Effects of Animal Fat, Flaxseed Oil, and Vitamin E on Growth Performance and Metabolic Profile of Finisher Pigs, Physical and Organoleptic Characteristics of Pork, and Gene Expression in Adipose and Muscle Tissues of Finisher Pigs

by

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Abstract

Producing high-quality pork is an integral part of successful and sustainable pig production. Lipid content and composition is central to the nutritional value and eating quality of pork. The objective of this project was to investigate the effect of dietary lipids and vitamin E supplementation on growth performance, serum metabolic profile, carcass characteristics, fatty acid (FA) profile, physical and organoleptic characteristics of pork, and expression of genes associated with lipid metabolism. A total of 96 pigs (initial body weight, 54 ± 3.4 kg) were used to investigate the effects of lipids [0, 1, 3, and 5% poultry fat (+ 1% flaxseed oil for diets with supplemental lipids)] and vitamin E (11 or 220 IU vitamin E/kg) in a 4 x 2 factorial arrangement with 3 gilt pens and 3 castrated male pens per treatment and 2 gilts or 2 castrated males per pen. Standardized ileal digestible Lys, Ca, and P were adjusted accordingly with the DE content. All diets were formulated to meet or exceed the 2012 NRC nutrient requirements. Blood samples were collected at the end of finisher 2 phase. Pigs were harvested at approximately 110 kg, and standard carcass data were collected. Loin chops were collected for the determination of physical and sensory characteristics of pork. Adipose and muscle samples were collected for gene expression analysis.

There were no lipid x vitamin E interactions on growth performance. Lipid supplementation improved gain:feed (linear, $P < 0.01$) during finisher 1, finisher 2, and overall phases, increased weight gain (linear; $P = 0.028$) during finisher-2, and increased (linear, $P < 0.05$) belly thickness. There were lipid x vitamin E interactions on fat-free lean percentage and

backfat thickness at 10th rib ($P < 0.05$). Vitamin E supplementation increased carcass leanness in pigs fed the 6% dietary lipids, but increased fatness in pigs fed the diets containing 2 and 4% lipids. Vitamin E supplementation increased serum α-tocopherol content ($P < 0.001$) and NPPC marbling score ($P < 0.05$). The percentage of the saturated FA and monounsaturated FA were reduced by lipid supplementation (linear, $P < 0.05$). Serum cholesterol and triglyceride increased (linear, $P < 0.01$) and the percentage of ω-6 polyunsaturated FA in muscle increased (linear, $P < 0.001$) with increased dietary lipids. Flaxseed oil supplementation increased ω-3 polyunsaturated FA and decreased the ω-6 to ω-3 ratio ($P < 0.001$). No clear differences were found on physical characteristics, belly firmness, oxidative stability, and sensory evaluation in pork, and gene expressions associated with lipid metabolism in adipose and muscle tissues. These results indicated that vitamin E supplementation increased NPPC marbling score, and animal fat and flaxseed oil could improve feed efficiency, and flaxseed oil was effective in improving the nutritional value of pork as indicated by ω-6 to ω-3 ratio without negative effect on organoleptic characteristics or oxidative stability of pork.

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CHAPTER I.

INTRODUCTION

Pork in General

Pork is the most widely consumed meat in the world, totaling about 112 million metric tons in 2013, and constituting over 37% of the total global meat intake (Table 1; FAO, 2017). China, the EU, and the United States are the largest pork producers. There has been tremendous development of technology in breeding, nutrition, and management, which lead to a tremendous increase in annual pork production. Improving the quality and organoleptic characteristics of pork is an integral part of successful and sustainable pig production (Adhikari et al., 2017).

Table 1 Global meat consumption and percentage of pork, 2010–2013 [a].

Year	2010	2011	2012	2013
Pork, 1,000 tonnes	105,727	106,174	109,937	112,104
Poultry meat, 1,000 tonnes	95,713	99,577	101,915	104,874
Bovine meat, 1,000 tonnes	64,522	64,399	64,816	65,218
Mutton & goat meat, 1,000 tonnes	12,779	12,871	12,973	13,387
Other meat, 1,000 tonnes	6,334	6,454	6,725	6,828
Total, 1,000 tonnes	285,074	289,475	296,365	302,411
Pork/total, %	37.1	36.7	37.1	37.1

[a] Data source: Authors' analysis based on FAO (2017).

Consumer Preference or Demands

The consumers' preference for a certain pork is influenced by appearance (e.g., color, marbling, fat, and drip) and (eating) quality (Font-i-Furnols et al., 2012; Ngapo et al., 2007). To respond to the market demands, the pork production system has been going through some changes. A successful example of consumer-driven innovation is the development of methods to satisfy the consumers' preference for lean pork (Blanchard et al., 2000; Ngapo et al., 2007). Recently, nutrition, health, environment, and animal welfare have been becoming more important factors when choosing pork products especially by European consumes (Verbeke et al., 2010). For instance, fat quality is more important than quantity when trying to reduce the risk of cardiovascular diseases.

Body Fat and Intramuscular Fat

Although mammals still need some essential fat deposition, any excess fat that is deposited into adipose tissues, other than the marbling fat, is undesirable and would reduce production efficiency of pigs. Fat deposition varies widely depending on genetic factors (i.e., breed, sex, and genotype) and nutrition. Much effort has been made to control body composition at slaughter, which determines the commercial values of the animal carcass. Genetic selection for increased leanness over the past 2 decades has led to a reduction of total carcass fat from 35 to 45% to less than 20% in modern pigs (Kouba and Sellier, 2011). Unfortunately, it has also resulted in a decrease of intramuscular fat (IMF) content from 8% to as low as 1%, which has adverse effects on overall acceptability (Apple, 2013; Hocquette et al., 2010; Wood et al., 2004). However, dietary modifications can offset those negative effects, and thus would undoubtedly have a positive impact on the pork industry (Apple, 2013).

Dietary Lipids and Lipogenesis

It is well established that dietary fat sources (plant oils or animal fat), or contents could affect the growth performance, pork quality, and fatty acid (FA) composition of muscle and adipose tissues of pigs (Lin et al., 2013; Wood et al., 2008). Although animal fat is an inexpensive source of energy (Swisher, 2017), pork producers are often hesitating to add fats to finisher diets because high-energy diets are thought to induce direct deposition of surplus energy into adipose tissues and, therefore, reduce carcass value.

The two major sources of body fat are de novo lipogenesis (the process of converting carbohydrates into fat), and direct deposition of dietary fat. Obviously, body fat would be increased by excess dietary energy or fat. Some studies have demonstrated that the IMF content could be increased by dietary lipids (Eggert et al., 2007; Huang et al., 2008b). On the other hand,

numerous studies have shown that de novo lipogenesis can be markedly reduced by dietary lipids in pigs (Allee et al., 1971a; Allee et al., 1971b). If the animal is on a "high-fat" diet and not consuming excess "non-fat" energy, overall leanness may be increased though the reduction of de novo lipogenesis.

Animal Fat

In the United States, 8.9 billion broilers and mature chickens, 243 million turkeys, 30 million cattle, and 118 million pigs were harvested in 2016 (Swisher, 2017). However, one-third to one-half of the weight of food-producing animals is not consumed by humans (Meeker and Hamilton, 2006). The US rendering industry produced approximately 4.6 million metric tons of animal protein meals, as well as 5.7 million metric tons of animal fats in 2016 (Swisher, 2017). Most of those rendered products are not fit for human consumptions, but they are invaluable feed ingredients for livestock, poultry, aquaculture, and companion animals (Meeker and Hamilton, 2006; Swisher, 2017). If animal fat can reduce carcass fat, it can increase further the market value of animal fat.

Omega-3 Fatty Acids and Pork Quality

Nutrient composition is also considered as one of the attributes of pork quality (Apple, 2013). The ω-3 polyunsaturated FA (PUFA) has beneficial effects on human health, such as development of brain, prevention of cardiovascular disease, cancers, and mental illnesses, and modulation of immune functions (Riediger et al., 2009). Researchers have attempted to fortify pork with ω-3 PUFA using fish oil or linseed oil (Guillevic et al., 2009a; Martínez-Ramírez et al., 2014a; Martínez-Ramírez et al., 2014b; Musella et al., 2009; Wood et al., 2008). Dietary ω-3 PUFA has been shown to improve IMF content in pork (Huang et al., 2008b), and alter expression of genes involved in adipogenesis (Luo et al., 2009). In addition, ω-3 FA can be

directly deposited into the membrane phospholipids, which is more sensitive to dietary manipulations compared with neutral lipids (Corino et al., 2014; Romans et al., 1995b). However, lipid oxidation and soft belly have been identified as the major problems in the effort to enhance the ω-3 FA content of pork by dietary manipulations (Romans et al., 1995b; Wood et al., 2003; Wood et al., 2008). On the other hand, animal fats have greater content of saturated and monounsaturated fatty acids, could increase belly firmness (Apple et al., 2007) and reduce de novo lipogenesis (Smith et al., 1996). Therefore, carcass fat may be reduced and the IMF and ω-3 FA contents of pork may be increased simultaneously by dietary supplementation with a combination of animal fat and flaxseed oil.

Furthermore, elucidating the fundamental cellular mechanisms associated with lipid metabolism is crucial in maximizing the quality and organoleptic characteristics of pork. Thus, the use of animal fat, in combination with flaxseed oil, and molecular biology to address pork quality issue in this research will have positive impacts on successful and sustainable pig production, as well as human health.

CHAPTER II.

LITERATURE REVIEW

FEEDING VALUES OF LIPIDS IN SWINE DIETS

Introduction

Energy is the most expensive component in swine diets. The dietary energy components include carbohydrate, protein, and lipids (Fig. 1). Lipid has the highest energy density, and the gross energy is approximately 2.25 times that of carbohydrate (Pettigrew and Moser, 1991; Stahly, 1984). It is impossible to investigate the effect of lipids without altering the ingredient composition of the diets. Thus, studies on dietary fat are inescapably linked to digestibility, ingredient composition, nutrient density, and energy system. The results of previous animal studies indicated that lipid supplementation had positive effects on growth performance (Azain, 2001; Pettigrew and Moser, 1991). Dietary lipids are of practical importance as well. For example, it greatly reduced aerial dust in swine buildings, most of which is derived from feed, and thus has beneficial effects on both human and pigs (Chiba et al., 1985). However, special attention should be paid to the possible reduced flowability of feed during mixing and distribution, increased risk of soft pellets, and increased oiliness of unlined bags, when fat is included in the diets (Pettigrew and Moser, 1991).

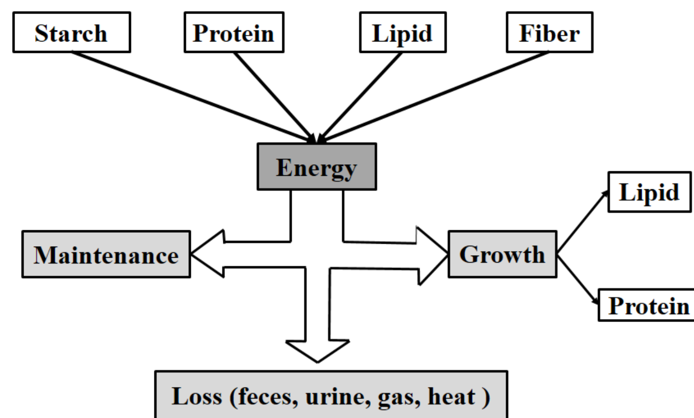

Fig. 1. Illustration of energy source and utilization in grower-finisher pigs. Lipid is one of energy sources, and has the highest energy density. Dietary energy is used for growth, maintenance, or lost in feces, urine, gas, and heat.

Dietary Source of Lipids

Lipids refer to a broad group of organic compounds that have low solubility in water and high solubility in nonpolar reagents. Lipids include fatty acids (FA), triacylglycerol (TAG), steroids, glycerophospholipids, sphingolipids, waxes, and others. Lipids are an excellent source of energy for swine and poultry. The quantitatively important components of dietary lipids are FA and TAG, although others are also of biological importance (Lin et al., 2013). The lipid source in swine diets can generally fall into 2 categories, 1) plant oils from oilseeds, such as soybean oil, rapeseed oil, sunflower oil, or corn oil, and 2) animal fats resulting from the rendering industry, such as tallow, lard, or poultry fat. In the United States, about 5.7 million metric tons of animal fats were produced in 2016, including tallow (44.8% of US rendered fats), poultry fat (19.5%), yellow grease/used cooking oil (16.0%), choice white grease (10.9%), lard (2.9%), and other greases (5.9%; Swisher, 2017). Most of those rendered fats are included in livestock feed and pet food. It is often ignored, but the basal feed ingredients also have a lipid component. For instance, the ether extract content of corn and soybean meal is 3.48 and 1.52%, respectively (NRC, 2012).

Over 90% of dietary lipids are TAG (Iqbal and Hussain, 2009), and the position of carbon on the glycerol is referred to as stereospecific numbering system: sn-1, sn-2, and sn-3 (Berry, 2009). In plant oils, unsaturated FA (oleic, linoleic acid (LA), α-linolenic acid (ALA)) are usually bound on the sn-2 position, whereas saturated FA (SFA; e.g., palmitic and stearic acid) are predominantly esterified at the sn-1, and sn-3 position (Bracco, 1994; Mattson and Volpenhein, 1963). Christie and Moore (1970) reported that most of porcine tissues, such as adipose, had predominantly palmitic acid at sn-2 position, whereas there were more unsaturated FA at the sn-2 positions of TAG in liver and blood. The melting temperature of FA is determined by carbon

chain length, degree of unsaturation, and position of FA (Michalski et al., 2013). Natural oils and fats have melting and crystallization ranges, rather than single melting points because they contain complex mixture of TAG (Michalski et al., 2013).

Digestion, Absorption, and Digestibility of Lipids

Intestinal digestion of dietary lipids is a complex process consisting of several steps (Hussain, 2014; Iqbal and Hussain, 2009; Kerr et al., 2015). In short, the process begins with emulsification from oral cavity to upper jejunum, where lipids mix with bile salts, lipase (lingual, gastric, and pancreatic), and co-lipase. The TAG are hydrolyzed into 2-monoacylglycerol (2-MAG) and free FA, and then they are taken up into enterocytes via transport proteins (e.g., CD36) or passive diffusion mechanisms. In the enterocytes, those absorbed lipids are bound to FA binding protein, transported to endoplasmic reticulum, where TAG is resynthesized and used for lipoprotein assembly, such as chylomicrons. Those lipoproteins are then transported through blood or lymph circulation. The process for cholesterol and phospholipids digestion is similar, but with different enzymes, transport proteins, and lipoproteins (Hussain, 2014; Iqbal and Hussain, 2009). The digestion of lipids is nearly completed at the terminal ileum (Jørgensen et al., 2000; Kerr et al., 2015).

Duran-Montgé et al. (2007) using ileo-rectally anastomosed pigs showed that the apparent ileal digestibility (AID) of supplemental tallow was 83.7 %, lower than that of high-oleic sunflower oil (90.8%), sunflower oil (90.4%), and linseed oil (92.6%). The same authors also measured apparent total tract digestibility (ATTD) of supplemental lipids using intact pigs, but no differences were observed among lipid sources, which may be due to the effect of the microflora in the hindgut (Duran-Montgé et al., 2007). Furthermore, small differences between ileal and fecal digestibility were observed for individual FA, except for 16:0 and 18:0, when fed

increasing contents of soybean oil (Jørgensen et al., 1993). The ATTD of 18:0 were negative for sunflower oil or linseed oil supplemented diets, whereas their AID values were above 70% (Duran-Montgé et al., 2007). The increase of 18:0 in fecal content can be explained by endogenous secretion of 18:0 and bio-hydrogenation of unsaturated FA by hindgut microflora (Jørgensen et al., 1993; Jørgensen et al., 2000). In contrast, ATTD of crude fat is similar to AID of crude fat (Jørgensen et al., 1992; Jørgensen et al., 2000; Duran-Montgé et al., 2007). Therefore, AID would be a better indicator of digestibility of individual FA, whereas ATTD can be used for digestibility of crude fat.

The true digestibility of lipids is usually not calculated due to the inconsistent values for endogenous losses of fat. Jørgensen et al. (1993) used the regression analysis to estimate endogenous fat, and showed that it was 4.74 and 4.41 g/kg DM intake at ileal end and fecal end, respectively. Noblet and Perez (1993) estimated the endogenous fat losses at zero fat intake, which was up to 8.5 g/kg DM. Recently, Kil et al. (2010) showed that true digestibility remained constant over different concentrations of dietary lipid.

Factors Affect Lipid Utilization in Swine Diets

Several factors were important in lipid digestibility and utilization, including physicochemical factors (e.g., carbon chain length, degree of saturation, free FA content, position of FA on the glycerol backbone, and hydrogenation), dietary factors (e.g., emulsifier, fat, crude protein, crude fiber, Ca, and Mg), and animal dependent factors (e.g., age, composition of endogenous fat, and microbial activity) (Bracco, 1994; Jørgensen et al., 1992; Kerr et al., 2015; Ravindran et al., 2016; Stahly, 1984).

***Physicochemical factors*.** For the individual FA, saturated FA (SFA) are less digestible than unsaturated FA in pigs (Duran-Montgé et al., 2007; Jørgensen et al., 2000). The AID of SFA

decreased with FA length, whereas the AID of polyunsaturated FA (PUFA) increased with the degree of unsaturation (Duran-Montgé et al., 2007; Jørgensen et al., 2000; Martínez-Ramírez et al., 2013). For the digestibility of added lipids, those with a ratio of unsaturated to saturated (U/S) FA above 1.5 have high digestibility, averaging 85 to 92% (Stahly, 1984). Lipids containing a U/S ratio less than 1.0 to 1.3 have relatively low digestibility, with widely ranging from 35 to 75% (Stahly, 1984). However, the digestibility of dietary saturated lipid is not always less than unsaturated lipid. For example, the ATTD of soybean oil and choice white grease were similar (Kil et al., 2011), and the AID of fish oil, rapeseed oil, and coconut oil were similar (Jørgensen et al., 2000).

Both dietary TAG and phospholipids can be hydrolyzed in the lumen of intestine by pancreatic lipase, more specifically on sn-1 and sn-3 positions, and release FFA and 2-MAG, the latter of which is readily reutilized for synthesis of chylomicron-TAG (Iqbal and Hussain, 2009; Michalski et al., 2013). Thus, the positioning of FA on the TAG molecule can affect the digestibility of FA (Bracco, 1994; Kerr et al., 2015). The FA, more particularly long chain (LC) FA, esterified at the sn-2 position of TAG is thought to be more efficiently absorbed than those at the sn-1 or sn-3 positions. For example, the low digestibility of cocoa butter can be partially explained by the stereospecific structure that long chain SFA (palmitic acid and stearic acid) are predominantly present in sn-1 and sn-3 positions (Bracco, 1994). Michalski et al. (2013) and Kerr et al. (2015) suggested that the effect of TAG structure on FA absorption is associated with interaction with divalent ions (Ca^{2+} and Mg^{2+}), and the melting temperature of the corresponding TAG. Commercially, the stereospecific structure of TAG can be redistributed by process of chemical and enzymatic inter-esterification (Berry, 2009). However, randomization of FA

position in TAG of either high- or low- PUFA lipids by chemically inter-esterification showed no influence on FA composition in depot fat of pigs (Scheeder et al., 2003).

Dietary factors. It has been showed that lipid digestibility was negatively affected by dietary fiber content (Just et al., 1980; Noblet and Perez, 1993; Stahly, 1984). For example, it was calculated that 1% increase in dietary crude fiber content resulted in about 3.8% reduction of ATTD of crude fat (Just et al., 1980), whereas NRC (2012) summarized that every 1% of additional dietary crude fiber decreased ATTD by 1.3 to 1.5%. Unexpectedly, Kil et al. (2010) found that supplementation of graded contents of purified NDF did not change apparent and true digestibility of fat. Chen et al. (2013) found that AID of acid hydrolyzed ether extract increased with increased content of dietary alfalfa meal, in which the insoluble fiber content accounts for 94% of total dietary fiber. Thus, the depression effect on lipid digestibility may be more attributable to characteristic of fiber, rather than dietary levels of fiber. Dietary minerals (Ca and Mg) and phytate may bind with released free FA in the lumen and form insoluble metallic soap, which reduced lipid digestibility in birds (Ravindran et al., 2016) and decreased ATTD of lipids in pigs (Jørgensen et al., 1992). However, increasing contents of lipid did not affect utilization of Ca, Mg, and P, indicating Ca-soap may be well utilized by pigs (Atteh and Leeson, 1983). Dietary crude protein content had minimal effects on digestibility of crude fat, but greater protein resulted in increased AID of C16:1 and C18:0 (Jørgensen et al., 1992; Just et al., 1980).

Anima dependent factors. Lipid digestibility increased with age in nursery pigs, and the increase was more pronounced for animal fat than plant oil (Cera et al., 1988; Adeola et al., 2013). Ravindran et al. (2016) reviewed that there were sex and specie effects on lipid utilization in poultry. Apparent lipid digestibility increased as dietary lipid increased (Just et al., 1980) because endogenous fat had greater influence on ATTD at low level of dietary fat than at high

levels (Adeola et al., 2013; Jørgensen et al., 1993). Kil et al. (2010) found that the true digestibility of extract corn oil (> 90%) at both ileal and fecal end were greater than that of intact fat from whole corn germ meal (varied from 76.7 to 87.5%), which was associated with less endogenous fat for pigs fed extract corn oil. In addition, the microflora activity in hindgut can affect individual lipid digestibility through bio-hydrogenation (Duran-Montgé et al., 2007; Jørgensen et al., 1993).

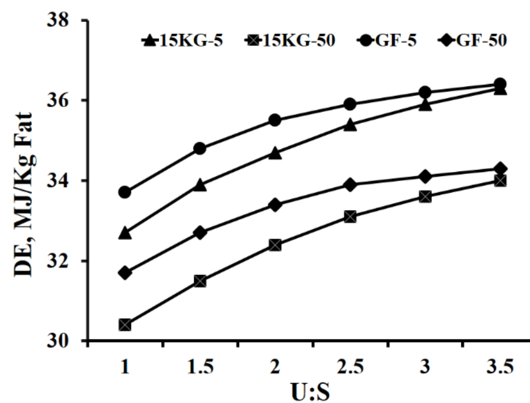

Fig. 2. Impact of U: S (ratio of unsaturated to saturated fatty acids in fat) and FFA (free fatty acid content of fat, g/kg) on digestible energy (DE) of pigs. 15KG-5 is pig of 15kg live weight fed with 5% FFA. 15KG-50 is 15kg live weight fed with 50% FFA. GF-5 is 30-85kg live weight fed with 5% FFA. GF-50 is 30-85kg live weight fed with 50% FFA. Figure was drawn based on Table 3b in Wiseman et al. (1998).

Energetic Value of Dietary Lipids

The digestible energy (DE) of lipids increases as the U:S increases, but decreases with the increase in free FA percentage (Fig. 2; Rosero et al., 2015; Wiseman et al., 1998). The NRC (2012) estimated the DE values of lipids based on U:S and FFA content using the same equations as Wiseman et al. (1998). The metabolizable energy (ME) was then estimated as 98 % of DE, and net energy (NE) was subsequently calculated as 88% of ME, as shown in Table 1. Su et al. (2015) suggested that the equation for predicating DE values may not be valid for all lipid sources, and they measured DE and ME values of 13 lipid sources using the difference

procedure, and proposed different prediction equations for DE and ME based on composition of fatty acids (Table 1).

Table 1 Predication equations for energy values of common lipid sources in swine diets[a,b].

Equation	Pig	R^2	Reference
DE, MJ/kg = 36.898 – [0.005×(FFA, g/kg)] − [7.330×exp(−0.906×U:S)]	30 to 85 kg	0.80	Wiseman et al. (1998); NRC (2012)
DE, MJ/kg = 37.890 – [0.005×(FFA, g/kg)] − [8.200×exp(−0.515×U:S)]	15 kg	0.77	Wiseman et al. (1998)
DE, MJ/kg = 34.15 + [0.07 × (PUFA, % of total FA)] + 0.21 × C18:0 − 0.04 × C18:1	39.4 kg	0.84	Su et al. (2015)
DE, Mcal/kg = 9.363 + [0.097 × (FFA, %)] − [0.016 × (ω-6: ω-3)] − [1.240 × (C20:0, %)] − [5.054 × (INIM, %)] + [0.014 × (C16:0, %)]	13 kg	0.82	Kellner and Patience (2017)
DE, Mcal/kg = 8.357 + [0.189 × U:S] − [0.195 × (FFA, %)] − [6.768 × (C22:0, %)] + [0.024 × (PUFA, %)]	50 kg	0.81	Kellner and Patience (2017)
DE, kcal/kg = [8,381 – (80.6 × FFA) + (0.4 × $(FFA)^2$) + (248.8 × U:S) – (28.1 × $(U:S)^2$) + (12.8 × FFA × U:S)]	Lactating sows	0.74	Rosero et al. (2015)
ME, MJ/kg = 33.37 + (0.07 × PUFA, % of total FA) + 0.20 × C18:0 − 0.04 × C18:1	39.4 kg	0.85	Su et al. (2015)
ME = DE × 98%	-	-	NRC (2012)
NE = ME × 88%	-	-	NRC (2012)

[a] FFA = free fatty acid, U:S = unsaturated : saturated fatty acid ratio, PUFA = polyunsaturated fatty acid, INIM = insoluble impurities.

[b] Inclusion level of lipid used in test diets ranged from 40 to 120 g/kg (Wiseman et al., 1998), whereas various source of lipids were used at level of 100 g/kg (Su et al., 2015), and 50 g/kg (Kellner and Patience, 2017).

The ratio of NE to ME, representing the efficiency of utilization of ME for NE, varied among lipid, protein, and starch, which were approximately 90, 60, and 82%, repectively (Noblet et al., 1994; Noblet and van Milgen, 2004). The DE and ME systems would underestimate the energetic value of lipids, whereas the NE system is superior to them (NRC, 2012). It indicated that the energy value of lipid-rich diets were usually underestimated under DE or ME system (Noblet et al., 1994; Noblet and van Milgen, 2004). Fat supplementation enhanced N utilization

and energy retention in growing pigs fed with identical DE and amino acid intakes (Bruininx et al., 2011). Wu et al. (2007) evaluated the efficiency of different energy systems for body weight gain with diets containing graded contents of tallow, and they found that NE system was more precise in predicting growth performance of pigs, as content of dietary fat did not change the net energy cost of weight gain.

For growing pigs, NE is usually calculated as the sum of the fasting heat production at zero activity and the retained energy (Noblet et al., 1994; Noblet and Van Milgen, 2013). However, measurement of NE is complex and expensive (Noblet and Van Milgen, 2013), and the NE value obtained depends on different NE systems (Kil et al., 2011; Noblet and van Milgen, 2004). Inconsistant NE values of lipids have been reported by several investigators over the years (Table 2), and thus, more accurate measurement of NE for lipids are needed in the future (Kerr et al., 2015).

Table 2 Net energy (NE) values of common lipid sources in swine diets, selected examples [a].

Lipid source	NE, kcal/kg	Contents of lipids, g/kg[a]	BW of pigs, kg[b]	Reference
Tallow	7,122	–	–	Sauvant et al. (2004)
Tallow	4,180	–	–	Galloway and Ewan (1989)
Beef tallow	6,895	–	–	NRC (2012)
Choice white grease	5,900	50 to 100	22 to 50, 85 to 130	Kil et al. (2011)
Choice white grease	7,149	–	–	NRC (2012)
Soybean oil	4,679	50 to 100	22 to 50, 85 to 130	Kil et al. (2011)
Soybean oil	7,545	–	–	NRC (2012)
Rapeseed oil	7,122	–	–	Sauvant et al. (2004)
Cottonseed oil	7,424	–	–	NRC (2012)

[a] Inclusion rate of lipid used in test diets
[b] BW = body weight

Energy Density and Voluntary Feed Intake

The voluntary feed intake in grower-finisher pigs is regulated by dietary, animal, and environmental factors, which was discussed in detail by Nyachoti et al. (2004) and recently by Li and Patience (2016). When those animal and environmental factors are absent, energy density of the diets will become the major dietary determinant for voluntary feed intake for pigs (Nyachoti et al., 2004; Li and Patience, 2016). However, daily energy intake, rather than energy density, is the true determinant for growth performance (Milgen et al., 2000; King et al., 2004).

It is generally assumed that pigs will adjust their feed intake to maintain a constant daily energy intake within a certain range of dietary energy concentrations (Cole et al., 1971). As shown in Fig. 3, a constant energy intake is achieved, as the feed intake decreased with increased dietary energy density. Although this concept has been around for a long time, the upper and lower limits of energy density are not well-defined for grower and finisher pigs, which vary because of genotypes, sex, body weight, and energy systems.

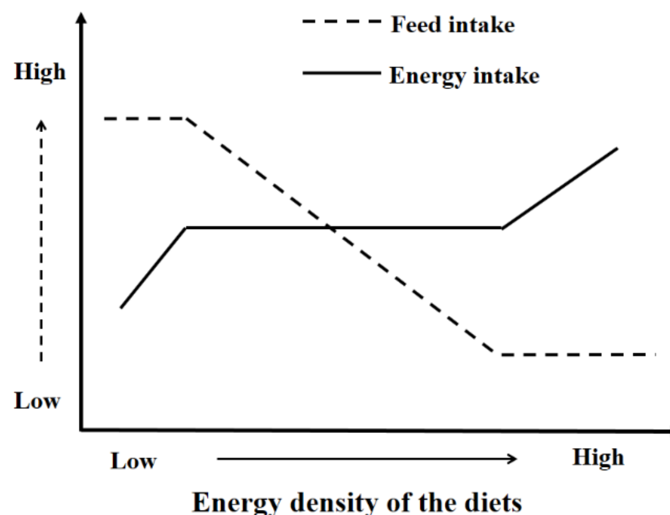

Fig. 3. Relationship between dietary energy density and daily intake of feed and energy. Pigs will adjust their feed intake in response to changes of dietary energy concentration to achieve a relatively constant energy intake, within a wide range of dietary energy concentrations. Redrawn from Cole et al. (1971).

Reduced energy density may decrease energy intake of pigs (Fig. 3), because pigs have a limited physical gut capacity and need to achieve a minimal gut fill (Black et al., 1986; Cole et al., 1971; Quiniou and Noblet, 2012). The maximum feed intake (FI_{max}, kg/d, 90% dry matter) for pigs weighing 10 to 205 kg can be predicted with an allometric equation: $FI_{max} = 0.115\ BW^{0.803}$ (Black et al., 1986), which was validated by Quiniou and Noblet (2012). Pigs weighting less than 20 kg may not be able to eat more feed to compensate for reduced energy density (Black et al., 1986). Pigs weighing 20 to 50 kg may not be able to compensate once the energy concentration was below 3.346 Mcal DE/kg (Black et al., 1986). Energy intake was reduced when ME decreased to 3.1 Mcal for pigs weighting 54 to 112 kg (Stein and Easter, 1996). Diets containing energy below the lower limits are usually rich in fibers and resulted in an increased gut fill and empty gut weight, and reduced dressing percentage (Kyriazakis and Emmans, 1995; Quiniou and Noblet, 2012; Stein and Easter, 1996). However, a potential advantage for those diets is to increase carcass leanness (Beaulieu et al., 2009; Stein and Easter, 1996; Smith et al., 1999).

Conversely, high energy diets may depress their feed intake, and, subsequently, maintain or slightly increase their energy intake (Fig. 3), possibly through nutrient-dependent feedback mechanisms from gut (Li and Patience, 2016). The DE intake remained constant for older pigs (50 to 115 kg) fed diets containing from 3.24 to 3.57 Mcal DE/kg, whereas it increased linearly in younger pigs (30 to 50 kg) in response to increased DE density (Beaulieu et al., 2009). Weaned pigs (9 to 25 kg) increased NE intake, but not DE intake, in response to increased NE concentration (Oresanya et al., 2008). Wu et al. (2007) showed DE, ME, and NE intake increased with increased NE content in the diets during 23 to 60 kg when digestible Lys to NE ratio was maintained. All things considered, it seems that the daily intake of ME or DE tends to increase

with increased energy content during grower phase (Wu et al., 2007; Beaulieu et al., 2009). More recently, Quiniou and Noblet (2012) fed individually penned pigs (35 to 110 kg) with 6 diets containing graded contents of NE ranging from 1.94 to 2.65 Mcal/kg, with a constant nutrients (i.e., SID Lys, Ca, and P) to NE ratio. They found that increased NE content would result in decrease in average daily feed intake (ADFI), but relatively similar in daily intake of NE when the NE content was above 2.22 Mcal/kg. In commercial conditions, De la Llata et al. (2001) failed to detect any difference on ME intake of pigs from 36 to 120 kg, when total Lys to ME ratio was maintained.

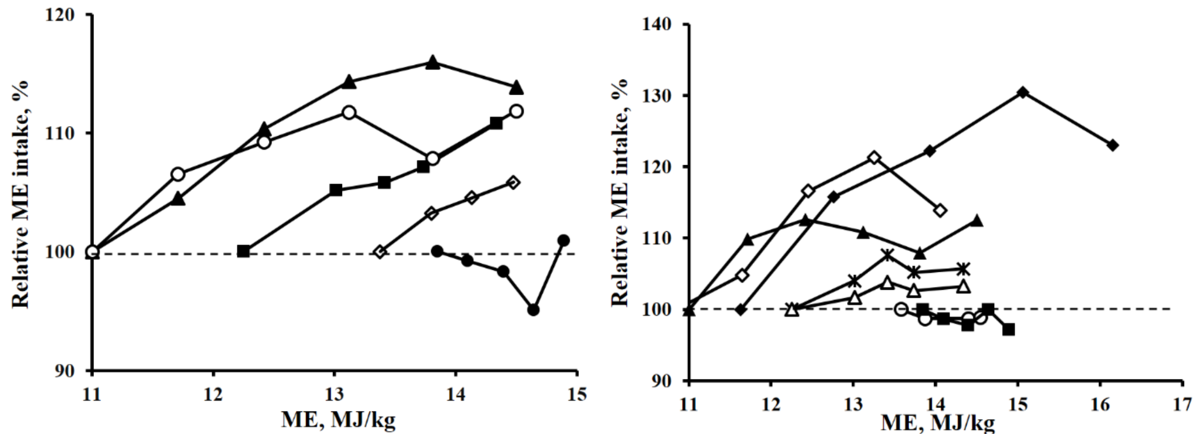

Fig. 4. Relationship between dietary energy density (ME, MJ/kg) and relative ME intake in grower (left graph) and finisher pigs (right graph). Relative daily ME intake is expressed as a percentage of the ME intake of the control or lowest energy density group in each study. Data adapted from (Stein and Easter, 1996; Smith et al., 1999; Wu et al., 2007; Beaulieu et al., 2009; Quiniou and Noblet, 2012; Cline et al., 2016). Each line represents individual trial.

Noblet and Van Milgen (2013) conducted a brief review to illustrate the relationship between DE concentration and daily DE intake using data published before year 2000. The same approach was used, and the graph was updated as shown in Fig. 4. As indicated in Noblet and Van Milgen (2013) and also in Fig. 4, there is an increase-plateau relationship if the lowest energy level is low in most studies, or the daily ME intake become relatively stable if the lowest energy level is quite high.

Energy Intake and Protein Deposition

A commonly used model for partitioning ME consumed is:

$ME = ME_m + PD/k_p + LD/k_f$

where ME_m = ME for maintenance (kJ/d), PD = protein deposition (kJ/d), LD = lipid deposition (kJ/d), and k_p and k_f are the corresponding energy efficiencies (NRC, 2012). The energy above maintenance will be used for protein and lipid deposition. Protein deposition depends on availability of amino acids, as well as supply of energy. It is generally assumed that PD increased linearly with increasing energy intake until reaching a plateau (PD_{max}), and the linear-plateau relationship depends on several intrinsic factors, such as genotype, BW, or age (Azain, 2001; Black et al., 1986; Campbell and Taverner, 1988; Quiniou et al., 1996). In addition, the PD_{max} decreased by 14% when ambient temperature increased from 23°C to 30°C (Bellego et al., 2002). Marginal deficiencies of amino acids would confound the energy metabolism because sufficient amino acids are necessary for maximum body protein deposition. The modelling of energy partitioning can be found in one review (van Milgen and Noblet, 2003).

The upper limit for PD was not always observed for high-performing pigs, such as pigs weighing 45 to 90 kg (Campbell and Taverner, 1988), and pigs weighing 40 to 100kg (Quiniou et al., 1996). It seems that genetic selections under ad libitum feeding may increase the maximum PD beyond the feed intake capacity of the pigs (Campbell and Taverner, 1988; van Milgen and Noblet, 2003). The NE intake did increase with increased dietary NE concentration in weaned pigs (Oresanya et al., 2008), and in grower-finisher pigs (Quiniou and Noblet, 2012). Perhaps, there might be beneficial effects on PD, if those pigs were fed high energy density diets to further increase their energy intake.

According to van Milgen and Noblet (2003), lighter pigs would be more sensitive to a changing energy supply compared with heavier pigs. King et al. (2004) investigated the effects of energy intake levels (ranging from 55 to 100% ad libitum) on carcass composition accretion in crossbred pigs (Large White × Landrace) weighing from 80 to 120 kg. The results showed that PD and LD linearly increased with increased dietary energy intake up to 100% ad libitum in both gilts and boars, whereas PD/LD ratio decreased with increased DE intake.

As previously discussed, energy intake can also be regulated by dietary energy density. However, the relationship between energy intake and protein deposition is largely based on studies in which energy intake was controlled through feed intake restriction. Oresanya et al. (2008) conducted an experiment with a 3 × 3 factorial arrangement, in which 3 contents of energy (2.15, 2.26, and 2.37 Mcal NE/kg) and 3 feed levels (100, 80, or 70% of ad libitum) were used for pigs weighing 9.5 to 25 kg. The results found no interactions on PD and total weight gain, but showed interactions on LD and LD:PD. Increased NE concentration resulted in increased NE intake, but did not change PD and total weight gain. It indicated that the response on growth and body composition may differ when daily energy intake was regulated from restriction or energy density. It is not clear whether such a physiological scenario exists for heavier pigs under ad libitum feeding.

If changes in dietary energy concentration affected voluntary feed intake, then amino acid intake would change as well. In addition, excess supply of protein will lead to increased release of ammonia into the environment and is not cost effective because of the low energetic efficiency of dietary protein utilization (van Milgen and Noblet, 2003). In fact, the idea of adjusting Lys content according to ME to optimize performance was supported by several studies (Bikker et al., 1994; Chiba et al., 1991a,b), assuming that relationship between PD and DE intake was

linear (Campbell and Taverner, 1988; Quiniou et al., 1996). Some studies investigated the interaction between DE content and Lys:DE, and no interactions were found for weaned pigs (Oresanya et al., 2007) and grower-finisher pigs (Smith et al., 1999). Quiniou and Noblet (2012) fed diets containing graded contents of NE to grower-finisher pigs, and found that the NE intake and average daily intake (ADG) linearly increased with increasing NE content, and there was a trend for backfat thickness to increase. The authors suggested that the effects of NE content on carcass characteristics would be depending on their response in LD and PD separately.

Evaluation of Dietary Lipids

Lipids have the greatest energy density, and the effect of lipid supplementation would be complicated if the energy intake was altered. Thus, dietary design is crucial for interpreting the effects of lipid supplementation. It is possible to formulate isocaloric and isolipidic diets when comparing different lipid sources (Kouba and Mourot, 1998). When graded contents of lipids were used, early investigators tended to formulate isocaloric and isonitrogenous diets by adding dilutors, or at the expense of other ingredients. Other nutrients, including amino acids and minerals, often meet or exceed the requirements. For example, sands was used as nonnutritive dilutors in semi-purified diets (Allee et al., 1971a; Allee et al., 1971c; Allee et al., 1972), or purified diets (Allee et al., 1971b). However, the synthetic diets with no fat have been shown to reduce daily gain (Allee et al., 1971a; Allee et al., 1972) and alter metabolic criteria, including blood insulin and thyroxine (Christensen and Just, 1988) compared with those fed typical barley-soy-based diets.

In some cases, investigators tried to maintain the energy concentration by increasing the quantities of fibrous ingredients. However, fiber source may influence the response to energy concentration, as the water-holding capacity of those bulky energy dilutors can be used to predict

the feed intake (g/kg live weight; Kyriazakis and Emmans, 1995). In addition, the fibrous ingredients contain high insoluble non-starch polysaccharides (NSP), which can reduce amino acid and energy digestibility and increase the rate of digesta passage (Noblet and Perez, 1993).

Some investigators substituted parts of cereal starch with lipid, and as a result, all diets were isonitrogenous, rather than isocaloric (Seerley et al., 1978; Smith et al., 1996). Although feed intake was not statistically analyzed in these studies, the addition of fat has been showed to reduce feed intake (Li and Patience, 2016; Pettigrew and Moser, 1991). Thus, it reduces intake of other nutrients, which resulted in inadequate intake of those nutrients, and eventually reduce ADG and feed efficiency, and increase carcass fatness (Pettigrew and Moser, 1991). It is possible to level off the difference of energy density by restricting the feed intake of those on a high-lipid treatment (Benítez et al., 2016; Christensen and Just, 1988; Williams et al., 1994). But, it may not be applicable in most normal conditions that uses ad libitum feeding where dietary energy concentration, instead of the daily energy intake, is under control.

It is now widely accepted that studies on lipid supplementation should maintain the ratio of nutrient to energy by gradual changes in the ingredient composition, i.e., decreasing low-energy ingredients while increasing high-energy ingredients (oil and fat; Beaulieu et al., 2009; De la Llata et al., 2001; Kellner et al., 2014; Smith et al., 1999; Wu et al., 2007). In most situations, the daily intake of ME or DE can remain constant (De la Llata et al., 2001; Smith et al., 1999; Wu et al., 2007), and consequently, the intake of other nutrients will remain constant as well. However, the effects of lipid are still confounded with the energy concentrations.

Effects of Lipids on Growth Performance

Pettigrew and Moser (1991) analyzed published data and categorized them as either protein:energy adjusted or not adjusted. They showed that addition of fat to the grower-finisher

pig diets decreased the ADFI, improved ADG and feed efficiency, and increased average backfat. Those responses were largely independent of whether the protein:energy was adjusted. In contrast, lipid supplementation during nursery phase did not increase ADG, but reduced ADFI. Since that review was published, much has changed, both in energy system and in genetic selection for improved gain to feed ratio (G:F) and leanness (Eggert et al., 2007). Therefore, a few more recent studies will be highlighted here.

Maintaining protein to energy ratio in grower-finisher pig diets. When the Lys to energy ratio was constant, lipid supplementation in grower and finisher diets consistently decreased ADFI and improved G:F (Beaulieu et al., 2009; Eggert et al., 2007; Engel et al., 2001; Myer and Combs, 1991; Quiniou and Noblet, 2012; Smith et al., 1999; Wu et al., 2007). For example, Smith et al. (1999) fed pigs (44.5 to 104 kg) with diets containing 1.5 to 6.0% choice white grease, and found that ADFI linearly decreased, whereas G:F linearly increased.

However, the growth response of grower-finisher pigs to lipid supplementation was not consistent. Some studies showed increased ADG for pig weighing 29.5 to 72.6 kg (Smith et al., 1999), 23.2 to 60.6 kg (Wu et al., 2007), and 41 to 123 kg (Benz et al., 2011b). Some studies showed that either fat source or concentration had negligible effect on ADG (Apple et al., 2009b; Engel et al., 2001; Realini et al., 2010). A majority of research has failed to show an effect on growth rate, for beef tallow (Apple et al., 2009b; Eggert et al., 2007; Weber et al., 2006), choice white grease (Engel et al., 2001; Myer and Combs, 1991; Weber et al., 2006), poultry fat (Apple et al., 2009b; Engel et al., 2001), and soybean oil (Apple et al., 2009b). No effect of choice white grease supplementation was observed on the ADG during 44.5 to 72.6 kg, and unexpectedly, it was quadratically decreased during 72.6 to 104.3 kg (Smith et al., 1999). Recently, Quiniou and

Noblet (2012) supplemented graded contents of NE on grower-finisher pig diets and found that ADG increased with increased NE content only from 35 to 50 kg BW.

In addition, several studies were conducted under commercial systems, where multiple concurrent stressors were present (e.g., stocking density, temperature, humidity, health status, and social environment). De La Llata et al. (2007) conducted 2 large-scale trials, including 1,200 gilts (27 to 120 kg BW) and 1,200 barrows (34 to 120 kg BW), respectively, to investigate the interactions between Lys to ME ratio and added fat (0 vs. 6%). In their gilt trial, they found no interactions on growth performance, whereas adding fat decreased ADFI, improved G:F, increased ADG from 27 to 75 kg BW, and did not change ADG from 75 to 120 kg BW. In their barrow trial, adding fat also decreased ADFI and improved G:F, but did not affect ADG. The increased daily intake of ME and total Lys during grower phase were observed in both gilt (27 to 45 kg BW) and barrow (34 to 60 kg BW) trials. Beaulieu et al. (2009) fed grower-finisher pigs with diets containing various contents of energy (3.12, 3.30, and 3.43 Mcal DE/kg) and found that ADG increased from 37 to 80 kg BW. Similarly, ADG increased from 36 to 59 kg BW, but no further improvement was observed from 59 to 120 kg BW (De la Llata et al., 2001). These results indicated that lighter pigs (< 80 kg BW) are more sensitive to increased energy density under commercial conditions.

All things considered, fat supplementation improved G:F and decreased feed intake on all phases, and had positive effects on daily gain in lighter pigs (< 80 kg BW). As previously mentioned, daily energy intake is the true determinant for animal growth performance. Predicting the pig's growth performance with dietary energy concentration is rather difficult under ad libitum feeding, unless the actual feed intake is known. Difference in daily energy intake may account for such an inconsistency. Furthermore, the energetic efficiency of lipids on BW gain

depends on several factors, such as body composition, breed, sex, and age (Noblet and van Milgen, 2013), and ambient temperature (Bellego et al., 2002). Precise determination of NE values for lipids would help to gain a better understanding of the varied responses in the future.

Maintaining protein to energy ratio in nursery pig diets. Pettigrew and Moser (1991) summarized that addition of fat could decrease ADFI and increase G:F, with greater response when protein:energy was adjusted. Tokach et al. (1995) evaluated lipid supplementation to weanling pigs in 6 experiments. The results indicated that lipid supplementation has no effect on growth performance from d 0 to d 14 after weanling, but it improved G:F from d 14 to d 35. Moreira and Mahan (2002) reported that daily gain and G:F increased with the addition of 5% lipids from 0 to 35 d after weaning, but there was no difference on feed intake. Oresanya et al. (2007) observed no effect of ADG, reduction in ADFI, and improvement of G:F for pigs fed a diet containing 3.6 Mcal DE/kg (added canola oil), compared those fed 3.4 Mcal DE/kg. Addition of lipids in nursery pig diets is of great practical importance in e.g., maintaining milk product quality and lubrication of the pellet die (Tokach et al., 1995).

Not maintaining protein to energy ratio in grower-finisher pig diets. Seerley et al. (1978) included 2.5 or 5% animal fat or poultry fat in grower-finisher diets with a constant protein content, and they found no effect on ADG, whereas fat supplementation improved G:F and 5% fat supplementation increased backfat thickness. Allee et al. (1971a) used isocaloric diets containing 1 or 13% corn oil, and found that fat supplementation improved ADG and G:F in grower-finisher pigs. Allee et al. (1971b) showed that addition of fat improved ADG and G:F in 5-wk-old piglets, and improved G:F in 2-wk-old piglets.

Utilization of Poultry Fat in Grower-Finisher Pig Diets

Poultry fat is high in oleic acid (37.4%), palmitic acid (21.6%), LA (19.5%), steric acid (6%), and palmitoleic acid (5.7%; NRC, 2012). The titer point for poultry fat is 31 to 35℃ and the iodine number is 77 to 88 (Azain, 2001). With a high content of unsaturated FA, poultry fat has a melting point of approximately 25℃, which is lower than lard and tallow (Kerr et al., 2015).

Poultry fat is rich in monounsaturated fatty acids (MUFA) and PUFA compared with lard and tallow. The LA content of *longissimus* muscle increased when poultry fat was fed, compared with animal fat and non-fat group (Seerley et al., 1978). Poultry fat linearly decreased 16:0 content of muscle and bellies, and increased 18:2 in muscle (Engel et al., 2001). The inclusion of 10% poultry fat did not affect the total MUFA, but increased 18:2 and 18:3 and decreased total SFA (16:0 and 18:0) in intramuscular fat compared with corn-soybean based control group (Shipp et al., 1997). Similarly, 5% poultry fat decreased total SFA, and increased C20:3n-6 and C20:4n-6 and maintained total MUFA in the *longissimus* compared with corn-soybean based control diet (Apple et al., 2009b). Poultry fat supplementation (5%) increased total unsaturated FA and decreased total SFA in both outer and inner layers of backfat, and decreased SFA to UFA ratio in belly, and consequently reduced belly firmness compared with no fat control (Eggert et al., 1998).

Inclusion of 5 to 6% poultry fat in grower-finisher pig diets showed reduced ADFI, improved G:F, when Lys to calorie ratio is maintained (Eggert et al., 1998; Engel et al., 2001; Woodworth et al., 1999). Many studies failed to detect any difference in ADG, carcass characteristics, meat quality of *longissimus* (Engel et al., 2001; Seerley et al., 1978), subjective color characteristics of *longissimus* and fat (Woodworth et al., 1999), belly quality (Engel et al., 2001), or carcass weight and dressing percentage (Apple et al., 2009b). In contrast, some studies

showed that adding poultry fat improved average backfat thickness (Apple et al., 2009b; Woodworth et al., 1999) and decreased subjective color (Eggert et al., 1998). Poultry fat may have negative effect on belly firmness (Eggert et al., 1998; Woodworth et al., 1999). Supplementation of beef tallow in finisher pig diets (80 to 115 kg) can compensate for the negative effect of poultry fat on belly firmness in lean pigs (Eggert et al., 1998).

DIETARY LIPID ON LIPID DEPOSITION AND PORK QUALITY

Introduction

Many aspects of lipid metabolism are not identical among human, pig, chicken, and rodents (Bergen and Mersmann, 2005). Efforts to understand the fundamental mechanisms associated with lipid metabolism and fat deposition are crucial in maximizing the quality and organoleptic characteristics of pork, which will increase pork acceptability by consumers. Under normal fed state for growing animals, the net results of anabolism (ingested fat and de novo lipogenesis) and catabolism (β-oxidation) is accumulation of body fat. The de novo lipogenesis is important in growing animals fed cereal-based diets because it accounts for most of the fat deposition. Dietary lipid is not only a highly available source of energy for food producing animals, but also it has other beneficial attributes. One of those attributes that is often overlooked is their effect on the reduction of body fat and increase in the IMF content of pork, the latter of which has a strong correlation with meat quality characteristics such as tenderness, juiciness, and flavor. Inconsistent results have been observed on carcass traits and meat quality when dietary lipids were included in swine diets (Azain, 2001; Pettigrew and Moser, 1991). Recent efforts have been concentrated on modification of pork fatty acid composition because of distinct biological effects of omega-3 fatty acids in human nutrition.

Pattern of Lipid Deposition

There are four main categories of body fat: subcutaneous fat, intermuscular fat, perirenal fat, and IMF. Among these fat depots, subcutaneous fat, intermuscular fat, and perirenal fat account of 60 to 70, 20 to 35, and about 5% of total body fat, respectively, in pigs (Kouba and Sellier, 2011), whereas IMF varies from 0.8 to 8.1% (Lonergan et al., 2007; Rincker et al., 2008). In pigs, many studies using classical allometric approach showed that fat deposition typically follows the order, in which perirenal fat is deposited fastest, followed by subcutaneous, intermuscular, and finally the IMF (Kouba and Sellier, 2011). The proportion of total fat in carcass weight increased from 16.1 to 33.6% at 30 kg to 140 kg live weight (Kouba and Bonneau, 2009). The P_2 backfat thickness can be used as indicator for body lipid content, which is linearly associated with body fat (as a proportion of empty body weight) for pigs weighing 20 to 90 kg (Black et al., 1986).

The first group of adipocytes are located at subcutaneous tissue, which can be detected between 50 to 75 d of gestation (Louveau et al., 2016). It is thought that the number of adipocytes is largely set before weaning (Azain, 2004). The expansion of adipose tissue occurs predominately during postnatal development, which is basically a result of filling adipocytes with lipids (Azain, 2004; Louveau et al., 2016). Compared with the obese genotype, the lean pigs had smaller adipocytes and more cells per gram of the backfat tissue (Scott et al., 1981).

Rate of lipid deposition is in the range of 30 to 50 g/d in the nursery, and in the range of 250 to 450 g/d in the finishing phase (Azain, 2001). However, the mechanisms associated with differential lipid accumulation in different fat depot remain unclear. Factors, such as diet, sex, genotype, and age, interact and contribute to the overall rate of fat buildup in pigs (Farnworth and Kramer, 1987). As previously discussed, after reaching the PD_{max}, any additional ME intake

will benefit exclusively lipid deposition. Adipose gain increased linearly with ME intake, which was calculated as 4 g/d for each additional MJ of ME intake (Bellego et al., 2002).

Anatomical location affects fatty acid composition, and, for instance, outer layer of backfat contains more unsaturated FA than inner layer (Irie and Sakimoto, 1992). Partitioning of FA between anatomical depots are affected by many factors, such as nutrition, genotype, temperature, sex, age, slaughter weight, and environmental temperature (Dunshea and D'Souza, 2003; Kloareg et al., 2007). The major storage form of FA in adipose tissue is TAG. In the TAG molecule, the glycerol backbone is synthesized from glucose. If pigs are consuming a high fat diet, preformed FA can be readily used after digestion (Dunshea and D'Souza, 2003). Fatty acids can also originate from de novo lipogenesis. There were many studies investigating de novo lipogenesis in the swine, but research on the actual synthesis of TAG itself is rather limited.

Overview of de novo Lipogenesis

In pigs, in vitro experiments showed that the preferred substrate for de novo lipogenesis in adipose tissue is glucose, although acetate, lactate, pyruvate, and propionate can be also readily utilized (O'Hea and Leveille, 1969b). The glucose is catabolized to pyruvate in glycolysis pathway, and then enters TCA cycle in the mitochondrion and exits as citrate. The citrate is cleaved to acetyl-CoA, which is the substrate for the first rate-limiting step in de novo lipogenesis pathway. The acetyl-CoA carboxylase (ACC) converts acetyl-CoA into malonyl-CoA, which is then used for biosynthesis of SFA via fatty acid synthase (FAS). The activity of ACC is stimulated by low citrate concentration and inhibited only by high levels of fatty acyl-CoA (Garrett and Grisham, 2012). The activity of FAS determines the maximal capacity of a tissue to synthesize FA (Kim et al., 2014). Malic enzyme (MEZ), glucose-6 phosphate dehydrogenase (G6PDH), and 6-phosphogluconate dehydrogenase are the main enzymes

involved in supplying the reducing equivalents. The major products of de novo lipogenesis are palmitic, stearic, and oleic acids, as well as small amounts of palmitoleic acid (Dunshea and D’Souza, 2003). Stearoyl CoA desaturate (SCD), also known as Δ9 desaturases, catalyzes the desaturation of SFA to synthesize MUFA, e.g., 18:0 to 18:1 or 16:0 to 16:1 (Nakamura and Nara, 2004).

Adipose tissue is the main site of de novo lipogenesis in pigs regardless of weight and breeds (Bee et al., 1999; Mourot et al., 1995; Mourot et al., 1996). After administration of ^{14}C-labelled glucose and acetate, a majority of the newly synthesized FA was found in adipose tissue of pigs (O'Hea and Leveille, 1969b). Lipogenic genes (ACC, FAS, and malic enzyme 1 (ME1)) are predominantly expressed in adipose tissue (Cánovas et al., 2009; Benítez et al., 2015), whereas lipogenic enzyme activities (or gene expression) and overall lipogenesis are low in the liver and muscle of pigs (O'Hea and Leveille, 1969b; Bee et al., 1999; Azain, 2004; Benítez et al., 2015). The SCD is predominantly expressed in subcutaneous adipose tissue, abdominal fat, kidney, and liver, whereas delta-6 desaturase were expressed in abdominal fat, kidney, adipose tissue, subcutaneous adipose tissue, heart, and liver (Cánovas et al., 2009). Specie differences are great for de novo lipogenesis, and it is difficult to make comparisons between different models. For instance, the substrate for de novo lipogenesis are glucose in pigs, chickens, rodents, and humans, whereas acetate is principal precursor in ruminant species (Bergen and Mersmann, 2005). Adipose tissue is the major site of de novo lipogenesis in pigs and ruminant species, whereas the liver plays a central role in lipogenesis for chickens, rodents, and humans (Bergen and Mersmann, 2005).

In the whole-body, the net lipid deposition = digested lipid + de novo lipogenesis - lipid oxidation. The contribution of de novo lipogenesis and lipid oxidation is not easy to determine.

Dunshea and D'Souza (2003) summarized that de novo lipogenesis from glucose accounts for 74 to 77% of lipids in the adipose tissue. Azain (2001) suggested that de novo lipogenesis accounted for more than half of the daily lipid deposition for pigs fed a typical corn-soybean meal diet with 3 to 5% fat. For pigs from 20 to 90 kg BW, de novo lipogenesis accounts for approximately 73% of body lipids (Madsen et al., 1992). Kloareg et al. (2007) conducted a serial slaughter study; pigs were fed wheat-maize-barley-soy-based diet containing 44 g lipids/kg and slaughtered at 90, 110, 130, and 150 kg. A regression analysis between nutrient intake and whole body deposition showed that the contribution of de novo synthesized FA was estimated to average 86% of the total non-essential FA deposition.

Numerous nutritional, genetic, and environmental factors affect de novo lipogenesis. For instance, food deprivation, dietary PUFA, and high-fat diets depress de novo lipogenesis (Allee et al., 1971c; Kersten, 2001). The activities of lipogenic enzymes, such as MEZ and citrate cleavage enzyme, were lower in gilts than barrows (Allee et al., 1971c; Allee et al., 1972). Barrows have a greater rate of endogenous fat synthesis (Ntawubizi et al., 2009), have more SFA (Juárez et al., 2010), and a greater fat deposition (Allee et al., 1971c). In contrast, gilts have a greater capacity for conversion of α-linolenic acid to LC ω-3 PUFA (Ntawubizi et al., 2009), and have more PUFA (Juárez et al., 2010). Obese pigs had greater lipogenic capacity and ACC activity compared with lean pigs (Scott et al., 1981). Large White pigs converted more ALA to eicosapentaenoic acid (EPA) than crossbred (13 vs. 6%; Kloareg et al., 2007). The ACC activity was greater in young Meishan pigs (< 40 kg), but greater in heavier Large White pigs (Mourot et al., 1996). In addition, desaturation of 18:0 to 18:1 in pigs reared at 30°C was less than those reared at 23°C (Kloareg et al., 2005). The lipogenic capacity and ACC activity increased up to 4

mo of age in crossbred pigs (Scott et al., 1981), or 40 to 60 kg in Large White barrows (Mourot et al., 1995), and then decreased thereafter in both crossbred pigs and Large White barrows.

Regulation of Lipid Metabolism by Fatty Acids

Energy homeostasis is regulated at both whole body and cellular levels (Lempradl et al., 2015). At the systemic level, brain, liver, pancreas, gut, muscle, and adipose tissue are important ones. Adipose tissue is not only an energy reservoir, but also an endocrine organ that secretes various types of adipokines, such as leptin and adiponectin (Luo and Liu, 2016). Leptin is a surrogate indicator of the abundance of lipid storage, and it is vital in regulating energy homeostasis by binding to leptin receptor in the central nervous system or peripheral organs (Efeyan et al., 2015; Luo and Liu, 2016). Circulating adiponectin concentration is negatively associated with lipid storage (Efeyan et al., 2015). In addition to adipokines, insulin and growth hormone are also involved in regulation of lipogenesis (Kersten, 2001).

At the cellular level, energy metabolism is regulated through various transcription factors, and peroxisome proliferator activated receptors (PPAR) seem to be master regulators of adipogenesis and lipid metabolism (Lempradl et al., 2015). The PPARα is central to up-regulation of FA oxidation, and is found in oxidative tissues (Grygiel-Górniak, 2014). The PPARδ is found in many cells and increases fatty acid oxidation in the skeletal muscle and lipolysis in the adipose tissue (Grygiel-Górniak, 2014; Yu et al., 2010). The PPARγ acts as a key regulator of adipogenesis, and is expressed in white adipose tissue (Georgiadi and Kersten, 2012; Grygiel-Górniak, 2014). Sterol regulatory element binding protein (SREBP) transcription factors have a critical role in regulating genes associated with fatty acid synthesis (Georgiadi and Gondret et al., 2001; Kersten, 2012; Price et al., 2000). However, the effects of SREBP on de

novo lipogenesis seem to be downstream of mTORC1 signaling cascades (Lempradl et al., 2015; Saxton and Sabatini, 2017).

In the long term, dietary fat can alter feed intake or lipid storage, which, therefore, alter energy homeostasis and may trigger release of adipokines or hormones (Duan et al., 2014; Yu et al., 2010). Effects of dietary fat on the number and size of adipocyte are possibly mediated by various transcription factors (Azain, 2004). In the short term, fluctuation in concentration of fatty acids can be recognized by cellular sensors. As previously discussed, dietary lipids are highly digestible, and are absorbed and transported to target tissues via circulatory system. Various sensing systems will respond to fluctuations of the concentrations of intra- and extracellular FA (Efeyan et al., 2015; Georgiadi and Kersten, 2012). For instance, G-protein-coupled receptors (e.g., GPR40 and GPR 120) and CD36 receptor are involved in FA signaling in the membrane (Efeyan et al., 2015).

Genes involved in lipogenesis (e.g., ACC, FAS, and ME1) were often down-regulated by dietary lipid supplementation (Benítez et al., 2016; De Tonnac et al., 2016; Duran-Montgé et al., 2009a,b; Kersten, 2001). The regulation seems to be mediated primarily at the transcriptional level. The best-studied nuclear receptors that are activated by FA are PPARs, which can bind various endogenous ligands, such as PUFA (Georgiadi and Kersten, 2012; Grygiel-Górniak, 2014). Fish oil feeding decreased fat deposition and expression of adipogenic genes, but increased expression of lipolysis and FA oxidation in porcine PPARδ transgenic mice (Yu et al., 2010). The PUFA (e.g., EPA and docosahexaenoic acid (DHA)) and their derivatives are high-affinity ligands for PPARγ; however, the binding is not necessary and sufficient for target gene expression (Georgiadi and Kersten, 2012; Grygiel-Górniak, 2014). Fish oil supplementation reduced fat deposition but increased plasma adiponectin and glucose uptake and expression of

adipogenic genes in porcine PPARγ transgenic mice (Yu et al., 2011). The PUFA, DHA, and its metabolite can activate PPARγ in vitro in a porcine model (Yu et al., 2008). The mRNA abundance of PPARγ increased linearly with longer duration of feeding linseed diet in muscle and spleen, whereas opposite responses occurred for TNF-α in muscle, adipose tissue, and spleen of pigs (Huang et al., 2008a). In their study, the IMF content was increased (Huang et al., 2008b).

It is well known that DHA can lower plasma TAG levels (Huang et al., 2017; Meadus et al., 2011). But, DHA increased total lipid content in the liver (De Tonnac et al., 2016), and induced adipocyte TAG storage, which leads to larger adipocytes in the adipose tissue of pigs (Huang et al., 2017). Supplementation of dietary DHA (9.4 g/d) in pigs for 25 d before harvest resulted in increased mRNA abundance of PPARα and PPARγ in the adipose tissue (Meadus et al., 2011). Pigs fed DHA for 30 d had elevated concertration of protein kinase A phosphorylation and increased gene expression of lipogenic (DGAT1 and ADRP) and fatty acid oxidation genes (CPT1) than those fed tallow or soy oil (Huang et al., 2017). The mRNA and protein content of PPARγ were markedly increased when dietary ω-6:ω-3 PUFA increased from 1:1 to 10:1 in pigs (Duan et al., 2014). In addition, dietary DHA often down-regulated SREBP1 (De Tonnac et al., 2016; Georgiadi and Kersten, 2012; Huang et al., 2017; Kersten, 2001; Yu et al., 2008), and, thus, may downregulate genes involved in lipogenesis (FAS, ME, and G6PDH) in pigs (De Tonnac et al., 2016). Those results indicated that excessive lipid overload in the whole body may induce lipid storage in adipose tissue through activation of PPARγ, which may protect non-adipose tissues against lipotoxicity (Georgiadi and Kersten, 2012).

Furthermore, PUFA can act as signaling molecules to suppress several desaturases, including SCD, delta-5 desaturase, and delta-6 desaturase (Nakamura and Nara, 2004). In pigs,

dietary ω-3 FA decreased SCD enzyme activity to 60% of its low-ω-3 control diets after 60 d of supplementation (Kouba et al., 2003). Reduction in SCD activity by dietary ω-6 FA was also observed in pigs (Kouba and Mourot, 1998). The SCD activity was similar between ω-3 FA- and ω-6 FA-rich diets (Guillevic et al., 2009b). Pigs fed PUFA (sunflower oil) diets had lower SCD mRNA abundance than those fed SFA (hydrogenated fat; Benítez et al., 2015). Both ω-3 and ω-6 PUFA are a potent inhibitor of SCD, whereas the product of SCD (18:1) is less effective (Nakamura and Nara, 2004). However, ω-3 PUFA (linseed oil and fish oil) showed stronger inhibitory effects on expression of SCD in liver and adipose tissue compared with ω-6 PUFA (Duran-Montgé et al., 2009b). Several mechanisms are involved in inhibitory role of PUFA. First, PUFA can deactivate SREBP1c, which is a regulator to activate the transcription of all genes for fatty acid synthesis (Nakamura and Nara, 2004). Second, PUFA may directly affect the SCD expression, or its effects may be mediated through PPARα (Nakamura and Nara, 2004). Third, the SFA treatment usually has a more substantial supply of substrates (16:0 and 18:0) compared with PUFA treatment and, thus, induce SCD expression (Benítez et al., 2015). However, dietary DHA did not affect expression of delta-6 desaturase and Elov5 (encoded elongase) in pigs (Meadus et al., 2011).

Methodology Considerations for Lipogenic Capacity

To assess the lipogenic capacity in the tissue, many investigators have measured radiolabeled incorporation of precursor (glucose-U-^{14}C) into FA using in vitro incubation system (e.g., biopsied adipose tissues; Allee et al., 1971b; Allee et al., 1972; Smith et al., 1996). The lipogenic enzyme activities, including ACC, FAS, MEZ, G6PD, 6GPD, and SCD can be measured by in vitro assay with appropriate substrates using adipose tissues of swine (Allee et al., 1971a; Kouba and Mourot, 1999). However, it is rather difficult to interpret the lipogenic

enzyme activities in the adipose tissue because the adipocyte cell size and number vary among dietary treatment, sex, age, and weight (Farnworth and Kramer, 1987). Therefore, metabolic activities on a per gram tissue basis can be misleading, and it is better to be expressed on per adipocyte basis rather than the tissue or soluble protein basis (Farnworth and Kramer, 1987; Hood and Allen, 1973; Mersmann and Smith, 2005; Smith et al., 1996).

Tissue handling and incubation conditions can markedly affect the metabolic rate (Mersmann and Hu, 1987; O'Hea and Leveille, 1969b). The nutritional status of the animal when samples are taken is also important. In some studies, the adipose tissue has been obtained immediately after harvest (Smith et al., 1996), whereas the use of the overnight fasting before harvest was reported for some studies. For instance, feed withdrawal for 48 h decreased the activity of SCD for more than 70% (Buller and Enser, 2009). Meal feeding had no effect on the lipogenic rate or the activities of lipogenic enzymes (MEZ, G6PDH, and 6-PGDH) in porcine adipose tissue as compared with animals fed ad libitum (O'Hea and Leveille, 1969a). In some instances, the backfat samples were obtained by biopsy technique (Allee et al., 1971a; Allee et al., 1971c; Allee et al., 1972). Furthermore, cautions should be taken when extrapolating the lipogenic activity in an adipose tissue sample to the entire depot because the adipocyte is not uniformly distributed across the fat depot.

Regression analysis between fatty acid intake and lipid deposition using comparative slaughter approach can be used to assess the contribution of de novo lipogenesis to whole body FA composition (Kloareg et al., 2005; Kloareg et al., 2007). However, it is not possible to measure the actual deposition rate for dietary non-essential FA, which originated from both de novo lipogenesis and diet. Instead, the oxidation rate of essential FA can be calculated and used for the estimation of non-essential FA. In vivo lipogenesis can be estimated using tritiated water

injection in broiler chickens (Crespo and Esteve-Garcia, 2002). Stable isotope method in lipid metabolism has been described in rodent models, as a more promising tool for food animal research (Murphy, 2006; Duarte et al., 2014).

Lipogenic capacity can also be estimated by measuring gene expression using qPCR. In addition to PPAR and SREBP-1, genes encoded key enzymes in lipid metabolism, such as ACC, SCD, glycerol-3 phosphate dehydrogenase, and FAS, can be measured (Benítez et al., 2016). The adipokines, leptin, and adiponectin, can be used as markers for overall adipose tissue metabolic status (Stern et al., 2016).

Dietary Fat Depresses Lipogenesis

Investigators studied the effect of dietary lipid on de novo lipogenesis using isocaloric diets with different levels or sources of dietary lipids earlier. The lipogenic capacity was markedly depressed by high-fat diets in both young and grower-finisher pigs (Allee et al., 1971a; Allee et al., 1971b, c; Allee et al., 1972). The specific activities of lipogenic enzymes in adipose tissue of weaned pigs, such as MEZ, G6PDH, and citrate cleavage enzyme, were reduced by fat addition (Allee et al., 1971b). Lipogenesis from glucose and activities of de novo lipogenesis enzymes were linearly reduced by increasing the dietary corn oil or tallow from 4 to 13% (Allee et al., 1971c). More recently, genes involved in lipogenesis (e.g., ACC, FAS, ME1) were often downregulated by dietary lipid supplementation (Benítez et al., 2016; De Tonnac et al., 2016; Duran-Montgé et al., 2009a,b; Kersten, 2001). Several factors can affect the degree of de novo lipogenesis inhibition by dietary fat.

Fat source. In pigs, Allee et al. (1972) demonstrated that 10% of corn oil, lard, coconut oil, and tallow were equally effective in depressing lipogenesis, and the reduction in FA synthesis capacity was approximately 50%. Fat sources (50 g/kg of linseed oil or animal fat) had no effects

on expression of ACC, FAS, lipoprotein lipase, and hormone sensitive lipase genes (Kim et al., 2014). Pigs fed linseed and sunflower oil diets showed the same total lipid content in adipose, muscle, and liver, but the effect on de novo lipogenesis enzyme activities (ACC, FAS, MEZ, and G6PDH) seemed to be tissue specific (Guillevic et al., 2009b).

Several investigators showed that SFA had stronger inhibitory effects on de novo lipogenesis than unsaturated FA. Smith et al. (1996) investigated the effect of specific fatty acids, such as 14:1/16:1, 16:0, 18:0. 18:1, and 18:2, on lipogenesis of weaned pigs. The results showed that 10% fat supplementation, regardless of sources, reduced lipogenesis from glucose relative to the control (10% cornstarch). The 16:0 exhibited greatest inhibitory effect on lipogenesis, followed by 14:1/16:1, 18:0, 18:2, and 18:1. Pigs fed diets containing 16:0 or 18:2 had greater percentage of smaller adipocytes, indicating that the magnitude of lipid filling in adipocytes may be reduced compared with those containing 18:0, 18:1 and 14:1/16:1(Smith et al., 1996). The enzyme activities of ACC, MEZ, and G6PDH, and total lipid content were greater in adipose tissue of pigs fed with high-linoleic acid diet (corn oil), compared with those fed tallow diet (Kouba and Mourot, 1999), which is consistent with Kouba and Mourot (1998). In contrast, some investigators showed that unsaturated FA had stronger inhibitory effects on de novo lipogenesis than SFA. For instance, pigs fed sunflower oil diets had lower ACC and ME1 mRNA levels than those fed hydrogenated fat (Benítez et al., 2015).

Therefore, it is difficult to determine whether the degree of inhibition of lipogenesis was dependent upon sources of dietary lipids. The variation observed may result from animal, age, genetics, tissue, or methodology. Azain (2004) suggested that the inhibitory effects of SFA and unsaturated FA are similar in pigs where adipose tissue is the primary site of de novo lipogenesis, whereas unsaturated FA is more inhibitory than SFA in rodents and chickens where liver is the

primary site of de novo lipogenesis. This conclusion is supported by more recent studies (Duran-Montgé et al., 2009a,b).

Energy density. When feed was offered based on BW, the activity of lipogenic enzymes (FAS, MEZ, and G6PDH) and carcass fat deposition were reduced by the restricted daily energy intake, which was independent of lipid sources (Bee et al., 2002). Both enzyme activities and fat deposition were similar for supplementation of 5% soy oil or tallow (Bee et al., 2002). When pigs had free access to diets, FAS activity was lowered by soy oil, and this effect was independently of dietary energy density (Bee et al., 1999). However, the MEZ activity was reduced by low dietary energy density (Bee et al., 1999). The difference can be explained by actual energy intake; the low-density diet only caused a 17% reduction in daily DE intake in an early study (Bee et al., 1999), whereas a 38% decrease was noted in a later study (Bee et al., 2002).

Species difference. It seems that the response to lipid supplementation in pigs is different from species that use the liver as their primary site of de novo lipogenesis. In rats, the hepatic lipogenic enzyme (ACC, FAS, MEZ, and G6PDH) activities were affected by fat supplementation, which increased in the order of marine oil < corn oil < tallow (Herzberg and Rogerson, 1988). Broiler chickens fed sunflower oil reduced abdominal fat by 13% and had a greater β-oxidation capacity (CPT1 and L-3-hydroxyacyl-CoA dehydrogenase in heart) and lower liver FAS activity compared with those fed beef tallow (Sanz et al., 2000). The percentage of abdominal, liver, and skin fats was lower in broiler chickens fed linseed oil than those fed tallow or sunflower oil; however, those fed linseed oil had greater de novo lipogenesis in the liver (Crespo and Esteve-Garcia, 2002; Ferrini et al., 2008). It is speculated that de novo lipogenesis only contribute a small percentage of total lipid deposition in chickens fed diets

supplemented with fat. Mice fed a high-fat diet showed depressed de novo lipogenesis but elevated rate of TAG esterification in hepatic and adipose tissues relative to those fed low-fat diets (Duarte et al., 2014). As previously discussed, the lipid deposition cannot be simply predicated by the de novo lipogenesis capacity. Perhaps, PUFA has more potent effect on lipid oxidation and de novo lipogenesis in rodents and chickens than pigs.

Effect of Lipid on Carcass Characteristics

There have been several comprehensive reviews about effects of dietary fat and fatty acids on meat quality and carcass fat (Madsen et al., 1992; Wood et al., 2003; Wood et al., 2008). A few more recent studies were included in this review of literature.

Not maintaining protein to energy ratio. As previously discussed, rate of lipid and protein deposition is difficult to predict when protein:energy is not maintained. For instance, dietary supplementation with 4 to 10% corn oil increased backfat thickness in pigs compared with 1% corn oil (Allee et al., 1971c; Allee et al., 1972). These results indicate that more lipids were directly deposited in the adipose tissue when lipids were included in the diets. In contrast, the percentage of flare fat in carcass, the percentages of separable fat (subcutaneous and intermuscular fat), and the fat:lean of loin, were less for pigs fed tallow than no fat-fed gilts (Realini et al., 2010).

Maintaining protein to energy ratio. Many studies reported that adding fat in grower-finisher pig diets increased carcass fatness when protein to energy ratio was maintained. Inclusion of 5% beef tallow, poultry fat, or soybean oil had greater average backfat thickness than pigs fed a control diet when equivalent lysine to calorie ratios are maintained (Apple et al., 2009b). Inclusion of 5% choice white grease (vs. beef tallow) increased backfat thickness (Weber et al., 2006). Including 5% beef tallow has minimal effects on carcass fat distribution, but

increased IMF content in the *longissimus* muscle 4.14 vs. 2.87%) in the average-lean pigs (Eggert et al., 2007). Adding 6% choice white grease tended to increase backfat thickness of barrows in the commercial environment (De La Llata et al., 2007). Increasing choice white grease led to increased 10th-rib backfat, and the magnitude of the increase depended on the cereal type (corn or sorghum) (Benz et al., 2011b). The addition of 5% beef tallow increased total weight of ham and total fat of ham (Eggert et al., 2007).

On the other hand, several investigators failed to demonstrate an effect of lipids on backfat thickness (Engel et al., 2001; Myer et al., 1992; Williams et al., 1994). Sometimes, inconsistent results, i.e., increased backfat in one experiment and no difference in another, were observed within the same study (Beaulieu et al., 2009; De la Llata et al., 2001). Reduced backfat thickness in pigs fed high-fat diets (4.5 to 6% choice white grease) was observed in one study (Smith et al., 1999). More recently, feeding graded contents of NE did not change backfat thickness, which was adjusted to hot carcass weight (HCW) (Quiniou and Noblet, 2012). Most importantly, dressing percentage (Beaulieu et al., 2009; Quiniou and Noblet, 2012) or carcass yield (Williams et al., 1994; Smith et al., 1999) was increased with increasing dietary lipid or energy content. This would be of economic importance when adding lipid to increase the energy density.

Fat source. Fat sources had no effects on the yield of the primal cuts, and the percentage of muscle, bone, and fat, whereas carcass from pigs fed beef tallow have a greater percentage of skin than those fed poultry fat and soybean oil (Apple et al., 2009a). The yield of the primal cuts was similar among dietary fat sources, with the exception that loin yield was lower in gilts fed fat blend than those fed the diet with no added fat (Realini et al., 2010). Feeding pigs with diets containing 10% animal fat, safflower oil, sunflower oil, and canola oil had similar backfat

thickness, loin area, and marbling score, whereas those fed safflower oil, sunflower oil, and canola oil had softer fat (Miller et al., 1990). It is the energy concentration, rather than fat source, that determines the growth performance and carcass traits (Bee et al., 2002). Backfat reduced as lysine to DE ratio increased, and 3.0 g lysine/Mcal DE was necessary to maximize the beneficial effects of fat addition to diets for 20 to 50 kg pigs (Chiba et al., 1991a).

Marbling Fat and Eating Quality

Intramuscular fat, often termed as marbling fat in beef, mainly consists of phospholipid, triacylglycerol, and cholesterol from all cells present in a meat sample (Wood et al., 2008; Hocquette et al., 2010). The amount of muscle phospholipid remains fairly constant, whereas neutral lipid (TAG) increases with increasing total fat content (Cannata et al., 2010; Fernandez et al., 1999a; Wood et al., 2008). Baghurst (2004) suggested that IMF is "the fat within the muscle fibers or cells, which is mostly structural phospholipids but with some triglycerides", while marbling fat (TAG or storage fat) is "found in the areas between muscle fibers or cells." Therefore, true IMF should be highly unsaturated and be an indicator of healthiness (Baghurst, 2004). To avoid confusion, we still use the term IMF to refer to the total extractable lipids because the lipid content in the reference papers examining relationship between fat content and pork quality was usually determined by ether extraction method in loin muscle after removing external fat and connective tissues (Blanchard et al., 2000; Brewer et al., 2001; Cannata et al., 2010; Fernandez et al., 1999a,b; Fortin et al., 2005; Lonergan et al., 2007; Rincker et al., 2008) or NIR (Font-i-Furnols et al., 2012).

The IMF content varies between and within breeds, and it is also affected by gender, age, and nutrition (Blanchard et al., 2000; DeVol et al., 1988; Hocquette et al., 2010). The IMF content was reported to be ranging from 0.14 to 9.3% in *longissimus* muscle (Blanchard et al.,

2000), 0.84 to 7.89% in *longissimus* muscle (Lonergan et al., 2007), or 0.76 to 8.09% in loin (Rincker et al., 2008), respectively. It is generally accepted that IMF content has positive influence on eating quality (tenderness, juiciness, and flavor) of pork (Wood et al., 2008) when evaluated by trained sensory panel (Cannata et al., 2010; DeVol et al., 1988; Fernandez et al., 1999a; Fortin et al., 2005) or by consumers (Brewer et al., 2001; Fernandez et al., 1999b; Font-i-Furnols et al., 2012). However, some studies showed only weak association between IMF content and eating quality (Blanchard et al., 2000) within defined classification of pH (Lonergan et al., 2007) or cooking temperature (Rincker et al., 2008). In addition, IMF has been shown to be negatively correlated with drip loss and cook loss (Cannata et al., 2010). Consumer acceptability is more complicated than eating quality. Brewer et al. (2001) showed that consumers were less likely to purchase highly marbled chops because of lighter color and less lean when they visually judged pork loin chops with low (1.05%), medium (2.33%), or high (3.46%) IMF content. Some studies have demonstrated that the acceptability of marbling varied among some countries (Ngapo et al., 2007) and among some consumers (Font-i-Furnols et al., 2012).

Several studies recommended that a minimal IMF content (threshold) is essential to ensure optimal eating quality. A Canadian study indicated that 1.5% IMF is required to avoid unsatisfactory tenderness (Fortin et al., 2005). A Spanish study showed that the minimum level of IMF is between 2.2 and 3.4% based on consumer’s score (Font-i-Furnols et al., 2012). A French study indicated that flavor and juiciness detected by sensory panel was enhanced when IMF percentage was above 2.5%, whereas the acceptability by consumers increased with increasing IMF content above 3.5% (Fernandez et al., 1999a,b). The threshold content of IMF in a US study was reported to be 2.5 to 3.0%, which was based on tenderness (DeVol et al., 1988).

Heritability of IMF content is relatively high (h^2 = 0.26 to 0.86; Ciobanu et al., 2011). The genetic correlation is positive with overall acceptability (r = 0.61), tenderness (r = 0.15), carcass fatness (r = 0.30), or negative with leanness (r = - 0.34; Ciobanu et al., 2011; Hocquette et al., 2010). The genetic selection for reduction in carcass fatness can, therefore, lead to a markedly reduced IMF content. However, improving the IMF content while maintaining leanness of pigs is still possible because subcutaneous fat only accounts for 1 to 5% of the variation in marbling (Jones et al., 1992). Although P_2 backfat thickness was negatively correlated with shear force, no correlation between P_2 backfat thickness and eating quality was found (Blanchard et al., 2000). Researcher have been trying to identify a biomarker to assess the status of IMF over the years. For instance, the SCD protein expression was correlated (r = 0.48) with IMF content and it has been proposed as a potential biomarker (Cánovas et al., 2009).

Effect of Lipid on Pork Quality

Lipid content and fatty acid composition is central to nutritional value and eating quality of pork. Wood et al. (2003) indicated that several aspects of pork quality may be affected by dietary lipids, including eating quality (tenderness, juiciness, and flavor), shelf life (lipid and pigment oxidation), and firmness (melting point of FA).

Intramuscular fat content. As previously mentioned, the eating quality of pork is affected by IMF content. Dietary supplementation of animal fat (5%) could increase the lipid content in *longissimus* muscle, picnic, and ham (Eggert et al., 2007), and increase NPPC marbling score of loin (Eggert et al., 1998) when lysine to calorie ratio is maintained. The lipid and cholesterol contents were greater in the muscle and adipose tissues of pigs fed corn oil than those fed tallow (Kouba and Mourot, 1999). Dietary ω-3 FA has been shown to improve IMF content of pork (Huang et al., 2008b) or marbling score (Juárez et al., 2011b), which may be

caused by alterations in the expression of genes involved in adipogenesis (Luo et al., 2009). However, highly marbled pork loins often had a lower PUFA content when samples were obtained from a commercial packing plant (Cannata et al., 2010).

Firmness. Fat hardness is becoming increasingly important in the pork industry in recent years because of the widespread use of distillers' co-products that is rich in unsaturated FA (Benz et al., 2011a; Johnston and Li, 2011). Consumers have become more aware of reducing SFA intake from meat products. But, the belly, as well as carcass, will be softer if unsaturated FA content is elevated (Wood et al., 2003). For instance, dietary lipids (2% poultry fat and 3% flaxseed oil) reduced belly firmness score in finisher pigs (Adhikari et al., 2017). Reduction of lean or fat firmness scores or both, were found with dietary sunflower oil and canola oil (Miller et al., 1990) and soybean oil (Averette Gatlin et al., 2002). In the meat industry, pork processors prefer firm fat to increase the speed of processing (Johnston and Li, 2011). Softer bellies may cause reduction of the fabrication and slicing efficiency, and have an oily appearance and a shorter product shelf life at retail (Soladoye et al., 2015).

Iodine value (IV) is a measure of the unsaturation of lipids and used as a standard indicator of carcass fat firmness (Benz et al., 2011a; Madsen et al., 1992). The maximum IV of backfat ranged from 70 to 75 g/100 g in US packing plants (Benz et al., 2011a). The carcass fat IV can be predicted by calculating the IV of diet (Madsen et al., 1992), but recent studies showed that linoleic acid content or intake was more accurate in the prediction of the carcass IV (Benz et al., 2011a; Kellner et al., 2014). To obtain a carcass IV of 74 g/100 g, linoleic acid concentration and intake should be limited to 3.4% and 88 g/d, respectively (Kellner et al., 2016). Feeding tallow would increase belly firmness and decrease IV (Apple et al., 2007; Browne et al., 2013). Withdrawal of unsaturated FA in the diets can reduce carcass IV, but the length of withdrawal

would be as long as 61 d in the high unsaturation treatment group (Kellner et al., 2015). However, these investigators did not take highly unsaturated FA (e.g., EPA and DHA) into account when calculating IV. More extensive equation has been proposed to calculate total IV (Meadus et al., 2010), which is vital in ω-3 PUFA enriched meat research.

Eating quality. Fatty acids are important in meat flavor development. During the cooking process, they act as flavor precursors of oxidation reactions, as well as solvent for volatile compounds (Calkins and Hodgen, 2007; Mottram, 1998). Particularly, phospholipids, which contain more unsaturated FA and generate aldehydes, can interact with Maillard reaction intermediates to produce many volatile compounds, and also control the production of sulfur compounds at optimum content (Mottram, 1998). On the other hand, lipid peroxidation contributes to off-flavor development, such as rancidity during a long-term storage and warmed-over flavor after reheating (Faustman et al., 2010; Mottram, 1998). Dietary lipid supplementation can alter the FA composition and fat deposition, and therefore, it likely changes pork flavor characteristics. Feeding canola oil (10%) increased off-flavor compared with those fed animal fat, safflower oil, and sunflower oil (Miller et al., 1990). Feeding choice white grease or poultry fat had no effect on physical and sensory quality of pork (Engel et al., 2001). When supplementing flaxseed in pig diets, off-flavors, such as fishy flavor, were sometimes detected by sensory panelists, and it was more pronounced for high-fat pork products and processed products than lean pork (Juárez et al., 2011a; Romans et al., 1995b). Wood et al. (2003) suggested that undesirable flavors occur if the pork contains more than 3% of ALA in total or neutral lipids when the processing conditions favor lipid oxidation.

As previously discussed, supplemented lipids may improve IMF content, which in turn has a positive impact on the juiciness and tenderness. Sensory traits were negatively affected in

fresh or frozen chops of pigs fed flaxseed diets (Juárez et al., 2011a). However, sensory traits became similar when supranutritional levels of vitamin E were supplemented in the flaxseed diets (Kouba et al., 2003; Sheard et al., 2000).

Effect of Lipid on Pork Fat Composition

The recommendation for PUFA to SFA ratio in human diets by nutritionists is above 0.4:1, and for ω6 to ω3 ratio is less than 4:1 (Wood et al., 2003). As previously mentioned, fatty acid composition in tissues is a result of de novo lipogenesis and direct deposition of dietary fat. The SFA and MUFA can originate from both exogenous and de novo lipogenesis, whereas PUFA deposition exclusively depends on dietary PUFA (Kloareg et al., 2007). The MUFA (16:1 and 18:1) increased with increasing tallow in the diets, whereas increased dietary 18:2 would decrease the MUFA content in tissues (Averette Gatlin et al., 2002). Lower MUFA content was usually found in pigs fed corn oil than those fed tallow, which may be caused by both lower oleic acid content in corn oil and the depression of SCD activity by high-PUFA diets (Kouba and Mourot, 1998; Kouba and Mourot, 1999). Dietary ω3 PUFA from linseed also decreased the MUFA content in muscle and reduced SCD activity compared with those fed the animal fat (Kouba et al., 2003).

Feeding tallow will increase SFA and MUFA contents (Apple et al., 2007; Browne et al., 2013). Feeding choice white grease or poultry fat linearly decreased 14:0 and 16:0 in muscle and 16:0 in belly, whereas it increased 18:2 in muscle and total unsaturated FA in belly (Engel et al., 2001). Modification of fatty acid composition by lipid supplementation is desirable to obtain healthier pork. For instance, pigs fed high-ω-3 PUFA diets had greater concentrations of PUFA and lower concentrations of SFA in tissues than those fed low-PUFA diets (Haak et al., 2008; Kim et al., 2014; Romans et al., 1995a; Romans et al., 1995b; Scheeder et al., 2003; Vossen et

al., 2017). Dietary ω-3 PUFA are more rapidly incorporated into phospholipids than neutral lipids (Romans et al., 1995b; Wood et al., 2008). Modification of pork fat composition and IV can be achieved within 6 wk before harvest (Averette Gatlin et al., 2002; Johnston and Li, 2011).

VITAMIN E IN SWINE NUTRITION

Introduction

Lipid oxidation has adverse effects on flavor, color, and nutritive value of meat products. Vitamin E functions as a well-known antioxidant at the cell membrane level (Brigelius-Flohe and Traber, 1999). Among 8 natural occurring forms of vitamin E (4 tocopherols and 4 tocotrienols), α-tocopherol is the biologically most active form and the most abundant naturally occurring form (Brigelius-Flohe and Traber, 1999). Supranutritional contents of α-tocopherol acetate in the diets have been shown to be effective in reducing lipid oxidation in meat products, and consequently, prolonging color stability and retail shelf life of pork (Asghar et al., 1991a; Guo et al., 2006a).

Vitamin E in Swine diets

Vitamin E is usually supplemented in the feed as its primary industrial form, i.e., DL-α-tocopheryl acetate, which is acetate ester of all-rac-α-tocopherol. One milligram of DL-α-tocopheryl acetate equals 1 IU of vitamin E (NRC, 2012). The typical inclusion rate in finisher diets is 23 IU vitamin E/kg (Flohr et al., 2015), which is slightly greater than the minimal requirement for growing and finishing pigs (11 IU vitamin E/kg for BW between 11 and 135 kg; NRC, 2012). Vitamin E toxicity has not been demonstrated in swine (NRC, 2012). There was no toxic effects of feeding pigs as high as 550 mg/kg in nursery diet (Bonnette et al., 1990), and as high as 700 mg/kg in finisher diet (Jensen et al., 1997; Kim et al., 2015).

DL-α-tocopheryl acetate is chemically stable, and 93% of its initial values remained in the corn-soybean meal-based complete swine diets after 90 d of storage at 23 to 26°C (Dove and

Ewan, 1991). However, high content of Cu or Fe increased the rate of α-tocopheryl acetate loss (Dove and Ewan, 1991). A more recent study showed that the concentration of vitamin E in vitamin premix with choline chloride decreased to 93, 75, and 50% of its initial value after 3, 6, and 9 mo, respectively, while samples without choline chloride decreased to 98, 78, and 59%, respectively (Tavčar-Kalcher and Vengušt, 2007).

Transfer of Vitamin E from Feed into Tissues

Dietary α-tocopheryl acetate is thought to be incorporated into mixed micelles and de-esterified in the gut lumen, and absorbed via chylomicrons or HDL into the lymph system and transported to the liver (Brigelius-Flohe and Traber, 1999; Galli et al., 2017; Rigotti, 2007). The liver serves as the major site for storage of vitamin E, and α-tocopherol transfer protein has a great binding affinity to α-tocopherol and controls the amount of circulating α-tocopherol (Rigotti, 2007; Galli et al., 2017). Vitamin E had short half-life in circulation (1.7 min after intravenous injection vs. 2.6 h after oral dosing) in pigs (van Kempen et al., 2016). The bioavailability of RRR-α-tocopheryl acetate in swine diets supplemented with 6% fat was about 20%, while that of all-rac-α-tocopheryl acetate is 12.5% (van Kempen et al., 2016).

Several studies reported that dietary supplementation of α-tocopheryl acetate resulted in an increase of tocopherol in plasma or tissues or both of pig (Asghar et al., 1991a; Boler et al., 2009; D'Arrigo et al., 2002; Guo et al., 2006a; Hasty et al., 2002; Hoving-Bolink et al., 1998; Hoz et al., 2003; Juárez et al., 2011b; Kim et al., 2015; Monahan et al., 1990; Phillips et al., 2001; Rey et al., 2001). For instance, pigs fed a diet containing 200 mg α-tocopheryl acetate/kg had 3 mg α-tocopheryl/kg, whereas those fed a diet containing 20 mg α-tocopheryl acetate/kg had less than 1 mg α-tocopheryl/kg in muscle tissues (Hoz et al., 2003). Linear responses to dietary vitamin E (from 0 to 200 IU/kg) were observed in serum, liver, heart, and adipose tissue

in weanling pigs (Moreira and Mahan, 2002). Increasing dietary vitamin E from 11 to 550 IU/kg increased the α-tocopherol concentration in the liver linearly and in serum quadratically (Bonnette et al., 1990). Recently, a meta-analysis established a nonlinear relationship between supplementary vitamin E and α-tocopherol accumulation in *longissimus* (Trefan et al., 2011). It indicated that the maximum concentration for α-tocopherol accumulation was between 6.3 and 7.3 µg/g loin tissue when dietary vitamin E was between 0 and 700 IU/kg (Trefan et al., 2011). Recently, Kim et al. (2015) observed a plateau of 6 µg/g in *longissimus thoracis et lumborum* of pigs fed 700 IU vitamin E/kg for 28 d. Feeding the same diet for 42 days did not further increase muscle vitamin E content.

Vitamin E on Pork Quality and Performance

Most studies reported no effects of vitamin E supplementation on growth performance (Cannon et al., 1996; Guo et al., 2006a; Hoving-Bolink et al., 1998; Kim et al., 2015) and carcass characteristics (Cannon et al., 1996; Guo et al., 2006a; Hoving-Bolink et al., 1998; Hasty et al., 2002) of pigs. In those studies, extra vitamin E may not be necessary for optimal growth performance. In some instances, positive effects on ADG and feed intake were reported though (Asghar et al., 1991b; Hasty et al., 2002).

Several investigators consistently reported that vitamin E at concentrations ranging from 100 to 700 IU/kg diet effectively alleviated lipid peroxidation in fresh whole-muscle meat (Asghar et al., 1991a; Boler et al., 2009; Bolink et al., 1998; Hoving- Kim et al., 2015; Jensen et al., 1997; Monahan et al., 1994a), or frozen chop (Guo et al., 2006b; Hoving-Bolink et al., 1998; Monahan et al., 1994a), precooked chop (Rey et al., 2001), or raw or cooked ground pork (Asghar et al., 1991a; Boler et al., 2009; Guo et al., 2006a; Guo et al., 2006b; Jensen et al., 1997; Juárez et al., 2011b; Monahan et al., 1990; Phillips et al., 2001). It can also reduce cholesterol

oxidation (Rey et al., 2001). Dunshea et al. (2005) reviewed that vitamin E supplementation resulted in 20 to 80% reduction of TBARS values in pork compared with control samples. The 2005 Dunshea's review was update and more recent data were included in Table 3, and it showed that the reduction ranged from 0 to 80% in pork. It seems that the vitamin E is more effective when more unsaturated FA is included in the diets. Trefan et al. (2011) conducted a meta-analysis and suggested that at least 100 IU vitamin E/kg diet was required to achieve a substantial decrease of TBARS values in *longissimus* muscle.

A threshold level of 0.5 to 1.0 mg MDA/kg tissue has been proposed for detecting rancidity and warmed-over off-flavor in pork by trained sensory panelists (Dunshea et al., 2005; Gray and Pearson, 1987). Therefore, vitamin E supplementation in grower and finisher pigs may reduce the off-flavor occurrence, especially when unsaturated FA was supplemented in the diets (Kouba et al., 2003; Sheard et al., 2000). Some studies did not find a positive effect of vitamin E on sensory characteristics of pigs fed diets containing corn oil (Cannon et al., 1996), or flaxseed (Juárez et al., 2011b). As vitamin E and lipid is transported to the muscle in a similar way, the IMF content may be also correlated to vitamin E content. But, this hypothesis was not confirmed by several researchers (Hoving-Bolink et al., 1998; Kim et al., 2015; Lahucky et al., 2007).

Table 3 Effects of vitamin E (VE) supplementation on lipid oxidation, color stability and drip loss of the pork [a]

Meat product	Product display time (d)	VE (mg/kg)	Feeding Duration (d)	Dietary lipid [b]	TBARS (%) [c]	Color (%) [d]	Drip loss (%) [e]	Reference
Whole-muscle pork								
Chop	6	100	98	3% SO	-68.2	+32.9	NS	Asghar et al. (1991a)
Chop	6	200	98	3% SO	-80.4	+42.9	-39.3	Asghar et al. (1991a)
Chop	5	100	84	5% CO	-44.6	NS	NS	Cannon et al. (1996)
Precooked chop	6	200	42	0.5% LN + 1.5% SUN	-24.2	–	–	Rey et al. (2001)
Precooked chop	6	200	42	2% OLV	-13.3	–	–	Rey et al. (2001)
Precooked chop	6	200	42	0.5% LN + 1.5% OLV	0	–	–	Rey et al. (2001)
Precooked chop	6	200	42	0.5%LN + 1.5% SUN	-24.2	–	–	Rey et al. (2001)
Chop		200	63	high oil corn		NS	NS	Guo et al. (2006a)
Chop	1	300	42	3.7% CAO	-50	NS	NS	Kim et al. (2015)
Processed pork								
Raw patties	8	200	14	–	-63.8	–	–	Monahan et al. (1990)
Precooked patties	6	200	14	–	-20.1	–	–	Monahan et al. (1990)
Raw patties	8	100	98	3% SO	-39.2	–	–	Asghar et al. (1991a)
Raw patties	8	200	98	3% SO	-70.4	–	–	Asghar et al. (1991a)
Raw patties	6	200	63	high oil corn	-21.8	NS	–	Guo et al. (2006a)
Precooked patties	6	200	63	high oil corn	-19.0	NS	–	Guo et al. (2006a)

[a] This table was modified from Dunshea and others (2005) and more recent data were included.

[b] SO = soy oil, CO = corn oil, LN= linseed oil, SUN = sunflower oil, OLV= olive oil, and CAO = canola oil.

[c] Decrease (-) or increase (+) in TBARS values (mg MDA/kg tissue) in supplemented group, relative to control group

[d] Decrease (-) or increase (+) in a-values (red color) in supplemented group, relative to control group.

[e] Decrease (-) or increase (+) in drip loss % in supplemented group, relative to control group.

Meat discoloration results from the oxidation of iron atom within myoglobin, when bright cherry red oxymyoglobin was converted to unattractive brownish metmyoglobin (Faustman et al., 2010). Lipid peroxidation and myoglobin oxidation seem to be closely related (each can exacerbate oxidation of the other; Faustman et al., 2010). Early studies showed that vitamin E supplementation can delay lipid and myoglobin oxidation (Monahan et al., 1990; Buckley et al., 1995), and consequently improve color stability and prolong retail shelf life of pork (Asghar et al., 1991a). Similar results were found in later studies (Hoving-Bolink et al., 1998; Monahan et al., 1994a). Guo et al. (2006b) demonstrated that feeding duration, rather than vitamin concentration, increases redness and yellowness of pork. However, a majority of studies failed to show any positive effects on pork color stability (Boler et al., 2009; Cannon et al., 1996; Guo et al., 2006a; Kim et al., 2015; Jensen et al., 1997; Phillips et al., 2001).

Some studies reported that vitamin E supplementation reduced drip loss from fresh pork (Cheah et al., 1995; Guo et al., 2006b; Monahan et al., 1994b) or from pork following frozen storage (Asghar et al., 1991a; Monahan et al., 1994a). The reduction in drip loss during storage can be explained by the mechanism that vitamin E increases physical stability of muscle cell membranes (Cheah et al., 1995; Monahan et al., 1994b). Recent evidences showed that vitamin E was accumulated in DHA-rich domains in the membrane bilayer, which is vital to stabilize and protect the cellular membrane (Raederstorff et al., 2015). Another mechanism is that vitamin E inhibited phospholipase A_2, resulting in reduction of Ca^{2+} release from the sarcoplasmatic reticulum (Cheah et al., 1995). However, several studies

failed to show any beneficial effects on water holding capacity (drip loss, purge loss, cook loss) of whole-muscle pork (Boler et al., 2009; Cannon et al., 1996; Guo et al., 2006a; Hasty et al., 2002; Hoving-Bolink et al., 1998; Jensen et al., 1997; Juárez et al., 2011b; Kim et al., 2015).

There are substantial discrepancies among existing data regarding lipid oxidation, meat color, and water holding capacity. This could be caused by differences in intrinsic and extrinsic factors (Faustman et al., 2010). First, no beneficial effect can be observed if the actual α-tocopherol content in control samples is above a critical value, e.g., 3.5 μg/g (Wood et al., 2003). Second, variance may come from muscle fiber type, which determines the α-tocopherol content and rate of discoloration (Jensen et al., 1998). Third, inconsistent result may be related to storage condition, such as temperature, pH, light, and oxygen pressure (Jensen et al., 1998).

Requirement of Vitamin E in Relation to Lipids

The bioavailability of vitamin E was affected by the type and amount of fatty acids in the diets. Compared with the diet rich in MUFA and SFA, either n-3 or n-6 PUFA-rich diets reduced concentrations of vitamin E in serum, muscle, liver, and adipose tissues (Prévéraud et al., 2014). Both dietary vitamin E and 5% choice white grease increased serum α-tocopherol concentrations in weanling pigs (Moreira and Mahan, 2002). There might be 2 mechanisms that can help explain the reduced availability of vitamin E with increased dietary PUFA. Firstly, as the primary lipid-soluble antioxidant, vitamin E is used to prevent these highly oxidizable PUFA in the membranes of cells from peroxidation (Trefan et al., 2011).

The second mechanism might be a decrease in the absorption rate of vitamin E (all-rac-tocopheryl acetate) in the gut by PUFA (Desmarchelier et al., 2013) because PUFA has been demonstrated to increase the size of mixed micelles and the zeta potential compared with SFA (Gleize et al., 2013).

The vitamin E requirement depends on the amount and the degree of unsaturation of PUFA in the diets (Raederstorff et al., 2015). Dietary PUFA led to accumulation of unsaturated FA in tissues, which are more vulnerable to lipid oxidation during storage (Sheard et al., 2000; Juárez et al., 2011a). As a natural antioxidant, vitamin E supplementation has positive effects on pork lipid oxidation and, thus, its requirement is increased for optimizing pork quality. Cannon et al. (1996) fed pigs diets containing 5% corn oil for 84 d and found reductions of TBA values in vitamin E treatment group (100 mg) during retail display and extended periods of vacuum packaging storage compared with no vitamin E treatment group. Dietary supplementation of vitamin E reduced pork lipid oxidation for pigs fed diets supplemented with high ω-3 PUFA (Hoz et al., 2003; Juárez et al., 2011b). Although it is impossible to determine a specific content of α-tocopherol that would be adequate to minimize lipid oxidation, Jensen et al. (1998) indicated that 200 IU vitamin E/kg diet was sufficient in most studies to maintain the TBARS values below the critical value, i.e., 0.5 mg MDA/kg.

Feeding oxidized lipids to pigs not only has deleterious effects on growth and health, but also results in loss of endogenous α-tocopherol (Liu et al., 2014; Kerr et al., 2015). Several indicators of metabolic oxidation status in biological samples have been proposed,

which include TBARS, hydrogen peroxide, conjugated dienes, 4-hydroxynonenal, hepatic transaminase enzymes, and endogenous antioxidants (Kerr et al., 2015; Liu et al., 2014). Therefore, risk of vitamin E deficiency should be considered when different source, content, and quality of lipids were included in the diets.

SUMMARY

The digestibility of plant oil is usually greater than animal fat. The AID would be a better indicator of the digestibility of individual FA. Some of the factors that could affect the lipid digestibility are: 1) physicochemical, 2) dietary, and 3) animal-dependent factors. More studies are needed to measure NE values of lipids because the DE and ME systems would underestimate the energetic value of lipids. Pigs can adjust their voluntary feed intake accordingly to energy density to achieve constant energy intake. There is an increase-plateau relationship if the lowest energy level is low in most studies. But, the daily ME intake become relatively stable if the lowest energy level is high. There is linear-plateau relationship between energy intake and PD, and beyond PD_{max}, energy will be more available to lipid deposition. It is now widely accepted that studies on lipid supplementation should maintain the ratio of nutrient to energy by gradual change in ingredient composition. Numerous studies have demonstrated that dietary lipids decrease feed intake and improve G:F at all phases, and may have positive effects on daily gain only in lighter pigs (eg., < 80 kg BW). However, more studies are needed to investigate effects of dietary fat under the NE system.

Lipids are not only energy sources that contribute to body fat and FA composition in pigs, but also bioactive compounds that are involved in overall lipid metabolism and cellular

functions. For instance, dietary PUFA can regulate lipid metabolism mediated by SREBP-1 and PPARs, and inhibit expression of SCD and other genes involved in de novo lipogenesis. Many studies showed that dietary SFA are equally effective as PUFA in depressing de novo lipogenesis. Dietary PUFA may activate PPARγ and induce lipid storage in adipose tissue, such as IMF. However, the addition of fat did not have consistent impact on backfat thickness. Energy density, lipid source and level, and species difference should be taken into consideration when investigating the inhibitory effect of lipids on de novo lipogenesis.

In recent years, more emphasis has been placed on improving eating quality and nutrition of pork. Dietary lipid supplementation is effective in improving ω-3 FA content, as well as IMF. Vitamin E is crucial for maintaining stability of PUFA in the body. Supranutritional contents of vitamin E have been shown to be effective in reducing lipid oxidation in meat products, but inconsistent results have been reported on the pork color and water-holding capacity. In most studies, 200 IU vitamin E/kg diet seems to be adequate in reducing the TBARS values, which are lower than the critical value, i.e., 0.5 mg MDA/kg. It is possible, therefore, that dietary lipid supplementation can decrease excess carcass fat by reduction of de novo lipogenesis in pigs and increase IMF content simultaneously to enhance eating quality of pork, and the lipid oxidation can be prevented by dietary vitamin E supplementation.

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CHAPTER III.

EFFECT OF ANIMAL FAT, FLAXSEED OIL, AND VITAMIN E SUPPLEMENTATION ON GROWTH PERFORMANCE, SERUM METABOLITES, AND CARCASS CHARACTERISTICS OF FINISHER PIGS, AND PHYSICAL CHARACTERISTICS OF PORK

Effect of animal fat, flaxseed oil, and vitamin E supplementation on growth performance, serum metabolites, and carcass characteristics of finisher pigs, and physical characteristics of pork

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A B S T R A C T

A total of 96 pigs [Yorkshire; 54.3 ± 3.4 kg of body weight (BW)] were used to determine the effect of dietary lipid (0, 2, 4, and 6%) and vitamin E (0, 2, 4, and 6%) supplementation in a 4 x 2 factorial arrangement of treatments on growth performance, carcass traits, and physical characteristics of finisher pigs. Forty eight pens containing 2 gilts or 2 castrated males per pen were randomly assigned to 8 diets with 3 gilt pens and 3 castrated male pens per treatment. For all diets with lipids, 1% flaxseed oil was included and the remaining was supplied by poultry fat. Pigs were switched from finisher-1 to finisher-2 diets when the average pen weight reached BW of 84.1 ± 4.0 kg. Standardized ileal digestible Lys and Ca and P were adjusted accordingly with the DE content to maintain constant Lys, Ca, and P to DE ratios. Pigs were harvested when they reached the target BW of 110 ± 3 kg. There were no lipid × vitamin E interactions on growth performance. The daily intake of Lys and DE were not affected by dietary treatments during the finisher-1 or 2 phases. Lipid supplementation linearly decreased average daily feed intake ($P < 0.05$), but improved gain:feed (G:F; $P < 0.01$) during the finisher-1, finisher-2, and overall phases. Pigs fed lipids had similar average daily gain (ADG) during the finisher-1 and overall phases, but they had greater ADG ($P = 0.028$) during the finisher-2 phase mostly because of a tendency toward an increase in the efficiency of Lys and DE utilization ($P < 0.10$). Unexpectedly, vitamin E reduced G:F ($P < 0.05$) during the finisher-1 and overall phases. Pigs fed 0, 2, and 4% lipids supplemented with vitamin E had thicker backfat at 10th rib (TRFT) and lower fat-free lean percentage (FFL) than those without vitamin E, whereas pigs fed 6% lipids with vitamin E

had thinner TRFT and greater FFL than those without vitamin E (lipid x vitamin E, $P < 0.05$). Increasing dietary lipid linearly increased ($P < 0.05$) belly thickness. Vitamin E supplementation increased NPPC marbling score ($P < 0.05$). Serum cholesterol and triglyceride increased (linear, $P < 0.01$) with increasing lipid supplementation. Dietary treatments had no effects on hot carcass weight, loin muscle area, dressing percentage, NPPC color and muscle scores, hunter color (L*, a*, b*), ultimate pH in ham and loin, and belly firmness. Dietary treatments did not affect drip loss, vacuum purge loss, marinade uptake, and proximate analysis of loin muscle, and serum urea N and glucose. These results indicated that lipid supplementation of a corn-soy based diet with constant nutrient:DE improved G:F from 54 to 110 kg, and increased ADG from 84 to 110 kg, and increased belly thickness. Vitamin E increased carcass leanness at 6% dietary lipid, but increased fatness when lower levels of lipids were included in the diets.

Keywords: swine, flaxseed oil, poultry fat, vitamin E, growth performance, carcass characteristics

1. Introduction

Animal by-products generated from meat industry can be further processed by rendering to produce high-quality fats and proteins, which are invaluable feed ingredients for food-producing animals and companion animals (Toldrá et al., 2016). Approximately 5.7 million metric tons of animal fats, including tallow, poultry fat, yellow grease, choice white grease, lard, and other grease, were produced in the United States in 2016 (Swisher, 2017).

Clearly, appropriate and efficient use of animal by-products, such as animal fat, is extremely important for the sustainable animal industry (Toldrá et al., 2016). In fact, adding animal fat in grower-finisher pig diets have many benefits, such as improving feed efficiency (Adhikari et al., 2017; Engel et al., 2001; Pettigrew and Moser, 1991; Smith et al., 1999) and dust control (Chiba et al., 1985).

One of the beneficial attributes for animal fat that is often overlooked is their effect on the reduction of body fat and increase in the intramuscular fat (IMF) content of pork. Numerous studies have shown that dietary lipids markedly reduced de novo lipogenesis (Allee et al., 1971a; Allee et al., 1971b; Smith et al., 1996), which accounts for about 74 to 77% of lipid deposition when pigs were fed cereal-based diets (Dunshea and D'Souza, 2003). Depending on the dietary energy status of the animal (Bee et al., 2002; Jakobsen and Thorbekt, 1993), dietary lipids can reduce de novo lipogenesis from carbohydrates or induce direct deposition of dietary fat. If animal is on "high-fat" diet with healthier fatty acid profile and not consuming excess "non-fat" energy, a desirable carcass fatty acid (FA) composition, as well as a reduced overall fatness, may be achieved (Engel et al., 2001; Smith et al., 1999). In addition, the chronic positive energy balance induced by lipid supplementation may promote IMF deposition in pigs (Eggert et al., 1998; Eggert et al., 2007).

There are many other fat sources or combinations, which may also affect de novo lipogenesis and fat distributions. For instance, ω-3 FA, which is beneficial for human health (Riediger et al., 2009), can be directly deposited into the membrane phospholipids (Corino et al., 2014; Romans et al., 1995). Dietary ω-3 FA has been shown to improve IMF content

(Huang et al., 2008) or marbling score (Juárez et al., 2011). Therefore, it is possible to satisfy consumer demands for reduced carcass fat and increase the contents of IMF and ω-3 FA in pork simultaneously by dietary supplementation with a combination of animal fat and flaxseed oil, which is high in ω-3 FA. Furthermore, the oxidative stability problems in pork associated with ω-3 FA can be alleviated by dietary supplementation with vitamin E (Guo et al., 2006; Jensen et al., 1998; Kim et al., 2015), which is a natural antioxidant.

A study was conducted to evaluate the effect of varying contents of dietary lipids (poultry fat with flaxseed oil) and vitamin E supplementation on: a) growth performance, b) serum metabolic profile, c) carcass characteristics, d) FA content and IMF content, e) FA profile, f) oxidative stability, g) physical and sensory characteristics, and h) expression of selected genes associated with lipid metabolism. In this paper, the results of growth performance, carcass characteristics, pork characteristics, and serum metabolites are reported, and other response criteria will be reported elsewhere.

2. Materials and methods

2.1. Animals and facilities

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Auburn University (Auburn, AL, US). A total of 96 (48 gilts and 48 castrated males) Yorkshire pigs weighing approximately 54.3 ± 3.4 kg body weight (BW) were selected and allocated to pens based on their litter, sex, and initial BW with 3 gilt pens and 3 castrated male pens per treatment. The experiment consisted of 2 trials because the availability of pigs and facilities was limited at one time. Each trial had 12 gilt pens and 12

castrated male pens and 2 trials were approximately 2 wk apart. In each trial, 24 pens were assigned randomly to 8 dietary treatments with 2 gilts or 2 castrated males per pen. Pig BW and feed consumption data were collected weekly to calculate average daily gain (ADG), average daily feed intake (ADFI), and gain to feed (G:F). When the average BW of the pigs in the pen reached 84.1 ± 4.0 kg BW, diets were changed from finisher 1 to finisher 2. The growth performance data for finisher-2 phase were collected from 84.1 ± 4.0 to 109.7 ± 1.8 kg. All pigs that reached the target BW of 110.0 ± 3.0 kg were kept in their pen and fed respective diets for 6 additional days until harvest. On the average, pigs were approximately 15, 19, and 22 wk of age for the beginning of the finisher-1, and finisher-2 phases, and at the end of the study, respectively.

Pigs were housed in pens with half-concrete and half-slatted floor in an open-sided grower-finisher building. Each pen was equipped with a feeder and a cup waterer to allow for ad libitum access to diets and water throughout the study. The average daily minimum and maximum temperatures were 15.8 and 25.5°C for Trial 1, and 9.1 and 20.4°C for Trial 2, respectively.

2.2. Dietary treatments

Corn-SBM based finisher-1 and finisher-2 diets were formulated to contain 0, 2, 4 or 6% lipids and 11 (NRC, 2012) or 220 IU vitamin E/kg in a 4 × 2 factorial arrangement of treatments (Tables 1 and 2). All the nutrients, including minerals and vitamins, were provided in amounts calculated to meet or exceed the NRC (2012) recommendations. The standardized ileal digestible (SID) Lys, and Ca, and P contents were adjusted accordingly to the changes in

the DE content of diets to maintain constant nutrient to DE ratios (Chiba et al., 1991a,b; Smith et al., 1999). Vitamin E, in the form of dl-α-tocopheryl acetate (BASF, Parsippany, NJ, US), was included in the diets by replacing the part of corn. Nutrient values for the ingredients were based on NRC (2012).

For all diets with lipids, 1% flaxseed oil was included and the remaining lipid was supplied by poultry fat. Filtered flaxseed oil was obtained from a commercial source (Healthy Oilseed LLC, Carrington, ND, US), and stored in plastic containers covered with N. The containers were stored in a walk-in cooler (4°C). Antioxidant was added to the flaxseed oil and mixed, and they were transported to the Auburn University Poultry and Animal Nutrition Center for diet preparation. The mixed feed was stored at room temperature in sealed paper bags (with plastic liners inside) after expelling air to limit the oxidation of unsaturated fatty acids. Batches of feed were used within one month after preparation.

2.3. Dietary chemical composition and fatty acid profile

Feed samples were collected from every batch of fee mixed and stored at -20°C until they were combined and subsampled for laboratory analysis. Dietary CP, Ca, and P content were determined according to AOAC (2000). Fatty acids of flaxseed oil and poultry fat were methylated according to O'Fallon et al. (2007; Table 3). The fatty acid methyl esters (FAME) were stored at -20°C until analysis using a gas chromatography (Shimadzu GC-2014; Shimadzu Co., Ltd., Kyoto, Japan) equipped with a split/splitless injector, a silica capillary column (Supelco SP 2560; Supelco, Bellefonte, PA., US; 100 m × 0.25 mm × 0.2 μm), and a flame ionization detector. Helium was used as carrier gas at a flow rate of 1.13 mL/min. The

split ratio was 30 to 1, and the injector and detector temperatures were kept at 250 and 260°C, respectively. The initial oven temperature was 100°C (held for 5 min); subsequently, the temperature was increased to 240°C at a rate of 4°C/min and held for 30 min. The FAME peaks were identified by comparison of their retention times with a FAME mix standard (47885-U; Supelco, Bellefonte, PA., US).

2.4. Carcass traits and physical characteristics

Pigs were harvested once a week, and each trial had 4 harvest days. Pigs were individually tattooed, and harvested at Auburn University Lambert-Powell Meats Laboratory under USDA inspection after an overnight fast. One pig in the second trial was removed from the study after collecting the blood samples because of hemorrhage from the left carotid artery. Therefore, only 95 pigs were used to collect the carcass data and tissues samples.

After exsanguination, backfat (intermediate/second layer) and loin muscle tissue samples were collected immediately at the last rib, frozen in liquid N, and stored at -80°C for the gene expression analysis, the results of which will be reported elsewhere. The eviscerated carcass was split longitudinally through the vertebrae midline, and hot carcass weight (HCW) was determined immediately after harvest. The dressing percentage was calculated based on HCW and the expected BW at harvest. The ADG obtained over the previous 2-wk was used to estimate the BW of each pig on the harvest day (Quiniou and Noblet, 2012), which was, again, 6 d after the end of the finishe-2 phase.

The carcass was then chilled for 24 h at 4 °C, and then carcasses were cut between the 10th and 11th rib. The following carcass characteristics were determined: backfat thickness at

the last rib (LRFT), backfat thickness at 10th rib (TRFT), and loin muscle area. Both LRFT and TRFT measurements included the skin. The proportion of fat-free lean (FFL) was estimated by the equation reported by NPPC (2000). Subjective evaluation for muscle score, color, marbling score was measured by a trained evaluator according to NPPC (2000).

Eight 2.54-cm thick loin chops were collected from the left side loin of each carcass, individually vacuum packaged in a 3-layer oxygen barrier bag (Sealed Air, Cryovac, Charlotte, NC, US), and frozen (-20 ± 2°C) for further analysis. After appropriate bloom time, the exposed loin muscle area was evaluated for instrumental color (L*, a*, and b*) from a mean of 2 random readings (Hunter Miniscan XE Plus, Model MSXP-4500C; Hunter Associates Laboratories, Reston, VA, US) with illuminant D65 at 10° observance and a 3.5-cm aperture. The ultimate pH (24 h postmortem) of the ham and loin at the 10th and 11th rib interface was measured using a portable pH meter with spear-tip (Waterproof Pocket pH Testrs; Oakton Instruments, Vernon Hills, IL, US).

After measuring the length, width, and temperature of each belly, subjective belly firmness was determined by suspending the center of the belly across a stainless steel bar and measuring the distance between belly ends with both skin-side up (SSU) and skin side down (SSD). Belly firmness score was calculated according to Whitney et al. (2006): $\cos^{-1}[(0.5 \times L^2 - D^2)/(0.5 \times L^2)]$, where L is the belly length and D is the distance between belly ends when suspended. Greater firmness score indicates firmer bellies. Fresh pork bellies were collected, vacuum-packaged, and stored at -20°C for further analysis.

2.5. Pork quality

Loin chops were used for measuring drip loss, vacuum purge loss, marinade uptake, marinade cook loss, proximate analysis. All the procedures of measurements for meat quality were described in detail elsewhere (Magee, 2017).

2.6. Serum metabolites

To assess metabolite profile, approximately 8 mL of blood was collected from each pig via anterior vena cava puncture using a sterile needle (16 gauge × 3 inches) and syringe 1 d after the end of the finisher-2 phase. All blood samples were collected between 0800 and 1000 h. Blood samples were allowed to clot and centrifuged at 1,500 × g for 15 min at room temperature to obtain cleaner serum samples, and an aliquot was frozen at -20°C until further analysis. Serum concentrations of albumin, globulin, urea N, total protein, glucose, triacylglycerol (TAG), and cholesterol were determined using an autoanalyzer (Boehringer Mannheim/Hitachi 911; Boehringer Manheim Corp, Indianapolis, IN, US; Adhikari et al., 2017) at Auburn University Clinical Pathology Laboratory (Auburn, AL, US). Serum from individual pig was used for albumin, globulin, urea N, total protein, glucose, TAG, and cholesterol analysis, and the average value from each pen was used for the statistical analysis.

Serum α-tocopherol content was analyzed at Iowa State University Veterinary Diagnostic Laboratory (Ames, Iowa, US). Because the serum TAG and cholesterol concentrations were affected by the dietary lipid treatments, the serum α-tocopherol concentration was adjusted for serum TAG or cholesterol concentration (Prévéraud et al., 2014). The serum samples were pooled within pen on an equal volume basis and were used for α-tocopherol measurements.

2.6. Statistical analysis

All data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) and the pen was used as the experimental unit. The statistical model initially included lipid, vitamin E, sex, trial, and all possible interactions. The analysis indicated that the trial × interactions were not important source of variation, thus, the dataset from 2 trials was combined. The initial and final BW for growth performance data, appropriate BW for serum metabolite data, and the HCW was used for carcass data were initially considered as a covariate or covariates. Belly thickness was used as a covariate for flop test and belly firmness score. The interactions and covariates which did not reach significant trend ($P > 0.10$) were then deleted from the final model. In addition to the main effect of vitamin E and interaction, 3 preplanned contrasts (0% flaxseed oil vs. 1% flaxseed oil, and linear and quadratic effects of supplemental lipids) were used to assess the effect of dietary lipids. The results are considered a statistically significant if $P \leq 0.05$ and a trend if $P \leq 0.10$.

3. Results

3.1. General

The analyzed CP contents were lower than calculated values of all experimental diets. But, the differences were relatively consistent, and on the average, analyzed values were 8.3 and 5.9% less than the intended values for the finisher 1 and finisher 2 diets, respectively. The Lys intake and the efficiency of Lys utilization for BW gain and fat-free lean gain were based on SID Lys. The effect of sex on pig performance had been well established over the years;

thus, the effect of sex was not assessed in the present study. However, an equal number of gilts and castrated males were used and the effect of sex were partitioned statistically.

3.2. Growth performance

There were no dietary lipid × vitamin E interactions on any of the growth performance criteria during the finisher-1, finisher-2, or overall phase (Table 4). Lipid supplementation decreased ADFI during the finisher-1, finisher-2, and overall phases (linear, $P < 0.05$). As expected, G:F was linearly increased with increasing the lipid content of the diets during the finisher-1, finisher-2, and overall phases ($P < 0.001$). Increasing dietary lipids improved ADG (linear, $P = 0.028$) during the finisher-2 phase, and therefore, tended to increase ADG during the overall phase (linear, $P = 0.053$). The efficiency of DE and Lys utilization for BW gain during the finisher-2 phase tended to increase as dietary lipid supplementation increased (linear, $P < 0.10$). There were no effects of dietary lipids on daily intakes of Lys and DE during the finisher-1, finisher-2, or overall phase. No differences were observed for the efficiency of Lys or DE utilization for BW gain during the finisher-1 or overall phase.

Flaxseed oil supplementation decreased ADFI and increased feed efficiency ($P < 0.031$) during the finisher-1, finisher-2, and overall phases. Pigs supplemented with 220 IU vitamin E/kg utilized feed, Lys, and DE less efficiently ($P < 0.035$) than those fed 11 IU vitamin E/kg diet during the finisher-1 and overall phases.

3.3. Carcass characteristics and pork characteristics

There were dietary lipid × vitamin E interactions ($P < 0.05$) on TRFT and FFL (Table 5). Pigs fed 0, 2, and 4% lipid diets supplemented with vitamin E had thicker TRFT and lower FFL than those without vitamin E, whereas those fed 6% lipids with vitamin E supplementation had thinner TRFT and greater FFL than those without vitamin E. Pigs supplemented with 220 IU vitamin E/kg diet had greater NPPC marbling score ($P = 0.042$) than those fed 11 IU vitamin E/kg diet. There were no difference on HCW, dressing percentage, loin muscle area, and NPPC color and muscle scores. No differences among dietary treatments in hunter color (L*, a*, and b*) and ultimate pH of ham and loin were observed at 24 h postmortem.

There were no dietary lipid × vitamin E supplementation interactions on any of the pork quality criteria. Similarly, neither lipid nor vitamin E had any effect on drip loss, vacuum purge loss, marinade uptake, and proximate analysis (collagen, fat, moisture, protein, and salt). Marinade cook loss decreased with increased dietary lipids (linear, $P = 0.024$; quadratic, $P = 0.090$).

The average temperature of belly was 3.94 ± 0.44°C in Trial 1, whereas it was 3.13 ± 0.56°C in Trial 2 (Table 6). The trial x treatment interaction was not observed though. Increasing dietary lipids linearly increased ($P = 0.040$) belly thickness. Dietary treatments had no effects on flop test and belly firmness score measured by SSU and SSD.

3.4. Serum metabolites

There were no dietary lipid × vitamin E interactions on serum total protein, albumin, albumin to globulin ratio (A:G), blood urea N (BUN), glucose, cholesterol, triglycerides, and

α-tocopherol (Table 7). At the end of the finisher-2 phase, serum cholesterol and TAG increased linearly ($P < 0.03$) with increasing dietary lipid content. Serum total protein tended to increase linear and quardartically ($P = 0.84$ and 0.095, respectively) with increasing dietary lipids. Serum albumin, BUN, glucose, and α-tocopherol were not affected by the dietary lipids. Supplementation of flaxseed oil increased ($P < 0.037$) serum total protein, albumin, cholesterol, and triglycerides, but it had no effects on BUN, glucose, and α-tocopherol content.

As expected, serum α-tocopherol content was greater in pigs fed 220 IU vitamin E/kg diets than those fed 11 IU vitamin E/kg diets ($P < 0.001$). But, vitamin E supplementation had no effects on other serum metabolites. There were dietary lipid × vitamin E supplementation interactions ($P < 0.049$) on serum globulin and A:G. Pigs fed the diets with no lipids and 2% lipids and 200 IU vitamin E/kg diets had lower serum globulin and greater A:G than those fed the diets without vitamin E, whereas pigs fed 4 and 6% lipids had greater serum globulin and lower A:G than those fed the diets without vitamin E.

4. Discussion

In our previous study, dietary lipids (2% poultry fat + 3% flaxseed oil) decreased feed intake during the grower phase and improved BW gain and G:F during the grower and finisher-1 phases (Adhikari et al., 2017). In the present study, lipid supplementation linearly decreased ADFI and improved G:F in both the finisher-1, finisher-2, and overall phases. An early review, which analyzed data from published studies, indicated that addition of fat to grower-finisher pig diets decreased the ADFI, improved ADG and feed efficiency, and

increased average backfat (Pettigrew and Moser, 1991). Numerous studies consistently showed that lipid supplementation can decrease ADFI and improve G:F when the Lys to energy ratio in the diet was constant (Beaulieu et al., 2009; Eggert et al., 2007; Engel et al., 2001; Myer and Combs, 1991; Quiniou and Noblet, 2012; Smith et al., 1999; Wu et al., 2007). The improved feed efficiency or increased growth rate or both was considered as a consequence of increased leanness of barrows fed 5% canola oil (vs. 2%; Dugan et al., 2001). The explanation for improved feed efficiency in this study will be addressed later.

In the current study, ADG was linearly increased only during the finisher 2 phase. In previous studies using a constant Lys:energy, the effect of dietary lipids on ADG has been rather inconsistent. For example, some studies showed increased ADG for pigs weighing 29.5 to 72.6 kg (Smith et al., 1999), 23.2 to 60.6 kg (Wu et al., 2007), and 41 and 123 kg (Benz et al., 2011). On the other hand, a majority of research has failed to show an effect of dietary supplementation with beef tallow (Apple et al., 2009b; Eggert et al., 2007; Weber et al., 2006), choice white grease (Engel et al., 2001; Myer and Combs, 1991; Weber et al., 2006), poultry fat (Apple et al., 2009b; Engel et al., 2001), and soybean oil (Apple et al., 2009b) on growth rate. Additionally, some studies investigated both the source and content of lipids and they found that neither of them had any effect on ADG (Apple et al., 2009b; Engel et al., 2001; Realini et al., 2010).

The energetic efficiency of lipids on BW gain depends on several factors, such as body composition, breed, sex, and age (Noblet and Van Milgen, 2013). No effect of choice white grease supplementation was observed on ADG from 44.5 to 72.6 kg, and unexpectedly,

it was quadratically decreased from 72.6 to 104.3 kg BW (Smith et al., 1999). Recently, Quiniou and Noblet (2012) used various NE contents on grower-finisher pig diets, and found ADG increased with increased NE content only from 35 to 50 kg BW. The specific linear-plateau relationships between protein deposition and energy intake may be different for pigs in these studies. Because whole body composition was not measured in this study, we could not calculate the protein deposition and lipid deposition rate. In the current experiment, a tendency to increase in the efficiency of Lys and DE utilization for BW gain by lipid supplementation were observed during the finisher 2 phase, but lipid supplementation had no effect on backfat thickness and FFL at slaughter. This indicated that the increased DE density or lipid supplementation may increase protein deposition and lipid deposition at a similar magnitude.

In the present study, a lipid by vitamin E interaction was found on carcass leanness, i.e., 220 IU vitamin E/kg decreased carcass leanness at 0, 2, and 4% lipids, while it increased carcass leanness at 6% lipids. The mechanism for this interaction is not immediately apparent at this time. Kim et al. (2015) found a decrease, then increase in backfat thickness when dietary vitamin E content increased from 35 to 700 IU/kg, and the crude fat was 7% in all diets. Guo et al. (2006) conducted a study investigating supplementation of lipids (normal corn, high-oil corn, and high-oil corn plus 4% choice white grease) and vitamin E (40 and 200 IU/kg), but they did not find any lipid × vitamin E interactions on growth performance and carcass traits.

Although animal fat is an inexpensive source of energy (Swisher, 2017), pork producers are often hesitant in adding fats in finisher pig diets because high-energy diets are thought to induce direct deposition of surplus energy into adipose tissues and, therefore, reduce carcass value. In the current study, the main effect of lipid supplementation on carcass leanness was not clear because of the interaction. When pigs were fed diets containing increased energy concentration while maintaining a constant Lys to ME ratio, many studies found no effects of lipid supplementation on carcass fatness (De la Llata et al., 2001; Engel et al., 2001; Quiniou and Noblet, 2012; Adhikari et al., 2017). However, Smith et al. (1999) found an increase then decrease in backfat thickness at a similar ME intake, whereas Cline et al. (2016) found that pigs reached a plateau of daily caloric intake and backfat thickness. Increased backfat thickness of pigs fed high-fat diets by lipid supplementation have also been reported (Pettigrew and Moser, 1991; Apple et al., 2009b; Benz et al., 2011). Inconsistent findings among studies on backfat thickness can be explained by the energy content of the control (Cline et al., 2016), degree of inhibition of de novo lipogenesis by dietary fatty acids (Allee et al., 1971a; Allee et al., 1971b), energy status of animals (Bee et al., 2002; Jakobsen and Thorbekt, 1993), and genetic factors (Eggert et al., 2007). In addition, previous studies showed that dietary supplementation of animal fat (5%) could increase the lipid content in *longissimus* muscle, picnic, and ham (Eggert et al., 2007), and increase NPPC marbling score of loin (Eggert et al., 1998) without affecting carcass leanness when Lys to calorie ratios in the diet were maintained. However, the current study did not identify differences in muscle fat content among treatment groups.

Lipid supplementation had no effect on dressing percentage, which is consistent with previous reports (Apple et al., 2009b; De la Llata et al., 2001; Engel et al., 2001). However, those findings differ from some other studies (Beaulieu et al., 2009; Quiniou and Noblet, 2012; Smith et al., 1999), which showed that increasing dietary lipids or energy density could result in greater dressing percentage. Loin muscle area and FFL were not affected by dietary lipids, which is consistent with previous reports (Adhikari et al., 2017; De la Llata et al., 2001; Engel et al., 2001; Smith et al., 1999). Dietary lipids did not affect objective color (L, a*, and b*) of loin muscle and pH of ham and loin, which agreed with previous report on the effect of poultry fat (Eggert et al., 1998; Engel et al., 2001). Similar to previous reports (Engel et al., 2001), dietary lipids did not affect objective belly firmness score in the current study. It is possible that the degree of unsaturation was still lower than the threshold, at which results in soft belly. However, several investigators found that poultry fat supplementation reduced subjective belly firmness (Eggert et al., 1998; Adhikari et al., 2017). In the current study, an increase in belly thickness from lipid supplementation was observed, which may be of economic importance. But, this phenomenon cannot be explained at this time. Apple et al. (2009a) reported that 5% poultry fat in finisher pig diets did not affect pork primal cut yields (including belly) compared to pigs fed diets without added fat.

Serum metabolite data can reflect the nutritional status of animals and metabolic activities (Adhikari et al., 2017). In the fed state, dietary lipids are readily digested and absorbed, and, eventually, enters the circulation (Kerr et al., 2015). As expected, serum TAG concentration was increased with increasing dietary lipids in the current study, as also

observed previously (Allan et al., 2001; Adhikari et al., 2017). In contrast, dietary lipid supplementation (3% flaxseed oil, coconut hydrogenated oil, or olive oil) had no effect on plasma TAG compared with those fed the basal diet without oil when pigs were fasted overnight prior to blood sampling (Prévéraud et al., 2014).

In the current study, serum cholesterol concentration was increased with increasing dietary lipids, which agreed with Allee et al. (1971c). Serum cholesterol has been shown to be greater in pigs fed 3% coconut hydrogenated oil but lower in pigs fed 3% flaxseed oil compared with those fed the basal diet with no oil (Prévéraud et al., 2014). In our previous study, the increased serum cholesterol concentration was only observed during the finisher-1 phase (Adhikari et al., 2017). The hypercholesterolemic effects of dietary lipid were more pronounced for saturated fatty acids than unsaturated fatty acids (Allan et al., 2001; Lauridsen, 2010; Prévéraud et al., 2014). However, the serum cholesterol and TAG were increased by flaxseed oil in the present study, which may be confounded with the effect of dietary poultry fat. Similar to previous report (Lauridsen, 2010), the dietary lipids or vitamin E had minimal effects on BUN and serum glucose.

Total protein and albumin contents are usually measured photometrically, and then the globulin fraction is calculated by subtracting the albumin from the total protein (Busher, 1990). Serum total protein, albumin, and A:G are indicators of adequate supply of protein (Lowrey et al., 1962). The increased total protein and albumin in this study were in response to the initial increment of lipid supplementation from 0 to 2%, and it remained constant with further increases. This increase reflects the improvement of growth rate of pigs in the

finisher-2 phase (Mule et al., 2006). In addition, the increased total protein and albumin may also reflect the increased demands because most of the non-esterified FA (especially long chain FA) are strongly bound by the albumin, which is the major protein component in the blood (Peters, 1995).

In the current study, serum α-tocopherol concentration was increased in pigs fed the diet supplemented with vitamin E, which is an indication of increased dietary α-tocopherol actate (Kim et al., 2015). Vitamin E supplementation did not affect ADG, loin muscle area, and FFL, which is consistent with previous reports (Cannon et al., 1996; Guo et al., 2006; Hoving-Bolink et al., 1998). Unexpectedly, pigs fed diets containing 220 IU vitamin E/kg reduced feed efficiency during the finisher-1 and overall phases, which is contrary to earlier reportsd (Cannon et al., 1996; Guo et al., 2006; Kim et al., 2015). The typical inclusion rate in finisher diets is 23 IU/kg (Flohr et al., 2015), which is slightly greater than the minimal requirement established by NRC (2012). Vitamin E toxicity has not been demonstrated in swine (NRC, 2012) and there have been no negative effects on growth performance when feeding finisher pigs as high as 700 mg vitamin E/kg diet (Kim et al., 2015).

In this experiment, vitamin E supplementation did not affect objective color (L, a*, and b*) of fresh loin muscle. Previous studies also found vitamin E supplementation (between 100 and 300 IU/kg) had no effects on instrumental color (Cannon et al., 1996; Guo et al., 2006; Hoving-Bolink et al., 1998; Kim et al., 2015). Vitamin E supplementation had no effect on ultimate pH of ham and loin, which agreed with previous reports (Guo et al., 2006; Hoving-Bolink et al., 1998; Kim et al., 2015). Negative correlation between drip loss and pH

at 24 and 48 h post-mortem has been well documented (Kim et al., 2015). Thus, it is not unexpected that vitamin E supplementation did not affect drip loss, vacuum purge loss, marinade uptake, or marinade cook loss of pre-frozen pork. It has been reported that 200 IU vitamin E/kg could lower the drip loss of pre-frozen pork (Asghar et al., 1991). However, this positive effect was inconsistent in earlier reports (reviewed by Jensen et al., 1998). A majority of more recent studies did not observe effects of vitamin E on water-holding capacity, measured by drip loss, vacuum purge loss, or cook loss (Cannon et al., 1996; Guo et al., 2006; Hoving-Bolink et al., 1998; Kim et al., 2015). In the present and other studies, no effects of vitamin E on lipid content in the muscle were observed (Hoving-Bolink et al., 1998; Kim et al., 2015).

5. Conclusion

As expected, dietary lipid supplementation improved feed efficiency throughout the finisher phase. In addition, lipid supplementation increased belly thickness without changing belly firmness. These data also indicated that vitamin E supplementation increased carcass leanness in pigs fed the 6% dietary lipids, but increased fatness in pigs fed the diets containing 2 and 4% lipids. The NPPC marbling score was enhanced by vitamin E supplementation. Dietary lipid supplementation increased serum cholesterol and TAG content at the end of finisher-2. Dietary treatment generally had no effect on pork quality. Thus, a combination of 6% lipids (5% poultry fat plus 1% flaxseed oil) with 220 IU vitamin E/kg diets can be used to optimize growth performance, carcass leanness, and belly characteristics.

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Table 1

Composition of experimental finisher 1 diets (as-fed basis) [1].

Item	Lipid, %:	0		2		4		6	
	VE, IU/kg:	11	220	11	220	11	220	11	220
Ingredient, g/kg									
Corn		700.2	699.8	667	666.6	634	633.6	601.3	600.9
SBM (47.5% CP)		273.5	273.5	285.9	285.9	298.1	298.1	310.0	310.0
Animal fat		-	-	10.0	10.0	30.0	30.0	50.0	50.0
Flaxseed oil		-	-	10.0	10.0	10.0	10.0	10.0	10.0
Dicalcium phosphate		11.90	11.90	12.78	12.78	13.75	13.75	14.70	14.70
Limestone		8.40	8.40	8.29	8.29	8.12	8.12	7.96	7.96
Salt		3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
Vitamin-mineral[2]		2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
VE acetate, 50%		-	0.44	-	0.44	-	0.44	-	0.44
Calculated composition[3]									
DE, Mcal/kg		3.41	3.41	3.51	3.51	3.61	3.61	3.71	3.71
ME, Mcal/kg		3.28	3.28	3.38	3.38	3.47	3.47	3.57	3.57
CP, g/kg		188	188	191	191	195	195	198	198
SID Lys, g/kg		8.5	8.5	8.8	8.8	9.0	9.0	9.3	9.3
Ca, g/kg		7.0	7.0	7.2	7.2	7.4	7.4	7.6	7.6
P, g/kg		6.0	6.0	6.2	6.2	6.4	6.4	6.5	6.5
Ca:P		1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17
SID Lys:DE, g/Mcal[3]		2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
SID Lys:ME, g/Mcal		2.59	2.59	2.60	2.60	2.60	2.60	2.60	2.60
Ca:DE, g/Mcal[3]		2.06	2.06	2.06	2.06	2.06	2.06	2.06	2.06
P:DE, g/Mcal[3]		1.76	1.76	1.76	1.76	1.76	1.76	1.76	1.76
Analyzed composition									
CP, g/kg		171	173	176	176	178	178	183	180
Ca, g/kg		6.8	6.9	6.8	7.5	7.9	7.0	7.6	7.6
P, g/kg		4.8	5.4	5.1	5.2	6.0	5.2	5.4	5.5

[1] Vitamin E (VE) premix (220 IU/kg) is included in place of corn. SBM = soybean meal, CP = crude protein, DE = digestible energy, ME = metabolizable energy, and SID = standard ileal digestible.

[2] Provide the following (unit/kg diet): Fe (ferrous sulfate), 150 mg; Zn (zinc oxide), 150 mg; Mn (manganous oxide), 37.5 mg; Cu (copper sulfate), 150 ppm; I (ethylenediamine dihydroiodide), 5 ppm; Se (sodium selenite), 3 ppm; vitamin A, 6,614 IU; vitamin D_3, 1,102 IU; VE, 11 IU; vitamin B_{12}, 0.03 mg; menadione (menadione Na bisulfite complex), 1 mg; riboflavin, 6 mg; D-pantothenic acid (D-Ca pantothenate), 45 mg; niacin, 28 mg; and choline (choline chloride), 110 mg.

[3] To maintain a constant ratio (Chiba et al., 1991a,b), Lys, Ca, or P content was adjusted for DE accordingly.

Table 2

Composition of experimental finisher 2 diets (as-fed basis) [1].

Item	Lipid, %:	0		2		4		6	
	VE, IU/kg:	11	220	11	220	11	220	11	220
Ingredient, g/kg									
Corn		748.3	747.9	717.1	716.7	685.3	684.9	654.5	654.1
SBM (47.5% CP)		224.6	224.6	235.0	235.0	246.0	246.0	256.0	256.0
Animal fat		-	-	10.0	10.0	30.0	30.0	50.0	50.0
Flaxseed oil		-	-	10.0	10.0	10.0	10.0	10.0	10.0
Dicalcium phosphate		13.10	13.10	14.10	14.10	15.00	15.00	16.00	16.00
Limestone		8.00	8.00	7.80	7.80	7.70	7.70	7.50	7.50
Salt		3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
Vitamin-mineral[2]		2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
VE acetate, 50%		-	0.44	-	0.44	-	0.44	-	0.44
Calculated composition[3]									
DE, Mcal/kg		3.40	3.40	3.50	3.50	3.60	3.60	3.70	3.70
ME, Mcal/kg		3.28	3.28	3.38	3.38	3.47	3.47	3.57	3.57
CP, g/kg		169	169	171	171	174	174	176	176
SID Lys, g/kg		7.3	7.3	7.5	7.5	7.8	7.8	8.0	8.0
Ca, g/kg		7.0	7.0	7.2	7.2	7.4	7.4	7.6	7.6
P, g/kg		6.0	6.0	6.2	6.2	6.4	6.4	6.5	6.5
Ca:P		1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17
SID Lys:DE, g/Mcal3		2.15	2.15	2.15	2.15	2.15	2.15	2.15	2.15
SID Lys:ME, g/Mcal		2.23	2.23	2.23	2.23	2.23	2.23	2.23	2.23
Ca:DE, g/Mcal[3]		2.06	2.06	2.06	2.06	2.06	2.06	2.06	2.06
P:DE, g/Mcal[3]		1.77	1.77	1.77	1.77	1.76	1.76	1.76	1.76
Analyzed composition									
CP, g/kg		162	156	163	163	165	160	166	164
Ca, g/kg		7.5	6.7	7.7	6.8	8.2	7.3	7.3	7.5
P, g/kg		5.2	4.8	5.7	5.1	5.9	5.5	5.6	6.0

[1] Vitamin E (VE) premix (220 IU/kg) is included in place of corn. SBM = soybean meal, CP = crude protein, DE = digestible energy, ME = metabolizable energy, and SID = standard ileal digestible.

[2] Provide the following (unit/kg diet): Fe (ferrous sulfate), 150 mg; Zn (zinc oxide), 150 mg; Mn (manganous oxide), 37.5 mg; Cu (copper sulfate), 150 ppm; I (ethylenediamine dihydroiodide), 5 ppm; Se (sodium selenite), 3 ppm; vitamin A, 6,614 IU; vitamin D_3, 1,102 IU; VE, 11 IU; vitamin B_{12}, 0.03 mg; menadione (menadione Na bisulfite complex), 1 mg; riboflavin, 6 mg; D-pantothenic acid (D-Ca pantothenate), 45 mg; niacin, 28 mg; and choline (choline chloride), 110 mg.

[3] To maintain a constant ratio (Chiba et al., 1991a,b), Lys, Ca, or P content was adjusted for DE accordingly.

Table 3

Fatty acid composition of poultry fat and flaxseed oil (g/100 g total fatty acids) [1,2].

Item	Poultry fat	Flaxseed oil
C16:0	25.05	4.59
C16:1	6.79	0.05
C18:0	6.13	3.30
C18:1n9	40.61	19.65
C18:2n6	18.99	14.98
C18:3n3	0.92	57.43
Other fatty acids	1.51	-

[1] Reported the values of 2 batches of samples analyzed in duplicate.

[2] Obtained flaxseed oil from a commercial source (Healthy Oilseed LLC, Carrington, ND, US).

Table 4
Effects of dietary lipids and vitamin E (VE) supplementation on grow performance of pigs during finisher-1, finisher-2 phases, and overall [1,2,3].

Item	Lipid, %: 0		2		4		6		SEM [4]	*P*-value				
VE, IU/kg:	11	220	11	220	11	220	11	220		Lipid, Ln	Lipid, Qd	VE	Lipid × VE	Flax, 0 vs.1%
Finisher-1 phase														
ADFI, g/d	2,785	3,006	2,766	2,878	2,699	2,759	2,625	2,635	81	0.002	0.828	0.092	0.610	0.017
Lys intake, g/d	23.7	25.6	24.3	25.2	24.4	24.9	24.4	24.5	0.7	0.768	0.731	0.102	0.659	0.983
DE intake, Mcal/d	9.49	10.24	9.71	10.10	9.76	9.95	9.74	9.79	0.29	0.714	0.760	0.106	0.658	0.923
ADG, g/d	1,098	1,072	1,110	1,055	1,154	1,062	1,138	1,079	41	0.467	0.951	0.053	0.882	0.671
G:F, g/kg	397	356	395	370	422	387	432	420	15	0.001	0.452	0.012	0.794	0.030
ADG:Lys intake, g/g	46.8	41.9	45.1	42.2	46.7	42.9	46.6	45.4	1.7	0.264	0.427	0.010	0.748	0.741
ADG:DE intake, g/Mcal	116.7	104.7	112.7	105.4	116.8	107.2	116.5	113.4	4.1	0.243	0.438	0.010	0.751	0.702
Finisher-2 phase														
ADFI, g/d	3,222	3,423	3,192	3,211	3,154	3,249	3,142	3,074	81	0.018	0.817	0.295	0.418	0.031
Lys intake, g/d	23.5	25.0	24.0	24.1	24.5	25.2	25.0	24.5	0.6	0.293	0.923	0.317	0.446	0.591
DE intake, Mcal/d	10.94	11.62	11.17	11.23	11.36	11.68	11.62	11.38	0.29	0.291	0.881	0.326	0.445	0.597
ADG, g/d	1,075	1,034	1,019	1,013	1,161	1,119	1,100	1,173	51	0.028	0.635	0.906	0.647	0.307
G:F, g/kg	333	301	319	319	367	346	350	377	15	0.001	0.789	0.543	0.227	0.021
ADG:Lys intake, g/g	45.6	41.3	42.4	42.4	47.4	44.6	44.1	47.5	1.9	0.092	0.754	0.516	0.214	0.429
ADG:DE intake, g/Mcal	98.1	88.8	91.1	91.2	101.9	96.1	94.7	102.1	4.1	0.093	0.776	0.520	0.218	0.428
Overall phase														
ADFI, g/d	2,984	3,177	3,012	3,027	2,950	2,945	2,862	2,815	73.0	0.002	0.648	0.463	0.391	0.020
Lys intake, g/d	23.5	25.0	24.6	24.7	24.9	24.7	24.6	24.4	0.6	0.667	0.470	0.512	0.481	0.464
DE intake, Mcal/d	10.15	10.80	10.55	10.60	10.63	10.61	10.60	10.44	0.26	0.823	0.583	0.494	0.426	0.640
ADG, g/d	1,085	1,048	1,071	1,034	1,164	1,082	1,118	1,121	36	0.053	0.837	0.150	0.717	0.291
G:F, g/kg	364	331	357	345	395	368	390	392	11.4	<0.001	0.716	0.036	0.439	0.006
ADG:Lys intake, g/g	46.3	42.0	43.8	42.4	46.7	44.0	45.4	45.2	1.4	0.193	0.603	0.035	0.519	0.701
ADG:DE intake, g/Mcal	107.0	97.3	101.8	98.5	109.5	102.3	105.4	105.8	3.2	0.119	0.716	0.034	0.420	0.506

[1] Ln = linear, Qd = quadratic, Flax = flaxseed oil, ADFI = average daily feed intake, ADG = average daily gain, G:F = gain to feed ratio, and DE = digestible energy.

[2] Least square means based on 6 pens containing 2 gilts or 2 castrated males/pen; finisher 1: 54.3 ± 3.4 to 84.1 ± 4.0 kg; finisher 2: 84.1 ± 4.0 to 109.7 ± 1.8 kg.

[3] Lys intake is based on standard ileal digestible (SID) Lys.

[4] SEM = pooled standard error of the mean.

Table 5
Effects of dietary lipids and vitamin E (VE) supplementation on carcass traits and pork characteristics [1,2].

Item	Lipid, %:	0		2		4		6		SEM [3]	*P*-value				
	VE, IU/kg:	11	220	11	220	11	220	11	220		Lipid, Ln	Lipid, Qd	VE	Lipid × VE	Flax, 0 vs.1%
Carcass traits															
HCW, kg		82.6	82.8	83.5	81.6	84.8	84.0	83.8	82.3	1.1	0.395	0.425	0.193	0.760	0.464
Dressing percentage, %		71.5	71.2	71.6	71.3	72.2	73.3	72.3	70.9	0.8	0.465	0.289	0.681	0.507	0.406
LRFT, mm		20.7	24.1	23.5	21.9	21.9	24.7	20.2	23.7	1.8	0.879	0.524	0.111	0.424	0.881
TRFT, mm		16.1	22.2	18.9	21.6	20.7	23.2	22.1	19.5	1.3	0.136	0.232	0.028	0.020	0.102
Loin muscle area, cm^2		44.3	40.4	44.2	42.5	41.1	41.8	38.9	43.9	1.9	0.445	0.693	0.963	0.126	0.869
FFL, %		54.9	50.8	53.5	51.8	52.0	50.9	50.7	53.1	1.0	0.196	0.624	0.126	0.017	0.285
Fresh pork characteristics															
NPPC color		3.2	3.0	3.0	2.9	3.0	2.9	3.1	3.0	0.2	0.855	0.498	0.498	0.997	0.584
NPPC marbling		1.6	1.9	1.4	1.9	1.6	1.9	1.4	1.8	0.3	0.605	0.817	0.042	0.983	0.689
Muscle score		2.6	2.3	2.4	2.3	2.6	2.7	2.3	2.6	0.1	0.348	0.673	0.673	0.174	0.626
L* of loin [4]		59.61	59.70	62.26	59.97	61.30	60.36	58.80	59.61	1.18	0.663	0.072	0.486	0.587	0.454
a* of loin [4]		8.74	8.95	8.75	8.42	8.74	8.63	8.88	8.70	0.43	0.963	0.545	0.739	0.934	0.650
b* of loin [4]		16.06	16.47	16.70	16.12	16.55	16.27	16.12	15.75	0.39	0.422	0.258	0.456	0.617	0.971
pH of ham, 24 h		5.69	5.71	5.68	5.68	5.70	5.70	5.67	5.68	0.04	0.617	0.898	0.835	0.975	0.616
pH of loin, 24 h		5.59	5.53	5.53	5.55	5.55	5.51	5.59	5.62	0.04	0.330	0.068	0.730	0.537	0.900
Characteristics of pre-frozen loin chops															
Drip loss, %		4.59	3.29	4.64	3.50	3.45	2.81	3.39	4.16	0.50	0.368	0.463	0.109	0.165	0.490
Vacuum purge loss, %		10.09	8.62	10.60	9.85	10.99	9.48	9.26	9.35	0.87	0.962	0.152	0.148	0.775	0.429
Marinade uptake, %		11.47	11.95	11.36	8.87	10.76	15.08	11.46	13.64	1.75	0.347	0.628	0.385	0.301	0.916
Marinade cook loss, %		20.55	21.62	21.98	22.51	21.96	18.90	19.68	16.50	1.29	0.024	0.090	0.249	0.286	0.469
Collagen, %		1.52	1.45	1.49	1.47	1.55	1.51	1.49	1.45	0.06	0.947	0.527	0.294	0.986	0.864
Fat, %		4.01	4.42	4.75	4.21	4.22	4.26	3.97	4.10	0.30	0.410	0.264	0.967	0.478	0.872
Moisture, %		76.42	75.60	75.44	75.96	75.77	75.42	76.00	76.04	0.33	0.939	0.131	0.520	0.244	0.389
Protein, %		26.29	26.13	26.47	26.30	26.45	26.52	26.25	26.32	0.22	0.636	0.224	0.774	0.895	0.325
Salt, %		0.778	0.726	0.701	0.744	0.689	0.706	0.705	0.711	0.037	0.189	0.452	0.888	0.623	0.168

[1] Ln = linear, Qd = quadratic; Flax = flaxseed oil, HCW = hot carcass weight, LRFT = last rib fat thickness, TRFT = tenth rib fat thickness, and FFL = percent fat free lean.

[2] Least square means based on 6 pens containing 2 gilts or 2 castrated males/pen.

[3] SEM = pooled standard error of the mean.

[4] L* (lightness), a* (redness), b* (yellowness): greater value indicates lighter, redder, more yellow, respectively.

Table 6
Effects of dietary lipids and vitamin E (VE) supplementation on quality characteristics of fresh pork belly [1,2].

Item	Lipid, %:	0		2		4		6		SEM [3]	*P*-value				
	VE, IU/kg:	11	220	11	220	11	220	11	220		Lipid, Ln	Lipid, Qd	VE	Lipid × VE	Flax, 0 vs.1%
Belly characteristics															
Temperature, °C		3.4	3.5	3.4	3.6	3.6	3.5	3.9	3.3	0.12	0.251	0.714	0.241	0.010	0.316
Length, cm		49.7	48.6	50.3	45.8	47.7	46.2	46.3	48.1	1.2	0.059	0.407	0.118	0.076	0.067
Width, cm		23.8	23.6	24.7	24.2	24.9	24.0	24.8	26.0	0.6	0.017	0.809	0.838	0.396	0.053
Thickness, mm		33.4	36.0	37.1	34.9	37.6	40.2	40.3	39.7	2.8	0.040	0.953	0.742	0.781	0.123
Flop test of belly [4]															
SSD, cm		10.8	14.1	10.6	11.4	12.5	12.4	11.1	11.5	1.4	0.676	0.883	0.294	0.643	0.467
SSU, cm		8.8	9.6	8.4	9.0	9.5	8.9	8.0	8.5	1.1	0.519	0.803	0.691	0.912	0.593
Belly firmness score [4,5]															
SSD, degrees		27.8	35.9	25.2	30.3	31.4	30.8	27.3	27.5	3.7	0.416	0.939	0.226	0.601	0.320
SSU, degrees		20.9	21.7	18.8	23.7	23.5	21.3	18.8	20.1	2.8	0.634	0.463	0.540	0.661	0.904

[1] Ln = linear, Qd = quadratic, Flax = flaxseed oil, SSD = skin-side down, SSU = skin-side up, L = linear, Q = quadratic.

[2] Least square means based on 6 pens containing 2 gilts or 2 castrated males/pen.

[3] SEM = standard error of the mean.

[4] Belly thickness was used as a covariate.

[5] Calculated as $\cos^{-1}[(0.5 \times L^2 - D^2) / (0.5 \times L^2)]$, where L is the belly length and D is the distance between belly ends when suspended. Greater angle indicates a firmer belly (Whitney et al., 2006).

Table 7
Effects of dietary lipids and vitamin E (VE) supplementation on serum metabolites and α-tocopherol at the end of finisher-2 phases [1].

Item	Lipid, %:	0		2		4		6		SEM [2]	*P*-value				
	VE, IU/kg:	11	220	11	220	11	220	11	220		Lipid, Ln	Lipid, Qd	VE	Lipid × VE	Flax, 0 vs.1%
Total protein [3], g/dL		5.91	5.80	6.09	6.22	6.07	6.26	6.02	6.22	0.14	0.084	0.095	0.314	0.662	0.017
Albumin [3], g/dL		4.10	4.04	4.14	4.45	4.24	4.31	4.29	4.21	0.11	0.167	0.126	0.449	0.331	0.037
Globulin [3], g/dL		1.81	1.77	1.97	1.77	1.82	1.96	1.74	2.02	0.08	0.261	0.455	0.455	0.033	0.185
A:G [4]		2.31	2.34	2.20	2.61	2.35	2.25	2.49	2.15	0.13	0.773	0.739	0.982	0.049	0.876
BUN [3,4], mg/dL		15.0	16.8	16.8	18.4	16.5	17.4	16.6	15.6	1.1	1.000	0.110	0.305	0.565	0.277
Glucose [3], mg/dL		90.2	87.8	90.1	96.9	89.2	92.5	93.9	94.8	3.6	0.240	0.850	0.391	0.626	0.188
Cholesterol [3], mg/dL		91.9	87.1	91.3	92.9	95.5	99.8	100.9	102.3	4.0	0.003	0.812	0.834	0.717	0.028
Triglycerides [3], mg/dL		33.7	24.4	30.8	34.5	39.0	33.9	41.9	38.6	2.9	<0.001	0.960	0.098	0.173	0.003
Alpha-tocopherol [5], μg/mL		0.2	1.5	0.4	0.9	0.3	1.3	0.7	1.0	0.3	0.908	0.706	<0.001	0.316	0.792

[1] Least square means based on 6 pens containing 2 gilts or 2 castrated males/pen.
[2] SEM = pooled standard error of the mean.
[3] For serum metabolites, the average value in each pen was used in statistical analysis.
[4] A:G = albumin to globulin ratio; and BUN = serum urea nitrogen.
[5] Pooled samples for each pen (2 pigs) were used for vitamin E analysis (detection limit = 0.2 μg/mL).

CHAPTER IV.

EFFECT OF ANIMAL FAT, FLAXSEED OIL, AND VITAMIN E SUPPLEMENTATION ON ORGANOLEPTIC CHARCATERISTICS AND FATTY ACID PROFILE OF PORK, AND GENE EXPRESSION IN ADIPOSE AND MUSCLE TISSUES

Effect of animal fat, flaxseed oil, and vitamin E supplementation on organoleptic characteristics and fatty acid profile of pork, and gene expression in adipose and muscle tissues

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A B S T R A C T

A total of 96 pigs were used to investigate the effects of dietary lipid (0, 1, 3, and 5% animal fat +1% flaxseed oil for diets with supplemental lipids) and vitamin E (11 or 220 IU vitamin E/kg) supplementation in a 4 × 2 factorial arrangement of treatments on pork quality, sensory characteristics, and fatty acid (FA) composition, and expression of genes associated with lipid metabolism. Forty-eight pens containing 2 gilts or 2 castrated males were randomly assigned to 8 treatments with 3 gilt pens and 3 castrated male pens per treatment. Pigs were slaughtered when they reached approximately 110 kg. Muscle and backfat samples at the last rib were collected to determine expression of genes associated with lipid metabolism. Loin muscle were collected for sensory characteristics and FA profile analysis. The percentage of the saturated FA and monounsaturated FA were reduced linearly ($P < 0.012$), while the percentage of ω-6 polyunsaturated FA (PUFA), total PUFA, and C18:2ω6c increased linearly ($P < 0.001$) with increased dietary lipids. Flaxseed oil supplementation increased 18:3ω3, 20:3ω3, and 20:5ω3, and decreased the ω-6 to ω-3 FA ratio and the linoleic acid to α-linolenic acid ratio ($P < 0.001$). The content of ω-3 PUFA and ω-3 highly unsaturated FA was greatest in pigs fed the diet containing 2% lipids (quadratic, $P < 0.001$). Serum α-tocopherol content were greater ($P < 0.001$) in pigs fed 220 IU vitamin E/kg than those fed 11 IU vitamin E/kg. Lipid supplementation had no effect on serum α-tocopherol content. Dietary treatments had no effects on Warner-Bratzler shear force, cook loss, lipid content, and thiobarbituric acid reactive substances content of loin muscle. No major differences were found on sensory characteristics and gene expression associated with lipid metabolism. In conclusion, feeding flaxseed oil and animal fat in finisher pigs is effective in improving the nutritional value of pork as indicated by ω-6 to ω-3 FA ratio without negative effect on organoleptic characteristics or oxidative stability of pork.

Keywords: pig, flaxseed oil, vitamin E, fatty acid composition, organoleptic characteristics, gene expression

1. Introduction

Modification of carcass fat content and fatty acid (FA) composition by lipid supplementation to optimize meat quality and healthiness to satisfy consumers' demand has received much attention in recent years. Typical grower-finisher pig diets contain high amounts of carbohydrate, and de novo lipogenesis from glucose accounts for 74 to 77% of adipose tissue triglyceride (Dunshea and D'Souza, 2003). Earlier studies indicated that the degree of inhibition of de novo lipogenesis was depended on the concentration (Allee et al., 1971c) and the source of lipids (Allee et al., 1972), even though it remains controversial whether saturated FA (SFA) and polyunsaturated FA (PUFA) are equally effective in suppressing de novo lipogenesis enzyme activities in pigs (Allee et al., 1972; Camara et al., 1996; Lin et al., 2013; Smith et al., 1996). After ingestion, the pre-formed FA from the diets are available for direct deposition (Duarte et al., 2014).

The optimal ratio of ω-6 FA to ω-3 FA is 1:1 for the human during evolution (Simopoulos, 2008), but the ratio in a typical pork product is more than 10:1 (Romans et al., 1995). Addition of flaxseed or flaxseed oil in the diet is an effective way to enrich ω-3 FA and lower the ratio of ω-6 to ω-3 in pork (Hoz et al., 2003; Juárez et al., 2011; Romans et al., 1995). Vitamin E is a natural antioxidant that protects cell membranes from oxidative damage (Raederstorff et al., 2015). The negative impact of dietary PUFA on pork quality can be alleviated by dietary supplementation with vitamin E (Guo et al., 2006a; Kim et al., 2015; Vossen et al., 2016). There have been a few studies describing the interaction between vitamin E and

lipid sources on pork quality, fatty acid composition, and lipid oxidation (Guo et al., 2006a; Hoz et al., 2003; Lauridsen et al., 2013; Lopez-Bote et al., 2003; Morel et al., 2006; Rey et al., 2001;). However, little attention has been paid to the possible inadequacy of vitamin E with increased lipids in pig diets (Raederstorff et al., 2015).

Energy metabolism is regulated at both the cellular and whole body through various transcriptional factors controlling gene expression (Lempradl et al., 2015). There is growing concern about the effects of dietary lipids on gene expression associated with lipid metabolism in pigs (Benítez et al., 2015; Benítez et al., 2016; Duran-Montgé et al., 2009a; Kellner et al., 2017; Kim et al., 2014). However, the interactions between dietary lipid and vitamin E on lipid metabolism in pigs is still unknown. The current study was conducted to investigate the effects of dietary lipids and vitamin E supplementation on pork quality, organoleptic characteristics, and fatty acid composition, and the expression of genes associated with lipid metabolism.

2. Materials and methods

2.1. Animals, diets, and sample collection

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Auburn University (Auburn, AL, US). Animal and diets have been previously described (Huang, 2017). In brief, a total of 96 pigs weighing 54 ± 3.4 kg was allocated to pens based on their weight, sex, and ancestry with 2 gilts or 2 castrated males per pen. Two trials were conducted, and in each trial, 24 pens (12 gilt pens and 12 castrated male pens) were assigned randomly to 8 dietary treatments in a 4 [0, 1, 3, and 5% animal fat (+ 1% flaxseed oil for diets with supplemental lipids)] × 2 (11 or 220 IU vitamin E/kg) factorial arrangement of treatments. In total, each treatment contains 3 gilt pens and 3 castrated male pens. At the end of the finisher period (BW: 110.0 ± 3.0 kg), pigs were harvested under USDA inspection at Auburn University

Lambert-Powell Meats Laboratory. After exsanguination, a sample of backfat and loin muscle tissue were collected immediately at the last rib by cutting through the hide. It has been reported that lipid metabolism was considerably different among backfat layers (Camara et al., 1996). Thus, specific tissues (muscle and backfat) were immediately dissected with a sharp razor blade where backfat was separated to get the intermediate (second) layer by two trained panelist in a lab adjacent to the abattoir. Processed samples were quick frozen in liquid N, and stored at -80°C for mRNA abundance analysis. The time elapsed from exsanguination to sample storing was within 5 to 10 min.

The carcass was then chilled for 24 h at 4°C and, then, it was ribbed between the 10th and 11th rib. Then, eight 2.54-cm thick loin chops were collected from the left side loin of each carcass, individually vacuum packaged in a 3-layer oxygen barrier bag (Sealed Air, Cryovac, Charlotte, NC, US), and frozen (-20°C) for pork quality and fatty acid profile analyses.

2.2. Pork quality traits

Loin chops were used for measuring cook loss, Warner-Bratzler Shear Force (WBS), proximate analysis, and thiobarbituric acid reactive substance (TBARS). All the procedures of measurements for meat quality were described in detail elsewhere (Magee, 2017).

2.3. Fatty acid profile determination

Fatty acid composition of muscle samples were determined according to O'Fallon et al. (2007). Vacuum-packed loin chops were thawed at 4°C overnight. After thawing, lean muscle tissue was physically separated from backfat and minced using a knife, and then ground for 15 s with a coffee grinder (Model 10029; Bella Cucina, Atlanta, GA, US). Ground lean muscle samples (1.00 g ± 0.01 g) were placed in a 15-mL culture tube with cap and Teflon liners (Pyrex 9825-16X; Corning Mexicana, San Nicolás, Nuevo León, Mexico) to which 5.3 mL of methanol,

0.7 mL of KOH (10 N, in water), and 0.5 mg of tridecanoic acid methyl ester (C13:0; Sigma-Aldrich, St. Louis, MO, US) were added prior to extraction. The methyl C13:0 was prepared as 0.5 mg/mL in methanol, and it was used as an internal standard. Then, the tubes were incubated in reciprocal shaking water bath (Model Precision 2870; Thermo Scientific, Waltham, MA, US) for 90 min at 55°C, with hand-shaking occasionally. After incubation, the tubes were removed and cooled using cold tap water. After cooling, 0.58 mL of H_2SO_4 (24 N) was added to each sample and mixed by inversion, followed by incubation for 90 min at 55°C. The tubes were cooled by tap water, and then 3 mL of hexane was added. After vortex-mixing for 5 min on a multi-tube vortex mixer (VWR Analog; VWR International, Radnor, PA, US) at a speed setting of 10 and centrifuged at 10°C for 5 min at 1,500 x g (Coulter Allegra X-15R Centrifuge; Beckman, Fullerton, CA, US), 1 mL of the top hexane layer containing fatty acid methyl esters (FAME) was placed into a new gas chromatography (GC) vial. The vials were capped and stored at –20 °C until GC analysis.

The FAME were analyzed using a gas chromatography (Shimadzu GC-2014; Shimadzu Co., Ltd., Kyoto, Japan) equipped with a split/splitless injector, a silica capillary column (100 m x 0.25 mm x 0.2 μm; Supelco SP2560; Supelco, Bellefonte, PA., US), and a flame ionization detector (Shimadzu Co., Ltd.). Helium was used as carrier gas at a linear velocity of 20 cm/s. The column flow rate is 1.18 mL/min, and the purge flow is 3 mL/min. The split ratio was 30:1, and the injection volume was 1 μL. The injector and detector temperatures were kept at 250 and 260°C, respectively. The initial oven temperature was 100°C (held for 5 min); subsequently, the temperature was increased to 240°C at a rate of 4°C/min and held for 30 min. Identification of FAME peak were conducted by comparison of their retention times with the standard (Supelco

37-Component FAME Mix Standard, 47885-U; Supelco, Bellefonte, PA., USA), which was ran once-daily. In this experiment, each loin chop was sampled and analyzed in duplicate.

2.4. Serum α-tocopherol concentration

To assess serum α-tocopherol content and metabolic profile, approximately 8 mL of blood was collected from each pig via anterior vena cava puncture using a sterile needle (16 gauge × 7.62 cm) and syringe, 1 d after the end of the finisher-2 phase. All blood samples were collected between 0800 and 1000 h. Blood samples were allowed to clot and centrifuged at 1,500 × g for 15 min at room temperature to obtain cleaner serum samples, and an aliquot was frozen at -20°C until further analysis. Serum α-tocopherol content was analyzed at Iowa State University Veterinary Diagnostic Laboratory (Ames, IA, US). Because the serum triacylglycerol (TAG) and cholesterol concentration were influenced by the dietary lipid treatments, the serum α-tocopherol was adjusted for serum cholesterol or TAG (Prévéraud et al., 2014). Serum concentrations of TAG and cholesterol were determined using an autoanalyzer (Boehringer Mannheim/Hitachi 911; Boehringer Manheim Corp, Indianapolis, IN, US) at Auburn University Clinical Pathology Laboratory (Auburn, AL, US). Serum from individual pigs were used for TAG and cholesterol analysis, whereas pooled samples of each pen (1 mL from each pig) were used for α-tocopherol measurements because of limited amount of each sample.

2.5. Sensory evaluation of pork

Auburn University Institutional Review Board for the Protection of Human Subjects approved the protocol for trained sensory panel evaluation. The evaluation was performed according to a standard protocol (AMSA, 2015). A total of 15 panelists were trained and evaluated initial and sustained juiciness, initial and sustained tenderness, pork flavor intensity, and off-flavor intensity using a 8-point scale (with 1 being extremely dry, extremely tough,

extremely bland, extreme off-flavor, respectively, and 8 being extremely juicy, extremely tender, extremely intense, no off-flavor, respectively). Two 2.54-cm loin chops from each pig were cooked to an internal temperature of 71°C, in the same manner as chops for WBS (Magee, 2017), and cut into 1.27 cm x 1.27 cm cubes. The evaluation was under red lighting, composed of 12 sessions, and no greater than 8 samples were served in each session. All the procedures of measurements for sensory characteristics were described in detail elsewhere (Magee, 2017).

2.6. Messenger RNA abundance analysis

2.6.1. Total RNA extraction and complementary DNA synthesis

Muscle and backfat (intermediate layer) sample were used for the gene expression analysis. Approximately 200 mg of frozen muscle was homogenized in a 5-mL tube containing 2-mL RNAzol RT (Molecular Research Center, Inc, Cincinnati, OH, US), whereas 3 g of frozen backfat was homogenized in 10-mL Tri Reagent (Molecular Research Center, Inc.). Total RNA were extracted and purified further by silica-membrane technology using RNAeasy mini kit (QIAGEN, Inc., Valencia, CA, US) following the manufactures' recommendations.

The RNA quality and quantity were determined using a microplate reader (BioTek Synergy 4 with the Take3 System; BioTek US, Winooski, VT, US). The corresponding optical density (OD) 260/280 ratios were between 2.0 and 2.2, while OD 260/230 ratio were between 1.8 and 2.2 for all samples. The sample purity was further confirmed by spectral scans (200 to 400 nm), which produced smooth curves exhibiting one peak at 260 nm for all RNA samples. Total RNA integrity was determined by analysis of the rRNA band on a 1.5% denaturing formaldehyde gel (GelRed; Biotium Inc., Fremont, CA, US). In short, 2.5 µg of RNA was loaded onto the gel, and subjected to electrophoresis at 5 V/cm until the dye migrated to 2/3 the length of the gel. The RNA band was visualized under UV using GelRed dye staining. The results

showed that all samples had sharp 28S and 18S ribosomal bands, with 28S rRNA band being approximately twice as intense as the 18S rRNA band.

Reverse transcription was subsequently performed (Promega ImPromII RT A3800 Kit; Promega Inc., Madison, WI, US) following the supplier's instruction. In short, a total volume of 5 μL of combination containing approximately 1 μg of purified RNA and 1 μL oligo-dT primer was denatured at 65°C for 5 min, and immediately chilled in ice for 5 min. Then, 15 μL of reverse transcription mix containing approximately 4.8 mM $MgCl_2$, 4 μL of 5 × buffer, 0.5 μL of ribonuclease inhibitor (RNasin; Promega Inc.), 1 μL of reverse transcriptase (ImProm-II; Promega Inc.) and 1 μL of dNTP mix was added to each reaction tube on ice. Finally, the RT reactions were incubated at 25°C for 5 min, followed by 42°C for 50 min, and then at 70°C for 15 min to inactivate the reverse transcriptase. The synthesized cDNA was stored at -20°C, and used as a template for real-time PCR.

2.6.2. Quantitative real-time PCR analysis

The amplification efficiency of primers was measured by the slope of the regression curve using 4 point of serial dilutions of the pooled cDNA template. All primer pairs (Table 1) were adapted from previous reports (Benítez et al., 2016; Bohan et al., 2014; Yan et al., 2013; Yan and Ajuwon, 2016), and those oligonucleotides were synthesized accordingly (Integrated DNA Technologies; Coralville, IA, US). Conventional PCR on cDNA were performed to test the primers and verify amplicon sizes. The specificity of primers was further confirmed by melting curve analysis. Fatty acid synthase (FAS), stearoyl CoA desaturate (SCD), and malic enzyme 1(ME1) were used as biomarkers for de novo lipogenesis. Sterol regulatory element binding protein-1 (SREBP1c) were used as biomarkers for both de novo lipogenesis and adipogenesis. The PPAR-γ was chosen as a marker for adipogenesis. Peroxisome proliferator-activated receptor

α (PPAR-α) was used as a marker for oxidative metabolism. Leptin and adiponectin were measured as indicators for overall adipose tissue metabolic status. Diacylglycerol O-acyltransferase 1 (DGAT1) and DGAT2 were gene markers for TAG synthesis (Yan and Ajuwon, 2016).

Real-time PCR reaction was performed (Roche LightCycler 480 Real-time PCR Machine; Roche Diagnostics, Indianapolis, IN, US). The 20 µL reaction mixture contained: 10 µL of reaction mix (LightCycler 480 SYBR Green I Master Mix (2×); Roche Applied Science, Indianapolis, IN, US), 1 µL of each primers (0.5 µM), 7 µL of distilled deionized H_2O, and 1 µL of cDNA. Sequence of primers and amplicon size are presented in Table 1. Non-template controls were performed in each plate. The PCR conditions were as follows: incubation for 5 min at 95 °C, followed by 45 cycles of 95°C for 10 s, 55 to 60°C for 10 s, and 72°C for 10 s. Finally, melting curve to test primer specificity was obtained by one cycle at 95°C for 5 s, 65°C for 1 min, and ramp up to 97°C at a rate of 0.11°C/s. Amplification data was analyzed with the software (LightCycler 480 SW1.5; Roche Diagnostics, Indianapolis, IN, US) and cycle threshold (Ct) values was obtained. The *S15* gene was validated as a suitable internal control for normalization because the efficiency of the *S15* primers was 100% (Table 2) and no difference was found between any groups on *S15* mRNA levels. Delta Ct values (ΔCt) for each sample were calculated (ΔCt = Ct of the target gene - Ct of the *S15* gene), and were used for the statistical analysis. Results were presented as fold changes, i.e., $2^{\Delta\Delta Ct}$ (ΔΔCt = ΔCt of treatment – ΔCt of control), relative to the data for control treatment group, which fed with 11 IU vitamin E and no lipids (Duran-Montgé et al., 2009a).

2.7. Statistical analysis

All data were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC, US). The pen was used as the experimental unit for pork quality, serum α-tocopherol concentration, sensory evaluation, and fatty acid profile data. The pig was used as the experimental unit for gene expression data. The statistical model initially included lipid, vitamin E, sex, trial, and all possible interactions. The initial analysis indicated that the trial × interactions were not important sources of variance, thus, the dataset from 2 trials was combined. The interactions and covariates, which did not reach significant trend ($P > 0.10$) were, then, deleted from the final model. In addition to the main effect of vitamin E and interaction, 3 preplanned contrasts (0 vs. 1% flaxseed oil, and linear and quadratic effects of supplemental lipids) were used to assess the effect of dietary lipids. The results are considered a statistically significant if $P \leq 0.05$ and a trend if $P \leq 0.10$.

3. Results

3.1. Pork quality and proximate analysis

The pork quality and proximate analysis of loin muscle were measured at the end of finisher-2 phase (Table 3). There were no dietary lipid × vitamin E supplementation interactions on any of the response criteria. Similarly, neither lipid nor vitamin E had any effect on WBS, cook loss, TBARS, and the content of fat and moisture in the muscle tissue.

3.2. Sensory characteristics

There were dietary lipid × vitamin E interactions ($P < 0.05$) on initial juiciness and pork flavor intensity (Table 3). Initial juiciness in pigs fed the diets with vitamin E supplementation increased with 2% lipids, but it decreased in pigs fed the diets with vitamin E supplementation with 4 and 6% lipids. Pork flavor intensity in pigs fed the diets with vitamin E supplementation increased with 2 and 4% lipids, but it decreased in those fed the diets with vitamin E

supplementation with 6% lipids. Vitamin E supplementation tended to increase sustained tenderness ($P = 0.056$). The off-flavor intensity decreased and, then, increased with dietary lipids (quadratic, $P < 0.05$). Lower off-flavor score in pigs fed the diets with 2% lipids had more intense off-flavor. There was no effect of dietary treatments on initial tenderness.

3.3. Fatty acid profile of loin muscles

Dietary lipids had a major impact on the lipid profile in the muscle (Table 4). The percentage of total PUFA (% of total FA), ω-6 PUFA, and C18:2ω6c increased linearly ($P < 0.001$) with increasing dietary lipids. The percentage of ω-3 PUFA and ω-3 HUFA increased quadratically ($P < 0.001$) with increasing dietary lipids because the ALA, 20:3ω3, and EPA fractions increased quadratically ($P < 0.001$). The content of ω-3 PUFA and ω-3 HUFA was greatest in pigs fed diet containing 2% lipids (quadratic, $P < 0.001$). The SFA declined linearly ($P < 0.01$) and MUFA declined linearly ($P < 0.05$) with increasing dietary lipids. The percentage of most of the SFA (i.e., C14:0, C16:0, C16:1, C17:0), and C18:1n9c decreased linearly ($P < 0.05$) with increasing dietary lipids. The ω-6 to ω-3 ratio and the LA to ALA ratio decreased quadratically with increasing dietary lipids ($P < 0.001$). The ratio of ω-6 to ω-3 was 5.18:1 in pigs fed diet containing 2% lipids and 220 IU vitamin E/kg diet, which was the lowest among all the dietary treatments.

Compared with pigs fed the basal diets, a lower LA to ALA ratio, as well as a lower ω-6 to ω-3 PUFA ratio, was obtained in those fed flaxseed oil diets (flax 0 vs.1%; $P < 0.001$). Feeding the flaxseed oil diets led to 126, 300, and 13% increase (flax 0 vs.1%; $P < 0.001$) in ω-3 PUFA, ω-3 HUFA, total PUFA, respectively, because of a greater ($P < 0.01$) proportion of C18:2ω6c, ALA, C20:3ω3, and EPA. The content of ALA, C20:3ω3, and EPA, increased to two-, four-, and eight- fold, respectively, with flaxseed oil supplementation. Similarly, a trend in

increasing the percentage of DHA to two-fold was observed in pigs fed the diets containing flaxseed oil ($P = 0.096$).

There were dietary lipid × vitamin E interactions on C20:3ω3 ($P < 0.05$) and C16:1 ($P = 0.062$). Compared with pigs fed the diets containing the same amount of lipids without vitamin E, those fed the diets with 6% lipids and supplemented with vitamin E had a lower percentage of C20:3ω3, whereas those fed 2 and 4% lipids with vitamin E supplementation had a greater percentage of C20:3ω3. Pigs receiving 2 and 6% lipids tended to have a lower percentage of C16:1 with vitamin E supplementation, whereas pigs fed the diets with no lipids and vitamin E supplementation had a greater proportion of C16:1. Vitamin E supplementation alone did not affect most of the FA content, except for a slight decrease ($P < 0.01$) in C18:1n9t and a tendency ($P = 0.08$) to decrease C22:0. However, dietary treatments did not affect the proportions of C10:0, C18:0, C18:2ω6t, C20:0, C18:3ω6, 20:1n9, 20:2ω6, 20:3ω6, and 20:4ω6.

3.4. Serum α-tocopherol concentration

The concentrations of serum α-tocopherol were analyzed at the end of the finisher-2 phase (Fig. 1A). Because the serum TAG and cholesterol concentration can be affected by the dietary treatments, serum α-tocopherol was adjusted for serum cholesterol (Fig. 1B) or TAG (Fig. 1C). There was no dietary lipid × vitamin E interaction on serum α-tocopherol. As expected, serum α-tocopherol content was greater ($P < 0.001$) in pigs fed the diets containing 220 IU vitamin E/kg than those fed the diets with 11 IU vitamin E/kg (Fig. 1A). Lipid supplementation had no effect on the serum α-tocopherol content. The adjustment of serum α-tocopherol with serum TAG and cholesterol concentration did not change the difference among treatments (Fig. 1B, C).

3.5. mRNA abundance in adipose tissue

In the adipose tissue, the expression of *SREBP1c* gene linearly ($P = 0.036$) increased with increasing dietary lipids (Table 5). The mRNA abundance of *leptin* tended ($P = 0.066$) to be increased linearly with increasing dietary lipids. Addition of flaxseed oil tended to increase the mRNA abundance of *leptin* ($P = 0.07$). The expression of *PPAR-α* gene tended to be greater ($P = 0.057$) in pigs fed the diets supplemented with vitamin E. There was dietary lipid × vitamin E supplementation interaction on the expression of *ME1* gene ($P < 0.01$). Compared with pigs fed the diets containing the same amount of lipids without vitamin E, those fed the diets with 0 or 6% lipids and vitamin E supplementation had a greater abundance of *ME1*, whereas those fed the diets with 2 and 4% lipids and vitamin E supplementation had a lower percentage of *ME1*. There were no dietary treatment effects on the expression of *FAS*, *SCD*, *PPAR-γ*, *adiponection*, *DGAT1,* and *DGAT2*.

3.6. mRNA abundance in muscle tissue

Vtamin E supplementation tended ($P = 0.06$) to lower the expression of *FAS* (Table 6). The expression of *SCD* was high in pigs fed the diets with 4% lipids, medium in pigs fed the diets with 0 and 6% lipids, and low in those fed the diets with 2% lipids (quadratic, $P = 0.016$). Similarly, the expression of *PPAR-γ* was high in pigs fed the diets 4% lipids, medium in pigs fed the diet with 0 and 6% lipids, and low is those fed the diets with 2% lipids (quadratic, $P < 0.05$). Addition of flaxseed oil tended to increase the mRNA abundance of *PPAR-γ* ($P = 0.055$). There were no dietary treatment effects on the expression of *FAS*, *SREBP1c,* and *PPAR-α*. In the muscle tissue, primer test results using pooled cDNA templates showed that *leptin*, *adiponectin*, *DGAT1*, and *DGAT2* mRNA abundance were low, and, thus, qPCR quantification on those genes as not conducted.

4. Discussion

Under practical conditions, the content and composition of body fat is the net result of lipid anabolism (direct deposition of digested lipids and de novo lipogenesis) and catabolism (β-oxidation of FA and their derivatives) in growing animals. The de novo lipogenesis accounts for 74 to 77% of adipose tissue TAG (Dunshea and D'Souza, 2003) when pigs were fed diets containing high amounts of carbohydrates. The primary products of de novo lipogenesis from glucose are SFA, and the major site of de novo lipogenesis is adipose tissues in pigs (O'Hea and Leveille, 1969). Poultry fat is a by-product from animal rendering, which contains approximately 41% of 18:1, 25% of 16:0, 19% of 18:2ω6, and 6.8% of 16:1 (Engel et al., 2001; Huang, 2017). When expressed as percentage of total FA, poultry fat supplemented diets usually have greater percentages of 16:0, 18:0, 16:1, and 18:1, with a lower proportion of 18:2ω6, compared with basal diets consisting mainly of corn and soybean meal (Apple et al., 2009; Seerley et al., 1978). In addition, those basal diets for finisher pigs typically contain 2 to 4% ether extract on an as-fed basis (Lin et al., 2013; Kellner et al., 2017; Quiniou and Noblet, 2012; Romans et al., 1995).

In the current experiment, total SFA, in particular 16:0, was linearly decreased with increasing dietary lipid, which is in agreement with previous report (Engel et al., 2001). Apple et al. (2009) reported a decrease in SFA and 16:0 when 5% poultry fat was added in the diets. Shipp et al. (1997) also showed a reduction in SFA, 16:0, and 18:0 in pigs fed 10% poultry fat compared with those fed the diets devoid of added fat. On the other hand, pigs fed the diets supplemented with poultry fat actually consumed greater amounts of SFA on a daily basis because of greater concentrations of those FA in the diets. Therefore, it is possible that SFA production from de novo lipogenesis was depressed to some extent in pigs fed poultry fat in our study. Interestingly, the content of 18:0 was similar among treatment groups. Thus, the lower

SFA content observed in muscle by lipid supplementation may result mostly from the difference in 16:0.

Numerous studies have shown that enzyme activities associated with de novo lipogenesis would be markedly reduced by a variety of dietary lipids in pigs (Allee et al., 1971a; Allee et al., 1971b; Kouba et al., 2003; Smith et al., 1996). In addition, increasing inclusion rate of lipids (corn oil or tallow) ranging from 1 to 10% resulted in a linear decrease in de novo lipogenesis and key enzyme activities (Allee et al., 1971c). Furthermore, most investigators demonstrated that lipid supplementation with various FA profiles decreased expression of *FAS* or *ME1 or both* compared with the control group (Duran-Montgé et al., 2009a; Kellner et al., 2017). In the current research, the expression of *FAS* and *ME1* was only numerically down-regulated in adipose tissue of pigs fed the diets with lipids.

In addition to the dietary origin, the MUFA (e.g., 16:1 and 18:1) in animals can also be synthesized from SFA (e.g., 16:0 and 18:0) by delta-9 destaturases, which are encoded by *SCD* gene (Nakamura and Nara, 2004). One possibility for the decreased proportion of SFA in the current study is that more SFA (e.g., 16:0 and 18:0) was, perhaps, further converted into MUFA (e.g., 16:1, 18:1) by delta-9 desaturases in pigs fed diets containing poultry fat. However, perhaps, it's not true because the total MUFA, 16:1, and 18:1 in muscle also linearly decreased with increased dietary poultry fat, which contains more than 40% of 18:1. It should be noted that several previous studies did not observe differences on total MUFA, particularly 16:1, in the muscle when poultry fat was included in the diets (Apple et al., 2009; Engel et al., 1998; Seerley et al., 1978). Considering the high content of 16:1 and 18:1 in poultry fat, it is speculated that a reduction of desaturation of 16:0 and 18:0 by delta-9 desaturases was induced in our study. In addition, both ω-3 and ω-6 PUFA are potent inhibitors of SCD (Nakamura and Nara, 2004).

Reduction in expression of *SCD* gene (Duran-Montgé et al., 2009b; Benítez et al., 2015) and enzyme activity (Kouba et al., 2003) have been reported by feeding ω-3 and ω-6 PUFA rich diets to pigs. However, the expression of *SCD* gene was similar in the adipose tissue, but in the muscle tissue, it increased when 4% lipid was included in the diets compared with other treatment groups. Similar results have been reported by Kellner et al. (2017) who showed that the expression of *SCD* gene did not differ by dietary lipid supplementation in the adipose tissue but increased in the liver by the diet supplemented with corn oil (containing 57% of 18:2ω6).

Another possibility for the decreased proportion of SFA and MUFA would be that they were diluted by the increased PUFA content in the muscle. Our results showed that the proportion of total PUFA, particularly 18:2ω6 and 18:3ω3, increased linearly with dietary lipids, indicating that surplus 18:2ω6 from poultry fat and 18:3ω3 from flaxseed oil induced direct deposition in the muscle. The ω6 FA (e.g., 18:2ω6) and ω3 FA are entirely derived from diets (Lin et al., 2013). Most published research have repeatedly reported an increase in 18:2ω6 in the muscle tissue when poultry fat was included in the diets (Seerley et al., 1978; Shipp et al., 1997; Engel et al., 2001; Apple et al., 2009) compared with pigs fed the basal diet with no lipids.

Dietary PUFA are known to interact with PPAR complex and SREBP1c, which mainly suppress the genes involved in de novo lipogenesis and activate the genes involved in oxidation (Georgiadi and Kersten, 2012). However, in vitro study in pigs showed that the expressions of SREBP1c and PPAR-α were not affected by dietary tallow, high-oleic sunflower, sunflower oil, flaxseed oil, fish oil, or their blend, in both the adipose and muscle tissues (Duran-Montgé et al., 2009a). In the current experiment, the mRNA abundance of SREBP1c was linearly increased with increasing dietary lipids, while no differences on PPAR-α was observed. Similar to our results, Kellner et al. (2017) reported that a diet supplemented with corn oil (containing 57% of

18:2ω6) increased mRNA abundance of SREBP1, and PPAR-α was not affected by dietary lipid supplementation. In the current study, flaxseed oil increased ω-3 HUFA content in muscle and tended to increase the expression of *SREBP1c* in the adipose tissue. The dietary ω-3 FA (e.g., flaxseed) has been shown to up-regulate genes involved in adipogenesis (e.g., PPAR-γ; Luo et al., 2009), and, consequently, improved the IMF content in pork (Huang et al., 2008) or marbling score (Juárez et al., 2011). On the contrary, flaxseed oil supplementation did not change ether exact content in muscle, with a tendency to decrease *PPAR*-γ gene in muscle in the current experiment. There were no major interactions between dietary lipids and vitamin E on FA profile and gene expression. Similarly, Lauridsen et al. (2013) found no interaction between dietary lipid sources and vitamin E supplementation, and vitamin E alone did not affect hepatic gene expression.

In adult mice model, a high-fat diet dramatically depressed de novo lipogenesis without affecting FA elogation, but TAG synthetic rate and liver fat content were elevated (Duarte et al., 2014). In the current experiment, no effect of dietary treatments on the expression of DGAT1 and DGAT2 was found in the adipose tissue. Lipid supplementation in pig diets have increased (Apple et al., 2009; Benz et al., 2011), unaffected (De la Llata et al., 2001; Engel et al., 2001; Quiniou and Noblet, 2012), or reduced backfat thickness (Smith et al., 1999). In those studies, Lys to calorie ratio were maintained, and the intake of calorie and Lys were generally similar. In our study, pigs were in growing phases, and dietary lipids had no effect on carcass fattness (Huang, 2017), which may reflect no difference on the TAG synthesis and deposition.

Omega-3 fatty acids can have distinct biological effects on human nutrition (Riediger et al., 2009). The ω-6 to ω-3 ratio is up to 16.7:1 in typical Western diets (Simopoulos, 2008), and, thus, producing pork with lower ω-6 to ω-3 ratio may reduce the risk for various chronic diseases

(Riediger et al., 2009; Simopoulos, 2008). In the current experiment, the ratio in porcine muscle tissue was as high as 12.4:1 in intramuscular fat when pigs fed the diet with no lipids, which was similar to 14:1 when pigs fed typical cereal-based diets (Romans et al., 1995). As expected, feeding the diets with flaxseed oil resulted in a reduction of ω-6 to ω-3 ratio from 12:1 to 5.2:1. The results are in agreement with previous studies (Hoz et al., 2003; Juárez et al., 2011; Luo et al., 2009; Romans et al., 1995), where flaxseed or flaxseed oil supplementation induced ω3 PUFA deposition in muscle. In addition, the increased level of poultry fat resulted in slight increases in ω-6 to ω-3 ratio (as well as LA:ALA) in pigs fed 4 and 6% lipids. Furthermore, the plateau and slight decrease in ω3 HUFA can be explained by the competition between ALA and LA on the desaturation and elongation pathway.

Vitamin E supplementation increased serum α-tocopherol concentration. Similar results have been reported in plasma of finisher pigs (Guo et al., 2006a; Kim et al., 2015), as well as in serum of weanling pigs (Lauridsen, 2010). The blood vitamin E concentration has been shown to be highly correlated with that in the diet and muscle, respectively (Kim et al., 2015; Prévéraud et al., 2014). Therefore, the dietary vitamin E supplementation is likely to increase its concentration in porcine tissues (e.g., adipose, muscle, and liver; Boler et al., 2009; Guo et al., 2006a; Kim et al., 2015; Prévéraud et al., 2014). In the current study, dietary lipids did not affect serum α-tocopherol concentration, contrary to some previous studies, in which PUFA decreased plasma α-tocopherol in grower pigs (Lauridsen, 2010; Prévéraud et al., 2014).

Vitamin E is a well-known antioxidant at the cell membrane level. Previous studies showed that vitamin E supplementation at levels ranged from 100 to 700 IU/kg reduced lipid peroxidation (as indicated by TBARS values) in fresh whole muscle (Kim et al., 2015) and pre-frozen loin chops (Asghar et al., 1991; Guo et al., 2006b; Hoving-Bolink et al., 1998; Monahan

et al., 1994). In contrast to those previous reports, vitamin E supplementation at 220 IU/kg did not affect TBARS values in frozen loin chops in the present experiment. In addition, dietary vitamin E alone had minimal effects on FA profile, which is similar to the results reported by Juárez et al. (2011). In contrast, some investigators reported that vitamin E supplementation reduced the percentage of SFA while increasing proportion of unsaturated FA (Guo et al., 2006b).

Enhancing ω3 FA has been known to compromise sensory characteristics of pork products (Romans et al., 1995). There were no major differences in sensory panel evaluations when pigs were fed diets containing up to 10% flaxseed (Matthews et al., 2000), which equals to 4% flaxseed oil because the oil content in flaxseed is approximately 40%. In the current study, flaxseed oil and vitamin E supplementation had little effect on pork quality and sensory characteristics. Previous studies demonstrated the protective effects of vitamin E supplementation on sensory characteristics (Kouba et al., 2003). In the present experiment, the TBARS values were below the threshold level of 0.5 to 1.0 mg MDA/kg tissue, which has been proposed for detecting rancidity and warmed-over off-flavor in pork by trained sensory panelists (Dunshea et al., 2005; Gray and Pearson, 1987). In addition, ALA was approximately 2% of total FA in the present study, and Wood et al. (2003) indicated that adverse effects on flavors can be detected when ALA approached 3% of total FA. It seem that the protective effects of vitamin E are more pronounced under conditions when FA are prone to peroxidation, such as ground pork (Asghar et al., 1991; Monahan et al., 1990), pre-frozen pork (Monahan et al., 1994), or greater amounts of unsaturated FA were included in the diets (Vossen et al., 2016).

5. Conclusion

Dietary lipid levels and composition, rather than vitamin E supplementation had major impact on the FA profile in the muscle tissues. Flaxseed oil (1%) supplementation largely

increased ω-3 PUFA content, reduced the percentage of SFA and MUFA, and reduced the ω-6 to ω-3 ratio from 12:1 to 5:1. Dietary supplementation with 220 IU vitamin E/kg increased serum α-tocopherol, but it did not change TBARS values in muscle. No major differences were found on pork quality, sensory characteristics, and expression of genes associated with lipid metabolism. Therefore, feeding flaxseed oil and animal fat in finisher pigs is effective in improving nutritional value of pork without negative effect on organoleptic characteristics or oxidative stability of pork.

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Table 1
List of primers for quantitative real-time PCR analysis [1].

Gene symbol	Gene bank accession number	Forward（5'- 3'）	Reverse（5'- 3'）	Size (bp)	Annealling temperature (°C)	Reference
S15	NM_214334.1	GGTAGGTGTCTACAATGGCAAGG	GGCCGGCCATGCTTC	114	55	Bohan et al. (2014)
FAS	NM001099930	GCAGGCGCGTGATGGGAATGGTG	GCCCGAGCCCGAGTGGATGAGCA	206	60	Benítez et al. (2016)
Leptin	GQ268935	GGCCCTATCTGTCCTACGTTGAAG	TGGAAGGCAGACTGGTGAGGAT	237	60	Benítez et al. (2016)
SCD	NM_213781.1	TCCCGACGTGGCTTTTTCTTCTC	CTTCACCCCAGCAATACCAG	205	60	Benítez et al. (2016)
PPAR-γ	NM_214379.1	GGCGAGGGCGATCTTGACAG	GATGCGAATGGCCACCTCTTT	148	60	Benítez et al. (2016)
ME1	XM_001924333.4	GCCGGCTTTATCCTCCTCT	TCAAGTTTGGTCTGTATTTTCTGG	223	60	Benítez et al. (2016)
SREBP-1c	NM_214157.1	ACCGCTCTTCCATCAATGAC	AATGTAGTCGATGGCCTTGC	106	55	Yan and Ajuwon (2016)
PPAR-α	NM_001044526.1	TGCCAGTATTGTCGTTTCCA	GGCCTTGACCTTGTTCATGT	219	55	Yan et al. (2013)
Adiponectin	NM_214370.1	TCTCGGCCAGGAAACCACCGA	CGGCCTGGGGTACCGTTGTG	117	60	Bohan et al. (2014)
DGAT1	AY116586.1	CCAGCAGAGGGTTCAAGACA	GACTAGCTGCCTCATCCAGG	109	55	Yan and Ajuwon (2016)
DGAT2	NM_001160080	CGAGACTACTTTCCCATCCA	GAACTTCTTGCTCACTTCGG	147	55	Bohan et al. (2014)

[1] *FAS* = fatty acid synthase; *SCD* = stearoyl CoA desaturate; *PPAR-γ* = peroxisome proliferator-activated receptor γ; *ME1* = malic enzyme 1; *SREBP1c* = sterol regulatory element binding protein-1c; *PPAR-α* = peroxisome proliferator-activated receptor α; *DGAT1* = diacylglycerol acyltransferase 1; and *DGAT2* = diacylglycerol O-acyltransferase 2.

Table 2
Gene Ct values and qPCR efficiency in the studied tissues [1].

Genes	Adipose tissue		Muscle Tissue	
	Average Ct ± SD	Efficiency (%)	Average Ct ± SD	Efficiency (%)
S15	19.8 ± 0.8	99	19.7 ± 0.8	100
FAS	19.9 ± 1.8	94	25.4 ± 1.3	89
Leptin	24.6 ± 1.7	100	–	–
SCD	18.7 ± 2.3	96	25.6 ± 1.5	94
PPAR-γ	21.0 ± 1.3	96	26.6 ± 0.7	91
ME1	21.1 ± 1.7	103	26.0 ± 1.2	96
Adiponectin	17.8 ± 1.3	98	–	–
SREBP1c	26.1 ± 2.2	102	26.6 ± 1.4	95
PPAR-α	27.0 ± 1.5	101	26.7 ± 1.0	94
DGAT1	28.1 ± 1.3	97	–	–
DGAT2	20.5 ± 1.5	94	–	–

[1] *FAS* = fatty acid synthase; *SCD* = stearoyl CoA desaturate; *PPAR-γ* = peroxisome proliferator-activated receptor γ; *ME1* = malic enzyme 1; *SREBP1c* = sterol regulatory element binding protein-1c; *PPAR-α* = peroxisome proliferator-activated receptor α; *DGAT1* = diacylglycerol acyltransferase 1; and *DGAT2* = diacylglycerol O-acyltransferase 2.

Table 3
Effects of dietary lipids and vitamin E (VE) supplementation on physical and sensory characteristics of pork [1,2].

Item	Lipid, %:	0		2		4		6		SEM [3]	*P*-value				
	VE, IU/kg:	11	220	11	220	11	220	11	220		Lipid, Ln	Lipid, Qd	VE	Lipid × VE	Flax, 0 vs. 1%
Pork characteristics															
WBS, kg		4.60	4.65	4.05	4.07	4.35	3.94	4.73	3.78	0.36	0.373	0.191	0.213	0.487	0.116
Cook loss, %		16.01	17.06	15.38	15.72	18.25	16.07	16.26	13.37	1.33	0.404	0.474	0.342	0.399	0.526
TBARS [4]		0.175	0.171	0.209	0.170	0.188	0.188	0.174	0.196	0.014	0.439	0.338	0.589	0.204	0.210
Fat, %		4.01	4.42	4.75	4.21	4.22	4.26	3.97	4.10	0.30	0.410	0.264	0.967	0.478	0.872
Moisture, %		76.42	75.60	75.44	75.96	75.77	75.42	76.00	76.04	0.33	0.939	0.131	0.520	0.244	0.389
Sensory characteristics [5]															
Initial juiciness		5.87	5.85	5.17	5.67	5.76	5.56	5.70	5.57	0.13	0.296	0.027	0.659	0.035	0.009
Sustained juiciness		5.66	5.67	5.08	5.54	5.62	5.41	5.46	5.47	0.14	0.361	0.123	0.469	0.105	0.040
Initial tenderness		5.21	5.61	4.93	5.40	5.49	5.53	5.27	5.10	0.16	0.518	0.734	0.112	0.155	0.343
Sustained tenderness		5.08	5.50	4.78	5.31	5.38	5.40	5.08	5.05	0.17	0.537	0.738	0.056	0.246	0.368
Pork flavor intensity		5.91	5.95	5.65	5.89	5.80	5.91	6.08	5.82	0.08	0.564	0.023	0.517	0.018	0.243
Off flavor intensity		7.73	7.87	7.57	7.72	7.59	7.76	7.82	7.72	0.08	0.810	0.048	0.146	0.361	0.144

[1] Ln = linear, Qd = quadratic, Flax = flaxseed oil, WBS = Warner-Bratzler shear force, and TBARS = thiobarbituric acid reactive substance.

[2] Least square means based on 6 pens containing 2 gilts or 2 castrated males/pen.

[3] SEM = pooled standard error of the mean.

[4] TBARS were expressed as mg malondialdehyde/kg meat.

[5] Based on an 8-point scale with 1 being extremely dry, extremely tough, extremely bland, extreme off-flavor, respectively, and 8 being extremely juicy, extremely tender, extremely intense, no off-flavor, of pork for initial and sustained juiciness, initial and sustained tenderness, pork flavor intensity, and off-flavor intensity, respectively.

Table 4

Effects of dietary lipids and vitamin E (VE) supplementation on fatty acid profile of loin muscle (% of total fatty acids) [1,2].

Item	Lipid, %: 0		2		4		6		SEM [3]	*P*-value				
	VE, IU/kg: 11	220	11	220	11	220	11	220		Lipid, Ln	Lipid, Qd	VE	Lipid × VE	Flax, 0 vs. 1%
C10:0	0.03	0.04	0.03	0.01	0.03	0.02	0.01	0.03	0.01	0.264	0.753	0.916	0.347	0.208
C12:0	0.04	0.03	0.03	0.04	0.02	0.02	0.01	0.02	0.01	0.082	0.667	0.863	0.926	0.323
C14:0	1.14	1.14	1.16	1.14	1.06	1.09	1.04	1.04	0.04	0.002	0.447	0.833	0.936	0.068
C16:0	24.63	25.15	24.81	24.25	24.40	24.26	23.73	24.06	0.38	0.011	0.895	0.895	0.489	0.045
C16:1	3.43	3.69	3.64	3.31	3.47	3.45	3.37	3.29	0.11	0.047	0.789	0.604	0.062	0.122
C17:0	0.22	0.21	0.20	0.18	0.18	0.18	0.17	0.17	0.02	0.013	0.391	0.551	0.923	0.014
C18:0	11.07	10.91	10.57	11.51	10.81	10.58	10.51	10.82	0.34	0.223	0.879	0.379	0.306	0.488
C18:1n9t	0.55	0.35	0.37	0.34	0.49	0.40	0.44	0.42	0.04	0.798	0.182	0.005	0.127	0.250
C18:1n9c	43.85	45.41	42.83	42.77	43.09	43.41	42.52	41.75	0.85	0.012	0.558	0.662	0.578	0.009
C18:2ω6t	0.01	0.01	0.08	0.02	0.01	0.00	0.00	0.04	0.03	0.865	0.518	0.704	0.335	0.511
C18:2ω6c	12.79	10.94	12.75	12.84	13.02	13.08	14.61	14.79	0.65	<0.001	0.442	0.417	0.351	0.004
C18:3ω6	0.07	0.08	0.06	0.05	0.07	0.06	0.06	0.08	0.02	0.768	0.412	1.000	0.889	0.394
C18:3ω3	0.97	0.99	1.99	2.00	1.93	1.98	2.00	2.05	0.07	<0.001	<0.001	0.488	0.982	<0.001
C20:0	0.13	0.12	0.17	0.14	0.16	0.13	0.15	0.13	0.02	0.543	0.198	0.143	0.814	0.162
C20:1n9	0.05	0.04	0.05	0.05	0.03	0.05	0.04	0.06	0.02	0.876	0.780	0.675	0.768	0.904
C20:2ω6	0.39	0.36	0.40	0.38	0.36	0.40	0.42	0.39	0.02	0.258	0.841	0.626	0.213	0.315
C20:3ω6	0.43	0.36	0.32	0.30	0.38	0.35	0.43	0.41	0.04	0.317	0.016	0.237	0.886	0.348
C20:3ω3	0.06	0.06	0.21	0.35	0.14	0.29	0.20	0.15	0.04	0.032	<0.001	0.032	0.029	<0.001
C20:4ω6	0.03	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.391	0.802	0.933	0.575	0.413
C20:5ω3	0.04	0.03	0.24	0.22	0.30	0.19	0.19	0.24	0.04	<0.001	<0.001	0.451	0.274	<0.001
C22:0	0.04	0.03	0.03	0.02	0.03	0.01	0.06	0.00	0.02	0.798	0.331	0.080	0.525	0.449
C22:6ω3	0.05	0.02	0.07	0.09	0.05	0.05	0.07	0.07	0.02	0.288	0.446	0.749	0.796	0.096
SFA	37.29	37.65	36.99	37.27	36.68	36.28	35.67	36.26	0.58	0.007	0.829	0.615	0.850	0.053
MUFA	47.87	49.50	46.89	46.46	47.08	47.31	46.36	45.51	0.92	0.012	0.566	0.825	0.562	0.009
ω-3 PUFA	1.12	1.10	2.51	2.65	2.41	2.51	2.45	2.51	0.09	<0.001	<0.001	0.311	0.870	<0.001
ω-3 HUFA	0.15	0.11	0.52	0.65	0.48	0.53	0.45	0.46	0.05	<0.001	<0.001	0.362	0.442	<0.001

ω-6 PUFA	13.72	11.76	13.61	13.61	13.85	13.90	15.52	15.71	0.79	0.001	0.440	0.447	0.482	0.016
Total PUFA	14.84	12.86	16.12	16.26	16.26	16.41	17.97	18.22	0.71	<0.001	0.569	0.479	0.345	<0.001
LA:ALA	13.23	11.06	6.45	6.48	6.78	6.64	7.38	7.37	0.54	<0.001	<0.001	0.139	0.132	<0.001
ω-6:ω-3	12.39	10.70	5.40	5.18	5.78	5.56	6.40	6.32	0.44	<0.001	<0.001	0.081	0.222	<0.001
PUFA:SFA	0.40	0.34	0.44	0.44	0.44	0.45	0.50	0.50	0.02	<0.001	0.707	0.510	0.490	<0.001

[1] Ln = linear, Qd = quadratic, Flax = flaxseed oil, SFA= saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; HUFA = highly unsaturated fatty acids; LA: ALA = linoleic acid (18:2ω6) to α-linolenic acid (18:3ω3) ratio; and ω-6:ω-3 = ω-3 fatty acids to ω-6 fatty acids ratio.

[2] Least square means based on 6 pens containing 2 gilts or 2 castrated males/pen.

[3] SEM = pooled standard error of the mean.

[4] ω-3 PUFA = C18:3ω3 + C20:3ω3 + C20:5ω3 + C22:6ω3; ω-3 HUFA = C20:3ω3 + C20:5ω3 + C22:6ω3; ω-6 PUFA = C18:2ω6t + C18:2ω6c + C20:2ω6 + C20:3ω6 + C20:4ω6; and total PUFA = ω-3 PUFA + ω-6 PUFA.

Table 5
Effects of dietary lipids and vitamin E (VE) supplementation on mRNA abundance in adipose tissue at the end of finisher-2 phases [1,2].

Item	Lipid, %:	0		2		4		6		SEM [3]	*P*-value				
	VE, IU/kg:	11	220	11	220	11	220	11	220		Lipid, Ln	Lipid, Qd	VE	Lipid × VE	Flax, 0 vs. 1%
FAS		1.08	0.58	0.71	0.64	1.04	0.44	1.05	0.71	0.21	0.142	0.792	0.482	0.514	0.223
SCD		1.00	1.01	1.68	1.21	0.84	1.04	0.66	0.95	0.34	0.208	0.570	0.113	0.324	0.529
PPAR-γ		1.00	0.30	0.72	0.57	0.28	0.56	0.55	0.68	0.22	1.000	0.637	0.422	0.572	0.741
ME1		1.05	1.50	0.88	0.78	0.99	0.71	1.08	1.41	0.34	0.962	0.946	0.116	0.009	0.899
SREBP1c		1.13	0.90	1.69	0.96	0.91	1.13	1.18	1.48	0.42	0.036	0.684	0.351	0.430	0.070
PPAR-α		1.00	0.84	0.61	1.25	0.86	0.96	1.06	1.54	0.30	0.209	0.492	0.057	0.134	0.505
Leptin		1.13	0.98	0.61	1.00	1.06	1.13	1.65	1.00	0.36	0.066	0.718	0.216	0.544	0.326
Adiponectin		1.06	0.45	1.49	0.84	0.73	1.01	0.80	1.18	0.32	0.379	0.999	0.510	0.217	0.487
DGAT1		1.00	0.50	2.21	1.33	0.61	1.29	0.78	1.32	0.41	0.148	0.966	0.147	0.735	0.378
DGAT2		1.00	0.77	1.10	1.13	1.16	0.81	0.80	1.49	0.37	0.178	0.425	0.100	0.565	0.163

[1] Least square means based on 12 pigs per treatments. Data were expressed relative to the group fed diets containing no lipid and 11 IU VE/kg.

[2] Ln = linear, Qd = quadratic, Flax = flaxseed oil, *FAS* = fatty acid synthase; *SCD* = stearoyl CoA desaturate; *PPAR-γ* = peroxisome proliferator-activated receptor γ; *ME1* = malic enzyme 1; *SREBP1c* = sterol regulatory element binding protein-1c; *PPAR-α* = peroxisome proliferator-activated receptor α; *DGAT1* = diacylglycerol acyltransferase 1; and *DGAT2* = diacylglycerol O-acyltransferase 2.

[3] SEM = pooled standard error of the mean.

Table 6
Effects of dietary lipids and vitamin E (VE) supplementation on mRNA abundance in muscle tissue at the end of finisher-2 phases [1,2].

Item	Lipid, %:	0		2		4		6		SEM [3]	*P*-value				
	VE, IU/kg:	11	220	11	220	11	220	11	220		Lipid, Ln	Lipid, Qd	VE	Lipid × VE	Flax, 0 vs.1%
FAS		1.00	1.07	0.77	0.60	0.70	0.55	0.33	0.52	0.38	0.906	0.194	0.060	0.112	0.614
SCD		1.00	1.39	1.31	0.85	1.86	1.70	1.34	0.98	0.46	0.704	0.016	0.240	0.179	0.127
PPAR-γ		1.00	1.34	0.87	0.90	1.48	0.97	1.73	0.27	0.40	0.458	0.018	0.976	0.396	0.055
ME1		0.97	1.35	2.33	0.97	0.78	0.52	0.84	2.19	0.68	0.117	0.473	0.852	0.582	0.234
SREBP1c		1.00	1.84	2.22	1.27	0.86	0.97	1.61	2.44	0.41	0.420	0.925	0.852	0.577	0.450
PPAR-α		1.00	1.26	0.72	0.82	0.55	0.51	0.87	0.80	0.40	0.153	0.924	0.379	0.813	0.176

[1] Least square means based on 12 pigs per treatments. Data were expressed relative to the group fed diets containing no lipid and 11 IU VE/kg.

[2] Ln = linear, Qd = quadratic, Flax = flaxseed oil, *FAS* = Fatty acid synthase; *SCD* = stearoyl CoA desaturate; *PPAR-γ* = peroxisome proliferator-activated receptor γ; *ME1* = malic enzyme 1; *SREBP1c* = sterol regulatory element binding protein-1; and *PPAR-α* = peroxisome proliferator-activated receptor α.

[3] SEM = pooled standard error of the mean.

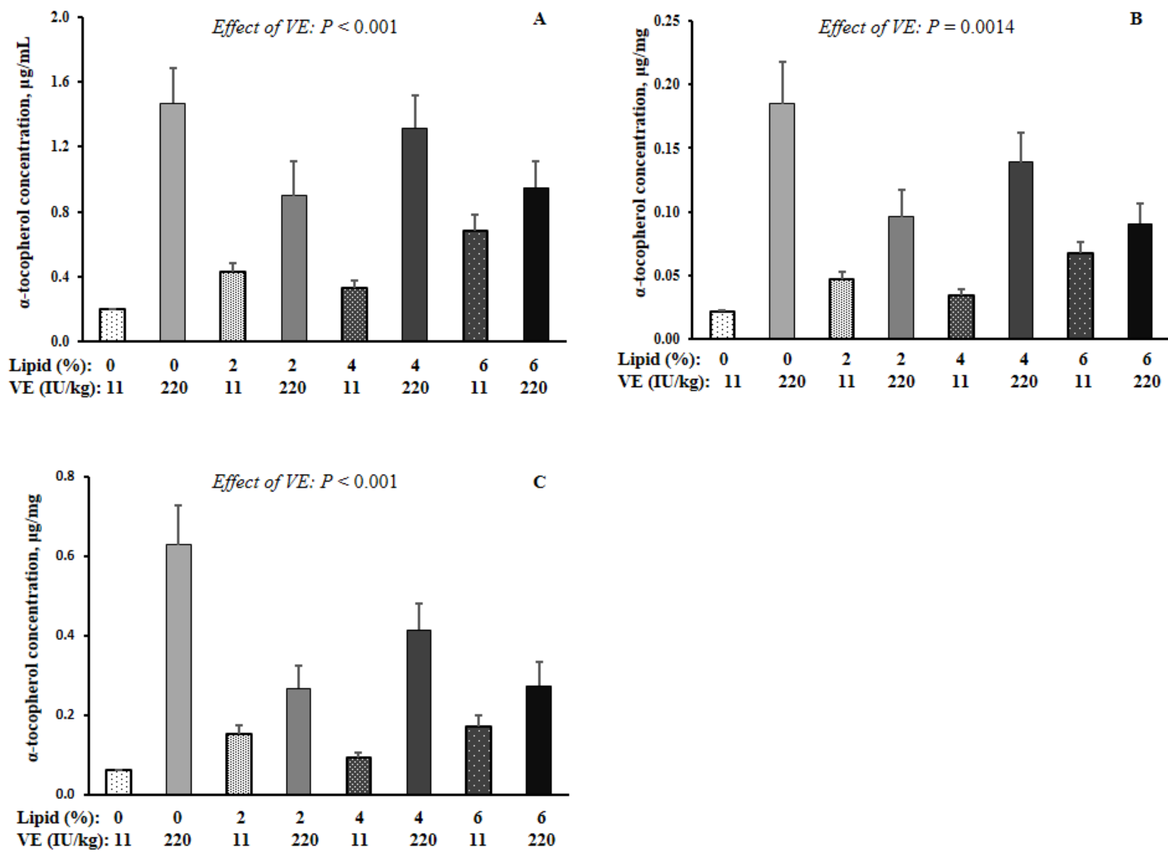

Fig. 1. Concentrations of vitamin E (VE; α-tocopherol) were expressed as µg/mL serum (A), µg/mg cholesterol (B), µg/mg triglyceride (C). Serum from individual pigs were used for cholesterol and triglyceride analysis, whereas pooled samples of each pen were used for vitamin E measurements. The pen was the experimental unit for statistical analysis. Each treatment had 6 pens (2 barrows or 2 gilts per pen) and values were expressed as the mean ± standard error.

CHAPTER V.

BIOAVAIABILITY, METABOLISM, DEPOSITION OF OMEGA-3 POLYUNSATURATED FATTY ACIDS IN PIGS: A REVIEW

Bioavailability, metabolism, deposition of omega-3 polyunsaturated fatty acids in pigs: A review

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A B S T R A C T

Although omega-3 fatty acids exhibit distinct biological effects on human nutrition, the global consumption of omega-3 fatty acids is still inadequate. Common foods such as meat products contain insufficient amount of omega-3 fatty acids, and, thus, the manipulation of lipid composition may be an effective way of adding value to pork products for the health conscious consumers. Several plant oilseeds are high in linolenic acid, whereas marine oils are usually high in long-chain omega-3 fatty acids. Lipid composition and configuration and other dietary factors have a large impact on fatty acid bioavailability. The enzymatic conversion pathway of α-linolenic acid to docosahexaenoic acid in mammals is tightly regulated, and this process seems to be relatively inefficient in pigs. In this review, we focus on bioavailability and metabolism and address the efficiency of different dietary sources of omega-3 fatty acids in omega-3 fatty acid-fortified pork production.

Keywords: omega-3 fatty acids, pig, pork, flaxseed, enrichment, lipid

1. Introduction

The ω-3 PUFA are a series of polyunsaturated fatty acids with the first double bond starting at the third carbon from the methyl end, including α-linolenic acid (ALA, 18:3ω3), stearidonic acid (SDA, 18:4ω3), eicosapentaenoic acid (EPA, 20:5ω3), docosapentaenoic acid (DPA, 22:5ω3), and docosahexaenoic acid (DHA, 22:6ω3), etc. Both the ω-3 and ω-6 PUFA have no less than 2 double bonds. All ω-3 PUFA are incorporated primarily into cell membrane phospholipids in most tissues, whereas DHA is the most abundant and is especially concentrated in the brain and retina (Arterburn et al., 2006; Blank et al., 2002). The ALA serves as a precursor for long chain (LC) ω-3 PUFA, including EPA and DHA. However, the bioconversion capacities

vary between and within mammalian species, and seem to be inefficient in humans (Arterburn et al., 2006; Plourde and Cunnane, 2007).

Since early studies observed an association between marine ω-3 PUFA and biological functions (Dyerberg et al., 1978; Dyerberg and Bang, 1979), the health benefits of LC ω-3 PUFA have been widely reported during the last 5 decades. Although some island nations consume more than optimal amounts, the dietary intake of ω-3 PUFA falls short of recommendations in most countries (Micha et al., 2014). Producing pork naturally fortified with ω-3 PUFA by feeding appropriate oil or oilseeds, is one of the potential ways to increase the intake of ω-3 PUFA in human diets without changing the behavior of consumers in certain populations.

Several reviews on the health benefit, feeding strategies and associated challenges in production of ω-3 PUFA-enriched pork have been published (Corino et al., 2014; Dugan et al., 2015; Ma et al., 2016). However, the authors of those reviews paid less attention to metabolism of ω-3 PUFA, whole body deposition efficiency, and regulation of incorporation of ALA into LC ω-3 PUFA. The aim of this article is to review the recent work on benefits and bioavailability of ω-3 fatty acid (FA), as well as the ω-3 biosynthetic pathway, to provide a better understanding on how to improve deposition of LC ω-3 PUFA in porcine tissues.

2. Human nutrition needs for omega-3 fatty acids

Early findings showed that Greenland Eskimos had a low incidence of ischemic heart disease despite high fat intake, and this may be due to their high intake of ω-3 PUFA from seafood (Bang et al., 1980; Dyerberg and Bang, 1979). Numerous studies have been conducted to evaluate the health benefits of ω-3 PUFA since then, which documented that dietary omega-3 fatty acids are important for mitigating risk for cardiovascular diseases (CVD; Mozaffarian and Wu, 2011; Riediger et al., 2009; von Schacky, 2015). In addition, the ω-3 PUFA may also have

long term health benefits in infant development, cancer, various mental illnesses, immune system, and bone health (Riediger et al., 2009; Simopoulos, 2008). The EPA acts as precursor for eicosanoids (3-series prostaglandins and 5-series leukotrienes), and has anti-inflammatory effects (Calder, 2015). The ω-3 PUFA increase insulin sensitivity through IGF-1, GLUT4, and adipokine associated pathways (Bhaswant et al., 2015), and reduce the risk of obesity (Simopoulos, 2016). A high ratio of ω-6 to ω-3 will promote many chronic diseases (Simopoulos, 2008) and obesity (Simopoulos, 2016). Unfortunately, this ratio increased from 1:1 during evolution to 17:1 or even higher in today's industrialized Western diets (Simopoulos, 2008).

The Institute of Medicine (2006) recommends that adequate intake of ALA for adult males and females is 1.6 and 1.1 g/d, respectively. Substantial evidence has led to the recommendation for the intake of EPA and DHA between 250 and 500 mg/d for general health (Harris et al., 2009). Several organizations, such as the American Heart Association, recommend consumption of 198 or 227 g of seafood (preferably, oily fish) per wk to ensure an ideal cardiovascular health (Lloyd-Jones et al., 2010). Recently, the American Heart Association recommended the treatment of patients with prevalent coronary heart disease and heart failure with ω-3 PUFA (Siscovick et al., 2017).

An underlying question is whether intake of ALA, the precursor ω-3 PUFA, can provide sufficient LC ω-3 PUFA to maintain optimal concentrations in humans. Several groups using in vitro assays established that the desaturation and chain elongation pathway unequivocally exists in human (Plourde and Cunnane, 2007). However, in vivo studies using stable isotopes in adult humans showed that ALA, ranging from < 0.1 to 9.0%, can be converted to EPA, and the conversion rate was 5% (Plourde and Cunnane, 2007). In contrast, most of the in vivo studies

indicated that the conversion of ALA to DHA is often negligible (< 0.5%) with few exceptions (Plourde and Cunnane, 2007; Harris et al., 2009).

Harris and von Schacky (2004) proposed the term “omega-3 index,” which is defined as the sum of EPA and DHA (% of total fatty acids) in erythrocyte membranes, to assess the LC ω-3 PUFA intake. It is considered as a better risk marker for CVD mortality, and the omega-3 index at 8% is cardio-protective target value, whereas the index less than 4% is at greater risk (Harris et al., 2009). A recent review using data from a total of 298 global studies, which examined the blood fatty acid profile of healthy adults, demonstrated that people in Western countries, such as United States, have a very low omega-3 index (< 4% wt of total FA; Stark et al., 2016). Oral supplementation of ALA, SDA, and EPA increased the omega-3 index, but did not change the DHA concentration (James et al., 2003; Harris et al., 2008).

3. The idea of ω-3 FA fortified pork production

The ALA is predominately present in plant sources, including flaxseed oil, canola oil, chia seed, camelina oil (Table 1), and some nuts. In some regions, they are not usually consumed consistently or not an available source of ω-3 FA in human diets (Micha et al., 2014). Besides, those plant originated oils are usually highly susceptible to oxidation and less stable at high temperatures; thus, they are not used for frying (Kamal-Eldin, 2006). Furthermore, humans have limited ability to convert plant ω-3 FA into LC ω-3 PUFA (Plourde and Cunnane, 2007), which are of particular relevance to human health (Mozaffarian and Wu, 2011; Simopoulos, 2016; von Schacky, 2015). It is reasonable that dietary intake of both ALA and LC ω-3 PUFA are desirable.

Marine fatty fish, seafood, and fish oil are common sources of LC ω-3 PUFA, especially EPA and DHA (Table 1). However, the world production capacity is not likely to be sufficient to meet the increasing demands for LC ω-3 PUFA. Over 66.8% of the world adult population in

more than 100 countries has very low intake of seafood omega-3 PUFA (< 100 mg/d; Micha et al., 2014). In addition, fish products are not widely accepted because of the fishy taste, relative high price, concerns about mercury accumulation, and high susceptibility to lipid peroxidation, which results in undesirable off-flavors (Ma et al., 2016).

On the other hand, pork is the most consumed meat in the world, accounting for over 37% of the total global meat intake (FAO, 2017). In Australia, 43% of the LC ω-3 PUFA intake originated from meat, poultry, and game (Howe et al., 2006). Unfortunately, pork from pigs fed cereal-based diets is low in ALA, and has an undesirably high ω-6 to ω-3 ratio (Table 2). In North America, typical swine diets in commercial production usually contain corn, soybean, and sometimes small grains (wheat/barley; Dugan et al., 2015). Pork is reported to have a PUFA to SFA ratio of 0.24:1 (Romans et al., 1995b), 0.32:1 (Juárez et al., 2011), or 0.33:1 (Engel et al., 2001), and with a ω-6 to ω-3 ratio of 4.5:1 (Juárez et al., 2011) or 14.3:1 (Romans et al., 1995b). However, pork is considered as a good candidate to provide ω-3 PUFA (Corino et al., 2014; Ma et al., 2016; Wood et al., 2008). In fact, the idea of developing ω-3 FA-fortified terrestrial meats is not new. Many studies have been conducted to produce ω-3 PUFA enriched pork by feeding high-ω-3 FA diets over the years (Dugan et al., 2015). This approach provides substantial amounts of ω-3 PUFA in human diets without changing the behavior of health-conscious consumers, and, thus, may be of great importance in certain populations with low fish intake.

Consumption of ω-3 enriched pork for 12 wk, which provided 185 mg LC ω-3 PUFA per day (vs. 41 mg/d for those eating regular pork), increased the omega-3 index to 5.1% (vs. 4.4% in control; Coates et al., 2009). Increased serum EPA concentrations were reported when providing eggs, pork, chicken meat, or dairy products from animals fed diets with extruded flaxseed (Weill et al., 2002). Harris and von Schacky (2004) raised the index from 4.7 to 7.9% in

healthy subjects with 500 mg EPA + DHA per day over 20 wk. A dose-response study for humans supplemented with fish oil for 5 mo showed that intake of 300 mg EPA and DHA per day increased the omega-3 index from 4.29 to 6.19% (Flock et al., 2013). Therefore, future studies with longer-term consumption of ω-3 enriched pork may further increase the omega-3 index.

4. Bioavailability of omega-3 fatty acids

Generally, the ω-3 FA are highly unsaturated, and are well utilized by animals and humans. The apparent ileal digestibility (AID) of individual FA should give a better estimation than apparent total tract digestibility (ATTD), because of possible biohydrogenation of unsaturated FA by hindgut microflora (Duran-Montgé et al., 2007). In growing pigs, the AID of linseed oil is 92.6% (Duran-Montgé et al., 2007), whereas the AID of ether extract in (coextruded) flaxseed ranged from 77.5 to 90.3% (Table 3). The AID of ALA from flaxseed oil is 98.9%, greater than those from coextruded flaxseed (77.5-90.3%) and ground flaxseed (89.8%) (Table 3). Martínez-Ramírez et al. (2013) indicated that dietary fiber levels, fat levels and source, host animals, other components (e.g. mucilage and cyanogenic glycosides in flaxseed) in the diets and even analytical procedures could contribute to the variation in the AID of FA. For instance, it is well documented that the digestibility of fat is reduced when the level of dietary fiber increased (Just et al., 1980). Htoo et al. (2008) found that coextrusion of flax and field pea improved the AID of ALA, DE and amino acid, compared with ground and not extruded flaxseed, and the magnitude of improvement depended on the conditions of extrusion.

Dietary ω-3 PUFA can be either esterified in triacylglycerol (TAG) or phospholipid (PL), or in the form of free FA (FFA). Over 90% of dietary lipids are TAG, and the position of carbon on the glycerol is referred to as stereospecific numbering system: sn-1, sn-2, and sn-3 (Michalski

et al., 2013). For instance, the PL contents decreased from 32.7 to 2.55% during development and maturity (Herchi et al., 2012), and it accounts for 1.8 to 2.5% of total lipids in mature flaxseed (Herchi et al., 2011). The PL would be removed from crude oils through degumming step, and, thus, refined oils mainly contain TAG (Shim et al., 2015).

Both dietary TAG and PL can be hydrolyzed in the lumen of intestine by pancreatic lipase, more specifically on sn-1, 3 position, and release FFA and 2-monoacylglycerols (2-MAG), the latter of which is readily reutilized for synthesis of chylomicron-TAG (Michalski et al., 2013; Kerr et al., 2015). Most of DHA in egg PL is present in sn-2 position, and thus they would be released as 2-MAG after digestion (Amate et al., 1999). On the contrary, only 49.5% of DHA in tuna TAG is esterified on sn-2 position (Amate et al., 1999). Amate et al. (2001) bottle fed infant piglets with formula containing DHA from either TAG form (tuna and fungal oil) or PL form (egg yolk) for 4 wks, and they found the percentage of DHA increased in the PL fraction of HDL and decreased in LDL when fed the PL form. In this study, the contents of TAG, PL, total cholesterol of plasma and lipoprotein were similar; however, the transportation of DHA via different lipoproteins may affect the tissue uptake (Amate et al., 2001). Awada et al. (2013) fed mice high fat diets, and found that the PL-bound LC ω-3 PUFA (krill oil) was more effective in reducing body fat deposition and adipocyte size, than in the form of TAG (tuna oil). The potential benefit of PL-DHA is promising in infant formula, but some conflicting results were also discussed in a recent review (Michalski et al., 2013). To our knowledge, no study was conducted to compare the efficiency of ω-3 deposition from either TAG or PL sources in pigs.

In plant oils, unsaturated FA (oleic, LA, ALA) are usually bound on the sn-2 position, whereas SFA (palmitic and stearic acid) are predominantly esterified at the sn-1, and sn-3 position (Mattson and Volpenhein, 1963; Bracco, 1994). About 72.6% of TAG in flaxseed oil has

ALA in the sn-2 position, whereas only 58.2% of total FA is ALA (Ciftci et al., 2012). The positioning of FA on the TAG molecule affects the digestibility of FA (Bracco, 1994; Michalski et al., 2013; Kerr et al., 2015). The FA (more particularly LC FA) esterified at the sn-2 position of TAG is thought to be more efficiently absorbed than those at the sn-1 or sn-3 positions. For example, supplementation of fish oil in human adults, with DHA mostly on sn-2 and EPA on sn-1,3 positions, resulted in more rapid incorporation of DHA than EPA in plasma TAG (Sadou et al., 1995). The low digestibility of cocoa butter can be partially explained by the stereospecific structure that long chain SFA (palmitic acid and stearic acid) are predominantly present in sn-1 and sn-3 position (Bracco, 1994). Michalski et al. (2013) and Kerr et al. (2015) indicated that the effect of TAG structure on FA absorption is associated with interaction with divalent ions (Ca^{2+} and Mg^{2+}), and the melting temperature of the corresponding TAG. Commercially, the stereospecific structure of TAG can be redistributed by process of chemical and enzymatic inter-esterification (Berry, 2009). However, randomization of FA position in TAG of either high or low- PUFA lipids by chemically inter-esterification showed no effect on FA composition in body fat of pigs (Scheeder et al., 2003).

5. Metabolism of ω-3 fatty acids in pigs

The LA (18:2 ω6) and ALA (18:3ω3) cannot be synthesized de novo, due to the lack of ω-6 (Δ12)-desaturase and ω-3 (Δ15)-desaturase, and, thus, they are dietary essential FA for humans and other mammals (Nakamura and Nara, 2004; Lin et al., 2013). The biosynthetic pathway (Fig. 1) involves successive desaturation and chain elongation steps occurring within endoplasmic reticulum, and β-oxidation process in peroxisome (Sprecher, 2000; Baker et al., 2016). This pathway is shared by both ω-3 and ω-6 PUFA, resulting in enzymatic competition between them (Baker et al., 2016). The ALA is desaturated to SDA (18:4ω3), and elongated to

eicosatetraenoic acid (20: 4ω3) to form EPA (20:5ω3) (Fig. 1). The EPA is further metabolized to eicosanoids or DHA (22:6ω3) by a series of elongation, desaturation, and β-oxidation.

The process of conversion of ALA to DHA is tightly regulated. Fatty acid desaturases, including Delta 5 desaturase and Delta 6 desaturase (FADS1), are nonheme iron-containing, oxygen-dependent, and membrane-bound proteins that use fatty acyl-CoAs as substrates introducing double bonds at a specific position in a FA chain (Nakamura and Nara, 2004). Delta 5 desaturase is encoded by fatty acid desaturase 2 (FADS1), rapidly catalyzing desaturation of 20:4ω3 and 20:3ω6 to 20:5ω3 and 20:4ω6, respectively (Sprecher, 2000; Nakamura and Nara, 2004). Delta 6 desaturase is encoded by fatty acid desaturase 2 (FADS2), and catalyzes the first and rate-limiting step for the biosynthesis pathway of LC PUFA (Sprecher, 2000; D'Andrea et al., 2002; Nakamura and Nara, 2004; Park et al., 2016). The D6D prefer ALA (Km value between 28.75 and 33.30) as a substrate over LA (Km value between 43.34 and 92.02) (Hrelia et al., 1990), and D6D is more active on ALA than on 24:5ω3 (D'Andrea et al., 2002). Interestingly, mammalian D6D is capable of introducing double bond at the position of Δ6, Δ4, Δ8, and using at least 10 substrates, including 18:3ω3, 18:2ω6, 24:5ω3, 24:6ω3, 18:1ω9, 16:0, 20:3ω3, 20:2ω6, 22:5ω3, and 22:4ω6 (Park et al., 2016). The D6D activity varies greatly in specific tissues. Recent data indicated that elongase 2 (ELOVL2), which convert 22:5ω3 to 24:5ω3, may be another control point in biosynthesis of DHA beyond D6D (Gregory et al., 2011).

The genomic structure of porcine FADS gene cluster, including FADS1, FADS2, and FADS3, spanning about 200 kbp on chromosome 2, have been recently identified (Taniguchi et al., 2015). There are two FADS1 isoforms: FADS1a (AB933145.1) that is orthologous to isoforms in mice and cattle, and a novel isoform named FADS1b (AB933146.1) which contains a first exon unique to the porcine genome (Taniguchi et al., 2015). In 5-mo-old gilts, FADS1a and

FADS2 were predominantly expressed in liver and in the outer layer of backfat tissue, whereas FADS1b and FADS3 were expressed in the inner layer of backfat tissue (Taniguchi et al., 2015). In addition to liver and adipose, intestinal mucosa has this desaturation and elongation capacity as well (Jacobi et al., 2011). Genetic variables of FADS1 and FADS2 seem to affect synthesis of LC PUFA in human population. There are limited data in swine, and one study showed that Large White pigs converted more ALA to EPA than crossbred (13 vs. 6%) (Kloareg et al., 2007). Gilts have a greater capacity for LC ω-3 PUFA synthesis (Ntawubizi et al., 2009) and deposit more PUFA (Haak et al., 2008; Juárez et al., 2010).

The ratio of ω-6 to ω-3 in the diets affects the production of LC ω-3 PUFA content, because they compete for the same enzyme system (Fig. 1). Smink et al. (2012) carried out a study in which pigs had high or low intake of LA or ALA, while daily intake of other fatty acid were constant. In this study, compared to pigs fed low LA diet, pigs fed high LA diet had greater ARA content and less LC ω-3 PUFA content in liver, and greater mRNA levels of FADS1 and FADS2 in liver. High ALA intake increased LC ω-3 PUFA content, except DHA, and had no effect on mRNA abundance of FADS1 and FADS2 in the liver. Blank et al. (2002) conducted a study in which increasing contents of ALA with constant LA led to linear increases of EPA and 22:5ω3, and quadratic increases of DHA in liver, plasma, and erythrocyte PL of piglets. Furthermore, there is potential feedback inhibition of this pathway by dietary pre-formed LC-PUFA (De Tonnac et al., 2016). Increasing levels of ARA with constant DHA in dietary lipids down-regulated the gene expression of FADS2 classic transcript in suckling piglets (Wijendran et al., 2013). In contrast, dietary ARA linearly up-regulated intestinal FADS2 gene expression in piglets during early development (Jacobi et al., 2011).

6. Supplementation of ALA in swine diets

Flaxseed and its oil extract are the richest natural source of the dietary ALA (> 50% of its lipid fraction; Table 1). The ingested ALA has several metabolic fates: incorporation into cell membrane and adipose tissue, conversion into LC ω-3 PUFA, β-oxidation in mitochondria, and carbon recycling into SFA and MUFA (Baker et al., 2016). As shown in Fig. 1, ALA is a metabolic precursor of LC ω-3 PUFA. Numerous studies showed that increasing dietary ALA from flaxseed or flaxseed oil results in substantial improvements in ALA and EPA in various tissues, e.g., muscle, adipose, and liver (Haak et al., 2008; Hoz et al., 2003; Juárez et al., 2010; Martínez-Ramírez et al., 2014b; Matthews et al., 2000; Romans et al., 1995a; Romans et al., 1995b; Vossen et al., 2017).

The net deposition efficiency of ALA from dietary ALA ranged from 50.4 to 69.0% in pigs weighing between 60 and 105 kg (Sobol et al., 2015). However, the whole body retention efficiency of ALA declined with time from 68.4 (d 0-15) to 52.6% (d 16-30), in pigs fed 10% flaxseed (15.5 g ALA/kg) weighing between 27 and 46 kg (Martínez-Ramírez et al., 2014a). The recovery of ALA as its ω-3 PUFA metabolites also decreased from 12.2 (d 0-15) to 7.5% (d 16-30) (Martínez-Ramírez et al., 2014a). Comparative slaughter study in pigs weighing between 90 to 150 kg showed that 24% of ingested ALA (1.5 g/kg) can be recovered as is and 13% was metabolized into its ω-3 PUFA metabolites (Kloareg et al., 2007). The 24-h recovery from oxidation of labeled ALA in growing pigs fed 10% flaxseed was measured to be 7.91% (Martínez-Ramírez et al., 2014a). The oxidation of LA and oleic acid in growing pigs is reported to be low (14 to 20%) in 48 h (Bruininx et al., 2011).The oxidation of ALA in human is most rapid among the 18-carbon FA, which was estimated to be 20 to 27% over 9 h (DeLany et al., 2000). Therefore, the deposition efficiency seems to vary widely, depending upon energy status

and amount and duration of ω3-PUFA supplementation, which in turn lead to different metabolic fates.

In some cases, intake of ALA can induce DHA accumulation in pig tissues. For instance, DHA content had a 20% to 51% increase in muscle and a 50% increase in adipose tissue of pigs fed diet supplemented with flaxseed (Enser et al., 2000). However, a majority of studies on DHA content after flaxseed or flaxseed oil supplementation in pigs found no change in muscle (Haak et al., 2008; Hoz et al., 2003; Juárez et al., 2010; Martínez-Ramírez et al., 2014b; Matthews et al., 2000; Romans et al., 1995a; Romans et al., 1995b; Vossen et al., 2017) and in brain tissue (Smink et al., 2012), or reduction in liver (Smink et al., 2012). Similar DHA synthesis-secretion rates were reported in rats fed adequate or excess ALA (Domenichiello et al., 2017). There are several reasons why accumulation of DHA in vivo reach plateau with increasing ALA supplementation. First, desaturation of 24:5ω3 was inhibited with increasing ALA concentration (Gregory et al., 2011; Portolesi et al., 2007). In addition, there are competitions on D6D for multiple substrates from both ω-3 and ω-6 series, such as C18 and C24 fatty acyl substrates (D'Andrea et al., 2002; Hrelia et al., 1990; Park et al., 2016). The LA to ALA ratio affect DHA accumulation (Blank et al., 2002; Smink et al., 2012). Second, the substrate (24:5ω3) for D6D is limited as elongase 2 (ELOVL2) becomes saturated with increased EPA (Gregory et al., 2011). Third, dietary DHA may depress expression of enzymes involved in DHA synthesis (De Tonnac et al., 2016).

Interestingly, pigs fed diets containing flaxseed had substantial amounts of eicosatrienoic acid (20:3ω3), which was the second largest proportion (8%) in whole-body content of total ω-3 PUFA after ALA (Martínez-Ramírez et al., 2014a). Corino et al. (2008), Juárez et al. (2010), and Martínez-Ramírez et al. (2014b) found similar results in adipose or muscle tissues when pigs fed

flaxseed supplemented diets. In vitro evidence showed that Baboon FADS2, which shares 97% identity and 99% similarity with human FADS2, actively catalyzed Δ8 desaturation of 20:3ω3 to 20:4ω3, which can enter Sprecher pathway (Park et al., 2009). An alternative pathway has been proposed: 18:3ω3→20:3ω3→20:4ω3 in other species (Fig. 1), but further studies are needed to determine the significance of this pathway in pigs (Martínez-Ramírez et al., 2014a; Park et al., 2009).

The ω-3 PUFA content can be enhanced by short-term dietary supplementation. Romans et al. (1995b) fed 15% flaxseed diets for 7, 14, 21, 28 d before harvest, and found ALA and EPA content in the muscle increased until 21 days. Juárez et al. (2010) investigated various levels (5, 10, and 15% co-extruded flaxseed) and different durations (4, 8 and 12 wks before harvest) of flaxseed feeding in pigs. Feeding 5% flaxseed for 4 wks resulted in reduction of ω-6:ω-3 from 6.1:1 to 2.6:1. The results indicated that higher levels of flaxseed for shorter periods provided greater efficiency in ω-3 PUFA deposition, whereas lower level of flaxseed for longer period resulted in lower variance in ω-3 PUFA content.

Utilization of flaxseed or flaxseed oil in livestock diet can be costly. Many processors produce their flaxseed oil by pressing or cold pressing without solvent, and thus the co-product (i.e., flaxseed meal) with a residual oil content of about 10% is suitable for animal diets (Shim et al., 2015). Eastwood et al. (2009) fed pigs (32 to 115 kg BW) with flaxseed meal at 5% increments from 0 to 15% of the diets, and observed linear increase in the ALA content in backfat and loin. In the same study, they found no effect of flaxseed meal supplementation on growth performance, because the nutritional profile of flaxseed meal was considered during diet formulation, i.e., low digestible Lys, high crude fiber, and phytic acid bounded P (Eastwood et al., 2009).

7. Supplementation of long chain ω-3 PUFA in swine diets

There is no doubt that consumption of preformed DHA, such as fish oil, is effective in raising EPA and DHA levels in muscle and subcutaneous fat (Haak et al., 2008; Leskanich et al., 1997; Sárraga et al., 2007). The incorporation of EPA and DHA in backfat reached plateau after 2 to 4 wks fish oil supplementation (Irie and Sakimoto, 1992). Although Howe et al. (2002) indicated that inclusion of 5 to 10% stabilized tuna fishmeal can improve LC ω-3 PUFA content without palatability problems, most studies reported that using fish products had adverse effects on sensory quality, i.e., fishy flavor (Leskanich et al., 1997; Ma et al., 2016; Sárraga et al., 2007). In addition, fishmeal or fish oil obtained from capture fisheries is unsustainable and may be no longer to be economical effective under commercial animal production system nowadays.

One of alternative sources of DHA is microalgae, such as *Crypthecodinium* and *Schizochytrium* (Spolaore et al., 2006). In fact, microalgae is a natural food source for most aquatic animals because fish cannot produce ω-3 PUFA by themselves. Microalgae is also important for large-scale production of EPA/DHA supplement in human nutrition, and now it is available as a commercial feed additive as source of ω-3 PUFA and protein for farm animals. Clear dose dependent response between dietary DHA and pork DHA was observed. For instance, Meadus et al. (2010) reported that feeding microalgae (containing 18% of DHA) to pigs for 25 days before harvest was effective in improving DHA content in belly, which resulted in deposition of 98 mg DHA/100g bacon for each additional g of DHA/kg feed. The DHA content in bacon reached 339 mg/100g for those fed 1.6% microalgae. However, increased lipid oxidation and off-flavor for those fed diets containing high DHA was reported in this study (Meadus et al., 2010). More recently, Vossen et al. (2017) supplemented diets with 0.3, 0.6, or 1.2% microalgae, which contained 26% of DHA, for 45 d before harvest, and found that the loin

and dry cured hams contained 20 and 56 mg DHA per 100 g tissue, respectively. Minimal adverse effect on sensory characteristics by microalgae supplementation was reported (Marriott et al., 2002; Vossen et al., 2017).

Several studies found that the LC ω-3 PUFA content in the muscle or adipose tissue were largely independent of timing of feeding ω3-PUFA-rich diets (Haak et al., 2008; Jaturasitha et al., 2009; Martínez-Ramírez et al., 2014b). Irie and Sakimoto (1992) reported that feeding 4% fish oil for 2 wks was more effective in deposition of EPA and DHA than feeding 2% fish oil for 4 wks, which was in agreement with Juárez et al. (2010). The net deposition efficiency of EPA + DHA from the dietary EPA + DHA was approximately 48.7% in pigs from 60 and 105 kg (Sobol et al., 2015).

8. Novel sources of omega-3 PUFA for animal diets

In addition to microalgae, higher plants are able to synthesize ω-3 and ω-6 PUFA, but most plant oils contain insignificant amounts of LC ω-3 PUFA (Table 1). The SDA is the product of ALA desaturation by D6D (Fig. 1). It is thought to be a better precursor than ALA, because supplementation of SDA can bypass the first and limiting step of biosynthesis of LC ω-3 PUFA involving D6D (Baker et al., 2016). Rats fed diet containing SDA had a two-fold increase in EPA and to a lesser extent increase in DHA compared with those fed diet containing equal amount of ALA (Yamazaki et al., 1992). The SDA, as well as ALA, can be found in the Boraginaceae, Cannabaceae, Primulaceae and Boraginaceae families (Kuhnt et al., 2012). *Echium vulgare*, which belongs to Boraginaceae, showed the highest amount of SDA relative to total FA (11.1%) (Kuhnt et al., 2012).

Numerous studies using SDA enriched echium oil have been conducted on Atlantic salmon and barramundi, but limited amount of LC ω-3 PUFA (especially DHA) was produced in

their flesh (Kitessa et al., 2014). In contrast, supplementing chickens with echium oil significantly increased the accumulation of ω-3 PUFA content in thigh and breast muscle, including ALA, SDA, EPA, and DPA (Kitessa and Young, 2008). The absolute changes of total ω-3 PUFA were of nutritional significance (676 vs. 265 mg/100 g in thigh muscle, 137 vs. 70 mg/100 g in breast muscle), in relation to those fed rapeseed oil (Kitessa and Young, 2008). The shortcoming of this study is that they used identical levels of echium oil and rapeseed oil, but both ALA and SDA contents were greater in echium oil diets. Kitessa et al. (2011) used equal total amounts of ALA plus SDA with different ALA:SDA, and found that higher SDA induced about 1.2-fold increase in LC ω-3 PUFA concentration in lamb muscle. The SDA-enriched soybean oil was more effective than flaxseed oil in increasing LC ω-3 PUFA content in egg yolk, excluding DHA, (Elkin et al., 2015). Similar results were reported in human studies (James et al., 2003; Harris et al., 2008). All things considered, the SDA may not be as effective as pre-formed DHA, to increase DHA contents in fish, poultry, or lamb. To our knowledge, only one study was reported using echium oil in pig diets (Tanghe et al., 2013). They found that 1% of flaxseed oil and echium oil were equally effectively in elevating EPA content (not DHA) in sow and piglet plasma and colostrum, but to a lesser extent than when fish oil was fed (Tanghe et al., 2013). It remains unclear whether SDA enriched oil is more effective than flaxseed oil in raising LC ω-3 PUFA concentration in growing-finishing pigs.

Over the years, progress has been made using genetic engineering to develop new and sustainable sources of the LC ω-3 PUFA oilseeds. The LC ω-3 PUFA-enriched vegetable oils derived from genetically modified plants has been reported in soybean, *Arabidopsis*, and *Camelina* (Kitessa et al., 2014). For instance, genetically modified seed oil of the *Camelina sativa*, has been developed to accumulate up to 12% EPA and 14% DHA, which is comparable to

fish oils (Ruiz-Lopez et al., 2014). Transgenic plants may play an important role in the future, to provide sustainable source of LC ω-3 PUFA for aquaculture, livestock, and human.

9. Non-dietary approaches

The ω-3 PUFA biosynthesis pathway in plants and lower organisms has been well studied. Genetic engineering biotechnology enables scientists to introduce ω-3 fatty acid desaturases as the key enzymes to animals. Considering high ω-6 to ω-3 ratio in feed and meat, transgenic animals that can produce ω-3 PUFA from its ω-6 PUFA analogs seem promising. Lai et al. (2006) successfully transferred *fat-1* gene from *C. elegans* to pigs, and found that the pigs were able to produce ω-3 PUFA, and, therefore, achieved an optimal ω-6 to ω-3 ratio in several tissues. Zhou et al. (2014) established transgenic pigs using *cbr-fat-1* gene from *C. briggsae,* which achieved 14.8 to 16.5% ω-3 PUFA, and 9% EPA of total FA in muscle.

Meadus et al. (2013) injected pork loin with 3.1% DHA in a tripolyphosphate brine solution and improved the DHA content to 116 mg/100 g raw loins, and 146 mg/100g after cooked. No changes were observed on subjective color, visual discoloration, off odors, marbling, and striping during display, as well as sensory evaluations.

10. Conclusions

Consumers are becoming more concerned about health-related aspects of food. It is generally accepted that improving LC ω-3 PUFA consumption has beneficial effects on public health, especially on cardiovascular health. Pork is the most consumed meat in the world, but commercial pork usually contains low amount of ω-3 PUFA. Technological challenges in producing ω-3 PUFA enriched pork using plant-originated ALA include relatively low deposition efficiency and low rate of conversion from ALA to LC ω-3 PUFA. Further experimental studies

will be valuable to further develop more appropriate, sustainable, and alternative source of LC ω-3 PUFA in animal feed.

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Table 1
Source of omega-3 PUFA[1].

Source	Ether extract (%)	ALA	EPA	DPA	DHA	∑n-3 [2]	PUFA	SFA	MUFA	Reference
		(% of total fatty acids)								
Flaxseed seed	44.8	58.2	-	-	-	58.2	73.6	7.9	18.5	Ciftci et al. (2012)
Perilla seed	40.0	60.9	-	-	-	60.9	75.9	7.6	16.6	Ciftci et al. (2012)
Chia seed	35.0	59.8	-	-	-	59.8	80.4	8.7	11.0	Ciftci et al. (2012)
Flaxseed meal	13.3	54.1				54.1	75.5	16.6	24.0	Eastwood et al. (2009)
Flaxseed oil	100	53.4	-	-	-	53.4	67.8	9.0	18.4	USDA (2015)
Fish oil, Menhaden	100	1.5	13.2	4.9	8.6	28.1	34.2	30.4	26.7	USDA (2015)
Fish oil, Salmon	100	1.1	13.0	3.0	18.2	35.3	40.3	19.9	29.0	USDA (2015)
Canola oil	100	9.1	-	-	-	9.1	28.1	7.4	63.3	USDA (2015)
Camelina oil	-	38.1	-	-	-	38.1	54.1	9.7	32.8	Dubois et al. (2007)
Soybean oil	100	6.5	-	-	-	6.5	57.3	15.3	22.7	USDA (2015)
Microalgae, g/100g	-	0.019	0.36	0.121	26.4	26.9	-	-	-	Vossen et al. (2017)

[1]ALA = alpha-linolenic acid; DHA = docosahexaenoic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid; and PUFA = polyunsaturated fatty acid.
[2] ∑n-3 = ALA + EPA + DPA + DHA.

Table 2
Example of the effect of diet on fatty acid composition in pork.

Item	Cereal:	Corn/wheat/soy [1]		Wheat/barley/soy [2]		Standard [3]	
	Fat added:	1 to 2% tallow		0.9% tallow		-	
	Unit:	mg/100 g tissue	%	mg/100 g tissue	%	mg/100 g tissue	%
C12:0		5.4	0.08	-	-	-	
C14:0		96.8	1.43	30.1	1.35	30	1.33
C16:0		1,747	25.9	538	24.1	526	23.2
C18:0		875	12.9	286	12.8	278	12.2
C16:1		172	2.54	74.4	3.33	62	2.71
C18:1		2,932	43.2	953	42.7	851	36.8
C18:2ω6		629	9.27	185	8.28	302	14.2
C18:3ω6		2.1	0.03	-	-	1.23	0.06
C20:2ω6		25.3	0.37	-	-	9.05	0.42
C20:3ω6		9.07	0.13	-	-	7.21	0.34
C20:4ω6		38.6	0.57	52.6	-	46.0	2.21
C22:4ω6		8.36	0.12	-	-	4.97	0.23
C18:3ω3		37	0.55	24	1.07	20.6	0.95
C20:3ω3		5.99	0.09	3.68	0.16	2.72	0.12
C20:4ω3		0.41	0.01	-	2.35	0.19	0.009
C20:5ω3		1.47	0.02	6.94	0.31	6.51	0.31
C22:5ω3		6.23	0.09	12.3	0.55	12.9	0.62
C22:6ω3		1.82	0.03	5.61	0.25	8.33	0.39
Total SFA		2,802	41.3	862	38.6	834	36.7
Total MUFA		3,204	47.2	1,060	47.4	913	39.5
Total PUFA		778	11.5	312	14.0	422	19.9
ω-6 PUFA		721	10.6	252	11.3	371	17.5
ω3 PUFA		56.6	0.83	57.7	2.58	51.3	2.40
PUFA: SFA		-	0.28	-	0.36	-	0.54
ω-6:ω-3		-	13.1	-	4.53	-	7.28

[1] Martínez-Ramírez et al. (2014b).
[2] Juárez et al. (2011).
[3] Enser et al. (1996).

Table 3
Digestibility of different sources of omega-3 polyunsaturated fatty acids.

Animal and condition	Oil, % in test diet	Item	Component	Value, %	Reference
50-kg, T-cannula	10% Flaxseed oil	AID	Supplemental fat	92.6	Duran-Montgé et al. (2007)
50-kg, T-cannula	10% Flaxseed oil	AID	ALA	98.9	Duran-Montgé et al. (2007)
40-kg, intact	10% Flaxseed oil	ATTD	Supplemental fat	95.9	Duran-Montgé et al. (2007)
40-kg, intact	10% Flaxseed oil	ATTD	ALA	98.6	Duran-Montgé et al. (2007)
110-kg, slaughter	6% Ground flaxseed	AID	Ether extract	75.6	Martínez-Ramírez et al. (2013)
110-kg, slaughter	6% Ground flaxseed	AID	ALA	89.8	Martínez-Ramírez et al. (2013)
23-kg, T-cannula	30% Flaxseed and field pea (50:50)	AID	ALA	77.5 to 90.3	Htoo et al. (2008)
45 to 80 kg, T-cannula	15% Fish oil	AID	Ether extract	91.6	Jørgensen et al. (2000)
45 to 80 kg, T-cannula	15% Fish oil	AID	ALA	96.2	Jørgensen et al. (2000)
45 to 80 kg, T-cannula	15% Fish oil	AID	EPA	98.1	Jørgensen et al. (2000)
45 to 80 kg, T-cannula	15% Fish oil	AID	DHA	96.8	Jørgensen et al. (2000)
45 to 80 kg, T-cannula	15% Rapeseed oil	AID	ALA	97.2	Jørgensen et al. (2000)

[1] AID = apparent ileal digestibility; ALA = alpha-linolenic acid; ATTD = apparent total tract digestibility; EPA = eicosapentaenoic acid; and DHA = docosahexaenoic acid.

[2] In these experiments, inert markers were used: 0.5% titanium dioxide (Duran-Montgé et al., 2007); 0.1% titanium dioxide (Martínez-Ramírez et al., 2013); and 0.2% chromic oxide (Jørgensen et al., 2000).

[3] Corrected subtracting the contribution of basal fat content.

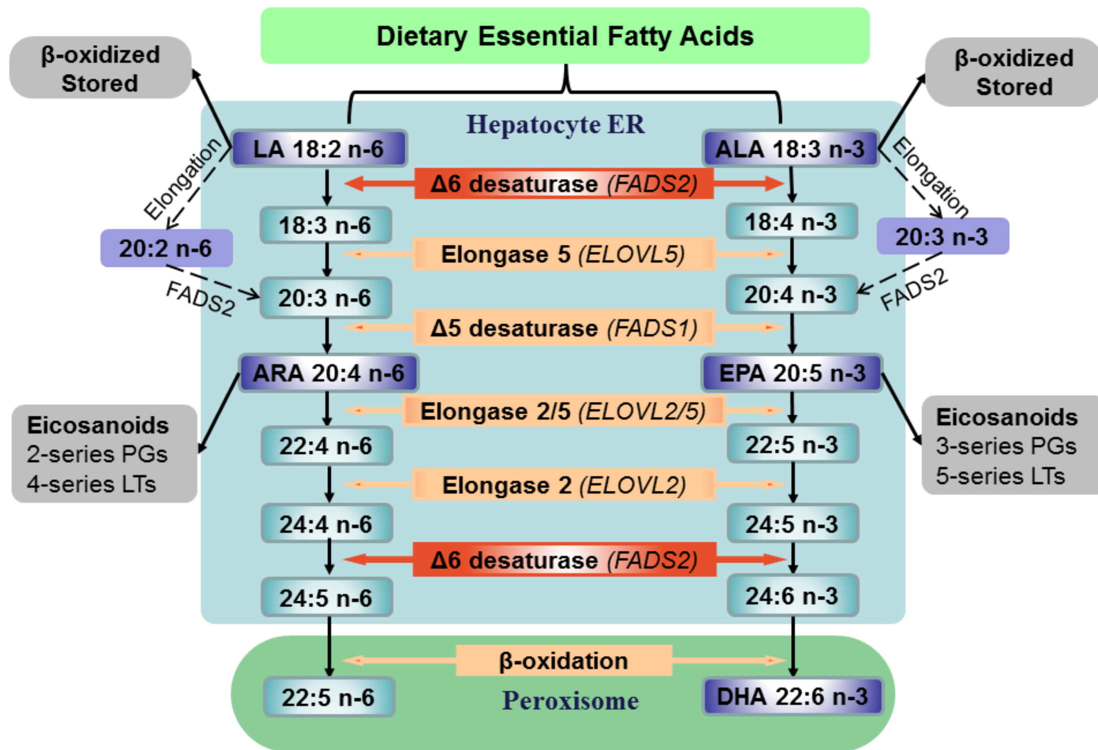


Fig. 1 Biosynthesis of LC PUFA from C18. Sharing the same enzyme system, both LA and ALA can be elongated and desaturated in endoplasmic reticulum of hepatocyte, and β-oxidized in peroxisome, in the conventional pathway (Sprecher, 2000; O'Neill and Minihane, 2017). An alternative pathway for the first two steps, via elongation-Δ8-desaturation, has been proposed (Park et al., 2009). LA, linoleic acid; ALA, α-linolenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

VI. APPENDICES

Appendix A

Daily minimum and maximum temperatures (°C) inside the building during the animal study [1]

Date	Min. T	Max.T	Date	Min. T	Max.T	Date	Min. T	Max.T
16-Sep	17.2	25.0	8-Oct	18.5	27	30-Oct	15	28
17-Sep	17.2	28.3	9-Oct	20	30	31-Oct	18	25
18-Sep	17.2	29.4	10-Oct	17	27	1-Nov	18.5	22.5
19-Sep	17.8	30.6	11-Oct	15	25.5	2-Nov	21.5	27
20-Sep	17.2	31.1	12-Oct	17.5	27	3-Nov	22	30
21-Sep	18.9	27.2	13-Oct	14	28.5	4-Nov	21	26
22-Sep	18.3	25.0	14-Oct	14	30	5-Nov	21	31
23-Sep	18.3	27.2	15-Oct	15	32	6-Nov	22	30
24-Sep	16.1	25.0	16-Oct	10.5	31.5	7-Nov	14	26
25-Sep	16.1	21.1	17-Oct	9.5	31	8-Nov	11.5	15
26-Sep	17.8	23.3	18-Oct	10	23.5	9-Nov	12	15
27-Sep	20.0	24.4	19-Oct	11	23	10-Nov	10.5	17
28-Sep	19.4	23.3	20-Oct	9.5	22	11-Nov	11.5	17
29-Sep	21.7	26.1	21-Oct	16	29	12-Nov	9	20
30-Sep	20.0	28.9	22-Oct	18	30	13-Nov	7	23
1-Oct	17.8	21.1	23-Oct	18	32	14-Nov	6	22
2-Oct	16.7	20.0	24-Oct	19	30	15-Nov	11	22
3-Oct	13.9	18.9	25-Oct	20	27	16-Nov	15.5	26
4-Oct	12.8	22.2	26-Oct	14	21.5	17-Nov	16	24
5-Oct	17.8	22.2	27-Oct	15	21	18-Nov	13	20
6-Oct	16.7	27.2	28-Oct	15	27	19-Nov	10	26.5
7-Oct	15.0	28.3	29-Oct	12	28	20-Nov	12	26

Date	Min. T	Max.T	Date	Min. T	Max.T	Date	Min. T	Max.T
21-Nov	7	26	18-Dec	1	20	14-Jan	10	21
22-Nov	2	19	19-Dec	5	21.5	15-Jan	10.5	15
23-Nov	3.5	20.5	20-Dec	11.5	21	16-Jan	9	25
24-Nov	8	19	21-Dec	16	19	17-Jan	2.5	21
25-Nov	11	23	22-Dec	18	20	18-Jan	-1.5	18
26-Nov	14	25	23-Dec	17.5	21	19-Jan	2	16
27-Nov	15	27	24-Dec	17	23	20-Jan	4.5	14
28-Nov	14	27	25-Dec	19	26	21-Jan	10.5	14
29-Nov	15	28	26-Dec	21	28	22-Jan	1	15
30-Nov	17	27	27-Dec	21	25.5	23-Jan	-3	9
1-Dec	16	25	28-Dec	16	24	24-Jan	1	17.5
2-Dec	8	18	29-Dec	18.5	26	25-Jan	10	20
3-Dec	5	17	30-Dec	16	20	26-Jan	11	16
4-Dec	7	21	31-Dec	9	18	27-Jan	7	11
5-Dec	9.5	23.5	1-Jan	6.5	12	28-Jan	1	13
6-Dec	10	24	2-Jan	6	18	29-Jan	4	22
7-Dec	8	29	3-Jan	5	21	30-Jan	8.5	23
8-Dec	8	23	4-Jan	3	19.5	31-Jan	13	23
9-Dec	12	26	5-Jan	4	16	1-Feb	16	19
10-Dec	18	25	6-Jan	9	20	2-Feb	15	24
11-Dec	14	27	7-Jan	13	20	3-Feb	6	16
12-Dec	16	26.5	8-Jan	14	16	4-Feb	1	20
13-Dec	17	25	9-Jan	8	14	5-Feb	1	17
14-Dec	11	23.5	10-Jan	1	14	6-Feb	1	18
15-Dec	10	29	11-Jan	2	18	7-Feb	2	20
16-Dec	16	22	12-Jan	3	20	8-Feb	-1	8
17-Dec	7.5	22	13-Jan	3	22	9-Feb	-3	8

[1] Min. T = minimum temperature. Max. T = maximum temperature

Appendix B

Fatty Acid Methylation

Reference: O'Fallon, J.V., Busboom, J.R., Nelson, M.L., Gaskins, C.T., 2007. A direct method for fatty acid methyl ester synthesis: Application to wet meat tissues, oils, and feedstuffs. J. Anim. Sci. 85:1511-1521.

A. Solutions and Chemicals

a) Hexane (GC grade)
b) Methanol (MeOH)
c) 10 *N* KOH
   - Weigh 56.11 g KOH (potassium hydroxide), dissolve and fill up to 100 mL with dd H_2O.
d) 24 *N* H_2SO_4
   - Measure 66.67 mL Sulfuric acid (H_2SO_4), and slowly diluted into dd H_2O, to make 100 mL solution.
e) Internal Standard: 0.5 mg of C13:0/mL of MeOH
   - Weigh 125 mg C13:0 accurately (97% purity=0.1289 g), fill up to 250 mL with MeOH
   - Stable at 4°C for 1 mo
f) FAME mix standard
   - Stable at 4°C for 1 mo

B. Direct Fatty Acid Methylation

a) Take vacuum-packed loin chop out of freezer, and thaw it at 4°C overnight.
b) Mince meat sample and weigh out 1 g. (For oil sample, pipette 40 μL)
c) Place 1 g meat into a 16 mL screw top Pyrex tube
d) Add 5.3 mL of MeOH, 0.7 mL of 10 *N* KOH, and 1 mL of C13:0 standard, to meat sample in tube. Place cap on tube and place in water bath at 55°C. If water bath has a shaker attachment, turn shaker attachment on to desired setting. If there is no shaker attachment, vortex samples for 5 s every 20 min. Incubate for 90 min.
e) After incubation, place samples in cold tap water and allow to cool to below room temperature.
f) After cooling, add 0.58 mL of 24 *N* H_2SO4. Mix tube by inversion and make sure K_2SO4 precipitate is present. Place tube back in water bath and incubate for 90 min at 55°C. If there is no shaker attachment, vortex samples for 5 s every 20 min.
g) Repeat step e, cool down.
h) After cooling, add 3 mL of hexane and vortex for 5 min.
i) Centrifuge tubes for 5 min at 1,500 x *g*.
j) Remove hexane layer (containing FAME), and place into a GC vial.
k) Be sure the sample is completely clear and free of any particulate matter, water bubbles etc.
l) Tightly capped, and place fatty acid vials in freezer at -20°C until analysis.

Appendix C

Operation Procedure of GC

Keep in Mind

- Keep the same procedure for all samples
- Keep clean, & wear gloves
- Gas is first on, and last off
- When finished, make sure all the temperature fall below 50°C before turning off the GC power.
- When finished, micro syringe should be washed (e.g., acetone) following procedures
- Injection-parts (septum, glass liner): should be changed after every 100 times.

Know your GC Machine

1. GC Machine: Shimadzu GC-2014 gas chromatograph
2. SUPELCO SP™ 2560 Fused silica capillary column 100m × 0.25 mm i.d. × 0.2 µm film thickness

    - Column Max. Temperature: 250°C

3. FID Detector temperature (high enough to avoid condensation of sample; FID ≥ 250°C)
4. Auto sampler: Shimadzu auto injector AOC-20i
5. High pressure gas cylinder and two-stage regulators

    - Main valve & gauge: should be at least 500 kpa
    - Outlet valve & gauge: control the GC pressure
    - Third value & gauge: on top and back of the GC
    - Only use high-purity gas

Turning on the Instrument

1. Open the valve of helium gas cylinder and set the pressure

    - Adjust the outlet/second knob slowly, and wait it increase:
        - ✓ Helium: 150 psi (fill up before 70 psi)
        - ✓ Hydrogen: 100 psi
        - ✓ Air: 100 psi

2. Turn on the GC2014 system. The switch located on the lower right front panel of the instrument.

    - After 20 min, ‘ready’ light is green

3. Turn on the PC, click the <GC solution> icon

    - Login, no password

Setting up the Analysis

1. Set up parameters

    - <AOC-20i>
        - ✓ Injection volume = 1 μL
    - <SPL1>
        - ✓ Inlet temperature: 250°C
        - ✓ Chose linear velocity mode
        - ✓ Linear velocity = 20 cm/s
        - ✓ Split ratio = 30:1
    - <Column>: set up the temperature program
    - <FID1> Temperature = 260°C
    - <General>: auto zero
    - Save the method as needed

2. Download the method

    - Open the method, and <download parameters>
    - Click <System on>
    - Make sure the flame on

3. Run a batch of samples

    - Use batch wizard to input the samples name and chose method files
    - Save the batch
    - Click the <run> button when the system is ready.
    - Click the <pause> button when editing was needed, after the batch started

Method Recommended by Supelco 37 FAME Mix (Separated by SP2560)

1. FID: 260°C
2. Inlet temperature: 250°C
3. Column: hold at 100°C for 5 min. ramp to 240°C at 4°C/min. Hold at 240°C for 30 min.
4. Flow rate

    - Carrier gas and flow rate: helium at 20 cm/s
    - Make-up gas and flow rate: nitrogen at 30 mL/min

5. Split ratio: 100:1
6. Sample injected: 1 μL

7. Mixed 500:500 μL

    - Sample concentration: 10 mg/mL in methylene chloride.
    - Internal standard: 1,000 μg/mL in methylene chloride.

Turn off the System

1. Click the <stop> button if the batch is running
2. Click <system off> to turn flame off
3. Wait after the oven and FID cool down to below 50°C

    - In batch analysis, we can run <shutdown.gcm>

4. Turn off the GC solution and PC
5. Turn off the GC2014 system power
6. Shut the carries gas

Post-Run Integration

1. Set up the following parameters

    - Click <edit>
    - [Slope]: more than the slope tested, can be change by "slope test".
    - [Width]: usually 2 to 3
    - [Drift]: 0 uV/min (automatic on)
    - [T-DBL]: 1000 (automatic off)
    - [Min Area]: set as needed
    - Manual integration is not encouraged.

2. Save the integration program
3. Make sure each data file under the same peak integration program.
4. Print reports (with desired format) and analyze the results

Appendix D

RNA Isolation (Adipose Tissue)

Modified from: TRI Reagent® RT - RNA, DNA, protein isolation reagent. Manufacturer's protocol (2008-2014), Molecular Research Center, Inc. Cincinnati, OH.

Overview

TRI Reagent® RT combines phenol and guanidine thiocyanate in a mono-phase solution to facilitate effective inhibition of RNase activity. A biological sample is homogenized or lysed in TRI Reagent® RT and the homogenate is separated into aqueous and organic phases by centrifugation. After phase separation, RNA remains in the aqueous phase while DNA and proteins are sequestered into the interphase and organic phase. RNA is precipitated from the aqueous phase by addition of isopropanol, washed with ethanol and solubilized.

Chemicals and Materials

a) TRI Reagent, Cat. No. RT 111(Molecular Research Center, Inc., Cincinnati, OH)
b) Chloroform (Mallinckrodt, St. Louis, MA)
c) Isopropanol (VWR, Radnor, PA)
d) Ethanol (Mallinckrodt, St. Louis, MA)
e) Water - Molecular Biology Grade (Thermo Fisher Scientific Inc., MA)
f) Conical tube (50 mL)
g) Corex centrifuge tube
h) Homogenizer
i) Pipette and tips
j) Aluminum foil
k) Centrifuge
l) Liquid N_2

Protocol

1. Homogenization

a) Install the homogenizer, and rinse the homogenizer-probe extremely well with Tri reagent for 1 min.
b) Take samples out from -80°C freezer and put them in liquid N.
c) Get a new conical tube (50 mL), then add 10 ml Tri reagent.

   - Use 50 to 100 mg tissue per 1 mL TRI Reagent RT.
   - Sample volume should not exceed 10% of the volume of TRI Reagent RT used for homogenization.

d) Quickly processed the tissue by crushing or grinding with hammer, and wrap some small particles of tissues with aluminum foil.

- Make sure avoiding thawing the samples in this step, and snap freeze several times to keep them frozen all the time.

e) Weigh 3 to 4 g fat tissue. Quickly homogenize each sample for 30 s. Make sure avoiding thawing the samples in this step.

- Between samples, rinse the probe with Tri reagent for 10 to 15 s.

f) Close the lid, mix and incubate for 5 to 10 min at room temperature.

2. Additional Steps to Remove Fat

a) Transfer the liquid to a Corex centrifuge tube (15 mL), covered with aluminum foil as lid.
b) Balanced precisely with lid on, then spin at 9,000 to 10,000 RPM (11,953 g) for 10 min at 4°C.

- After spin, a fatty surface layer that should be discarded and a clear aqueous phase which should be put into a new clean tube.

c) Transfer the lower clear supernatant to a fresh 50 mL tube.

- The clear supernatant is about 5 to 6 mL.
- Before next step, this supernatant can be stored at -80°C for 1 mo, or -20°C for 1 wk.

3. Phase Separation

a) Add 2 ml Chloroform. (0.2 mL per mL of TRI reagent)
b) Cover the sample tightly, shake vigorously for 30 s.

- This is very important - manual shaking results in a better emulsification and a higher volume of aqueous phase later!

c) Allow to stand for 15 min at room temperature.
d) Divided into 2 tubes (Corex tube, 15 mL), with aluminum foil covered as lid.
e) Balanced with Tri reagent, then spin the resulting mixture at 10,000 RPM for 15 min at 4°C.

- Spin separates the mixture into 3 phases: a colorless upper aqueous phase (RNA, ~ 60% Volume TRI), an interphase (containing DNA) and a red organic phase (containing Protein).
- Avoid shaking after spinning the samples.

f) Carefully transfer 5 ml of the clear aqueous phase to a fresh Conical tube (50 mL).

- During the collection, leave the remaining aqueous phase as an undisturbed layer to avoid collection of DNA from the interphase.
- 0.5 mL/mL TRI

4. RNA Precipitation

a) Add 5 mL of isopropanol to precipitate RNA, mixing the aqueous phase.

   - (0.5 mL/mL TRI).

b) Gently mix and stand for 10 min at room temperature.
c) Transfer samples to a Corex centrifuge tube (15 mL), using pipette.
d) Balanced the tubes and spin at 10,000 g for 10 min at 4 to 10°C.
e) Take them out, and circle the precipitation area on the tubes via marker, so we will know the location of RNA in the RNA wash step.

   - RNA precipitate (often invisible before centrifugation) forms a gel-like or white pellet on the side and bottom of the tube.

5. RNA Wash

a) Remove the supernatant and wash the RNA pellet with 10 mL of 75% ethanol (1 mL/mL).
b) Spin at 10,000 g for 5 min at 4°C.

   - If the RNA pellet accumulates on the side of the tube or tends to float, sediment the pellet at 12,000 g.
   - The ethanol wash can be repeated to improve 260/280 ratio of the isolated RNA.

c) The RNA (in 75% ethanol) can be stored at 4°C for 1 wk, or -20°C for one year.

6. RNA Solubilization

a) Remove the ethanol wash and dry RNA pellet for 10 to 20 min on clean paper tissue.

   - Pour out ethanol at one time.
   - Avoid drying the pellet completely, as this will decrease its solubility.

b) Dissolve RNA with 100 μL RNase free H_2O by passing the solution through a pipette tip few times.

   - Typically, amount of water used should be sufficient to obtain RNA concentration ranging 0.5 to 2 μg/mL.

c) If stopped here, store the RNA samples at -80°C.
d) If not, put RNA samples on ice box. Spin down (pulse) for a few seconds, and proceed to purification steps with RNeasy mini kit, and measure OD260/OD280.

Appendix E

RNA Isolation (Muscle Tissue)

Modified from: RNAzol®RT Brochure, 2015 (Molecular Research Center Inc., Cincinnati, OH.)

Overview

RNAzol®RT separates RNA from other molecules in a single-step based on the interaction of phenol and guanidine with cellular components. No chloroform-induced phase separation is necessary to obtain pure RNA. A biological sample is homogenized or lysed in RNAzol®RT. DNA, proteins, polysaccharides and other molecules are precipitated from the homogenate/lysate by the addition of water and removed by centrifugation. The pure RNA is isolated from the resulting supernatant by alcohol precipitation, followed by washing and solubilization.

Chemicals and Materials

- RNAzol ®RT, Catalog No: RN 190 (Molecular Research Center, Inc., Cincinnati, OH)
- Isopropanol (VWR, Radnor, PA)
- Water - Molecular Biology Grade (Thermo Fisher Scientific Inc., MA)
- 5ml centrifuge PE tube
- Homogenizer
- Pipette and tips (5 mL, 1 mL), RNase free
- Aluminum foil
- Liquid nitrogen
- High-speed centrifuge

Protocol

1. Homogenization

a) Install the homogenizer, and rinse the homogenizer-probe extremely well with Tri reagent for 1 min.
b) Take samples out from -80°C freezer, and put them in liquid N.
c) Add 2 mL RNAzol to a 5-mL centrifuge tube. Place it on a balance and tare it.
d) Quickly pulverize or crushing the frozen tissue with hammer into powder, put back to liquid N.

    o Make sure avoiding thawing the samples in this step, and snap freeze several times to keep them frozen all the time.

e) Weigh about 200 mg muscle tissue into the 5-mL tube. Do not allow it to thaw!

- Sample volume should not exceed 10% of the volume of RNAzol used for homogenization.

f) Immediately homogenize the sample by the Polytron homogenizer, for 2 min at high-speed (4 to 5 times, 10 s each time).

- Between samples, rinse the probe with RNAzol reagent for 10 to 15 s.

g) After 15 min, votex for 15 s. Snap frozen in liquid N, and stored at -80°C, up to 1 yr.

- The sample homogenate, before addition of water, can be stored overnight at 4°C or for at least one year at -20°C.

2. DNA/Protein Precipitation

a) To thaw the samples, put them at room temperature around 30 min.

- In the manual: we can incubate them at 37 to 40°C for 5 min with periodic mixing.

b) Add 0.8 mL Thermo biology grade water, vortex for 30 s.
c) Stand for 15 min at room temperature.
d) Centrifuge 12,000 g x 15 min, in a 4°C refrigerator.

- Tips: After addition of water and centrifugation, the homogenate forms two phases: the lower blue solid precipitation phase and the blue/colorless upper aqueous phase whereas DNA and proteins are in the interphase and organic phase. A volume of the aqueous phase is about 80% of the initial volume of RNAzol plus a volume of tissue used for homogenization.

3. RNA Precipitation

a) Transfer supernatant (2 mL) to a new tube.
b) Precipitate RNA by adding 2 mL isopropanol (1 vol.), mix by inverting the tube several times, let stand for 15 min at room temperature.
c) Centrifuge at 12,000 g x 10 min.
d) Pour off the liquid against the RNA precipitation.

- RNA precipitate (often invisible before centrifugation) forms a white pellet at the bottom al the tube.

4. RNA Wash

a) Add 0.8 mL 75% ethanol (4°C cold).
b) Centrifuge 10,000 g x 3 min; Pour off the ethanol; wash twice.

- The RNA precipitate can be stored in 75% ethanol overnight at room temperature (RNA wash), for at least one week at 4°C, or at least one year at -20°C.

c) At the end of the procedure, dry the pellet briefly 5 to 10 min. It is important not to let the RNA pellet dry completely, as it will greatly decrease its solubility.

- Can pulse spin and pipette the ethanol away.
- Do it twice.

5. RNA Solubilization

a) Solubilize the RNA pellet in 100 µL RNase-free water.

- Stand on the ice for 5 min.
- Tap the tube gently with fingers to help it soluble.

b) Finish step 5, RNA stored at -80°C

Appendix F

RNA Cleanup

Modified from: RNAeasy mini kit (QIAGEN, Inc., Valencia, CA)

Procedure

1. Adjust the sample to a volume of 100 µL with RNase-free water. Add 350 µL Buffer RLT, and mix well.

2. Add 250 µL ethanol (96 to 100%) to the diluted RNA, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 3.

3. Transfer the sample (700 µL) to an RNeasy Mini spin column placed in a 2 mL collection tube (supplied). Close the lid gently, and centrifuge for 15 s at ≥ 8,000 x g. Discard the flow-through.

4. Add 500 µL Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at ≥ 8,000 x g to wash the spin column membrane. Discard the flow-through.

5. Add 500 µL Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min at ≥ 8,000 x g (≥ 10,000 rpm) to wash the spin column membrane.

6. Place the RNeasy spin column in a new 1.5 mL collection tube (supplied). Add 30 to 50 µL RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at ≥ 8,000 x g to elute the RNA.

7. If the expected RNA yield is > 30 µg, repeat step 7 using another 30 to 50 µL RNase free water, or using the eluate from step 6 (if high RNA concentration is required). Reuse the collection tube from step 6.

Appendix G

Protocol and Images of Total RNA Resolved on the Denaturing Agarose Gel

Overview: Total RNA integrity was assessed by resolving 2.5 µg of RNA on a 1.5% gel, and confirming the 28s and 18s rRNA bands were intact and sharp.

Protocol was modified from reference:

https://www.thermofisher.com/us/en/home/references/protocols/nucleic-acid-purification-and-analysis/rna-protocol/agarose-gel-electrophoresis-of-rna.html

Chemicals and Materials

- Ambion® NorthernMax® Formaldehyde Load Dye (Thermo Fisher Scientific Inc., MA)
- Gel red (Biotium Inc., Fremont, CA).
- Milli-Q water (purified by Milli-Q® Integral Water Purification System)
- Water - Molecular Biology Grade (Thermo Fisher Scientific Inc., MA)
- RNase Away
- 10× MOPS
- 37% Formaldehyde
- Dry bath incubator (Thermo Fisher Scientific Inc., MA)
- Gel tank

Protocol

1. Wash the Gel Tank

a) Wash with tap water, and then soaked with RNase Away for 1 h.
b) Then rinse thoroughly 10 times with Milli-Q water.
c) Put it on clean towel paper, and let the tank completely dry.

2. Prepare the Gel

a) Weigh 0.5 g agarose in a flask, and then add 36 mL water (molecular biology grade).
b) Using Microwaves, heat the agarose until dissolved (30s to 10s), then cool to 60°C.
c) Add 5 mL 10X MOPS running buffer, and 9 mL 37% formaldehyde (12.3 M), in a chemical fume hood.
d) Put the dry, clean gel tank on a clean bench.
e) Pour the gel, using a comb that will form wells large enough to accommodate at least 25 µL.
f) After 45 min, the gel was done. Then remove the comb.
g) Assemble the gel in the tank, and add enough (~ 300 mL) 1X MOPS running buffer, allowing for covering the gel by a few millimeters, but make sure it is not above the fill line!

3. Prepare the RNA Sample

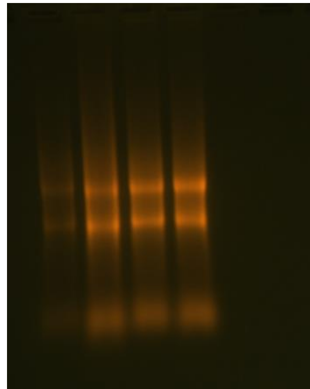
a) Take RNA samples out from -80°C freezer, and thaw for 30 min on ice box.
b) Take 2 µg RNA to 1.5ml new tube, and add 1 volume's Formaldehyde Load Dye.
c) Denature the samples at 65-70° C for 5 min on a dry bath incubator.
d) Take samples out immediately, and put them back on the ice box.
e) Use P10 or P2 Pipette, take 0.5 µL gel red, and mix well with the sample.

4. Electrophoresis

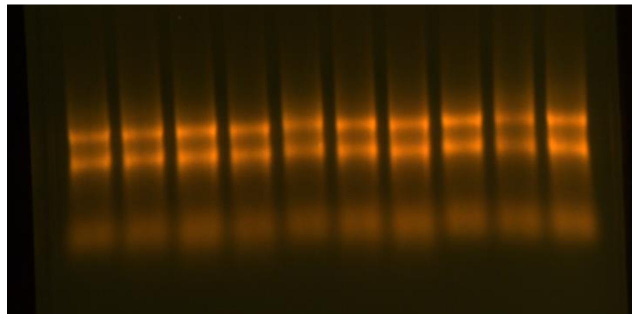
a) Load the samples by pipetting into the sample wells.
b) Run electrophorese at 5 V/cm until the bromophenol blue (the faster migrating dye) has migrated at least 2 to 3 cm into the gel, or as far as 2/3 the length of the gel (about 1.5 h).

5. Image Analysis

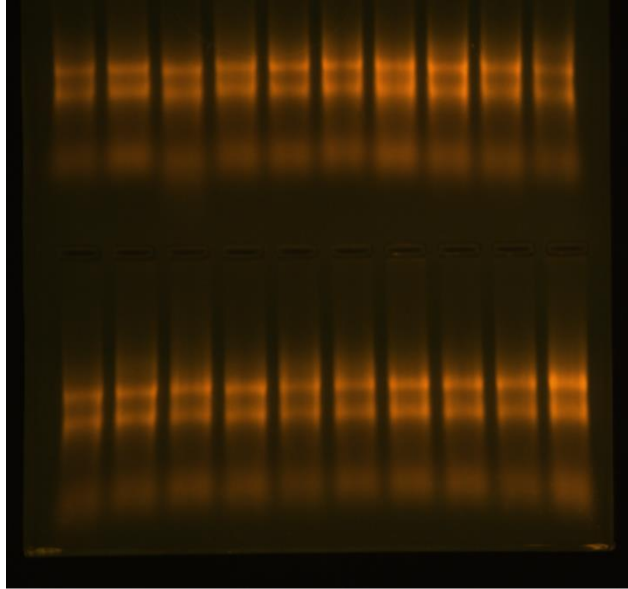
a) Visualize the gel on a UV trans illuminator.
b) Images were captured and showed below.



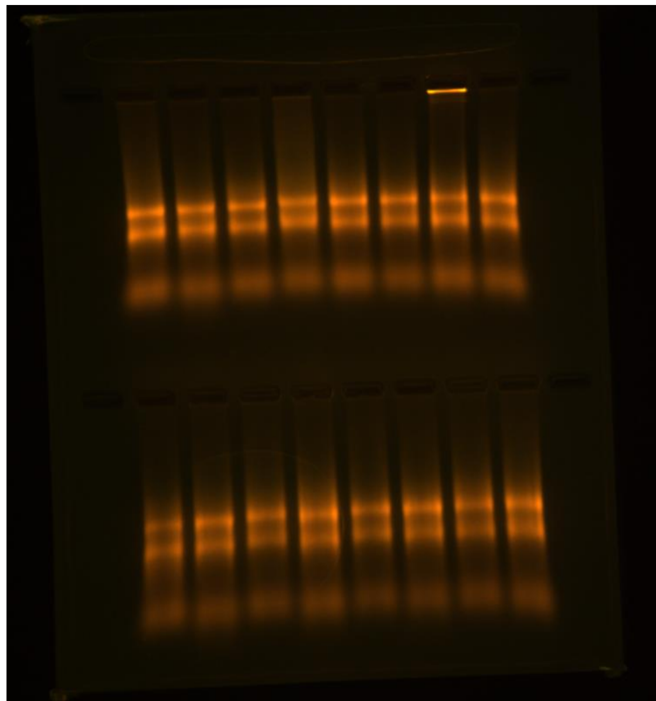
Images of RNAs from muscle tissues developed on 4/20/2016, before RNAs were purified. The lanes from left to right are: practice sample #a, practice sample #b, #17, #18.



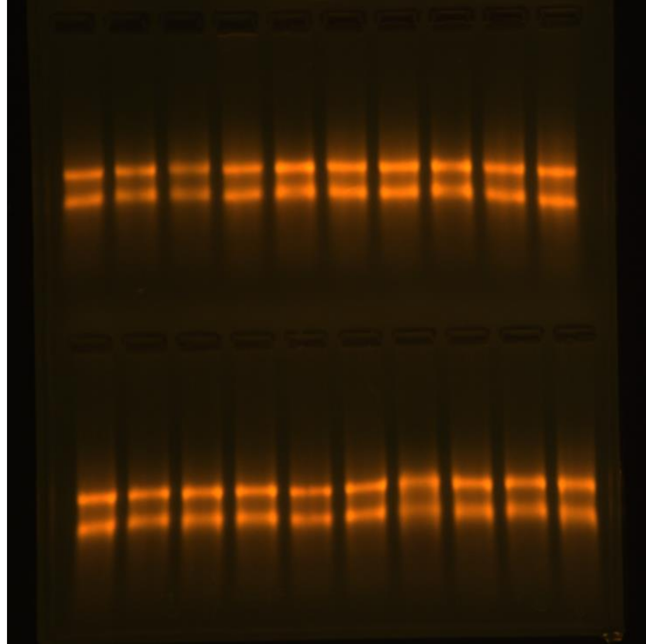
Images of RNAs from muscle tissues developed on 4/29/2016, before RNAs were purified. The lanes from left to right are: #9, #12, #13, #19, #24, #25, #31, #33, #38, #40.



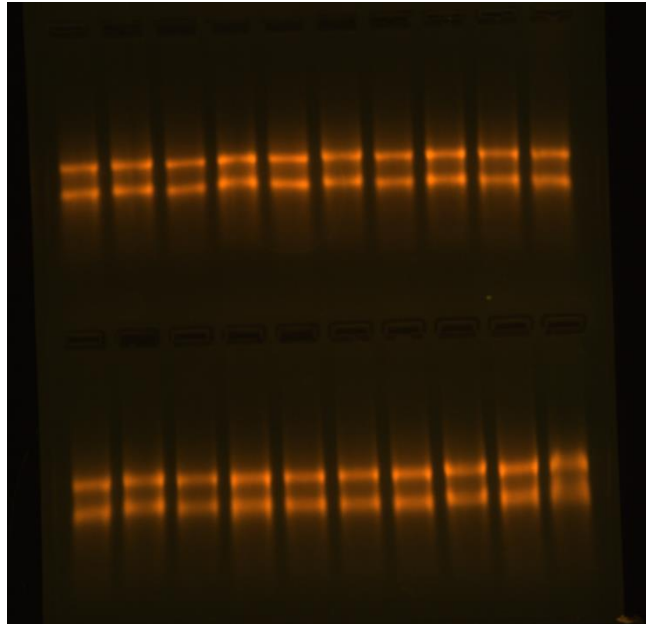
Images of RNAs from muscle tissues developed on 5/16/2016, before RNAs were purified. The upper lanes from left to right are: #2, #6, #10, #15, #20, #22, #23, #26, #14, #30; the lower lanes from left to right are: #32, #34, #35, #39, #41, #42, #43, #45, #46, #16.



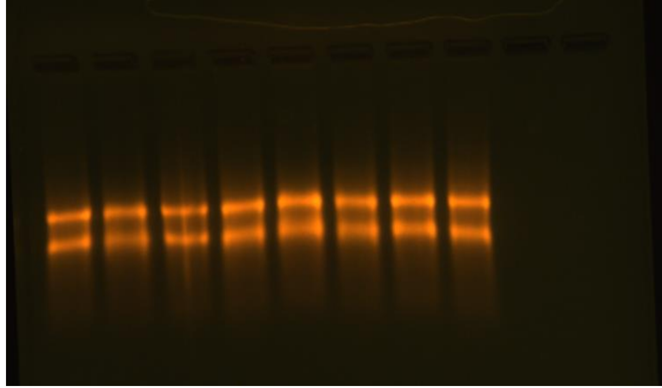
Images of RNAs from muscle tissues developed on 5/17/2016, before RNAs were purified. The upper lanes from left to right are: #11, #27, #29, #47, #48, #55, #57, #58; the lower lanes from left to right are: #60, #69, #75, #84, #85, #86, #95, #96.



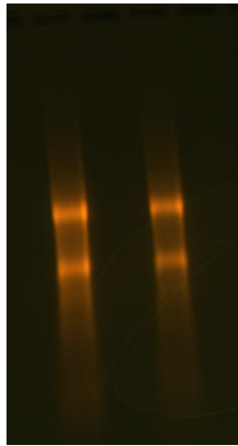
Images of RNAs from muscle tissues developed on 5/27/2016, after RNAs were purified by RNAeasy mini kit. The upper lanes from left to right are: #5, #52, #56, #59, #61, #65, #68, #71, #72, #79; the lower lanes from left to right are: #81, #82, #83, #90, #91, #92, #1, #77, #78, #80.



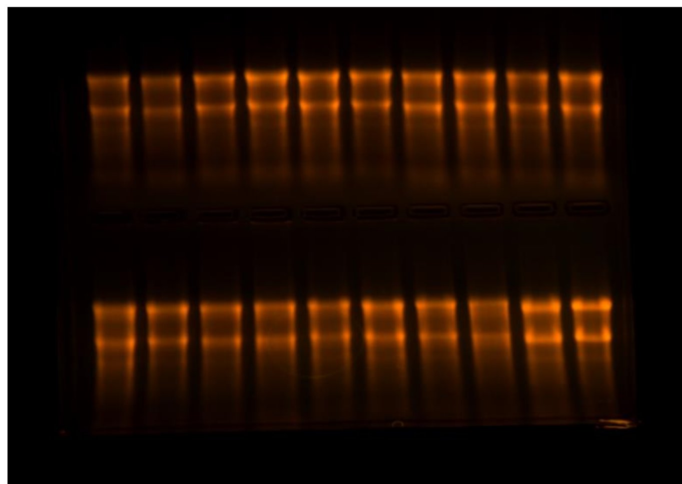
Images of RNAs from muscle tissues developed on 6/2/2016, after RNAs were purified by RNAeasy mini kit. The upper lanes from left to right are: #3, #4, #7, #21, #28, #37, #44, #49, #50, #51; the lower lanes from left to right are: #53, #54, #63, #64, #66, #67, #73, #87, #88, #89.



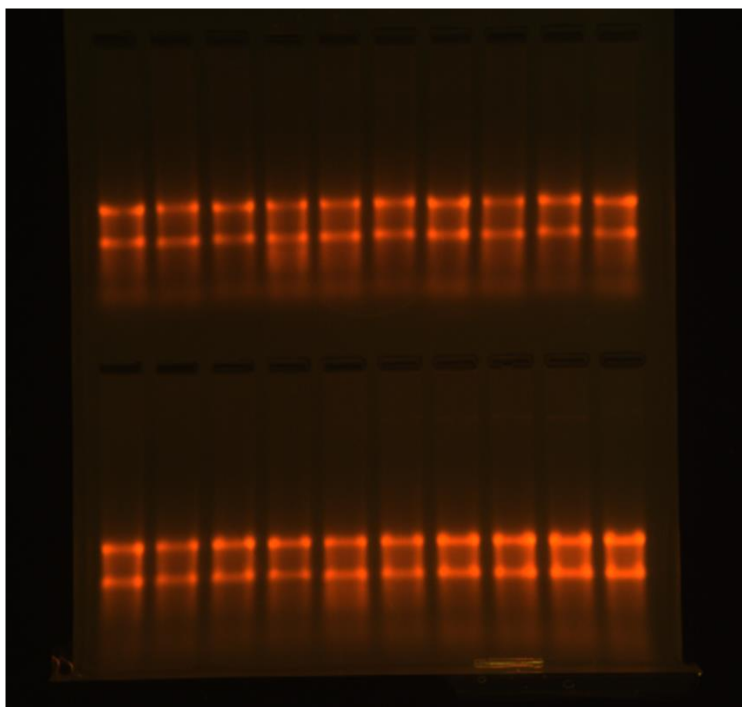
Images of RNAs from muscle tissues developed on 6/3/2016, after RNAs were purified by RNAeasy mini kit. The lanes from left to right are: #8, #12, #36, #62, #74, #76, #93, #94.



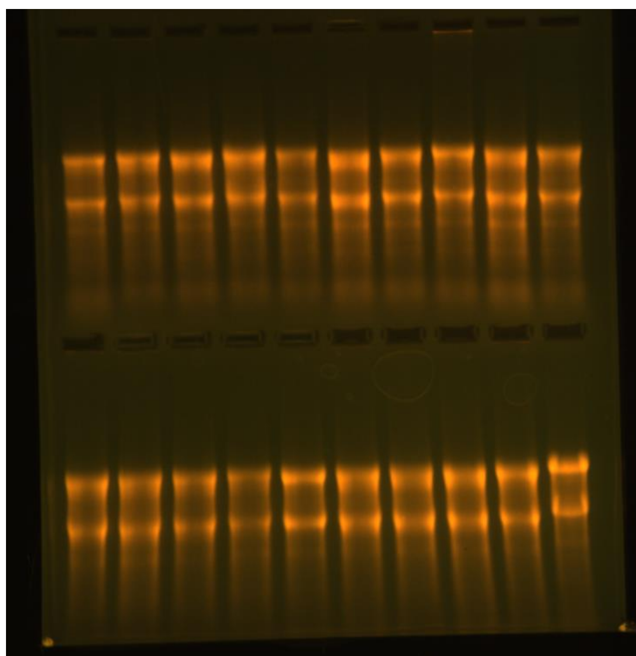
Images of RNAs from backfat tissues developed on 7/21/2016, after RNAs were purified by RNAeasy mini kit. The lanes from left to right are: #45, #47.



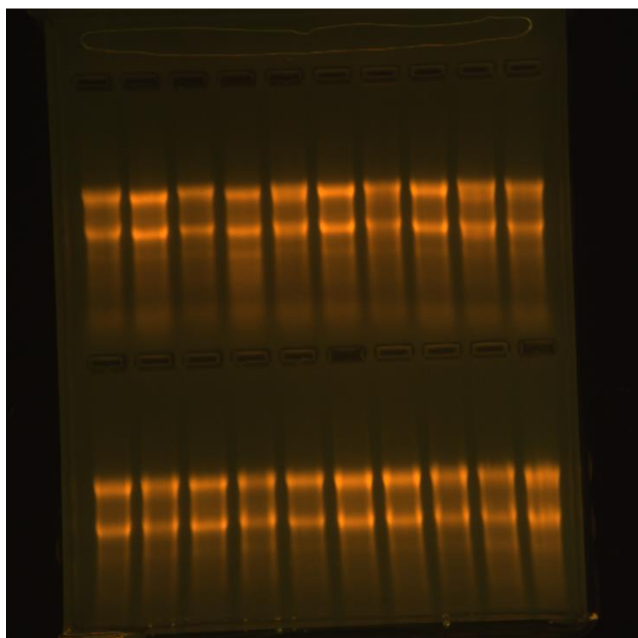
Images of RNAs from backfat tissues developed on 8/18/2016, after RNAs were purified by RNAeasy mini kit. The upper lanes from left to right are: #2, #4, #6, #11, #14, #15, #16, #21, #22, #23; the lower lanes from left to right are: #26, #29, #32, #33, #34, #36, #37, #38, #44, #46.



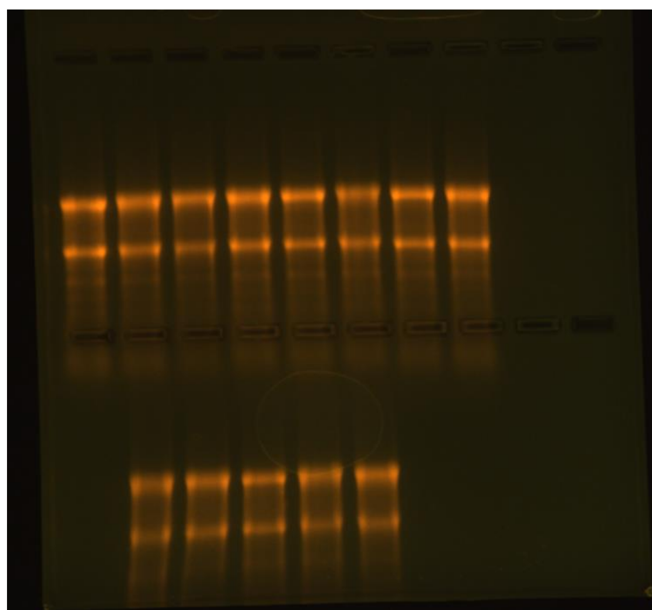
Images of RNAs from backfat tissues developed on 8/22/2016, after RNAs were purified by RNAeasy mini kit. The upper lanes from left to right are: #1, #3, #5, #7, #10, #12, #13, #17, #20, #24; the lower lanes from left to right are: #27, #30, #39, #42, #43, #48, #67, #71, #74, #83.



Images of RNAs from backfat tissues developed on 8/25/2016, after RNAs were purified by RNAeasy mini kit. The upper lanes from left to right are: #8, #18, #19, #25, #28, #31, #35, #40, #41, #50; the lower lanes from left to right are: #52, #53, #61, #64, #62, #66, #75, #76, #83, #73.



Images of RNAs from backfat tissues developed on 9/1/2016, after RNAs were purified by RNAeasy mini kit. The upper lanes from left to right are: #49, #51, #54, #55, #56, #57, #58, #59, #60, #63; the lower lanes from left to right are: #78, #82, #85, #88, #90, #93, #94, #95, #96, #69.



Images of RNAs from backfat tissues developed on 9/2/2016, after RNAs were purified by RNAeasy mini kit. The upper lanes from left to right are: #9, #65, #68, #72, #77, #79, #81, #84; the lower lanes from left to right are: #86, #87, #89, #91, #92.

Appendix H

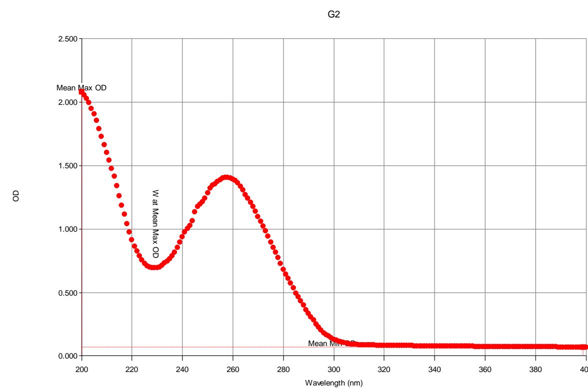
Images of RNA Spectral Scans

Instrument: BioTek Synergy 4 microplate reader with the Take3 system (BioTek US, Winooski, VT).

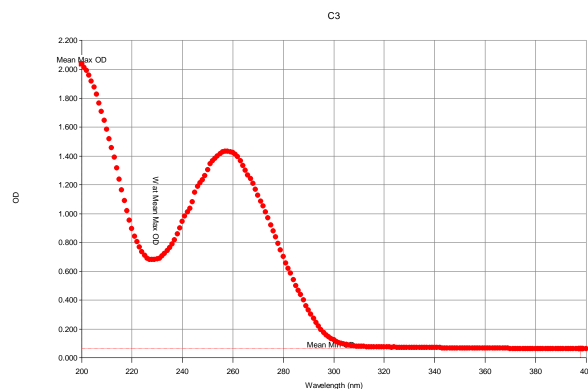
Principle of Measurements

- Spectral scans (200 to 400 nm) to verify sample purity. In a buffer at neutral pH, a good RNA quality need to be: OD260/OD280 > 1.8 and OD260/OD230 > 2.0.
- Absorbance at 260 nm is used to measure the amount of nucleic acid present in the sample.
- Aromatic amino acids absorb light at 280 nm, so absorbance measurements at that wavelength are used to estimate the amount of protein in the sample.
- Measurements at 230 nm are used to determine the amount of other contaminants that may be present in the samples, such as guanidine thiocyanate.

Images of Spectral Scans (Examples)



Representative image of RNA spectral scans from muscle tissue (pig # 50), developed on 6/2/2016



Representative image of RNA spectral scans from adipose tissue (pig # 15), developed on 8/6/2016

Appendix I

Protocol for cDNA Synthesis

Reference: Technical manual for the ImProm-II™ Reverse Transcription System, 2016 (Promega Co., Madison, WI)

Keep in Mind

1. The ImProm-II™ Reverse Transcriptase, ImProm-II™ 5X Reaction Buffer, dNTP Mix and Control RNA should be kept chilled before use. Thaw on ice; do not heat in a warming block to thaw.
2. Oligo(dt) primers initiate reverse transcription at the 3´ end of the transcript, difficult secondary structure may lead to incomplete cDNA synthesis. Oligo(dT) primers are not recommended as the only primer for cDNA synthesis if 18S rRNA is used for normalization in a real-time PCR experiment as the oligo(dt) primer will not anneal.
3. Mg^{2+}: longer messages more efficient at lower [Mg^{2+}], while shorter messages are transcribed more efficiently at higher [Mg^{2+}]. Promega recommend 1.5 mM to 8 mM (mean = 4.75; we use 6 mM as per positive control)

General Laboratory Precautions

1. Use designated work areas and pipettors for pre- and post-amplifi cation steps. This precaution is intended to minimize the potential for cross-contamination between samples and prevent carryover of nucleic acid (DNA and RNA) from one experiment to the next.
2. Wear gloves and change them often.
3. Prevent contamination by using barrier or positive displacement pipette tips.
4. Use sterile, nuclease-free thin-walled reaction tubes.

Need:

1. Kit: Promega ImPromII RT (A3800), 100 reactions
   Storage Conditions: Store all system components at -20°C. Thaw and maintain the ImProm-II™ 5X Reaction Buffer, the ImProm-II™ Reverse Transcriptase, dNTP Mix and the 1.2kb Kanamycin Positive Control RNA on ice during use. For long-term storage, the 1.2kb Kanamycin Positive Control RNA may be stored at -70°C in single-use aliquots. See the expiration date on the system label.
2. Ice bucket
3. Eppendorf PCR-Cooler
4. Pipette (10, 200 uL) and barrier tips
5. RNase free tubes (1.5 mL & PCR tube)
6. Gloves
7. Centrifuge (1.5 mL tube).
8. MJR PTC-100 Programmable Thermal Controller (96-well PCR Thermal Cycler).

Procedure

1. Thaw on ice (30 min for RNA, 1.5 h for others)

    - RNA samples
    - Oligo dt, 5XBuffer, dNTP Mix, Positive Control RNA, Reverse Transcriptase (**RT**), RNAsin

2. Thaw at room temperature

    - $MgCl_2$, water

3. Turn on and preheated 65°C heat block.
4. Set the procedure (RTJB) on PTC-100 Thermal Controller.

    - Set: anneal 5 min at 25°C then incubate @ 42°C for 50 min then heat 70°C for 15 min, then extend at 4°C. Processed-Pause.

5. Label new 1.5 ml tubes. On ice, combine the experimental RNA (up to 1 μg) and the cDNA primer in Nuclease-Free Water for a final volume of 5 μL per RT reaction.

    - Build one tube of Positive Control and negative control in each set if necessary.

6. Close each tube of RNA tightly. Place the tubes into a preheated 65°C heat block for 5 min. Immediately chill in ice-water for at least 5 min.
7. Spin each tube for 10 s in a micro-centrifuge to collect the condensate and maintain the original volume. Keep the tubes closed and on ice until the reverse transcription reaction mix is added.
8. Prepare the reverse transcription reaction mix (n + 1 volume). Gently mix, and keep on ice prior to dispensing into the reaction tubes

.

	1x volume	12x volume
E. 5x Buffer (4 uL)	4	48
F. MgCL (4.8 uL)	4.8	57.6
G. dNTP (1 uL)	1	12
H. RNAsin (0.5 uL)	0.5	6
I. water (3.7 uL)	3.7	44.4
J. reverse trans. (1 uL)	1	12

  - DO Not add RT in the mix immediately. Add it right before we aliquot into each tube.

9. Add 15 μL aliquots of the reverse transcription reaction mix to each PCR reaction tube on ice. Be careful to prevent cross-contamination. Add 5 μL of RNA and primer mix to each reaction for a final reaction volume of 20 μL per tube.
10. Mix RX/template by finger flip, and then put them on the PTC-100.

- Incubated at 42°C for 1 h and then inactivated at 70°C for 15 min.

11. Close the lid tightly, then turn 1/4 more circle.
12. Press “proceed” to start.
13. After done, take out the PCR tubes and store the cDNA samples at -20°C.

Appendix J

Protocol for Real-time qPCR with LightCycler480

The Roche LightCycler® 480 Real-Time PCR System is a 96 well-plate based real-time PCR platform that is used for highly accurate qualitative and quantitative detection of nucleic acids and genotyping. Fluorescent signals measured during cycling are correlated with the amount of PCR product in the reaction, allowing the calculation of input copy number of the target nucleic acid.

Keep in Mind

1. Roche Lightcycler 480 SYBR green I Master (Cat.No. 04707516001)

    - Once the kit is opened, keep out of light, stored at -15 to -25°C.
    - After the first thawing, the master mix may be stored for up to 4 weeks at +2°C to +8°C in blue container. Avoid repeated freezing and thawing.

2. cDNA thaw on ice.
3. Primer

    - 0.4X dilution
    - Thaw at room temperature
    - Matching forward and reverse primers

4. To reduce variance, practice the pipette skills.

Need

1. Matching forward and reverse primers
2. Pure water (provided in Roche kit)
3. cDNA serial dilutions for dose curve (i.e., 12.5 ng, 25 ng, 50 ng)
4. White plates and covers sheets (Roche Lightcycler)
5. Ice block (Eppendorf PCR-Cooler)
6. Foil
7. Ice bucket

Procedure

1. Set up the LightCycler480 program and warm up.
2. Thaw primers and water at room temperature (> 1 h in 4°C).
3. Thaw SYBR green I Master, and cDNA on ice or 4°C.
4. Calculate the mix volume (10% more). For 1X mix: (19 µL)

	1X mix	58X mix	106X
Water	7 µL	406 µL	742
Forward primer	1 µL	58 µL	106
Reverse primer	1 µL	58 µL	106
SYBR Green	10 µL	580 µL	1060

5. Start mix for each primer mix set by adding water, forward primer and reverse primer.
6. Turn off the lights, add the SYBR Green to the primer mix.

   - Mix carefully by pipetting up and down, or flip up/down. **Do not vortex**!

7. Then aliquot 19 µL into each well containing sample.

   - Try to keep the plate out of light as much as possible.
   - The complete PCR mix (SYBR + primers + template) is stable for up to 24 h at room temperature. Keep the PCR mix away from light!

8. Pipette 1 µL cDNA samples onto each well.

   - Use a 1 ml pipette tip as indicator, to tell which well we already added.

9. Place cover slip on the plate (easiest to roll the cover from left to right of plate, then smooth with plastic tube holder lid), then cover with aluminum foil (to avoid light).
10. Take upstairs and spin once in lettuce dryer (careful not to spin too hard or it will spatter the samples out of the wells).
11. Then place the plate in the machine and run the program (set up beforehand)
12. General template for saving files:

    - [Name][date] [sample + primer]
    - Chunxi - 3 Aug 16 Muscle +S15

13. Program takes around 1 h and 15 min to run.
14. Plate can be thrown away once the program has finished.
15. Need flash drive to collect the data tables.

Appendix Table 1

Effects of dietary lipids and vitamin E (VE) supplementation on fatty acid content of loin muscle tissues in finisher pigs (mg/100 g wet tissue) [1,2].

Item	Lipid, %: 0		2		4		6		SEM [3]	*P*-value				
VE, IU/kg:	11	220	11	220	11	220	11	220		Lipid, Ln	Lipid, Qd	VE	Lipid × VE	Flax, 0 vs.1%
C10:0	0.7	1.9	0.9	0.6	0.7	0.7	0.4	0.6	0.4	0.051	0.568	0.225	0.227	0.048
C12:0	0.8	1.5	1.0	1.1	0.5	0.8	0.3	0.5	0.4	0.036	0.752	0.198	0.906	0.177
C14:0	24.4	37.7	33.6	30.9	24.9	30.3	23.1	24.8	4.0	0.047	0.397	0.127	0.250	0.346
C16:0	520	810	703	640	564	665	527	558	77	0.090	0.479	0.110	0.151	0.383
C16:1	72.6	118.3	104.0	89.5	80.4	95.8	75.0	77.8	12.0	0.091	0.447	0.154	0.099	0.400
C17:0	4.6	6.2	5.3	4.7	4.0	4.4	3.9	3.5	0.5	0.001	0.896	0.526	0.125	0.010
C18:0	233	354	298	296	248	287	233	249	33	0.079	0.514	0.067	0.258	0.359
C18:1n9t	11.5	10.4	9.8	8.5	11.0	10.4	9.6	9.4	1.0	0.385	0.686	0.259	0.944	0.167
C18:1n9c	943	1,460	1,263	1,137	1,003	1,197	953	986	146	0.092	0.535	0.142	0.168	0.354
C18:2ω6t	0.2	0.4	1.1	0.3	0.2	0.0	0.0	0.7	0.3	0.622	0.702	0.883	0.247	0.793
C18:2ω6c	260	323	355	313	287	330	322	319	27	0.480	0.419	0.427	0.213	0.185
C18:3ω6	1.7	2.0	1.6	1.6	1.4	1.7	1.3	1.6	0.5	0.380	0.792	0.573	0.987	0.384
C18:3ω3	20.7	32.0	57.9	51.8	44.0	53.6	45.0	47.9	5.5	0.004	<0.001	0.267	0.400	<0.001
C20:0	2.8	4.4	4.7	3.8	3.5	4.0	3.3	3.4	0.7	0.581	0.259	0.543	0.338	0.715
C20:1n9	1.1	1.5	1.6	1.4	0.8	1.2	0.9	1.2	0.4	0.334	0.870	0.434	0.850	0.675
C20:2ω6	8.1	10.8	11.3	9.5	8.2	10.9	9.2	9.2	1.2	0.698	0.455	0.328	0.220	0.777
C20:3ω6	8.7	9.2	8.8	6.9	8.1	8.6	9.3	8.5	0.7	0.947	0.103	0.441	0.320	0.285
C20:3ω3	1.4	1.5	5.9	8.4	3.5	9.5	4.4	3.5	1.7	0.201	0.001	0.107	0.177	0.002
C20:4ω6	0.6	0.2	0.2	0.6	0.2	0.4	0.3	0.1	0.3	0.446	0.829	0.957	0.713	0.646
C20:5ω3	0.7	0.7	6.1	4.8	5.6	4.2	4.1	4.6	0.5	<0.001	<0.001	0.149	0.241	<0.001
C22:0	0.8	0.8	0.8	0.4	0.6	0.3	1.2	0.1	0.4	0.621	0.437	0.113	0.547	0.453
C22:6ω3	0.9	0.6	1.8	1.6	1.2	1.1	1.6	1.3	0.6	0.401	0.440	0.583	0.998	0.149
SFA	787	1,216	1,048	977	846	993	792	840	115	0.081	0.486	0.096	0.176	0.366
MUFA	1,028	1,591	1,378	1,236	1,096	1,305	1,038	1,075	158	0.091	0.531	0.145	0.163	0.353
ω-3 PUFA	23.7	34.8	71.7	66.6	54.3	68.3	55.1	57.3	6.5	0.001	<0.001	0.236	0.457	<0.001
ω-3 HUFA	3.0	2.9	13.8	14.8	10.3	14.7	10.1	9.3	1.7	0.001	<0.001	0.339	0.407	<0.001

ω-6 PUFA	279	346	378	332	305	351	342	338	28	0.535	0.450	0.435	0.204		0.223
Total PUFA	303	380	450	398	359	419	397	396	34	0.237	0.124	0.380	0.227		0.032
LA: ALA	12.7	10.4	6.2	6.3	6.7	6.3	7.3	7.3	0.5	<0.001	<0.001	0.073	0.064		<0.001
ω-6: ω-3	11.9	10.2	5.3	5.1	5.7	5.3	6.4	6.3	0.4	<0.001	<0.001	0.047	0.172		<0.001
Total FAME	2,118	3,187	2,876	2,612	2,301	2,717	2,227	2,310	302	0.154	0.443	0.135	0.169		0.558

[1] Ln = linear, Qd = quadratic, Flax = flaxseed oil, SFA= saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; HUFA = highly unsaturated fatty acids; LA: ALA = linoleic acid (18:2ω6) to α-linolenic acid (18:3ω3) ratio; ω-6: ω-3 = ω-3 fatty acids to ω-6 fatty acids ratio; FAME = fatty acids methylated esters.

[2] Least square means based on 6 pens; each pen contained 2 gilts or 2 castrated males.

[3] SEM = pooled standard error of the mean.

[4] ω-3 PUFA = C18:3ω3 + C20:3ω3 + C20:5ω3 + C22:6ω3; ω-3 HUFA = C20:3ω3 + C20:5ω3 + C22:6ω3; ω-6 PUFA = C18:2ω6t + C18:2ω6c + C20:2ω6 + C20:3ω6 + C20:4ω6; total PUFA = ω-3 PUFA + ω-6 PUFA.

Appendix Table 2

Pearson's correlation coefficients (*r*) between muscle fatty acid composition (% of total fatty acids) and mRNA abundance of genes in muscle and adipose tissue [1,2].

Item	ω-3 PUFA	ω-3 HUFA	ω-6 PUFA	ω-6:ω-3	SFA	MUFA	PUFA	UFA	PUFA:SFA	MUFA:SFA	MUFA:PUFA
Adipose tissue											
FAS	NS	NS	0.29**	NS	NS	-0.37**	0.27**	NS	0.20*	-0.35**	-0.29**
SCD	NS	NS	0.20*	NS	NS	-0.20*	0.19*	NS	NS	NS	NS
PPAR-γ	NS	NS	NS	NS	0.18*	NS	NS	-0.18*	NS	NS	NS
ME1	NS	NS	NS	NS	NS	-0.18*	NS	NS	NS	-0.19*	NS
SREBP1c	NS	NS	0.33**	NS	NS	-0.32**	0.33**	NS	0.28**	-0.22**	-0.31**
PPAR-α	NS	NS	0.20*	NS	NS	-0.19*	0.18*	NS	NS	NS	NS
Leptin	NS	-0.18*	NS	NS	0.29**	NS	NS	-0.29**	NS	-0.24**	NS
Adiponectin	NS	NS	0.19*	NS	NS	-0.23**	NS	NS	NS	-0.21**	-0.19*
DGAT1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
DGAT2	NS	NS	0.18*	NS	NS	NS	0.18*	NS	0.18*	NS	NS
Muscle tissue											
FAS	0.28**	0.31**	0.25**	NS	NS	-0.28**	0.30**	NS	0.27**	-0.19*	-0.29**
SCD	NS	NS	0.22**	NS	NS	-0.18*	0.21**	NS	0.20**	NS	-0.19*
PPAR-γ	NS	NS	0.26**	NS	NS	-0.21**	0.24**	NS	0.21*	NS	-0.27**
ME1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SREBP	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
PPAR-α	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.20*

[1] Data from individual pigs were used for correlation analysis using the SAS PROC CORR procedure (SAS Ins. Inc., Cary, NC).

[2] NS = not significant, *FAS* = fatty acid synthase; *SCD* = stearoyl CoA desaturate; *PPAR-γ* = peroxisome proliferator-activated receptor γ; *ME1* = malic enzyme 1; *SREBP1c* = sterol regulatory element binding protein-1c; *PPAR-α* = peroxisome proliferator-activated receptor α; *DGAT1* = diacylglycerol acyltransferase 1; and *DGAT2* = diacylglycerol O-acyltransferase 2.

* Probability value of obtaining the observed coefficient ($0.051 \le P \le 0.100$).

** Probability value of obtaining the observed coefficient ($P \le 0.050$).

Appendix Table 3

Pearson's correlation coefficients (r) between muscle fatty acid composition (% of total fatty acids) and serum metabolites [1,2].

Item	ω-3 PUFA	ω-3 HUFA	ω-6 PUFA	ω-6:ω-3	SFA	MUFA	PUFA	UFA	PUFA:SFA	MUFA:SFA	MUFA:PUFA
Total protein [3], g/dL	0.21**	NS	NS	NS	NS	NS	0.19*	NS	0.20*	NS	-0.22**
Albumin [3], g/dL	0.20*	0.23**	0.26**	NS	NS	-0.24**	0.28**	NS	0.26**	NS	-0.32**
Globulin [3], g/dL	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
A:G Ratio	NS	NS	0.18*	NS	NS	-0.25**	NS	NS	NS	-0.25**	-0.18*
BUN [3,5], mg/dL	NS	NS	-0.21**	NS	0.29**	NS	-0.18*	-0.29**	-0.24**	NS	NS
Glucose [3], mg/dL	NS	NS	NS	NS	NS	NS	NS	NS	0.17*	NS	NS
Cholesterol [3], mg/dL	NS	NS	-0.24**	NS	NS	-0.26**	0.25**	NS	0.23**	-0.21**	-0.30**
Triglycerides [3], mg/dL	0.31**	0.29**	-0.32**	NS	NS	-0.25**	0.36**	NS	0.37**	NS	-0.34**

[1] Data from individual pigs were used for correlation analysis using SAS Proc Corr procedure.

[2] NS = not significant.

* Probability value of obtaining the observed coefficient ($0.051 \le P \le 0.100$).

** Probability value of obtaining the observed coefficient ($P \le 0.050$).