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The composition of dietary fat alters the transcriptional profile of pathways associated with lipid metabolism in the liver and adipose tissue in the pig

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Abstract

The objective was to investigate the of effect chemical composition of dietary fat on transcription of genes involved in lipid metabolism in adipose tissue and liver via transcriptional profiling in growing pigs. A total of 48 Genetiporc 6.0 × Genetiporc F25 (PIC, Inc., Hendersonville, TN) barrows (initial BW of 44.1 ± 1.2 kg) were randomly allotted to 1 of 6 dietary treatments. Each experimental diet included 95% of a corn-soybean meal basal diet and 5% of either: corn starch (CNTR), animal-vegetable blend (AV), coconut oil (COCO), corn oil (COIL), fish oil (FO), or tallow (TAL). Pigs were sacrificed on d 10 (final BW of 51.2 ± 1.7 kg) to collect tissues. Expression normalization across samples was performed by calculating a delta Ct (ΔCt; cycle threshold) value using RPL32. Delta delta Ct values (ΔΔCt) were expressed relative to the CNTR treatment. In adipose tissue adding dietary fat regardless of source decreased the mRNA abundance of FASN compared to CNTR fed pigs (P = 0.014). Of the dietary fat sources tested, pigs fed a COIL based diet tended to have greater adipose tissue expression of FASN (P = 0.071). Abundance of PRKAG-1 mRNA was greater in adipose tissue of barrows a fed COIL based diet than barrows fed CNTR or FO diets (P = 0.047). In liver adding dietary fat regardless of source increased the mRNA abundance of ACACA, ATGL, INSR, PPAR-α, PRKAG-1, and SCD (P ≤ 0.020) and tended to have greater abundance of HSL (P = 0.071) and SREBP-1 (P = 0.086) compared to CNTR fed barrows. Pigs fed a TAL based diet had greater hepatic transcription of HSL than pigs fed CNTR, COCO, or FO diets (P = 0.013). Hepatic transcription of FASN tended to be greater in pigs fed COCO than pigs fed other dietary fat sources (P = 0.074). Dietary omega-3 fatty acid content tended to negatively correlate with mRNA abundance of PRKAG-1 (P = 0.065) in adipose tissue and ATGL (P = 0.063) in liver. Dietary fat SFA content was negatively correlated with PPAR-α in liver (P ≤ 0.039). Dietary fat MUFA content tended to be positively correlated with ACACA, PPAR-α, PRKAG-1 mRNA abundance in liver (P ≤ 0.100). To conclude, the intake of omega-3 fatty acids suppressed the mRNA abundance of genes involved in lipolysis in both adipose tissue and liver. Dietary SFAs are greater inhibitors of lipogenesis in adipose tissue than omega-6 fatty acids. Intake of medium chain fatty acids alter hepatic lipid metabolism differently than intake of long chain fatty acids.

Keywords

dietary fat, lipogenesis, lipolysis, adipose, liver, swine

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Running Head: Dietary fat alters lipid metabolism in pigs

The composition of dietary fat alters the transcriptional profile of pathways associated with lipid metabolism in the liver and adipose tissue in the pig[1]

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ABSTRACT: The objective was to investigate the of effect chemical composition of dietary fat on transcription of genes involved in lipid metabolism in adipose tissue and liver via transcriptional profiling in growing pigs. A total of 48 Genetiporc 6.0 × Genetiporc F25 (PIC, Inc., Hendersonville, TN) barrows (initial BW of 44.1 ± 1.2 kg) were randomly allotted to 1 of 6 dietary treatments. Each experimental diet included 95% of a corn-soybean meal basal diet and 5% of either: corn starch (CNTR), animal-vegetable blend (AV), coconut oil (COCO), corn oil (COIL), fish oil (FO), or tallow (TAL). Pigs were sacrificed on d 10 (final BW of 51.2 ± 1.7 kg) to collect tissues. Expression normalization across samples was performed by calculating a delta Ct (ΔCt; cycle threshold) value using *RPL32*. Delta delta Ct values (ΔΔCt) were expressed relative to the CNTR treatment. In adipose tissue adding dietary fat regardless of source decreased the mRNA abundance of *FASN* compared to CNTR fed pigs ($P = 0.014$). Of the dietary fat sources tested, pigs fed a COIL based diet tended to have greater adipose tissue expression of *FASN* ($P = 0.071$). Abundance of *PRKAG-1* mRNA was greater in adipose tissue of barrows a fed COIL based diet than barrows fed CNTR or FO diets ($P = 0.047$). In liver adding dietary fat regardless of source increased the mRNA abundance of *ACACA*, *ATGL*, *INSR*, *PPAR-α*, *PRKAG-1*, and *SCD* ($P \leq 0.020$) and tended to have greater abundance of *HSL* ($P = 0.071$) and *SREBP-1* ($P = 0.086$) compared to CNTR fed barrows. Pigs fed a TAL based diet had greater hepatic transcription of *HSL* than pigs fed CNTR, COCO, or FO diets ($P = 0.013$). Hepatic transcription of *FASN* tended to be greater in pigs fed COCO than pigs fed other dietary fat sources ($P = 0.074$). Dietary omega-3 fatty acid content tended to negatively correlate with mRNA abundance of *PRKAG-1* ($P = 0.065$) in adipose tissue and *ATGL* ($P = 0.063$) in liver. Dietary fat SFA content was negatively correlated with *PPAR-α* in liver ($P \leq 0.039$). Dietary fat MUFA content tended to be positively correlated with *ACACA*, *PPAR-α*, *PRKAG-1* mRNA

abundance in liver ($P \leq 0.100$). To conclude, the intake of omega-3 fatty acids suppressed the mRNA abundance of genes involved in lipolysis in both adipose tissue and liver. Dietary SFAs are greater inhibitors of lipogenesis in adipose tissue than omega-6 fatty acids. Intake of medium chain fatty acids alter hepatic lipid metabolism differently than intake of long chain fatty acids.

Key words: dietary fat, lipogenesis, lipolysis, adipose, liver, swine

INTRODUCTION

Increased inclusion of dietary fat is known to suppress lipogenesis in adipose tissue of pigs (Bortz et al., 1963; Allee et al., 1971). How dietary fat sources that differ in their fatty acid composition alter the transcription of genes involved in lipid metabolism is less known (Jump, 2002; Duran-Montge et al., 2009). Sources of dietary fat are diverse in fatty acid chain length and degree of unsaturation (Powles et al, 1995; NRC, 2012). Quantifying the effect of dietary fat composition on gene expression associated with lipid partitioning can provide insight into changes in post-absorptive lipid metabolism. This, in turn, would lead to a more accurate prediction of the pig's response to inclusion of fat in the diet.

Most published studies on lipid metabolism have employed human or rodent subjects (Bergen and Mersmann, 2005), and of the few in growing pigs, most have measured the expression of genes involved in hepatic lipogenesis (Duran-Montge et al., 2009). Thus, there are few data to describe the effects of dietary fat source on lipogenesis and lipolysis in adipose tissue, which in the pig, is the most important organ involved in fatty acid synthesis (O'Hea and Leveille, 1969). The current understanding derived from these few porcine studies is that SFA inhibit adipose lipogenesis more than omega-6 fatty acids (Smith et al., 1996; Kellner et al., 2016a). However, suppression of lipogenesis due to the intake of SFA is not a consistent finding (Allee et al., 1971). It has also been reported that dietary MUFA are positively correlated with increased hepatic lipogenesis (Duran-Montge et al., 2009). Thus, the objective was to investigate the effect of chemical composition of dietary fat sources on transcriptional profiling of genes involved in lipid metabolism in adipose tissue and liver in growing pigs.

MATERIALS AND METHODS

All experimental procedures adhered to guidelines for the ethical and humane use of animals for research, and were approved by the Iowa State University Institutional Animal Care and Use Committee (#2-16-8201-S).

Animals, Housing, and Experimental Design

A total of 48 Genetiporc 6.0 × F25 (PIC, Inc., Hendersonville, TN) barrows (in 2 sequential cohorts of 24 barrows each) with an average initial BW of 44.1 ± 1.2 kg were randomly allotted to 1 of 6 dietary treatments in a 10 d experiment. Pigs were housed individually throughout the experiment, in a room in which each pen provided 1.83 m^2 of floor space, a nipple drinker, and a composite feeder and had slatted concrete flooring. The length of experiment was based on previous data from Kellner et al. (2015, 2016a) showing that the mRNA abundance of genes involved in lipid metabolism and the fatty acid composition of depot fat in pigs can be altered by dietary fat intake within 7 to 14 d.

Diets and Feeding

Each experimental diet (Table 1) consisted of a corn-soybean meal diet with either 5% cornstarch (Control [**CNTR**] or 1 of 5 dietary fat sources: animal-vegetable blend (**AV** with iodine value [**IV**] = 68.7 g/100 g; Darling Pro Ingredients, Wahoo, NE), coconut oil (**COCO** with IV = 1.0 g/100 g; Bulk Apothecary, Aurora, OH), corn oil (**COIL** with IV = 126.3 g/100 g; Feed Energy Co., Des Moines, IA), fish oil (**FO** with IV = 137.4 g/100 g; Double S Liquid Feed Services, Danville, IL), or tallow (**TAL** with IV = 44.0 g/100 g; Darling Pro Ingredients, Omaha, NE). Dietary fat sources were selected to provide a diverse range of fatty acid profiles and degree of unsaturation. More specifically, COCO was selected to provide intake of saturated medium chain fatty acids. The COIL source was selected to provide a high intake of omega-6 fatty acids, while FO provide a high intake of omega-3 fatty acids. A TAL source was selected

to provide a high intake of saturated and mono-unsaturated long chain fatty acids and a low intake of PUFA. Finally, an AV source was selected to provide a combination of SFA, MUFA, and PUFA intake. The chemical composition and the fatty acid profiles of the dietary fats are presented in Tables 2 and 3, respectively.

Feed was provided at 3.2 times maintenance (NRC, 2012). The daily feed allowance was provided in 2 equal meals at 0800 h and 1600 h. If any feed remained in the feeders at 0800 h, it was measured and discarded before the next allotment of feed was added. Daily energy intake (kcal of NE/d) was determined using the following equation: $[(BW^{0.6}) \times 197] \times 3.2$ (NRC, 2012). Prior to the initiation of the study, pigs were fed a common diet. Water was provided ab libitum.

Data and Sample Collection

Pigs were individually weighed on d 0, 7 and 10. Pigs were sacrificed on d 10 (final BW = 51.2 ± 1.7 kg) to collect tissue samples. Adipose tissue was collected using a cork bore (12.7 mm; Flinn Scientific, Bativia, IL) from the 10^{th} rib back fat (ensured all back-fat layers of adipose tissue were represented as in the pig). Immediately following collection, all skin and lean tissue were removed from the core. Jejunum tissue was collected by removing the small intestine and collecting a 10 cm section 5.0 to 5.1 m from the pyloric sphincter; the tissue was immediately rinsed with buffered saline to remove all digesta. Liver was collected by taking a 1 × 1 cm cross section from the middle of the right lobe. Post-collection, all tissue samples were immediately placed in a 7.6 × 17.8 cm labelled sterile sample bags (Fisher Science, Hanover Park, IL), snap frozen in liquid nitrogen, and stored at -80°C for later analysis.

Diet Analysis

Dietary fat sources were analyzed in duplicate at a commercial laboratory (Barrow-Agee Laboratories, Memphis, TN) for fatty acid content (method Ce 1-62; AOCS, 2009), FFA (Ca 5a-

40; AOCS, 2009), moisture and volatile matter (Ca 2c-25; AOCS, 2009), insoluble impurities (Ca 3a-46, AOCS, 2009), unsaponifiable matter (Cb-53, AOCS, 2009), and initial peroxide value (Cd 8b-90; AOCS, 2009). Iodine value was calculated from the fatty acid profile using the following equation: IV = [C16:1] × (0.95) + [C18:1] × (0.86) + [C18:2] × (1.732) + [C18:3] × (2.616) + [C20:1] × (0.795) + [C20:2] × (1.57) + [C20:3] × (2.38) + [C20:4] × (3.19) + [C20:5] × (4.01) + [C22:4] × (2.93) + [C22:5] × (3.68) + [C22:6] × (4.64); brackets indicate percentage concentration (Meadus et al., 2010).

Feed samples were homogenized and then finely ground through a 1 mm screen in a Retsch grinder (model ZMI; Retsch Inc., Newtown, PA). Acid hydrolyzed ether extract (method 2003.06; AOAC, 2007) was determined using a SoxCap SC 247 hydrolyzer and a Soxtec 255 semiautomatic extractor (FOSS North America, Eden Prairie, MN). Dry matter was determined by drying samples in an oven at 105°C to a constant weight. Gross energy was determined using an isoperibolic bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL). Benzoic acid (6.318 Mcal/kg; Parr Instrument Co., Moline, IL) was used as the standard for calibration and determined to contain 6.321 ± 0.007 Mcal of GE/kg. All feed analyses were performed in duplicate and repeated when the intra-duplicate CV was greater than 1%.

mRNA Abundance

Adipose tissue, liver, and jejunum were homogenized using a PowerGen 700D homogenizer (Fisher Science, Hanover Park, IL). Total RNA was then isolated using TRIzol reagent (Fisher Science, Hanover Park, IL) following the manufacturer's protocol with the modification of repeating the RNA pellet wash step to reduce contaminants. The concentration and quality of RNA was quantified using a spectrophotometer (ND-100, NanoDrop Technologies, Rockland, DE). All samples had 260/280 nm ratios above 1.8 and the integrity of

the RNA was further verified by visualization of the 18S and 28S ribosomal bands via a SYBR Safe DNA gel stain (Life Technologies, Carlsbad, CA) after running 2 ug RNA by electrophoresis on a 2% agarose gel. Isolated RNA was then used for cDNA synthesis using the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. A spectrophotometer (ND-100, NanoDrop Technologies, Rockland, DE) quantified synthesis of cDNA.

To determine mRNA abundance, quantitative real time PCR was performed using 20 µL reactions prepared according to the manufacturer's instructions using iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA), 1 µL of each forward and reverse primer (diluted with RNAase free H_2O to 100 µ*M*; Table 4), and 1 µL of cDNA (diluted 200 ng/µL). Fluorescence of SYBR Green was quantified with a single color MyiQ optical module (Bio-Rad Laboratories, Hercules, CA). Each assay plate contained no-reverse transcriptase negative controls and pooled reference samples. The quantitative real time PCR cycling conditions included a 30 second step at 95°C, and then 38 PCR cycles were run, with each cycle consisting of 3 stages (95°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec). Optical detection was performed at 55°C. Analyses of amplification plots were performed with the MyiQ Optical System Software version 1.0 (Bio-Rad Laboratories Inc., Hercules, CA) and cycle threshold (**Ct**) values for each reaction obtained. All mRNA abundance analyses were performed in triplicate and repeated when the intra-triplicate CV was greater than 2%. Expression normalization across samples within tissue was performed by calculating a delta Ct value (**ΔCt =** Ct of the target gene – Ct of the housekeeping gene) for each sample using ribosomal protein-L32 (***RPL32***), as transcript abundance proved to be similar among treatments within tissue ($P = 0.518$). Thus, *RPL32* was considered a suitable housekeeping gene. Delta delta Ct values (**ΔΔCt**) were expressed relative to the CNTR

treatment by the following equation: ΔΔCt = ΔCt of dietary fat treatment – ΔCt of CNTR (Pfaffl, 2001; Duran-Montge et al., 2009). Thus, all ΔΔCt values of CNTR are equal to 1.

Statistical Analysis

The response to the 6 dietary treatments were analyzed using PROC MIXED (SAS 9.4; SAS Inst. Inc., Cary, NC) with treatment as a fixed effect, replicate (2 cohorts of 24 barrows each) as a random effect, and pig as the experimental unit. Determination of the effect of dietary fat regardless of source on mRNA abundance was conducted via a contrast statement between CNTR and the 5 dietary fat treatments (Kaps and Lamberson, 2004; Oehlert, 2010). Determination of the correlation between dietary fatty acid concentration and mRNA abundance was analyzed using PROC CORR (SAS 9.4; Duran-Montge et al., 2009). Non-detectable fatty acid concentrations were treated in all statistical analyses as 0. All *P*-values ≤ 0.05 were considered significant and *P*-values > 0.05 and ≤ 0.10 were considered trends.

RESULTS

Moisture, impurities, and unsaponifiables of the 5 dietary fat sources were ≤ 1.1% (Table 2). Analyzed FFA level of the 5 dietary fat sources ranged from 0.08 to 12.80%. Initial peroxide value of FO was 13.8 mEq/kg. The other 4 dietary fat sources had an initial peroxide value of ≤ 1.3 mEq/kg. Fatty acid composition of the 5 sources (Table 3) confirmed the selection of sources detailed previously. Thus, the 5 dietary sources were of high quality and provided a diverse array of fatty acid intake.

Due to feed intake being limited, no differences were evident among the 6 dietary fat treatments for feed intake or BW (data not reported; $P \geq 0.753$). In the jejunum, no differences

were evident among the 6 dietary fat treatments for expression of fatty acid binding protein-2 (***FABP-2***) and fatty acid transport protein-4 (***FATP-4***; data not reported; $P \geq 0.175$).

Effects of dietary fat on mRNA abundance in adipose tissue

Pigs fed dietary fat regardless of source decreased fatty acid synthase (***FASN***) mRNA abundance compared to pigs fed a diet with no added dietary fat ($P = 0.014$; Table 5). Of the dietary fat treatments, pigs fed the COIL-based diet tended to have greater abundance of *FASN* mRNA ($P = 0.071$). Protein kinase, AMP-activated, gamma-1 non-catalytic subunit (***PRKAG-1***) abundance was greater in barrows fed a COIL-based diet than barrows fed CNTR or FO diets ($P = 0.047$). Pigs fed a COIL-based diet had increased abundance of sterol regulatory element-binding protein-1 (***SREBP-1***) compared to pigs fed CNTR or FO diets ($P = 0.025$). There was no impact of dietary treatments on mRNA abundance of acetyl CoA carboxylase (***ACACA***), ATP citrate lyase (***ACLY***), adipose triglyceride lipase (***ATGL***), hormone sensitive lipase (***HSL***), peroxisome proliferator activated receptor-alpha (***PPAR-α***), or stearoyl CoA desaturase (***SCD***) ($P \leq 0.125$).

Effects of dietary fat on mRNA abundance in liver

Regardless of source, including fat in the diet increased abundance of *ACACA*, *ATGL*, *PPAR-α*, *PRKAG-1*, and *SCD* in liver ($P \leq 0.020$; Table 6) and tended to increase abundance of *HSL* ($P = 0.071$) and *SREBP-1* ($P = 0.086$). Hepatic transcription of *ACACA* was greater in pigs fed AV- or TAL-based diets than pigs fed CNTR or FO diets ($P = 0.011$). Pigs fed a TAL-based diet had greater abundance of *ATGL* mRNA than pigs fed CNTR or FO diets ($P = 0.013$). Abundance of *HSL* was greater in pigs fed a TAL-based diet than those fed CNTR, COCO, or FO diets ($P = 0.013$). Pigs fed a COIL-based diet had greater mRNA abundance of *PPAR-α* than those fed CNTR, COCO, or FO diets ($P < 0.001$). Hepatic *PRKAG-1* transcription was greater

in pigs fed AV- or TAL-based diets than pigs fed CNTR, COCO, or FO ($P = 0.004$). Pigs fed AV-, COIL-, or TAL-based diets had greater *SCD* mRNA abundance than those fed CNTR or FO diets ($P = 0.025$). Barrows fed AV-, COIL-, or TAL-based diets tended to have increased mRNA abundance for *ACLY* ($P = 0.098$) and *SREBP-1* ($P = 0.069$) than those fed CNTR, COCO, or FO diets. Abundance of *FASN* tended to be decreased for all pigs fed any of the fat supplemented diets, except for COCO ($P = 0.074$). There was no effect of diet on the abundance of mRNA for fatty acid binding protein-1 (***FABP-1***; $P = 0.914$).

Correlation between dietary fatty acid composition and transcription of genes involved in lipid metabolism

In genes that were affected by dietary treatment ($P \leq 0.050$; Table 7), omega-3 concentration was negatively correlated with *SCD* hepatic transcription ($P = 0.042$). Omega-3 concentration also tended to be negatively correlated with adipose tissue *PRKAG*-1 expression ($P = 0.065$) and hepatic *ATGL* expression ($P = 0.063$). Dietary fat source omega-6:omega-3 was positively correlated with adipose tissue *PRKAG-1* abundance ($P = 0.034$). Additionally, omega-6:omega-3 tended to be positively correlated with adipose tissue *SREBP-1* abundance ($P = 0.082$). Dietary fat SFA content was negatively correlated with hepatic transcription of *INSR* and *PPAR-α* ($P \leq 0.039$). Dietary fat MUFA content tended to be positively correlated with hepatic *ACACA*, *PPAR-α*, and *PRKAG-1* mRNA abundance ($P \leq 0.100$). Dietary fat MUFA:SFA was positively correlated with *PPAR-α* abundance in liver ($P = 0.046$). Additionally, dietary fat MUFA:SFA tended to be positively correlated with *SREBP-1* abundance in adipose tissue ($P = 0.093$).

In genes that tended to be affected by dietary treatment ($P \leq 0.10$; Table 8), dietary omega-6 concentration tended to be positively correlated with *FASN* abundance in adipose tissue

($P = 0.085$). Dietary fat SFA concentration was positively correlated with abundance of *FASN* mRNA in liver ($P = 0.050$). Dietary fat MUFA content tended to be positively correlated with hepatic transcription of *ACLY* and *SREBP-1* and negativity correlated with *FASN* abundance ($P \leq 0.100$). Dietary fat PUFA:SFA tended to be positively correlated with *FASN* mRNA abundance in adipose tissue ($P = 0.099$).

DISCUSSION

Changes in adipose tissue lipid metabolism

Unlike in humans or in rodents where de novo lipogenesis primarily occurs in the liver, in pigs lipogenesis occurs in adipose tissue (O'Hea and Leveille, 1969). The addition of 5% dietary fat decreasing *FASN* abundance compared to CNTR reported herein, supports the generally accepted view that increasing the level of dietary fat suppresses fatty acid synthase function (a multi-faceted enzyme that synthesizes palmitic acid from malonyl CoA in the cytosol of the adipocytes in pigs [Beld et al., 2015]) and reduces the rate of de novo lipogenesis in adipose tissue (Allee et al., 1971; Smith et al., 1996). As a consequence, the fatty acid profile of the carcass reflects that of the diet (Kellner et al., 2014, 2016b). These mRNA abundance data further suggest that the suppression of de novo lipogenesis via mRNA abundance of *FASN* and the transcription factor *SREBP-1* (regulates the expression of key enzymes involved in the lipogenesis pathway [Kim and Spiegleman, 1996; Yahagi et al., 1999]) is reduced when the dietary fat source (i.e. COIL) is high in linoleic acid an omega-6 fatty acid. Omega-6 fatty acids being a less potent inhibitor of de novo lipogenesis in comparison to other fatty acids is supported in the literature. Duran-Montge et al. (2009) reported a positive correlation between *FASN* mRNA abundance and increased dietary fat omega-6 content and omega-6:omega-3.

Kellner et al. (2016a) reported greater mRNA abundance of *FASN* in pigs fed 3% COIL than 3% TAL. Kouba and Mourot (1998) reported greater *ACACA* (a biotin-dependent enzyme which produces malonyl CoA from actetyl CoA in an irreversible reaction which is the rate limiting step of de novo lipogenesis [Volpe and Vagelos, 1976]) and *FASN* expression in COIL-fed pigs than TAL-fed pigs. Smith et al. (1996) observed a greater rate of lipogenesis in cultured porcine adipocytes with a linoleic acid enriched diet versus an oleic acid enriched diet. In contrast, Allee et al. (1971) found that the suppression of lipogenesis was not different in growing pigs fed 10% COIL or TAL.

The observation in adipose tissue that SFAs are a more potent inhibitor of de novo lipogenesis than omega-6 fatty acids (linoleic acid in particular) relates to the fact that dietary fatty acids are largely unmodified in composition (chain length and degree of unsaturation) from ingestion to deposition (Ellis and Isbell, 1926; Kellner et al., 2014). De novo synthesized fatty acids are SFA (i.e. palmitic and stearic acid) or MUFAs (i.e. palmitoleic or oleic acid; Kloareg et al., 2007). Thus, if the pig consumes and deposits SFA of dietary origin, there is less need for the adipocyte to synthesize fatty acids of similar chemical structure (i.e. palmitic, palmitoleic, stearic, or oleic acid). In contrast, if the pig consumes and deposits omega-6 fatty acids (i.e. linoleic acid) the negative feedback on de novo lipogenesis in the adipocyte does not apply to same the degree. The finding that increased dietary PUFA intake does not decrease body fat in pigs is in contrast to the response to dietary PUFA in growing chickens where the primary site of lipogenesis is in liver (Crespo et al., 2002). In chickens, increased PUFA intake resulted in decreased body fat (Crespo et al., 2002).

Growing pigs are normally in a positive energy balance; therefore, the pig's reliance on the breakdown of stored lipids via lipolysis for sources of fuel is minimal (Enser, 1984).

However, these data indicate a suppression of *PRKAG-1* (kinase responsible for phosphorylation and activation of proteins involved in the lipolytic cascade such as adipose triglycericde lipase and hormone sensitive lipase [Bijland et al., 2013]) in pigs fed FO (high in omega-3 fatty acids), while all other dietary fat sources increased the mRNA abundance of *PRKAG-1*. Feeding long chain omega-3 fatty acids (i.e., docosahexanoic acid) are known to reduce plasma triglycerides in pigs (Meadus et al., 2011). Currently there are no porcine *PRKAG-1* abundance data directly to support or contrast this finding. However, supporting evidence of increased omega-3 fatty acid intake causing decreased *PRKAG-1* mRNA and lipolysis rate can be found in experiments with human or rodent subjects, where the liver is the primary site of de novo lipogenesis. Dietary intake of FO in is known to decrease adiposity in rodents fed high fat diets (Belzung et al., 1993; Shearer et al., 2012). Furthermore, rats fed a diet with both FO and TAL versus just TAL decreased plasma NEFA levels and basal intracellular lipolysis by ~50% (Rustan et al., 1993). Intake of omega-3 fatty acids in humans has been shown to suppress protein kinase A in cancer cells in mammary tissue (Moore et al., 2001), and in primary macrophages (Fournier et al., 2016). The suppression of protein kinase A may be due to cell-membrane incorporation of omega-3 fatty acids impairing the upstream signaling pathway to activate protein kinase A (Fournier et al., 2016).

Changes in hepatic lipid metabolism

Though liver is not the primary site of lipogenesis in the pig. Liver still plays a crucial role in lipid metabolism via lipid transportation, fatty acid oxidation, synthesis of cholesterol and phospholipids, and ketogenesis (Odle et al., 1995). Hepatic lipid metabolism changes were largely correlated to MUFA or SFA content. Dietary fat sources high in MUFA were positively correlated with *ACACA* and tended to be positively correlated with *ACLY* and *SREBP-1*, but

were negatively correlated with *FASN*. The explanation of medium chain SFA (C6:0 through C12:0) intake resulting in an increase of *FASN* mRNA abundance in liver and not in adipose tissue is possibly due to its metabolic endpoint (Foufelle et al., 1992). Medium chain fatty acids once absorbed into the enterocyte enter portal capillaries and are transported via the portal vein to the liver (Odle, 1997). This is in contrast to longer chain SFA, MUFA, and PUFA which are packaged into chylomicrons, directed through the lymphatic system, and then circulated to target peripheral tissues (i.e. adipose and muscle; Bach and Babayan, 1982; Odle, 1997). Thus, the exposure of digested and absorbed medium chain fatty acids from COCO is greater in liver than adipose tissue (Foufelle, 1992; Odle, 1997).

Duran-Montge et al. (2009) reported in growing pigs a similar positive correlation between dietary MUFA concentration and mRNA abundance of *ACACA* and *SREBP-1*, but found no significant correlation between dietary MUFA concentration and mRNA abundance of *FASN*. The differences between the Duran-Montge et al. (2009) correlations and the correlations reported herein, may be due to chain length and degree of saturation levels of dietary fat (TAL vs. COCO).

Liver uptake and accumulation of medium chain fatty acids could increase the fed-state signal and reduce the need for lipid oxidation. Hepatic expression of *PPAR-α* (transcription factor of fatty acid oxidation [Lee et al., 1995; Duran-Montge et al., 2009]), and *PRKAG-1* was also decreased in pigs fed COCO. These decreases of mRNA abundance in COCO-fed pigs may explain the positive correlations between MUFA content and *PPAR-α* and *PRKAG-1* and negative correlations with SFA *PPAR-α* mRNA abundances.

In animals where hepatic lipogenesis occurs at a greater proportion than found in pigs. Hepatic transcription of *FASN* was increased in rainbow trout fed 5% COCO compared to 5%

FO (Figueiredo-Silva et al., 2011). Hepatic transcription of *FASN* was also increased in rats fed 33% palm oil (a fat source comprised mainly of medium chain length, SFAs; NRC, 2012) versus rats fed 32% COIL (Foufelle et al., 1992). Foufelle et al. (1992) also found that mRNA abundance of *ACACA* was higher in palm oil versus COIL.

As reported in adipose tissue, omega-3 fatty acid intake decreased the transcription of genes related to lipolysis in the liver. In comparison to the other dietary fat treatments, pigs fed FO had reduced mRNA abundance of *PRKAG-1*, *ATGL*, and *HSL*. Omega-3 fatty acids are known to lower plasma triglycerides and non-esterified fatty acid levels (Rustan et al., 1993; Shearer et al., 2012). This is due to omega-3 fatty acids from FO increasing the activity of lipoprotein lipase in adipose and muscle and stimulating β-oxidation in muscle (Shearer et al., 2012). Thus, in comparison to the other long chained dietary fat sources tested in this experiment, FO could reduce fatty acids being delivered to the liver. This may explain why the transcription of genes involved in hepatic lipolysis were decreased. This explanation is supported by Rustan et al. (1993) who reported reduced whole body lipid utilization in rats fed omega-3 fatty acids compared with lard. In contrast, Sun et al. (2011) reported an increase in hepatic lipolysis and expression of *HSL* in mice fed increasing amounts of docosahexaenoic acid (omega-3 fatty acid).

These mRNA abundances could possibly be decreased by omega-3 incorporation into cellular membrane phospholipids, causing a disruption of membrane protein function and resulting in a suppression of the lipolytic cascade (Fournier et al., 2016). Clearly, more work is needed in determining the impact of omega-3 fatty acids on the rate of lipid breakdown and porcine hepatic tissue.

Conclusion

Added dietary fat will generally suppress the expression of genes involved in lipogenesis and increase the expression of lipolysis related genes. Intake of omega-3 fatty acids suppresses the transcription of genes involved in lipolysis in both adipose tissue and liver. Dietary SFA are more potent inhibitors than omega-6 fatty acids of the transcription of genes involved in de novo lipogenesis in adipose tissue. Due to their metabolic endpoint in the liver versus peripheral tissues, medium chain fatty acids have different effects than longer chain fatty acids on hepatic transcription of lipid metabolism genes.

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Table 1. Ingredient and nutrient composition (as-fed basis) of experimental diets d 0 to 10

Item	Control	Animal-vegetable blend	Coconut oil	Corn oil	Fish oil	Tallow
Ingredient, %						
Corn	68.41	68.41	68.41	68.41	68.41	68.41
Soybean meal (46.5% CP)	22.50	22.50	22.50	22.50	22.50	22.50
Corn starch	5.00	-	-	-	-	-
Experimental dietary fat	-	5.00	5.00	5.00	5.00	5.00
Limestone	0.96	0.96	0.96	0.96	0.96	0.96
Monocalcium phosphate (21%)	1.22	1.22	1.22	1.22	1.22	1.22
Salt	0.50	0.50	0.50	0.50	0.50	0.50
L-lysine HCL	0.33	0.33	0.33	0.33	0.33	0.33
DL-methionine	0.10	0.10	0.10	0.10	0.10	0.10
L-threonine	0.12	0.12	0.12	0.12	0.12	0.12
Trace mineral premix[1]	0.20	0.20	0.20	0.20	0.20	0.20
Vitamin premix[2]	0.20	0.20	0.20	0.20	0.20	0.20
Santoquin[3]	0.06	0.06	0.06	0.06	0.06	0.06
Titainium dioxide	0.40	0.40	0.40	0.40	0.40	0.40
Analyzed composition						
DM, %	86.66	87.35	87.77	86.79	87.61	87.45
GE, Mcal/kg	3.89	4.07	4.06	4.05	4.06	4.09
Acid hydrolyzed ether extract, %	2.97	9.32	8.94	8.55	9.14	9.21

[1]Provided 165 mg Zn (zinc sulfate), 165 mg Fe (iron sulfate), 39 mg Mn (manganese sulfate), 17 mg Cu (copper sulfate), 0.3 mg I (calcium iodate), and 0.3 mg Se (sodium selenite) per kilogram of diet.

[2]Provided 6,614 IU vitamin A, 827 IU vitamin D, 26 IU vitamin E, 2.6 mg vitamin K, 29.8 mg niacin, 16.5 mg pantothenic acid, 5.0 mg riboflavin, and 0.023 mg vitamin B12 per kilogram of diet.

[3]Santoquin Mixture 6 (feed and forage antioxidant; Novus International, St. Charles, MO).

Table 2. Analyzed chemical composition of dietary fat sources[1]

Item	Animal-vegetable blend[2]	Coconut oil[3]	Corn oil[4]	Fish oil[5]	Tallow[6]
Free fatty acid, %	7.00	0.08	12.80	2.80	3.60
Moisture and volatile matter, %	0.06	0.02	0.42	0.34	0.06
Insoluble impurities, %	0.02	0.02	0.02	0.06	0.06
Unsaponifiable matter, %	0.41	0.23	0.47	0.69	0.31
MIU,[7] %	0.49	0.27	0.91	1.09	0.43
Initial peroxide value, mEq/kg	0.30	0.20	0.60	13.80	1.30

[1]Analysis via Barrow Agee Laboratories (Memphis, TN).
[2]Sourced via Darling Pro Ingredients (Wahoo, NE).
[3]Sourced via Bulk Apothecary (Aurora, OH).
[4]Sourced via Feed Energy Co. (Des Moines, IA).
[5]Sourced via Double S Liquid Feed Serves, Inc. (Danville, IL).
[6]Sourced via Darling Pro Ingredients (Omaha, NE).
[7]MIU = moisture, impurities, and unsaponifiables.

Table 3. Analyzed fatty acid composition of dietary fat sources[1]

Item	Animal-vegetable blend[2]	Coconut oil[3]	Corn oil[4]	Fish oil[5]	Tallow[6]
Fatty acid, %					
Caprylic acid (C8:0)	ND[9]	6.17	ND	ND	ND
Capric acid (C10:0)	ND	5.39	ND	ND	ND
Lauric acid (C12:0)	ND	48.46	ND	0.11	ND
Myrsitic acid (C14:0)	1.63	19.75	ND	9.88	2.78
Palmitc acid (C16:0)	22.39	9.44	11.92	20.33	24.08
Palmitoleic acid (C16:1 n-9)	2.92	ND	0.09	11.66	2.48
Hexadecadienoic acid (C16:2 n-4)	ND	ND	ND	ND	1.43
Margaric acid (C17:0)	0.46	ND	ND	0.82	1.22
Stearic acid (C18:0)	10.45	9.08	1.71	3.49	20.29
Oleic acid (C18:1 n-9)	45.25	1.07	27.20	9.28	41.59
Linoleic acid (C18:2 n-6)	13.41	0.06	56.84	1.15	2.81
Linolenic acid (C18:3 n-3)	0.62	ND	1.35	1.34	0.31
Octadecatetraenoic acid (C18:4 n-3)	ND	ND	ND	2.01	ND
Arachidonic acid (C20:4 n-6)	0.24	ND	ND	1.36	ND
Eicosapentaenoic acid (C20:5 n-3)	ND	ND	ND	14.32	ND
Docosapentaenoic (C22:5 n-3)	ND	ND	0.16	2.81	ND
Docosahexaenoic acid (C22:6 n-3)	ND	ND	ND	8.22	ND
Other fatty acids	2.61	0.58	0.75	11.78	4.44
Omega-3, %	0.62	0.00	1.51	29.08	0.31
Omega-6, %	14.22	0.06	56.84	2.94	2.81
Omega-6/Omega-3	22.94	NC[10]	37.64	0.10	9.06
MUFA, %	49.46	1.07	27.55	22.82	44.95
PUFA, %	14.84	0.06	58.35	33.85	3.12
SFA, %	35.22	98.87	14.12	35.76	48.92
MUFA/PUFA	3.33	17.83	0.47	0.67	14.41
MUFA/SFA	1.40	0.01	1.95	0.64	0.92
PUFA/SFA	0.42	0.00	4.13	0.95	0.06
Iodine value[7]	68.7	1.0	126.3	137.4	44.0
U:S[8]	1.83	0.01	6.08	1.58	0.98

[1]Analysis via Barrow Agee Laboratories (Memphis, TN).
[2]Sourced via Darling Pro Ingredients (Wahoo, NE).
[3]Sourced via Bulk Apothecary (Aurora, OH).
[4]Sourced via Feed Energy Co. (Des Moines, IA).
[5]Sourced via Double S Liquid Feed Serves, Inc. (Danville, IL).
[6]Sourced via Darling Pro Ingredients (Omaha, NE).
[7]Iodine value calculated from fatty acid composition: IV = [C16:1] × (0.95) + [C18:1] × (0.86) + [C18:2] × (1.732) + [C18:3] × (2.616) + [C20:1] × (0.795) + [C20:2] × (1.57) + [C20:3] × (2.38) + [C20:4] × (3.19) + [C20:5] × (4.01) + [C22:4] × (2.93) + [C22:5] × (3.68) + [C22:6] × (4.64); brackets indicate percentage concentration (Meadus et al., 2010).
[8]Unsaturated to saturated fatty acid ratio.
[9]Non-dectable.
[10]Non-calcuable.

Table 4. Forward and reverse primer sequences

Gene	Description	Forward primer 5'-3'	Reverse primer 5'-3'
ACACA	Acetyl CoA carboxylase	ATGGATGAACCGTCTCCC	TGTAAGGCCAAGCCATCC
ACLY	ATP citrate lyase	AGGAGGAGTTCTATGTCTGC	CAACAGGTGTTTCTTGATGGCC
ATGL (PNPLA2)	Adipose triglyceride lipase (Patatin-like phospholipase domain containing 2)	ATCATAACCCACTTCGCC	ACACGGGAATGAAGGTGC
FABP-1	Fatty acid binding protein 1	ACATCAAGGGGACATCGG	GTCTCCATCTCACACTCC
FABP-2	Fatty acid binding protein 2	GGTAAAGAGGAAACTTGC	AGTGAGTTCAGTTCCGTCTGC
FATP-4	Fatty acid transport protein 4	AGCTCTTCTACATCTACACG	AATCCGTAGTACACCAGG
FASN	Fatty acid synthase	CACAACTCCAAAGACACG	AGGAACTCGGACATAGCG
HSL	Hormone sensitive lipase	AACGCAATGAAACAGGCC	TGTATGATCCGCTCAACTCG
PPAR-α	Peroxisome proliferator activated receptor-alpha	AACGGCATCCAGAACAAG	CATCACAGAGGACAGCATGG
PRKAG-1	Protein kinase, AMP-activated, gamma 1 non-catalytic subunit	TTGGTGACTAATGGTGTCCG	TGAAATCAGTGATGGTCAGC
SCD	Stearoyl CoA desaturase	TACTATCTGCTGAGTGCTGTGG	CTGGAATGCCATCGTGTTGG
SREBP-1	Sterol regulatory element-binding protein 1	TGGCGCTTCTCTTTGTCTATGG	GTGCTAGAGAGTCAGTGG

Table 5. Effects of dietary fat source on mRNA abundance in adipose tissue[1]

		Dietary treatment[2], ΔΔCt[3]							*P*-value	
Gene	Description	CNTR	AV	COCO	COIL	FO	TAL	SEM	TRT[4]	CNTR vs. DF[5]
ACACA	Acetyl CoA carboxylase	1.00	1.00	1.74	2.59	-0.33	0.32	0.91	0.249	0.946
ACLY	ATP citrate lyase	1.00	1.94	1.27	2.74	-0.14	0.80	0.98	0.422	0.764
ATGL (PNPLA2)	Adipose triglyceride lipase (Patatin-like phospholipase domain containing 2)	1.00	1.81	2.45	3.97	0.20	3.14	1.00	0.125	0.236
FASN	Fatty acid synthase	1.00	-1.37	-0.74	0.25	-1.44	-0.99	1.25	0.071	0.014
HSL	Hormone sensitive lipase	1.00	2.86	2.99	2.46	0.47	2.98	1.07	0.223	0.186
PPAR-α	Peroxisome proliferator activated receptor-alpha	1.00	3.27	2.10	3.57	2.27	2.38	1.13	0.635	0.163
PRKAG-1	Protein kinase, AMP-activated, gamma 1 non-catalytic subunit	1.00^{bc}	2.72^{ab}	3.08^{ab}	4.00^{a}	-0.22^{c}	1.80^{abc}	0.97	0.047	0.235
SCD	Stearoyl CoA desaturase	1.00	0.20	0.61	1.43	-0.23	-0.12	1.06	0.797	0.552
SREBP-1	Sterol regulatory element-binding protein 1	1.00^{c}	3.78^{a}	1.29^{bc}	3.65^{ab}	0.45^{c}	1.51^{abc}	0.83	0.025	0.219

^{a,b,c}Within a row, least square means lacking a common superscript differ ($P < 0.05$).

[1]Total of 48 barrows (8 per treatment) with an initial BW of 44.1 ± 1.2 kg and a final (d 10) BW of 51.2 ± 1.7 kg.

[2]Each experimental diet included 95% of a corn-soybean meal basal diet and then 5% of either: corn starch (control; CNTR), animal-vegetable blend (AV), coconut oil (COCO), corn oil (COIL), fish oil (FO), or tallow (TAL).

[3]Delta delta cycle threshold (Ct).

[4]Probability value of obtaining the observed difference among the 6 dietary treatments.

[5]Probability value of obtaining the observed difference between CNTR and 5 dietary fat (DF) treatments.

Table 6. Effects of dietary fat source on mRNA abundance in liver[1]

		Dietary treatment[2], ΔΔCt[3]							*P*-value	
Gene	Description	CNTR	AV	COCO	COIL	FO	TAL	SEM	TRT[4]	CNTR vs. DF[5]
ACACA	Acetyl CoA carboxylase	1.00^{c}	5.12^{a}	2.90^{abc}	3.96^{ab}	2.09^{bc}	5.15^{a}	1.05	0.011	0.006
ACLY	ATP citrate lyase	1.00	3.67	1.13	3.03	0.47	3.80	1.05	0.098	0.222
ATGL (PNPLA2)	Adipose triglyceride lipase (Patatin-like phospholipase domain containing 2)	1.00^{c}	4.05^{ab}	3.59^{ab}	4.37^{ab}	2.36^{bc}	4.92^{a}	1.11	0.013	0.002
FABP-1	Fatty acid binding protein 1	1.00	1.06	1.01	0.39	0.56	0.20	1.92	0.914	0.455
FASN	Fatty acid synthase	1.00	-2.01	2.64	-0.87	-1.49	-0.78	2.00	0.074	0.255
HSL	Hormone sensitive lipase	1.00^{b}	2.31^{ab}	1.37^{b}	2.30^{ab}	0.93^{b}	3.36^{a}	0.52	0.013	0.071
PPAR-α	Peroxisome proliferator activated receptor-alpha	1.00^{c}	7.33^{ab}	1.78^{c}	8.54^{a}	5.67^{b}	7.93^{ab}	0.89	<0.001	<0.001
PRKAG-1	Protein kinase, AMP-activated, gamma 1 non-catalytic subunit	1.00^{b}	4.00^{a}	1.94^{b}	2.70^{ab}	1.34^{b}	3.95^{a}	0.87	0.004	0.014
SCD	Stearoyl CoA desaturase	1.00^{b}	4.21^{a}	2.76^{ab}	3.92^{a}	1.09^{b}	3.94^{a}	1.16	0.025	0.020
SREBP-1	Sterol regulatory element-binding protein 1	1.00	4.33	1.82	3.07	1.33	3.68	0.98	0.069	0.086

^{a,b,c}Within a row, least square means lacking a common superscript differ ($P < 0.05$).

[1]Total of 48 barrows (8 per treatment) with an initial BW of 44.1 ± 1.2 kg and a final BW (d 10) of 51.2 ± 1.7 kg.

[2]Each experimental diet included 95% of a corn-soybean meal basal diet and then 5% of either: corn starch (control; CNTR), animal-vegetable blend (AV), coconut oil (COCO), corn oil (COIL), fish oil (FO), or tallow (TAL).

[3]Delta delta cycle threshold (Ct).

[4]Probability value of obtaining the observed difference among the 6 dietary treatments.

[5]Probability value of obtaining the observed difference between CNTR and 5 dietary fat (DF) treatments.

Table 7. Correlation coefficients (*r*) between dietary fatty acid composition and mRNA abundance of genes that were affected by dietary fat treatment in adipose (AT) and liver (LT)

	Gene[1]							
	PRKAG-1	*SREBP-1*	*ACACA*	*ATGL*	*HSL*	*PPAR-α*	*PRKAG-1*	*SCD*
Item	AT	AT	LT	LT	LT	LT	LT	LT
Omega-3	-0.885*	NS[2]	NS	-0.859*	NS	NS	NS	-0.891**
Omega-6	NS	NS	NS	NS	NS	NS	NS	NS
Omega-6/Omega-3	0.966**	0.918*	NS	NS	NS	NS	NS	NS
SFA	NS	NS	NS	NS	NS	-0.898**	NS	NS
MUFA	NS	NS	0.803*	NS	NS	0.828*	0.829*	NS
PUFA	NS	NS	NS	NS	NS	NS	NS	NS
MUFA/PUFA	NS	NS	NS	NS	NS	NS	NS	NS
MUFA/SFA	NS	0.815*	NS	NS	NS	0.885**	NS	NS
PUFA/SFA	NS	NS	NS	NS	NS	NS	NS	NS
U:S	NS	NS	NS	NS	NS	NS	NS	NS
Iodine Value	NS	NS	NS	NS	NS	NS	NS	NS

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

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

[1]Description of genes: *ACACA*: acetyl CoA carboxylase, *ATGL*: adipose triglyceride lipase (*PNPLA2* [Patatin-like phospholipase domain containing 2]), *HSL*: hormone sensitive lipase, *PPAR-α* peroxisome proliferator activated receptor-alpha, *PRKAG-1*: protein kinase, AMP-activated, gamma 1 non-catalytic subunit, *SCD*: stearoyl CoA desaturase, *SREBP-1*: sterol regulatory element-binding protein 1.

[2]Non-significant ($P \geq 0.101$).

Table 8. Correlation coefficients (*r*) between dietary fatty acid composition and mRNA abundance of genes that tended to be affected by dietary fat treatment in adipose (AT) and liver (LT)

	Gene			
	FASN	*ACLY*	*FASN*	*SREBP-1*
Item	AT	LT	LT	LT
Omega-3	NS[2]	NS	NS	NS
Omega-6	0.826*	NS	NS	NS
Omega-6/Omega-3	NS	NS	NS	NS
SFA	NS	NS	0.876**	NS
MUFA	NS	0.804*	-0.825*	0.839*
PUFA	NS	NS	NS	NS
MUFA/PUFA	NS	NS	NS	NS
MUFA/SFA	NS	NS	NS	NS
PUFA/SFA	0.806*	NS	NS	NS
U:S	NS	NS	NS	NS
Iodine Value	NS	NS	NS	NS

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

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

[1]Description of genes: *ACLY*: ATP citrate lyase, FASN: fatty acid synthase, *SREBP-1*: sterol regulatory element-binding protein 1.

[2]Non-significant ($P \geq 0.101$).