokes pain, fever and vascular contraction. It also inhibits T-cell proliferation, IL-2 and IFN-ɣ synthesis and induces production of IgE (Sijben and Calder, 2007). PGE_2 produced by ovaries, uterus and embryonic membranes also acts on oviducts and corpus luteum, it controls ovulation and corpus luteum secretion of progesterone. $PGF_{2\alpha}$ produced in endometrium promotes luteolysis, uterine contractions and supports ovulation (Senger, 2005).

Moreover, leukotrienes and lipoxins are products of the lipoxygenase pathway, in which oxygen is introduced on different positions of ARA leading to formation of hydroperoxides, such as 5-HPETE. 5-HPETE is further dehydrated to form LTs. In like manner, lipoxins are synthesized by the action of combined lipoxygenase enzymes (Martin, 2015)

Leukotriene B_4 induces chemotaxis of leukocytes during inflammatory processes, while leukotrienes C_4, D_4 and E_4 mediate inflammation through smooth muscle contraction, vascular permeability, mucus secretion and bronchoconstriction (Martin, 2015).

Omega-3 fatty acids and respective eicosanoids

Eicosanoids derived from n-3 PUFAs (mainly EPA and DHA) have been described as acting antagonistically to ARA-derived molecules, either by competing with ARA as substrate for eicosanoid synthesis, or by modifying expression of inflammatory genes (Palmquist, 2009; Calder, 2012; Siriwardhana et al., 2013). Omega-3 derived resolvins and protectins are also key molecules in resolution of inflammatory processes (Serhan et al., 2004).

Many in vitro studies evidenced differences in potency of ARA- and EPA-derived mediators, such as PG2 and PG3 (Bagga et al., 2003), as well as LTB4 and LTB5 (Lee et al., 1984) and more recently, PGD2 and PGD3 (Tull et al., 2009). Generally, EPA-derived series

3 PGs and TXs and series 5 LTs are less potent than ARA-derived series 2 PGs and TXs and series 4 LTs, with a few exceptions (Calder, 2012).

Other anti-inflammatory properties attributed to n-3 PUFAs regard to their ability to activate peroxisome proliferator-activated receptors (PPARs) and G-protein coupled receptor 120 (GPR120) (Calder, 2012; Siriwardhana et al., 2013) and reduce the activation of pro-inflammatory transcription factor NF-κB (Novak et al., 2003).

The anti-inflammatory effects of n-3 PUFAs have been vastly investigated in vitro. Weldon et al. (2007) described a reduction in pro-inflammatory cytokines (TNF-α, IL-1B and IL-6) in LPS-induced macrophages treated with DHA and EPA separately. Likewise, Oliver et al. (2012) observed increased anti-inflammatory IL-10 production and reduced NF-κB activation in LPS-induced macrophages after treatment with DHA. Murine LPS-induced dendritic cells treated with EPA and DHA had reduced NF-κB activity, and anti-inflammatory effects seemed to occur independently of PPARγ activation (Draper et al., 2011). Moreover, DHA was shown to inhibit TNF-α-induced ICAM-1 mRNA and protein expression by inhibition of the IKK/NF-κB pathway and activation of the Nrf2/HO-1 pathway in cultured human endothelial cells (Yang et al., 2013b).

Additionally, Tomasdottir et al. (2013) studied the effects of dietary fish oil (2.8% of diet) in mice and observed reduced number of neutrophils, cytokines and chemokines, increased NK cells and differences in gene expression in macrophages throughout the inflammatory process. The authors concluded that fish oil attenuated the inflammatory response in antigen-induced peritonitis and stimulated and intensified the resolution phase of inflammation.

Consequently, there is emerging interest in n-3 PUFAs in treatment of inflammatory diseases. The use of omega-3 rich fish oil to treat cardiovascular disease has been extensively studied, and despite many controversial results, there is clear evidence of beneficial effects from fish oil administration. The topic has been reviewed broadly (Herold and Kinsella, 1986; Horrocks and Yeo, 1999; Kris-Etherton et al., 2002; Wang et al., 2006; Kromhout et al., 2012).

Rheumatoid arthritis is another infamous disease in which omega-3 FAs have been widely employed. Several meta-analyses were conducted on this theme, and they conjointly divulge favorable outcomes from treatment with omega-3 PUFAs (Fortin et al., 1995; MacLean et al., 2004).

Moreover, omega 3 fatty acids have been shown to have anticancer properties (Yang et al., 2013a; Wu et al., 2012; Faber et al., 2013; Abdi et al., 2014) and several studies have been conducted investigating its effects on adipose tissue inflammation (Kalupahana et al., 2010; Siriwardhana et al., 2013) and adipocyte differentiation (Hsu et al., 2004; Liu et al., 2005; Tseng et al., 2010).

Polyunsaturated fatty acids in swine diets

The supplementation of fish oil to swine diets has been studied broadly, and flaxseed has been often employed to increase the omega-3 content of diets.

In a comprehensive study by Liu et al. (2012; 2013a,b), weanling pigs were fed 5% fish oil and challenged with LPS injection in order to investigate potential anti-inflammatory and neuroendocrine effects of n-3 PUFAS. Pig tissues (small intestine, LD muscle, hypothalamus, pituitary and adrenal glands, thymus and spleen) had increased n-3 (ALA, DHA, EPA) and reduced n-6 (LA, ARA) PUFAs in the fish oil treatment. Jejunum and ileum had increased villi height and villus height to crypt depth ratio with fish oil supplementation, as well as increased expression of tight junction proteins. Jejunum and muscle concentrations of TGF-α and PGE2 were reduced by fish oil supplementation, as well as decreased expression of TLR4 and NOD2, which are associated with initiation of inflammatory responses. The authors also found increased protein concentration and protein:DNA ratio in muscles of pigs fed fish oil, and decreased expression of ligases responsible for protein degradation. Fish oil also decreased abundance of mRNA of TLR4 and other signaling pro-inflammatory molecules in neuroendocrine tissues (Liu et al., 2013b).

Upadhaya et al. (2015) fed linseed oil at 0.75% of the diet of LPS challenged pigs and reported lower count of white blood cells and decreased serum cortisol, TNF-α and PGE2, along with a tendency for reduced serum IgG and improved ADG and carcass lean percent.

Furthermore, Hess at al. (2008) studied the effects of feeding neonatal pigs with increasing levels of ARA (0, 0.5, 2.5 and 5%) and EPA (5%) and found increasing amounts of ARA in intestinal mucosa that accompanied decreasing levels of LA, with maximum enrichment obtained at 8 days of supplementation. Similarly, 5% EPA treatment enriched intestinal mucosa with EPA and DHA, with no impact on intestinal morphology or piglet performance. Further analyses (Jacobi et al., 2011) indicated no major effects of ARA in liver

PUFA metabolism, but increasing levels of ARA increased activity of Δ6-desaturase in intestinal mucosa. Following an ischemic period (Jacobi et al., 2012), ilea of pigs receiving 5% ARA treatment produced lower percentage of denuded villus surface area. Treatment with 5% EPA had the lowest levels of PGE2, even though EPA treatment caused increase in COX-2 mRNA expression. Both 5% ARA and EPA treatments showed improved transepithelial resistance in ischemia-injured ileum; but treatments acted distinctly on epithelial barrier function (indicated by mannitol and inulin flux).

Additionally, neonatal pigs receiving total parenteral nutrition on a high PUFA diet (11% ARA and 5% DHA of total FA) had increased concentration of CPT-I mRNA in liver, but not increased enzyme activity, while greater glucose oxidation was reported in liver homogenates of pigs fed the high PUFA solution (Campbell et al., 2010).

Collectively, these results indicate that dietary PUFAs are successfully incorporated in pig tissues and may modulate inflammatory responses and alter the metabolism of young pigs.

Moreover, dietary PUFAs fed to sows have shown to alter milk fatty acid composition and incite different responses in nursing piglets. Fritsche et al. (1993) reported increases in sow milk n-3 fatty acids and in sow and piglets' serum when feeding 3.5 and 7% fish oil to lactating sows. This increase in n-3 was accompanied by a decrease in serum ARA for both sows and piglets. Similarly, Arbuckle and Innis (1993) found increased DHA and EPA in sow milk, piglet plasma and liver when feeding lactating sows with 1% fish oil. Piglet ARA was decreased in plasma and liver, but growth performance was unaffected by fish oil treatment. By feeding increasing levels of salmon oil (0.5, 1 and 2%) to lactating sows, Rooke et al. (2001) described increase n-3 PUFA in piglets' tissue (brain, retina and liver), plasma and colostrum. These were followed by decrease in n-6 PUFAs, leading to a lower n-6:n-3 ratio.

Furthermore, dietary flaxseed oil improved sow milk ALA and DHA content (with no variation in milk ARA) and increased n-3 PUFAs in tissues of 14 d old suckling pigs (Bazinet et al., 2003). In like manner, Farmer and Petit (2009) fed different forms of flax to gestating and lactating sows and observed increased ALA and EPA in sow plasma and milk, but not DHA, and these observations followed a decrease in ARA. In 1 d old piglets, carcasses from flax treatments contained increasing levels of ALA and EPA, while piglets' brains had higher concentrations of DHA, but not EPA, along with decreased levels of ARA. For all these measures, flaxseed oil at 3.5% and ground flaxseed at 1% of the diet displayed more

pronounced differences than inclusion of 6.5% flaxseed meal. Oppositely, flaxseed meal increased glycogen concentration in 1d old piglet carcass and BW gain at d 56 (Farmer et al., 2010). Mortality was also reduced by flax treatments and serum antibodies to ovalbumin were greater in piglets that consumed colostrum at d 2, suggesting a role for n-3 PUFAS to improve immunity and/or nutrient absorption in the gut (Farmer et al., 2010).

Also, Tanghe et al. (2013) observed enrichment of colostrum, sow and piglet plasma with EPA and DHA, which was followed by a decrease in ARA by feeding sows 1% fish oil from 73 d of gestation throughout lactation. Flaxseed and echium oil provided at 1% of the diet in the same experiment increased amount of α-linolenic acid in colostrum, sow and piglet plasma. In the same study, fish oil depressed pre-weaning growth of piglets, which the authors attribute to low concentrations of ARA in these pigs. Conversely, even though ADG was not altered, improved intestinal glucose transport and glycogen concentrations were reported in piglets from sows fed 1% fish oil and 0.14% of an algal DHA product added to lactation diets, as well as increased DHA (but not EPA) in sow milk and piglet tissue (jejunum and l. dorsi) (Gabler et al., 2007).

In like manner, studies have been conducted to investigate effects of PUFAs on subsequent reproductive performance of sows. Mateo et al. (2009) fed sows 1% fish oil from d 60 of gestation throughout lactation and reported improved piglet ADG throughout lactation in the first and in the subsequent cycle of sows. Milk content of IgG, DHA and EPA were increased with fish oil treatment, along with a decrease in milk ALA (18:3n-3) levels. Smits et al. (2011) found that feeding lactating sows 0.3% fish oil had positive effects on subsequent litter size, without affecting other reproductive performance traits. The authors attribute this response to better oocyte quality and follicular development.

Additionally, Eastwood et al. (2014) studied the effects of different n6:n3 ratios on diets of gestating and lactating sows. Number of pigs born and piglets born alive were unaffected by treatments, and weaning weights were decreased in 1:1 plant based and 5:1 fish oil treatment. On subsequent parity, pigs from sows on fish oil treatment had lower birth and weaning weights and decreased pre-wean ADG. Sow serum ALA concentration was highest on 1:1 plant treatment, while levels of EPA and DHA were greater in fish oil treatment; same FA profile pattern was observed in sow milk, with no alterations in IgA or IgG concentrations in piglet serum or colostrum.

More recently, Rosero et al. (2015b) analyzed composition of milk from lactating sows fed different combinations of linseed and canola oils, to reach levels of 2.1% or 3.3% LA (18:2n-6) and 0.15% and 0.45% of α-linolenic acid (18:3n-3) of the diet. Linoleic acid tended to increase ARA secretion in milk, while ALA tended to decrease ARA concentrations and increase EPA content in milk. As in agreement with most other studies, no effects on piglet growth performance were detected. The reported estimated negative balance of essential FAs in sows consuming diets without added lipids in that study is viewed by the authors as potentially harmful for subsequent reproductive performance of sows. Indeed, in dairy cows it has been shown that pro-inflammatory eicosanoids (n-6) can be beneficial during post-partum immunity, while anti-inflammatory derivatives (n-3) can promote embryo survival and improve pregnancy rates during breeding and early pregnancy (Thatcher et al., 2010).

In a similar and more up-to-date experiment by Rosero et al. (2016), feeding 3 levels of ALA (0.15, 0.30, 0.45%) and 3 levels of LA (2.1, 2.7, 3.3%) showed positive results on subsequent reproductive traits of sows. Linoleic acid at 3.3% improved farrowing rate of parity 1 sows, while ALA tended to decrease wean-to-estrus interval in parity 3 sows or older. Furthermore, total pigs born and pigs born alive increased linearly with increasing levels of LA. The authors speculate these may result from improved follicular development and oocyte quality.

In conclusion, the use of lipids in swine diets can provide many benefits to pigs in all stages of production. Special attention should be given to quality and probable peroxidation of highly unsaturated lipids, which can cause adverse effects when fed to animals. Antioxidants have been utilized in attempts to correct these effects. Finally, whether they are used as alternative energy sources or for their particular fatty acid content, lipids have many facets that need to be further explored.

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CHAPTER II: IMPACT OF LIPID PEROXIDATION AND ANTIOXIDANTS ON NURSERY PIG PERFORMANCE AND HEALTH

ABSTRACT

Lipids are commonly added to swine diets to increase energy density and improve feed efficiency. However, highly unsaturated lipids are prone to peroxidation, products of which can be detrimental when ingested by pigs. The purpose of the current study was to determine the impact of peroxidized corn oil with or without addition of a synthetic antioxidant blend on growth performance, oxidative status and response to vaccination in nursery pigs. A total of 176 nursery pigs (1-week post-weaning; initial BW= 9.11±0.4 kg) were housed in pens with 4 pigs/pen in a RCBD and assigned to 4 dietary treatments. Treatments consisted of a corn-soybean meal based basal mix that was supplemented with 6% of either control corn oil (IV=123.2, FFA=0.09%, anisidine value=2.2, peroxide value=0.4 mEq/kg oil) or peroxidized corn oil (IV=116.9, FFA=0.11%, anisidine value=164.4, peroxide value=146 mEq/kg oil) with or without an antioxidant blend containing ethoxyquin (minimum 3%), butylated hydroxytoluene and butylated hydroxyanisole (Endox Dry, Kemin Industries, Inc). Nursery diets consisted of 2 phases; Phase 1 fed for 14 days and Phase 2 fed for 16 days. Pigs were vaccinated with porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* (Mhyo) killed vaccine (Circumvent PCV M, Intervet Inc.) on d 3 and d 17 of the study. Blood samples were collected from 2 pigs per pen prior to vaccination on d 3, d 17 and at the end of the trial (d 31) to determine antibody titers to vaccinations, oxidative status, and vitamin E concentrations. There were no differences in performance among treatments. Serum malondialdehyde concentration, a marker for oxidative stress, did not differ ($P = 0.30$; mean = 3.53 ± 0.2 mmol/L) among treatments. Antibody titers to Mhyo increased post-vaccination (interaction, $P < 0.001$; -0.05, 0.19 and 1.68 for d 3, d 17 and d 31 in vaccinated pigs) and antibody titers to PCV2 increased following the second vaccination, but not the first vaccination (interaction, $P < 0.001$; 0.12, 0.15 and 0.89 for d 3, d 17, and d 31 in vaccinated pigs), but there were no differences in response to vaccination due to dietary treatments. Serum vitamin E concentrations decreased in pigs fed peroxidized oil by 29% on d 17 (0.79 vs. 1.11 µg/mL for peroxidized oil and control, resp.) and 36% (1.06 vs. 1.65 µg/mL) on d 31 (interaction, $P < 0.001$). Supplementation of antioxidant increased serum vitamin E concentration ($P < 0.001$; 1.28 vs 1.01 µg/mL) and this effect tended (interaction, $P = 0.06$) to be greater in pigs fed control oil. Results indicate that pig performance and response to vaccine were not affected by peroxidized corn oil or supplementation of antioxidant. However, serum

vitamin E status was reduced by consumption of peroxidized oils, which could be counteracted by the use of antioxidants in the diet. This reduction in vitamin E concentration may be an early indicator of oxidative stress; hence, a longer feeding period of peroxidized oils may be required to cause detrimental effects on performance**.**

INTRODUCTION

Supplemental lipids are widely used in swine diets as a means to increase energy density and improve feed efficiency. Lipids that are highly unsaturated, such as corn oil, are prone to peroxidation, which leads to formation of free radicals, hydroperoxides and other oxidation products (AOCS, 2005). Peroxidized lipids have been shown to decrease performance of swine and broilers and induce oxidative stress in most cases (Lu et al., 2014; Liang et al., 2015; Rosero et al., 2015; Shurson et al., 2015).

Oxidative stress is regarded as an imbalance between the production of reactive oxygen species and the biological ability to clear reactive intermediates. Excessive accumulation of reactive oxygen species can cause damage to DNA, proteins and lipid components of cells (Valko et al., 2007). Consequently, inclusion of antioxidants may be especially critical under these circumstances. Indeed, many studies reported reductions in markers of oxidative stress, improved performance, and enhanced meat quality when feeding synthetic antioxidants (Harrell et al., 2010; Tavárez et al., 2011; Boler et al., 2012; Lu et al., 2014).

Nevertheless, controversy still remains on how peroxidized lipids may affect the immune system in livestock. Dibner et al. (1996) demonstrated altered distribution of IgA in the large intestine of broilers and reduced lymphocyte proliferation in pigs fed oxidized poultry fat. Similar results were observed by Liang et al. (2015), in which concentrations of IgA and CD4 and CD8 molecules were reduced in the jejunum of broilers fed oxidized soy oil. Conversely, feeding oxidized lipids to pigs did not affect serum haptoglobin, IgA or IgG (Liu et al., 2014b) or alter DNA-binding activity of transcription factor nuclear factor-kappa B (NF-kB) (Ringseis et al., 2007); even though a tendency to increase DNA-binding activity of transcription factor peroxisome proliferator-activated receptor gamma (PPARγ) was observed by the same authors. In addition, a coccidiosis challenge study in broilers did not show significant differences in performance when feeding lipids with increasing peroxide values (McGill et al., 2011).

We hypothesized that feeding nursery pigs with peroxidized corn oil will decrease growth performance and response to vaccination, while supplementation of antioxidants will ameliorate these negative effects. Thus, the objectives of this study were to determine the effects of peroxidized oil and antioxidant supplementation on growth performance, oxidative status and response to vaccination of nursery pigs.

MATERIALS AND METHODS

Animals and dietary treatments

All animal protocols were approved by the North Carolina State University Institutional Animal Care and Use Committee. Nursery pigs (SPG; n = 176; mean BW = 9.11±0.4 kg) that were weaned at 3 weeks of age and had been fed a common nursery diet for 1 week were allotted to a RCBD based on initial BW, sex and litter of origin and randomly assigned within blocks to 4 dietary treatments. Pigs were housed in pens with 4 pigs/pen (2 castrated males and 2 females) and pens were 2.8 m^2 in dimension and contained one 2-hole feeder and 2 nipple drinkers. Pigs had ad libitum access to feed and water.

Dietary treatments were arranged in a 2 x 2 factorial with factors consisting of peroxidation status of corn oil and antioxidant supplementation. Diets were corn-soybean meal based and supplemented with 6% of an edible, refined corn oil (WebstaurantStore, Lancaster, PA) or the same oil which had been peroxidized. Each of these diets were either not supplemented or supplemented with 0.1% of a synthetic antioxidant blend containing butylated hydroxytoluene, butylated hydroxyanisole and minimum of 3.0% ethoxyquin (Endox® Dry; Kemin Industries, Inc., Des Moines, IA). Peroxidized corn oil was obtained by exposing control corn oil to heat (80°C) with constant oxygen supply (15.4 mL/min per kg of lipid) for 12 days. Subsequently, both control and peroxidized oil were stabilized with 0.1% of liquid antioxidant containing tertiary butyl hydroquinone (TBHQ) (Rendox® CQ, Kemin Industries, Inc.) to prevent further oxidation. Representative oil samples were obtained and submitted to a commercial laboratory (New Jersey Feed Laboratory Inc., Trenton NJ) and lipids were analyzed for their chemical composition and peroxidation measures (Table 2) according to AOAC (2007) and AOCS (2010).

Pigs were fed 2 nursery diet phases; the Phase 1 diets were offered from d 0 to d 14, followed by the Phase 2 diets from d 15 to d 31 of the study (Table 1). Diets were formulated

to meet or exceed NRC (2012) nutrient recommendations. All pigs and feeders were weighed at placement (d 0), when switching diets at d 15, and at the end of the study (d 31) for growth performance calculations.

Antibody titers and oxidative status

Two randomly selected pigs (1 female and 1 castrated male) per pen were vaccinated with porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* (Mhyo) killed vaccine (2 mL dose of Circumvent PCV M, Intervet Inc., Omaha, NE) on d 3 and d 17 of the study. Blood samples were collected from 2 pigs per pen (1 vaccinated pig and 1 control pig) prior to vaccination on d 3, prior to the second vaccination on d 17, and at the end of the study (d 31) to determine antibody titers to Mhyo and PCV2. Samples collected on d 17 and d 31 were also used to assess oxidative status and serum vitamin E concentrations. Blood samples were centrifuged at 1,500 × g for 20 min at 20°C to obtain serum. Serum samples were frozen at –20°C for further analyses.

Oxidative status was determined as serum concentrations of malondialdehyde (MDA) using a commercially available kit (OxiSelect TBARS Assay Kit, Cell Biolabs Inc., San Diego, USA). In addition, serum samples were submitted to the Veterinary Diagnostic Laboratory at Iowa State University (Ames, IA) for analyses of antibody titers and vitamin E concentrations. Antibody titers to PCV2 and Mhyo were determined by ELISA (INgezim Circo IgG, Ingenasa, Madrid, Spain and IDEXX M. hyo. Ab Test, Idexx Laboratories Inc., Westbrook, USA, for PCV2 and Mhyo, respectively). Titer results are presented as the sample to positive (S/P) ratio, calculated as the sample absorbance minus the negative control mean, divided by the difference between the positive and negative control means. Serum vitamin E concentrations were determined by HPLC using UV detection after extraction with a mixture of hexane and chloroform. Separation was achieved using a Brownlee C18 (4.6 x 33 mm) column (PerkinElmer, Inc., Waltham, MA) and a mobile phase with 95% of a 90/10 methanol/chloroform mixture and 5% water.

Statistical analysis

Growth performance data were analyzed using PROC Mixed of SAS (v. 9.4; SAS Inst. Inc., Cary, NC), testing for fixed effects of peroxidation, antioxidant supplementation and their interaction. Pen was the experimental unit and weight block was used as random effect. Mortality data were analyzed using PROC FREQ of SAS. Antibody titers, serum MDA and vitamin E concentrations were analyzed by PROC Mixed of SAS. The model included fixed effects of vaccination, day of sampling, peroxidation, antioxidant supplementation and relevant interactions. Pig was considered the experimental unit and pig nested within pen was used as random effect. Antibody titers were analyzed as repeated measures on d 3, d 17 and d 31; serum MDA and vitamin E were analyzed as repeated measures on d 17 and d 31. Mean values were compared by Tukey-Kramer test. Differences between treatments were considered significant at $P < 0.05$ and tendencies at $0.05 \leq P < 0.10$.

RESULTS

Chemical analysis of the oils indicated increased peroxide values, anisidine value, secondary aldehydes (hexanal and 2,4-decadienal) and decreased oxidative stability index in peroxidized oil. Moisture, insoluble impurities, unsaponifiable matter and total and free fatty acids were similar amongst control and peroxidized oils (Table 2).

Pig mortality was relatively high at 10.2%, but did not differ between dietary treatments. No significant differences were detected in growth performance throughout the experimental period (Table 3). However, ADFI tended to increase (1.06 vs. 1.02 kg/d; $P < 0.07$) while G:F tended to decrease (0.68 vs. 0.70 kg/kg; $P < 0.10$) during Phase 2 in pigs supplemented with antioxidant.

Antibody titers to Mhyo increased post-vaccination (interaction, $P < 0.001$; -0.05, 0.19 and 1.68 for d 3, d 17 and d 31 in vaccinated pigs) and antibody titers to PCV2 increased following the second vaccination, but not the first vaccination (interaction, $P < 0.001$; 0.12, 0.15 and 0.89 for d 3, d 17, and d 31 in vaccinated pigs), yet there were no differences in response to vaccination due to dietary treatments (Table 4). Serum MDA concentration, a marker for oxidative stress, was not impacted by peroxidation or antioxidant supplementation (Table 4). MDA concentrations displayed a tendency ($P < 0.07$) for a day by vaccination

interaction, in which control pigs on day 31 had the highest serum MDA concentrations and vaccinated pigs on d 31 had the lowest serum MDA levels.

Serum vitamin E concentrations decreased in pigs fed peroxidized oil by 29% on d 17 (0.78 vs. 1.11 μg/mL for peroxidized oil and control oil, respectively) and 36% (1.05 vs. 1.65 μg/mL) on d 31 (interaction, $P < 0.01$; Figure 1A). Supplementation of antioxidant increased serum vitamin E concentrations ($P < 0.001$; 1.28 vs 1.01 μg/mL) and this effect tended (interaction, $P = 0.06$) to be greater in pigs fed control oil (Figure 1B).

DISCUSSION

Growth performance. The measures of peroxidation achieved by forcing peroxidation of the corn oil by using heat and oxygen exposure are comparable to other recent publications (Boler et al., 2012; Liu et al., 2014a; Rosero et al., 2015). However, while others have reported decreases in growth performance (DeRouchey et al., 2004; Harrell et al., 2010; Liu et al., 2014c; Rosero et al., 2015), the expected decline due to consumption of peroxidized oil was not observed in the present study. Similar to our results, Hanson et al. (2015) did not find differences in growth performance when nursery pigs were fed diets containing oxidized DDGS (PV = 84.1 mEq/kg oil) during a 7-week period, and these authors suggested that the final PV of the diet (calculated as 1.7 mEq/kg of feed) was not sufficiently high to impart detrimental effects in performance. Furthermore, Lu et al. (2014) did not observe effects in growth performance during a 26 d period of feeding 5% peroxidized soybean oil (PV = 180 mEq/kg of oil and PV = 9 mEq/kg of diet) to nursery pigs (starting pig BW was 10.9 kg). Comparably, Boler et al. (2012) were only able to distinguish changes in performance after a 28 d period of feeding peroxidized corn oil (PV = 150 mEq/kg oil and PV = 7.5 mEq/kg in the diet) to finishing pigs (starting pig BW was 80.3 kg).

In the current study, diets containing peroxidized corn oil were calculated to have a PV of 8.7 mEq/kg of diet. Nonetheless, it has been documented that PV of lipids decreased after being added to diets, as demonstrated by Shi-Bin et al. (2007). Indeed, our analyzed PV for diets containing peroxidized oil ranged from 3.1 (diets without antioxidant) to 1.1 mEq/kg of diet (diets supplemented with antioxidant) compared to control diets (mean value was 0.44 mEq/kg). It is worth noting that these analyses were performed after over one year of diets being stored at -4°C, a period in which primary products of oxidation could have declined

substantially. Because several compounds are produced during lipid peroxidation, different analytical tests should be used to measure peroxidation, including primary (peroxides) and secondary (e.g. anisidine value and thiobarbituric acid reactive substances (TBARS)), which are customarily performed in the industry (Kerr et al., 2015). Nonetheless, these and other assays have limitations (NRC, 2012; Liu et al., 2014a; Kerr et al., 2015; Shurson et al., 2015), which further complicates the estimation of peroxidation and predicates the need for novel approaches.

Inclusion of antioxidant did not influence growth performance in the present study. These results are in agreement with Boler et al. (2012), in which antioxidant (a blend of TBHQ and ethoxyquin) supplementation did not improve performance of finishing pigs. Similarly, Lu et al. (2014) reported that antioxidant (ethoxyquin and propyl gallate at 135 mg/kg) treatment did not affect pig performance during the first 26 d of supplementation. However, from d 26 onwards and during the overall trial period (118 d), antioxidant treatment was able to restore growth performance of pigs fed peroxidized lipids to levels comparable to that of pigs fed a control diet without peroxidized oil (40% increase in ADG, 25% increase in ADFI and 16% improvement in G:F for the overall 118 d period compared to pigs fed peroxidized oil). Moreover, Harrell et al. (2010) observed a 2.5% increase in ADFI and a tendency to improve ADG by 2.5% in pigs supplemented with antioxidant containing TBHQ and ethoxyquin during a 42 d feeding period. Nonetheless, these authors did not find significant interactions between dietary peroxidized oil and supplementation of antioxidant. We speculate that the 31 d trial period in the present study may not have been long enough to demonstrate adverse effects of peroxidation, limiting the potential benefit of supplemental antioxidant.

Serology. The observed increase in antibody titers following vaccination was anticipated and agrees with the literature (Morrow et al., 1994; Horlen et al., 2008). Although lacking in our current investigation, the detrimental effects of peroxidation on the immune capacity of broilers have been previously reported. Dibner et al. (1996) observed reduced lymphocyte proliferation in pigs and reduced IgA concentration in intestinal tissue of broilers fed peroxidized poultry fat. Liang et al. (2015) recently demonstrated a 48% reduction in secretory IgA and a reduction of approximately 40% in CD4 molecules in jejunal mucosa, but not serum, of broilers fed peroxidized soybean oil (PV= 8.97 mEq/kg diet). Consistent with our results,

Liu et al. (2014b) did not find differences in serum IgA and IgG in pigs fed 10% peroxidized lipids. To our knowledge, the immunomodulatory effects of peroxidized lipids on the response to vaccination have not been previously described in pigs. Thus, more studies investigating the immune response of animals fed peroxidized lipids are necessary to better understand the extent to which animals are affected and the mechanisms involved.

A common assay for products of oxidative damage, the thiobarbituric acid reactive substances (TBARS) assay, measures the production of a conjugated-double-bond compound formed from the reaction of TBARS with malondialdehyde (MDA). In contrast to previous publications (Ringseis et al., 2007; Shi-bin et al., 2007; Boler et al., 2012; Liu et al., 2014b; Lu et al., 2014; Liang et al., 2015; Rosero et al., 2015), serum MDA concentrations were not distinguishable between treatments in the present study. Similar results have been reported by Hanson et al. (2015), in which serum MDA concentrations did not differ due to supplementation of oxidized DDGS. Nonetheless, serum vitamin E concentrations did vary in response to dietary treatments. Vitamin E is a chain-breaking antioxidant with protective effects against membrane damage (Wiseman, 1996). A decrease in serum vitamin E due to peroxidation of oil detected in our study is in agreement with findings by Ringseis et al. (2007), in which growing pigs receiving peroxidized (PV= 10 mEq/kg oil) sunflower oil at 9% of the diet had lower (approximately 30% decrease) α-tocopherol concentrations in intestinal epithelial cells compared to pigs receiving fresh oil (PV= 2.5 mEq/kg oil). Comparable results have been reported in broilers. Boler et al. (2012) reported a 54% decrease in plasma vitamin E when feeding 5% peroxidized (PV= 150 mEq/kg) corn oil to growing pigs. In the same study, however, addition of antioxidant did not influence plasma vitamin E concentrations. Moreover, Tavárez et al. (2011) observed a 58% decrease in vitamin E in serum of birds fed peroxidized (PV= 180 mEq/kg) soybean oil. Addition of antioxidant blend increased serum vitamin E concentrations by 14%, which is in agreement with our findings of a 28% increase in serum vitamin E concentration.

Despite the lack of differences in MDA concentrations, serum vitamin E results indicate that peroxidized oil may have disturbed oxidative systems in pigs, but not to the extent where performance was affected. Concurrently, the presence of antioxidant seemed to exert a sparing effect on vitamin E.

In conclusion, pig performance and response to vaccine were not affected by peroxidized corn oil or supplementation of antioxidant. However, serum vitamin E status was reduced by consumption of peroxidized oils and increased with the addition of antioxidant. We propose that a longer feeding period of peroxidized lipids may be necessary to demonstrate adverse effects on growth performance, while a decline in vitamin E status may be an early indicator of induced oxidative stress in pigs fed peroxidized lipids.

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Table 1. Composition of experimental diets, as-fed basis[1]

Item	Phase 1	Phase 2
Ingredient, %		
Corn, yellow dent	57.56	60.23
Soybean meal, 47.5% CP	33.10	30.71
Corn oil[2]	6.00	6.00
L-lysine HCl	0.37	0.35
DL-methionine	0.16	0.11
L-threonine	0.14	0.09
Monocalcium phosphate, 21% P	0.83	0.75
Limestone	1.13	1.05
Salt	0.50	0.50
Phytase[3]	0.02	0.02
Vitamin premix[4]	0.04	0.04
Trace mineral premix[5]	0.15	0.15
Antioxidant[6]	0 or 0.1	0 or 0.1
Calculated composition, %		
ME (kcal/kg)	3629.0	3633.8
Crude Protein	21.05	21.05
Crude Fat	9.24	9.27
Total lysine	1.44	1.36
Calcium	0.70	0.65
Total Phosphorus	0.56	0.54
Analyzed composition, %		
Moisture	12.93	12.60
Crude Protein	20.35	18.96
Crude Fat	8.60	8.46
Calcium	0.73	0.70
Phosphorus	0.55	0.51
Ethoxyquin[7]	<10 (24) ppm	<10 (18.5) ppm

Table 2. Continued.

[1]Diets were formulated to meet or exceed NRC (2012) requirements. Phase 1 diets were offered from d 0 to d 14, followed by the Phase 2 diets from d 15 to d 31 of the study.

[2]Control or peroxidized corn oil. Peroxidized corn oil was created by heating the control oil (80°C) while bubbling O_2 through the lipid at a constant rate of supply (15.4 mL/min per kg of lipid) for 12 d. Lipids were stabilized with liquid antioxidant (TBHQ) after peroxidation.

[3]Ronozyme HiPhos 2500 (DSM Nutritional Products, Parsippany, NJ). Provided 400 FYT of phytase activity/kg of complete diet.

[4]Supplied per kilogram of complete diet: 8,227 IU of vitamin A, 1,172 IU of vitamin D_3 as D-activated animal sterol, 47.0 IU of vitamin E, 0.03 mg of vitamin, B_{12}, 5.8 mg of riboflavin, 35.2 mg of niacin, 23.5 mg of d-pantothenic acid as calcium pantothenate, 3.8 mg of vitamin K as menadione dimethylpyrimidinol bisulfate, 1.7 mg of folic acid, and 0.23 mg of d-biotin.

[5]Supplied per kilogram of complete diet: 16.5 mg Cu as $CuSO_4$, 0.30 mg I as ethylenediamine dihydriodide, 165 mg Fe as $FeSO_4$, 40 mg Mn as $MnSO_4$, 0.30 mg Se as Na_2SeO_3, and 165 mg Zn as ZnO.

[6]Added to half of the experimental treatments; Endox® Dry; Kemin Industries, Inc.

[7]Values in parenthesis are ethoxyquin concentrations of diets supplemented with antioxidant.

Table 3. Composition of experimental oils[1]

Item	Control	Peroxidized
Moisture, %	0.5	0.5
Insoluble impurities, %	0.13	0.10
Unsaponifiable matter, %	0.78	0.56
Free fatty acids, %	0.09	0.11
Total fatty acids, %	93.0	90.2
Iodine value	123.2	116.9
Peroxide value, mEq/kg		
Initial	0.4	146
4 h AOM[2]	3	290
20 h AOM	443	539
Anisidine value[3]	2.2	164.4
Oxidative Stability Index (OSI), hours	20.63	2.95
Hexanal, ppm	<10.0	345
2,4-Decadienal, ppm	7	1622

[1]Antioxidant (Rendox® CQ, Kemin Industries, Inc.) was added at 0.1% after lipids were peroxidized to control oil and peroxidized oil to prevent further degradation.

[2]AOM, active oxygen method.

[3]Anisidine value is a relative measure used to determine aldehyde content of peroxidized oils.

Table 4. Effects of peroxidized oil and antioxidant supplementation on growth performance and mortality of nursery pigs[1]

	Control oil		Peroxidized oil			*P*-VALUE		
Item	**No Antioxidant**	**With Antioxidant**	**No Antioxidant**	**With Antioxidant**	**SEM**	**Peroxidation[2]**	**Antioxidant[3]**	**Interaction**
BW, kg								
d 1	9.11	9.11	9.11	9.11	0.428	0.940	0.985	1.000
d 14	12.88	12.64	12.81	12.57	0.643	0.814	0.414	0.989
d 31	24.21	25.11	24.13	24.05	0.890	0.153	0.298	0.216
ADG, kg								
d 1 to 14	0.244	0.232	0.221	0.227	0.025	0.502	0.883	0.654
d 15 to 31	0.707	0.740	0.709	0.714	0.021	0.341	0.154	0.277
d 1 to 31	0.486	0.489	0.470	0.481	0.022	0.433	0.628	0.770
ADFI, kg								
d 1 to 14	0.459	0.463	0.472	0.455	0.027	0.915	0.746	0.596
d 15 to 31	1.023	1.097	1.017	1.033	0.037	0.149	0.070	0.238
d 1 to 31	0.760	0.817	0.762	0.763	0.031	0.213	0.165	0.177
G:F								
d 1 to 14	0.521	0.496	0.461	0.490	0.039	0.356	0.944	0.454
d 15 to 31	0.693	0.676	0.700	0.692	0.008	0.135	0.096	0.561
d 1 to 31	0.640	0.599	0.618	0.629	0.016	0.801	0.333	0.104
Mortality, n	3	6	6	3	-	1.000	1.000	0.526

[1]Values represent least squares means of 44 pens with 4 pigs (2 females, 2 castrated males) per pen.

[2]Peroxidized corn oil was heated at 80°C with constant O_2 supply (15.4 mL/min per kg of lipid) for 12 d. Oil was stabilized with liquid antioxidant (TBHQ) after peroxidation.

[3]Antioxidant (Endox® Dry, Kemin Industries) was added at 0.1% to half of the experimental diets.

Table 5. Effects of peroxidized oil and antioxidant supplementation on serum oxidative status and antibody titers of nursery pigs[1]

	Control oil		Peroxidized[2] oil		
Item	**No Antioxidant**	**With Antioxidant[3]**	**No Antioxidant**	**With Antioxidant[3]**	**SEM**
MDA, mmol/L[4]	3.49	3.61	3.66	3.35	0.21
PCV2[5]					
d 3	0.15	0.11	0.15	0.11	0.05
d 17	0.13	0.12	0.10	0.10	0.04
d 31	0.44	0.46	0.49	0.54	0.05
Mhyo[5]					
d 3	-0.05	-0.06	-0.05	-0.05	0.04
d 17	0.00	-0.01	-0.02	-0.01	0.04
d 31	0.86	0.78	0.81	0.86	0.04

[1]Values represent means of control and vaccinated pigs.

[2]Peroxidized corn oil heated (80°C) with constant O_2 supply (15.4 mL/min per kg of lipid) for 12 d. Oil was stabilized with liquid antioxidant (TBHQ) after peroxidation.

[3]Antioxidant (Endox® Dry, Kemin Industries) was added at 0.1% to half of experimental diets.

[4]Main effect of peroxidation, antioxidant and their interaction was not significant ($P>0.10$). Day by vaccination interaction tended ($P<0.07$) to differ, in which control pigs on day 31 had highest serum MDA and vaccinated pigs on d 31 had lowest serum MDA levels.

[5]Main effect of day, vaccination and day by vaccination interaction ($P<0.001$). Main effect of peroxidation, antioxidant and their interaction was not significant ($P>0.10$).

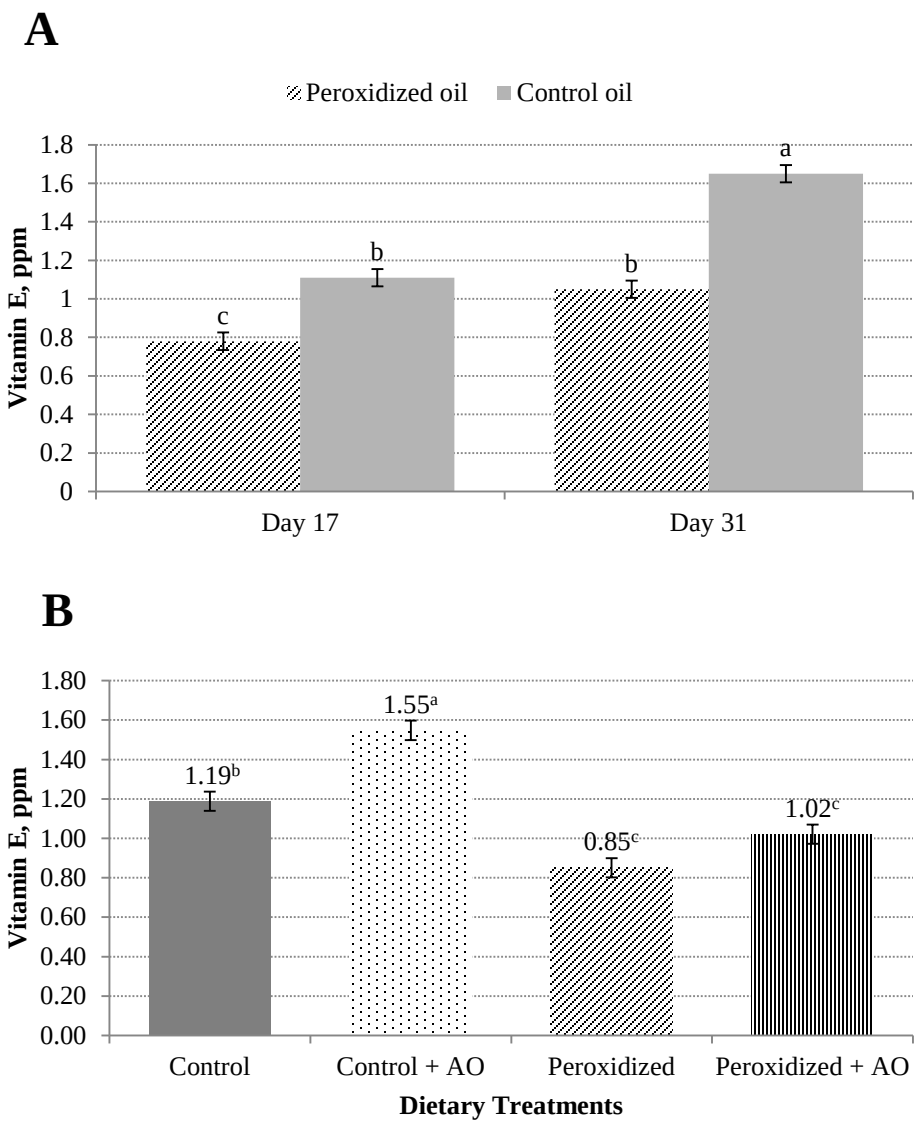

Figure 1. Serum vitamin E concentrations in pigs fed diets containing either 6% control oil or 6% peroxidized oil supplemented either without or with antioxidant (+AO) at 0.1%. Peroxidation decreased (P<0.001) serum vitamin E concentrations and this effect was greater on day 31 than day 17 of the study (Panel A). Antioxidant supplementation increased (P<0.001) vitamin E concentrations and this effect tended to be greater in pigs fed the diet with control oil (Panel B; interaction, $P < 0.06$). $^{abc}P < 0.05$.

CHAPTER III: IMPACT OF LIPID PEROXIDATION ON GROWTH PERFORMANCE AND VIABILITY OF NURSERY PIGS

ABSTRACT

Peroxidized lipids can be detrimental to pig performance and induce oxidative stress. However, no information exists with regard to the impact of lipid peroxidation on health, viability, and mortality of pigs housed under the rigors of commercial conditions, such as high population density and immune challenge. The objective of this study was to investigate the impact of lipid peroxidation in a dose-dependent manner on performance, health, medical treatment, viability and oxidative status of nursery pigs. A total of 2,200 nursery pigs (initial BW = 5.95±0.2 kg) were housed in 100 pens with 22 pigs/pen in a RCBD based on initial BW and sex and randomly assigned within blocks to 5 dietary treatments. Treatments consisted of a corn-soybean meal based supplemented with 5% of either control corn oil (IV=117.5, FFA=0.06%, anisidine value=2.5, peroxide value=4.8 mEq/kg oil) or peroxidized corn oil (IV=119.5, FFA=0.35%, anisidine value=30.4, peroxide value=162.6 mEq/kg oil). These 2 diets (0% and 100% peroxidation of oil) were blended to obtain 5 different concentrations of peroxidation. Final treatments consisted of diets with 0 (diet with 5% control oil), 25, 50, 75, and 100% (diet with peroxidized corn oil) peroxidation. Nursery diets consisted of 3 phases; Phase 1 fed for 7 days; Phase 2 fed for 13 days and Phase 3 fed for 23 days. Pigs were vaccinated with PRRS modified live virus vaccine mixed with a *Mycoplasma hyopneumoniae* (Mhyo) bacterin vaccine (Ingelvac PRRS® MLV and Ingelvac MycoFLEX®, Boehringer Ingelheim Vetmedica, Inc.) on d 0 and a PCV2 and Mhyo combination vaccine (Ingelvac FLEXcombo, Boehringer Ingelheim Vetmedica, Inc.) at approximately 9 weeks of age. Blood samples were collected from 20 pigs per treatment (10 light- and 10 heavy-weight pigs) prior to (on d 33) and 14 d after the second vaccine dose to determine antibody titers to vaccination, oxidative stress and vitamin E concentrations in serum. Pig G:F decreased linearly ($P < 0.03$) with increasing dietary peroxidation level. Final pen gain, considering mortality and pigs culled, decreased linearly ($P < 0.01$) with increasing level of peroxidation (341, 317, 311, 303 and 305 kg, for 0, 25, 50, 75 and 100% level). Antibody titers to Mhyo and PCV2 increased post-vaccination ($P <0.01$; 0.06 vs. 0.22 for Mhyo and 0.26 vs. 0.35 for PCV2, before and after second vaccination, respectively) but there were no differences due to dietary treatment, BW category (light vs. heavy pigs) or their interaction. Serum concentrations of 8-OHdG and protein carbonyls were not affected by peroxidation level, BW category or their interaction. Serum MDA was 19% higher in heavy BW pigs than in light weight pigs ($P < 0.01$), but

concentrations did not differ due to peroxidation level or peroxidation by BW interaction. Total antioxidant capacity in serum increased 10% in heavy BW pigs compared to light pigs ($P < 0.03$), and decreased linearly ($P=0.05$) as peroxidation level increased (100.0, 97.1, 89.1, 84.7, 92.9 µM Trolox eq/mL for 0, 25, 50, 75 and 100% peroxidation). Vitamin E concentrations decreased ($P = 0.01$) linearly (1.24, 1.32, 1.23, 0.99, 1.15 ppm for 0, 25, 50, 75 and 100% peroxidation) with increasing level of peroxidation. Concentrations were 10% higher in heavy compared to light BW pigs ($P < 0.04$). Data indicated a progressive negative impact of lipid peroxidation on pig productivity measured under field population conditions, which was primarily related to increased mortality, number of pigs medicated, and number of pigs that were excessively light. These results underscore the importance of proper assessment of lipid peroxidation and quality control in order to improve profitability of pork production.

INTRODUCTION

Improving the efficiency of swine production is essential for the economic viability and the environmental sustainability of the swine industry. Because the energy component of swine diets is one of the costliest, inexpensive, energy dense ingredient sources such as rendered fats and DDGS oil are becoming more prevalent. However, quality and peroxide values of these sources are highly variable, and could potentially be detrimental to animal health and productivity (van Kempen and McComas, 2002; Song et al., 2014; Kerr et al., 2015).

Indeed, peroxidized lipids have shown to decrease performance of swine and broilers and induce oxidative stress in most cases (Lu et al., 2014; Shurson et al., 2015). This response appeared to be linear with inclusion of lipids with increasing peroxidation values (DeRouchey et al., 2004; Liang et al., 2015; Rosero et al., 2015; Hanson et al., 2016).

Nevertheless, effects of peroxidized lipids on the immune system of livestock animals remains to be elucidated. The ultimate impact of lipid peroxidation on health, viability, and mortality of pigs that are housed under the rigors of commercial conditions, such as high population density and greater immune challenge, is not known. Therefore, we designed and conducted a large field study to assess the effects of feeding pigs with peroxidized oils on pig productivity and herd health.

We hypothesized that feeding nursery pigs with peroxidized corn oil would decrease growth performance, compromise health status and promote oxidative stress, and these effects

could impose a higher toll on light-weight pigs. Thus, the objective of this study was to investigate the impact of lipid peroxidation on performance, health, medical treatment, viability and oxidative status of nursery pigs in a dose-dependent manner.

MATERIALS AND METHODS

Animals and dietary treatments

All animal protocols were approved and conducted under the supervision of licensed veterinarians. This experiment was conducted in a commercial research facility located in White Hall, IL, owned and operated by The Hanor Family of Companies. Nursery pigs (PIC TR-4 sire x Camborough derivative; n = 2,200; mean BW = 5.95 ± 0.2 kg) were allotted to a RCBD based on initial BW and sex and randomly assigned within blocks to 5 dietary treatments. Pigs were housed in pens with 22 pigs/pen using 100 pens (20 pens per treatment). Pens were 5.6 m^2 in dimension and contained one 6-hole feeder and 2 nipple drinkers. Pigs had ad libitum access to feed and water.

Dietary treatments consisted of 5 degrees of lipid peroxidation to present a dose response challenge of increasing peroxidation and treatments were administered for the duration of the nursery phase (43 days). Diets were corn-soybean meal based and supplemented with 5% of either an edible, refined corn oil (WebstaurantStore, Lancaster, PA) or the same oil that was peroxidized. These 2 treatments (0% and 100% peroxidation of oil) were manufactured by a commercial feed mill (Greenfield, IL) owned and operated by The Hanor Family of Companies. The remaining dietary treatments were obtained by mixing those 2 diets on farm by using an automated feeding system (Feedlogic Corporation, Wilmar, MN) capable of blending, weighing, and recording feed delivered to individual pens. Final treatments consisted of diets with 0 (diet with 5% control oil), 25, 50, 75, and 100% (diet with peroxidized corn oil) peroxidation.

Peroxidized corn oil was obtained by exposing corn oil to heat (65°C) and forcing air through the oil at a constant rate of 20 L/min for 12 days. This was done in a 3,785 L capacity stainless steel tank, provided with 2 heaters located on each end of the tank. Air was distributed through the oil through PVC pipes with multiple 1 mm diameter holes to allow for increased exposure of oil to air. Throughout the peroxidation process, oil samples were taken and analyzed for 2-thiobarbituric acid value (TBA) and peroxide value (PV) according to AOCS

(method Cd 19-9, 2010) and AOAC (method 965.33, 2007), respectively. After 12 days of peroxidation, a TBA value of 0.08 units and 150 mEq/kg of oil for peroxide value were reached, and the peroxidation processed was stopped. Lipids were subsequently stabilized with liquid antioxidant containing tertiary butyl hydroquinone (TBHQ) (Rendox® CQ, Kemin Industries, Inc.; added at 0.1% of the lipid) to prevent further peroxidation.

Pigs were fed three nursery diet phases, using a Phase 1 diet offered from d 0 to d 7, a Phase 2 diet from d 8 to d 20, followed by phase 3 diet fed from d 21 to 43 (Table 1). All pigs and feeders were weighed on d 0, when switching diets on d 7 and d 20, and at the end of the study on d 43 for growth performance calculations. Calculations of ADG and ADFI accounted for dead pig weights and the number of days they were present in the pen. Total pen gain was calculated as the difference of the total weight of pigs in the pen at the end of the study and the total weight of pigs in the pen at the start of the study. Thus, this measure considers only the pigs that finished the study and does not include pigs that died or were culled, which is practically relevant.

The decision to remove or treat a pig was based on poor or declining body condition, lameness, injury or noticeable respiratory distress. Pigs removed from test were weighed, tagged and recorded with removal reason and date. Removed and tagged pigs were maintained on their designated dietary treatments. Outcome of pigs in sick pens was tracked by tag number. Pigs weighing less than or equal to 13.6 kg at the end of the trial were considered in the non-full-value category and were culled. Deaths and all light-weight or substandard pigs were recorded at the end of the nursery period to consider viability of pigs (full-value pigs, FVP).

To determine the ability of pigs to respond to an immune challenge, pigs were vaccinated according to the established protocol designed by the veterinary department of the farm, including a PRRS modified live virus vaccine mixed with a *Mycoplasma hyopneumoniae* (Mhyo) bacterin vaccine (0.5 mL dose; Ingelvac PRRS® MLV and Ingelvac MycoFLEX®, Boehringer Ingelheim Vetmedica, Inc., St Joseph, MO) at placement and a PCV2 and Mhyo combination vaccine (1 mL dose; Ingelvac FLEXcombo, Boehringer Ingelheim Vetmedica, Inc., St Joseph, MO) at approximately 9 weeks of age.

Blood samples were collected from 20 pigs per treatment (10 light- and 10 heavy-weight pigs; selected from the 20 lightest and 20 heaviest pens) prior to (on d 33) and 14 d

after the second vaccine dose to determine antibody titers to vaccination. The second blood collection was done 4 days after all pigs had been sent to the finisher barns (on d 43 of the study), but sampled pigs remained in the nursery rooms and continued to receive their assigned diets until the second bleeding day. Blood samples collected on d 33 were also used to assess oxidative status (total antioxidant capacity, malondialdehyde, nucleic acid damage, protein carbonyl) and vitamin E concentrations in serum. Immediately after collection, blood samples were placed at 4°C and allowed to clot for approximately 12 h before serum separation. Serum was obtained by centrifugation at 1,000 × g for 12 min at room temperature and subsequently frozen at -20°C for further analyses.

Chemical analysis

Representative oil samples were submitted to commercial laboratories (Kemin Industries, Inc., Des Moines, IA and New Jersey Feed Laboratory Inc., Trenton, NJ) and lipids were analyzed for their chemical composition and peroxidation measures (Table 2) according to AOAC (2007) and AOCS (2010).

Serum samples were sent to the Veterinary Diagnostic Laboratory at Iowa State University (Ames, IA) for analyses of antibody titers and vitamin E concentrations. Antibody titers to PCV2 and Mhyo were determined by ELISA (INgezim Circo IgG, Ingenasa, Madrid, Spain for PCV2; IDEXX M. hyo. Ab Test, Idexx Laboratories Inc., Westbrook, USA for Mhyo). Titer results are presented as the sample to positive (S/P) ratio, calculated as the sample absorbance minus the negative control mean, divided by the difference between the positive and negative control means. Serum vitamin E concentrations were determined by HPLC. Briefly, serum samples underwent ethanol and hexane/chloroform extractions and dissolved in a chromatographic mobile phase (95:5; 95% being 90:10 methanol:chloroform, 5% water), then concentrations were measured by HPLC (Dionex PDA-3000, Dionex Corp., Sunnyvale, CA) set at a wavelength of 292 nm. Separation was achieved using a reverse phase Pecosphere C18 (4.6 x 33 mm) column (PerkinElmer, Inc., Waltham, MA).

Serum concentrations of malondialdehyde (MDA) were measured using a commercially available kit, following manufacturer's instructions (OxiSelect TBARS Assay Kit, Cell Biolabs Inc., San Diego, USA). Total antioxidant capacity (TAC) in serum was determined according to a colorimetric method previously described by Erel (2004).

Concentrations of 8-hydroxy-2'-deoxyguanosine (8-OHdG) were measured in serum using a commercially available kit, according to manufacturer's instructions (DNA/RNA Oxidative Damage EIA Kit; Cayman Chemical Company, Ann Arbor, MI). Protein carbonyls were quantified in serum using a commercial kit (Protein Carbonyl Colorimetric Assay Kit, Cayman Chemical Company, Ann Arbor, MI) with modifications. Briefly, serum samples were diluted 1:3 with 0.01 M phosphate-buffered saline (pH 7.0) to obtain protein levels in the range of 1 to 10 mg/ml, then incubated with 10 mM dinitrophenylhydrazine (DNPH) in 2.5 M HCl (for serum samples) or 2.5 M HCl alone (for sample blanks) for 1 hour in the dark. Trichloroacetic acid (TCA, 10% final concentration) was added to precipitate proteins in the DNPH samples and their appropriate blanks. The DNPH samples and sample blanks were washed with 10% TCA, which was followed by 3 ice-cold acetone washes. Finally, DNPH samples and their blanks were resuspended with 6 M guanidine hydrochloride (pH 6.5) and aliquoted into 96 well plates. Absorbances were measured at 360 nm in a multi-detection microplate reader (Synergy HT, BioTek Instruments, Inc., Winooski, VT). Carbonyl content (nmol/ml) was determined with the corrected absorbances (DNPH sample absorbance minus sample blank absorbance) and the extinction coefficient of DNPH. To normalize the carbonyl content to the amount of protein present in the resuspended 6 M guanidine HCl samples, protein concentrations were determined using a bicinchoninic acid assay kit (Pierce BCA Protein Assay Kit, Thermo Fisher Scientific Inc., Rockford, IL). Final carbonyl content was expressed as nmol carbonyl/mg protein.

All analyses were conducted in duplicate; intra- and inter-assay CV were 1.9 and 1.3% for MDA, 2.2 and 3.5% for TAC, 9.3 and 12.4% for 8-OHdG, 0.7 and 0.8% for protein carbonyls, respectively.

Statistical analysis

Two pens were removed from the study due to issues with the feed delivery system at the beginning of the trial. Growth performance, health status (percentage of sick pigs, pigs receiving medical treatment, culling rate), number of full-value pigs at nursery close-out and mortality data were analyzed using PROC Mixed of SAS (v. 9.4; SAS Inst. Inc., Cary, NC), testing for fixed effects of peroxidation. Pen was the experimental unit and weight block was used as random effect.

Antibody titers, markers of oxidative stress and vitamin E concentrations were analyzed by PROC Mixed of SAS. The model included fixed effects of peroxidation, day of sampling, BW category (heavy- vs light-weight) and relevant interactions. Pig was considered the experimental unit and pig nested within pen was used as random effect (for antibody titers only). Antibody titers were analyzed as repeated measures using day of sampling.

Mean values were compared by Tukey-Kramer test. Orthogonal contrasts were used to determine linear and quadratic effects of peroxidation levels. Significant differences between treatments were considered at $P < 0.05$ and tendencies at $0.05 \leq P < 0.10$.

RESULTS

Growth performance and health status. No significant differences were detected in ADG or ADFI throughout the experimental period (Table 3). Feed efficiency (G:F) decreased linearly with increasing levels of peroxidation in the feed ($P < 0.02$) during the first 20 d of the study and for the overall period. Final pen body weight gain, accounting for death losses and culled pigs, decreased linearly ($P = 0.002$) with increasing peroxidation (Figure 1).

Percentage of pigs removed, culled and treated increased linearly ($P < 0.03$) with increasing level of peroxidation. Mortality increased linearly ($P < 0.04$) while percentage of full-value pigs decreased linearly ($P < 0.01$) with increasing dietary peroxidation level (Table 3).

Serology. Antibody titers to Mhyo and PCV2 increased after the second vaccination ($P < 0.01$; 0.06 vs. 0.22 for Mhyo and 0.26 vs. 0.35 for PCV2, on d 33 and d 47, respectively), yet there were no differences due to dietary treatment, BW category or their interaction (Table 4). Serum concentrations of 8-OHdG and protein carbonyls were not affected by peroxidation level, BW category or their interaction. Serum MDA was 19% higher ($P < 0.01$) in heavy BW pigs than in light weight pigs, but concentrations did not differ due to peroxidation level or peroxidation by BW interaction. Total antioxidant capacity in serum increased by 10% ($P < 0.03$) in heavy BW pigs compared to light weight pigs and reduced linearly ($P = 0.05$) as peroxidation level increased. Vitamin E concentrations decreased linearly ($P = 0.01$) with increasing peroxidation level of the diet. Concentrations were 10% higher ($P < 0.04$) in heavy weight pigs compared to light weight pigs (Table 4).

DISCUSSION

Several compounds are produced during lipid peroxidation and various analytical tests can be employed to measure oxidation, such as peroxide value, anisidine value, thiobarbituric acid reactive substances (TBARS), and these are customarily performed in the industry (Kerr et al., 2015). In the current study, peroxidation of corn oil as measured by primary (peroxides) and secondary (aldehydes) oxidation compounds was comparable to values reported previously in the literature (Liu et al., 2014a; Rosero et al., 2015). While these and other studies (DeRouchey et al., 2004; Shi-bin et al., 2007; Lu et al., 2014; Hanson et al., 2016) have demonstrated adverse effects on pig growth performance, the health status and viability of pigs fed peroxidized oils in a commercial setting have not been documented hitherto.

In the present study, feed efficiency of pigs was reduced as peroxidation level in the diet increased. Moreover, health status of pigs was clearly compromised by increasing peroxidation level in diets, which was evidenced by greater percentage of pigs removed and treated and reinforced by decreased viability of pigs at the end of the study. When considering mortality and pig viability in the calculation of total pig gain for each pen, total gain per pen at the end of the trial was significantly reduced with increasing levels of peroxidation by up to 11%. These data clearly indicate the negative impact of peroxidation on pig productivity and survival. To our knowledge, this is the first study that quantified peroxidation level of supplemental lipids in relation to the negative impact on viability of pigs, and presents population responses to peroxidation, including removed pigs, excessively light pigs, mortality, and medical treatments, that are otherwise often ignored. This measure is particularly pertinent to the industry, because the financial value of retaining viable pigs is a powerful driver of return over investment.

To investigate possible mechanisms of peroxidation on the immune system of pigs, we measured serum antibody titers to vaccines in the heaviest and lightest pigs; however, these variables were unaffected by peroxidation or BW of pigs. Toxicity of dietary lipid peroxides (as indicated by enlarged livers) has been reported in rats (Kanazawa et al., 1985) and pigs (Liu et al., 2014c; Lu et al., 2014). Reduced immune capacity has been described by Dibner et al. (1996), who observed reduced lymphocyte proliferation in pigs and reduced IgA concentration in intestinal tissue of broilers fed oxidized poultry fat. Liang et al. (2015) recently demonstrated a 48% reduction in secretory IgA and reduction of approximately 40%

in CD4 molecules in jejunal mucosa of broilers fed oxidized soy oil. On the other hand, Liu et al. (2014b) did not find differences in serum IgA and IgG in pigs fed 10% oxidized lipids. Despite the lack of effects on antibody titers due to dietary treatments in the present study, the increase in number of pigs that received medical treatment and were removed or culled indicates that pigs were sensitive to peroxidation, and perhaps immunocompromised.

Poor health status may be related to oxidative stress (Sordillo and Aitken, 2009). Consumption of peroxidized lipids induced oxidative stress in pigs and broilers, as indicated by increased concentrations of MDA as measured by TBARS (Ringseis et al., 2007; Shi-bin et al., 2007; Tavárez et al., 2011; Delles et al., 2014; Liu et al., 2014bç Lu et al., 2014; Rosero et al., 2015; Liang et al., 2015; Hanson et al., 2016), protein carbonyls (Delles et al., 2014 ; Lu et al., 2014) and altered activities of antioxidant enzymes, such as catalase, superoxide dismutase and glutathione peroxidase (Luci et al., 2007; Ringseis et al., 2007; Shi-bin et al., 2007; Boler et al., 2012; Delles et al., 2014; Liang et al., 2015). Frequently used as a marker of oxidative stress, the TBARS assay measures the production of a conjugated-double-bond compound formed from the reaction of TBARS with malondialdehyde (MDA). In contrast to afore mentioned publications, serum MDA concentrations were not affected by dietary peroxidation level, but concentrations were increased in heavy BW pigs compared to their lighter counterparts.

It was expected that heavier pigs would be more capable to overcome dietary induced oxidative stress than lighter pigs, which was supported by greater total antioxidant capacity (TAC) and vitamin E status in these pigs. Thus, the observed increase in MDA concentrations in this category of pigs was unexpected and conflicts with further serology data provided in this study.

8-hydroxy-2'-deoxyguanosine (8-OHdG) is the product of reactive oxygen species (ROS) attack on purines and is an indicator of nucleic acid damage caused by oxidative imbalance (Sen and Chakraborty, 2011). The expected increase in this marker in pigs fed peroxidized lipids was not detected and it conforms with results obtained for serum protein carbonyls. Carbonyls are derived from the reaction of proteins with ROS and have been found to increase in pigs due to consumption of peroxidized lipids (Lu et al., 2014). Despite the lack of differences in these oxidative stress markers, there was a reduction in serum TAC and vitamin E concentrations in pigs fed increasing dietary peroxidation levels. This suggests that

TAC along with the antioxidant properties of vitamin E were able to minimize oxidative stress to levels similar across dietary treatments. Linear decreases in TAC were also detected by Liang et al. (2015) when feeding broilers with increasing dietary PV levels and Rosero et al. (2015) in pigs fed oils with increasing levels of peroxidation.

Vitamin E has been reported to decrease in animals fed peroxidized lipids (Luci et al., 2007; Ringseis et al., 2007; Boler et al., 2012; Hanson et al., 2016) and is consistent with our findings. In contrast, feeding oxidized DDGS increased serum vitamin E concentrations in pigs (Hanson et al., 2015). It has been demonstrated that vitamin E decreases in complete diets ensuing addition of peroxidized oils (Luci et al., 2007; Ringseis et al., 2007; Hanson et al., 2016). In the current study, vitamin E was not analyzed in oils or in the complete diet; hence, there is a possibility that part of the observed decrease in serum vitamin E reflected a reduced vitamin E intake due to destruction of vitamin E in the diet. Hanson et al. (2016) reported that dietary vitamin E concentration was reduced by approximately 16% in diets with peroxidized oil compared to control diets, but serum vitamin E concentrations were reduced by approximately 70% in pigs fed peroxidized lipids.

In conclusion, we clearly demonstrated in a large field study that increasing levels of peroxidation significantly increased mortality and decreased health status of pigs, as indicated by greater number of pigs medicated and pigs that were excessively light, resulting in reduced total pig viability at the end of the nursery. Feed efficiency and total pen gain were impaired with increasing levels of peroxidation in diets. Response to vaccine and indicators of oxidative stress in serum (MDA, 8-OHdG and protein carbonyls) were not affected by increasing levels of lipid peroxidation in the diets. However, serum vitamin E status and total antioxidant capacity were reduced by increasing levels of peroxidized lipids, which may have contributed to the pigs' ability to maintain oxidative stress markers to similar levels across treatments. Thus, quantification of peroxidation level of lipid sources for swine is critically important to design quality control programs for oil and fat sources and to increase profitability of pork production, especially for weaned pigs that are expected to be the most vulnerable to poor lipid quality.

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Table 1. Composition of experimental diets, as-fed basis

Item	Phase 1	Phase 2	Phase 3
Ingredient, %			
Corn, 8.5% CP	8.55	40.29	41.03
Soybean meal	23.54	26.75	35.40
Corn DDGS, 6.5% fat	6.00	8.00	16.00
Oat groats, steamed	20.00	0.00	0.00
Whey permeate[1]	20.00	7.00	0.00
Plant based protein product[2]	7.50	5.50	0.00
Nursery concentrate[3]	6.50	3.50	0.00
Corn oil[4]	5.00	5.00	5.00
L-Lysine	0.48	0.43	0.25
DL-Methionine	0.22	0.17	0.05
L-Threonine	0.10	0.07	0.00
Limestone	0.30	1.15	1.15
Monocalcium phosphate, 21% P	0.31	0.80	0.40
Salt	0.35	0.40	0.43
Cu chloride	0.03	0.03	0.00
Zn oxide	0.42	0.42	0.00
Vitamin mineral premix[5]	0.15	0.15	0.15
Organic acid blend[6]	0.30	0.10	0.10
Sorbent	0.20	0.20	0.00
Choline chloride, 60%	0.07	0.07	0.00
Enzyme blend	0.00	0.00	0.06
Analyzed composition, %			
Moisture	8.40	9.63	10.19
Crude protein	25.80	25.78	24.47
Crude fat	8.55	6.97	9.32
Ca	0.46	0.80	0.68
P	0.56	0.59	0.53

[1]DairyLac 80 (International Ingredients Corp., Monett, MO).

[2]PEP NO-PORC CP 65 (TechMix, LLC., Stewart, MN).

[3]Corn gluten meal, 45%; dextrose, 35%; soy hulls, 5.6%; amino acid blend, 4%; phytase, 0.7%; others.

[4]Control or peroxidized corn oil. Peroxidized corn oil was heated (65°C) with constant air supply (20 L/min) for 12 d. Lipids were stabilized with liquid antioxidant (TBHQ) after peroxidation.

[5]Supplied per kg of complete diet: 105.0 mg of zinc, 100.0 mg of iron, 45.0 mg of manganese, 15.0 of cooper, 0.7 mg of iodine, 0.3 of selenium, 9,920.7 IU of vitamin A, 1,653.5 IU of D3, 77.2 mg of vitamin E, 3.9 mg of vitamin K, 44.1 mg of vitamin B12, 9.9 mg of riboflavin, 33.1 mg of d-pantothenate, 55.1 mg of niacin, 3.3 mg of thiamine, 5.5 mg of pyridoxine, 0.9 mg of folic acid, and 0.3 mg of biotin.

[6]AviPlus (Vetagro Inc., Chicago, IL).

[7]Flo-Bond (Agri-Tec, Amarillo, TX).

[8]Supplied per kg of complete diet: phytase, 1200 FTU (Quantum Blue, AB Vista, Marlborough, UK), and xylanase, 8000 units (Porzyme, Danisco Animal Nutrition, Marlborough, UK).

Table 2. Composition of experimental oils

Item	Control	Peroxidized
Moisture, %	0.07	0.36
Insoluble Impurities, %	0.02	0.01
Unsaponifiable Matter, %	0.82	0.85
Free Fatty Acids, %	0.06	0.35
Iodine Value	117.5	119.5
Peroxide Value, mEq/kg fat		
Initial[1]	3.3	162.6
4 h AOM[2]	4.6	148
20 h AOM	6.8	442
Anisidine Value[3]	2.5	30.4
Oxidative Stability Index[4], h	47.8	0.78
Hexanal, ppm	<5	61
2,4-Decadienal, ppm	15	1080
TBHQ[5], ppm	196.8	17.6

[1]Average values from analysis provided by New Jersey Feed Laboratory Inc. (method 965.33; AOAC, 2007) and Kemin Industries, Inc.

[2]Active Oxygen Method, Cd 12-57 (AOCS, 2010).

[3]Anisidine value is a relative measure used to determine aldehyde content of peroxidized oils.

[4]OSI, method Cd 12B-92 (AOCS, 2010).

[5]Tertiary butyl hydroquinone, liquid antioxidant added at 0.1% of lipid.

Table 3. Effects of lipid peroxidation on growth performance, health status and viability of pigs fed increasing levels of peroxidation[1]

	Level of Peroxidation								
	1	2	3	4	5		*P*-value		
Item	0%	25%	50%	75%	100%	SEM	Perox[2]	Linear	Quad
BW, kg									
d 1	5.95	5.95	5.95	5.94	5.95	0.17	0.998	0.794	0.830
d 7	6.93	6.69	6.72	6.74	6.74	0.21	0.606	0.370	0.273
d 20	12.03	11.98	11.85	11.97	12.01	0.28	0.759	0.878	0.275
d 43	22.61	22.35	22.60	22.33	22.56	0.48	0.889	0.880	0.635
ADG, kg									
d 1 to 7	0.097	0.090	0.089	0.093	0.090	0.008	0.945	0.655	0.614
d 7 to 20	0.409	0.406	0.399	0.399	0.408	0.008	0.664	0.702	0.181
d 1 to 20	0.299	0.290	0.281	0.285	0.290	0.007	0.264	0.242	0.060
d 20 to 43	0.460	0.449	0.465	0.451	0.454	0.012	0.795	0.760	0.957
d 1 to 43	0.386	0.373	0.377	0.372	0.377	0.008	0.510	0.298	0.264
ADFI, kg									
d 1 to 7	0.102	0.098	0.097	0.098	0.100	0.005	0.924	0.811	0.370
d 7 to 20	0.487	0.476	0.479	0.482	0.490	0.010	0.305	0.444	0.052
d 1 to 20	0.349	0.338	0.338	0.341	0.346	0.008	0.396	0.855	0.057
d 20 to 43	0.786	0.761	0.777	0.768	0.780	0.017	0.578	0.906	0.254
d 1 to 43	0.581	0.561	0.568	0.565	0.574	0.012	0.299	0.656	0.076
G:F									
d 1 to 7	0.960	0.910	0.888	0.891	0.884	0.058	0.873	0.340	0.595
d 7 to 20	0.840	0.853	0.829	0.831	0.822	0.010	0.188	0.068	0.600
d 1 to 20	0.856	0.860	0.830	0.840	0.831	0.010	0.068	0.020	0.687
d 20 to 43	0.586	0.588	0.598	0.587	0.583	0.007	0.624	0.716	0.226
d 1 to 43	0.665	0.669	0.664	0.659	0.652	0.005	0.144	0.023	0.216
Removed, %[3]	5.18	7.95	9.32	10.49	10.10	1.24	0.019	0.002	0.151
Treated, %[4]	5.37	9.42	11.82	11.42	10.34	1.31	0.003	0.003	0.006
Culls, %[5]	0.21	0.72	0.91	1.90	1.18	0.45	0.074	0.021	0.286
Mortality, %[6]	0.97	1.36	2.50	2.73	2.88	0.73	0.270	0.033	0.620
Full Value Pigs, %[7]	98.85	97.82	96.59	95.22	95.95	0.94	0.049	0.005	0.325

[1]Values represent least squares means of 20 pens with 22 pigs per pen.

[2]Peroxidized corn oil heated (65°C) with constant air supply (20 L/min) for 12 d. Oil was stabilized with liquid antioxidant (TBHQ) after peroxidation.

[3]Pigs removed from original pens due to sickness or small size.

[4]Pigs in original and sick pens that were treated with injectable medicine.

[5]Pigs 13.6kg or less at the end of the trial period.

[6]Pigs dead in original and sick pens.

[7]All pigs at the end of the trial period minus culls pigs and mortality.

Table 4. Effects of lipid peroxidation on serum oxidative status and antibody titers of pigs fed increasing levels of peroxidation[1]

	Level of Peroxidation						BW Group			*P*-value				
	1	2	3	4	5									
Item	**0%**	**25%**	**50%**	**75%**	**100%**	**SEM**	**Light**	**Heavy**	**SEM**	**Perox[1]**	**Linear**	**Quad**	**BW**	**Perox*BW**
Vitamin E, ppm	1.24	1.32	1.23	0.99	1.15	0.06	1.12	1.24	0.04	0.004	0.011	1.000	0.033	0.275
8OHdG, pg/mL	1798.6	2033.7	1947.6	2002.2	1686.4	164.3	1986.7	1800.7	103.9	0.530	0.623	0.120	0.209	0.636
TAC, µM Trolox eq/mL	100.0	97.1	89.09	84.73	92.95	4.13	88.41	97.11	2.60	0.069	0.051	0.102	0.021	0.842
MDA, µm	9.08	10.93	10.20	9.26	9.25	0.54	8.90	10.58	0.38	0.153	0.487	0.087	0.003	0.757
Protein Carbonyl, pmol/mg	1213.8	1236.9	1199.6	1200.8	1189.3	29.2	1203.2	1212.9	18.5	0.810	0.363	0.780	0.711	0.411
Mhyo, S/P ratio[2]	0.164	0.100	0.12	0.13	0.17	0.02	0.13	0.15	0.01	0.177	0.600	0.032	0.265	0.663
PCV2, S/P ratio[2]	0.280	0.302	0.31	0.30	0.33	0.02	0.32	0.29	0.01	0.589	0.177	0.984	0.111	0.107

[1]Peroxidized corn oil heated (65°C) with constant air supply (20 L/min) for 12 d. Oil was stabilized with liquid antioxidant (TBHQ) after peroxidation.

[2]Main effect of sampling day, P<0.001.

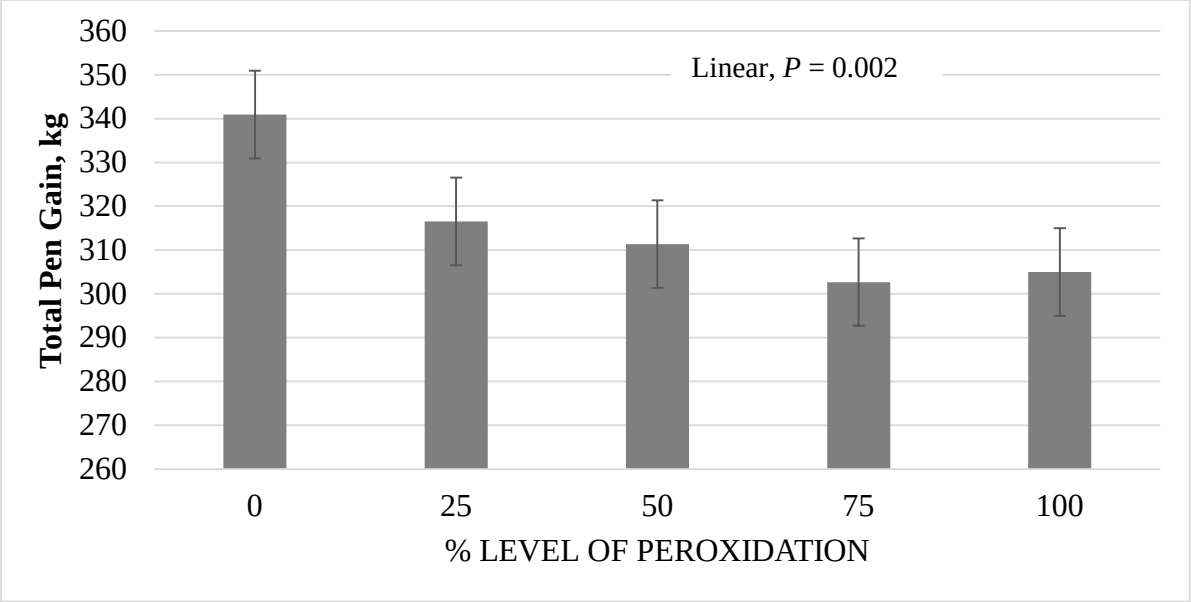

Figure 1. Impact of lipid peroxidation on total gain of pens of pigs during the 43-day nursery period. Each value represents the mean of 20 pens with 22 pigs per pen (at the start of the study). Pen gain was calculated as the sum of weights of pigs present at the end of the study minus the sum of the weights of pigs at the start of the study.

CHAPTER IV: PRACTICAL EVALUATION OF LINOLEIC ACID AND ANTIOXIDANT SUPPLEMENTATION FOR LACTATING SOWS HOUSED UNDER HIGH AMBIENT TEMPERATURES

ABSTRACT

Heat stress imposes significant challenges to lactating sows, including decreased feed intake and impaired reproductive performance. Linoleic acid, as a precursor of eicosanoids, has been shown to improve subsequent reproduction of sows. High producing lactating sows are under oxidative stress, and the use of antioxidants could ameliorate adverse effects associated with it. The purpose of the current study was to determine and verify the impact of linoleic acid supplementation on reproductive performance of sows under practical field conditions and evaluate the impact of commercial antioxidants on oxidative stress markers and sow performance. A total of 605 sows entered the farrowing room and finished lactation in groups of 22 to 24 sows per group. Sows were allotted to a RCBD balanced by parity and assigned within groups to 4 dietary treatments in a 2 x 2 factorial design. Factors consisted of levels of linoleic acid (LA; 1.4 or 3.3%) and antioxidant supplementation (0 or 0.1%). Sows were fed dietary treatments from farrowing until weaning. The first 14 groups (n = 313 sows) of sows were used to assess sow and litter performance. Milk and blood samples were collected from 16 sows per treatment prior to weaning for oxidative stress status assessment. Sow BW change and ADFI were not affected by dietary treatments. Sow BW at 21 d of lactation tended (P = 0.09) to be higher for mature (parity 3 and over) sows consuming 1.4% LA than other treatments. Sow G:F was improved (P = 0.03) by 6% in sows consuming 1.4% rather than 3.3% LA in their diets. Mature sows had higher BW and lower BW loss (change) at d 21 of lactation (P < 0.001). Mature sows consumed (P < 0.001) more feed and had lower feed efficiency than young (parity 1 and 2) sows at d 21 of lactation. Litter performance, number of pigs weaned and pre-wean mortality were not affected by dietary treatment or parity group. Total antioxidant capacity in sow serum was 9% higher (P = 0.02) and protein carbonyls tended (P = 0.07) to be 5% lower in sows fed 1.4% LA compared to sows fed 3.3% LA. Concentrations of MDA increased 20% in sows fed 1.4% LA compared to 3.3% and tended (P = 0.05) to be higher in mature sows compared to young sows. Serum 8OHdG increased (P < 0.01) 28% in mature sows compared to young sows. Vitamin E concentrations in serum were highest in mature sows fed 1.4% LA compared to other treatments (LA x parity group interaction, P < 0.01). Antioxidant supplementation tended (P = 0.07) to increase serum vitamin E by 13% compared to dietary treatments without antioxidant. Vitamin E was 17% higher (P < 0.01) in milk from sows fed 1.4% LA. All 27 groups (n=603) of sows were used to measure subsequent

reproductive performance. Wean-to-estrus interval was not affected by dietary treatment, but mature sows came into estrus almost one day earlier than young sows ($P < 0.001$). Percentage of sows bred, sows returning to estrus and wean-to-farrow interval were not affected by dietary treatment, parity group or their interactions. Farrowing rate tended ($P = 0.07$) to be higher in mature sows fed 3.3% LA with antioxidant than in young sows fed the same dietary treatment (93.4 vs. 82.0%, respectively). Culling rate was 11 percentage points higher in young sows fed 3.3% LA with antioxidant than in mature sows fed the same diet and young sows fed tallow with antioxidant (antioxidant x LA x parity group interaction, $P < 0.02$). Number of pigs born alive was not affected by diet or parity group, but total number of pigs born tended ($P = 0.08$) to increase in mature sows fed 3.3% LA with antioxidant compared to young sows fed the same diet. Supplementation of antioxidant during lactation did not improve oxidative stress status of sows, and did not affect performance of sows and litters during lactation or subsequent reproductive performance. Furthermore, inclusion of 3.3% linoleic acid in lactation diets did not improve performance during lactation or subsequent reproductive performance of sows.

INTRODUCTION

High ambient temperatures during summer months negatively impact animal performance in production systems. During this period, swine producers face significant economic losses due to animals experiencing heat stress (St-Pierre et al., 2003). Sows under high heat have decreased milk production, which mainly reflects the reduced feed intake under these circumstances, but also because in order to dissipate heat, altered blood flow can lead to insufficient nutrient supply to mammary glands (Renaudeau et al., 2012). Moreover, sows experience elevated oxidative stress during mid-late gestation and throughout lactation, and challenges associated with heat stress further increased oxidative stress status in sows (Mahan et al., 2007; Berchieri-Ronchi et al. 2011, Zhao et al., 2011).

Sow reproductive performance also suffers during high temperatures, as shown by delayed or failure to return to estrus, leading to lower farrowing rates in sows bred during summer months (Hennessy and Williamson, 1984). Furthermore, reduced feed consumption when sows are exposed to heat stress negatively affects their daily intake of essential fatty acids (EFA) leading to a negative EFA balance, as demonstrated previously by Rosero et al. (2012a,b). Adequate intake of EFA, namely linoleic (C18:2n-6) and α-linolenic acids (C18:3n-

3), during lactation is of particular interest since these fatty acids are precursors of prostanoids, which can potentially impact reproduction of sows (Thatcher et al., 2010). Indeed, Rosero et al. (2016a) found improved farrowing rate, decreased wean-to-estrus interval and greater subsequent litter size in sows consuming linoleic acid at 3.3% of the diet. However, inclusion of highly unsaturated fatty acids, such as corn oil, further increases oxidative and metabolic stress in the sow; rendering the use of antioxidants especially critical under these circumstances.

Therefore, we hypothesized that supplementation of linoleic acid during lactation will improve reproductive performance of sows housed under high ambient temperatures. Further, oxidative stress associated with lactation and the impact of unsaturated dietary lipids on redox status of the sows may be alleviated by the inclusion of antioxidants in the feed. Thus, the objectives of this study were to determine and verify the impact of linoleic acid supplementation on reproductive performance of sows under practical field conditions and evaluate the impact of commercial antioxidants on oxidative stress markers and sow performance.

MATERIALS AND METHODS

Animals and dietary treatments

All animal protocols were approved and conducted under the supervision of licensed veterinarians. This experiment was conducted in a commercial research facility owned and operated by The Hanor Family of Companies, located in Mooreland, OK, during the months of June to October.

A total of 605 sows (Camborough, PIC, Hendersonville, TN) entered the farrowing room and finished lactation in groups of 22 to 24 sows per group. A total of 149, 155 and 301 sows representing parity 1, 2 and 3 to 6 were used in the study. Parity 1 and 2 sows were considered young sows, and parity 3 to 6 were regarded as mature sows. Sows were allotted to a RCBD balanced by parity and assigned within groups to 4 dietary treatments in a 2 x 2 factorial design. Factors consisted of levels of linoleic acid (LA) and antioxidant supplementation. Diets were corn-soybean meal based with 12% wheat middlings. Levels of LA were obtained by adding 3.75% of either tallow (3% LA content) or corn oil (54% LA content) for a final dietary concentration of 1.4 and 3.3% LA, respectively. Each of these diets

were either not supplemented or supplemented with 0.1% of a synthetic antioxidant blend containing butylated hydroxytoluene, butylated hydroxyanisole and minimum of 3.0% ethoxyquin (Endox® Dry; Kemin Industries, Inc., Des Moines, IA). Diets were formulated to meet or exceed NRC (2012) nutrient recommendations and were manufactured by a commercial feed mill (Enid, OK) owned and operated by The Hanor Family of Companies (Table 1). Diets were color coded for visual confirmation; feed samples were collected weekly on farm and at the feed mill for every new batch. Representative feed samples were submitted to the Agricultural Experiment Station Chemical Laboratories, University of Missouri (Columbia, MO) and proximate analyses were conducted following AOAC (2007) methods.

For the first 14 groups (n = 313 sows), sows were weighed individually when entering the farrowing room, at approximately 110 d of gestation, and again at exactly 21 d of lactation. Sow BW change during lactation was calculated as the difference between sow BW at d 21 of lactation and the estimated post-farrowing BW, using the following equation by Rosero et al. (2013):

Post-farrow BW (kg) = -8.246 + 0.981 * BW at approx. 110 d gestation (kg) - 0.679 * total pigs born.

Sow feed efficiency (G:F) was calculated as the sum of total litter gain (kg) and sow BW change (kg), divided by ADFI (kg). Sows were fed a common lactation diet between placement and farrowing. Experimental diets were provided on the day of farrowing until weaning (23 ± 1 d) and feed offered and refusals were recorded daily for ADFI calculations. Sows were fed to satiety twice daily in the morning and afternoon. Feed refusals were removed once daily in the morning. At farrowing, number of pigs born alive, stillborn pigs and mummies were recorded. Cross-fostering was performed 18 to 24 h after birth to allow for colostrum intake from their dam before moving pigs. All litters were standardized to 12 piglets and initial litter weights and weaning weights were recorded for litter gain calculations. Handling and processing of litters were performed according to standard farm practices under the recommendation of licensed veterinarians. Mortality of pigs throughout lactation was recorded and all litters were weighed at exactly 21 d of lactation. Pigs weighing less than 3.62 kg at weaning were considered no-value pigs. For the remaining 13 groups (n = 292), the same practices were performed, except for measurements of sow BW, litter weights and feed intake.

After weaning (23.1 ± 0.5 days), all sows were moved to breeding rooms and housed

in individual stalls. Sows had fence-line contact daily with a boar to aid in estrus detection. Once sows came into heat, artificial insemination was performed according to standard farm practices. Sows were checked routinely for vaginal discharge and had daily fence-line contact with a boar to detect any sows returning to estrus. Sows were fed a common gestation diet twice daily. Breeding information was recorded and included days to estrus, return to estrus and sows removed by culling. Subsequent reproductive performance data were also collected and included gestation length, abortions, number of pigs born alive, stillborn pigs and mummies.

During the first two months of the study, ambient temperatures of the farrowing room were recorded hourly using data recorders (LogTag®, LogTag Recorders Ltd., Auckland, New Zealand).

Oxidative status

Blood samples were collected from 16 sows per treatment (n = 64; equally balanced by parity) at weaning and at approximately 23 d of lactation. Blood samples were used to assess oxidative status (total antioxidant capacity, malondialdehyde, 8-hydroxy-2'-deoxyguanosine, and protein carbonyl) and vitamin E concentrations in serum. Immediately after collection, serum was obtained by centrifugation at 1,000 × g for 12 min at room temperature and subsequently frozen at -20°C for further analyses.

Milk samples were collected from the same 64 sows one day prior to weaning, at approximately d 22 of lactation, from at least 4 functional glands (anterior, middle and posterior). Oxytocin (1 mL) was injected intramuscularly to facilitate milk let down. Samples were immediately frozen at -20°C for analyses of vitamin E concentrations.

Serum and milk samples were sent to the Veterinary Diagnostic Laboratory at Iowa State University (Ames, IA) for analysis of vitamin E concentrations. Serum and milk vitamin E concentrations were determined by HPLC. Briefly, samples underwent ethanol and hexane/chloroform extractions and were dissolved in a chromatographic mobile phase (95:5; 95% being 90:10 methanol:chloroform, 5% water), then concentrations were measured by HPLC (Dionex PDA-3000, Dionex Corp., Sunnyvale, CA) set at a wavelength of 292 nm. Separation was achieved using a reverse phase Pecosphere C18 (4.6 x 33 mm) column (PerkinElmer, Inc., Waltham, MA).

Serum concentrations of MDA were measured using a commercially available kit, following manufacturer's instructions (OxiSelect TBARS Assay Kit, Cell Biolabs Inc., San Diego, USA). Total antioxidant capacity in serum was determined according to a colorimetric method previously described by Erel (2004). Concentrations of 8-hydroxy-2'-deoxyguanosine (8-OHdG) were measured in serum using a commercially available kit, according to manufacturer's instructions (DNA/RNA Oxidative Damage EIA Kit; Cayman Chemical Company, Ann Arbor, MI). Protein carbonyls were quantified in serum using a commercial kit (Protein Carbonyl Colorimetric Assay Kit, Cayman Chemical Company, Ann Arbor, MI) with modifications. Briefly, serum samples were diluted 1:3 with 0.01 M phosphate-buffered saline (pH 7.0) to obtain protein levels in the range of 1 to 10 mg/ml, then incubated with 10 mM dinitrophenylhydrazine (DNPH) in 2.5 M HCl (for serum samples) or 2.5 M HCl alone (for sample blanks) for 1 hour in the dark. Trichloroacetic acid (TCA, 10% final concentration) was added to precipitate proteins in the DNPH samples and their appropriate blanks. The DNPH samples and sample blanks were washed with 10% TCA, which was followed by 3 ice-cold acetone washes. Finally, DNPH samples and their blanks were resuspended with 6 M guanidine hydrochloride (pH 6.5) and aliquoted into 96 well plates. Absorbances were measured at 360 nm in a multi-detection microplate reader (Synergy HT, BioTek Instruments, Inc., Winooski, VT). Carbonyl content (nmol/ml) was determined with the corrected absorbances (DNPH sample absorbance minus sample blank absorbance) and the extinction coefficient of DNPH. To normalize the carbonyl content to the amount of protein present in the resuspended 6 M guanidine HCl samples, protein concentrations were determined using a bicinchoninic acid assay kit (Pierce BCA Protein Assay Kit, Thermo Fisher Scientific Inc., Rockford, IL). Final carbonyl content was expressed as nmol carbonyl/mg protein.

All analyses were conducted in duplicate; intra- and inter-assay CV were 6.7 and 3.7% for MDA, 17.1 and 7.7% for TAC, 8.1 and 27.7% for 8-OHdG, 0.7 and 0.02% for protein carbonyls, respectively.

Statistical analysis

Sow, litter performance, markers of oxidative stress and vitamin E concentrations in serum and milk were analyzed using PROC Mixed of SAS (v. 9.4; SAS Inst. Inc., Cary, NC). The model included fixed effects of linoleic acid level, antioxidant supplementation, parity

group (young vs. mature) and relevant interactions. Sow groups entering the farrowing room together were used as random effect. Sow initial BW (at approx. 110 d) and litter weights after cross-fostering were used as covariates for performance analyses. Number of pigs weaned, mortality and no-value pigs were analyzed by fitting the data to a Poisson regression using PROC Glimmix of SAS (v. 9.4; SAS Inst. Inc., Cary, NC). Observations with response means greater than three and a half standard deviations from the mean were excluded from the analysis.

Subsequent reproductive performance data were analyzed using mixed linear models according to the data distribution. One sow was culled and one euthanized after weaning and were not moved to the breeding rooms; therefore, these sows were excluded from subsequent reproductive performance analysis. Wean-to-estrus and wean-to-farrow interval data were analyzed by fitting the data to a Poisson regression using PROC Glimmix of SAS with the Laplace method of estimation. For dichotomous variables (sows bred, farrowed, returns, culls) data were analyzed using PROC Glimmix of SAS with a Bernoulli distribution. Breeding, farrowing and return to estrus rates were computed using data from sows that were bred within 18 days after weaning. Total number of pigs born, pigs born alive and stillborn pigs were analyzed using PROC Mixed of SAS, and these variables also excluded data from sows that were bred later than 18 days post-weaning. All models included fixed effects of linoleic acid level, antioxidant supplementation, parity group and relevant interactions. Group of sows was used as the random effect, and group by linoleic acid by antioxidant interaction was also included in the random statement in all models, except for models with Poisson regression and for the variable of sows bred. Observations with response means greater than three and a half standard deviations from the mean were excluded from the analysis.

Mean values were compared pairwise by simple effects least squares mean differences. Differences between treatments were considered significant at $P < 0.05$ and tendencies at $0.05 \leq P < 0.10$.

RESULTS

Sow and litter performance during lactation. Average temperature in the farrowing room during the months of June-August was 25.4°C; the minimum average temperature recorded was 18.1°C and maximum was 31.0°C during this period. The following results include sows

from the first 14 groups placed on test (n=313). Sow BW change and ADFI were not affected by dietary treatments (Table 2). Sow BW at 21 d of lactation tended (LA x parity group interaction, *P*=0.09;) to be higher for mature sows consuming 1.4% LA compared to those fed 3.3% LA, but this was not the case for young sows. Sow G:F was improved (*P* = 0.03) by 6% in sows consuming 1.4% LA compared to 3.3% LA in their diets. Sow parity group significantly (*P* < 0.001) affected sow performance, in which mature (parity 3 and over) sows had higher BW and lower BW loss (change) at d 21 of lactation. Mature sows consumed (*P* < 0.001) more feed and had lower feed efficiency than young (parity 1 and 2) sows at d 21 of lactation. Litter performance, number of pigs weaned and pre-weaning mortality were not affected by dietary treatment or parity group (Table 2). Number of no-value pigs was not affected by LA, antioxidant supplementation, or parity group.

Moreover, when analyzing data from all sows placed on test (n=605), number of pigs weaned, number of no-value pigs at weaning and mortality displayed similar results to the ones reported above, in which main effects of linoleic acid level, antioxidant supplementation, parity group and their interactions did not affect these variables (Table 3).

Serology and milk. Total antioxidant capacity was 9% higher (*P* = 0.02) and protein carbonyls tended (*P* = 0.07) to be 5% lower in sows fed 1.4 compared to sows fed 3.3% LA, while antioxidant supplementation, parity group and their interactions did not affect serum TAC or protein carbonyl concentrations (Table 4). Concentrations of MDA increased 20% in sows fed 1.4% LA compared to 3.3% LA and tended (*P* = 0.05) to be higher in mature sows compared to young sows (Table 4). Antioxidant supplementation did not impact serum MDA. Serum 8OHdG increased (*P* < 0.01) 28% in mature sows compared to young sows, but dietary treatment and interactions did not impact this marker.

Vitamin E concentrations in serum were highest in mature sows fed 1.4% LA compared to other treatments (LA x parity group interaction, *P* < 0.01). Antioxidant supplementation tended (*P* = 0.07) to increase serum vitamin E by 13% compared to dietary treatments without antioxidant. Vitamin E was 17% higher (*P* < 0.01) in milk from sows fed 1.4% LA, but was not impacted by antioxidant supplementation, parity group and their interactions (Table 4).

Subsequent reproductive performance. Wean-to-estrus interval was not affected by dietary treatment (Table 5), but mature sows came into estrus almost one day earlier than young sows (4.9 vs. 5.8 days for mature and young, respectively; $P < 0.001$). Percentage of sows bred within the first 18 d post-weaning and percentage of sows that returned to estrus after being bred within 18 d post-weaning were not affected by dietary treatment, parity group or their interactions (Table 5).

Wean-to farrow interval and percentage of sows that farrowed from sows bred within 18 days of weaning were not affected by dietary treatment, parity group or their interactions (Table 5). However, farrowing rate considering all weaned sows displayed a tendency (antioxidant x LA x parity group interaction, $P = 0.07$) in which mature sows fed 3.3% LA with antioxidant had a higher farrowing rate than young sows fed the same dietary treatment (93.4 vs. 82.0%, respectively). Culling rate was 11 percentage points higher in young sows fed 3.3% LA with antioxidant than in mature sows fed the same diet and young sows fed 1.4% LA with antioxidant (antioxidant x LA x parity group interaction, $P < 0.02$).

Number of pigs born alive and stillborn pigs were not affected by diet or parity group, but total number of pigs born tended (antioxidant x LA x parity group interaction, $P = 0.08$) to increase in mature sows fed 3.3% LA with antioxidant compared to young sows fed the same diet (Table 5).

DISCUSSION

High producing sows are under catabolic and oxidative stress status during lactation and these can potentially impair litter performance (Kim et al., 2013). Moreover, reduced feed intake during high ambient temperatures can aggravate these conditions. Proper nutrition of the sow during this period is hence extremely important, and this study explored several concepts to address these issues.

The supplementation of synthetic antioxidants to sows in the current study did not influence sow or litter performance during lactation or in the subsequent reproductive cycle. Correspondingly, oxidative stress markers in serum of sows taken during lactation were not impacted by antioxidant supplementation, with the exception of a tendency to increase serum vitamin E concentrations. Indeed, the synthetic antioxidants used in this study (BHA, BHT and ethoxyquin) were not expected to have a systemic effect in the sows, because these are rapidly

absorbed and metabolized to be excreted in most animals (Madhavi and Salunkhe, 1996). Therefore, we can infer that the inclusion of highly unsaturated corn oil did not affect peroxidation of the feed and, we can speculate that antioxidant supplementation did not impact oxidative status in the intestinal tract. Indeed, we have previously shown that supplementation of antioxidant increased vitamin E concentrations in serum of nursery pigs, but had no impact in serum MDA (Chang and van Heugten, 2016).

Dietary supplementation of sows with the natural antioxidant vitamin E increased vitamin E in sow plasma (Pinelli-Saavedra and Scaife, 2005), but these authors did not report measures of other oxidative markers. Contrary to our results, Szczubiał (2015) reported improved oxidative status of sows, as indicated by lower plasma TBARS and increased activity of antioxidant enzymes, when feeding sows with a blend of vitamin E, C and β-carotene injection. However, in both studies described above, sows were fed supplemented diets during gestation (Szczubiał, 2015) and from gestation throughout lactation (Pinelli-Saavedra and Scaife, 2005).

Sows fed diets containing 3.3% LA (corn oil based diets) had decreased TAC, vitamin E, and higher protein carbonyls, which could be related to the increased susceptibility of corn oil to peroxidation. Lipids with a higher degree of unsaturation are more prone to peroxidation than saturated lipids (Kerr et al., 2015). Consumption of peroxidized lipids has been shown to induce oxidative stress in pigs (Lu et al., 2014; Rosero et al., 2015; Shurson et al., 2015). Nonetheless, the increase in serum MDA in sows fed 1.4% LA (tallow based diet) is surprising and does not agree with the expected increase in MDA as an oxidative stress marker reported in other studies when peroxidized lipids were fed. Moreover, supplementation of antioxidant to our lactation diets would be expected to protect against lipid peroxidation.

Mature sows seemed to be in increased oxidative stress when compared to young sows as indicated by higher MDA and 8OHdG in serum. 8-hydroxy-2'-deoxyguanosine (8-OHdG) is the product of reactive oxygen species (ROS) attack on purines and is an indicator of nucleic acid damage caused by oxidative imbalance (Sen and Chakraborty, 2011). On the other hand, serum vitamin E was greater in mature vs. young sows suggesting greater antioxidant capacity in mature sows.

Milk vitamin E concentrations increased in sows fed 1.4% LA (tallow), which agrees with results observed in serum. However, this increase did not impact litter performance.

Similarly, Pinelli-Saavedra and Scaife (2005) did not report improvements in litter performance due to increased vitamin E content of sow milk.

Sow performance during lactation was improved when sows were fed 1.4% LA (tallow based diets), as indicated by improved feed efficiency and heavier sow body weight at weaning. Moreover, mature sows consuming tallow tended to lose less weight at the end of lactation than sows fed the other dietary treatments. These results were not expected, because ME, protein, lysine, and fat content of diets were formulated to be similar among all treatments. However, feed analysis indicated a lower fat content in diets containing corn oil (average 4.8% vs. 7.1% in corn and tallow diets, respectively), and this difference could explain, in part, the decreased performance of sows consuming these diets. Furthermore, the increased degree of saturation in tallow was expected to have a lower digestibility than more unsaturated corn oil, as reviewed by Rosero et al. (2016b).

As predicted, mature sows were heavier at placement and at weaning, lost less weight during lactation and consumed more feed than young sows. However, young sows had better G:F than mature sows, which is somewhat unexpected because litter performance, which was used to calculate efficiency, was not affected by parity. Therefore, it appears that increased feed intake of mature sows prevailed over their reduced weight loss, making them less efficient than young sows.

Supplementation with increasing level of LA did not impact litter performance or piglet mortality during lactation, and these results agree with Rosero et al. (2016a), in which performance of lactating sows fed increasing levels of linoleic acid were minimal. However, based on results found by the same authors, we expected an improvement in subsequent reproductive performance of sows. The minimum level of 3.3% linoleic acid in lactation diets was reported by Rosero et al. (2016a) to be required to maximize subsequent reproduction of sows. Linoleic acid is a precursor of prostaglandins that have important roles in reproduction, such as $PGF_{2\alpha}$ and PGE_2 (Weems et al., 2006). Indeed, linoleic has shown to be beneficial during post-partum immunity of dairy cows (Thatcher et al., 2010).

Nonetheless, we failed to detect improvements in subsequent reproductive performance of sows with increased linoleic acid concentration in the diet. Similar to Rosero et al. (2016a), we did not find differences in wean-to-estrus interval, proportion of sows farrowing relative to sows bred, and proportion of sows bred within 18 days after weaning due to linoleic acid

supplementation. The improvements in farrowing rate and culling rate in young sows fed linoleic acid reported by these authors conflict with our findings, in which young sows fed 3.3% LA with antioxidant tended to have the lowest farrowing rate and highest culling rate. However, the observed tendency towards an increase in total pigs born in mature sows fed 3.3% LA with antioxidant agrees with findings by Rosero et al. (2016a).

Even though our study was solely focused on supplementation of this particular omega 6 fatty acid, other authors have explored the effects on subsequent reproductive performance of sows fed fish oils, which are rich in omega 3 fatty acids. Smits et al. (2011) found that feeding lactating sows 0.3% fish oil had positive effects on subsequent litter size, without affecting other reproductive performance traits. Mateo et al. (2009) fed sows 1% fish oil from d 60 of gestation throughout lactation and reported improved piglet ADG throughout lactation in the first and in the subsequent cycle of sows, but did not observe an increase in subsequent litter size.

Collectively, these data indicate that supplementation of antioxidant during lactation did not impact oxidative stress status of sows and did not affect performance of sows and litters during lactation or sow subsequent reproductive cycle. Furthermore, inclusion of corn oil to achieve 3.3% linoleic acid level in lactation diets did not improve performance during lactation or subsequent reproductive performance of sows. Thus, we were unable to duplicate the results found by Rosero et al. (2016a).

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Table 1. Composition of experimental diets, as-fed basis[1]

Item	1.4% LA	1.4% LA + AO	3.3% LA	3.3% LA + AO
Ingredient, %				
Corn, 8.5% CP	49.59	49.49	49.58	49.48
Soybean meal	31.00	31.00	31.00	31.00
Wheat middlings	12.00	12.00	12.00	12.00
Corn oil	0.00	0.00	3.75	3.75
Tallow	3.75	3.75	0.00	0.00
L-Lysine	0.10	0.10	0.10	0.10
L-Threonine	0.06	0.06	0.07	0.07
Limestone	1.11	1.11	1.11	1.11
Monocalcium phosphate, 21% P	0.77	0.77	0.77	0.77
Salt	0.40	0.40	0.40	0.40
Potassium, magnesium sulfate[2]	0.50	0.50	0.50	0.50
Organic mineral source [Zn-Mn-Cu][3]	0.08	0.08	0.08	0.08
Vitamin mineral premix[4]	0.20	0.20	0.20	0.20
Betaine[5]	0.20	0.20	0.20	0.20
Anti-caking aid[6]	0.10	0.10	0.10	0.10
Choline chloride, 60%	0.13	0.13	0.13	0.13
Enzyme blend	0.04	0.04	0.04	0.04
Antioxidant[7]	0.00	0.10	0.00	0.10
Calculated composition, %				
ME (kcal/kg)	3428.8	3425.5	3435.9	3432.6
Crude protein	21.16	21.15	21.14	21.13
Total lysine	1.21	1.21	1.20	1.20
Ca	0.82	0.82	0.82	0.82
Total P	0.57	0.57	0.57	0.57
Linoleic acid, 18:2n-6	1.41	1.41	3.30	3.30
Analyzed composition, %				
Moisture	11.15	10.79	11.23	11.07
Crude protein	20.75	21.28	21.93	21.32
Crude fat	7.10	7.06	4.67	4.92
Ca	0.79	0.67	0.83	0.79
P	0.62	0.66	0.75	0.64
Ethoxyquin (ppm)	59.7	67.6	41.3	57.1

Table 1. Continued.

[1]Diets were formulated to meet or exceed NRC (2012) nutrient recommendations. LA, linoleic acid; AO, antioxidant.

[2]Added as a laxative (Dynamate, The Mosaic Company, Plymouth, MN).

[3]Supplied per kg of complete diet: zinc, 50 mg from zinc amino acid complex; manganese, 20 mg from manganese amino acid complex, and copper, 10 mg from copper amino acid complex (Availa, Zinpro Corporation, Eden Prairie, MN).

[4]Supplied per kg of complete diet: Zn, 125 mg; Fe, 100 mg; Mn, 50 mg; Cu, 25 mg; I, 0.7 mg; Se, 0.3 mg; vitamin A, 11,023 IU; vitamin D3, 1,764 IU; vitamin E, 77 IU; vitamin K, 4.4 mg; vitamin B12, 0.044 mg; riboflavin, 8.8 mg; d-pantothenate, 26.5 mg; niacin 55.1 mg; thiamine, 3.3 mg; pyridoxine, 3.3 mg; folicacid, 1.21 mg; biotin, 0.28 mg; phytase, 476 FTU (Quantum Blue, AB Vista, Marlborough, UK), and chromium, 0.4 mg/kg.

[5]Betafin (Danisco Animal Nutrition, Marlborough, UK).

[6]KALLSIL (Kemin Inudstries, Inc., Des Moines, IA).

[7]Supplied per kg of complete diet: phytase, 1200 FTU (Quantum Blue, AB Vista, Marlborough, UK), and xylanase, 8000 units (Porzyme, Danisco Animal Nutrition, Marlborough, UK).

[8]Endox® Dry (Kemin Industries, Inc., Des Moines, IA).

Table 2. Effects of supplemental levels of linoleic acid and antioxidant on performance of lactating sows[1]

Item	Parity 1 and 2				Parity 3+				SEM	*P*-value			
	1.4% LA	1.4% LA + AO	3.3% LA	3.3% LA + AO	1.4% LA	1.4% LA + AO	3.3% LA	3.3% LA + AO		LA	AO	LA*AO	Parity Group
Sows, n[2]	38	40	41	38	39	39	37	41					
BW at 110 d gestation, kg	226.2	228.5	233.3	226.7	286.1	294.7	293.6	282.4	4.2	0.97	0.56	0.02	<0.001
BW at farrowing, kg[3]	236.9	236.7	236.9	236.5	236.1	236.5	236.5	236.1	0.4	0.76	0.56	0.26	0.21
BW at d 21 lactation, kg[4]	223.3	221.4	223.6	220.6	234.5	234.9	227.9	229.5	2.8	0.08	0.69	0.99	<0.001
BW change, kg	-13.7	-13.3	-11.6	-13.8	-3.3	-3.9	-8.5	-6.8	2.3	0.25	0.89	0.96	<0.001
ADFI, kg/d	4.12	4.01	4.23	4.27	4.81	4.81	4.79	4.88	0.17	0.31	0.95	0.56	<0.001
Sow G:F[5]	0.51	0.50	0.47	0.46	0.45	0.44	0.42	0.43	0.02	0.03	0.83	0.65	<0.001
Litter weight after cross-fostering, kg[6]	17.80	17.33	18.06	17.48	19.14	18.84	20.47	19.51	0.43	0.05	0.06	0.52	<0.001
Litter weight at d 21 lactation, kg	72.93	71.69	69.62	71.43	71.28	71.80	70.72	71.29	1.64	0.26	0.69	0.45	0.89
Litter gain, kg	54.36	53.13	51.09	52.86	52.73	54.17	52.10	54.17	1.60	0.16	0.52	0.59	0.95
Pigs weaned, n	11.16	11.30	11.17	11.03	10.82	10.63	10.83	10.68	0.53	0.92	0.86	0.90	0.46
Piglet mortality, n	0.84	0.68	0.83	1.03	1.18	1.37	1.19	1.32	0.16	0.56	0.70	0.55	0.17
No-value pigs, n[7]	0.02	0.08	0.08	0.02	0.09	0.10	0.02	0.08	0.04	0.44	0.56	0.51	0.37

[1]Diets were supplemented with 3.75% of either tallow (1.4% LA) or corn oil (3.3% LA), with or without supplementation of antioxidant (0.1% inclusion; Endox® Dry, Kemin Industries). LA, linoleic acid; AO, antioxidant.

[2]Data collected from the first 14 groups of sows placed on test.

[3]Sow BW after farrowing was calculated using the equation by Rosero et al. (2013).

[4]Linoleic acid by parity group interaction tendency (*P*=0.09).

[5]Sow feed efficiency was calculated as sow BW change plus litter gain at weaning, divided by ADFI.

[6]Litters were standardized to 12 pigs per litter.

[7]Three-way interaction (LA*AO*PARITY) tendency (*P*=0.06).

Table 3. Effects of supplemental levels of linoleic acid and antioxidant on performance of lactating sows[1]

	Parity 1 and 2				Parity 3+					*P*-value			
Item	1.4% LA	1.4% LA + AO	3.3% LA	3.3% LA + AO	1.4% LA	1.4% LA + AO	3.3% LA	3.3% LA + AO	SEM	LA	AO	LA*AO	Parity Group
Sows, n	78	77	76	73	75	75	75	76					
Pigs weaned, n	11.06	11.25	11.12	10.94	10.62	10.65	10.64	10.61	0.38	0.84	1.00	0.77	0.33
Piglet mortality, n	0.94	0.75	0.88	1.06	1.38	1.35	1.36	1.39	0.12	0.52	0.93	0.39	0.12
No-value pigs, n[3]	0.01	0.03	0.04	0.01	0.04	0.04	0.02	0.04	0.02	0.82	0.77	0.28	0.18

[1]Diets were supplemented with 3.75% of either tallow (1.4% LA) or corn oil (3.3% LA), with or without supplementation of antioxidant (0.1% inclusion; Endox® Dry, Kemin Industries). LA, linoleic acid; AO, antioxidant.

[2]Data collected from all 27 groups of sows placed on test.

[3]Three-way interaction (LA*AO*PARITY) tendency (*P*=0.05).

Table 4. Effects of supplemental levels of linoleic acid and antioxidant on serum oxidative status and milk vitamin E concentrations[1]

	Parity 1 and 2				Parity 3+					*P*-value			
Item	1.4% LA	1.4% LA + AO	3.3% LA	3.3% LA + AO	1.4% LA	1.4% LA + AO	3.3% LA	3.3% LA + AO	SEM	LA	AO	LA*AO	Parity Group
TAC, µM Trolox eq/mL	100.1	100.2	90.8	94.4	101.0	100.7	85.4	96.5	5.2	0.023	0.332	0.318	0.899
MDA, µmol/L	12.9	9.9	10.5	10.3	13.5	14.9	10.9	10.7	1.2	<0.01	0.534	0.688	0.051
8OHdG, pg/mL	1344.3	1264.3	1280.0	1431.0	1382.4	1670.3	1876.8	1902.7	175.8	0.102	0.443	0.951	<0.01
Protein Carbonyls, pmol/mg	939.4	902.2	948.3	939.8	928.3	860.1	960.5	977.1	39.5	0.070	0.364	0.292	0.973
Serum Vitamin E, ppm	2.56	2.45	2.40	3.03	4.05	4.38	2.65	3.39	0.30	0.024	0.067	0.185	<0.001
Milk Vitamin E, ppm	3.56	3.52	3.10	3.25	3.86	4.19	3.01	3.53	0.33	<0.01	0.219	0.632	0.158

[1]Diets were supplemented with 3.75% of either tallow (1.4% LA) or corn oil (3.3% LA), with or without supplementation of antioxidant (0.1% inclusion; Endox® Dry, Kemin Industries). LA, linoleic acid; AO, antioxidant.

Table 5. Effects of supplemental levels of linoleic acid and antioxidant on the subsequent reproductive cycle of sows[1]

	Parity 1 and 2				Parity 3+					*P*-value			
Item	1.4% LA	1.4% LA + AO	3.3% LA	3.3% LA + AO	1.4% LA	1.4% LA + AO	3.3% LA	3.3% LA + AO	SEM	LA	AO	LA*AO	Parity Group
Sows, n	78	77	76	72	75	75	74	76					
Wean-to-Estrus interval, d†	5.5	6.3	5.6	5.7	5.2	4.7	4.9	4.6	0.3	0.252	0.940	0.454	<0.001
Wean-to-Farrow interval, d†	124.3	125.9	124.1	124.5	122.8	123.6	124.2	123.6	1.4	0.972	0.575	0.497	0.230
Bred within 18 d:Weaned, %†[2]	93.3	97.2	98.6	95.7	97.2	90.5	95.8	96.0	2.3	0.373	0.417	0.699	0.455
Returns:Bred within 18 d, %‡	10.5	12.0	9.1	18.1	10.9	8.4	14.2	8.7	4.2	0.591	0.922	0.757	0.528
Sows farrowed:Bred within 18 d, %‡	94.3	92.7	91.4	84.9	91.4	94.0	91.3	97.2	3.1	0.739	0.626	0.763	0.253
Sows farrowed:Weaned, %†[3]	87.2^{ab}	89.6^{ab}	89.5^{ab}	82.0^{b}	89.3^{ab}	85.3^{ab}	87.9^{ab}	93.4^{a}	3.7	0.755	0.938	0.868	0.441
Culling rate, %†[4]	11.6^{ab}	5.2^{b}	6.6^{ab}	16.7^{a}	9.3^{ab}	12.0^{ab}	9.4^{ab}	5.3^{b}	3.3	0.863	0.885	0.406	0.855
Total pigs born, n‡	13.0^{ab}	13.5^{ab}	13.3^{ab}	12.6^{b}	13.8^{ab}	13.2^{ab}	13.6^{ab}	14.0^{a}	0.5	0.939	0.801	0.896	0.092
Pigs born alive, n‡	12.5	12.7	12.6	12.0	12.4	11.9	12.4	12.6	0.4	0.931	0.492	0.892	0.665
Stillborn pigs, n‡[5]	0.3^{d}	0.8^{bc}	0.7^{c}	0.4^{dc}	1.2^{a}	1.2^{a}	1.1^{ab}	1.1^{ab}	0.1	0.823	0.678	0.073	<0.001

[1]Diets were supplemented with 3.75% of either tallow (1.4% LA) or corn oil (3.3% LA), with or without supplementation of antioxidant (0.1% inclusion; Endox® Dry, Kemin Industries). LA, linoleic acid; AO, antioxidant.

[2]Three-way interaction (LA*AO*PARITY) tendency, *P*=0.068.

[3]Three-way interaction (LA*AO*PARITY) tendency, *P*=0.069.

[4]Three-way interaction (LA*AO*PARITY), *P*=0.018.

[5]Three-way interaction (LA*AO*PARITY), *P*=0.043.

†Relative to all weaned sows (n=603).

‡Relative to sows that were bred within 18 d post-weaning (n=553).

CHAPTER V: IMPROVING GASTRO-INTESTINAL HEALTH AND SURVIVAL OF PIGLETS BY FEEDING LONG CHAIN POLYUNSATURATED FATTY ACIDS TO LACTATING SOWS

ABSTRACT

The challenges associated with pre-weaning performance provide the greatest opportunity for improving whole herd efficiency. Supplementation of long-chain polyunsaturated fatty acids (LCPUFA) to lactating sows has shown positive effects on litters and feeding pigs directly with LCPUFA improved intestinal health. The purpose of the current study was to measure enrichment of sow milk and intestinal tissue of piglets from sows fed multiple levels of eicosapentaenoic acid (EPA) or arachidonic acid (ARA) for various lengths of time, and to determine the impact of LCPUFA supplementation on pre- and post-weaning pig performance. A total of 60 sows were selected at approximately 110 d of gestation and assigned to 5 dietary treatments in a RCBD. Treatments consisted of: control (palm kernel oil; PKO); 1.0 and 2.0% (w/w of diet) ARA; 1.0 and 2.0% (w/w of diet) EPA. Supplementation started 2 weeks prior to and ended at weaning; sows were fed oil treatments as a top-dress once daily immediately prior to the morning feeding. Milk samples were collected on d 6 and d 13 of supplementation for FA profile analysis. One pig per sow per treatment (total of 60 pigs for each time point) was euthanized and ileal mucosa scrapings were collected for FA profile analysis on d 7 and d 14 of supplementation, and at 1 week post supplementation. Milk ARA increased 32% from d 6 to d 13 in sows supplemented with 2% ARA (interaction, $P<0.04$). Sows supplemented with 1% ARA had similar levels of ARA concentration in milk for both days (3.5% of fatty acids, w/w), and remaining treatments displayed significantly lower ARA concentrations in milk (0.64%). Milk EPA concentrations were highest ($P<0.001$) for sows fed the 2% EPA treatment (6.25%), followed by 1% EPA supplementation (3.76%), while amounts in control and ARA fed sows were lowest (1%). Concentrations of DHA in milk were highest ($P<0.001$) in sows fed 2% EPA, followed by 1% EPA level, and were similar among the remaining treatments. Concentrations of EPA in intestinal mucosa of pigs from sows fed 2% EPA increased to 6.5% during the supplementation period, and decreased to 2.33% 1 week after weaning (interaction, $P<0.001$). Supplementation of sows with 1% EPA increased mucosal EPA concentrations compared to control PKO, but concentrations remained similar throughout the 14 d supplementation period and one week after weaning. Concentrations of DHA in intestinal mucosa of piglets increased when sows were supplemented with 2% EPA treatment, and enrichment decreased over time (interaction, $P<0.02$). Concentrations of ARA in intestinal mucosa of piglets were highest ($P<0.001$) when ARA was supplemented to sows at either 1 or

2% ARA level, compared to other treatments. Sows fed control palm kernel oil and 1% EPA treatments weaned piglets 10% heavier and with 18% improved ADG at weaning than pigs from sows fed 2% ARA ($P<0.05$). Pig BW and ADG post-weaning were not affected by dietary supplementation of sows. Mortality, total pig gain and weight produced at the end of the study (30 d into the nursery) were not affected by fatty acid supplementation. Dietary supplementation of sows with LCPUFA increased milk ARA and EPA progressively, which directly influenced ARA and EPA profile in piglet intestinal mucosa. However, intestinal enrichment with LCPUFA did not affect performance of pigs post-weaning.

INTRODUCTION

Reducing piglet mortality pre- and post-weaning and improving the transition of pigs into the nursery have major implications on the profitability of pork production. Throughout lactation, a piglet's only source of nutrition comes from the sow's milk, which is followed by a dramatic change at weaning, when pigs are separated from their dams and usually offered a dry diet as their only means of nutrition. Therefore, the stress accompanied by mixing litters and switching diets takes a huge toll on the gastro-intestinal health of the pig, leading to an increased susceptibility to inflammation and infection (Wijtten et al., 2011; Kick et al, 2012). This decreases growth performance post-weaning, increases the number of low value pigs and increases veterinary intervention to treat pigs suffering from diarrhea.

Long-chain polyunsaturated fatty acids (LCPUFA), as precursors of prostaglandins, have been shown to improve intestinal barrier function and recovery following intestinal injury (Blikslager et al., 2007; Jacobi et al., 2012; Liu et al., 2012). A dose-response enrichment of intestinal phospholipids of suckling piglets fed LCPUFA was successfully determined by Hess et al. (2008) and Jacobi et al. (2012), and the latter further demonstrated the protective effects of prophylactic enrichment against acute intestinal injury.

Moreover, many studies support the fact that sow colostrum and sow milk can be enriched with essential fatty acids through dietary supplementation, which then increases concentrations in piglet serum and tissues (Fritsche et al., 1993; Rooke et al., 2001; Gabler et al., 2007; Mateo et al., 2009; Tanghe et al., 2013).

Therefore, we propose that supplementation of the sow diet with LCPUFA will enrich sow milk and subsequently the intestinal tissue of the nursing piglet with LCPUFA. We

hypothesized that feeding lactating sows with a diet rich in essential fatty acids, specifically arachidonic acid (ARA, C20:4 n-6) and eicosapentaenoic acid (EPA, C20:5 n-3), will improve pre- and post-weaning performance of pigs. By delivering these fatty acids through the milk, we expected to prophylactically promote gut health, thereby improving survival and performance. Thus, the objectives of this study were to determine the dose and duration of ARA and EPA supplementation required to efficiently enrich sow milk and intestinal tissue of suckling piglets and to determine its effects on pre- and post-weaning performance of pigs in a commercial production flow.

MATERIALS AND METHODS

Animals and dietary treatments

All animal protocols were approved and conducted under the supervision of licensed veterinarians. Experiments were conducted in commercial farms in North Carolina.

A total of 60 sows were selected at approximately day 110 of gestation and allocated to 1 of 5 dietary treatments. Sows were selected in 3 groups of 20 sows, which was 1 week of production at the sow farm; average sow parity was 3.9. Sows were supplemented during lactation for the last 14 days prior to weaning. The duration of supplementation was chosen based on Hess et al. (2008), who observed significant enterocyte enrichment of piglets with LCPUFA after 8 days of dietary supplementation with ARA and EPA. Treatments consisted of: control (palm kernel oil; PKO); 1.0 and 2.0% (w/w of diet) arachidonic acid (ARA); 1.0 and 2.0% (w/w of diet) eicosapentaenoic acid (EPA). The total daily amount of product offered to sows for each treatment was: 344 g of PKO in the control treatment; 127 g of EPA oil + 217 g PKO in 1% EPA treatment; 254 g of EPA oil + 90 g PKO in 2% EPA treatment; 172 g of ARA oil + 172 g PKO in 1% ARA treatment; and 344 g of ARA in 2% ARA treatment. The daily amount supplied to sows was calculated based on a 7.3 kg/d feed intake for the last 2 weeks of lactation and the analyzed concentration of the targeted LCPUFA (Table 1). The inclusion rates of ARA and EPA were determined based on Hess et al. (2008).

All sows were supplemented once daily in the morning with the same amount (344 g/d) of oils and oil mixtures to avoid possible confounding effects due to variable energy intake among treatments. To encourage complete consumption of the added lipid, lipid sources were mixed with a small portion of the dry lactation feed (approx. 1.5 kg) and offered to sows prior

to their morning feeding. Thus, lipid sources were provided at known daily amounts and therefore, sow daily feed intake was not measured.

At two weeks prior to weaning, when sow supplementation started, average litter size was 11.8 pigs per sow. At 7 and 14 days (weaning) of supplementation, 1 pig per sow per treatment (total of 60 pigs for each time point) was euthanized by electric current (head to tail) stunning followed by immediate exsanguination. Ileal segments were excised, cut lengthwise, flushed with PBS, gently blotted dry with paper towels and mucosa was scraped using glass microscope slides. Mucosa scrapings were subsequently frozen at -20°C for further fatty acid profile analysis. Lastly, 1 pig per sow per treatment was kept on the farm for one week after weaning and was euthanized for collection of ileal mucosa following the same procedures described above.

Milk samples (approx. 30 mL) were collected twice by hand from randomly selected glands at day 6 and 13 of supplementation. Oxytocin (1 mL) was injected intramuscularly to facilitate milk let down. Samples were immediately frozen at -20°C for further analyses of fatty acid profile. All pigs were ear-tagged with an individual number and different colored tag according to their respective treatment. Piglets were weighed at the start of supplementation on d 1, before sampling, and at the end of supplementation, on d 14, for growth performance calculations.

At weaning, all pigs (n= 628) were transported 240 km to a commercial nursery in NC. Pigs were randomly distributed to pens according to size. Pigs were weighed at approx. 16 days into the nursery, and again 14 d later. Groups of pigs were started and weaned at different days at the sow farm, but all pigs were weighed in the nursery on the same days. Therefore, ADG calculations in the nursery were done for 15, 16 and 18 d post weaning for group 1, 2, and 3, respectively, and once more 14 d later (29, 30 and 32 d post weaning). Feed intake during the nursery was not recorded. Mortality was recorded by farm personnel throughout the study.

Chemical analysis

Ileal mucosa scrapings were analyzed for fatty acid composition by GC/MS. Approximately 100 mg of mucosa scrapings were mixed with hexane, methanol and 3 N methanolic-HCl. Samples were placed in a water bath at 95°C for 1 h, cooled and mixed with

0.88% (w/v) NaCl solution, followed by hexane addition. After centrifugation, the top layer was collected and evaporated under N_2. Methyl esters were dissolved in 50 µL of hexane and 1 µL of this solution was manually injected into a gas chromatograph (Agilent 6890N Network Gas Chromatograph, Agilent Technologies, Santa Clara, CA) equipped with a mass spectrometric detector (5973Network Mass Selective Detector, Agilent Technologies, Santa Clara, CA). Separation was achieved using a 30-m capillary column (0.25 mm x 0.3 µm film thickness; HP-23 cis/trans FAME CR). For electron ionization (EI) analysis, the temperature was programmed from 50 to 100°C at 10°C/min, then to 200°C at 4°C/min, held for 2 min, and finally to 245°C at 4°C/min, held for 10 min. The average helium velocity was 36 cm/sec and the split ratio was 100:1. Fatty acid amounts were determined by the areas of the ions for each fatty acid relative to the total area of all fatty acids detected in each sample.

Milk fatty acid profile was determined by GC/FID. Lipids were extracted using a modified method (Radin, 1981). Briefly, an internal standard of heptadecanoic acid (C17:0) dissolved in alcohol was added to milk samples, followed by hexane/isopropanol (4:1) mixture and saturated NaCl solution. Contents were vortexed and centrifuged and the supernatant was collected. The remaining bottom layer was extracted 3 more times with hexane and combined supernatants were evaporated under N_2. Fatty acid methylation was conducted by adding 3.75 M NaOH dissolved in methanol/water (1:1) mixture and heating contents in a boiling water bath for 5 min, then vortexing and returning to the water bath for 25 min. Samples were cooled by plunging into cool water and a methyl alcohol/6.0 N HCl (1.7:1) mixture was added. Samples were returned into a boiling water bath for 10 min, cooled by plunging into cool water and a methyl tert-butyl ether/hexane (1:1) mixture was added. Samples were quickly vortexed and placed into a water bath shaking for 10 min. Following centrifugation, the lower aqueous phase was discarded and a 0.3 M NaOH solution was added to the remaining organic layer. After centrifugation, the supernatant was collected and evaporated under N_2. Methyl esters were dissolved in 100 µL of hexane and 1 µL of this solution was injected into a gas chromatograph (HP 5890 Series II, Hewlett-Packard Co., Wilmington, DE) equipped with a 100-m capillary column (0.25 mm x 0.2 µm film thickness; SP-2560, Supelco Bellefonte, PA), a flame ionization detector, and an auto sampler (HP 6890 Series Injector, Agilent Technologies, Santa Clara, CA). Peaks were identified by comparison of retention times with authentic fatty acid methyl esters (Sigma-Aldrich Co., St Louis, MO).

Statistical analysis

Fatty acid concentrations in intestinal mucosa were analyzed using the Mixed procedure of SAS (v. 9.4; SAS Inst. Inc., Cary, NC). The model included diet (palm kernel oil control, ARA level, and EPA level), day of sampling (7 and 14 d of supplementation and 1 wk post-supplementation), and their interaction. Group of sows was used as the random effect. For milk fatty acid profile, data were analyzed using the Mixed procedure of SAS, with fixed effects of diet, day, and their interaction in the model. Day was used as a repeated measure and group of sows was used as the random effect.

Growth performance data were analyzed using PROC Mixed of SAS using individual pig observations. The model included fixed effects of diet and initial BW of pigs was used as a covariate. Sow and group of sows were included as random effects. Additionally, data were analyzed on a sow basis, considering the total pig weight for each sow at the start of the study and the final pig weight produced per sow. The final pig weight considered pigs that died, therefore, sows with greater death losses produced less final pig body weight. These and mortality data were analyzed using PROC Mixed of SAS with fixed effect of diet and random effect as group of sows in the model. Differences between treatments were considered significant at $P \leq 0.05$ and were considered tendencies at $0.05 < P \leq 0.10$.

RESULTS

Sow milk and pig intestinal mucosa fatty acid profile. Concentrations of ARA in sow milk (Table 2, Figure 1) increased substantially following supplementation of ARA to the sow and this increase was greater after 13 d of supplementation for the 2% ARA treatment compared to 6 d of supplementation (interaction, $P < 0.04$). Milk ARA levels averaged 7.13% for the 2% ARA treatment and 3.49% for the 1% ARA treatment, compared to 0.52% for the control treatment, on both collection days (Figure 2). Milk EPA concentrations were highest ($P < 0.001$) for the 2% EPA treatment, at 6.25%, followed by 1% EPA supplementation, at 3.76%, while remaining treatments had values less than 1% (Table 2; Figure 2). Concentrations of docosahexaenoic acid (DHA, C22:6 n-3) in milk were highest ($P < 0.001$) in sows fed 2% EPA, followed by 1% EPA, and were similar among the remaining treatments (Table 2; Figure 2). Milk DHA concentrations tended ($P < 0.1$) to increase after 13 d of supplementation in all treatments.

Enrichment of intestinal mucosa was significantly impacted by LCPUFA sources (Table 3). Concentrations of EPA in intestinal mucosa increased as EPA in the diet increased and this response differed depending on when pigs were sampled relative to sow supplementation (interaction, $P < 0.001$; Figure 3). The 2% EPA level of sow supplementation increased piglet mucosal EPA concentrations to 6.59 and 5.55% at 7 and 14 d of supplementation, and decreased to 2.33% 1 week after weaning. Supplementation of sows with 1% EPA increased mucosal EPA concentrations compared to control PKO, but concentrations remained similar throughout the 14 d supplementation period and one week after weaning.

Similarly, concentrations of DHA in mucosa samples were increased with increasing EPA supplementation and concentrations were greatest with the 2% EPA treatment, and enrichment decreased over time (interaction, $P < 0.02$; Figure 4).

Concentrations of ARA in intestinal mucosa of piglets were highest ($P < 0.001$) when ARA was supplemented to sows at either 1 or 2% ARA level, compared to other treatments (Table 3; Figure 5).

Pig performance during lactation and post-weaning. A total of 55 pigs died during the course of the study and 31 pigs were missing due to either lost ear tags or due to lost records on the farm. There were 441 pigs represented at the end of the study.

Pig BW at weaning (at d 14 of supplementation) and pre-wean ADG were significantly impacted by sow supplementation of LCPUFA (Table 4). Sows fed control palm kernel oil and 1% EPA treatments weaned piglets that were approximately 10% heavier and had 18% improved ADG at weaning than pigs from sows fed 2% ARA ($P < 0.05$). Pig BW and ADG post-weaning were not affected by dietary supplementation of the sow. Total pig gain and weight produced per sow at the end of the study (approx. 30 days into the nursery) were not affected by fatty acid supplementation (Table 5). Overall mortality for the duration of the study was relatively high at 11.1%, but was not influenced by dietary treatment.

Growth rates were determined for pigs that were euthanized for sample collection and these results agree with data considering all pigs (Table 6). Pig BW at weaning (2 weeks of supplementation) and ADG from initiation of the study until weaning tended ($P < 0.1$) to be lower when sows were fed 2% ARA compared to sows fed PKO and 1% EPA. However, no differences were observed in pig BW or ADG post-weaning or overall.

DISCUSSION

The ARA and EPA sources used in the present study contained 42.5% ARA and 57.2% EPA, respectively. Although the ARA source contained a small amount of EPA (3.5%) and the EPA source contained DHA (10%), we did not attempt to balance all fatty acids between treatments to maintain a more practical approach. Nonetheless, control sows were supplemented with palm kernel oil and all sows received a total of 343 g/d of added lipid, such that energy provided by supplemental lipids was similar between treatments. This approach allowed us to feed our targeted concentrations of ARA and EPA, while maintaining similar energy intake. The duration and level of supplementation was chosen based on Hess et al. (2008), who observed significant enterocyte enrichment of piglets with LCPUFA after 8 days of dietary supplementation with 2.5% ARA and 5% EPA (w/w of total fatty acids).

Extensive research has been conducted with sow supplementation of fish oils, which normally contain high levels of omega 3 (n-3) fatty acids (mainly EPA and DHA), and enrichment of sow milk with these LCPUFA has been consistent (Fritsche et al., 1993; Arbuckle and Innis, 1993; Rooke et al., 2001; Gabler et al., 2007; Mateo et al., 2009; Tanghe et al., 2013).

To our knowledge, no studies have been conducted with feeding sows directly with ARA. Products rich in this particular fatty acid are used in infant formulas, and are not readily or cheaply available to the industry. However, a few studies have investigated the effects of feeding sows with a higher n-6:n-3 FA ratio, which usually includes a higher percentage of corn oil to increase levels of linoleic acid (LA, C18:2n-6). Linoleic acid can be converted to ARA in the body by action of elongase and desaturase enzymes (Palmquist, 2009). Addition of approximately 3% corn and canola oil mixture to sow diets with a 9:1 n-6:n-3 ratio increased the ARA content in colostrum of sows by 166%, and these sows weaned heavier piglets compared to sows fed a 5:1 n-6:n-3 ratio diet with fish oil (Eastwood et al., 2014). Conversely, Yao et al. (2012) did not find differences in milk ARA content by feeding sows with increasing ratios of n-6:n-3 in lactation diets with 4% inclusion of corn and linseed oil mixtures. Interestingly, however, these authors reported a decrease in sow milk EPA and plasma DHA of piglets from sows fed 9:1 and 13:1 n-6:n-3 ratios, compared to a 3:1 ratio, but litters from sows fed the 9:1 ratio tended to gain more weight than other treatments.

More recently, Rosero et al. (2015) analyzed composition of milk from lactating sows fed different combinations of linseed and canola oils, targeting levels of 2.1% or 3.3% LA and 0.15% or 0.45% α-linolenic acid (ALA, C18:3 n-3) of the diet. Higher levels of linoleic acid supplementation tended to increase ARA secretion in milk by 20%, while LNA tended to decrease ARA concentrations and increased EPA content in milk by 37%. As in agreement with most other studies, no effects on piglet growth performance were detected by these authors.

Similar to our observed milk FA profile, the intestinal mucosa of piglets was effectively enriched with ARA and EPA, and these effects were still evident after piglets were weaned, when the supply of ARA and EPA from sow milk had ceased. Intestinal absorption of lipids occurs mainly in the upper portion of the small intestine, however, in the present study, ileum fatty acid profile was analyzed based on increased enrichment in ileum rather than jejunum found by Hess et a. (2008). Moreover, since pigs were not fasting when they were euthanized, ileum would be a better indicator of tissue incorporation of these FAs, whilst the upper small intestine portion could be potentially biased by being the site of enterocyte absorption of fatty acids. The increase in mucosal ARA found in pigs from sows fed the 2% ARA treatment was 37% higher than the control PKO treatment, and is significantly lower compared to the increase of approximately 70% (comparing to sow milk) reported by Hess et al. (2008) in ileum of pigs fed 2.5% ARA for 16 days. However, these authors fed pigs directly with enriched milk replacers, which could explain the disparity between results. Moreover, visual observations of reduced feed intake patterns in sows fed ARA treatments (discussed below) could explain the relative lower enrichment in piglet mucosa. Our findings that ARA levels in mucosa of piglets from sows supplemented with 2% EPA were similar to pigs from sows fed the control treatment are in agreement with those found by Hess et al. (2008).

Similar to Hess et al., mucosal enrichment with EPA in piglets from sows fed the 2% EPA treatment plateaued after 7 days of supplementation, and levels of enrichment increased from less than 1% in control treatment to 6% on average at d 7 and 14 of supplementation, decreasing to similar levels of pigs from sows fed 1% EPA treatment one week post supplementation. Concentrations of DHA in piglet mucosa were highest when sows were supplemented with 2% EPA treatment, which was expected due to the higher content of DHA in EPA product (10%), compared to zero content in the ARA product.

Long-chain PUFAs, particularly eicosanoids (20-carbon PUFAs), are precursors for important molecules, such as prostanoids, that play key roles in inflammatory processes. These have been extensively studied and have found to be particularly important in maintaining intestinal health (Blikslager et al., 2007; Willemsen et al., 2008). Moreover, intestinal enrichment with LCPUFA has shown to improve recovery of pigs after ischemic injury in the ileum and after imposed detrimental effects of malnutrition. Malnourished pigs demonstrated improved recovery from lesions induced by dietary restriction in the small intestine when fed LCPUFAs (Lopez-Pedrosa et al., 1999). Pigs fed 5% ARA had increased PGE_2 production and decreased percentage of denuded villus surface area in the ileum, whereas pigs fed 5% EPA had the lowest production of PGE_2, and both 5% ARA and 5% EPA treatments displayed improved transepithelial electrical resistance in the ileum following ischemic injury (Jacobi et al., 2012).

Weaning is perhaps the most drastic event in a pig's life cycle, and it takes huge tolls on intestinal integrity and health. By conducting this study in a commercial facility, we were able to promote more rigorous conditions, such as increased population density and greater immune challenge, than provided in a research-controlled environment. Based on the afore mentioned studies, we expected the observed enrichment with LCPUFA in piglet mucosa to positively affect post-weaning performance. Furthermore, the relatively high mortality indicates that these pigs were housed under challenging conditions and these should have allowed an opportunity to detect differences between treatments.

Pigs from sows fed 1% EPA treatment had similar performance at weaning than pigs from sows fed the control treatment, whereas pigs from sows fed 2% ARA treatment had the lightest BW at weaning and lowest pre-wean ADG. However, we did not detect any differences in performance of pigs after weaning. We further explored the potential impact of dietary treatments on total pig weight and pig gain produced per sow. These measurements take into account death losses and pig weight gain and are directly representative of the total output of pork produced by the sow (in this case until d 32 of the nursery). No differences were observed due to ARA or EPA supplementation on the total amount of pig weight produced per sow.

Our results disagree with Mateo et al. (2009), in which pigs from sows supplemented with omega 3 fatty acids had increased BW and pre-wean ADG compared to control treatment, but these authors did not measure pig performance post weaning. Moreover, Leonard et al.

(2010) observed improved ADG and feed efficiency during the second week post-weaning in pigs from sows fed fish oil, and increased expression of inflammatory cytokines in the colon, as well as increased villi height to crypt depth ratio in the ileum of pigs from sows fed fish oil late during gestation and lactation. Lack of differences in piglet performance until weaning were reported by others (Fritsche et al., 1993; Arbuckle and Innis, 1993; Tanghe et al., 2013), when sows were fed fish oil from gestation throughout lactation.

It is worth noting that there was no adaptation period prior to the initiation of the study, and even though sow feed intake was not measured, based on visual observations, sows fed ARA treatments appeared to refuse more feed than control sows or sows fed EPA, presumably due to reduced palatability of the feed when the ARA product was top-dressed. This could explain, in part, the reduction in piglet growth rates for pigs from sows fed ARA, and the fact that these differences faded after pigs were no longer under indirect supplementation, after weaning.

In conclusion, we successfully demonstrated that sow milk concentrations of ARA and EPA can be increased progressively by supplementing these LCPUFA in sow diets. Increased ARA and EPA in milk directly influenced ARA and EPA by increasing enrichment in the intestinal mucosa of piglets. This enrichment with ARA and EPA did not appear to improve mortality, growth rate, or total weight of pigs produced by the sow. Palatability of LCPUFA products, especially ARA, needs to be considered in order to avoid potential bias in sow feed intake.

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Table 1. Composition of supplemental long-chain polyunsaturated fatty acid sources

		Fatty acid product source[1]		
Item	**Name**	**ARA**	**EPA**	**PKO**
Fatty acids, %				
8:0	Caprylic acid	0	0	3.7
10:0	Capric acid	0	0	4
12:0	Lauric acid	0	0	44
14:0	Myristic acid	0.3	0.4	16
16:0	Palmitic acid	8.8	1	8.5
16:1	Palmitoleic acid	0.1	0.4	0
18:0	Stearic acid	8	3.8	2
18:1(n-7)	Vaccenic acid	0.4	2.1	0
18:1(n-9)	Oleic acid	21.9	5.1	19
18:2(n-6)	Linoleic acid	7.1	0.7	2
18:3(n-3)	α-linolenic acid	0.1	0.5	0
18:3(n-6)	γ-linolenic acid	2.8	0.5	0
18:4(n-3)	Stearidonic acid	0	1.3	0
20:0	Arachidic acid	0.7	0.6	0.1
20:1(n-9)	Gondoic acid	0.4	3.3	0.2
20:2(n-6)	Eicosadienoic acid	0.6	0.6	0
20:3(n-6)	Dihomo-γ-linolenic acid	3.1	0.6	0
20:4(n-3)	Eicosatetraenoic acid	0	2.5	0
20:4(n-6)	**Arachidonic acid**	**42.3**	**3.5**	**0**
20:5(n-3)	**Eicosapentaenoic acid**	**0**	**57.2**	**0**
21:5(n-3)	Heneicosapentaenoic acid	0	1.3	0
22:0	Behenic acid	1.4	0	0
22:1(n-11)	Cetoleic acid	0	1.1	0
22:5(n-3)	Docosapentaenoic acid	0	1.7	0
22:6(n-3)	Docosahexaenoic acid	0	10	0
24:0	Lignoceric acid	1.1	0	0.3
Others[2]		0.9	1.8	0.2

[1]Arachidonic acid (ARA) and eicosapentaenoic acid (EPA) were obtained from the products ARASCO and MEG-3 oil, which were graciously donated by DSM Nutritional Products, Inc. Palm kernel oil (PKO) was donated by Cargill, Inc., Minneapolis, MN.

[2]Other fatty acids not specified included C14:1, C15:0, C17:0, and C22:5(n-6).

Table 2. Impact of supplementation of palm kernel oil (PKO; control lipid source), arachidonic acid (ARA), or eicosapentaenoic acid (EPA) on fatty acid composition of sow milk[1]

	6 DAYS OF SUPPLEMENTATION					13 DAYS OF SUPPLEMENTATION						*P*-VALUE		
Item	**PKO**	**1% ARA**	**2% ARA**	**1% EPA**	**2% EPA**	**PKO**	**1% ARA**	**2% ARA**	**1% EPA**	**2% EPA**	**SEM**	**TRT**	**DAY**	**TRT* DAY**
Fatty acids, %														
C12:0	5.86	3.21	0.31	3.17	2.12	7.03	3.53	0.30	4.54	2.66	0.70	<0.001	0.091	0.783
C14:0	7.11	4.67	2.53	5.07	4.45	7.86	4.85	2.99	6.26	4.99	0.59	<0.001	0.038	0.858
C16:0	28.61	25.33	27.88	27.73	28.65	28.08	25.59	27.88	28.83	30.33	1.23	0.030	0.092	0.515
C16:1	1.36	0.55	0.93	0.52	0.96	0.48	1.33	0.48	1.18	0.37	0.51	0.975	0.756	0.336
C18:0	4.22	4.34	5.09	4.62	4.81	4.26	7.69	4.52	4.29	4.48	1.14	0.568	0.548	0.391
C18:1c	32.23	36.43	36.71	31.66	30.71	31.82	32.19	32.69	30.20	27.04	2.15	0.025	0.008	0.678
C18:2c	16.52	17.67	18.09	17.30	16.81	16.13	16.91	15.56	16.23	16.19	0.95	0.768	0.124	0.865
C18:3n3	0.70	0.83	0.74	0.84	0.77	0.74	0.78	0.92	0.76	0.84	0.11	0.858	0.639	0.765
C20:4n6	0.53	3.50	6.14	0.64	0.82	0.50	3.50	8.12	0.51	0.86	0.46	<0.001	0.128	0.038
C20:5n3	0.05	0.05	0.10	4.45	5.27	0.02	0.04	0.05	3.07	7.24	0.81	<0.001	0.837	0.295
C22:6	0.04	0.06	0.05	0.47	1.10	0.06	0.04	0.02	0.58	1.29	0.11	<0.001	0.097	0.174

[1]Sows (n=60) were supplemented once daily in the morning with the same amount (344 g/d) of different oil treatments. Supplementation started 2 weeks before weaning.

Table 3. Impact of supplementation of palm kernel oil (PKO; control lipid source), arachidonic acid (ARA), or eicosapentaenoic acid (EPA) on fatty acid composition of intestinal mucosa of suckling pigs (7 and 14 days of supplementation of the sow) and weanling pigs (1 week post-weaning)[1]

	7 DAYS OF SUPPLEMENTATION					14 DAYS OF SUPPLEMENTATION					1 WEEK POST SUPPLEMENTATION						*P*-VALUE		
Item	PKO	1% ARA	2% ARA	1% EPA	2% EPA	PKO	1% ARA	2% ARA	1% EPA	2% EPA	PKO	1% ARA	2% ARA	1% EPA	2% EPA	SEM	TRT	DAY	TRT* DAY
Fatty acids, %																			
C12:0	0.88	0.40	0.12	0.37	0.23	0.34	0.22	0.06	0.31	0.15	0.11	0.02	0.08	0.17	0.16	0.11	<.0.001	<.0.001	0.029
C14:0	2.42	1.57	1.35	1.52	1.17	1.73	1.39	1.05	1.62	1.28	1.10	0.87	0.59	0.66	0.92	0.24	<.0.001	<.0.001	0.429
C16:0	24.95	31.13	29.06	30.96	29.27	26.12	30.48	27.42	33.68	29.00	30.51	31.34	35.15	30.29	30.10	1.89	0.047	0.099	0.206
C16:1	2.29	1.87	2.40	1.85	1.61	2.11	1.54	1.51	1.21	1.89	2.04	1.69	1.24	1.67	1.89	0.28	0.113	0.076	0.232
C18:0	18.12	15.71	10.08	14.75	17.92	15.45	16.16	13.49	13.29	14.86	16.66	17.53	18.38	17.71	16.88	1.84	0.286	0.053	0.249
C18:1c	18.67	19.39	26.26	20.71	13.06	18.74	16.85	24.90	16.00	15.14	21.57	22.10	19.19	21.73	21.64	2.06	0.002	0.092	0.011
C18:2c	12.65	11.75	9.80	12.23	11.39	13.69	10.04	9.49	10.09	10.95	8.40	10.93	8.35	6.61	10.12	2.48	0.477	0.060	0.797
C18:3n3	0.34	0.20	0.40	0.20	0.19	0.15	0.17	0.25	0.31	0.16	0.35	0.24	0.44	0.36	0.48	0.10	0.086	0.002	0.210
C20:4n6	9.55	12.88	11.83	5.68	6.86	10.64	14.90	15.89	6.82	4.81	8.57	9.98	11.34	7.86	6.26	1.28	<.0.001	0.081	0.103
C20:5n3	0.15	0.06	0.83	2.38	6.59	0.16	1.08	0.04	3.14	5.55	0.06	0.07	0.04	1.50	2.33	0.48	<.0.001	<.0.001	<.0.001
C22:6	1.12	0.59	0.56	1.82	3.64	1.62	0.57	0.38	2.03	3.18	0.88	0.39	0.61	2.38	2.04	0.29	<.0.001	0.196	0.020

[1]One pig per sow per treatment was euthanized at each time point. Sows were supplemented daily with different oil treatments. Supplementation started 2 weeks before weaning and finished at weaning.

Table 4. Impact of supplementation of palm kernel oil (PKO; control lipid source), arachidonic acid (ARA), or eicosapentaenoic acid (EPA) to sows on performance of pigs[1]

		ARA		EPA			TRT
Item	**PKO**	**1%**	**2%**	**1%**	**2%**	**SEM**	***P*-value**
BW, kg							
Initial[2]	2.30	2.71	2.30	2.68	2.45	0.22	0.214
Weaning	5.95[a]	5.66[ab]	5.40[b]	5.91[a]	5.76[ab]	0.15	0.048
Nursery (day 15)[3]	7.74	7.73	7.55	8.07	7.99	0.27	0.147
Nursery (day 30)[3]	13.47	13.57	13.30	14.13	13.56	0.23	0.177
ADG, kg/d							
Start to weaning	0.253[a]	0.232[ab]	0.213[b]	0.251[a]	0.239[ab]	0.014	0.046
Nursery (d 0 to 15)	0.107	0.118	0.122	0.127	0.117	0.013	0.609
Nursery (d 15 to 30)	0.398	0.405	0.400	0.420	0.393	0.018	0.496
Nursery (d 0 to 30)	0.246	0.255	0.256	0.267	0.246	0.010	0.354
Initial to Nursery d 30	0.247	0.248	0.243	0.262	0.248	0.008	0.152

[1]Included all pigs on test.

[2]Initial BW was used as a covariate in the statistical analysis (mean initial BW was 2.49 kg; the study was initiated 2 weeks prior to weaning; supplementation ended at weaning).

[3]Pigs were weighed after 15, 16 or 18 days in the nursery for the first measurement and were weighed again 14 days subsequently.

[ABC]Means without common superscripts differ ($P < 0.05$).

Table 5. Impact of supplementation of palm kernel oil (PKO; control lipid source), arachidonic acid (ARA), or eicosapentaenoic acid (EPA) to sows on total pig weight produced per sow[1]

		ARA		EPA			TRT
Item	**PKO**	**1%**	**2%**	**1%**	**2%**	**SEM**	***P*-value**
Total initial pig weight, kg	19.91	21.96	18.94	23.21	19.62	2.11	0.329
Total final pig weight, kg	99.22	105.18	93.61	114.38	88.59	9.71	0.138
Total pig weight gain, kg	79.31	83.22	74.66	91.18	68.97	8.15	0.156
% Mortality	10.65	7.77	10.88	9.52	16.62	4.25	0.572

[1]Each mean represents 12 sows. Data were calculated as total pig weights produced per sows. Pigs that were euthanized for sample collection were omitted from these data.

Table 6. Impact of supplementation of palm kernel oil (PKO; control lipid source), arachidonic acid (ARA), or eicosapentaenoic acid (EPA) on performance of pigs that were euthanized for tissue collection[1]

Item	PKO	ARA		EPA		SEM	TRT *P*-value
		1%	2%	1%	2%		
BW, kg							
Initial[2]	2.31	2.71	2.30	2.70	2.42	0.19	0.001
7 d of supplementation	4.22	3.85	3.89	4.07	3.99	0.14	0.237
14 d of supplementation	5.99	5.88	5.58	6.11	5.93	0.17	0.093
1 week post supplementation[3]	6.03	6.03	5.58	5.99	6.00	0.22	0.483
ADG, kg/d							
d 0 to d 7 of supplementation	0.261	0.206	0.211	0.241	0.223	0.019	0.226
d 0 to d 14 of supplementation	0.256	0.249	0.225	0.266	0.250	0.013	0.084
d 0 to 1 wk post supplementation	0.171	0.170	0.149	0.169	0.169	0.012	0.500
d 14 to 1 wk post supplementation[4]	0.005	0.017	-0.016	-0.015	0.013	0.020	0.450

[1]Values are least square means of 36, 12, 24, and 12 pigs for initial, 7 d, 14 d of supplementation and 1 week after supplementation BW, respectively.

[2]Initial BW was used as a covariate in the statistical analysis (mean initial BW was 2.49 kg; the study was initiated 2 weeks prior to weaning).

[3]Measurements taken 1 week post weaning, after sow supplementation had ceased.

[4]Growth rate during the week after weaning, after sow supplementation had ceased.

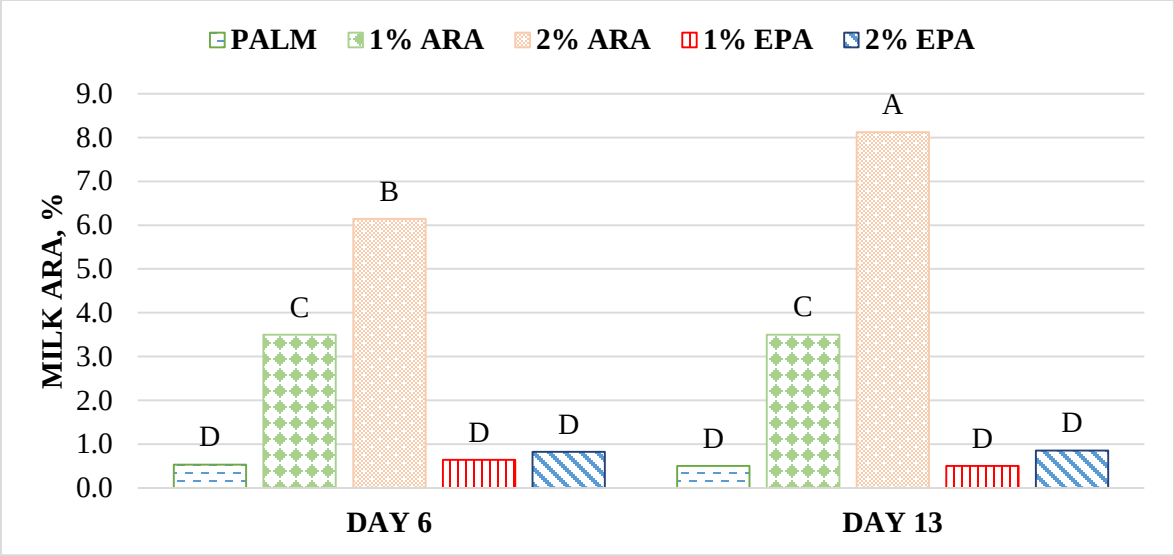

Figure 1. Impact of supplementation of palm kernel oil (PKO; control lipid source), arachidonic acid (ARA), or eicosapentaenoic acid (EPA) on concentrations of ARA (% of total fatty acids) in sow milk after 6 and 13 days of supplementation. [ABC]Means without common superscripts differ ($P < 0.05$).

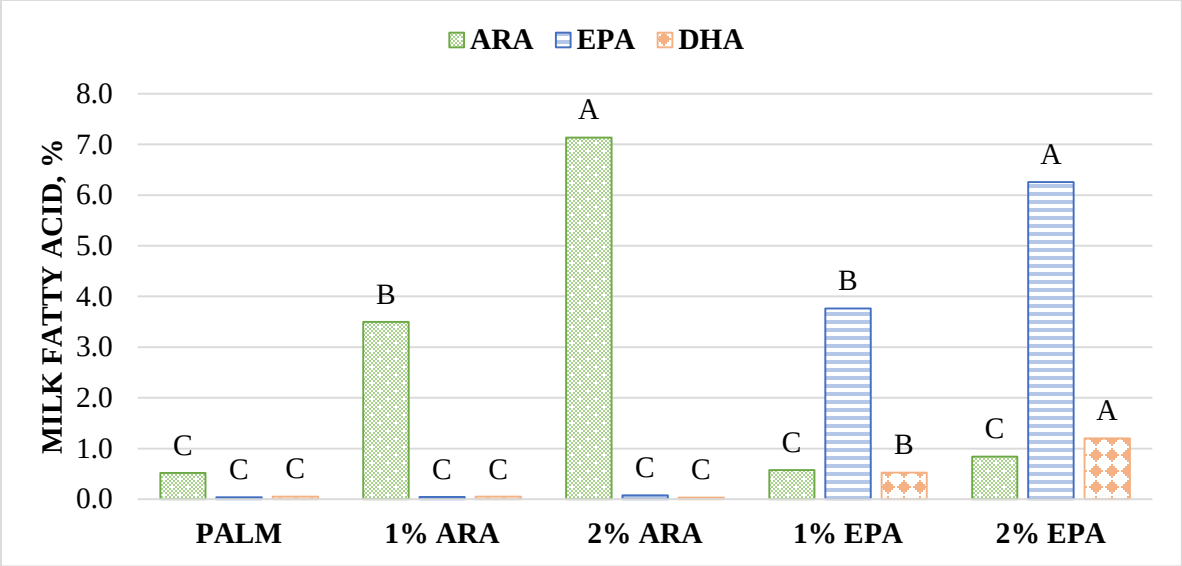

Figure 2. Impact of supplementation of palm kernel oil (PKO; control lipid source), arachidonic acid (ARA), or eicosapentaenoic acid (EPA) on concentrations of ARA, EPA, and DHA (% of total fatty acids) in sow milk. ^{ABC}Means without common superscripts differ ($P <$ 0.05).

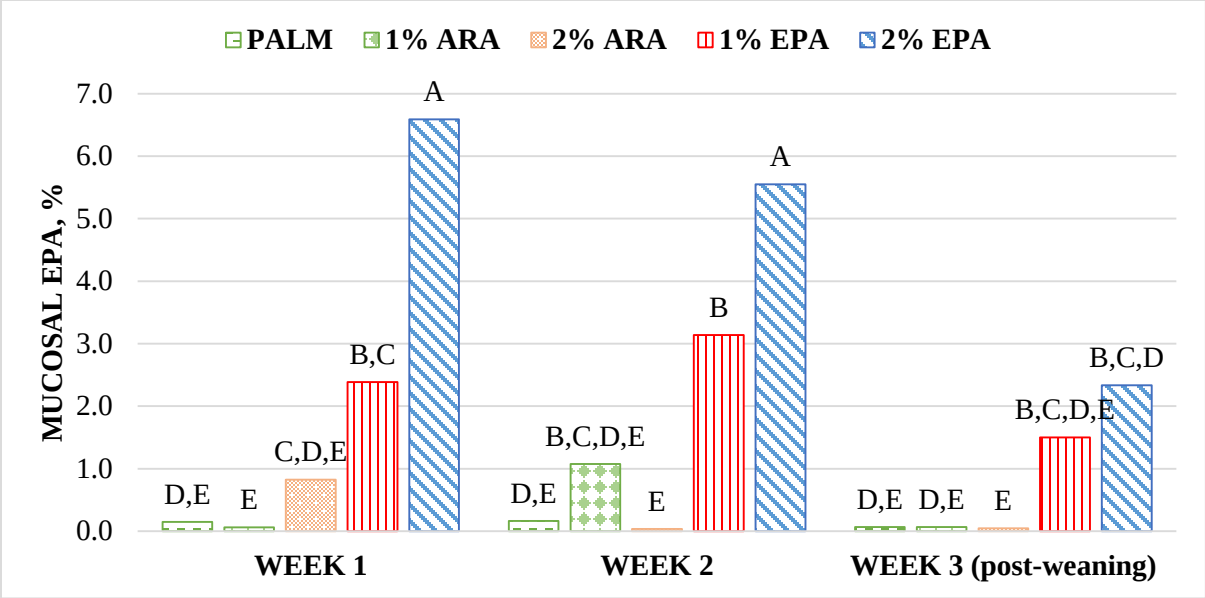

Figure 3. Impact of supplementation of palm kernel oil (PKO; control lipid source), arachidonic acid (ARA), or eicosapentaenoic acid (EPA) on concentrations of EPA (% of total fatty acids) in the mucosa of suckling pigs after 7 and 14 d of supplementation and 1 week post-supplementation. ^{ABC}Means without common superscripts differ ($P < 0.05$).

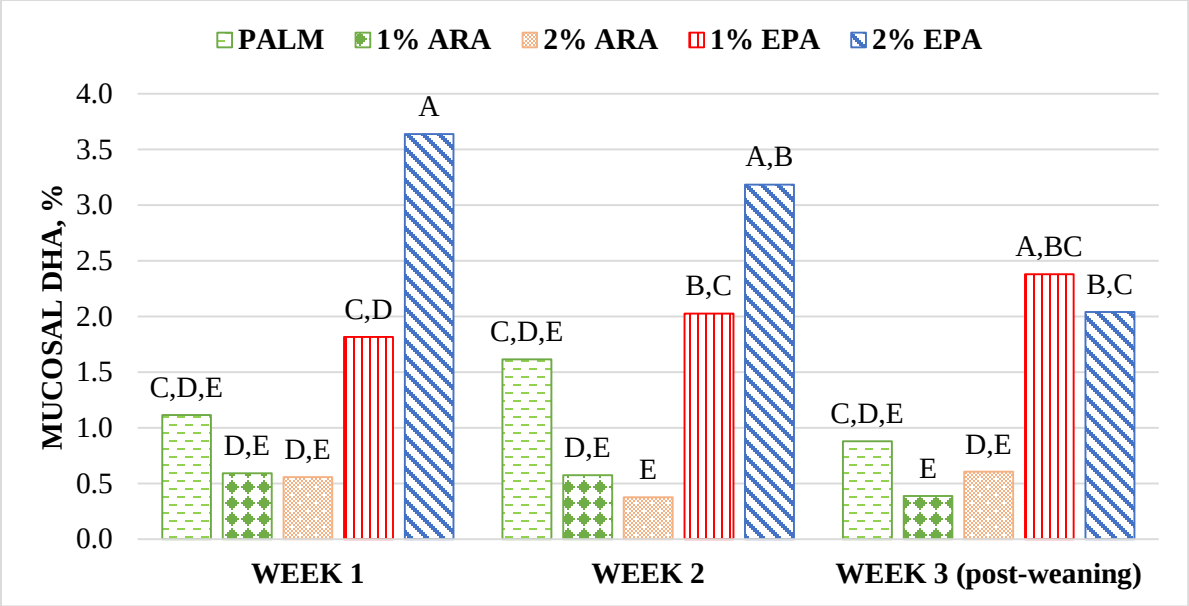

Figure 4. Impact of supplementation of palm kernel oil (PKO; control lipid source), arachidonic acid (ARA), or eicosapentaenoic acid (EPA) on concentrations of DHA (% of total fatty acids) in the mucosa of suckling pigs after 7 and 14 d of supplementation and 1 week post-supplementation. [ABC]Means without common superscripts differ ($P < 0.05$).

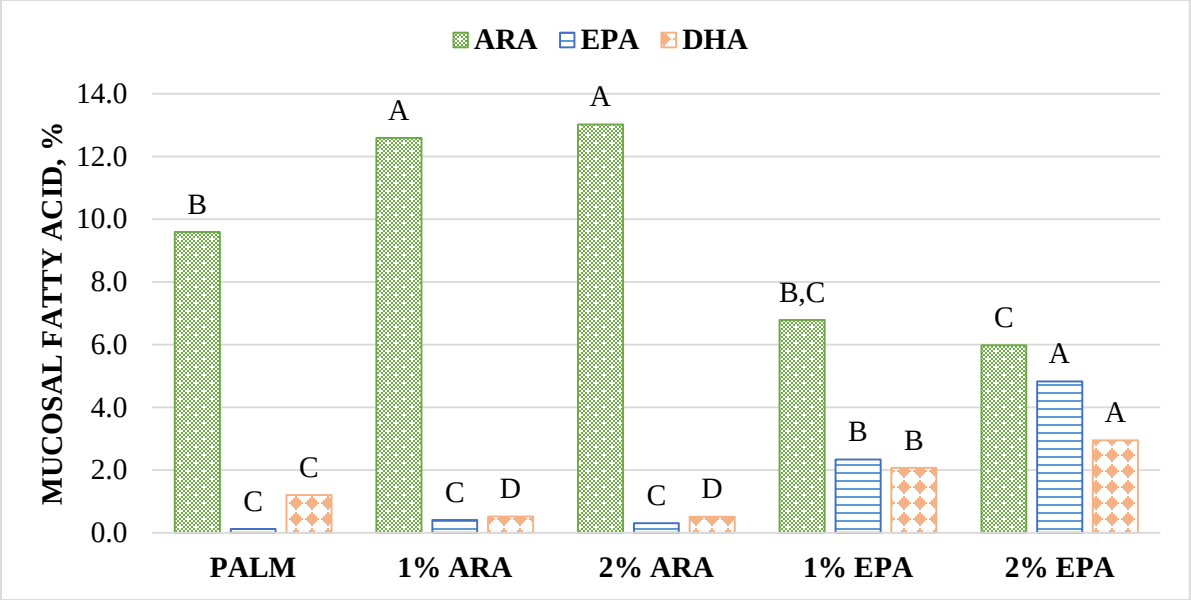

Figure 5. Impact of supplementation of palm kernel oil (PKO; control lipid source), arachidonic acid (ARA), or eicosapentaenoic acid (EPA) on concentrations of ARA, EPA, and DHA (% of total fatty acids) in the mucosa of suckling pigs. ^{ABC}Means without common superscripts differ ($P < 0.05$).