RESEARCH REPORT
PORK QUALITY

Title: Determining the temporal and spatial regulation of marbling development in the longissimus muscle of porcine offspring from weaning through finishing – NPB #18-068

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Industry Summary

Intramuscular fat, also known as marbling, positively impacts the palatability and tenderness of meat, which contributes to eating quality. The goal for pork producers is to improve meat quality and production efficiency to satisfy consumers and increase carcass value. In pigs the loin muscle is the most economically important tissue given that the quality of the carcass including the total muscle and fat percentage are assigned based on loin muscle (**LM**) measurements. Still, this anatomically protracted muscle is not homogeneous in composition which can impact the predicted quality and quantity of lean pork produced. The objective of this study was to determine which adipogenic genes are responsible for the spatial and temporal development of marbling in response to different diets. A total of 80 pigs were randomly assigned to 1 of 4 diets: lysine deficient (**LysDef**), vitamin A deficient (**VitADef**), lysine and vitamin A deficient (**LysVitADef**), and a control group who received the NRC recommended requirements (**CON**) from weaning though the finishing phase. At day 0, 84, and 150 of the trial, biopsies were taken from the proximal, medial, and distal locations of the LM. The biopsies were subjected to quantitative PCR to determine location-specific adipogenic gene expression. The animals were then harvested to assess carcass characteristics. The current data indicate that restriction of dietary lysine increased the expression of genes involved in the de novo synthesis of fatty acids from glucose and other carbon sources via Acetyl-CoA Carboxylase and Fatty Acid Synthase upregulation. The location along the loin muscle and the phase of feeding had the greatest impact on expression of the genes of interest. Expression of these genes was relatively low during the nursery phase which is to be expected because the young pig diverting nutrients to the growth and development of skeletal muscle and bone and not towards fat deposition during this phase. For most genes, the expression increased during the feeder phase and in many cases was sustained through the finisher phase. These expression profiles are consistent with a shift in metabolism towards muscle and fat deposition during this time. The fatty acid composition was also impacted by the location along the loin where the percentage of saturated

fatty acids decreased from the proximal to distal loin muscle and the percentage of unsaturated fatty acids increased from the proximal to distal loin muscle, irrespective of dietary treatment. These data indicate a spatial and temporal separation of lipid development and metabolism in the loin muscle and future studies should investigate the cellularity and activity of these intramuscular adipocytes in efforts to manipulate this tissue niche towards a more consistent and predictable pork product with respect to the amount and distribution of fat in the loin muscle.

Key Findings:

1. Gene Expression data showed temporal and spatial regulation of the target genes
2. Dietary treatments did not impact marbling score, color, or loin eye area.
3. **Location** in the loin muscle had the greatest impact on marbling score, color, and loin eye area.
4. The proximal portion of the loin had higher percentages of saturated fatty acids while the distal portion had higher percentage of unsaturated fatty acids
5. The valuation of pork carcasses based on the loin muscle quality parameters should take into consideration differences along the length of the loin.

Scientific Abstract
A total of 80 pigs were subjected to one of 4 dietary treatments from wean to finish to determine the temporal and spatial regulation of marbling deposition in the loin muscle of the developing market hog. : lysine deficient (LysDef), vitamin A deficient (VitADef), lysine and vitamin A deficient (LysVitADef), and a control group who received the NRC recommended requirements (CON) from weaning though the finishing phase. At day 0, 84, and 150 of the trial, biopsies were taken from the proximal, medial, and distal locations of the LM. The biopsies were subjected to quantitative PCR to determine location-specific adipogenic gene expression. The animals were then harvested to assess carcass characteristics. The current data indicate that restriction of dietary lysine increased the expression of genes involved in the de novo synthesis of fatty acids from glucose and other carbon sources via Acetyl-CoA Carboxylase and Fatty Acid Synthase upregulation. The location along the loin muscle and the phase of feeding had the greatest impact on expression of the genes of interest. Numerically, the final body weight was greatest in the control group and the LysDef and LysVitADef groups had the lightest final body weights however these differences were not statistically significant ($P \geq 0.15$). There were no treatment x phase interactions for the expression of the adipogenic and lipogenic genes of interest ($P \geq 0.07$). ACC expression was greatest ($P < 0.05$) in the CON and LysDef diets compared to that of the VitADef and LysVitADef diet which had similar ($P > 0.05$) expression. Fatty acid synthase expression was also greater ($P \leq 0.01$) in the LysDef diet compared to the CON and LysVitADef diet group. The LysDEF group was similar ($P = 0.24$) to that of the VitADef treatment group. The expression of SCD was similar in the LysDef and VitADef groups ($P = 0.82$) which were both increased ($P \leq 0.03$) compared to the LysVitADef group and were similar ($P \geq 0.09$) to that of the CON group. Treatment had no effect ($P = 0.14$) on the percentage of SFA, however the percentage of saturated fatty acids decreased ($P <0.001$) as location went from the proximal to distal portions of the loin muscle. In contrast, the percentage of polyunsaturated fatty acids increased ($P <0.001$) from the proximal to distal portions of the loin muscle. The biological data generated from the current study are informative in terms of the spatial and temporal regulation of marbling development in the LM of the growing pig.

Introduction

Consumers are becoming more aware about the eating quality and nutritional properties of their meat choices, which directly influences their demand for these products. One of the most important factors for purchasing decisions, perception of quality, and actual eating quality is the amount of intramuscular fat or "marbling" that is present (Wood et al., 2008). Marbling positively influences sensory characteristics in pork including taste, flavor, juiciness, and perceived tenderness (Wood et al., 2008). While consumers remain health conscious about the overall leanness of their pork products, there has been a noticeable shift in demand towards consistent, high quality pork products with sufficient marbling to maximize the taste and eating experience. With increasing demand domestically and internationally, the quality of US pork will remain a determining factor for the competitiveness of the industry. These conditions mean that pork producers will need to deliver a more consistent, high quality product while meeting the demand and productivity targets of the industry.

The *Longissimus dorsi* or loin muscle (**LM**) in pigs represents upwards of 25 percent of the total carcass value and is considered a premium pork cut by consumers. This makes the size and quality of the LM on each carcass of critical economic importance to pork producers. Anatomically, the LM is expansive in length and bridges the hindquarter with the forequarter of the pig. The LM develops along both spatial and temporal continuums and, as a result, exhibits cellular and molecular differences in its composition over time and along the length of the muscle. Because of this dynamic developmental paradigm, the amount and composition of marbling in the LM is not uniform in the live animal or in the final LM products. In the live animal, marbling develops between bundles of muscle fibers over the course of muscle growth and development. Marbling develops from a combination of increases in the number and size of fat cells within the muscle, which develop during late prenatal and early postnatal life and then begin to fill with dietary and endogenous fat throughout the productive lifespan of the market-destined hog (Hausman et al., 2009; Harper and Pethick, 2004). It is known that this developmental pattern of lipid filling occurs over time and is tightly regulated by molecular factors in response to systemic cues and those within the local muscle niche. However, the specific timing and activity of these molecular factors across the lengthy landscape of the LM is less understood and is critical to modulating the marbling content to improve quality and consistency across the muscle. The impending changes to pork quality grading system make it a high priority to understand the basic cellular biology that undergirds the applied nutritional and management practices aimed at increasing the uniform marbling quality of pork products

The local context in which the marbling develops influences the lipid content and composition at different locations within a muscle. This variable content is exacerbated in a protracted muscle such as the LM, which essentially bridges the fore and hindquarters of the animal. This translates to differential meat quality within this economically valuable muscle. Strategies to improve the uniformity of marbling cross the LM will be dependent on manipulating the number and metabolic disposition of intramuscular adipocytes, which are under the control of adipogenic and lipogenic transcription factors. Faucitano et al., (2004) reported that both total IMF content and marbling scores were anatomical location dependent with the highest values corresponding to the middle section of the thoracic region of the LM. Chop location impacts LM quality with the middle 30-80% of the LM being the most uniform and the anterior and posterior portions of loin exhibiting different color and amounts of marbling (Homm et al., 2006). This means at least 50% of the LM does not exhibit this same uniformity and these discrepancies can have large economic impacts on a commercial scale.

Objectives

The overarching objective of the proposed research is to determine the spatial and temporal paradigms through which IMF or "marbling" develops in the LM of pigs. Using two nutritional models known to increase marbling (Lysine deficiency and Vitamin A restriction), we profiled the histological, and molecular development of marbling over time and along the length of the LM in growing pigs from weaning through to the final carcass. To be clear, these objectives were not intended to revisit Lysine or Vitamin A requirements in growing pigs, but rather to use the known phenotypic outcomes from these nutritional scenarios as models to study the biology of marbling development over time. We hypothesized that the spatial (along the length of the muscle) and temporal (over the course of production) patterns of marbling development in the LM are regulated by molecular regulatory factors involved in adipogenesis and lipogenesis that determine the timing and location of LM marbling development in response to nutritional regimens and management practices.

The **specific objectives to test this hypothesis** were to:

1. Collect wean to finish muscle biopsies from the proximal, medial, and distal LM to profile the temporal and spatial pattern of marbling deposition in two porcine marbling models.

2. Also using these biopsies, determine the molecular regulatory factors that are responsible for the localized marbling deposition in the LM across production phases.

3. Compare the final marbling scores to the longitudinal data from objectives 1 and 2 to identify developmental time points and biomarkers that correspond to the LM phenotypes at harvest.

Materials and Methods

Animals: All animal procedures were approved by the Mississippi State University Institutional Animal Care and Use Committee. A total of 80 commercial barrows were stratified by weaning weight and assigned to one of four phase specific, post-weaning dietary treatment groups (n = 20 per treatment) consisting of:

1. A phase-specific control diet (**CON; n = 20**),
2. A phase-specific lysine deficient diet (**-LYS; n = 20**),
3. A phase-specific Vitamin A restricted diet (**-VitA; n = 20**),
4. A phase-specific lysine & vitamin A deficient diet (-**LYS/-VitA; n = 20**).

The latter three diet formulations increased marbling deposition in the LM in previous studies. The CON and –VitA diets were formulated to a constant concentration of SID Lys:ME to meet NRC requirements for Lys within each phase. The –VitA diet was created by not adding Vitamin A to the premix for each phase-specific diet. The –LYS and –LYS/–VitA diets were formulated at 20% below the NRC Lys:ME requirements for pigs based on their body weight. Pigs were housed individually in an environmentally controlled facility in pens equipped with a nipple water dispenser and a single-sided, dry self-feeder. Pigs will have ad libitum access to

feed, water and were weighed and feed disappearance measured every 14 days for determination of ADG, ADFI and calculation of F/G. Pigs were weighed individually and transported to the Mississippi State University Meat Science and Muscle Biology Laboratory for carcass data and tissue sample collection at the end of the trial. Hot carcass weights were measured immediately after evisceration and each carcass will evaluated for percentage yield, backfat, loin depth, and percent lean.

Muscle Biopsies: Loin muscle biopsies were collected from the proximal, medial, and distal ends of the LM using thoracic vertebrae number 7, the last rib, and lumbar vertebrae number 3 as landmarks for the respective regions. These samples were collected weaning for a baseline determination, and then after the nursery phase, and at the end of the feeder-finisher phases of production to garner a longitudinal molecular signature for marbling development over time. Approximately 200 mg of tissue were collected from each location at each of the three time points. We have employed this biopsy technique in pigs (Stephenson et al., 2016). This approach creates a model of marbling development to investigate the spatial and temporal regulation of marbling development along the LM over the course of the nursery, feeder, and finisher phases of production. At the conclusion of the feeding trial, the animals were harvested to determine carcass characteristics and tissues were collected for further molecular and histological analysis.

Tissue Collection at Harvest: From the left side of the carcass, the whole loin (IMPS Item No. 410) were separated from the shoulder, ham, and belly. The skin, bone, cartilage, and tenderloin will then be removed to yield a boneless pork loin (IMPS Item no. 413). The length of the boneless loin was measured and then cut into thirds at the anterior, medial, and posterior ends (**Figure 2**). Marbling scores were assigned to each location based on the NPPC photographic marbling standard scales. Next, a one-inch chop from each location were trimmed of all fat and then ground for fatty acid profile determination.

Tissue Analysis: Fatty acid analysis and real-time PCR were conducted as previously described by us in Burnett et al., 2016. For each biopsy sample, RNA was extracted for real-time quantitative PCR to measure the mRNA expression of 9 genes involved in de novo lipogenesis, fatty acid desaturation, and fatty acid turnover. These genes are presented in Table 6 and include: acetyl-CoA carboxylase (**ACC**; rate limiting for de novo synthesis of fatty acids), acyl-CoA oxidase **(ACOX;** involved in mitochondrial beta oxidation of fatty acids)**,** adiponectin (**AdipoQ**; adipokine involved in glucose homeostasis and lipid metabolism), CAAT enhancer binding protein alpha (**CEBPA**; responsible for early adipogenesis in concert with PPARg) carnitine palmitoyl transferase 1-b (**CPT1b;** involved in transfer of fatty acids into the mitochondria for oxidation)**,** preadipocyte Factor 1 (**DLK1;** gatekeeper for progression of preadipocytes to mature adipocytes), fatty acid synthase (**FASN**; fatty acid synthesis), stearoyl-CoA desaturase (**SCD**; responsible for desaturation of long chain fatty acids), and peroxisome proliferator activator protein gamma (**PPARg**; master regulator of adipogenesis).

Results

Growth Performance

There was no difference in initial, nursery, feeder, or finisher bodyweight between the treatments ($P \geq 0.54$). Numerically, the final body weight was greatest in the control group and the LysDef and LysVitADef groups had the lightest final body weights however these differences were not statistically significant ($P \geq 0.15$). Similar patterns were observed with the

average daily gain for the feeder, finisher, and total average daily gain between the treatment groups, although again these differences were not statistically significant ($P \geq 0.15$).

Gene Expression

Gene expression data are presented in Table 8 and Figures 1-9. There were no treatment x phase interactions for the expression of the adipogenic and lipogenic genes of interest ($P \geq 0.07$). ACC expression was greatest ($P < 0.05$) in the CON and LysDef diets compared to that of the VitADef and LysVitADef diet which had similar ($P > 0.05$) expression. Fatty acid synthase expression was also greater ($P \leq 0.01$) in the LysDef diet compared to the CON and LysVitADef diet group. The LysDEF group was similar ($P = 0.24$) to that of the VitADef treatment group. The expression of SCD was similar in the LysDef and VitADef groups ($P = 0.82$) which were both increased ($P \leq 0.03$) compared to the LysVitADef group and were similar ($P \geq 0.09$) to that of the CON group.

With regards to overall expression, there was increased expression of ACC ($P \leq 0.001$) during the finisher phase of production compared to the feeder and nursery phases. The Feeder phase was also increased ($P < 0.001$) compared to the nursery phase. The expression of ACOX increased ($P < 0.03$) from the nursery to feeder phases and decreased ($P = 0.0014$) from the feeder to the finisher phase of production. CEBA followed a similar pattern of increase ($P \leq 0.001$) from nursery to feeder followed by a decrease ($P = 0.02$) from feeder to finisher. For DLK1 the Nursery and Finisher phases did not differ ($P = 0.15$). The feeder phase tended to have increased ($P = 0.06$) expression of DLK1 compared to the finisher phase and was significantly greater ($P = 0.001$) that the nursery phase. FASN expression was increased ($P = 0.003$) in the feeder phase compared to the nursery phase, while there was no difference between the feeder and finisher, or nursery and finisher phases ($P \geq 0.10$). Similarly, SCD expression was increased ($P = 0.005$) in the feeder phase compared to the nursery phase, while there was no difference between the feeder and finisher, or nursery and finisher phases ($P \geq 0.13$). These expression patterns indicate a transient increase in lipogenic activity during the feeder phase of production that in some cases was sustained through the feeder phase.

There was a loin muscle location by feeding phase interaction for expression of each of the target genes of interest ($P \leq 0.001$). A description of each of these interactions follows. For ACC (Figure 1) there was no difference in expression in the nursery phase between the proximal, medial, and distal portions of the LM ($P \geq 0.54$), however during the feeder phase, the medial portion of the LM had increased expression compared to the proximal and distal LM ($P \leq 0.003$). In the finisher phase, the proximal LM had the greatest expression of ACC ($P < 0.0001$) while the distal portion had the decreased expression of ACC compared to the proximal and medial portions ($P \leq 0.0001$). During the nursery phase, there was no difference ($P = 0.531$) in ACOX expression (Figure 2) between the medial and distal portions of the LM while the proximal location had increased ($P <0.0001$) compared to the other two locations. During the feeder phase the medial ACOX expression increased ($P < 0.0001$) and proximal ACOX expression decreased ($P < 0.0001$). Distal ACOX expression was intermediate ($P <0.0001$) between the two. There was no difference ($P = 0.293$) between the finisher medial and finisher

proximal ACOX expression, while the finisher distal location had increased ($P \leq .023$) expression compared to the other two locations.

In the nursery phase, AdipoQ expression (Figrure 3) was increased ($P \leq .0001$) in the proximal LM compared to the medial and distal LM which had similar ($P = 0.383$) expression levels to one another. In the feeder phase, AdipoQ expression increased ($P \leq .0001$) in the medial LM compared to the proximal and distal LM which only tended to differ between the two ($P = 0.051$). By the finisher phase, there was no difference ($P = 0.961$) between the medial and distal AdipoQ expression while the proximal location had increased ($P \leq 0.038$) expression compared to the other two. The expression of CEBPA (Figure 4) was similar ($P \geq 0.234$) for all locations during the nursery phase. Expression was greatest ($P \leq 0.0001$) in the medial LM and least ($P \leq 0.0001$) in the proximal LM during the feeder phase. There was no difference ($P = 0.383$) between the medial and distal LM during the finisher phase, while it was increased ($P \leq 0.02$) in the proximal LM.

During the nursery phase CPT1b expression (Figure 5) was greatest ($P \leq 0.04$) in the proximal LM and least ($P \leq 0.014$) in the distal LM. Expression increased ($P \leq 0.0001$) in the medial LM in the feeder phase and decreased ($P \leq 0.0001$) in the proximal LM while the distal LM expression was intermediate. In the finisher phase, there was no difference ($P \geq 0.263$) between the distal LM and the proximal or medial LM, however the medial CPT1b expression was greater ($P = 0.040$) in the medial LM compared to the proximal LM. Expression of DLK1 (Figure 6) was greater ($P \leq 0.001$) in the proximal LM during the nursery phase compared to the medial and distal portions which were not different ($P = 0.77$). In the feeder phase, the medial LM had increased ($P < 0.0001$) expression compared to the proximal location while the distal location was increased ($P < 0.0001$) compared to the proximal LM but not different ($P = 0.277$) compared to the medial LM. In the finisher phase there was no difference ($P = 0.431$) between the proximal and medial LM DLK1 expression while distal LM had increased ($P \leq 0.001$) expression compared to the other two locations.

During the nursery phase, expression of FASN (Figure 7) was greatest ($P \leq 0.005$) in the proximal LM while there was no difference ($P = 0.515$) between the medial and distal LM. In the nursery phase medial LM expression increased and was greater ($P \leq 0.0001$) than that of proximal and distal LM, and distal LM expression was greater ($P = 0.006$) than expression in the proximal LM at this timepoint. In the finisher phase, distal and medial LM expression levels were not different ($P = 0.955$) and were both decreased ($P \leq 0.013$) compared to that of the proximal LM. Nursery expression of SCD (Figure 8) was similar ($P \geq 0.265$) in the proximal, medial, and distal portions of the LM. The medial and distal LM had similar ($P = 0.556$) expression in the feeder phase and were both greater ($P \leq 0.0007$) than that of the proximal LM. There were no differences ($P \geq 0.157$) between any of the locations in the finisher phase. Each location had similar ($P \geq 0.675$) expression of PPARg (Figure 9) in the nursery phase. Expression of PPARg increased in the medial LM during the feeder phase and was greater ($P \leq 0.001$) than that of the proximal and distal LM. The proximal LM had decreased expression compared to the distal location which was intermediate between the other two locations. In the finisher phase the proximal, medial and distal LM had similar ($P \geq 0.152$) PPARg expression.

Carcass Cutout and Morphometrics

Carcass cutout weights and morphometric data were largely unaffected by dietary treatments. Hot carcass data weights were numerically decreased in the LysDef, VitADef, and LysVitADef groups compared to the control but these differences were not statistically significant. These numerical differences were also reflected in the carcass cutouts with the exception of the belly weights which were numerically increased in the LysDef treatment group compared to the other treatment groups.

Loin Quality Characteristics

There were no treatment x location interactions for the loin quality characteristics including loin eye area, marbling score, and lean color were not affected by treatment ($P > 0.05$). Dietary treatment did not affect LEA, marbling score or lean color ($P > 0.07$). However, location in the loin impacted these parameters with the medial location having the greatest LEA ($P < 0.001$), the proximal location having the greatest marbling score ($P = 0.002$), and the distal location having the greatest lean color score ($P < 0.001$).

Fatty acid composition

There were no treatment x location interactions for the percentage of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), or branched chain fatty acids ($P > 0.70$). Additionally, there were no treatment by location interactions for the ratio of omega 3 to omega 6 fatty acids, polyunsaturated to saturated fatty acids, or the saturation index ($P > 0.24$). Treatment had no effect ($P = 0.14$) on the percentage of SFA, however the percentage of saturated fatty acids decreased ($P < 0.001$) as location went from the proximal to distal portions of the loin muscle. In contrast, the percentage of polyunsaturated fatty acids increased ($P < 0.001$) from the proximal to distal portions of the loin muscle. The saturation index, which is defined as the ratio of stearic acid to oleic acid decreased ($P < 0.001$) from the proximal to distal portions of the loin muscle, while treatment had no effect ($P = 0.11$). There was no location effect ($P = 0.01$) for monounsaturated fatty acids, however the LysDef diet had an increased percentage of MUFA ($P = 0.03$) compared to the Control, Vitamin A deficient, and Vitamin A + Lysine deficient diets. The LysDef also had a decreased percentage of PUFA ($P < 0.0001$) compared to the other 3 diets. This resulted in a decreased ratio of PUFA:SFA ($P < 0.0001$) in the LysDef diet compared to the other three diets as well.

Discussion

Adipose tissue plays a central role in energy metabolism and is a direct contributor to the eating quality and valuation of pork carcasses and products. Adipose tissue depots serve as on-demand repositories for cellular energy and are capable of repurposing end products of digestion and metabolism into storage forms for future use. While accumulation of lipid in the subcutaneous, and visceral adipose tissue depots is often discounted and detracts from carcass value, the development of lipid filled adipose tissue within skeletal muscle is a tightly regulated biological process that has positive economic implications for pork producers and quality implications for consumers. The temporal and spatial regulation of development of intramuscular lipid through adipogenesis and subsequent lipogenesis and lipid metabolism are critical to the efficient, and sufficient deposition of marbling to satisfy consumer demands and to achieve price premiums for pork producers. However, these processes have not been thoroughly investigated in the contemporary market destined hog and provided the impetus for the current study.

Adipogenesis is tightly controlled by the master regulator PPARg and is reinforced by expression of CEBPA. Together these transcription factors promote the expression of other genes involved in the adipogenic program to establish adipocytes in the canonical adipose tissue depots. DLK1 is involved in preadipocyte metabolism and prevents these cells from differentiating into mature, lipid storing adipocytes thereby serving as important gatekeeper for the development of adipose tissue depots. Its expression would be expected to be higher in during the early phases of production and then as the animal matures and substrates for lipid filling become more available, DLK1 expression would decrease to allow for adipocyte maturation and metabolism. In this case the expression levels were lower during the nursery phase which may have been a result of lack of preadipocyte formation or metabolism during this early period. However, expression increased in the feeder phase and then tended to decrease into the finisher phase which may have been indicative of adipocyte maturation and lipid filling in these later phases.

The expression of lipogenic and adipogenic genes was expectedly low in the nursery phase of production which represents a period of increased lean deposition and limited fat accumulation in the muscle and other depots. During the nursery and feeder phases, nutrients are being diverted to muscle protein synthesis and only nutrients in excess of this requirement are diverted to fat synthesis. As the pig begins to approach the inflection point in its growth curve and maximal muscle growth begins to cease, there is a shift towards fat deposition in the later feeder and finisher phase.

Once intramuscular adipocytes have been established, they then need to fill with lipids to contribute to the commercial trait of marbling. These lipids can come from exogenous (dietary) or endogenous (synthesized from metabolic precursors) sources depending on the nature of the diet and physiological phase of production the pig is in (Hausman et al., 2009). The combination of these sources results in the expansion of the intracellular lipid droplet and contributes to the visible appearance of marbling in pork products (Hocquette et al., 2010). The rate-limiting step in de novo fatty acid synthesis is catalyzed by ACC and this enzyme is an important gatekeeper for metabolic substrates from the citric acid cycle and products of amino acid metabolism.

Because ACC is involved in de novo fatty acid synthesis it is likely that this increase resulted from the increased use of carbons from glucose and/or amino acids for fatty acid synthesis as the efficiency of protein synthesis decreased with time. The FASN enzyme complex works in concert with ACC to accomplish de novo lipogenesis of long-chain fatty acids. Once ACC catalyzes the initial formation of the malonyl-CoA intermediate from carboxylation of acetate, FASN is responsible for subsequent addition of carbon units to the growing hydrocarbon chain during lipogenesis.

The current data indicate that restriction of dietary lysine increased the expression of genes involved in the de novo synthesis of fatty acids from glucose and other carbon sources via ACC and FASN upregulation. This diet also resulted in an increase in SCD expression which is involved in the rate-limiting desaturation of long chain fatty acids. Together these data indicate increased lipogenic activity in response to dietary lysine restriction which may result from the redirection of nutrients away from protein synthesis and towards fatty synthesis due to the lack of sufficient Lysine, an essential amino acid for muscle growth. This is supported by the findings of Doran et al., (2006) who reported that a reduced protein diet in pigs resulted in a targeted increase in SCD expression in the IMF depot but not in subcutaneous adipose tissue. To reiterate, the goal of this proposal was not to revisit lysine or vitamin A requirements but these scenarios did help to understand the responsiveness and interrelationships between lean muscle and intramuscular adipose tissue metabolism in this tissue-level niche.

In terms of the final pork product, location along the LM had a measurable impact on the composition of fatty acids present. Marbling scores, and the amount of saturation of the fatty acids present, decreased from the proximal to distal portions of the LM and the amount of amount of PUFA increased from proximal to distal. As mentioned before, SCD expression increased in the medial and distal portions of the LM during the feeder phase and may have contributed to the increased profile of unsaturated FA in these locations in the LM at harvest. In contrast, in beef animals selected for high or low marbling, the presence of large marbling flecks was highest in the center portion of the LM compared to the proximal LM (Lee et al., 2018). In the current study lean color increased from the proximal to distal LM which was opposite, and may also be reflective of, the distribution of marbling in that same direction. Diet caused moderate changes in the composition of fatty acids and this effect was not location specific. Lysine deficiency increased the percentage of MUFA in the LM and decreased the percentage of PUFA which could lead to changes in the sensory and shelf-life characteristics of the pork products. PUFA are subject to oxidation and the decrease in PUFA may extend the shelf life and warrants further investigation. Olivares et al. 2009, reported that while dietary vitamin A levels impacted FA composition in subcutaneous adipose tissue, vitamin A did not impact the composition of intramuscular lipids which is what we observed in the analysis of the IMF in the current study.

The biological data generated from the current study are informative in terms of the spatial and temporal regulation of marbling development in the LM of the growing pig. The observed spatial and temporal separation of adipose development and lipid metabolism in the loin muscle have generated new hypotheses and future studies will focus on the characterizing

the cellularity and subcellular activity of these intramuscular adipocytes in efforts to manipulate this valuable tissue niche towards a more consistent and predictable pork product with respect to the amount and distribution of fat in the LM.

Table 1. Vitamin Premix Formulated with Vitamin A to produce Control and Lysine Deficient Diets

Nutrient	Unit of Measure	Units per kg	Units per Lb.
Vitamin A	IU	1,653,441.10	750,000.80
Vitamin A	IU	2,755,730	1,249,999.10
Vitamin D	IU	551,146	249,999.80
Vitamin E	IU	17,636.70	8,000
Menadione	mg	1,763.70	800
Niacin	mg	1,234.50	559.9
Niacin	mg	18,606.70	8,440
Riboflavin	mg	3,306.90	1,500
Vitamin B12	mg	15.4	6.9
Pantothenic Acid	mg	11,023	5,000
Mineral Oil	mg	10,000	4,536
Rice Hulls	mg	459,750	208,542.60
Calcium Carbonate	mg	445,000	201,852

[1]Swine Vitamin Px NP26873 (DSM Nutritional Products Canada Inc.)

Table 2. Vitamin premix[1] formulated without Vitamin A to produce Vitamin A deficient and Lysine + Vitamin A deficient diets

Nutrient	Unit of Measure	Units per kg	Units per Lb.
Vitamin D	IU	551,146	249,999.80
Vitamin E	IU	17,636.70	8,000
Menadione	mg	1,763.70	800
Niacin	mg	1,234.50	559.9
Niacin	mg	18,606.70	8,440
Riboflavin	mg	3,306.90	1,500
Vitamin B12	mg	15.4	6.9
Pantothenic Acid	mg	11,023	5,000
Mineral Oil	mg	10,000	4,536
Rice Hulls	mg	463,057	210,042.60
Calcium Carbonate	mg	445,000	201,852

[1]Swine Vitamin Px NP26817 (DSM Nutritional Products Canada Inc.)

Table 3. Composition of Nursery Diets

Ingredients	Composition (%)			
	Control	LysDef	VitADef	LysVitADef
Corn, Yellow Dent	45.330	45.680	45.330	45.680
Soybean meal	10.000	10.000	10.000	10.000
Milk, Whey Powder	32.000	32.000	32.000	32.000
Fish meal	10.000	10.000	10.000	10.000
L-Lysine-HCl	0.350		0.350	
DL-Methionine	0.140	0.140	0.140	0.140
L-Threonine	0.100	0.100	0.100	0.100
L-Tryptophan	0.040	0.040	0.040	0.040
L-Histidine, 99%	0.050	0.050	0.050	0.050
L-Phenylalanine	0.090	0.090	0.090	0.090
L-Valine	0.030	0.030	0.030	0.030
Limestone	0.200	0.200	0.200	0.200
Dicalcium phosphate	0.050	0.050	0.050	0.050
Salt	0.020	0.020	0.020	0.020
Mineral premix[a]	0.100	0.100	0.100	0.100
Vitamin premix A[b]	0.100	0.100		
Vitamin premix B[c]			0.100	0.100
Zinc oxide	1.400	1.400	1.400	1.400

[a]Swine trace mineral (NB-8534; Nutra Blend, Llc)

[b]Swine Vitamin Px NP26873 (DSM Nutritional Products Canada Inc.)

[c]Swine Vitamin Px NP26817 (DSM Nutritional Products Canada Inc.)

Table 4. Composition of Feeder Diets

	Composition (%)			
Ingredients	Control	LysDef	VitADef	LysVitADef
Corn	75.272	75.672	75.272	75.672
Soybean meal	17.500	17.500	17.500	17.500
Poultry fat	4.200	4.200	4.200	4.200
L-Lysine-HCl	0.400		0.400	
DL-Methionine	0.100	0.100	0.100	0.100
L-Threonine	0.120	0.120	0.120	0.120
L-Tryptophan	0.028	0.028	0.028	0.028
Limestone	0.780	0.780	0.780	0.780
Dicalcium phosphate	1.300	1.300	1.300	1.300
Salt, Iodized	0.180	0.180	0.180	0.180
Mineral premix[a]	0.050	0.050	0.050	0.050
Vitamin premix A[b]	0.040	0.040		
Vitamin premix B[c]			0.040	0.040

[a]Swine trace mineral (NB-8534; Nutra Blend, Llc)

[b]Swine Vitamin Px NP26873 (DSM Nutritional Products Canada Inc.)

[c]Swine Vitamin Px NP26817 (DSM Nutritional Products Canada Inc.)

Table 5. Composition of Finisher Diets

Ingredients	Composition (%)			
	Control	LysDef	VitADef	LysVitADef
Corn	85.852	86.202	85.852	86.202
Soybean meal	8.200	8.200	8.200	8.200
Poultry fat	3.500	3.500	3.500	3.500
L-Lysine-HCl	0.350		0.350	
DL-Methionine	0.030	0.030	0.030	0.030
L-Threonine	0.110	0.110	0.110	0.110
L-Tryptophan	0.028	0.028	0.028	0.028
L-Valine	0.020	0.020	0.020	0.020
Limestone	0.650	0.650	0.650	0.650
Dicalcium phosphate	1.000	1.000	1.000	1.000
Salt, Iodized	0.190	0.190	0.190	0.190
Mineral premix[a]	0.035	0.035	0.035	0.035
Vitamin premix A[b]	0.035	0.035		
Vitamin premix B[c]			0.035	0.035

[a]Swine trace mineral (NB-8534; Nutra Blend, Llc)

[b]Swine Vitamin Px NP26873 (DSM Nutritional Products Canada Inc.)

[c]Swine Vitamin Px NP26817 (DSM Nutritional Products Canada Inc.)

Table 6. Genes of interest and TaqMAN Gene Expression Assays to determine mRNA abundance of Adipogenic Regulatory Factors involved in marbling development in the Longissimus muscle			
Target Gene of Interest	**Abbreviation**	**TaqMAN Assay No.**	**Description**
Acetyl CoA-Carboxylase	ACC	Ss03389963	Rate-limiting enzyme in the synthesis of long chain fatty acids
Acyl-CoA Oxidase	ACOX	SS03386405	Initial enzyme in the beta oxidation pathway for long chain fatty acids
Adiponectin	AdipoQ	Ss03384375	Protein hormone involved in glucose homeostasis and lipid metabolism
CCAAT/enhancer-binding protein alpha	CEBPA	Ss03373315	Transcription factor involved in adipogenesis
Carnitine palmitoyl transferase-1B	CPT1b	Ss03378792	Rate-limiting for oxidation of fatty acids in the mitochondria
Fatty Acid Synthase	FAS	Ss03386194	Enzyme complex that catalyzes fatty acid synthesis
Preadipocyte Factor 1	DLK1	Ss03388081	Gatekeeper in adipogenesis; prevents differentiation of preadipocytes
Peroxisome Proliferator Activated Receptor Gamma	PPARg	Ss03394829	Master regulator of adipogenesis
Stearoyl-CoA desaturase	SCD	Ss03392313	Delta 9 desaturase; rate-limiting for monounsaturated fatty acid synthesis
Beta Actin	ACTB	Ss03376081	Housekeeping

Table 7. Growth performance of pigs from wean to finish subjected to one of four dietary treatments[1]

	Control	LysDef	VitADef	LysVitADef	SEM	P-value
Initial Nursery Body Weight	25.1	22.35	22.2	24.11	1.41	0.55
Nursery Phase Average Daily Gain	1.99	1.97	1.90	1.90	0.07	0.70
Initial Feeder Body Weight	55.85	55.37	53.15	53.15	2.09	0.70
Feeder Phase Average Daily Gain	1.75	1.67	1.72	1.69	0.04	0.45
Initial Finisher_BW	153.70	148.80	149.20	147.90	3.53	0.54
Finisher Phase Average Daily Gain	1.78	1.55	1.68	1.53	0.09	0.18
Final Body Weight	253.80	236	243.40	233.60	6.65	0.15
Total Average Daily Gain (150 days)	1.58	1.48	1.52	1.46	0.04	0.15

[1]Weaned pigs were received and acclimated to commercially available Ware Milling 18% pig starter pellet diet for 14 days. The pigs were then randomly assigned to pens and pens were randomly assigned to 1 of 4 dietary treatments. Body weights were determined after 28 days in the nursery phase, 56 days in the feeder phase, and 56 days in the finisher phase.

Table 8. Effect of Dietary Treatment and Phase of Production on Relative Expression of Adipogenic and Lipogenic Genes in Loin muscle biopsies of growing pigs collected during the nursery, feeder, and finisher phases of production.

	Dietary Treatment					Feeding Phase				*P* <		
Gene of Interest	**Control**	**Lysine Deficient**	**Vitamin A Deficient**	**Lys & Vitamin A Deficient**	**SEM**	**Nursery**	**Feeder**	**Finisher**	**SEM**	**Treatment**	**Phase**	**Treatment x Phase**
ACC	0.70^{a}	0.81^{a}	0.48^{b}	0.46^{b}	0.08	0.08^{x}	0.70^{y}	1.10^{z}	0.06	0.0032	<0.001	0.07
ACOX	0.87	0.87	0.88	0.83	0.12	0.80^{x}	1.13^{y}	0.66^{z}	0.1	0.99	0.00	0.76
AdipoQ	0.62	0.79	0.61	0.50	0.11	0.71	0.63	0.56	0.09	0.27	0.51	0.62
CEBPA	0.73	0.76	0.63	0.60	0.08	0.16^{x}	1.06^{y}	0.82^{z}	0.07	0.40	<0.001	0.40
CPT1B	0.73	0.76	0.79	0.81	0.09	0.8	1.05	0.46	0.08	0.96	<0.001	0.66
DLK1	0.38	0.50	0.36	0.35	0.06	0.28^{x}	0.53^{y}	0.39^{z}	0.05	0.30	0.01	0.68
FASN	0.57^{a}	0.90^{b}	0.75^{ab}	0.35^{a}	0.09	0.46^{x}	0.81^{y}	0.65^{xy}	0.08	<0.001	0.01	0.43
PPARg	1.04^{a}	0.99^{b}	0.92^{a}	0.76^{ab}	0.15	0.09	1.49	1.2	0.13	0.59	<0.001	0.93
SCD	0.62^{ab}	1.18^{a}	1.11^{a}	0.37^{b}	0.23	0.40^{x}	1.22^{y}	0.84^{xy}	0.2	0.05	0.02	0.78

Data presented as least square means and SEM for the main effects of treatment and feeding phase
^{a,b,c} Dietary treatment Means within a row that do not bear a common superscript differ ($P < 0.05$)
^{x,y,z} For feeding phase, means within a row that do not bear a common superscript differ ($P < 0.05$)
ACC: Acetyl CoA-Carboxylase
ACOX: Acyl-CoA Oxidase
AdipoQ: Adiponectin
CEBPA: CCAAT/enhancer-binding protein alpha
CPT1b: Carnitine Palmitoyl Transferase-1b
DLK1: Preadipocyte Factor 1
FAS: Fatty Acid Synthase
PPARg: Peroxisome Proliferator Activted Receptor-gamma
SCD: Stearol CoA-Desaturase

Figure 1. Temporal Expression of Acetyl-CoA Carboxylase in Proximal, Medial and Distal Loin Muscle of Growing Pigs

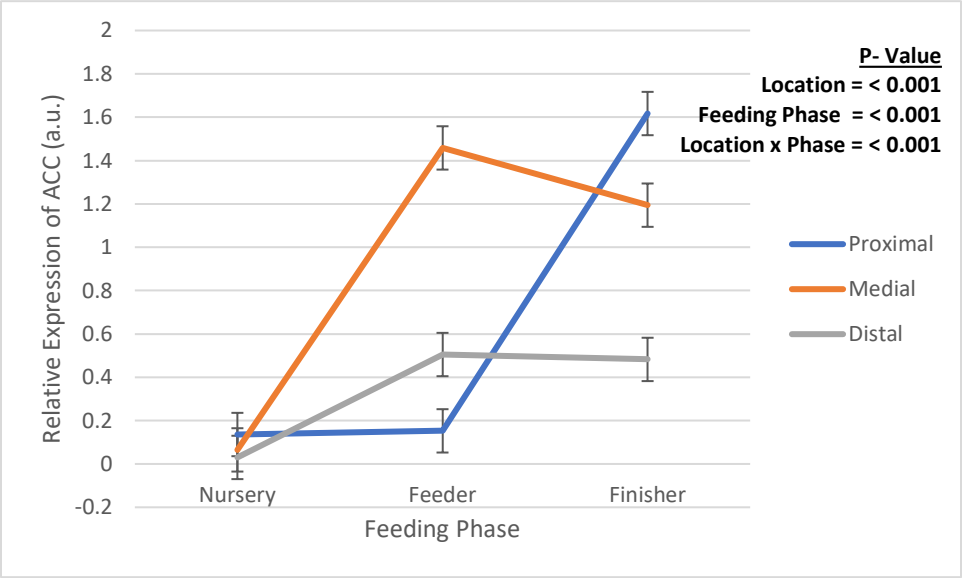

Figure 3. Temporal Expression of Acyl-CoA Oxidase in Proximal, Medial and Distal LM of Growing Pigs

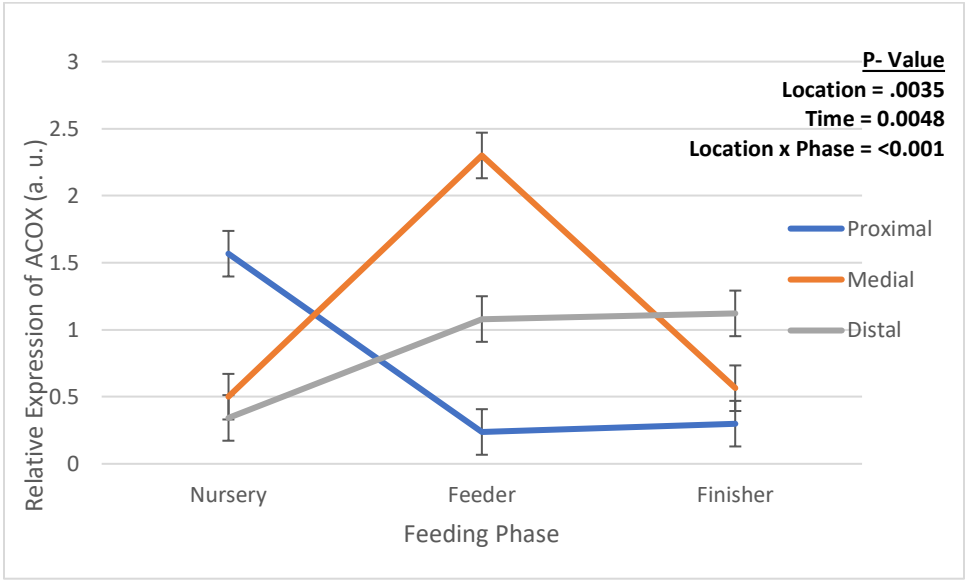

Figure 2. Temporal Expression of Adiponectin in Proximal, Medial and Distal LM of Growing Pigs

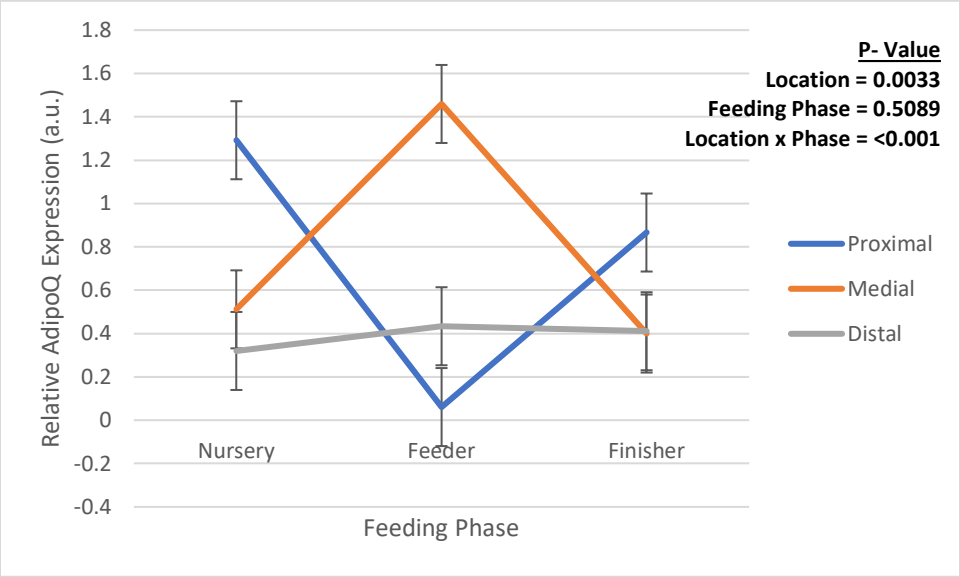

Figure 4. Temporal Expression of CEBPA in Proximal, medial and Distal LM of Growing Pigs

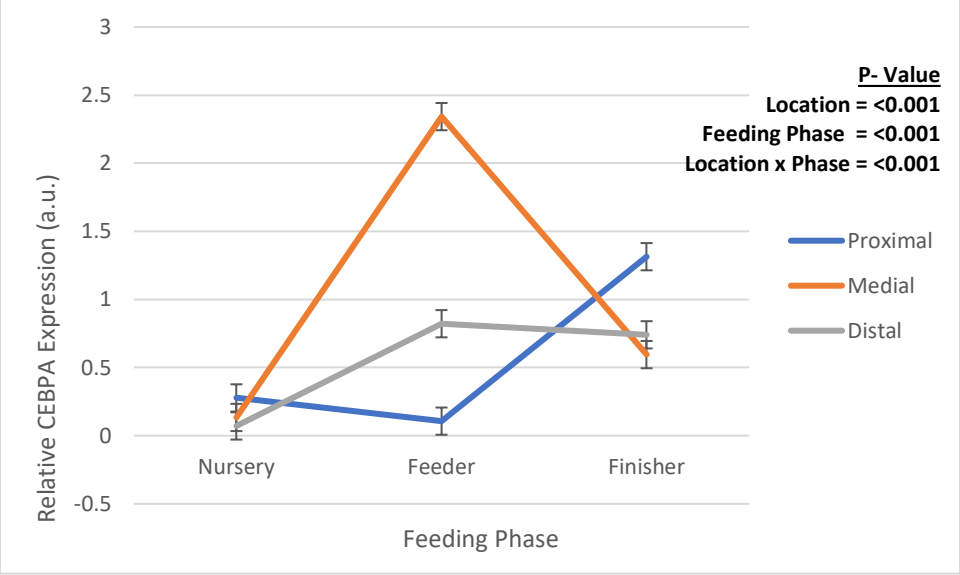

Figure 5. Temporal Expression of CPT-1b in Proximal, medial and Distal LM of Growing Pigs

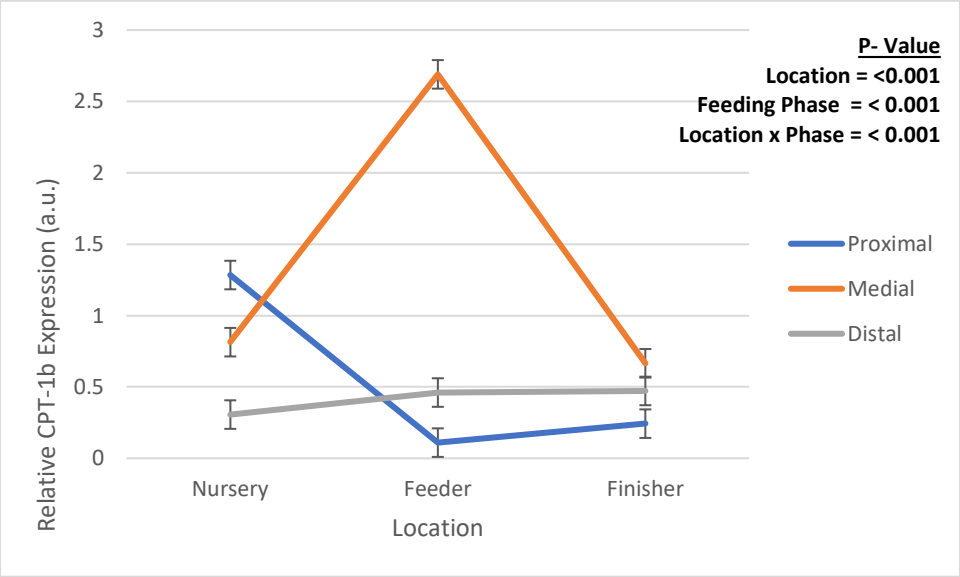

Figure 7. Temporal Expression of Dlk-1 in Proximal, Medial and Distal LM of Growing Pigs

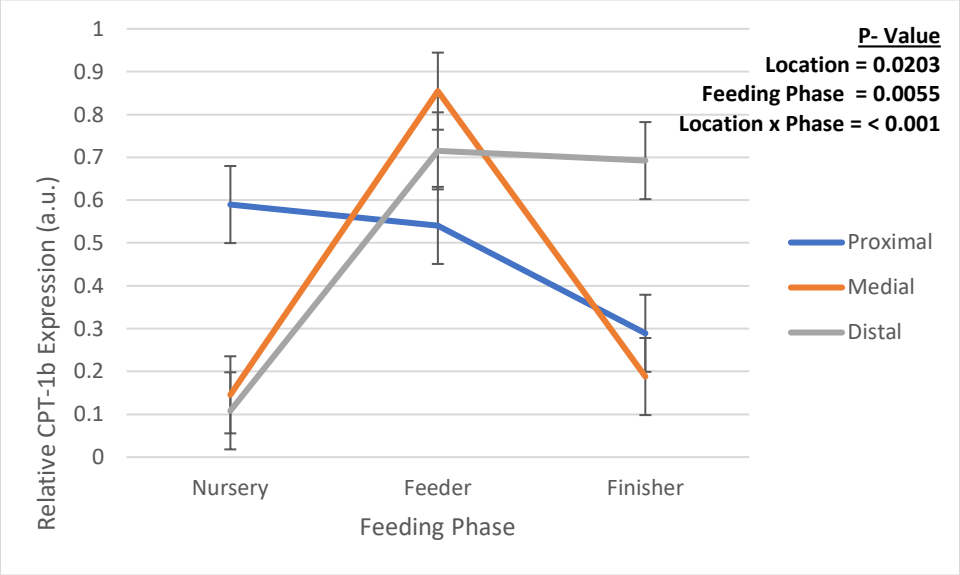

Figure 6. Temporal Expression of FASN in Proximal, Medial and Distal LM of Growing Pigs

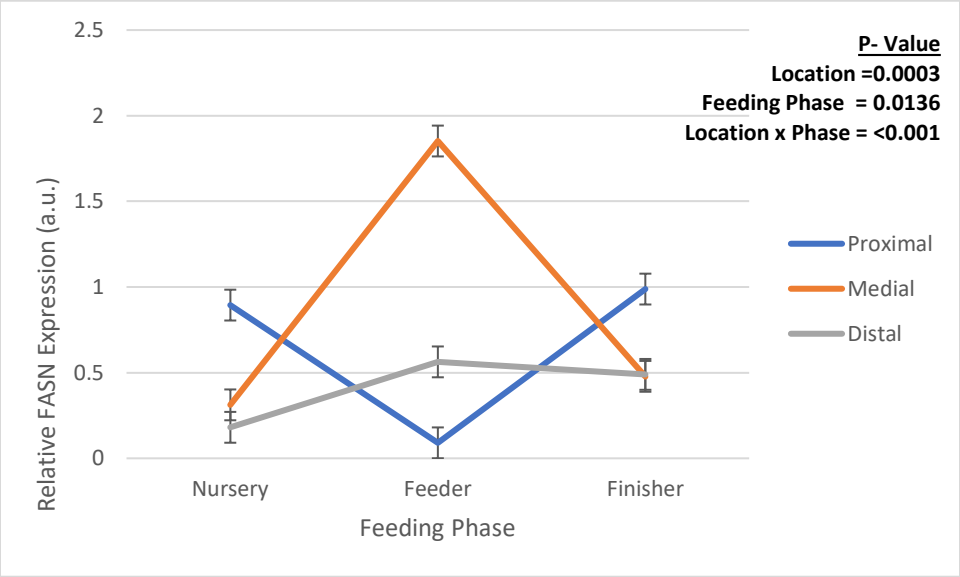

Figure 8. Temporal Expression of SCD in Proximal, Medial and Distal LM of Growing Pigs

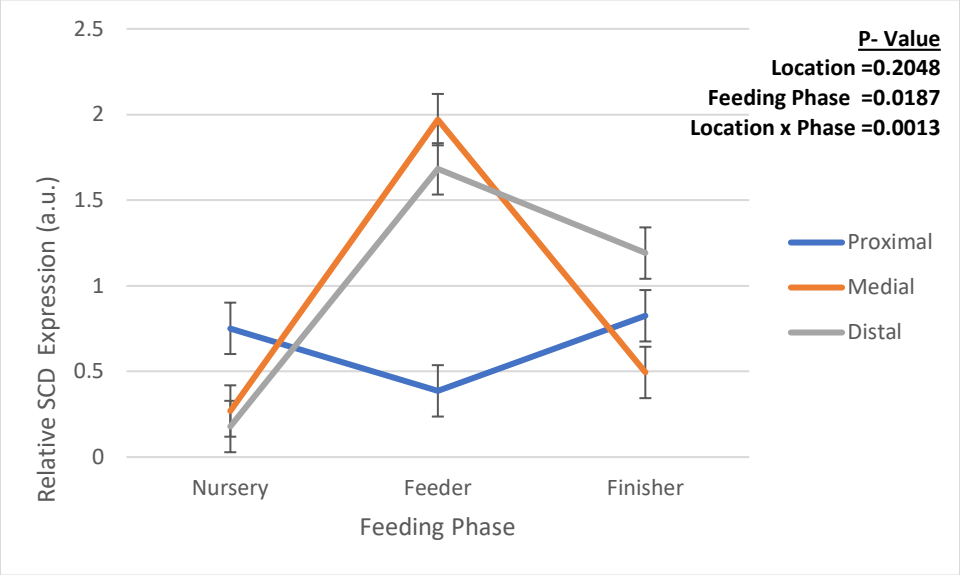

Figure 9. Temporal Expression of PPARg in Proximal, Medial and Distal LM of Growing Pigs

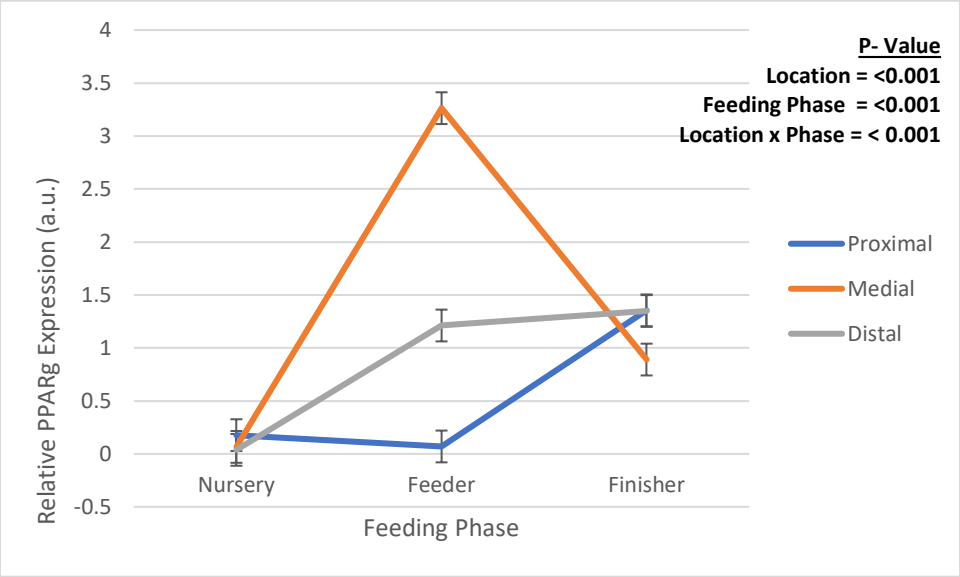

Table 8. Carcass Cutout and Morphometric Data

	Control	LysDef	VitADef	LysVitADef	**SEM**	**P-value**
HCW	190.6	173.9	183	173.7	5.53	0.11
Dressing Percentage	74.99	73.62	75.21	74.33	0.6	0.25
Loin Weight	19.17	17.54	19.55	18.04	1.1	0.53
Carcass Length	31.99	31.68	31.76	31.66	0.29	0.84
1st Rib Backfat	1.18	1.08	1.22	1.05	0.08	0.44
10th Rib Backfat	0.76	0.77	0.92	0.73	0.06	0.15
Last Lumbar Vertebrae Backfat	0.65	0.65	0.63	0.53	0.07	0.53
Belly Weight	8.86	9.24	8.11	8.23	0.48	0.7
Whole Shoulder Weight	17.32	15.43	16.62	16.3	0.71	0.31
Picnic Weight	9.35	7.86	8.71	8.56	0.41	0.11
Boston Butt Weight	7.97	7.57	7.91	7.74	0.44	0.92
Ham Weight	20.48	18.62	19.32	19.31	0.83	0.47
Trim Weight (lean)	3.39	3.17	3.3	2.78	0.25	0.33
Trim Weight (fat)	7.51	8.34	8.39	7.79	0.52	0.57

Table 9. Loin Quality Characteristics at the proximal, medial, and distal locations of the longissimus muscle harvested from Finished Hogs

	Treatment				Location			Probability		
	Control	LysDef	VitADef	LysVitADef	Proximal	Medial	Distal	Treatment	Location	Treatment*Location
Loin Eye Area (in^2)	5.2	4.8	4.6	4.66	5.37^a	6.26^b	2.74^c	0.35	<0.001	0.13
Marbling Score	2.5	3.1	2.7	2.8	3.10^a	2.45^b	2.70^b	0.07	0.0024	0.58
Lean Color	2.7	2.7	2.4	2.8	2.10^a	2.60^b	3.20^c	0.73	0.0001	0.05

[a-c]Means not bearing a common superscript differ ($P < 0.05$)

Marbling score determined by visual observation using the Pork Checkoff marbling score color scoring guide

Lean color score determined by visual observation using the Pork Checkoff lean color score scoring guide which has values ranging from 1-5

Table 10. The effect of dietary treatment and sample location on the percentage of fatty acids present in loin muscle collected from finished pork carcasses

	Treatment					Location				*P*-Value		
	Control	LysDef	VitADef	LysVitADef	SEM	Proximal	Medial	Distal	SEM	Treatment	Location	Treatment*Location
Saturated Fatty Acids	39.34	40.04	39.55	39.28	0.28	40.9[a]	38.55 [b]	39.14 [b]	0.24	0.14	<0.001	0.99
Monounsaturated Fatty Acids	39.86[a]	42.17[b]	40.36[a]	40.98[a]	0.58	41.7	40.22	40.62	0.49	0.03	0.1	0.7
Polyunsaturated Fatty Acids	19.63[a]	16.61[b]	18.94[a]	18.75[a]	0.66	15.96[a]	20.22[b]	19.27[b]	0.57	0.009	<0.001	0.89
Branched Chain Fatty Acids	1.16	1.16	1.15	1.08	0.58	1.44[a]	1.02[b]	0.97[b]	0.05	0.73	<0.001	0.94
n3:n6[1]	0.04	0.04	0.04	0.04	0.0009	0.04[a]	0.03[b]	0.04[a]	0.0008	0.98	<0.001	0.24
P:S[2]	0.5[a]	0.42[b]	0.48[a]	0.48[a]	0.02	0.39[a]	0.53[b]	0.5[b]	0.02	0.01	<0.001	0.93
Saturation Index[3]	0.66	0.68	0.67	0.66	0.008	0.71[a]	0.64[b]	0.65[b]	0.007	0.11	<0.001	0.98

[a-c]Within Treatment or Location, Means not bearing a common superscript differ ($P < 0.05$).

[1]n3:n6 defined as the ratio of omega 3 to omega 6 fatty acids.

[2]P:S defined as the ratio of polyunsaturated to saturated fatty acids.

[3]Saturation Index defined as the ratio of stearic acid to oleic acid.

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