

NOVEL FEED ADDITIVES TO IMPROVE LIPID PROFILES IN PIGS

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By

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NOVEL FEED ADDITIVES TO IMPROVE LIPID PROFILES IN PIGS

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ABSTRACT

Opportunities to export high quality pork products to trade partners like Japan are economically favorable outlets for US pork producers. Domestic consumers also rely heavily on fresh pork appearance, especially color and marbling, to make initial and repeat purchasing decisions. Two studies were performed to elucidate novel ways to improve pork quality, specifically by altering the lipid profile and increasing intramuscular fat (i.m.).

In experiment 1, forty individually reared barrows (100 kg \pm 3 kg, PIC C23 x 337) were randomly assigned to 1 of 5 treatments containing dried distillers grains with solubles and 3% added fat to determine the resulting carcass characteristics and fresh pork quality. The treatments included 1) a positive control containing a corn and soybean meal base with 3% choice white grease (PCON), 2) 30% high protein dried distillers grain and 3% choice white grease (HPDDG), or 30% DDGS with 3) no added fat (DDGS), 4) 3% choice white grease (DDGS + CWG), or 5) 3% butter oil (DDGS + BO). Postmortem carcasses characteristics and quality attributes were not different between diets ($P > 0.08$) except the semimembranosus muscle from pigs fed HPDDGS had the most basic ultimate pH ($P = 0.01$) suggesting pigs fed HPDDGS had less glycolytic potential at the time of slaughter. Including DDGS in the diet decreased ($P < 0.01$)

MUFA and increased ($P < 0.01$) PUFA. Butter oil increased ($P < 0.01$) CLA content in fat, but did not increase SFA or iodine value. Although added fat in the diet altered fatty acid composition in multiple fat depots, adding additional saturated fat to the diet in the form of butter oil did not mitigate the unsaturation of DDGS.

Skycis[®] (narasin, NAR) is a swine performance ionophore labeled to increase rate of gain in the last 4 weeks of the finishing phase. The objective of experiment 2 was to evaluate inclusion of NAR in pig diets and determine the source of carcass yield, specifically dressing percentage, improvements observed in previous studies. Barrows ($n=50$) and gilts ($n=50$) of PIC C22 x 337 genetics were randomly assigned to a diet containing 0 or 15 ppm NAR initiated at 39.5 kg of body weight and continued until slaughter. Barrows tended ($P = 0.09$) to have heavier viscera, significantly ($P < 0.01$) more fat at the 10th rib, and a greater ($P = 0.01$) percentage of i.m. fat compared to gilts. Gilts were heavier muscled than barrows as evidenced by many gender differences ($P < 0.05$) observed in the cutability phase of the study, specifically larger LM ($P < 0.01$). Heavy muscled, lean animals such as gilts may have increased organ mass from up regulated metabolic activity, but pigs with greater intake will also have heavier intestinal tract compensating for the increased consumption suggesting barrows in this study ate more, especially late in the growth curve. Pigs fed NAR had heavier ($P < 0.01$) hearts, but few treatment differences were observed in carcass quality and cutability. In general, fat content increased and made up a larger percentage of total carcass weight in pigs fed NAR. The current results do not confirm the source of previously observed differences in DP in pigs fed NAR, but could be attributed to the tendency of NAR to increase carcass fatness.

CHAPTER 1

LITERATURE REVIEW

Global Pork Demand

Globally speaking, pork accounts for the majority of worldwide meat supply at 40% followed by poultry at 34% (USDA-FAS, 2016). In the next decade or so, global pork consumption is expected to continue to rise, largely due to China's growing population and popularity of pork cuisine in their culture (Hansen and Gale, 2014). Countries like China and Russia continue to increase domestic production and some analysts (FAO, 2015) predict in the long term, world trade of pork exports will decline. However, China faces rising production costs, disease, environmental regulations and resource constraints that may actually slow domestic production and increase imports from countries like the US, Canada and European Union (Hansen and Gale, 2014).

Domestic demand for protein is also growing from a renewed interest in meat products from US consumers. There is a strong demand for pork and other meat items in the US as the country has begun recovering from a recession and disposable income has moderately increased for meat consumers who are allotting a greater portion of income to animal protein in their budgets (NPB, 2014). Plus, with high beef prices and unexpected increases in poultry prices, pork products are an attractive grocery store item (NBP, 2014). Furthermore, volumes of hidden and forgotten research pushed aside during erroneous and unjustifiable dietary trends during the 1970s to 1980s have revealed fat, particularly saturated animal fat, is the not the enemy once accused to be (Teicholz,

2014). Not to mention, the local food movement has brought about a staggering increase in demand for products grown close to home. Ultimately, there is and will continue to be an international and domestic demand for pork.

Marketing US Pork

International Trade. In 2015, the US exported 2.1 million metric tons of pork worth \$5.575 billion (USMEF, 2016). Mexico accounts for the largest volume of pork exports including variety meats from the US, but in 2015 the Japanese imported \$1.5 million of pork, which is worth more than several other US pork importers combined (USMEF, 2016). The Japanese have consistently been the most valuable importer of US pork in the last 10 years (USMEF, 2016), indicating their willingness to pay for high quality, white table cloth cuts that meet strict specifications. Other US trade partners include China, Canada, South Korea and Central and South America (USMEF, 2016).

Consumer Demands. Improving pork quality is essential to the future success of the pork industry as trade partners like Japan, and local US consumers have been demanding higher quality products more strongly than ever before. In particular, color and marbling are two quality attributes in desperate need of improvement in pork. Color is the sole indicator a consumer can use as an indicator of wholesomeness, quality and freshness at the time of purchase (Mancini and Hunt, 2005; Suman and Joseph, 2013). Any deviation from the expected bright red color leads to product rejection (Mancini and Hunt, 2005; Suman and Joseph, 2013). Furthermore, Asian markets including Japan have been shown to prefer higher quality pork than typical US domestic markets (Ryan et al., 2010). Japanese consumers prefer fresh pork with a darker, more uniform color (Chen et al., 2010) and greater marbling and fat content (Martinez and Zering, 2004; Dransfield,

2008) compared to US consumers. In the last several decades fat has largely been seen as the enemy to US consumers from a diet and health standpoint (Teicholz, 2014) so producers responded by selecting and finishing genetically leaner pigs. However, not only do lipids play an integral role in flavor development (Calkins and Hodgen, 2007; Dransfield, 2008), intramuscular fat (i.m.) has a considerable impact on perceived palatability of meat products and tenderness (Smith et al., 1984).

The Working Model

The modern US pig is genetically superior to even the most recent ancestors and worldly counterparts and continues to change with each generation. According to the National Pork Board (NPB) (2014), US hog producers have consistently improved reproductive efficiency, growth performance, and carcass yield each year in the last several decades. The NPB (2005) describes the ideal market hog profitable for every industry segment in Symbol III, which includes science based standards and producer practices to achieve a phenotypically desirable animal with ideal live animal production performance and carcass characteristics. According to Symbol III, pig carcasses should have a color score of 4, 3% intramuscular fat (i.m), 24 h pH of 5.9, minimum drip loss of 2.5%, 15 to 18 mm (0.6 to 0.7 in) backfat, and 25 mm (1.0 in) thick bellies (NPB, 2005).

The most recent version of a pork quality audit reported a tremendous range in the retail value and quality of fresh and processed pork products (Wright et al., 2005).

According to the survey, pork loin chops scored on average 3.52 for color and contained 2.37% i.m. based on the National Pork Producer Council (NPPC) (1999) standards, had 1.82% drip loss and a pH of 5.64. Despite meeting some of the Symbol III requirements, 12.5% of boneless chops were considered “low quality” (Wright et al., 2005). A large

portion of pork chops are sold as an enhanced product to improve palatability which rate higher with trained sensory panels for every palatability trait including tenderness, juiciness, flavor intensity, but also off-flavor (Wright et al., 2005). Generally speaking, processed products were similar in terms of quality and palatability, but differed substantially in terms of price or retail value (Wright et al., 2005). Despite the industry focus and emphasis to improve pork quality, there is still opportunity to improve fresh pork and processed product quality and consistency for consumers.

Despite the fact that producers are working with a genetically superior model that is an efficient pork producing machine with more muscle and less fat on a pounds heavier carcass, lax management, poor nutrition, and abnormal environmental factors can prevent these animals from reaching their maximum genetic potential and achieving high quality status as a carcass. Not to mention, pigs are being finished at a much higher end weight than ever before, which changes how we manage them and consider their biological functions. Pork carcass composition and quality is easily manipulated via dietary feedstuffs, feed additives, management and environmental conditions. Current producers have an opportunity and an obligation to meet current demands with higher quality pork and pork products. Using a combination of biological type, nutrition, and management, hog producers have an opportunity to manipulate the lipid profile of pigs and ultimately improve pork quality.

Pork Quality

Tenderness, juiciness and flavor are three areas typically associated with meat quality (Martin et al., 2003). Pork quality is largely the result of both ante and

postmortem events (Ryan et al., 2010), particularly factors that effect postmortem pH decline, which in turn largely influences fresh meat quality (Hambrecht et al., 2003; Scheffler and Gerrard, 2007). Pre-harvest factors that affect quality include but are not limited to nutrition, genetic predisposition, management and handling, lairage time, as well as long and short-term stress (Aberle et al., 2012). Events at the time of harvest and shortly after such as stunning method, exsanguination, carcass chill rate, and chilling temperature can also impact meat quality (Aberle et al., 2012). Thus, the quality of fresh meat is a direct result of the postmortem conversion of muscle to meat and the intricate interworking of postmortem biochemical reactions.

Temperature and pH

Events including animal handling, ambient temperature, pen space, lairage time, stunning method and exsanguination that influence meat quality tend to manifest in biochemical changes postmortem (Aberle et al., 2012). Tissue temperature and pH are quintessential contributors to meat quality that singly or together effect virtually every chemical reaction studied in meat science and consequently, any deviations in temperature and pH during the conversion of muscle to meat will impact meat quality (Aberle et al., 2012). In a living animal the pH is 7.4, but under normal conditions that pH will decline to 5.6 to 5.7 in 6 to 8 hours postmortem, but by 24 h, will have reached an ultimate pH of 5.3 to 5.7 (Aberle et al., 2012). Normal body temperature for animals is around 38°C (100°F), which declines to around 0°C (32°F) by 24 h postmortem (Aberle et al., 2012). This tremendous drop in temperature affects the physical nature of carcasses as well as the chemical reactions that take place during slaughter (Aberle et al., 2012). However, during the slaughter process, the body loses its ability to regulate

temperature and certain slaughtering procedures like scalding and singeing add heat to the carcass (Aberle et al., 2012). Uncontrolled heat elevation may complicate pH decline and effect meat quality if the carcass is not chilled in timely fashion.

Rigor mortis is the phenomenon of stiffening carcasses as permanent cross bridges are formed between actin and myosin. Without an ATP supply, relaxation is impossible and the cross bridges stay linked, hence the permanent contraction, or stiffening of muscles (Aberle et al., 2012). Resolution of rigor occurs only when proteolytic degradation breaks down proteins in the Z-disk including desmin, nebulin, and titin, causing the sarcomere to lose structural integrity (Aberle et al., 2012). Rigor mortis and pH decline are closely related because both are related to the amount of stored energy in the biological system at the time of death (Aberle et al., 2012).

Postmortem, the body attempts to maintain homeostasis by shifting to anaerobic metabolism when oxygen stores are depleted and due to the body's inability to remove the lactic acid by-product to the liver, lactic acid builds up in the muscle and causes a pH decline in the tissue. Glycolytic potential is a measure of all the compounds available in a muscle that can be converted to lactic acid and is a way to measure the capacity of postmortem glycolysis and potential extent of pH decline (Hamilton et al., 2003). If energy supplies are low or metabolized quickly, the onset of rigor is rapid, but this has opposing results on pH; low energy supplies yield a high pH (6.5 to 6.8), but if the available energy is metabolized quickly, the pH is abnormally low (5.2 to 5.4). Optimal pH and meat quality is achieved when rigor is delayed while glycogen stores are depleted by ongoing postmortem muscle contraction (Aberle et al., 2012). Some of the major

quality factors related to pH decline are color, water holding capacity (WHC), drip loss and moisture loss during cooking (Aberle et al., 2012).

Pork Color

Of all the fresh meat characteristics, color is the most important one influencing purchase decisions because consumers are unable to feel the texture or smell the odor through packages (Mancini and Hunt, 2005). Consequently, consumers rely on color as the sole indicator of freshness and wholesomeness at the point of sale and any deviations from the bright red color leads to product rejection and revenue loss (Mancini and Hunt, 2005; Suman and Joseph, 2013). Discolored products can be repurposed into ground products, but some are thrown out long before their microbiological integrity is compromised (Faustman and Cassens, 1990). Either way, the end result is more than one billion dollars in lost revenue annually (Smith et al., 2000) and a deplorable waste of edible protein products (Faustman and Cassens, 1990).

There are two pigments in meat responsible for meat color, myoglobin (Mb) and hemoglobin (Aberle et al., 2012; Suman and Joseph, 2013). In living muscle, Mb binds and delivers O₂ to mitochondria so the tissue can maintain physiological functions (Wittenberg and Wittenberg, 2003). In meat, Mb serves as the main color pigment that produces the bright red color (Suman and Joseph, 2013). Aberle et al. (2012) estimate Mb accounts for 80 to 90% of the red color, but even in well-bled animals hemoglobin can account for about 10% of the red color. Structurally speaking, Mb is a globin moiety with eight helical segments that wrap around a heme prosthetic group (Suman and Joseph, 2013). The heme group resides in the hydrophobic pocket of the protein globin (Mancini and Hunt, 2005) and provides water solubility and protects the heme iron from

oxidation, (Suman and Joseph, 2013). The single iron atom in the heme ring makes six bonds; four with nitrogen atoms in the ring, one with histidine-93 in the globin and the final spot is available to reversibly bind ligands including O₂, CO, or NO (Mancini and Hunt, 2005; Suman and Joseph, 2013). Myoglobin absorbs color and functions as a pigment because the heme group has conjugated double bonds that absorb light (Suman and Joseph, 2013).

Myoglobin can exist in one of four distinct states including deoxymyoglobin (DeoxyMb), oxymyoglobin (OxyMb), carboxymyoglobin (COMb) or metmyoglobin (MetMb) (Mancini and Hunt, 2005) all depending on the oxidation state of the heme iron and packaging environment (Suman and Joseph, 2013). Deoxymyoglobin produces a dark purplish red color when no ligand is bound to the reduced, ferrous iron atom (Mancini and Hunt, 2005; Suman and Joseph, 2013). Both OxyMb and COMb appear bright red when O₂ or CO bind, respectfully, to the iron atom in a ferrous, or reduced state (Suman and Joseph, 2013). Carboxymyoglobin is much more stable than OxyMb because the iron has a much stronger affinity for CO compared to O₂ (Suman and Joseph, 2013). Oxidation of the three ferrous states yields the ferric state, MetMb, which is associated with meat discoloration (Suman and Joseph, 2013). Cooking denatures Mb and heat induced denaturation of Mb causes the dull brown color of meats (King and Whyte, 2006).

Color can be affected by endogenous factors like pH, muscle source, antioxidants, lipid oxidation and mitochondrial activity (Mancini and Hunt, 2005) or exogenous factors such as live animal management, diet, and genetics (Faustman and Cassens, 1990). When animals are stressed prior to slaughter, body temperature increases, glycolysis

rapidly uses up ATP stores quickly building up hydrogen protons culminating in early rigor onset and exaggerated conversion of muscle to meat (Aberle et al., 2012). The very rapid pH decline from over abundance of hydrogen protons coupled with raised body temperatures during early onset of rigor, increases protein degradation and results in a serious quality defect called Pale, Soft, and Exudative, or PSE (Aberle et al., 2012). Pale soft and exudative pork has a very light color, poor water holding capacity, and reduced tenderness and juiciness (Aberle et al., 2012). Dark cutting beef and DFD, or Dry, Firm, and Dark pork, result from a glycogen deficiency at death. In this case, postmortem metabolism is limited and diminished accumulation of hydrogen protons results in higher ultimate pH, but improved water holding capacity (Aberle et al., 2012). In addition to the palatability issues with PSE, the biggest concern with both defects is the color is unattractive to consumers and will consequently be rejected (Aberle et al., 2012).

Studies have linked meat discoloration to specific enzymes (Arihara et al., 1995) but Joseph et al. (2012) indicated the abundant presence of antioxidant and chaperone proteins that protect Mb from oxidation and improve color stability. Lipid oxidation generates secondary products like ketones and aldehydes that cause off odors (Pearson et al., 1977). However, the reactive products of lipid oxidation also compromise meat color by accelerating Mb oxidation, thus reducing lipid oxidation may help improve meat quality two fold (Faustman et al., 2010). Also, as mitochondrial activity persists, Mb will be outcompeted for O₂ resulting in dark meat (Ramanathan et al., 2009). Because mitochondria respiration creates an anaerobic environment and helps reduce MetMb to OxyMb, harnessing mitochondrial respiration substrates that favor MetMb reduction could improve color stability as well (Suman and Joseph, 2013).

Color has also been correlated to glycolytic potential in several studies (Meadus and MacInnis, 2000; Hamilton et al., 2003; Moeller et al., 2003). According to Meadus and MacInnis (2000), L^* ($r = 0.40$) and b^* ($r = 0.43$) are correlated to glycolytic potential suggesting increased glycolytic potential promotes acidity, paleness and yellowness. Both Hamilton et al. (2003) and Moeller et al. (2003) found positive correlations ($r = 0.23$ and 0.33 , respectively) between LM L^* and glycolytic potential. In separate studies, Rosenvold et al. (2001a, b) strategically fed pigs to lower glycolytic potential. Pigs either tended to have darker loins with higher 45 min pH (Rosenvold et al., 2001a) or altered the glycogen stores enough to significantly darken LM color without affecting pH (Rosenvold et al., 2001b). Collectively, these data imply reducing glycolytic potential may improve pork quality (Mancini and Hunt, 2005).

According to Apple et al. (2004a) supplementing magnesium mica improved a^* , but not L^* or b^* . After 14 d storage, Wiegand et al. (2002a) reported L^* decreased and a^* increased in pork from pigs supplemented short term with vitamin D₃. Naturally associated pigments in grains and oil may contribute to meat and fat color as well (Maw et al., 2003). Research has attributed dietary changes to alter things like glycogen storage or antioxidant accumulation which ultimately relates to fundamental intrinsic color traits, pH, oxygen consumption and MetMb reducing ability as previously discussed (Mancini and Hunt, 2005).

Fresh pork color is widely accepted as detrimentally affected by the presence of the halothane (Nn or nn) allele (Channon et al., 2000; Fisher et al., 2000; Eggert et al., 2002). Loins from Nn pigs have lower pH and are paler than NN pigs (Eggert et al., 2002) and pigs with the nn genotype are PSE 100% of the time compared to NN pigs,

which exhibit PSE 8% of the time (Fisher et al., 2000). Genetic issues are easily solved by selecting for the NN genotype and utilizing breeds that are well known for their quality attributes such as Durocs (Wood et al., 2004) and Berkshires (Lee et al., 2012).

Measuring Color

In their review, Mancini and Hunt (2005) describe three general ways to measure color: computer vision, instrumental color or visual color. Computer vision uses the analysis of digital images to evaluate color. O'Sullivan et al. (2003) describes advantages of computer vision analysis as only needing one image that accounts for surface variation in Mb redox state and the information can be converted to many instrumental color measurements. There are several options for instrumental color found in a variety of colorimeters and spectrophotometers, which can make deciding which tool to use for research difficult. To further complicate the issue, each instrument has options to choose the color system (Hunter, CIE, or tristimulus), Illuminants (A, C, D65, or Ultralume), observer angle and aperture size (Mancini and Hunt, 2005). Visual color can be determined by subjectively comparing meat to NPPC or Japanese color standards. Although this method most mimics consumers, subjective color is also highly variable.

Ultimately, Mancini and Hunt (2005) recommend choosing the option that best suits the needs of the experimental objectives. One of the most common color methods used for meat science research is instrumental color (Hunter or CIE), which use reflectance technologies that shine a light source of known energy onto a sample and measures the amount of energy that is reflected back. Instrumental technologies report three color values (Hunter = L, a, b; CIE = L*, a*, b) expressed numerically on a three dimensional axis based on the opponent color theory that assumes the human eye

perceives color in three pairs: light to dark, red to green and blue to yellow (Hunter Lab, 2012). Lightness, L or L*, ranges from 0 to 100 with 0 indicating total absence of light (dark) and 100 indicating pure white (light). Redness, a or a*, is indicated on the red to green scale with negative values indicating more green and positive values indicating more red color. Finally, blueness and yellowness is quantified by b or b*. Negative values indicate more blue while positive values indicate more yellow color (Hunter Lab, 2012).

Water Holding Capacity

Water holding capacity is the ability of meat to retain water when an external force is applied and affects nearly every attribute of fresh meat quality including color, texture, firmness of raw meat and the juiciness and tenderness of cooked meat (Aberle et al., 2012). Purge is accumulated moisture that is economically significant because of the weight lost in escaped water and also, purge contains quality meat proteins (Aberle et al., 2012). Fresh meat WHC capacity is crucial for further processing into sausages, cured, and heat processed products because WHC affects yield, palatability and shrink during storage (Aberle et al., 2012). Not surprisingly, unacceptable WHC costs the meat industry millions of dollars each year (Huff-Lonergan and Lonergan, 2005).

Water is a crucial element in muscle. Not only is lean muscle 75% water (Huff-Lonergan and Lonergan, 2005), water also acts as a lubricant in the very fast, very repetitive movements of muscle contraction in the myofibrillar protein system (Puolanne and Halone, 2010). Water is found within the structure of muscles as well within muscle cells. Specifically, water is held between myofibrils, myofibrils and the sarcolemma, and between muscle cells and muscle bundles (Huff-Lonergan and Lonergan, 2005).

Although there is an immense number of publications that study WHC, few have markedly increased the knowledge or understanding on the foundation of WHC and research has yet to discover the fundamental explanation for the bulk water holding in muscle (Puolanne and Halonen, 2010).

According to Hamm (1972), water holding in muscle is caused by electrostatic repulsion between myofibrillar proteins that cause swelling of myofibrils. Because water is dipolar, water is attracted to charge species like protein (Huff-Lonergan and Lonergan, 2005; Pearce et al., 2011). There are three types of water, majority of which resides in the intra and extramyofibrillar spaces (Huff-Lonergan and Lonergan, 2005). Bound water makes up a very small fraction of water in muscle cells. Bound water exists in the vicinity of non-aqueous constituents and has decreased mobility, and is resistant to freezing and being driven off during heating (Fennema, 1985). Immobilized water is held in place by steric effects or attraction to bound water (Fennema, 1985). However, since immobilized water is not directly bound to protein, but simply held within the muscle, this type of water does not flow freely from meat, but is driven out by drying and is susceptible to freezing (Huff-Lonergan and Lonergan, 2005). Thus, immobilized water is the type of water most affected during the conversion of muscle to meat and the goal of meat processors is to retain as much of this water as possible (Huff-Lonergan and Lonergan, 2005). The last type of water is free water held in meat by weak forces and flows from tissues unimpeded (Fennema, 1985).

The rate and extent of pH decline, proteolysis and protein oxidation are key factors in determining WHC (Huff-Lonergan and Lonergan, 2005). As rigor progresses postmortem, the space for water in myofibrils decreases and fluid is forced into

extramyofibrillar space and easily lost as drip and purge (Huff-Lonergan and Lonergan, 2005). During the conversion of muscle to meat, lactic acid builds up and lowers pH. At the isoelectric point (pI = 5.4) net charges on protein are zero. Since proteins are then attracted to each other, when the positive and negative charges are equal, the amount of water held by the protein decreases (Huff-Lonergan and Lonergan, 2005). Additionally, the space between myofibrils is reduced because there is no longer a repulsion between the like charges on proteins forcing the structure to close together and force water out (Huff-Lonergan and Lonergan, 2005). The longitudinal and latitudinal contraction essentially squeezes water out of space between myofibrils and into the extramyofibrillar space (Pearce et al., 2011).

Rapid pH decline that results in the ultimate pH value while the carcass is still at warm temperatures denatures proteins including the water binding proteins (Huff-Lonergan and Lonergan, 2005). The most severe example is PSE when pigs inherit a mutation in the halothane gene responsible for the ryanodine receptor and calcium release channel (Fujii et al., 1991). In the case of the halothane gene mutation, the biological system is unable to control Ca^{2+} release in the sarcoplasm, particularly in times of stress when there is increased muscle contraction and increased rate of muscle metabolism leading to an increased rate of pH decline (Bendall and Wisemer-Pedersen, 1962; Lundstrom et al., 1989).

Marbling and Tenderness

Intramuscular fat, also known as marbling, plays a critical role in the physical properties, aesthetic appearance of meat, and most importantly, the palatability and eating experience for the consumer. Marbling is heavily used in the beef industry to assign

USDA Quality Grades and sort carcasses based on value, however, neither subjective or objective marbling measures are used in other major industries to help segregate carcasses. Marbling is something consumers can physically see in packages and may purchase or reject products based on marbling content (Aberle et al., 2012), however, some consumers actually tend to stray away from fatty products for health concerns. The problem with consumers' negative perception on fat lies in the fact that fat plays a vital role in improving meat tenderness, juiciness and flavor (Savell and Cross, 1988).

Tenderness is the single most important attribute contributing to the palatability of beef (Smith, 1972) but likely other species as well (Bratzler, 1971). Tenderness is intimately associated with juiciness and tender meat releases juices and stays moist through sustained mastication (Smith and Carpenter, 1974). According to Smith et al. (1973), differences in tenderness result from combined effects of numerous traits that loosely fit into one of three categories: actomyosin effects, background effects or bulk density and lubrication effects.

Actomyosin effects relate to the contractile state of actin and myosin and the structural integrity of the Z-disk (Smith et al., 1973). The degree of alternation and weakening of myofibrillar structures has largely been attributed to endogenous proteolytic enzymes (Sentandreu et al., 2002) and oxidation (Huff-Lonergan et al., 2010). The calpain system largely influences post-mortem proteolysis (Koochmaraie and Geesink, 2006). Both calpains and the calpain inhibitor calpastatin have important roles in tenderization (Kemp et al., 2010). The proteolytic enzyme calpain and the inhibitor calpastatin both require Ca^{2+} to function to break down the structural proteins titin, nebulin, desmin and troponin T, but do not break down myosin and actin (Huff-Lonergan

et al., 2010). Holding carcasses postmortem in refrigeration for an extended period of time is called aging and is performed to improve meat tenderness (Aberle et al., 2012). Tenderization rates vary by species (beef < lamb < pork) because of the ratio of calpastatin:calpain (beef > lamb > pork) (Koochmaraie et al., 1991).

Actomyosin effects that impact tenderness also include muscle fiber diameter and sarcomere length (Berry et al., 1974). Longer muscle fibers with smaller diameters are easier to bite through than short fibers with large diameters. Background effects are the consequence of connective tissue and collagen presence in meat (Smith et al., 1973). Decreasing collagen content and increasing soluble collagen content improves tenderness (Berry et al., 1974).

The amount, the distribution, as well as the chemical and physical state of i.m. fat changes the bulk density of meat and add to lubrication effects (Smith et al., 1973). There are multiple theories concerning how marbling impacts tenderness. According to Smith et al. (1973), in any given bite of meat, the presence of marbling decreases the mass per unit volume, lowers the bulk density of the bite, and increases apparent tenderness because fat is less resistant to shear than protein. Jeremiah et al. (1970) suggests considering marbling as a dilution factor that helps provide fewer muscle fibers to chew in a bite. Marbling also infiltrates connective tissue by depositing in between the perimysium and endomysium (Smith and Carpenter, 1974) which loosens the structure of connective tissue, aids in heat penetration, solubilization and thus collagen breakdown (Carpenter, 1962).

Intramuscular fat surrounds muscle fibers and lubricates fibers seemingly making the product juicier and thus more tender (Carpenter, 1962), especially during heating

when i.m. fat solubilizes under heating and becomes part of the juices (Jeremiah et al., 1970). Uniformly dispersed marbling is particularly desirable and will enhance juiciness by lubricating the maximum number of fibers (Briskey and Kauffman, 1971). Increased marbling also provides insurance during the use of high, dry heat cooking methods and advance degrees of doneness that the meat will still appear tender and juicy (Smith and Carpenter, 1974). Finally, Smith et al. (1974) propose fat, either SC, intermuscular, or intramuscular, insulates muscle fibers and protects fibers from cold-shock during chilling and prevents abnormal sarcomere shortening. Smith and Carpenter (1974) further suggest fat insulation also helps keep proteolytic enzymes active longer.

Intramuscular fat is especially affected later in growth due to fat deposition patterns in the pig. Dietary ingredients including protein and fat sources as well as content can influence the amount and FA profile of i.m. fat. According to Apple et al. (2004b), the ME:Lys ratio may affect IMF development. Low protein feeding strategies (18% vs 20%) increased the total lipid content from 1.7% to 2.8% (Teye et al., 2006). Durocs are also known to have increased marbling relative to SC fat and furthermore, Durocs and Berkshires have greater lipid amounts in LM compared to Large White and Tamworth breeds (Wood et al., 2004).

Temperature and pH decline in postmortem muscle have a significant role in tenderness development as well (Locker and Hagyard, 1963; Marsh, 1985). Moderate pH decline and rate of decline are optimal for tenderness because pH may exert an influence on proteolytic enzymes (Marsh et al., 1987; Carlin et al., 2006). However, in low pH, such as in PSE cases, proteolysis is arrested and tenderization occurs at a very slow rate (Barbut et al., 2008). Moreover, the calpains are susceptible to oxidation and subsequent

inactivation (Lametsch et al., 2008), specifically, m-calpain (Carlin et al., 2006).

Oxidation decreases the ability of calpastatin to inhibit mu-calpain, but calpain activity and inhibition of calpain by calpastatin varies on the environmental conditions like pH and inhibition of mu-calpain by calpastatin is actually diminished by oxidation (Carlin et al., 2006).

Smith et al.'s reports on the relationship between beef marbling scores (1984) and USDA Quality Grades (1987) both indicate greater marbling and consequential improved Quality Grade influence and positively impact palatability and consumer eating experience. Although there is not a magic number, generally speaking 3% i.m. fat on an uncooked basis is required for acceptable palatability (Savell and Cross, 1988). Furthermore, adequate aging allows time for postmortem proteolytic enzymes to breakdown myofibrillar proteins consequently improving tenderness (Koochmaraie and Geesink, 2006; Aberle et al., 2012). The pork industry has ample opportunity to enhance pork quality and consumer acceptance of commercial pork by improving i.m. content and subsequently tenderness.

Muscle Fiber Type

Muscles are heterogeneous mixtures of different types of myofibers (Klont et al., 1998; Aberle et al., 2012). Type of muscle, location and function determine fiber composition (Lefaucheur and Gerrard, 2000), which is an important factor that influences biochemical processes like energy metabolism in the living animal, postmortem conversion of muscle to meat and the resulting meat quality (Klont et al., 1998; Choe et al., 2008). Muscle fibers can be classified by their color (red or white), ATPase activity, preferred metabolism (oxidative or glycolytic), contraction speed (slow or fast), or

myosin isoform (Type I or II) (Klont et al., 1998; Lefaucher and Gerrard, 2000; Aberle et al., 2012). Although some properties overlap between fiber types, there are four myosin isoforms, Type I, IIa, IIx(d) and IIb, that can generally account for all fiber types.

Type I muscle fibers, also known as red fibers, contain high concentrations of Mb which is responsible for the red color observed in Type I fibers. Due to the increased Mb content, Type I fibers also have higher O₂ levels. Additionally, Type I fibers have a greater number of mitochondria and are more vascular. Therefore, Type I fibers are largely oxidative and noted for slower contraction rates, but can sustain contraction for longer periods of time. Type I fibers tend to have greater lipid content which may serve as an energy store that contributes to sustained contraction. Posture muscles, like the LM, contain large quantities of Type I fibers (Aberle et al., 2012).

White muscle fibers, or Type II, contain high levels of glycolytic enzymes and prefer aerobic or anaerobic glycolytic metabolism over oxidative. With a decreased capillary density, but extensive development of the sarcoplasmic reticulum and T-tubules, Type II fibers provide rapid, phasic contractions, however, they will fatigue very easily. Type IIa fibers are classified as fast twitch like Type IIb, but resemble red muscle fibers in other characteristics including color, Mb content, fiber diameter, mitochondria size and content as well as metabolism type (Aberle et al., 2012). When Type IIb fibers increase and Type I fibers decrease, faster glycolytic rates increase lactate production and cause a rapid pH decline and paler postmortem muscle with higher drip loss (Choe et al., 2008). Conversely, increased Type I fibers and fewer Type IIb would increase oxidative metabolism and lead to a higher pH and potentially improve pork quality.

Research has shown the number of fibers, the area of fibers, and the composition of fiber type in any given muscle impacts quality (Rehfeldt et al., 2008; Ryu et al., 2008; Choi et al., 2013). In turn, fiber type is modified by muscle type, species, breed, genetics, fetal and postnatal nutrition, ambient temperature, exercise and growth promoting agents (Lefaucheur and Gerrard, 2000). For some time, the swine industry has used genetic selection to improve growth rate and lean yield but, selection for growth and yield made concomitant changes in muscle characteristics including muscle fiber traits (Rehfeldt et al., 2008). In a comparison of domestic and wild type pigs, Ruusunen and Puolanne (2004) stated the LM, semimembranosus (SM), gluteus superficialis (GS) and infra spinam of wild pigs contained a greater amount of Type IIa and less Type IIb than domestic pigs and muscles in wild pigs had twice as much capillary density indicating a greater oxidative potential. Ruusunen and Puolanne (2004) suggest domestication and selection of heavier muscled, leaner animals shifted muscle fiber composition towards a glycolytic model and inadvertently selected for pigs with less desirable pork quality.

There are clear differences in muscle fiber type by breed (Ryu et al., 2008; Lee et al., 2012). Berkshires exhibited higher 45 min and 24 h pH, the lowest drip loss and L* values, implying the oxidative nature of LM in Berkshires (Ryu et al., 2008). So, not surprisingly, compared to Landrace, Yorkshires and crossbreds, Berkshires have increased number and percent area of Type I fibers and decreased Type IIb content (Ryu et al., 2008). In agreement, Lee et al. (2012) confirmed Berkshires have an increase in the cross section area (total area:total number of fibers), increased fiber density (number of fibers per mm²) and increased number (density x LEA) of Type 1 fibers. Although the percent area (area of fiber type:total cross section) was similar between Berkshires,

Durocs, Landrace and Yorkshires for Type I and IIb fibers, Berkshires and Durocs had increased percentages of Type IIa fibers (Lee et al., 2012). Although Landrace pigs demonstrated a larger LEA, Durocs and Berkshires had significantly higher NPPC color and marbling scores and according to trained panelists, Berkshires and Durocs are more tender and have stronger pork flavor (Lee et al., 2012). Multiple regression analysis revealed fresh pork quality traits and muscle fiber characteristics explain differences in meat quality, but quality characteristics explain a greater proportion of variability than muscle fiber type (Lee et al., 2012).

Differences in muscle fiber type can be attributed to differences in mature size that differ by breed, but can also depend on the age and weight of pigs (Lefaucher and Gerrard, 2000). In a study by Choi et al. (2013), heavy pigs sorted at 86 d of age had an increased fiber number and decreased fiber density as well as a larger LEA compared to light weight pigs, regardless of Type I fiber percentage in light pigs. Furthermore, pigs that had a higher percentage of Type I fibers had a larger LEA. Heavy pigs harboring less Type I fibers had a faster pH decline compared to heavy pigs with a greater amount of Type I fibers (Choi et al., 2013). The selection of pigs based on weight at the same age and Type I fiber content could help improve and control quality without reducing growth and carcass performance (Choi et al., 2013).

When pigs are born, muscles are predominantly made of Type I fibers that shift to Type IIa and IIb/x as development continues. However, increased activity shifts fibers from IIb to IIx to IIa to I and decreased activity will reverse the pathway (Lefaucher and Gerrard, 2000). Vestergaard et al. (2000) observed darker color and increased pigmentation in loose housed bulls fed roughage based diets compared to tie stall bulls

fed ad libitum concentrate. There was no difference in ultimate pH between the treatments and color differences were attributed to physical activity rather than feeding level and diet composition (Vestergaard et al., 2000). Loose housing increased the prevalence of slow contracting fibers, vascularization, and oxidative potential. Increased oxidative potential could decrease lactate production (Vestergaard et al., 2000) thereby improving meat quality.

Similar results are observed in pigs with access to large (Peterson et al., 1998) or outdoor pens (Gentry et al., 2004) where “spontaneous exercise” may occur. According to Peterson et al. (1998) compared to daily treadmill training or no added exercise spontaneous activity induced slow twitch fiber hypertrophy in five muscles and shifted the ratio of Type IIb to IIa in both LM and SM muscles. Interestingly, in the psoas major, an increase in fast twitch muscles was observed at the expense of slow twitch fibers (Peterson et al., 1998). Pigs born in outdoor environments harbored a greater amount of Type I than IIa in LM and pigs finished in outdoor settings had greater IIa than IIb in both the SM and LM (Gentry et al., 2004). Authors speculate outdoor rearing may delay or prevent the shift of IIa fibers to IIb and larger pens allowed for spontaneous exercise that may have shifted LM and SM fibers to IIa (Gentry et al., 2004). Yet, Gentry et al. (2002) found even though pigs in larger pens walked more, exercise did not alter muscle fiber distribution, similar to results seen by Lewis et al. (1989).

Lipids, Triglycerides, and Fatty Acids

Biological lipids are a chemically and functionally diverse group of compounds defined by their insolubility in water (Nelson and Cox, 2013). Lipids can be classified

according to function as storage lipids, structural lipids, lipid signals (hormones), enzyme cofactors, or pigments (Nelson and Cox, 2013). Structurally speaking, there are eight general classifications of lipids including fatty acids (FA), glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids and polyketides (Nelson and Cox, 2013). Fatty acids are hydrocarbon derivatives; they are composed of a carboxylic acid with hydrocarbon chains ranging from 4 to 36 carbons long. The carbon chains may be fully saturated, contain one or more double bonds, or contain rings and branches (Nelson and Cox, 2013).

The simplest lipid constructed from FA are triacylglycerols, also known as triglycerides, fats, or neutral fats. Triacylglycerols (TAG) are composed of a three-carbon glycerol backbone and three FA connected by an ester linkage. Triacylglycerols are non-polar, hydrophobic molecules with lower gravities than water allowing lipids to float on water (Nelson and Cox, 2013). Additionally, most pure lipids are colorless, odorless and flavorless (Allen et al., 1976). The properties of TAG and individual FA are pertinent to later conversations about FA extraction and saturation levels of pork fat.

Triacylglycerols are stored in specialized cells called adipocytes, or fat cells, and serve as a major energy reservoir (Nelson and Cox, 2013). Using TAG for fuel is incredibly advantageous when compared to polysaccharides like glycogen. Carbon atoms in FA tails are more reduced than sugars and oxidation of TAG yields a significant larger (more than twice as much) amount of energy, gram for gram, than oxidation of carbohydrates. Furthermore, TAG are hydrophobic and do not have the added water weight associated with stored polysaccharides (Nelson and Cox, 2013). Although carbohydrates like glucose are quick sources of readily available energy, TAG can store

enough energy for months compared to only hours or days available in glycogen (Nelson and Cox, 2013).

Triacylglycerols stored in adipocytes are part of a much larger biological system. Adipose tissue is a type of connective tissue that surrounds lipids like TAG that serve as heat insulators or energy stores (Allen et al., 1976). Fat is a collection of adipose cells suspended in a connective tissue matrix along with other cytoplasmic lipids and water (Allen et al., 1976). Although terms like “fat” and “lipid” are used interchangeably, fatty or adipose tissue contains lipids, however, lipids do not contain connective tissue, water and other enzymes present in fat. Lipids are simply a component of fat (Allen et al., 1976) and play active roles in the various biological activities of adipose tissue including energy storage, structural elements of membranes, insulation, organ padding, and releasing hormones in the endocrine system (Allen et al., 1976; Nelson and Cox, 2013).

Given the usefulness of TAG for energy, it is not surprising the primary purpose of adipose tissue is energy storage in the form of fat or oil (Nelson and Cox, 2013; Allen et al., 1976). Adipose tissue is profusely infiltrated with a capillary network and nervous system to control transport of lipids and provide constant energy despite intermittent intake of nutrients (Allen et al., 1976). However, it is the building blocks of TAG, FA, that are responsible for holding and releasing energy.

Fatty acid oxidation is the energy-yielding pathway for many organisms. The process of β -oxidation breaks down FA into acetyl-CoA, which passes through the Citric Acid Cycle to produce electron carriers, who then pass electrons through the Respiratory Electron Transport chain to finally yield energy in the form of ATP (Nelson and Cox, 2013). Fatty acids themselves can be obtained from the diet, de novo synthesis when

internal energy sources are abundant, or from TAG stored in adipocytes as fat droplets (Nelson and Cox, 2013). The quantity and individual FA composition are influenced by a number of factors including the stage of growth and development, nutrition, hormones, anatomical location, genetics, sex, exercise, and environmental stressors (Allen et al., 1976).

Lipid Biosynthesis

De novo synthesis of lipids or lipid biosynthesis refers to the building of lipids from simple molecules using inherent biological systems. Lipid synthesis begins with the formation of individual FA, which takes place in the cell cytosol. The rate-limiting step of de novo FA synthesis is the formation of malonyl-CoA from acetyl-CoA catalyzed by the enzyme acetyl-CoA carboxylase. Using the 3-carbon molecule malonyl-CoA to molecule to start the process and carry CO₂ is what makes the reaction to make FA chains thermodynamically favorable.

Fatty acid chains are formed by a repeating 4-step sequence catalyzed by the enzyme fatty acid synthase (FAS). Each pass through the cycle adds a pair of carbons to the chain from malonyl Co-A. Fatty acid synthase I (FAS I) is a large molecule specific to vertebrates and mammalian FAS I contains seven active sites in different domains for all the reactions that build FA chains. A single product, palmitic acid (16:0), leaves cycle each time with no production of intermediates (Nelson and Cox, 2013). In pigs, the primary products of de novo synthesis include 16:0, 16:1, 18:0, and 18:1. During times of rapid growth FA will contribute to cell membrane formation, but in times of ample food supply and maintenance, FA will be stored as TAG. Triglyceride biosynthesis and degradation is regulated by hormones including insulin that signals to store FA in TAG or

glucagon and epinephrine that signal the mobilization of FA stored in TAG (Nelson and Cox, 2013).

Several other enzymes are crucial to the formation of FA. Malic enzyme generates cytosolic NADPH, which is required in FA synthesis as an electron donor. Fatty acid elongation systems in smooth endoplasmic reticulum and mitochondria add acetyl groups to lengthen palmitic acid. Fatty acid desaturases are responsible for introducing double bonds to specific locations on long chain fatty acids and are regulated at the transcriptional level (Nakamura and Nara, 2004). Stearoyl-CoA desaturase (Δ^9 desaturase) catalyzes the synthesis of monounsaturated FA (MUFA), including oleic acid which has a double bond on the ninth carbon from the carboxyl end of the FA. Other enzymes including Δ^5 and Δ^6 desaturases are required to make polyunsaturated FA (PUFA) (Nakamura and Nara, 2004). Although mammals can readily make double bonds in the Δ^9 position of fatty acids, they do not have the desaturase enzymes to make double bonds between C-10 and the methyl end of FA chains. Hence, mammals are unable to make their own linoleic and linolenic acids, which are precursors for other long chain FA, but plant species can. Therefore, mammals must obtain these “essential” FA by consuming them in plant products. Linoleic acid is the precursor for omega 6 FA like, arachidonic acid (20:4n6), which in turn is the precursor for eicosanoids. Linolenic acid is the precursor for omega 3 FA like eicosapentaenoic acid (EPA, 20:5n3) and docosahexaenoic (DHA, 22:6n3) (Nelson and Cox, 2013).

Sources Of Fatty Acids

The three main sources of fatty acids are the diet, stored fat in lipid droplets and fats synthesized in one organ for export to another (Nelson and Cox, 2013). Although

fatty acids in the form of triglycerides are very efficient energy stores, tapping the energy is difficult due to the hydrophobic nature of fatty acids. Triglycerides provided in the diet must first be emulsified by bile salts that act like a detergent to break down large triglycerides molecules and convert them to micelles. In micelles, the fatty acid tails are oriented towards the center creating a non-polar core surrounded by the salts, which drastically increases the accessibility of lipases to triglycerides. Pancreatic lipases degrades the triglycerides into monoglycerides, diglycerides, free fatty acids and glycerol which easily diffuse through intestinal mucosa where they are reconverted back into triglycerides and packaged as lipoprotein aggregates called chylomicrons (Nelson and Cox, 2013).

Alipoprotein C-II binds lipids in blood and help chylomicrons travel to adipose and muscle tissues. Lipoprotein lipase hydrolyzes TAG to FA and glycerol, which are then taken up by cells in target tissues. Fatty acids can be oxidized immediately in muscle for energy or in a satisfied or fed state, excess FA are reesterified into triglycerides in adipose cells. Stored fatty acids may be used later in an energy deficient state, such as during exercise, when the body uses up glycogen stores. As aforementioned, the hormones epinephrine and glucagon can signal TAG out of storage and initiate transport of TAG to muscles for oxidation. Free FA released from TAG in adipose tissue will bind to serum albumin in blood with transports FA to target tissues to be oxidized (Nelson and Cox, 2013).

The process of FA catabolism is called β -oxidation and takes place in the mitochondria of cells in the target tissue. As the reverse process of synthesis, β -oxidation successively removed two carbon unites in the form of acetyl-CoA which is then

oxidized to CO₂ in the Citric Acid Cycle. The NADH and FADH₂ from the first two steps of β -oxidation donates electrons to the mitochondrial respiratory chain, which phosphorylates ADP to ATP, thus the energy released by FA oxidation is conserved as ATP (Nelson and Cox, 2013).

Adipose Tissue in Meat Animals

Fat is located everywhere in major “white” fat locations including subcutaneous fat (SC), i.m., and mesentery fat (Allen et al., 1976), but fat is also in visceral organs, the digestive tract, skeleton, head, feet and tail (Leat, 1983). Despite the biological importance and relevance of some of these depots, they are not considered integral parts of the carcass and have little economic value to the meats industry (Lizardo et al., 2002). The nutritional quality and value, chemical and physical properties as well as the processing potential of meat and fat are related to the FA composition of TAG (Lizardo et al., 2002).

Historically, it has been widely accepted that the FA profiles of pigs and their fat depots largely reflects dietary fat content (Ellis and Isbell, 1926; Wood et al., 2008), but FA profile of tissues may not totally reflect dietary fat due to the availability of FA at the site of incorporation (Carrol, 1965). Hence the FA of fat depots relies on de novo synthesis and endogenous enzyme activity as well (Wiseman and Agunbiade, 1998). Furthermore, shifts in body composition can be driven by nutritional plane and genetic predisposition (Wiegand et al., 2011) and the degree of fatness is directly related to the degree of saturation in tissues (Martin et al., 1972).

Fat quality

Fat quality is measured by composition, hardness, color, impurities and oxidative stability, which is largely defined by the FA profile (Azain, 2001). The physical characteristics of fat are directly related to the physical properties of FA, which, is largely determined by the length and degree of unsaturation in the hydrocarbon chain (Maw et al., 2003; Nelson and Cox, 2013). Melting points of FA are particularly relevant soft fat conversations pertaining to pork quality and determine the hardness of fat, often identified by the iodine value (IV).

Differences in melting points and resulting hardness are attributed to different degrees of “packing” of FA molecules which is dependent on the length and unsaturation of the FA tails (Nelson and Cox, 2013). As the most stable form, saturated FA chains are able to freely rotate around the carbon bonds and achieve a fully extended carbon chain, which encounters the least steric hindrance (Nelson and Cox, 2013). Saturated FA are then able to pack tightly together and align in nearly crystalline arrays (Nelson and Cox, 2013), resulting in very firm, hard fat, which is more desirable. However, when a double bond is present between two carbon atoms, this creates a kink in the carbon chain and inhibits FA from packing together (Nelson and Cox, 2013). Less thermal energy is needed to disorder poorly packed unsaturated FA and thus, unsaturated FA have much lower melting points than saturated FA with the same length carbon chain (Nelson and Cox, 2013). Consequently, unsaturated fats are soft and oily making them unsightly, difficult to cut, and more susceptible to oxidative rancidity (Maw et al., 2003).

In regards to color, aliphatic FA in their purest form are colorless, but natural fats and oils from plants and animals have associated pigments (O’Conner, 1960). Maw et

al. (2003) found linoleic acid explained 30% variation ($P < 0.05$) of yellow backfat, however, since linoleic is an essential FA, all levels in tissues are from the diet and the associated pigments in the diet are responsible for the color. Fatty acids are not good predictors of red color (Maw et al., 2003), but fat becomes increasingly ($P < 0.001$) translucent as levels of myristic, palmitic and stearic acid decrease and linoleic and linolenic (Maw et al., 2003).

The presence of moisture, impurities (i.e. hair, bone, soil, plastic) and unsaponifiables (i.e. cholesterol, vitamins, waxes) is called the MIU index and is a more informative index of quality (Azain, 2001). A final factor in determining fat quality is the ability to resist oxidative breakdown. Fat with high concentrations of PUFA tend to have low stability due to the high probability of oxidative breakdown of the double bonds (Azain, 2001). According to Wood et al., (2003), PUFA have more double bonds and are more easily oxidized, producing undesirable flavors, tastes, and colors, ultimately reducing product shelf life. Consequently, dietary ingredients like added fat and DDGS are very important in determining the quality of the end product because pork tissues tends to greatly reflect the FA profile of the diet (Wood et al., 2003). However, different anatomical sites have distinct responses to dietary fat due to varying lipogenic activity in the respective fat depot (Leszczynski et al., 1992) or in response to a specific dietary fat (Allee et al., 1972).

Metabolic Modifiers

Metabolic modifiers are technologies developed to increase efficiency and cutability of market animals to enhance profitability of animal production. Metabolic

modifiers are compounds fed, injected or implanted in animals to improve live performance, efficiency, carcass yield, and meat quality (Dikeman, 2007) by increasing protein and muscle accretion while simultaneously decreasing fat deposition (Dunshea et al., 2005). Of the available metabolic modifiers used today, most are implemented in feeding programs to assist growth performance and carcass composition with relatively few compounds being fed to improve meat quality (Dikeman, 2007). In fact, the focus on improving live performance and yield via selection and use of metabolic modifiers may actually have been detrimental to meat quality because of heavy selection to decrease carcass fat. Nonetheless, extensive research on metabolic modifiers has been recorded in recent years defining the role of various compounds in multiple livestock species. Examples of metabolic modifiers include but are not limited to anabolic steroids, somatotropin, beta agonists, vitamins and vitamin like compounds fed at supra-nutritional levels, and designer lipids including, conjugate linoleic acid (CLA).

Ractopamine Hydrochloride

Ractopamine hydrochloride (RAC) is a β -adrenergic agonist approved by the FDA in 1999 for use in finishing swine 69 to 109 kg. In May of 2006, the FDA approved a new label that permitted the use of 5 to 10 ppm RAC in the last 20.4 to 40.8 kg prior to slaughter (Carr et al., 2009). Ractopamine hydrochloride (RAC) has been marketed under the trade names Paylean[®] for pigs and Optaflexx[®] for cattle by Elanco Animal Health. In a review published by Apple et al. (2007a), the authors concluded RAC inclusion in the diet increased growth rate and growth efficiency with no detrimental effects on fresh pork color, firmness, water holding capacity or intramuscular fat.

Ractopamine and other β -agonists indirectly lead to decreased lipogenesis and increased lipolysis (Mersmann, 1998). Notably, the effectiveness of RAC and magnitude of change in composition depends on the dose, feeding duration, inclusion level, species and nutrient concentration in the diet (Mersmann, 1998; Moody et al., 2000). Over time, RAC response also diminishes from down regulation or desensitization of β_1 -adrenergic receptors (Moody et al., 2000).

The most obvious observation after RAC administration is increased muscle mass and decreased carcass fattness (Mersmann, 1998). Ractopamine increases lean yield by repartitioning nutrients away from adipose tissue accretion to lean tissue deposition (Moody et al., 2000; Carr et al., 2005a; Apple et al., 2007a). Specifically, feeding RAC in pigs decreased leaf fat, 10th rib fat, last rib fat, and belly firmness, but increased dressing percentage and loin eye area (Carr et al., 2005b). Although cimaterol is effective at decreasing i.m. fat, increasing shear force and drip loss, generally speaking, RAC does not change i.m. fat content (Dunshea et al., 2005). In the same study by Carr et al. (2005b), feeding RAC reduced tenderness and increased linoleic acid concentrations in subcutaneous fat (Carr et al., 2005b), but Carr et al. (2005a) found RAC had little effect on pork juiciness and flavor. Ractopamine can also successfully be fed to pigs that by today's standards are considered heavy without negatively affecting desired carcass characteristics given the current market demands (Carr et al., 2009).

According to Carr et al. (2005b) the type of cereal grain (corn, wheat, or barely) fed with or without RAC does not change carcass, meat, or fat quality attributes as long as diets contain equal Lys content. Adeola et al. (1990) reported a significant interaction between RAC and protein level and implied both growth and carcass leanness are

improved at higher protein levels (17%), but at 13% crude protein, only carcass leanness is improved. Apple et al. (2004b) showed pigs fed RAC on a high energy diet (3.48 Mcal of ME/kg) were fatter compared to low energy diets (3.30 Mcal of ME/kg), but carcass cutability improved when the Lys:ME ratio increased from 1.7 to 3.1 g/Mcal. If the Lys:ME ratio plays a role in carcass leanness, Wiegand et al. (2011) suggest feeding high energy level diets do not make RAC more efficient as a repartitioning agent. Additionally, Apple et al. (2007b) found RAC exacerbated effects of feeding soybean oil compared to beef tallow on pork fat unsaturation levels.

Conjugated Linoleic Acid

Conjugated linoleic acid (CLA), coined a “designer lipid,” has been a popular research topic because of potential health benefits that result from increasing CLA in the diet. Conjugated linoleic acid is mixture of positional and geometric isomers of linoleic acid with conjugated double bonds at positions 7,9-, 8,10-, 9,11-, 10,12-, or 11,13- (Dunshea et al., 2005; Schmid et al., 2006). The main isomers found in meat are cis-9, trans-11 and trans-10, cis-12, both of which have human benefits (Dikeman, 2007). The primary source of CLA is from Δ^9 desaturase activity on trans vaccenic acid during rumen biohydrogenation (Griinari et al., 2000), so expectedly food sources originating from ruminant animals like cattle and sheep have markedly higher levels of CLA compared to monogastrics (Schmid et al., 2006). Conjugate linoleic acid content can vary by individual tissues or animals, breed or genetics, season, production practices and perhaps most importantly, CLA content largely varies by diet (Schmid et al., 2006). In monogastrics such as pigs, only the direct supplementation of CLA or the precursor, trans-vaccenic acid, are effective in elevating CLA in tissues (Schmid et al., 2006).

Like other metabolic modifiers, CLA successfully improves live animal performance and repartitions nutrients to concurrently increase lean mass and decrease fat content in pork carcasses. Several studies (Thiel-Cooper et al., 2001; Wiegand et al., 2001, 2002b; Weber et al., 2006; Dugan et al., 1997) have shown supplementing CLA enhances pig performance with overwhelming evidence CLA significantly improves G:F. However, other studies have failed to detect differences in performance (White et al., 2009), BW (Barnes et al., 2012), ADG (Thiel-Cooper et al., 2001; Wiegand et al., 2001, 2002b), or feed intake (Wiegand et al., 2001, 2002b; Barnes et al., 2012) with CLA supplementation. Performance improved linearly as CLA increased from 0 to 1%, but optimum reduction of carcass fat was achieved at 0.5% or less of CLA (Thiel-Cooper, et al., 2001). According to Weber et al. (2006), CLA, added fat, and RAC work in an additive manner to enhance pig growth and carcass quality.

Dietary inclusion of up to 1.0% CLA repeatedly decreased backfat (Dugan et al., 1997; Thiel-Cooper et al., 2001; Wiegand et al., 2001, 2002b; Barnes et al., 2012) but the impact on muscle accretion is less clear. Dietary CLA up to 1.0% has reduced LM (Thiel-Cooper et al., 2001; Barnes et al., 2012), increased LM (Wiegand et al., 2002b; Weber et al., 2006), or failed to change LM size at all (Wiegand et al., 2001; White et al., 2009). Interestingly, despite the fact CLA reduced backfat, studies have also shown CLA significantly (Dugan et al., 1999; Wiegand et al., 2002b; Joo et al., 2002) and tended (Barnes et al., 2012) to increase i.m. fat. In a meta-analysis, carcass backfat is decreased on average by 6% (1.2 mm), but i.m. fat and marbling increase by 7% and 11%, respectively (Dunshea et al., 2005). This is a crucial finding as reduced backfat is often associated with decreased marbling or i.m. fat content and subsequent negative eating

experiences. Increasing marbling may be advantageous as greater marbling is associated with increased palatability, specifically tenderness and juiciness (Brewer et al., 2001; Wood et al., 2004).

Many factors can affect the ability of CLA to reduce backfat. According to Azain (2003), added dietary fat (added fat tends to reduce CLA effect), initial backfat depth of pigs (pigs with > 23 mm of backfat have more advantageous response compared to pigs with < 20 mm) and gender (barrows are fatter and typically have greater response) can influence the ability of CLA to reduce SC fat. Dunshea et al. (2005) surmised a relationship between the backfat of pigs fed CLA and control pigs. According to this relationship, the magnitude of reduction in backfat from CLA is related to the initial fat depth and CLA may provide greater efficacy in pigs with a propensity for obesity (Dunshea et al., 2005). Smith et al. (2002) suggest CLA depresses the activity of steroyl coenzyme-A and may be the responsible mechanism for reducing adiposity in pigs fed CLA.

Dietary CLA also shifts the FA profile creating a more saturated profile throughout the entire carcass (Weber et al., 2006; White et al., 2009; Barnes et al., 2012). The shift towards SFA has numerous downstream effects throughout fabricating and further processing of pork. Unsurprisingly, dietary CLA not only increases SFA, but increases belly firmness (Thiel-Cooper et al., 2001; Weber et al., 2006). Joo et al. (2002) showed dietary CLA improved WHC in addition to decreasing linoleic acid and increasing CLA in the FA profile of i.m. fat. According to Dunshea et al. (2005), CLA does not change ultimate pH of the loin, but drip loss is improved by 5%. Dietary CLA does not appear to improve pork color (Wiegand et al., 2002b; White et al., 2009; Barnes

et al., 2012). With greater proportions of SFA, lipid oxidation is reduced (Wiegand et al., 2002b) and authors further suggest color stability improved with changing the FA profile and inhibited lipid oxidation (Joo et al., 2002). Furthermore, 0.6% CLA for 10 d minimized the negative effects of 20% DDGS inclusion, but not 40% DDGS but increasing the SFA content in carcass fat (White et al., 2009).

In addition to increasing SFA, dietary CLA is incorporated into SC and i.m fat depots (Ostrowska et al., 2003) offering an opportunity to increase CLA in meat products for human consumption. Ostrowska et al. (2003) revealed an obvious selectivity for the intake and incorporation of CLA isomers in fat, particularly cis/trans. The 9-cis, 11-trans isomer was incorporated most efficiently into backfat (46.4%) while i.m. preferentially absorbed cis-11, trans-13 (0.74%) (Ostrowska et al., 2003). Currently dairy and beef products are the main sources of CLA (2 to 24 mg/g of fat), but there is potential to substantially increase CLA content, particularly cis/trans isomers in pork (Dunshea et al., 2005). Literature suggests the cis-9, trans-11 isomer has multiple health benefits, however, majority of studies have been conducted using rodent models and have not been thoroughly tested on humans. The literature indicates CLA possesses anticarcinogenic and antiatherogenic benefits, body composition maintenance and weight loss properties, and the ability to regulate the immune system preventing cachexia and hypertensive reactions (Whigham et al., 2000). However, the use of meat as a CLA vessel is quite impractical due to the massive quantity required to intake recommended CLA levels.

Pasture feeding and vegetable oils increase CLA content in meat, largely by providing PUFA in the diet (Schmid et al., 2006). In particular, linseed oil can increase CLA content in i.m. fat (Enser et al., 1999). Cattle finished on pasture have increased

CLA content in cooked meat compared to cattle finished in a feedlot, albeit the CLA content was different between muscles (Lorenzen et al., 2007). Although diet can be used to up regulated the CLA content of meat even throughout cooking, feeding CLA to genetically lean pigs has limited use in the current US industry (Dikeman, 2007) as selection pressures for meat type hogs may have nullified any practical applications for CLA in pigs, unless pork quality is rewarded at the plant level.

Ionophores

Ionophores were originally developed as anticoccidial feed additives for poultry produced by strains of *Streptomyces* and included molecules such as monensin, lasalocid, salinomycin, and narasin (Bergen and Bates, 1984). Ionophores improve production by increasing the efficiency of energy metabolism, improving nitrogen metabolism and impede development of feedlot disorders including bloat and acidosis (Bergen and Bates, 1984). Ionophores act favorable in cattle by fermenting dietary carbohydrates into propionate, a more energy efficient compound with reduced methane production compared to acetic and butyric acid production (Wolin, 1981).

Skycis[®] (Narasin) is high performance ionophore labeled as a swine premix for the increased rate of gain when fed during the least 4 weeks of the finishing phase. The active ingredient in Skycis[®] is narasin (NAR), an ionophore used in food production. Skycis[®] is available over the counter and is intended for oral administration to growing/finishing swine (FDA, 2012). The FDA (2012) has approved two doses including 16.6 to 27.2 g/ton to increase rate of weight gain and 18.1 to 27.2 g/ton to increase rate of weight gain as well as improve feed efficiency. Skycis has a zero day

withdraw and is also accepted in worldwide markets including Japan. Although NAR is technically an antibiotic, there are currently no human applications for ionophores, so the use of ionophores in animal production is a favorable alternative when considering antibiotic resistance concerns. Not to mention, ionophores are exempt from veterinary feed directives (VFDs) implemented January 1, 2017.

Normally bacteria are impermeable to anions and use inherent ion gradients to transport nutrients across membranes (Callaway et al., 2003). However, ionophores are anions capable of disrupting normal bacterial cell membrane physiology (Bergen and Bates, 1984) by transporting sodium, potassium and hydrogen protons across cell membranes thereby inverting the alkaline environment inside the cell wreaking havoc on cell functions and cell homeostasis (Russell, 1987). Ionophores are highly lipophilic compounds that rapidly dissolve into bacterial cell membranes taking with them ions and shielding the ionic charges allowing translocation of ions across the bacterial membrane (Pressman, 1976). The potassium gradient, which transports potassium ions out and hydrogen ions into cells, is much stronger than the sodium gradient, which transports sodium ions into cells and hydrogen ions out. The influx of hydrogen protons reverses the pH gradient building an acidic interior environment. The bacterium responds by activating the ATPase pump to remove hydrogen protons from inside the cell (Booth, 1985). In addition to ATPase, other pumps to remove sodium and potassium are activated uncoupling ATP hydrolysis from cell division and fermentation functions, which in turn depletes cellular ATP and leads to cell death (Russell 1987; Russell and Strobel, 1989).

Interestingly, ionophores are selective in targeting gram-positive cells (Russell and Strobel, 1989). The peptidoglycan layer of gram-positive bacteria is porous and allows small molecules to pass through and reach the cytoplasmic membrane where the ionophore can dissolve, whereas gram-negative bacteria are surrounded by a lipopolysaccharide layer (Russell et al., 1987; Calloway et al., 2003). Bacteria sensitive to ionophores produce lactate, butyrate, and formate, while bacteria resistant to ionophores produce propionate and succinate as end fermentation products (Chen and Wolin; 1979; Nagaraja and Taylor, 1987). Additionally, ionophore sensitive bacteria degrade amino acids in the small intestine (Dierick et al., 1986). Increasing the proportion of propionate producing bacteria is favorable because propionate is efficiently fermented and is a gluconeogenic precursor (Russell and Strobel, 1989). Molnar et al. (1987) argue ionophores may also be effective at preventing dysentery in swine because narasin also inhibits lactic acid producing bacteria that can abnormally lower pH and cause gastrointestinal distress.

Nitrogen (N), as well known, is crucial to life as a component of amino acids, the building blocks of protein, as well as to reproduction of life itself as an element in the nucleic acids of DNA. With cell homeostasis altered, the proportion of effective gram-positive bacteria is reduced and protein utilization improves (Dierick et al., 1986). In work from Wuethrich et al. (1998), fecal N decreased ($P < 0.05$), apparent N digestibility increased ($P < 0.05$), and the ratio of propionic to acetic and butyric acid increased ($P < 0.05$) in NAR fed barrows. Growth performance is thus improved by an altered digestion, absorption and retention of N in the gastrointestinal tract of pigs (Dierick et al., 1986).

Recent work has repeatedly shown NAR improves efficiency, but also has the opportunity to change carcass composition (Arentson et al., 2014; Arentson and Chewning, 2015, 2016; Arkfeld et al., 2015). Combined pooled data in a meta-analysis of four studies by Arentson et al. (2014) found pigs fed Skycis[®] had higher ADG, G:F ratio and HCW compared to control pigs. Growing pigs fed 15 or 30 ppm NAR greater ($P < 0.01$) ADG and ADFI compared to control, but only 30 ppm increased ($P < 0.05$) G:F ratio compared to control pigs (Arentson et al., 2016). According to Arkfeld et al. (2015), Skycis[®] dietary inclusion has been shown to increase feed efficiency of barrows and gilts when fed during the last 85 days of feeding without negatively impacting carcass composition. Although only barrows increased ADG ($P < 0.01$) by 2% when fed NAR, regardless of sex, pooled results from three marketing cuts found pigs fed NAR had improved ($P = 0.03$) G:F, but also tended ($P = 0.08$) to have reduced LM depth compared to controls (Arkfeld et al., 2015). However, in two studies by Arentson and Chewning (2015, 2016), NAR improved gain efficiency, but also increased HCW, loin depth and carcass yield. Additionally, NAR may be helpful combatting poor performance from heat stress. Knauer et al. (2015) found supplementing NAR during the summer significantly ($P < 0.05$) improves growth performance (ADG, G:F) and carcass characteristics (HCW, yield) in finishing pigs. In 12 to 25 kg pigs, NAR alone improves growth performance compared to control and *Saccharomyces cerevisiae* fermentation products (YFP), but when NAR is fed with YFP and tribasic copper chloride, results were additive and resulted in the greatest improvement of ADG, ADGI and G:F (Früge et al., 2016).

Altering Pig Performance, Carcass Composition, and Pork Quality

Current hog producers recently persevered through record setting production costs in 2013, which resulted from dramatic increases in ethanol production, consequential skyrocketing corn prices, and an untimely drought in 2012. Along with the return of normal weather patterns, increased global corn production and reduced corn prices, average yearly production costs have been driven down and the pork market has returned to “normal” conditions (NBP, 2014), albeit current market prices are not exactly producing large margins.

Accounting for more than two-thirds of production costs, feed is a major contributor to production costs (Ewan, 2001; NPB, 2014). The cost of raising pigs has risen from \$120/head in the early 1990s to more than \$180/head in 2013 (NPB, 2014). Naturally, producers strive to lower production costs by adding concentrated sources of energy and fat to diets by adapting fat replacement strategies and using alternative and sometimes unconventional, but less expensive plant seed oils and saturated animal fats. This is particularly true when facing adverse conditions like high corn prices, drought or unfavorable market conditions.

Pigs are quite flexible in their ability to consume and even thrive on a range of fat sources with varied saturation levels (Wood et al., 2008). However, changing dietary fat in pigs alters the FA profiles of tissues to directly reflect the FA profile of the diet (Wood et al., 2008; Apple et al., 2009a, b, c). Other research has focused on the appropriate energy content in swine diets in combination with added dietary fat (Bee et al., 2002), the appropriate lysine:ME (Apple et al., 2004b) and with metabolic modifiers (Wiegand et al., 2011). However, due to pigs’ malleability, the end consequences on live growth

performance, carcass composition, and lean meat and fat quality must be evaluated and taken into consideration when making feeding recommendations and choices, in addition to the price and availability of feedstuffs.

Dietary Fat

A pig's appetite and feed intake may limit growth potential, so increasing dietary energy with added fats or oils may increase energy intake and positively influence the growth curve (Lizardo et al., 2002). Studies have evaluated the effect of poultry fat (Apple et al., 2009a, b, c), choice white grease (Kellner et al., 2014), yellow grease (Stephenson et al., 2016), palm oil and palm kernel oil (Teye et al., 2006), beef tallow (De La Llata, et al., 2001), canola/rapeseed oil (Wiseman and Agunbiade, 1998), coconut oil (Allee et al., 1972), and glycerol (Lee et al., 2013) amongst other added fat sources on pig performance, carcass composition, and carcass quality. Some research has indicated adding fat at some point in the finishing phase improves feed efficiency compared to no added fat (Weber et al., 2006; Benz et al., 2011; Stephenson et al., 2016). However, others have shown the type of added fat or oil does not influence growth performance (Teye et al., 2006; Kellner et al., 2014) or carcass characteristics (Stephenson et al., 2016), nor does adding fat at all change parameters compared to not adding fat (Averette Gatlin et al., 2002; Apple et al., 2009a).

Growth Performance. Lee et al. (2013) fed added fat from corn germ, tallow, palm oil and glycerol with 30% DDGS for 88 d to find no difference in BW or ADG, but in the late finishing phase, pigs fed palm oil had lower ($P < 0.05$) ADFI compared to control diets and overall, palm oil diets tended ($P = 0.06$) to reduce ADFI. Phase feeding beef tallow and yellow grease over five time periods produced similar ($P > 0.06$)

performance results, but ADG and ADFI also increased ($P < 0.05$) in quadratic fashion as time on beef tallow increased (Browne et al., 2013). Over 84 d, added dietary fat improved G:F compared to diets with no added fat (Stephenson et al., 2016). Although Kellner et al. (2014) found the type of added fat did not impact ($P = 0.53$) ADG, ADFI, or G:F, when comparing amount of added fat, pigs who received 6% fat compared to 3% fat for 55 d did improve ADG ($P = 0.04$) and G:F ($P = 0.006$), but ADFI was not different. Although added fat at 6% CWG improved feed efficiency of pigs in all phases of commercial production, this only occurred when pigs were in an energy dependent state of growth (De la Llata et al., 2001).

Alternatively, as the level of dietary fat increased, intake and feed:gain decreased linearly ($P < 0.05$) (Averette Gatlin, et al., 2002). Apple et al. (2009a) also identified ADG, ADFI and G:F did not change ($P > 0.35$) regardless of added fat (5%), fat source (beef tallow, poultry fat or soybean oil), or slaughter weight (28.1 to 113.6 kg). Different oil types including palm oil, palm kernel oil and soybean oil did not change ADG, feed conversion ratio or final BW (Teye et al., 2006). Interestingly, as soy oil was replaced by beef tallow, growth rate was unaffected, but both ADFI and feed:gain increased linearly ($P < 0.0001$), suggesting animal fat is not as easily digested by pigs as plant oils are (Averette Gatlin, et al., 2002).

Clearly, caloric density, digestibility, energy state and even palatability of fat sources play a small role in live animal performance. Not to mention, source and nutritional value of added fat is undoubtedly variable, collectively represented by research with confounding results and feeding recommendations. Oils appear to have a higher caloric density and are easily digestible compared to animal fats as seen by less

consumption, but equal or improved gain (Averette Gatlin, et al., 2002). The energy state of the pig should be considered as well. Stephenson et al., (2016) improved ADG in the early finishing phase, indicating an energy dependent stage in the animals' lifetime. By adding dietary fat, more energy is provided in the feed which allows for increased protein accretion compared to pigs with no added fat at early growth and finishing stages (Stephenson et al., 2016) when more energy is requirement for growth rather than simply maintaining the body. Lastly, regardless of how energy dense a fat sources is, if the product is not palatable according to pigs senses, feeding attempts to improve efficiency and cut costs will be fruitless.

Carcass composition. A number of studies have shown added fat in the diet does not change carcass slaughter weight (Averette Gatlin et al., 2002), HCW (Apple et al., 2009a), back fat (Lee et al., 2013) or LM area (Stephenson et al., 2016). Feeding 5% tallow, poultry fat or soybean did not change ($P > 0.29$) primal yields, nor did fat source change percent bone ($P = 0.55$), muscle ($P = 0.213$), or fat ($P = 0.50$) (Apple et al., 2009c). Despite when added CWG or corn oil were fed at 2, 4 and 6% for an extended period of time (134 d), Kellner et al. (2016) failed to see significant ($P > 0.33$) changes in carcass composition and increasing fat content in the diet only revealed a tendency ($P = 0.07$) to increase carcass yield. Phase feeding beef tallow and yellow grease over five time periods produced no dietary differences between slaughter weight, HCW, 10th rib fat or lean muscle yields indicating feeding duration (37 to 103 d) of fat had no effect ($P > 0.23$) on carcass characteristics (Browne et al., 2013).

However, including beef tallow in the last three of five feeding phases increased ($P = 0.03$) LM depth compared to yellow grease and as feeding duration of yellow grease

increased, fat free lean yield linearly increased ($P = 0.02$) compared to beef tallow (Browne et al., 2013). When 4% beef tallow, soybean oil, or a 50:50 combination was fed for three different durations during the finishing phase (0 to 42 d; 42 to 84 d; 0 to 84 d), there were no changes in carcass characteristics (HCW, LEA, percent yield) between fat sources, however, adding dietary fat over the entire feeding period increased ($P = 0.03$) backfat and tended ($P = 0.08$) to reduce fat free lean index compared pigs fed diets with no added fat (Stephenson et al., 2016).

Carcass quality. Teye et al. (2006) indicated feeding oils with varying saturation levels did not impact LM color, pH, drip loss or shear force or thiobarbituric acid-reacting substances (TBARS). Blended fat containing animal and plant sources did not influence drip loss, pH, or loin and belly color (Averette Gatlin et al., 2002). These data are further confirmed by Weber et al. (2006) who found neither addition of fat or fat source (CWG or beef tallow) influenced ($P > 0.14$) carcass pH, subjective color and marbling, drip loss, or percent IMF and moisture.

Belly quality. Belly quality is an increasingly important factor in the pork world due to the incredible demand for bacon. Quality defects, such as thin and soft bellies, identified by packers are detrimental to profitability in the processing sector because of reduced yields and inferior final products (Person, et al., 2005; Apple, 2010). Although “thick” bellies are optimal for processors to increase yields, consumers often discriminate against thick bellies for being too fatty and prefer bacon from “average” to “thin” bellies (Person et al., 2005). An ideal belly would be average thickness to meet both demands, but also be fairly saturated to avoid soft fat issues including oxidation potential (Azain,

2001) and consequential reduced shelf life (Wood et al., 2003), reduced yield and “No. 1” slice production (Apple, 2010) and reduced export potential (Carr et al., 2005b).

Hypothetically, feeding saturated added fat should increase SFA in tissues and help improve belly softness. Lee et al. (2013) reported dietary added fat and fat source did not change belly length, width or weight and amongst five different diets. Belly flop distance was the greatest in bellies from pigs fed the control diets, but flop distance was not different between fat sources (Lee et al., 2013). Yet, increasing the concentration of dietary fat tended to increase belly weight and saturated fat in the form of tallow yield firmer ($P < 0.05$) bellies that tended to be thicker ($P < 0.10$) (Kellner et al., 2014). Despite variability ($P < 0.05$) in belly flexibility based on dietary treatment including CWG and high oil corn compared to a control diet with no added fat, there were no significant differences ($P > 0.05$) in smokehouse yield and sliceability between bellies (Rentfrow et al., 2003).

Dried Distillers Grains

Although distillers grains co-products have been fed to livestock for the better part of 50 years (Shurson et al., 2012), the growth of the ethanol industry in the US has greatly influenced the availability and incorporation of ethanol co-products in swine and other livestock industries as feedstuffs (Stein and Shurson, 2009). Co-products from modern ethanol plants are attractive to producers when corn prices are high, but also because co-products are highly concentrated sources of protein and energy and contain relatively high levels of digestible phosphorus and amino acids (Shurson et al., 2003).

The processing regimen as well as the type and quality of the starting product determines the nutritional quality and composition of the co-product. Dry grind plants

and wet mills are the two main types of ethanol production facilities (Shurson et al., 2012). Both processes process maize and mix it with yeast to convert the starch to ethanol and carbon dioxide (Shurson et al., 2012). The ethanol is distilled off and the residual co-products left behind are centrifuged and dried before being released as feedstuffs for livestock (Shurson et al., 2012). After distillation, the remaining grain fraction is centrifuged. The liquid portion is evaporated and the remaining fraction is known as the condensed distillers solubles (Shurson et al., 2012). The coarse solids are also dried and simply called dried distillers grains (DDG). Majority of DDG are recombined with at least 75% of the solubles, aptly named dried distillers grains with solubles (DDGS) (Shurson et al., 2012), and can be included in pig diets up to a 30% inclusion rate before live growth performance is hindered (Stein and Shurson, 2009).

High protein DDG (HPDDG) may be used in pig growing and finishing diets to replace soybean meal and maize germ (Shurson et al., 2012). The biorefining technology known as BFrac dehulls and degerms corn prior to fermentation resulting in two products, corn germ and dried distillers grains that are not mixed back with solubles (Widmer et al., 2007). This end product is referred to as HPDDG and contains more protein, but less fat, ADF, NDF and phosphorus (P), than DDGS (Widmer et al., 2007). According to Xu et al. (2010), DDGS contains 6 to 12% oil or fat and from a review of data, Shurson et al. (2012) state DDGS contains 10.2% crude fat, while HPDDG has 3.7% crude fat. On the other hand, DDGS is approximately 27.5% protein, 25.3% NDF, 9.9% ADF and HPDDG contains 41.1% protein and only 16.4% NDF and 8.7% ADF (Shurson et al., 2012).

Growth performance. There is substantial disagreement on the net effect of high inclusion levels of DDGS in swine grower-finisher diets. Nutrient composition and digestibility varies among DDGS sources (Shurson et al., 2012) and according to Stein and Shurson (2009), discrepancies in pig performance could be attributed to differences in the quality DDGS. Although, DDGS may be incorporated into swine diets at any phase of production, in grower-finisher rations, acceptable growth can be accomplished with inclusion levels up to 30% (Stein and Shurson, 2009). Dahlen et al. (2011) reported inclusion of 20% DDGS in the diet did not negatively impact initial or final BW, ADG or ADFI and gain efficiency was similar compared to a control corn and soybean meal diet. Similarly, Shircliff et al. (2015), Widmar et al. (2008), Drescher et al. (2008), and Duttlinger et al. (2008) all concur swine diets with up to 20% DDGS do not alter growth performance.

However, there are studies that concluded high inclusion levels of DDGS negatively impact growth parameters during the growing and finishing phase. Although Cromwell et al. (2011) found ADFI and G:F were not affected, ADG was linearly reduced as pigs were fed increasing levels of DDGS (0 to 45%). For pigs fed increasing levels of DDGS (0 to 30%) for 56 d, there was a linear tendency for ADG and ADFI to decrease and ADG and ADFI decreased linearly in pigs fed DDGS up to 20% for 78 d (Linneen et al., 2008). Whitney et al. (2006) reported pigs fed 20 or 30% DDGS had reduced ADG compared to 0 or 10% DDGS and G:F was decreased in pigs fed 30% DDGS compared to 0, 10 or 20% DDGS. Hinson et al. (2007) also described inclusion of DDGS at 10 or 20% in the diet reduced ADG, ADFI and final BW.

Carcass composition. Several studies have noted inclusion of DDGS in the diet has no impact on carcass composition traits including HCW (Xu et al., 2010; Dahlen et al., 2011; McClelland et al., 2012), LEA (Pompeu et al., 2013) or 10th rib backfat (McClelland et al., 2012, Lee et al., 2013). In contrast, according to Whitney et al. (2006) as the level of DDGS increases in the diet, HCW and LM area are linearly decreased. Furthermore, Rojo et al. (2016) found carcass yield and LM depth both decreased linearly as HPDDG or DDGS was increased 30% in pig diets. Widmer et al. (2008) reported including HPDDG did not change HCW or 10th rib backfat, but LM area and depth decreased linearly with HPDDG addition to the diets. Nutrient concentration and digestibility may vary between sources of DDGS (Stein and Shurson, 2009) and may be a contributing factor related to discrepancies reported related to DDGS and carcass composition.

Carcass quality. Whitney et al. (2006) reported visual firmness, marbling, WHC, drip loss, cooking loss and shear force were not impacted by including DDGS in the diet. However, inclusion of DDGS has been shown to decrease marbling and LM firmness (Xu et al., 2010). Most importantly, feeding DDGS has also been widely associated with soft bellies (Widmer et al., 2008; Cromwell et al., 2011; Dahlen et al., 2011), which can easily be explained by the amount of PUFA in tissues from feeding DDGS. Belly flop decreased linearly ($P < 0.05$) from 23.9 to 17.6 and 22.6 to 19.0 cm for pigs fed 20% HPDDG and 30% DDGS, respectively (Rojo et al., 2016).

Feeding DDGS may be a contributing factor to color differences in pork. Egg yolk color intensity increased linearly as DDGS in laying hen diets increased from 0-25%, largely attributed to the highly available xanthophylls in DDGS, which is a major

yellow carotenoid (Masa'deh et al., 2011). Moreover, increased yellow color has been associated with increased linoleic and α -linolenic fatty acid content (Maw et al., 2003). Since these fatty acids cannot be synthesized in mammals and presence in tissues is directly from dietary intake and fatty acids themselves are colorless, meaning, the yellow color must be attributed to associated carotenoids in the feed (Maw et al., 2003).

Although LM L*, a*, and b* were not significantly different in pigs fed DDGS and added fat, the L* value of backfat from pigs fed a control diet with no DDGS or added fat was numerically greater, indicating a lighter color, than diets containing DDGS (Lee et al., 2013). According to Xu et al., 2010, increasing DDGS content in the diet did not change belly fat Minolta or Japanese color scores, but did decrease LM a* and b*. Further, increasing DDGS to 20 to 30% resulted in darker backfat and yellowness increased linearly with increasing DDGS (Xu et al., 2010). This may indicate a higher inclusion percentage or more time on feed could change fat color. From a retail perspective, various forms of added fat and DDGS can be included in the diet without negatively impacting pork color or creating discernable differences for the consumer.

Dietary Energy

Growth performance. Growth performance is significantly changed by energy content of pig diets (Bee et al., 2002; Apple et al., 2004b; Suarez-Belloch et al., 2013). Pigs fed low energy diets have been shown to increase feed intake, reduce average daily gain, and reduce feed utilization (Bee et al., 2002). However, according to Suarez-Belloch et al. (2012), ADFI decreases ($P < 0.05$) with increasing dietary energy and feed conversion tended ($P < 0.10$) increase. Apple et al. (2004b) concluded energy density did not change ADG or ADFI, but pigs fed greater metabolizable energy (ME) were more

efficient ($P < 0.02$) as seen when increasing the Lys: ME ratio linearly increased ($P < 0.01$) ADG and G:F.

Carcass composition. High energy diets can increase HCW, backfat and the percentage of loin, ham and shoulder in the carcass (Bee et al., 2002). Yet, Suarez-Belloch et al. (2013) reported carcass weight, ham size and yield were not different as energy increased in the diet. Although Apple et al. (2004b) did not see a change in muscle as metabolizable energy increased either, studies have reported as energy increases, back fat also increases (Apple et al., 2004b; Suarez-Belloch et al., 2013) and ultimately overall yield changed (Apple et al., 2004b). Not surprisingly, as energy in the diet decreases, backfat follows suit (Apple et al., 2004b; Hinson et al., 2011). However, as the ME:Lys ratio increased, backfat decreased, LM depth and area increased, ham yield increased resulting in the fat free lean yield to increase (Apple et al., 2004b).

Logically, increased lean growth in pigs from inclusion of ractopamine hydrochloride in the diet requires increased dietary protein to sustain protein synthesis and accretion (Adeola et al., 1992; Dunshea et al., 1993). Evidence from Apple et al. (2004b) shows increasing the Lys:ME ratio increases muscle, but decreases fat supports this theory that ractopamine fed pigs require more lysine to meet their maximum potential for muscle accretion because as Lys:ME increases, cutability also increases.

Carcass quality. Dietary energy density does not appear to change carcass quality (Apple et al., 2004b; De la Llata et al., 2001; Suarez-Belloch et al., 2013) including color, percent i.m. fat, shear force or cook loss (Suarez-Belloch et al., 2013). The Lys:ME ratio did not change LM pH, drip loss, LM color or LM firmness, but as the Lys:ME ratio increased subjective marbling and LM lipid content decreased ($P < 0.01$)

and shear force linearly increased ($P < 0.01$) (Apple et al., 2004b). Moreover, L^* quadratically decreased ($P < 0.03$) as the Lys:Me ratio increased (Apple et al., 2004b). Diets low in protein and lysine content will limit protein synthesis and increase the energy available for fat deposition, which corresponds to increased fat, particularly IMF, which develops late in the growth curve (Teye et al., 2006). Although the level of lysine will affect the ractopamine response, increasing the lysine content of diets to achieve improved carcass cutability may be done at the expense of carcass quality in terms of color, marbling, and tenderness (Apple et al., 2004b).

Altering Lipid Profiles In Pigs

Despite studies revealing changing dietary fat and energy have little to no consequence on overall pig performance and carcass composition (Averette Gatlin et al., 2002; Apple et al., 2009a; Kellner et al., 2014; Stephenson et al., 2016), the changes in fatty acids profiles in response to dietary modifications is widely accepted (Wood et al., 2003; Wood et al., 2008). Generally, diets containing high concentrations of PUFA, especially long chain FA like the essentials linoleic and linolenic, are largely reflected in tissues. This idea was long ago established by Ellis and Isbell (1926) and subsequently confirmed by many others (Koch et al., 1968; Irie and Sakimoto, 1992; Wiseman and Agunbiade 1998; Averette Gatlin et al., 2002; Apple et al., 2009a, b, c). However, the fatty acid profile of fat may not totally reflect dietary fat because incorporation of FA into tissues relies on FA available at the site of incorporation (Carroll, 1965). Fat depots are dynamic and play a vital role in energy metabolism (Martin et al., 1972). Thus, FA profiles depend not only on the diet, but also on de novo synthesis and interconversions

by endogenous enzymes (Wiseman and Agunbiade, 1998). Furthermore, FA proportions and the resulting degree of saturation are also a function of breeding, background, sex and body composition (Martin et al., 1972).

As pigs fatten, SFA and MUFA levels increase, but PUFA decreases (Wood et al., 1984, 1986). This change can be attributed to increased desaturation of 16:0 and 18:0 or preferential incorporation of dietary 18:1 or perhaps a combination of both concepts (Apple et al., 2009c). Wiseman and Agunbiade (1998) importantly noted 60 to 70% of changes in the FA composition occur within just 2 weeks of changing the diet. Long chain FA including EPA and DHA can be incorporated into SC backfat within 4 weeks with the most significant changes occurring in the first 2 weeks (Irie and Sakimoto, 1992). Specifically, the concentration of particular FA in SC fat depends on the initial concentration, rate of change, capacity for depletion or deposition in a specified back fat layer or other fat depot, and if the FA is essential and derived strictly from a dietary source or synthesized endogenously (Wiseman and Agunbiade, 1998).

Lipogenesis and Enzymatic Activity

Lipogenesis and lipolysis are tightly regulated mechanisms that largely depend on the challenges facing individual pigs on a daily basis. Not surprisingly, as pigs reach maturity, total fat content increases and the proportions of FA change (Wood et al., 2008). Wood et al. (1986) reported as backfat increased from 8 mm to 18 mm, 18:0 increased and 18:2n6 decreased and fatter carcasses were firmer and exhibited less fat separation. According to Wood et al. (2008), as pigs accumulate fat mass and protein accretion slows, de novo synthesis has an increasing role in the synthesis of SFA and MUFA and direct incorporation of 18:2n6 from the diet declines. Moreover, de novo

synthesis is reduced in genetically lean pigs compared to pigs predisposed for fat deposition (Steele et al., 1974). Thus, as pigs fatten, their FA profiles become more saturated due to increased production and incorporation of SFA including 16:0 and reduced deposition of PUFA, as indicated by increased fat firmness.

Remarkably, incorporation of 18:2n6 early in growth and development actually inhibits MUFA generation from stearoyl-CoA carboxylase in SC fat (Kouba and Mourot, 1999). Contrastingly, Pascual et al. (2006) proposed as growth continues, 18:2n6 may in fact enhance de novo synthesis. Data provided by Allee et al. (1972) stated regardless of saturation level, 10% added fat was effective in inhibiting FA synthesis. Both malic enzyme and citrate enzyme activity were significantly reduced ($P < 0.05$) by dietary fat including corn oil, lard, coconut oil and tallow (Allee et al., 1972). Although FA profiles were not provided, added fat did significantly increase backfat (Allee et al., 1972) and presumably saturation level and firmness.

Kouba et al. (2003) fed linseed oil as an unsaturated fat source for 60 d to pigs and found a 40% reduction ($P < 0.001$) in Δ^9 desaturase activity as well as a decrease ($P < 0.01$) in oleic acid, but only in fat tissue and not muscle. As time on feed increased, PUFA decreased ($P < 0.01$) and SFA increased ($P < 0.01$) in both muscle and fat tissues (Kouba et al., 2003). Low protein diets limit muscle accretion and the energy reserved for muscle synthesis is diverted to fat synthesis and Teye et al. (2006) found feeding a low protein diet increased 18:1 and decreased n-3 and n-6 FA. According to Doran et al. (2006), low protein diets increase the expression of Δ^9 desaturase in LM and a linear relationship exists between levels of 18:1 and expression of Δ^9 desaturase in LM, but not fat.

Notably, research has shown increasing the concentration of certain FA is done at the expense of others, specifically, as linoleic acid increases, oleic acid decreases (Koch et al., 1968; Wiseman and Agunbiade 1998). Dietary linoleic acid has an inhibitory effect on Δ^9 desaturase (Kouba and Mourot, 1998; Kouba et al., 2003) and MUFA reduction can be attributed to reduced Δ^9 desaturase activity (Kouba et al., 2003). Although Allee et al. (1971) found as dietary fat content increased (1, 4, 7, or 13%), FA synthesis, malic enzyme and citrate cleavage enzyme activity decreased. In disagreement, Kouba et al. (2003) found incorporation of dietary fat did not change the activities of other lipogenesis enzymes including acetyl Co-A carboxylase, malic enzyme or glucose-6-phosphate dehydrogenase in muscle or fat regardless of time on feed.

Although we can clearly see differences in enzymatic activity, few pieces of evidence provide the mechanism of how lipolysis can be regulated with dietary alterations. When FA synthesis decreases, a simultaneous decrease in hepatic lipogenic fatty acid synthase and acetyl-CoA carboxylase proteins occurs (Toussant et al., 1981) and decreases in fatty acid synthase is associated with a decrease in fatty acid synthase mRNA (Clarke et al., 1990). Clarke et al. (1990) believed dietary PUFA uniquely regulate gene expression of lipogenic enzymes by using an entity from long chain polygenic FA as a coactivator in transcription thereby reducing the abundance of mRNA for lipogenic proteins. According to Allee et al. (1971), increasing dietary fat increases free FA in plasma suggesting free FA have a role in control of FA synthesis. Although studies mark PUFA as potent inhibitors of FA synthesis in rodents (Clarke et al., 1977; Toussant et al., 1981), added fat regardless of saturation level and 18:2n6 content was seemingly effective at inhibiting FA synthesis in pigs (Allee et al., 1972).

Dietary Fat

Control diets with no added fat are often the most saturated (Apple et al., 2009b; Kellner et al., 2014; Lee et al., 2015). Beef tallow is more saturated than poultry fat which is more saturated than SBO, which can be seen in the FA profiles of BF (Apple et al., 2009) and composite carcass samples (Apple et al., 2009c). Soybean oil is very unsaturated and markedly ($P < 0.05$) reduces SFA, MUFA but increase PUFA in BF (Apple et al., 2009) and composite carcass samples (Apple et al., 2009c). Beef tallow is 1.4 times more saturated than yellow grease while yellow grease contains five times more PUFA which caused pigs fed beef tallow to have greater proportions of SFA and MUFA and lower PUFA in s. c. fat compared to pigs fed yellow grease (Browne et al., 2013). At the same inclusion level, beef tallow is the most saturated fat source while corn oil is the most unsaturated and CWG falls in the middle (Kellner et al., 2014). Unsurprisingly, as the unsaturation increased in the dietary fat source from tallow to CWG to oil, the IV of belly, jowl and backfat also increased ($P < 0.05$) (Kellner et al., 2014). Notably, although decreasing PUFA fat sources lowered 18:2 and IV, Averette Gatlin et al. (2002) found the magnitude of improvement was less than expected demonstrating animal fat may have a lower digestibility compared to plant oils.

Dried Distillers Grains with Solubles

Regardless of inclusion level or depot, DDGS increases PUFA and raises IV of pork fat (Whitney et al., 2006; Xu et al., 2010; Shircliff et al., 2015). A cooperative study reported while SFA and MUFA levels decreased in subcutaneous fat, PUFA levels increased with increasing (15%, 30% and 45%) levels DDGS in the diet (Cromwell et al., 2011). Xu et al. (2010) demonstrated SFA decreased in belly and subcutaneous fat with

increased (10%, 20% and 30%) levels of DDGS, but the content of PUFA increased. In a study by McClelland et al. (2012), when DDGS increased up to 45% in the diet, SFA and MUFA levels in subcutaneous and belly fat decreased, while PUFA increased. Iodine value has been well documented to increase with DDGS inclusion largely attributed to the increase in PUFA. IV of s. c., belly and loin fat depots increased linearly from 58.4 to 72.4, 61.4 to 72.3 and 54.8 to 57.7, respectively, when 0 to 30% DDGS was included in the diet (Xu et al., 2010). This is further supported by data from Whitney et al. (2006), Benz et al. (2010), and McClelland et al. (2012) who all reported IV increased with DDGS inclusion up to 45% in multiple fat depots including the belly, jowl, IMF and 2 s. c. fat layers.

Dietary Energy

Lipogenesis is the first limiting pathway in storage of body fat and is also the most tightly regulated by energy intake (Vernon et al., 1999). When pigs are in a positive energy balance, they do not rely on stored fat for energy (Enser, 1984). However, during fasting induced energy depletion, mobilization of fatty acids from stored fat is selective and leads to profound remodeling of adipose tissue composition (Raclot et al., 2005). Furthermore, reduced feed intake reduces TAG synthesis in fat (Mersmann et al., 1981). So, unsurprisingly, the extent in which tissue FA are altered from dietary fat depends on the energy level in the diet (Bee et al., 2002).

Dietary fat source did not change the activity of lipogenic enzymes, but diets were equal in digestible energy, so this is not surprising (Bee et al., 2002) despite other research claiming PUFA intake decreases lipid content in adipose tissue by reducing lipogenic activity (Kouba and Mourot, 1999). Low energy diets did, however, decrease

($P < 0.01$) carcass fat deposition and reduce ($P < 0.01$) activity of lipogenic enzymes (Bee et al., 2002). Regardless of fat source, low energy diets increased PUFA and decrease SFA and MUFA, but the desaturation index (18:1:18:0) increased in low energy diets (Bee et al., 2002). Kouba and Mouroto (1998) found dietary PUFA, particularly, omega-6 FA, impair the activity Δ^9 desaturase. Key enzymes including glucose-6-dehydrogenase, malic enzyme, and fatty acid synthase are also reduced when dietary energy is restricted, which is consistent with decreased fat deposition (Bee et al., 2002) because leaner pigs are known to have reduced de novo synthesis (Wood et al., 1985; Correa et al., 2008). Although lipogenesis decreases in low energy diets from impaired enzyme activity, if the SFA substrate increases from the diet, the innate metabolic response might be to increase Δ^9 desaturase activity to compensate and Bee et al. (2002) further speculated desaturation of fat tissue in low energy diets increased because of upregulated Δ^9 desaturase activity.

Fat Depots

Carcass composite samples. Using unsaturated dietary fat does increase PUFA in entire carcass composite samples, which ultimately has economic ramifications for soft pork (Apple et al., 2009c). Initially, pigs start the growing and finishing stages of production with the same concentration of 16:0 and regardless of dietary treatment, the concentration of 18:0 increases as pigs reach maturity (Apple et al., 2009c). According to Wood et al. (1989), as the amount of carcass fat increases, MUFA concentration increases and PUFA concentration decreases. Moreover, MUFA concentrations increase with increasing body weight (Apple et al., 2009c). According to Eder et al. (2001), as pigs' body weight increases, they show a preferential absorption and incorporation of

18:1 at the expense of 16:0 and 18:0. Apple et al. (2009c) considered increased desaturation of 18:0 into 18:1 is occurring alone or perhaps concurrently with the preferential incorporation of 18:1, causing PUFA concentration to decrease and MUFA concentration to increase (Apple et al., 2009c).

Subcutaneous fat. Apple et al. (2009b) describes in detail 5% added fat not only changes the fatty acid profiles of SC fat, but individual fat layers are distinguishable as well with the inner layer of backfat being more saturated than the outer layer. Both layers particularly reflect dietary levels of n-3 and n-6 FA families, especially linoleic and linolenic acids (Bee et al., 2002). The IV of the outer layer of SC is greater compared to the inner layer indicating the outer layer is more unsaturated (Apple et al., 2009b). Furthermore, IV differed ($P < 0.01$) by dietary fat treatment in both layers (Apple et al., 2009b). Wiseman and Agunbiade (1998) also found dietary rapeseed, soybean oil, and tallow are reflected in the FA pattern of backfat and concur the inner layer of shoulder fat was more saturated than the outer layer. The inner fat layer is the most dynamic with the greatest lipogenic activity (Leymaster and Mersmann, 1991; Warnants et al., 1999), and consequently has a high proportion of SFA and MUFA from de novo synthesis (Warnants et al., 1999). Malmforms et al. (1978) suggested the temperature gradient across SC fat forces SFA to deposit on the inner layer to a warmer environment and unsaturated FA to deposit in a cooler out layer.

Interestingly, Lee et al. (2013) fed 30% DDGS and added fat, but did not find treatment differences in FA composition or IV of backfat. This could be attributed to feeding fat with high concentrations of DDGS and the high PUFA concentrations in DDGS negating or overpowering the differences from the fat sources. The FA

composition of SC fat does reflect the dietary source that is fed in the last 2 to 3 finishing phases as shown by backfat, however, the deposition rate of specific fatty acids were dependent on the length of time pigs were fed a specific fat source (Browne et al., 2013). In general, adding fat to the diet will decrease SFA, MUFA, and increase PUFA and IV, but feeding duration and fat source may interact and affect fatty acid composition in backfat (Stephenson et al., 2016).

Intramuscular fat. Averette Gatlin et al. (2003) failed to see differences in LM IV, despite increasing the IV of the dietary fat source from 20 to 100. Apple et al. (2009a) reported as slaughter weight increases, SFA and MUFA generally increase and PUFA decrease in LM. Although Apple et al. (2009a) showed the FA profile of LM is susceptible to dietary fat changes and can be altered rather quickly, Wiegand et al. (2011) reported regardless of energy content in the diet, FA composition and IV was not affected in i.m. Wiegand et al. (2011) further suggested finishing pigs likely deposit fat in the jowl and over the front shoulder prior to the loin and belly region. Leszczynski et al. (1992) reported dietary fat has a greater affect on belly fat than loin muscle indicating fat depots have different sensitivities to direct incorporation of linoleic and linolenic acids. Thus, if i.m. is the last fat to accumulate near the end of a food animal's growth curve and live span, it is likely this depot is undergoing greater lipogenic activity at the time of slaughter and halted prior to reaching biological maturity. Dietary fat is not a likely contributor to i.m. FA profiles and increased SFA and MUFA content in i.m. is a direct result of de novo synthesis.

Belly fat. Not surprisingly, several studies have shown inclusion of DDGS in the diet increases belly IV (Whitney et al., 2006; Benz et al., 2010; McClelland et al., 2012)

regardless of IV determination method (Shircliff et al., 2015). Variations of added fat will change IV depending on the saturation of the source with CWG increasing IV compared to beef tallow, however, when fat was added at 6%, IV did not differ regardless of fat sources or fat depot (Kellner et al., 2014). This may be indicative of a threshold for added fat concentration to inhibit de novo synthesis and dilute differences based on dietary intake (Kellner et al., 2014). Despite differences in MUFA concentrations, when 30% DDGS was fed with beef tallow, palm oil or glycerol, belly IV did not change (Lee et al., 2013).

Several researches speculate high IV and soft bellies that result from PUFA in diets from sources like DDGS, may be prevented by including saturated fats like beef tallow or palm kernel oil in pig diets (Averette Gatlin et al., 2002; Lizardo et al., 2002; Teye et al., 2006). Feeding a supplemental fat source for 6 to 8 weeks was sufficient time to alter the FA profile in pigs and improve pork processing potential (Averette Gatlin et al., 2002). Browne et al. (2013) suggest feeding saturated fat in the latter production phases may also prevent fat quality issues that develop when PUFA are fed in the late finishing phase like DDGS. However, when Salyer et al. (2012) combined CWG and wheat middlings in pig diets, CWG mitigated the effect of wheat middlings on live performance, but carcass yield and IV were not overcome. Lee et al. (2013) were also unable to improve fat quality with different fat sources and recommended using a higher concentration of dietary fat, but also caution the economic viability of increasing fat content from particular sources. Interestingly, Pompeu et al. (2013) reported feeding CLA diminished the negative effects of DDGS by increasing SFA and decreasing IV in

both belly and jowl fat indicating fat supplementation in the form of CLA can positively effect FA profiles.

Jowl fat. Typically, jowl fat will have a higher IV compared to other fat depots and this is attributed to fat deposition patterns and differing physiological maturity of fat depots (Wiegand et al., 2011; Shircliff et al., 2015). Because correlations are weak between fat depots when pigs are slaughtered at the same weight, Wiegand et al. (2011) suggest pigs slaughtered at the same weight may have different physiological maturities or be at different points on their respective growth curves. Not only are pigs different individually, but the fat depots within the same animal have different physiological maturities as well (Wiegand et al., 2011) with jowl fat being more mature compared to belly fat (Shircliff et al., 2015).

As pigs accumulate fat mass and protein accretion slows, the influence of de novo synthesis becomes increasingly prevalent (Wood et al., 2008). Iodine value was greatest in jowl fat compared to belly and SC fat when 3% tallow, CWG or corn oil was fed, but not when 6% added fat was used indicating high fat levels may inhibit de novo synthesis and dilute any possible differences based on dietary intake (Kellner et al., 2014). Furthermore, the jowl is one of the first locations on a pig to accumulate fat mass (Hammond, 1932) and the jowl region is subject to a longer turnover rate of adipose tissue compared to belly and loin fat and is not easily impacted by changes in fat source or feeding duration (Stephenson et al. 2016). Therefore, PUFA fed early in the growing phases stay in fat long term and are not easily diluted by saturated fat in finishing diets or de novo synthesis.

Fat For Thought

Science and producers must consider the extraneous circumstances that occur beyond a pig's "normal" conditions and daily routine, if that is normal can reasonably be defined. Factors including dietary fat, dietary energy, ambient temperatures, and disease state can all influence both lipolysis and lipogenesis. Illness and hormone fluctuations can easily impact feed intake, feed utilization and the body's endogenous response to FA metabolism. Ultimately, the biological system is quite complex and FA metabolism is dependent on several exogenous as well as endogenous factors. The nature and extent of fatty tissue deposits may be vitally related to the animals' ability to compete or be buffered against environmental stress (Martin et al., 1972). Physiologically speaking, pigs will naturally strive to maintain homeostasis and achieve normal status at all costs complicating our ability to understand mechanisms.

Perhaps the most influential factor to consider is the evolution of the current market pig. The slaughter weight of the average pig today is 136 to 145 kg (300 to 320 pounds), which is considerably larger than just a decade ago (Carr et al., 2009). Much of the data from pigs provided in the early literature base were harvested at a much lower weight than pigs harvested in more recent years. Arguably pigs, as well as other livestock, have morphed into different biological types that perform differently than animals in the 20th Century. The fact that pigs have been slaughtered at different points in the growth curve may account for variability in the literature and the inability to truly understand the mechanisms behind regulating lipogenesis with diet from the current data.

Rational

Opportunities to export high quality pork products to trade partners like Japan are economically favorable outlets for US pork producers. Additionally, domestic consumers rely heavily on the fresh pork appearance, especially color and marbling, to make both initial and repeat purchasing decisions. Continuing to improve pork quality is not only an obtainable goal, but one that will ensure the continuation of happy, satisfied customers.

In the day and age of having technology at our finger tips, consumers are more (ill) informed than ever and aware of common livestock production practices. Marketing schemes including non-GMO, no added hormones, antibiotic free, all natural, local, organic, etc., have exploded in the retail scene. Consequently, producers, packers and retailers are looking for new and novel ways to raise, fabricate, process, sell and market safe, wholesome food products that meet consumer expectations. Live pig performance is gravely affected by management, diet, and handling at slaughter. Taking into account consumer concerns in terms of dietary supplements, feed additives and animal welfare two studies were performed to elucidate novel ways to improve pork quality, specifically by altering the lipid profile and increasing i.m. fat.

CHAPTER 2

ADDED FAT FED WITH 30% DRIED DISTILLERS GRAINS WITH SOLUBLES TO PIGS ALTERS FATTY ACID COMPOSITION IN FOUR FAT DEPOTS BUT DOES NOT CHANGE CARCASS COMPOSITION OR QUALITY

ABSTRACT

High inclusion levels of unsaturated dietary fat sources negatively impacts pork fat quality, but supplementing swine diets with saturated animal fats may help improve the fatty acid profile of pork carcasses. Forty individually reared barrows (100 kg \pm 3 kg, PIC C23 x 337) were randomly assigned to one of five treatments containing dried distillers grains with solubles and 3% added fat to determine the resulting carcass characteristics and fresh pork quality. The treatments included (1) a positive control containing a corn and soybean meal base with 3% choice white grease (PCON), (2) 30% high protein dried distillers grain and 3% choice white grease (HPDDG), or 30% DDGS with (3) no added fat (DDGS), (4) 3% choice white grease (DDGS + CWG), or (5) 3% butter oil (DDGS + BO). Postmortem carcasses characteristics and quality attributes were not different between diets ($P > 0.08$) except the semimembranosus muscle from pigs fed HPDDGS had a more basic ultimate pH ($P = 0.01$) compared to all other treatments. Diet changed ($P < 0.01$) SFA, MUFA and PUFA in subcutaneous, intramuscular, belly and jowl fat depots. Including 30% DDGS in the diet decreased MUFA and increased PUFA concentrations in backfat, belly and jowl fat. Although

added fat in the diet altered fatty acid composition in multiple fat depots, adding additional saturated fat to the diet did not significantly improve other measures of carcass quality.

Key words: pigs, DDGS, choice white grease, butter oil, pork quality

INTRODUCTION

The fatty acid profile of pork directly reflect the dietary fat source fed to the live animal (Wood et al., 2008). Feed is a major cost to producers accounting for two-thirds of production expenses (Ewan, 2001; Hollis and Curtis, 2001), and naturally producers prefer lowering operating costs by adopting fat replacement strategies in the form of oils. Dried distillers grains with solubles (DDGS) is a popular feed choice for pigs at all stages of production (Stein and Shurson, 2009), but increasing levels of DDGS inevitably increases the unsaturation level in pork fat (Cromwell et al., 2011; McClelland et al., 2012), which can negatively influence further processing characteristics and fresh pork export potential (Carr et al., 2005b; Apple 2010).

Added fat in growing-finishing swine diets typically improves gain and feed efficiency (Azain, 2001). Animal fats are more saturated than plant oils and can potentially be fed in combination with DDGS to improve fat quality without negatively impacting (Apple et al., 2009a; Browne et al., 2013) and possibly improving (Stephenson et al., 2016) growth efficiency. Previous studies suggests fat in the form of conjugated linoleic acid (CLA) supplementation can positively effect fatty acid profiles diminishing the negative effects of DDGS by increasing SFA content and decreasing iodine value (IV) in belly and jowl fat (Pompeu et al., 2013). The objective of this study was to determine the influence of added fat on muscle and fat quality of pigs fed diets containing 30% DDGS and 3% added fat in the form of choice white grease or butter oil.

MATERIALS AND METHODS

The University Animal Care and Use Committee approved animal care and experimental protocols prior to the initiation of this experiment.

Experimental Design

Forty barrows (100 kg \pm 3, PIC C23 x 337) were individually reared and randomly assigned to one of five dietary treatments including 1) a positive control containing a corn and soybean meal base with 3% choice white grease (PCON), 2) 30% high protein dried distillers grain and 3% choice white grease (HPDDG), or 30% dried distillers grain with 3) no added fat (DDGS), 4) 3% choice white grease (DDGS + CWG), or 5) 3% butter oil (DDGS + BO). Experimental diets were formulated to meet or exceed NRC nutritional requirements (NRC, 2012) and balanced on the equivalent ME:Lys ratio. All pigs were provided ad libitum access to feed and water until time of slaughter. On the last day of the finishing phase when pigs were 125 \pm 3 kg, feed was removed 15 h ante mortem and the animals were transported 5 km to the University of Missouri Red Meats Abattoir and Meat Processing Facility. Pigs were humanely slaughtered following standard US pork industry practices and USDA-FSIS inspection criteria.

Data Collection

Carcass composition and muscle quality. Hot carcass weight was recorded immediately following slaughter and inspection processes and prior to entering the chill

box. At 45 min postmortem, initial pH was measured between the 10th and 11th ribs in the longissimus muscle (LM) and posterior to the aitch bone in the semimembranosus muscle (SM) using a portable spear-type pH meter (Meat Probes, Inc., Topeka, KS, USA). Initial temperature of the LM and SM was measured in the geographic center of the muscle using a handheld digital thermometer (Traceable Digital Thermometer, Thermo Fisher Scientific). After a 24 h chill, carcasses were ribbed between the 10th and 11th ribs and allowed to bloom for 30 min. Loin muscle area was calculated using a hand held grid (Iowa State University, Ames, IA). Fat thickness was measured perpendicular to the skin at the 10th rib and last rib including skin in the measurement. Also, at 24 h postmortem, pH and temperature of the LM at the 10th rib and SM posterior to the aitch bone was measured. Objective color values for L*, a*, and b* were measured on the 10th rib cut surface of the LM using a Minolta Chroma Meter CR-410 (Konica Minolta, Sensing, Inc., Japan). Objective fat color was also recorded 48 h postmortem on a skinless loin (IMPS #410; NAMI, 2014) along the dorsal side posterior to the 11th rib. A portion of the LM from the 11th rib region on each carcass was reserved for moisture and fat percentage and placed in Whirlpac bags, labeled and stored at -20°C until further analysis. A second chop from the 12th region on each carcass was also reserved for fatty acid analysis, placed in Whirlpac bags, labeled and stored at -20°C until further analysis.

Moisture and intramuscular fat percentage. As previously described by Keeton et al. (2003) and Dow et al. (2011), moisture and fat percentages were determined using CEM SMART Trac rapid analysis system. Initially, two CEM sample pads were dried and 3.75 to 4.5 g of LM sample were smeared across one pad. The second pad was placed over the sample sandwiching the sample between both pads. Moisture percentage

was determined by using the weight and CEM Moisture/Solids Analyzer. The dried sample was wrapped in TRAC paper and packed into the bottom of a CEM TRAC tube. The tube was placed into the CEM Rapid Fat Analyzer. Intramuscular fat percentages were determined on a dry basis using nuclear magnetic resonance and converted to a wet basis.

Drip loss. Samples for drip loss were collected 24 h postmortem on a chilled carcass. Approximately 10 g of a single cube of LM was weighed and the exact weight was recorded. The meat cube was hung from a fishhook attached to string that ran through a plastic vesicle. A Whirl-pack bag was placed around the plastic vesicle to catch the drip. The plastic vesicle allowed the sample to hang freely without touching the surface of the bag. The samples were allowed to hang for 24 h at 4°C. The samples were removed from the hooks taking careful consideration to entirely remove the sample and not leave any meat on the hook and the sample was weighed again. The initial and final weight was used to calculate a percent loss according to the following equation: $((\text{initial weight} - \text{final weight})/\text{initial weight}) * 100$.

Water holding capacity. Water holding capacity (WHC) was measured using the Carver Press Method according to Kauffman et al. (1986). Again, samples of LM were removed 24 h postmortem from a chilled carcass. A 0.3 g sample was weighed and placed on a piece of filter paper. The sample and filter paper were placed between two plastic plates and placed in the Carver press under 5,000 psi of pressure for 3 min. Two rings were revealed after removing the filter paper from the press. The inner ring represented the area of the pressed sample and the outer ring was the area of the expressed moisture. The rings were traced using a planimeter (PLANIX 5, Tamaya

Technics, Inc., Shinagawa-Ku, Tokyo, Japan) and the area of the inner and outer rings were determined and used to calculate water holding capacity by dividing the outside area by the inside area. Water holding capacity is thus expressed as the ratio of water expelled per unit of meat.

Belly fabrication and flexibility measurements. Fresh bellies (IMPS #408; NAMI, 2014) were removed from the carcass at 48 h postmortem and used to evaluate horizontal and vertical flexibility of the belly post sparerib and associated cartilage removal. Skin-on bellies were weighed and used to measure flexibility according to a modified procedure described by Rentfrow et al. (2003). In accordance with Rentfrow et al. (2003), fresh bellies were suspended over a polyvinyl chloride pipe mounted on a board marked with a 2.54 cm grid matrix. Horizontal and vertical flex were determined by measuring the distance the belly ends traveled on the grid matrix when oriented either horizontally or vertically over the pipe. However, contrary to Rentfrow et al. (2003), horizontal flex was measured as the distance between belly ends when the belly was draped over the pipe and vertical flexibility as the distance from the belly ends to the zero line. (The zero line was defined as the top line on the grid matrix in which a belly is perfectly rigid and has zero flexibility.) Thus, a low horizontal score and high vertical score indicates a soft, more flexible belly while a high horizontal score and low vertical score indicates a firmer, more rigid belly.

Fat tissue collection. Fat tissue samples were collected after a 24 h chill from four fat depots including the jowl, belly, subcutaneous back fat (SC), and intramuscular fat (i.m.). Approximately 5 x 5 cm square samples were collected at the cranial tip of the jowl region at the site of head removal, a region on the evisceration midline caudal to the

sternum but anterior to mammary tissue, and three-quarters the distance around the LM at the 10th rib. Subcutaneous back fat was separated into inner and outer layers prior to fatty acid analysis. Fat samples were placed in Whirlpac bags, labeled and stored at -20°C until fatty acid profile determination analysis.

Fatty acid profiles. Fatty acid profiles were determined as previously described by Wiegand et al. (2011) using a chloroform/methanol method for lipid extraction. Prior to pulverization and extraction, SC fat was separated into two layers denoted as inside and outside SC and jowl fat was separated from any lymph and connective tissue. A core was taken from the geographic center of a reserved chop from the 12th rib region, pulverized and used to extract a fatty acid profile of intramuscular fat. Briefly, tissue was homogenized (TissueMaster, Biospec Products, Inc., in chloroform:methanol (CHCl₃:CH₃OH, 2:1, v/v), homogenates were filtered and subsequently vortexed with potassium chloride (KCl) to separate aqueous and lipid phases. Following a 2 h separation, lipids were saponified with potassium hydroxide (KOH) in methanol (MeOH). Free FA were then methylated in a transesterification reaction using boron trifluoride (BF₃) as an acid catalyst. Dehydrated fatty acid methyl esters (FAMES) were loaded into a Varian 3,800 gas chromatographer (Varian, Pala Alto, CA) to analyze fatty acid profiles. Helium served as the carrier gas in a fused silica capillary column (SPTM – 2,560; 100 m x 0.25 mm x 0.2 µm film thickness; Supelco, Bellefonte, PA). Individual fatty acids were normalized and the area under each peak represented a percentage of the total fatty acid profile. IV from fatty acid profiles were determined according to the equation described by AOCS (1998): $IV = (0.95 \times [C16:1]) + (0.86 \times ([C18:1n9t] +$

[C18:1n9c])) + (1.732 x ([C18:2n6t] + [C18:2n6c])) + (2.616 x [C18:3n3]) + (0.785 x [C20:1]), with the brackets indicating concentrations of fatty acids.

Statistical Analysis

Statistical analysis for carcass characteristics and fatty acid profiles was performed using the GLM procedure of SAS 9.3 (SAS Inst., Cary, NC) with pig as the experimental unit. The statistical model included the fixed effects dietary treatment. Least squares means and standard errors were estimated using the PDIF option. Level of significance was predetermined at $P < 0.05$.

RESULTS AND DISCUSSION

Carcass Composition

Dietary treatment did not significantly ($P > 0.41$) change carcass characteristics including HCW, LEA, last rib fat, or 10th rib fat (Table 1). In agreement with the current data, several studies have noted inclusion of DDGS in the diet has no impact on carcass traits including HCW (Xu et al., 2010; Dahlen et al., 2011; McClelland et al., 2012), LEA (Pompeu et al., 2013) or 10th rib backfat (McClelland et al., 2012, Lee et al., 2013). In contrast, according to Whitney et al. (2006) as the level of DDGS increases in the diet, HCW and LM area are linearly decreased. Furthermore, Rojo et al. (2016) found carcass yield and LM depth both decreased linearly as HPDDG or DDGS was increased 30% in pig diets. Widmer et al. (2008) reported including HPDDG did not change HCW or 10th rib backfat, but LM area and depth decreased linearly with HPDDG addition to the diets.

Nutrient concentration and digestibility may vary between sources of DDGS (Stein and Shurson, 2009) and may be a contributing factor related to discrepancies reported related to DDGS and carcass composition.

Although pigs in the current study were fed only 3% fat for the last 25 kg of gain, a number of studies have shown added fat in the diet does not change carcass slaughter weight (Averette Gatlin et al., 2002; Apple et al., 2009a), HCW (Kellner et al., 2016), back fat (Lee et al., 2013) or LM area (Stephenson et al., 2016). Despite when added CWG or corn oil were fed at 2, 4 and 6% for an extended period of time, Kellner et al. (2016) failed to see significant changes in carcass composition and increasing fat content in the diet only revealed a tendency to increase carcass yield. Phase feeding added fat over five time periods produced no dietary differences between slaughter weight, HCW, or 10th rib fat (Browne et al., 2013). However, including beef tallow in the last three feeding phases increased LM depth compared to yellow grease and as feeding duration of yellow grease increased, fat free lean yield linearly increased compared to beef tallow (Browne et al., 2013). When 4% beef tallow, soybean or a 50:50 combination was fed for the first 42 d, last 42 d or the entire 84 d feeding period, there were no changes in carcass characteristics between fat sources, however, adding dietary fat over the entire feeding period increased backfat and reduced fat free lean index compared pigs fed diets with no added fat (Stephenson et al., 2016).

Muscle and Fat Quality

The final pH of SM was different ($P = 0.01$) between dietary treatments (Table 1), but no other differences in temperature or pH of LM or SM were detected. This is contrary to several studies that found including DDGS (Whitney et al., 2006), DDGS and

added fat (Lee et al., 2013) or dietary oils (Teye et al., 2006) did not affect pH of the LM. Pigs fed HPDDGS had the greatest ultimate pH value, 6.20 followed by DDGS and DDGS + BO (5.96), DDGS + CWG (5.94) and the pigs fed CON diet had the lowest pH at 5.86.

A biorefining technology known as Bfrac dehulls and degerms corn prior to fermentation resulting in two products, corn germ and dried distillers grains that are not mixed with solubles (Widmer et al., 2007). This product is referred to as high protein dried distillers (HPDDG) and contains more protein, but less fat, ADF, NDF and phosphorus (P), than DDGS (Widmer et al., 2007). Postmortem, the body attempts to maintain homeostasis by shifting to anaerobic metabolism when oxygen stores are depleted and due to the body's inability to remove the lactic acid by product to the liver, lactic acid builds up in the muscle and causes a pH decline in the tissue. Glycolytic potential is a measure of all the compounds available in a muscle that can be converted to lactic acid and is a way to measure the capacity of postmortem glycolysis and potential extent of pH decline (Hamilton et al., 2003). The diet containing HPDDG was a high protein, but low energy diet and possibly resulted in pigs with less glycolytic potential at the time of death and thus, were unable to reach lower ultimate pH in SM likely due to the size of the SM muscle compared to the LM.

Surprisingly, there were no color differences ($P > 0.25$) in LM or SC fat between treatments (Table 2.1). Egg yolk color intensity increased linearly as DDGS in laying hen diets increased from 0 to 25%, largely attributed to the highly available xanthophylls in DDGS, which is a major yellow carotenoid (Masa'deh et al., 2011). Moreover, increased yellow color has been associated with increased linoleic and α -linolenic fatty

acid content (Maw et al., 2003). Since these fatty acids cannot be synthesized in mammals and presence in tissues is directly from dietary intake and fatty acids themselves are colorless, meaning, the yellow color must be attributed to associated carotenoids in the feed (Maw et al., 2003). Although LM L*, a*, and b* were not significantly different in pigs fed DDGS and added fat, the L* value of backfat from pigs fed a control diet with no DDGS or added fat was numerically greater, indicating a lighter color, than diets containing DDGS (Lee et al., 2013). According to Xu et al. (2010) increasing DDGS content in the diet did not change belly fat Minolta or Japanese color scores, but did decrease LM a* and b*. Further, increasing DDGS to 20 to 30% resulted in darker backfat and yellowness increased linearly with increasing DDGS (Xu et al., 2010). Numerically speaking in the current data, the PCON diet revealed the lowest b* value in backfat compared to other diets and may indicate a higher inclusion percentage or more time on feed could change fat color. From a retail perspective, fat can be added to the diet in a variety of forms without negatively impacting pork color or creating discernable differences for the consumer.

Other quality indicators including IMF, moisture content, drip loss, water holding capacity and belly quality were not significantly different between treatments (Table 1). In agreement with our data, Whitney et al. (2006) reported visual firmness, marbling, WHC, drip loss, cooking loss and shear force were not impacted by including DDGS in the diet. Teye et al. (2006) indicated feeding oils with varying saturation levels did not impact LM drip loss or shear force values. However, inclusion of DDGS has been shown to decrease marbling and LM firmness (Xu et al., 2010). Diets low in protein and lysine content will limit protein synthesis and increase the energy available for fat deposition,

which corresponds to increased fat, particularly IMF, which develops late in the growth curve (Teye et al., 2006). According to Apple et al. (2004b), the ME:Lys ratio may affect IMF development, however, since the treatment diets were balanced on a ME:Lys ratio, significant differences were not detected.

Feeding DDGS has been associated with soft bellies (Widmer et al., 2008; Cromwell et al., 2011; Dahlen et al., 2011), which can easily be explained by the amount of PUFA in tissues from feeding DDGS. Hypothetically, feeding saturated added fat should increase SFA in tissues and help improve belly softness. Although Lee et al. (2013) found control diets had the greatest flop distance by at least 7.8 cm, no differences between fat five added sources were detected. Yet, increasing the concentration of dietary fat tended to increase belly weight and saturated fat in the form of tallow yield firmer ($P < 0.05$) bellies that tended to be thicker ($P < 0.10$) (Kellner et al., 2014). Diets in the current study containing DDGS easily yielded the softest bellies, but of those diets, belly weight tended ($P = 0.12$) to be the heaviest and flexibility was the most desirable from pigs fed butter oil. Butter oil is a saturated fat source has potential to increase belly weight and saturation if given in a higher concentration or longer period of time.

Fatty Acid Composition

Subcutaneous fat. Apple et al. (2009b) describes in detail 5% added fat not only changes the fatty acid profiles of SC fat, but individual fat layers are distinguishable as well. Interestingly, Lee et al. (2013) fed 30% DDGS and added fat, but did not find treatment differences in FA composition or IV of backfat. In general, adding fat to the diet will decrease SFA, MUFA, and increase PUFA and IV, but feeding duration and fat

source may interact and affect fatty acid composition in backfat (Stephenson et al., 2016). The IV of backfat layers will also increase with increasing levels of DDGS (McClelland et al., 2012).

In the inner SC fat layer, SFA and MUFA decreased ($P < 0.01$) with DDGS inclusion, but PUFA concentrations increased ($P < 0.01$) compared to CON and HPDDG (Table 2.2). In the outer SC fat layer, PUFA concentration increased ($P < 0.01$), but MUFA decreased ($P < 0.01$) with inclusion of DDGS (Table 2.3). Outer SC fat had the lowest ($P < 0.01$) proportion of SFA in pigs fed DDGS + CWG compared to all other diets. In both inner and outer SC fat layers, pigs fed the PCON diet had the most saturated inner and outer SC fat, while pigs fed DDGS + CWG had the least saturated inner and outer SC ($P < 0.01$). Not surprisingly, the iodine values of SC fat from pigs fed DDGS were higher ($P < 0.01$) than pigs without.

In agreement with Apple et al. (2009b), the IVs for each diet were greater numerically in the outer layer compared to the inner layer indicating the outer layer was more unsaturated and IV differed ($P < 0.01$) by dietary fat treatment in both layers. The inner fat layer is the most dynamic with the greatest lipogenic activity (Leymaster and Mersmann, 1991; Warnants et al., 1999), and consequently has a high proportion of SFA and MUFA from de novo synthesis (Warnants et al., 1999).

Intramuscular fat. Intramuscular fat largely consisted of MUFA (Table 2.4). Contrary to other depots, diets with DDGS revealed increased ($P < 0.01$) SFA and MUFA, but PUFA concentrations only tended ($P = 0.09$) to differ with HPDDG containing the greatest proportion of PUFA. Additionally, IV was not different ($P = 0.19$) between diets. Wiegand et al. (2011) reported regardless of energy content in the

diet, FA composition and IV was not affected in i.m. Wiegand et al. (2011) further suggests finishing pigs likely deposit fat in the jowl and over the front shoulder prior to the loin and belly region. Thus, if i.m. is the last fat to accumulate near the end of a food animal's growth curve and live span, it is likely this depot is undergoing greater lipogenic activity at the time of slaughter when protein accretion has plateaued and fat begins accumulating. As fat depth increases, fatty acid profiles become more saturated from increased lipogenic activity and dietary fatty acids are no longer preferentially absorbed by tissues (Wood et al., 1989). As a result, i.m. is largely composed of SFA and MUFA from de novo synthesis.

Belly fat. Belly fat from pigs fed DDGS had less ($P < 0.01$) MUFA, but greater ($P < 0.01$) proportions of PUFA (Table 2.5). The PCON diet was the most saturated ($P < 0.01$) compared to all other diets, while the DDGS + CWG diet had the lowest ($P < 0.01$) proportion of SFA in belly fat. Iodine value was significantly ($P < 0.01$) different by diet with the PCON diet exhibiting the lowest IV and DDGS + CWG the highest.

Not surprisingly, several studies have shown inclusion of DDGS in the diet increases belly IV (Whitney et al., 2006; Benz et al., 2010; McClelland et al., 2012) regardless of IV determination method (Shircliff et al., 2015). Variations of added fat will change IV depending on the saturation of the source with CWG increasing IV compared to beef tallow, however, when fat was added at 6%, IV did not differ regardless of fat sources or fat depot (Kellner et al., 2014). This may be indicative of a threshold for added fat concentration to inhibit de novo synthesis and dilute differences based on dietary intake (Kellner et al., 2014). Despite differences in MUFA concentrations, when 30% DDGS was fed with beef tallow, palm oil or glycerol, belly IV did not change (Lee

et al., 2013). We expect saturated animal fats, like butter oil, to increase saturation and harden fat, however, our data suggests BO is not sufficient to overcome 30% inclusion of DDGS. Similar to findings by Salyer et al. (2012) who combined CWG and wheat middlings to find CWG mitigated effect of wheat middlings on live performance, but carcass yield and IV were not overcome, BO was not able to alleviate any negative effects of DDGS on fat quality in any depot.

Jowl fat. Pigs fed DDGS had greater ($P < 0.01$) PUFA concentrations, but lower ($P < 0.01$) MUFA concentrations in jowl fat compared to the PCON and HPDDG (Table 6). Saturated FA were different ($P < 0.01$) in jowl fat, but the pattern is less clear. Iodine values were different ($P < 0.01$) between diets and were numerically the greatest of all fat depots.

The influence of diet on fatty acid composition in animal tissues has been long established (Ellis and Isbell, 1926; Wood et al., 2008). Research has also suggested majority of fatty acid turnover in porcine tissue will occur in the first 14 d of changing dietary fat source, concentration, or both (Irie and Sakimoto, 1992; Wiseman and Agunbiade, 1998). Typically, jowl fat will have a higher IV compared to other fat depots and this is attributed to fat deposition patterns and physiological maturity of fat depots (Wiegand et al., 2011; Shircliff et al., 2015). The jowl region is subject to a longer turnover rate of adipose tissue compared to belly and loin fat and is not easily impacted by changes in fat source or feeding duration (Stephenson et al., 2016).

Butter oil and CLA. The primary source of CLA is from Δ^9 desaturase activity on trans vaccenic acid during rumen biohydrogenation (Griinari et al., 2000), so expectedly food sources originating from ruminant animals like cattle and sheep have

markedly higher levels of CLA compared to monogastrics and only the direct supplementation of CLA or the precursor, trans-vaccenic acid, are effective in elevating CLA in tissues in animals like pigs (Schmid et al., 2006). Butter oil in the diet was effective at increasing ($P < 0.01$) CLA content, particularly the cis-9, trans-11 isomer, nearly doubling CLA content in both layers of backfat, belly and jowl fat depots compared to all other treatments. Additionally, HPDDGS numerically increased CLA content in fat depots, likely due to the PUFA fat content in HPDDGS.

Similar to metabolic modifiers, CLA successfully improves live animal performance and repartitions nutrients to concurrently increase lean mass and decrease fat content. Several studies (Thiel-Cooper et al., 2001; Wiegand et al., 2001, 2002b; Weber et al., 2006; Dugan et al., 1997) have shown supplementing CLA enhances pig performance with overwhelming evidence CLA significantly improves G:F. Moreover, CLA supplementation repeatedly decreased backfat (Dugan et al., 1997; Thiel-Cooper et al., 2001; Wiegand et al., 2001, 2002b, Barnes et al., 2012) but the impact on muscle accretion is less clear. Dietary CLA up to 1.0% has reduced LM (Thiel-Cooper et al., 2001; Barnes et al., 2012), increased LM (Wiegand et al., 2002b; Weber et al., 2006), or failed to change LM size at all (Wiegand et al., 2001; White et al., 2009). In our data, CLA in the form of butter oil numerically reduced last and tenth rib fat.

Dietary CLA shifts the FA profile creating a more saturated profile throughout the entire carcass (Weber et al., 2006; White et al., 2009; Barnes et al., 2012). White et al. (2009) reported 0.6% CLA for 10 d minimized the negative effects of 20% DDGS inclusion, but not 40% and CLA also increased SFA content in carcass fat. Pompeu et al. (2013) also found 0.6% supplementation of CLA was effective at diminishing negative

effects of 30% DDGS by increasing SFA. However, 3% BO did not mitigate the effects of DDGS on fat quality as seen by no differences in SFA or IV compared to pigs fed DDGS with no added fat. Including 3% BO likely did not provide CLA in a high enough concentration to dilute the effects of DDGS.

Interestingly, despite the fact CLA reduced backfat, studies have also shown CLA significantly (Dugan et al., 1999; Wiegand et al., 2002b; Joo et al., 2002) and tended (Barnes et al., 2012) to increase i.m. fat. Reduced backfat is often associated with decreased marbling or i.m. fat content and subsequent negative eating experiences. Increasing marbling may be advantageous as greater marbling is associated with increased palatability, specifically tenderness and juiciness (Brewer et al., 2001; Wood et al., 2004). Although not significantly different, pigs fed butter oil contained the greatest amount of marbling, 3%, and were the only treatment to meet marbling standards on an uncooked basis for acceptable palatability as described by Savell and Cross (1988). Butter oil may be a viable option to increase pork quality by increasing i.m. fat and CLA content in pork products.

IMPLICATIONS

As expected, SFA, MUFA and PUFA were different between all diets in all fat depots. Specifically, the three diets containing DDGS had greater proportions of PUFA and lower proportions of MUFA in belly, jowl and SC fat. Despite evidence the FA composition of fat depots are perceptive to changes in dietary fat source and concentration, 3% BO was not capable of overcoming the unsaturated content of DDGS

in pig diets. Butter oil may be a unique alternative to supplement swine diets to increased CLA content in pork, but further research is needed to describe thresholds for the effectiveness of alternative fat sources in terms of source, concentration, feeding duration and economic feasibility.

Table 2.1. Carcass composition and muscle and fat quality of pigs fed five different diets containing 30% dried distillers grains and added fat in the form of choice white grease or butter oil

Item	Dietary Treatment ¹					SEM	P-value
	PCON	HPDDG	DDGS	DDGS + CWG	DDGS + BO		
Carcass Composition							
HCW ² , kg	95.76	96.50	93.89	94.52	92.59	1.53	0.41
LM ³ area, cm ²	49.27	47.98	46.53	48.79	47.10	1.56	0.71
Last rib fat, mm	2.70	2.94	2.73	2.84	2.54	0.19	0.65
Tenth rib fat, mm	2.27	2.21	2.29	2.24	2.13	0.15	0.95
Muscle and Fat Quality							
Initial LM temperature, °C	36.26	39.53	38.42	38.17	37.58	0.79	0.08
Final LM temperature, °C	3.06	3.26	3.04	3.05	3.03	0.12	0.61
Initial SM ⁴ temperature, °C	42.26	41.72	41.97	41.81	42.01	0.18	0.26
Final SM temperature, °C	4.55	4.74	4.94	4.48	5.21	0.33	0.53
Initial LM pH	6.14	6.29	6.21	6.36	6.14	0.11	0.58
Final LM pH	5.74	5.81	5.76	5.83	5.70	0.03	0.09
Initial SM pH	5.93	6.10	6.08	6.04	5.94	0.08	0.42
Final SM pH	5.86	6.20	5.96	5.94	5.96	0.06	0.01
LM L* ⁵	49.77	52.10	48.52	48.97	48.99	1.29	0.31
LM a* ⁵	14.06	13.25	13.53	14.06	14.13	0.33	0.25
LM b* ⁵	6.24	6.36	5.69	5.85	6.20	0.37	0.67
SC ⁶ L*	74.81	74.87	75.31	74.73	74.23	0.48	0.63
SC a*	6.10	5.99	5.80	5.72	6.33	0.26	0.48
SC b*	3.85	4.08	4.01	3.99	4.09	0.15	0.82

Table 2.1 (con'd). Carcass composition and muscle and fat quality of pigs fed five different diets containing 30% dried distillers grains and added fat in the form of choice white grease or butter oil

Item	Dietary Treatment ¹					SEM	P-value
	PCON	HPDDG	DDGS	DDGS + CWG	DDGS + BO		
Muscle and Fat Quality							
i.m. ⁷ , %	2.69	2.82	2.84	2.84	3.06	0.27	0.91
Moisture, %	72.55	72.26	72.67	72.77	72.43	0.31	0.81
Drip Loss, %	2.12	2.13	1.48	1.87	1.78	0.34	0.64
WHC ⁸	2.21	2.35	2.20	2.72	2.11	0.34	0.73
Belly weight, kg	4.86	4.96	4.36	4.77	5.08	0.20	0.12
Horizontal flex ⁹ , cm	10.48	12.07	7.94	8.57	9.21	1.61	0.40
Vertical flex ¹⁰ , cm	20.96	20.64	21.27	21.59	21.91	0.75	0.77

¹PCON = positive control corn and soybean meal + 3% choice white grease (CWG), HPDDG = 30% high protein + 3% CWG, DDGS = 30% Dried distillers grains with solubles (DDGS), DDGS + CWG = 30% DDGS + 3% CWG, DDGS + BO = 30% DDGS + 3% butter oil.

²HCW = hot carcass weight.

³LM = longissimus dorsi muscle.

⁴SC = subcutaneous back fat.

⁵Objective color scores; L* = lightness to darkness; a* = red to green; b* = blue to yellow.

⁶SC = subcutaneous back fat.

⁷i.m. = intramuscular fat.

⁸WHC = water holding capacity.

⁹Horizontal belly flexibility measured as the distance between belly ends.

¹⁰Vertical belly flexibility measure as the distance from the belly end to a zero line.

Table 2.2. Fatty acid composition of the inner subcutaneous fat layer from pigs fed five different diets containing 30% dried distillers grains and added fat in the form of choice white grease or butter oil

Fatty Acid	Dietary Treatment ¹					SEM	P-value
	PCON	HPDDG	DDGS	DDGS + CWG	DDGS + BO		
C14:0 (Myristic acid)	1.29	1.22	1.14	1.09	1.52	0.04	< 0.01
C16:0 (Palmitic acid)	24.30	23.46	22.68	21.39	22.70	0.32	< 0.01
C18:0 (Stearic acid)	13.50	12.28	12.56	11.58	11.87	0.33	< 0.01
C16:1 (Palmitoleic acid)	1.50	1.57	1.10	1.22	1.33	0.06	< 0.01
C18:1n9c (Oleic acid)	40.85	41.39	37.47	38.03	36.80	0.47	< 0.01
C18:1n7c (Oleic acid)	1.47	1.56	0.91	1.28	1.21	0.10	< 0.01
C18:2n6c (Linoleic acid)	13.46	14.95	20.57	21.85	20.88	0.57	< 0.01
C18:9c11t CLA	0.07	0.09	0.06	0.08	0.14	0.01	< 0.01
C18:9t11t CLA	0.01	ND ³	<0.01	0.01	0.02	0.01	0.06
C18:3n3 (α -Linolenic acid)	0.98	0.85	0.77	0.82	0.68	0.09	0.16
C20:4n6 (Arachidonic acid)	0.21	0.21	0.20	0.25	0.24	0.01	0.02
C22:5n3 (Docosapentaenoic acid)	0.03	0.00	0.01	0.03	0.01	0.01	< 0.01
Total SFA	40.03	37.90	37.26	34.85	37.11	0.60	< 0.01
Total MUFA	44.28	45.01	39.85	40.91	39.82	0.51	< 0.01
Total PUFA	15.69	17.09	22.89	24.24	23.06	0.59	< 0.01
PUFA:SFA	0.37	0.43	0.59	0.67	0.60	0.02	< 0.01
Omega-6	14.51	16.08	21.95	23.22	22.14	0.60	< 0.01
Omega-3	1.10	0.92	0.87	0.93	0.76	0.09	< 0.01
O6:O3	13.88	22.37	26.28	25.13	29.36	2.43	< 0.01
IV ²	62.44	65.21	70.92	73.86	70.93	0.87	< 0.01

¹PCON = positive control corn and soybean meal + 3% choice white grease (CWG), HPDDG = 30% high protein + 3% CWG, DDGS = 30% Dried distillers grains with solubles (DDGS), DDGS + CWG = 30% DDGS + 3% CWG, DDGS + BO = 30% DDGS + 3% butter oil

²IV = iodine value.

³ND = not detectable.

Table 2.3. Fatty acid composition of the outside subcutaneous fat layer from pigs fed five different diets containing 30% dried distillers grains and added fat in the form of choice white grease or butter oil

Fatty Acid	Dietary Treatment ¹					SEM	P-value
	PCON	HPDDG	DDGS	DDGS + CWG	DDGS + BO		
C14:0 (Myristic acid)	1.26	1.20	1.11	1.06	1.47	0.04	< 0.01
C16:0 (Palmitic acid)	22.55	21.74	20.96	19.89	21.24	0.36	< 0.01
C18:0 (Stearic acid)	11.78	10.95	12.08	10.81	11.29	0.47	0.28
C16:1 (Palmitoleic acid)	1.76	1.81	1.25	1.39	1.55	0.08	< 0.01
C18:1n9c (Oleic acid)	42.81	42.87	38.23	39.94	38.62	0.54	< 0.01
C18:1n7c (Oleic acid)	1.69	1.43	1.17	1.31	1.18	0.11	0.01
C18:2n6c (Linoleic acid)	14.42	16.06	21.44	21.85	20.83	0.56	< 0.01
C18:9c11t CLA	0.08	0.13	0.09	0.10	0.19	0.02	< 0.01
C18:9t11t CLA	ND ³	0.01	ND ³	ND ³	0.01	0.01	0.15
C18:3n3 (α -Linolenic acid)	0.85	0.95	0.77	0.84	0.76	0.05	0.04
C20:4n6 (Arachidonic acid)	0.24	0.25	0.21	0.27	0.27	0.02	0.14
C22:5n3 (Docosapentaenoic acid)	0.03	ND ³	0.02	0.03	0.02	0.01	< 0.01
Total SFA	36.53	34.80	34.99	32.51	34.95	0.71	0.01
Total MUFA	46.83	46.71	41.10	43.08	41.82	0.62	< 0.01
Total PUFA	16.63	18.49	23.90	24.41	23.24	0.59	< 0.01
PUFA:SFA	0.43	0.51	0.66	0.72	0.64	0.03	< 0.01
Omega-6	15.58	17.32	22.91	23.33	22.18	0.59	< 0.01
Omega-3	0.97	1.03	0.91	0.97	0.87	0.05	0.12
O6:O3	16.19	17.22	25.71	24.74	25.95	1.53	< 0.01
IV ²	65.69	68.91	73.23	75.71	72.74	0.91	< 0.01

¹PCON = positive control corn and soybean meal + 3% choice white grease (CWG), HPDDG = 30% high protein + 3% CWG, DDGS = 30% Dried distillers grains with solubles (DDGS), DDGS + CWG = 30% DDGS + 3% CWG, DDGS + BO = 30% DDGS + 3% butter oil.

²IV = iodine value.

³ND = not detectable.

Table 2.4. Fatty acid composition of intramuscular fat from pigs fed five different diets containing 30% dried distillers grains and added fat in the form of choice white grease or butter oil

Fatty Acid	Dietary Treatment ¹					SEM	P-value
	PCON	HPDDG	DDGS	DDGS + CWG	DDGS + BO		
C14:0 (Myristic acid)	1.33	1.27	1.26	1.27	1.37	0.06	0.62
C16:0 (Palmitic acid)	ND ³	0.01	ND ³	ND ³	ND ³	0.01	0.42
C18:0 (Stearic acid)	0.23	0.21	0.15	0.12	0.15	0.04	0.35
C16:1 (Palmitoleic acid)	25.76	25.19	25.41	24.70	25.03	0.37	0.35
C18:1n9c (Oleic acid)	12.40	11.31	11.89	13.05	13.01	0.51	0.09
C18:1n7c (Oleic acid)	0.16	0.09	0.08	0.05	0.01	0.04	0.06
C18:2n6c (Linoleic acid)	3.67	3.70	3.16	3.08	3.02	0.12	<0.01
C18:9c11t CLA	ND ³	0.01	ND ³	ND ³	ND ³	0.01	0.42
C18:9t11t CLA	0.25	0.25	0.39	0.41	0.37	0.02	<0.01
C18:3n3 (α -Linolenic acid)	0.61	0.82	0.56	0.65	0.62	0.06	0.07
C20:4n6 (Arachidonic acid)	39.97	38.27	39.03	39.47	39.91	0.70	0.42
C22:5n3 (Docosapentaenoic acid)	11.91	11.76	15.10	16.20	15.56	0.85	<0.01
Total SFA	0.25	0.27	0.35	0.36	0.34	0.02	<0.01
Total MUFA	11.27	10.93	14.54	15.55	14.92	0.87	<0.01
Total PUFA	0.64	0.82	0.56	0.65	0.64	0.07	0.09
PUFA:SFA	23.41	14.89	40.16	24.32	23.58	6.79	0.15
Omega-6	55.58	57.79	59.98	60.20	59.53	0.90	<0.01
Omega-3	12.55	12.58	15.66	16.85	16.20	0.83	<0.01
O6:O3	4.47	4.79	3.90	3.63	3.70	0.20	<0.01
IV ²	43.23	42.35	41.43	41.87	41.97	0.53	0.19

¹PCON = positive control corn and soybean meal + 3% choice white grease (CWG), HPDDG = 30% high protein + 3% CWG, DDGS = 30% Dried distillers grains with solubles (DDGS), DDGS + CWG = 30% DDGS + 3% CWG, DDGS + BO = 30% DDGS + 3% butter oil.

²IV = iodine value.

³ND = not detectable.

Table 2.5. Fatty acid composition of belly fat from from pigs fed five different diets containing 30% dried distillers grains and added fat in the form of choice white grease or butter oil

Fatty Acid	Dietary Treatment ¹					SEM	P-value
	PCON	HPDDG	DDGS	DDGS + CWG	DDGS + BO		
C14:0 (Myristic acid)	1.45	1.39	1.30	1.27	1.61	0.05	< 0.01
C16:0 (Palmitic acid)	23.24	22.08	21.75	20.84	22.01	0.32	< 0.01
C18:0 (Stearic acid)	9.87	8.61	9.52	8.96	9.29	0.30	0.05
C16:1 (Palmitoleic acid)	2.20	2.33	1.60	1.79	2.10	0.14	< 0.01
C18:1n9c (Oleic acid)	45.20	44.64	41.42	42.26	41.87	0.69	< 0.01
C18:1n7c (Oleic acid)	2.17	2.32	1.82	1.95	1.82	0.10	< 0.01
C18:2n6c (Linoleic acid)	12.33	14.89	18.95	19.40	17.73	0.65	< 0.01
C18:9c11t CLA	0.06	0.08	0.05	0.07	0.14	0.01	< 0.01
C18:9t11t CLA	0.01	0.02	0.01	<0.01	<0.01	0.01	0.22
C18:3n3 (α -Linolenic acid)	0.92	0.91	0.86	0.82	0.75	0.05	0.14
C20:4n6 (Arachidonic acid)	0.24	0.27	0.26	0.30	0.29	0.02	0.20
C22:5n3 (Docosapentaenoic acid)	0.02	ND ³	0.01	0.01	0.02	0.01	0.16
Total SFA	35.40	32.93	33.34	31.79	33.76	0.53	< 0.01
Total MUFA	50.13	49.93	45.31	46.44	46.28	0.78	< 0.01
Total PUFA	14.47	17.14	21.35	21.77	19.97	0.65	< 0.01
PUFA:SFA	0.38	0.50	0.61	0.65	0.56	0.02	< 0.01
Omega-6	13.39	16.07	20.34	20.78	18.97	0.68	< 0.01
Omega-3	1.02	0.97	0.95	0.91	0.84	0.05	0.19
O6:O3	13.38	17.36	22.05	23.17	23.28	1.71	< 0.01
IV ²	64.72	68.79	72.21	73.79	70.68	0.70	< 0.01

¹PCON = positive control corn and soybean meal + 3% choice white grease (CWG), HPDDG = 30% high protein + 3% CWG, DDGS = 30% Dried distillers grains with solubles (DDGS), DDGS + CWG = 30% DDGS + 3% CWG, DDGS + BO = 30% DDGS + 3% butter oil.

²IV = iodine value.

³ND = not detectable.

Table 2.6. Fatty acid composition of jowl fat from pigs fed five different diets containing 30% dried distillers grains and added fat in the form of choice white grease or butter oil

Fatty Acid	Dietary Treatment ¹					SEM	P-value
	PCON	HPDDG	DDGS	DDGS + CWG	DDGS + BO		
C14:0 (Myristic acid)	1.34	1.26	1.22	1.20	1.44	0.05	0.01
C16:0 (Palmitic acid)	22.24	20.87	21.01	20.32	21.49	0.31	< 0.01
C18:0 (Stearic acid)	9.57	8.32	9.46	9.16	9.09	0.27	0.02
C16:1 (Palmitoleic acid)	2.10	2.28	1.61	1.76	1.98	0.12	< 0.01
C18:1n9c (Oleic acid)	46.10	46.67	43.01	43.63	43.40	0.51	< 0.01
C18:1n7c (Oleic acid)	1.98	2.06	1.57	1.49	1.69	0.11	< 0.01
C18:2n6c (Linoleic acid)	12.81	14.44	18.06	18.45	17.10	0.52	< 0.01
C18:9c11t CLA	0.09	0.13	0.09	0.10	0.17	0.01	< 0.01
C18:9t11t CLA	0.01	0.01	0.01	0.01	0.01	0.01	0.79
C18:3n3 (α -Linolenic acid)	1.10	1.15	1.11	1.07	0.92	0.09	0.39
C20:4n6 (Arachidonic acid)	0.24	0.25	0.24	0.28	0.27	0.01	0.16
C22:5n3 (Docosapentaenoic acid)	0.03	0.02	0.02	0.04	0.04	0.01	< 0.01
Total SFA	33.97	31.27	32.45	31.41	32.80	0.48	< 0.01
Total MUFA	50.75	51.65	46.67	47.36	47.55	0.58	< 0.01
Total PUFA	15.28	17.09	20.89	21.23	19.64	0.52	< 0.01
PUFA:SFA	0.42	0.51	0.61	0.64	0.57	0.02	< 0.01
Omega-6	13.95	15.71	19.56	19.92	18.42	0.55	< 0.01
Omega-3	1.23	1.24	1.23	1.20	1.05	0.09	0.49
O6:O3	11.82	13.26	16.40	17.49	18.26	1.32	0.01
IV ²	66.72	70.34	72.71	73.95	71.23	0.63	< 0.01

¹PCON = positive control corn and soybean meal + 3% choice white grease (CWG), HPDDG = 30% high protein + 3% CWG, DDGS = 30% Dried distillers grains with solubles (DDGS), DDGS + CWG = 30% DDGS + 3% CWG, DDGS + BO = 30% DDGS + 3% butter oil.

²IV = iodine value.

CHAPTER 3

Pigs fed 15 ppm Skycis[®] (narsin) have similar carcass composition and lean quality compared to controls, but prominent gender differences exist in carcass composition

ABSTRACT

The objective of this study was to evaluate inclusion of Skycis[®] (NAR) in the diets of pigs and determine the source of carcass yield, specifically dressing percentage, improvements observed in previous studies. Barrows (n = 50) and gilts (n = 50) of PIC C22 x 337 genetics were randomly assigned to a diet containing 0 (CON) or 15 ppm NAR initiated at 39.5 kg of body weight and continued until slaughter. Pigs (n = 47 barrows, n = 46 gilts) were slaughtered in two groups at which time individual organ weights were recorded. At 24 h postmortem carcass composition and carcass quality data was obtained. At 48 h postmortem, one side of each carcass was fabricated into wholesale cuts according to North American Meat Institute specifications. Data points three standard deviations from the mean as determined by PROC UNIVARIATE were removed and remaining data was analyzed by using the PROC MIXED procedure of SAS 9.3. Barrows tended ($P = 0.09$) to have heavier viscera, significantly ($P < 0.01$) more fat at the 10th rib, a greater ($P = 0.01$) percentage of i.m. fat compared to gilts. Gilts were heavier muscled as evidenced by many gender differences ($P < 0.05$) observed in the cutability phase of the study and larger LM ($P < 0.01$). Pigs fed NAR had heavier ($P < 0.01$) hearts, but few treatment differences were observed in carcass quality and cutability. Heavy muscled, lean animals such as gilts may have increased organ mass

from up regulated metabolic activity, but pigs with greater intake will also have heavier intestinal tract compensating for the increased consumption suggesting barrows in this study ate more, especially late in the growth curve. In general, fat content increased and made up a larger percentage of total carcass weight in pigs fed NAR. The current results do not confirm the source of differences previously observed in HCW and DP in pigs fed NAR, but could be attributed to the tendency of NAR to increase carcass fatness.

Key words: Skycis®, naracin, pigs, carcass composition, pork quality, metabolism

INTRODUCTION

Skycis[®] (Narasin) is high performance ionophore labeled as a swine premix for increased rate of gain when fed during the least 4 weeks of the finishing phase. The active ingredient in Skycis[®] is narasin (NAR), an ionophore used in food production. Skycis[®] is available over the counter and is intended for oral administration to growing/finishing swine (FDA, 2012). The FDA (2012) has approved two doses including 16.6 to 27.2 g/ton to increase rate of weight gain and 18.1 to 27.2 g/ton to increase rate of weight gain as well as improve feed efficiency. Skycis has a zero day withdraw and is also accepted in worldwide markets including Japan. Although NAR is technically an antibiotic, there are currently no human applications for ionophores, so the use of ionophores in animal production is a favorable alternative when considering antibiotic resistance conversations.

According to Wuethrich et al. (1998), fecal N decreased ($P < 0.05$), apparent N digestibility increased ($P < 0.05$), and the ratio of propionic to acetic and butyric acid increased ($P < 0.05$) in NAR fed barrows. Increasing the proportion of propionate producing bacteria is favorable because propionate is efficiently fermented and is a gluconeogenic precursor (Russel and Strobel, 1989). Growth performance is thus improved by an altered digestion, absorption and retention of N in the gastrointestinal tract of pigs (Dierick et al., 1986).

Ionophores act favorable in cattle by fermenting dietary carbs into propionate, a more energy efficient compound with reduced methane production compared to acetic and butyric acid production (Wolin, 1981) and have application in swine to improve

efficiency and carcass composition (Arentson et al., 2014; Arentson and Chewning, 2015, 2016; Arkfeld et al., 2015). The objective of this study was to evaluate inclusion of Skycis[®] in the diets of pigs and determine the source of carcass yield, specifically dressing percentage, improvements, previously observed in other experiments.

MATERIALS AND METHODS

Animals and Experimental Design

The study was organized as a completely randomized design. Barrows (n=50) and gilts (n=50) of PIC C22 x 337 genetics were randomly assigned to a dietary treatment containing either 0 (CON) or 15 ppm NAR (13.6 grams/ton). Dietary treatment was initiated at 39.5 kg of body weight and continued until slaughter. Pigs (n=47 barrows, n=46 gilts) were slaughtered in two groups. At each marketing cut, pigs were uniquely tattooed and transported to the University of Missouri Red Meats Abattoir where pigs were humanely slaughtered following standard U.S. pork industry practices and USDA-FSIS inspection criteria. Immediately prior to slaughter, a final body weight of each pig was recorded.

Data Collection

Visceral organs. During slaughter, total intestine, stripped intestine, leaf fat, liver, spleen, pancreas, heart, lungs, kidneys, mesentery fat, and HCW data were recorded for each carcass. To obtain intestinal weights, the small and large intestine were segmented from the stomach and reproductive organs. The intestines were weighed with

digesta, flushed with water, rested for 10 min in a plastic lug and re-weighed empty. Leaf fat was collected on the slaughter floor by the same technician for all pigs to standardize the method. A licensed veterinarian was present to subjectively evaluate the pluck and liver for any major abnormalities.

Carcass measurements. Chilled carcass weights were collected after a 24 h postmortem chill. Also at 24 h postmortem, the left side of all carcasses were ribbed between the 10th and 11th ribs and allowed to bloom for 30 min. Fat thickness was measured perpendicular to the skin surface at the last and 10th ribs including the skin in the measurement. Loin muscle area was calculated using a hand held grid (Iowa State University, Ames, IA). Objective color values for L*, a*, and b* were measured on the 10th rib cut surface of the LM using a HunterLab Mini Scan model 45/0 LAV (Hunter Associates Laboratory, Virginia, USA) with a D65 light source, 1.27 cm aperture and a physical standard. A one-inch chop taken from the 10th rib was reserved from each carcass to perform moisture and fat analysis as previously described by Keeton et al. (2003) and Dow et al. (2011) using the CEM SMART Trac rapid analysis system.

Carcass cutouts. At 48 h postmortem, the right side of each carcass was fabricated into primal cuts and weighed according to the North American Meat Institute (NAMI) (2014) specifications as follows. The wholesale ham (IMPS #401) was removed from the side in a straight cut approximately perpendicular to a line parallel to the shank 3.8 cm from the anterior edge of the aitch bone and the shank was removed at the hock joint. The ham was skinned, fat trimmed to 0.32 cm and further processed into the inside ham (IMPS #402F), outside ham (IMPS #402D), knuckle (IMPS #402H), and shank trim. The pork shoulder (IMPS #403) was separated from the side in a straight cut between the

2nd and 3rd ribs and the foot was removed immediately dorsal to the knee joint. The boston butt (IMPS #406) and picnic shoulder (IMPS #405) were separated by a straight cut perpendicular to the backbone and 2.5 cm from the outermost curvature of the thoracic vertebrae. Both the boston butt and picnic shoulder were skinned, fat trimmed to 0.32 cm and finished to boneless roasts (IMPS #406A and 405A, respectively). The entire pork loin (IMPS #410) was removed from the belly by a straight cut from a point on the shoulder end where the first rib met the backbone to a point immediately ventral to the psoas major. The whole loin was skinned, fat trimmed to 0.32 cm and the psoas major (IMPS #415) was removed. The sirloin was removed at a point in between the last and second to last lumbar vertebrae, perpendicular to the backbone. The vertebrae, ribs, blade bone, associated muscles, and remaining fat were removed from the loin to create a boneless loin (IMPS #413C) and the aitch bone was removed from the sirloin to create a boneless sirloin (IMPS #413C). Finally, the belly (IMPS #408) was finished by removing the spareribs and squaring the flank edge and ventral midline.

Statistical Methods

Data points greater than three standard deviations from the mean as determined by PROC UNIVARIATE were removed from the data set. Remaining data was analyzed by using the PROC MIXED procedure of SAS 9.3 (SAS Inst., Cary, NC) using carcass as the experimental unit. The model included the fixed effect of gender and diet as well as the random effect of group. Least squares means and standard errors were estimated using the PDIF option. Significance was determined as $P < 0.05$.

RESULTS

Visceral Organs

Data collected during the slaughter procedure is presented as organ weights (Table 3.1), organ weights as a percentage of HCW (Tables 3.2), organs as a percentage of total viscera weight (Table 3.3) and the amount of intestinal gut fill by weight and gut fill as a percentage of total viscera weight, live weight, and HCW (Table 3.4). There was a significant interaction between gender and dietary treatment for lung weight ($P = 0.02$), heart weight as a percentage of HCW ($P = 0.01$) and lung weight as a percentage of HCW ($P < 0.01$).

There were several significant gender differences in organ weights and organ weights as a percentage of HCW, total viscera, and gut fill. In general, barrows tended ($P = 0.09$) to have heavier viscera compared to gilts. Barrows had more leaf ($P = 0.01$) and tended to have more mesentery fat ($P = 0.08$) that made up a greater percentage of HCW ($P = 0.03$ and 0.02 , respectively) compared to gilts. Leaf fat in barrows also contributed 1.66% more weight to total viscera weight compared to gilts ($P = 0.01$). Barrows tended ($P = 0.10$) to have heavier full intestines, but both full ($P = 0.02$) and empty intestines ($P = 0.05$) of barrows made up a greater percentage of total weight when expressed as a percentage of HCW. Barrows contained significantly more gut fill ($P < 0.01$), which unsurprisingly made up a greater percentage of total viscera weight ($P = 0.05$), live weight ($P < 0.01$), and HCW ($P < 0.01$). Gilts had heavier spleens ($P < 0.01$) which also contributed to 0.03% more ($P < 0.01$) of HCW.

There was only one significant treatment difference. Pigs fed NAR had heavier ($P < 0.01$) hearts. However, NAR tended to increase leaf fat ($P = 0.09$) and kidney weight ($P = 0.07$). Narasin also tended to increase leaf fat ($P = 0.10$) and heart weight ($P = 0.08$) as a percentage of HCW as well as liver ($P = 0.06$) and lung ($P = 0.06$) weights as a percentage of total viscera weights.

Carcass Measurements

Carcass characteristics are presented in Table 3.5. There was a significant interaction between gender and treatment for live weight ($P = 0.03$), HCW ($P = 0.05$), and CCW ($P = 0.05$) and last rib fat ($P = 0.01$). Barrows had more ($P < 0.01$) fat at the 10th rib, but gilts had heavier muscled LM ($P < 0.01$). Barrows also had a greater ($P = 0.01$) percentage of i.m. fat, but there were no significant ($P > 0.33$) color differences by gender.

There were no significant differences by treatment in carcass composition or quality. However, pigs fed NAR had a 0.35% numerical ($P = 0.26$) increase in dressing percentage (77.42% vs 77.07%). In terms of color, NAR fed pigs had numerically lower L* values ($P = 0.17$) and higher a* values ($P = 0.23$).

Carcass Cutouts

Data is presented as total side weight for each cut (Table 3.6) and the percent of each cut by HCW (Table 3.7) and percent of each cut by chilled side weight (Table 3.8). There were multiple gender by treatment interactions including skinned loin ($P = 0.05$), boneless sirloin ($P = 0.04$), whole ham ($P = 0.04$), inside ham ($P = 0.03$), and square

belly ($P < 0.01$) weights as well as the bone in, skin on picnic as a percentage of HCW ($P = 0.05$) and square belly and spareribs as a percentage of HCW ($P < 0.01$) and CCW ($P < 0.01$ and $P = 0.03$ respectively).

Many gender differences ($P < 0.05$) observed in the cutability phase of the study. Gilts had heavier boneless loins ($P < 0.01$), skinned hams ($P < 0.01$), outside hams ($P < 0.01$), knuckles ($P < 0.01$), skinned picnics ($P = 0.05$), and boneless picnics ($P < 0.01$). The skinned loin ($P = 0.04$), boneless loin ($P < 0.01$), whole ham, skinned ham, and various individual ham muscles ($P < 0.01$), skinned picnic ($P = 0.02$), and boneless picnic ($P < 0.01$) all accounted for a greater percentage of HCW in gilts compared to barrows. Lastly, the skinned loin ($P = 0.02$), boneless loins ($P < 0.01$), whole ham ($P < 0.01$), skinned ham ($P < 0.01$), various individual ham muscles ($P < 0.01$), and boneless picnic ($P < 0.01$), all accounted for a greater percent of CCW in gilts compared to barrows.

Narasin tended ($P = 0.09$) to increase whole loin and belly weights. Ham knuckle as a percent of HCW ($P < 0.05$) and CCW ($P = 0.01$) was reduced for NAR fed pigs compared to the control fed pigs.

DISCUSSION

Heart and liver weights expressed on a BW or percent of body weight basis were heavier in genetically lean pigs suggesting pigs with greater rates of lean deposition not only metabolize more protein, but there is a corresponding increase in the tissues active in the metabolism and distribution of amino acids (Wiseman et al., 2007). However, in

the current study, not only did barrows deposit more fat in several depots, they also carried greater weight in the gastrointestinal tract as both gut fill and tissue accretion. Similarly, Wiseman et al. (2007) also reported barrows had heavier livers ($P < 0.01$), empty intestinal tract weights ($P < 0.01$) and tended to have greater kidney fat ($P < 0.06$).

Feed intake increases as animals mature and late in the growth curve intake exceeds requirements and increases fat, but decreases protein accretion (Friesen et al., 1994). Yet, genetically lean pigs require added intake to account for increased protein accretion and is also reflected in increased organ size (Mersmann, 1991). Pigs, however, are largely being slaughtered at heavier weights (Carr et al., 2009) allowing pigs to reach more advanced physiological maturity ultimately changing the dynamics at the end of the growth curve. In particular, females are not only given the opportunity to reach puberty, but also cycle through estrus at least once if not multiple times before slaughter. With each of these cycles, gilts will decrease feed intake compared to barrows, who are expected to consistently intake more feed throughout the finishing period. Regardless of NAR treatment, barrows had a greater ADG, ADFI than gilts, but gilts had a greater G:F (Arkfeld et al., 2015). Barrows in the current data had more gut fill and increased intestinal mass compared to gilts suggesting increased intake. Thus, although genetically lean pigs may have heavier organs from increased metabolic activity, pigs with greater intake will also have heavier intestinal tract and liver weights (Wiseman et al., 2007) compensating for the increased consumption.

Regardless of genetic background, gilts have less backfat, larger loin eyes, higher percent fat free lean, and also less seam and i.m. fat compared barrows (Martin et al., 1972). Friesen et al. (1994) finished pigs to 127 kg and gilts had larger LM, greater

protein accretion and less backfat. Interestingly, gilts reached their optimal lean to fat ratio at 104 kg and as Friesen et al. (1994) keenly point out genetically lean pigs do not necessarily make superior carcasses, but simply reach the desired market weight faster.

In agreement with our study, Arkfeld et al. (2015) also reported at the end of the finishing period, regardless of NAR treatment, gilts were leaner at the 10th rib ($P < 0.001$) and have a larger LM area ($P = 0.05$).

Recent work has repeatedly shown NAR improves efficiency (Arkfeld et al., 2015; Knauer et al., 2015; Fruge et al., 2016), but also has the opportunity to change carcass composition (Arentson et al., 2014; Arentson and Chewning, 2015, 2016).

Ionophores were originally developed as anticoccidial feed additives for poultry produced by strains of *Streptomyces* and included molecules such as monensin, lasalocid, salinomycin, and NAR (Bergen and Bates, 1984). Ionophores are anions capable of disrupting normal bacterial cell membrane physiology (Bergen and Bates, 1984) by transporting sodium, potassium and hydrogen protons across cell membranes thereby inverting the alkaline environment inside the cell inflicting chaos on cell functions and cell homeostasis (Russell, 1987).

Interestingly, NAR is selective in targeting gram-positive cells (Russell and Strobel, 1989) which, produce lactate, butyrate, and formate, while bacteria resistant to ionophores produce propionate and succinate as end fermentation products (Chen and Wolin; 1979; Nagaraja and Taylor, 1987). Additionally, ionophore sensitive bacteria degrade amino acids in the small intestine (Dierick et al., 1986). With cell homeostasis altered by NAR action, the proportion of effective gram-positive bacteria is reduced (Dierick et al., 1986), which favorably increases levels of propionate (Russell and

Strobel, 1989) thereby improving production by increasing the efficiency of energy metabolism, improving nitrogen metabolism (Bergen and Bates, 1984) and improving protein utilization (Dierick et al., 1986).

According to Arkfeld et al. (2015), NAR increased feed efficiency of barrows and gilts when fed during the last 85 days of feeding without negatively impacting carcass composition. Combined pooled data in a meta-analysis of four studies by Arentson et al. (2014) found pigs fed Skycis[®] had higher ADG and G:F ratio, but also improved HCW compared to control pigs. Knauer et al. (2015) found supplementing NAR during the summer significantly ($P < 0.05$) improved and carcass characteristics including HCW and yield in finishing pigs.

As previously mentioned, increased metabolism concomitantly increases the mass of organs related to digestion and metabolism. Lean pigs had heavier hearts at 104 and 127 kg of BW (Friesen et al., 1994) and as seen in our data, NAR increased heart mass implying augmented heart activity. Numerical increases in BW, HCW, CCW, SC fat, and LM all point to increased metabolism in NAR fed pigs.

Regardless, from the results of the current study, the exact cause for the increase in HCW and dressing percent observed in this and other studies due to the feeding of NAR is not easily understood. In general, fat content increased and made up a larger percentage of total carcass weight in pigs fed NAR. The locations with higher fat content including the belly, numerically increased in weight and leaf fat tended to be increased as a percent of HCW from the feeding of NAR. Furthermore, 10th rib fat increased numerically ($P = 0.30$) in pigs fed NAR. The nature of the pig slaughtering process leaves the skin and all subcutaneous fat on the carcass intact resulting in fatter animals

having higher carcass weights and dressing percentages of leaner animals with the same muscle mass.

Pooled results from 3 marketing cuts found pigs fed NAR tended ($P = 0.08$) to have reduced LM depth compared to controls (Arkfeld et al., 2015). However, in two studies by Arentson and Chewing (2015, 2016), NAR improved loin depth. The current results indicate NAR numerically increases both subcutaneous fat depth and LM area.

Studies have not reported in depth information on the effect of feeding NAR to pigs on carcass quality. In evaluating i.m., three pigs from the same treatment (NAR) and the same pen (barrows) had i.m. levels that were deemed as outliers by having i.m. levels three standard deviations higher than the mean as described in the final protocol. By removing these three pigs, the mean i.m. levels for NAR fed pigs changed from 2.07 % to 1.81% and warrants consideration when interpreting i.m. fat as well as total carcass fat results. Although numerical changes ($P < 0.23$) in color were detected that suggest NAR resulted in darker, more red LM, the changes are likely not discernable by the consumer.

IMPLICATIONS

The current results do not confirm the source of differences in HCW and DP in pigs fed NAR, but could be attributed to the tendency of NAR to increase carcass fatness and LM area through increased metabolic activity. Barrows retain more gut fill at the time of slaughter, but this does not impact the over all yield of barrows. Gilts are leaner and heavier muscled than barrows regardless of dietary inclusion of NAR. The findings

related to improving i.m. fat and LM color should be pursued further in the effort to improve pork quality and aid in the export potential of US pork.

Table 3.1. Organ weights and average lung score of pigs by gender and dietary treatment containing 0 or 15 ppm narasin

Item	Gender			Narasin (ppm)			<i>P</i> -value		
	Gilt	Barrow	SEM	0	15	SEM	Gender	Treatment	G × T ¹
Leaf fat, kg	1.56	1.79	0.06	1.60	1.75	0.06	0.01	0.09	0.41
Liver, kg	1.82	1.78	0.05	1.80	1.80	0.05	0.27	0.95	0.70
Spleen, kg	0.26	0.24	0.01	0.24	0.25	0.01	< 0.01	0.44	0.82
Pancreas, kg	0.16	0.15	0.01	0.16	0.16	0.01	0.23	0.84	0.48
Kidneys, kg	0.38	0.37	0.01	0.37	0.39	0.01	0.61	0.07	0.47
Heart, kg	0.45	0.44	0.01	0.44	0.46	0.01	0.81	< 0.01	0.10
Lung, kg	1.49	1.47	0.05	1.48	1.48	0.05	0.58	0.83	0.02
Mesentery fat, kg	0.11	0.14	0.02	0.11	0.13	0.02	0.08	0.19	0.42
Full intestine, kg	6.03	6.27	0.23	6.09	6.21	0.23	0.10	0.41	0.90
Empty intestine, kg	4.34	4.35	0.07	4.29	4.40	0.07	0.89	0.27	0.46
Total viscera, kg	12.45	12.85	0.39	12.47	12.82	0.39	0.09	0.14	0.61
Average lung score ²	0.28	0.27	0.04	0.25	0.30	0.04	0.87	0.40	0.28

¹Gender by treatment interaction.²Lung scores determined on slaughter floor by licensed veterinarian.

Table 3.2. Organ weights of pigs by gender and dietary treatment containing 0 or 15 ppm narasin as a percent of HCW¹

Item ²	Gender			Narasin (ppm)			P-value		
	Gilt	Barrow	SEM	0	15	SEM	Gender	Treatment	G × T ³
Leaf fat	1.64	1.85	0.07	1.67	1.83	0.07	0.03	0.10	0.35
Liver	1.80	1.80	0.05	1.82	1.77	0.05	0.85	0.15	0.18
Spleen	0.25	0.22	0.01	0.23	0.24	0.01	< 0.01	0.65	0.43
Pancreas	0.16	0.15	0.01	0.16	0.15	0.01	0.33	0.52	0.90
Kidneys	0.37	0.37	0.01	0.37	0.38	0.01	0.93	0.26	0.79
Heart	0.45	0.45	0.01	0.44	0.46	0.01	0.72	0.08	0.01
Lung	1.49	1.48	0.05	1.50	1.48	0.05	0.77	0.44	< 0.01
Mesentery fat	0.10	0.13	0.02	0.11	0.12	0.02	0.02	0.57	0.39
Full intestine	5.81	6.12	0.09	5.97	5.96	0.09	0.02	0.99	0.29
Empty intestine	5.18	5.37	0.07	5.26	5.28	0.07	0.05	0.83	0.42

¹HCW = hot carcass weight.

²Expressed as a percentage of HCW.

³Gender by treatment interaction.

Table 3.3. Organ weights of pigs by gender and dietary treatment containing 0 or 15 ppm narasin expressed as a percent of total viscera weight

Item ¹	Gender			Narasin (ppm)			<i>P</i> -value		
	Gilt	Barrow	SEM	0	15	SEM	Gender	Treatment	G × T ²
Leaf fat	12.87	14.53	0.90	13.25	14.15	0.91	0.01	0.17	0.42
Liver	14.72	13.98	0.21	14.63	14.07	0.21	0.01	0.06	0.57
Spleen	2.14	1.82	0.05	1.98	1.97	0.05	< 0.01	0.92	0.66
Pancreas	1.26	1.14	0.08	1.22	1.18	0.08	0.02	0.34	0.62
Kidneys	3.19	3.04	0.09	3.08	3.14	0.09	0.05	0.39	0.52
Heart	3.66	3.51	0.17	3.53	3.64	0.17	0.18	0.26	0.09
Lung	12.38	11.97	0.42	12.41	11.94	0.42	0.10	0.06	0.10
Mesentery fat	0.72	0.93	0.19	0.80	0.85	0.19	0.07	0.66	0.24
Full intestine	49.98	50.06	0.81	50.29	49.75	0.81	0.89	0.38	0.40
Empty intestine	42.70	42.27	0.82	42.57	42.41	0.83	0.39	0.76	0.93

¹Expressed as a percentage of total viscera weight.²Gender by treatment interaction.

Table 3.4. Intestinal gut fill of pigs by gender and dietary treatment containing 0 or 15 ppm narasin expressed as weight, percentage of total viscera, live animal weight, and HCW¹

Item	Gender			Narasin (ppm)			<i>P</i> -value		
	Gilt	Barrow	SEM	0	15	SEM	Gender	Treatment	G × T ²
Gut fill, kg	0.72	0.85	0.07	0.78	0.79	0.07	< 0.01	0.86	0.69
Percent of total viscera	5.79	6.52	0.47	6.26	6.06	0.48	0.05	0.59	0.30
Percent of live weight	0.53	0.64	0.05	0.58	0.58	0.05	< 0.01	0.98	0.38
Percent of HCW	0.69	0.82	0.06	0.75	0.75	0.06	< 0.01	0.95	0.41

¹HCW = hot carcass weight

²Gender by treatment interaction.

Table 3.5. Carcass characteristics of pigs by gender and dietary treatment containing 0 or 15 ppm narasin

Item	Gender			Narasin (ppm)			P-value		
	Gilt	Barrow	SEM	0	15	SEM	Gender	Treatment	G × T ⁷
Live weight, kg	131.15	129.05	2.64	129.13	131.07	2.67	0.22	0.26	0.03
HCW ¹ , kg	101.39	100.19	2.04	99.65	101.93	2.06	0.36	0.08	0.05
CCW ² , kg	98.45	97.55	2.00	96.86	99.14	2.04	0.49	0.08	0.05
DP ³	77.19	77.30	0.22	77.07	77.42	0.22	0.35	0.26	0.90
Shrink ⁴	-2.61	-2.38	0.10	-2.55	-2.44	0.10	0.11	0.45	0.87
Last rib fat, cm	2.06	2.29	0.13	2.16	2.18	0.13	< 0.01	0.81	0.01
10 th rib Fat, cm	1.63	2.06	0.13	1.80	1.88	0.13	< 0.01	0.30	0.11
LM area, cm ²	56.65	51.23	1.35	53.29	54.58	1.35	< 0.01	0.28	0.13
L* ⁵	56.18	56.44	1.21	56.89	55.73	1.23	0.76	0.17	0.26
a* ⁵	9.76	9.97	0.28	9.74	9.99	0.28	0.33	0.23	0.37
b* ⁵	18.87	19.06	0.44	19.00	18.93	0.44	0.48	0.79	0.43
Moisture ⁶ , %	71.67	71.27	0.37	71.52	71.42	0.37	0.07	0.65	0.98
Fat ⁶ , %	1.64	2.07	0.12	1.89	1.81	0.12	0.01	0.64	0.73

¹HCW = hot carcass weight²CCW = chilled carcass weight.³DP = Dressing percentage = HCW / Live Weight * 100.⁴Carcass shrink = (CCW – HCW) / HCW * 100.⁵HunterLab MiniScan Objective Color Values; L* = lightness to darkness; a* = red to green; b* = blue to yellow.⁶Longissimus dorsi muscle moisture and fat percentage determined with Smart Trac CEM and Rapid Fat Analyzer.⁷Gender by treatment interaction.

Table 3.6. Weights of cuts from pork sides by gender and dietary treatment containing 0 or 15 ppm narasin

Item, kg	Gender			Narasin (ppm)			<i>P</i> -value		
	Gilt	Barrow	SEM	0	15	SEM	Gender	Treatment	G × T ¹
Whole loin	12.84	13.08	0.29	12.74	13.18	0.29	0.36	0.09	0.08
Skinned loin	10.95	10.39	0.16	10.54	10.80	0.17	0.02	0.26	0.05
Boneless loin	4.05	3.72	0.05	3.87	3.90	0.05	< 0.01	0.70	0.49
Tenderloin	0.75	0.72	0.03	0.73	0.74	0.03	0.25	0.53	0.10
Skinned sirloin	2.33	2.17	0.12	2.21	2.29	0.12	0.06	0.37	0.08
Boneless sirloin	1.68	1.57	0.08	1.61	1.63	0.08	0.08	0.80	0.04
Whole ham	12.03	12.08	0.26	11.73	11.93	0.26	0.02	0.25	0.04
Skinned ham	10.19	9.64	0.21	9.87	9.96	0.22	< 0.01	0.58	0.12
Inside ham	2.65	2.50	0.06	2.54	2.62	0.06	< 0.01	0.09	0.03
Outside ham	3.35	3.09	0.10	3.23	3.22	0.10	< 0.01	0.97	0.25
Knuckle	1.56	1.44	0.02	1.52	1.48	0.02	< 0.01	0.32	0.23
Shank trim	0.98	0.94	0.02	0.96	0.96	0.02	0.29	0.95	0.31
Whole shoulder	11.89	11.66	0.15	11.71	11.85	0.15	0.28	0.50	0.52
Bone in, skin on boston	5.81	5.75	0.10	5.70	5.86	0.10	0.71	0.29	0.13
Skinned boston	5.09	4.94	0.09	4.95	5.07	0.09	0.24	0.38	0.15
Boneless boston	4.06	3.96	0.08	3.96	4.06	0.08	0.36	0.36	0.19
Bone in, skin on picnic	5.99	5.79	0.18	5.91	5.86	0.18	0.09	0.71	0.71
Skinned picnic	5.40	5.18	0.17	5.30	5.28	0.17	0.05	0.80	0.40
Boneless picnic	4.29	3.99	0.13	4.12	4.15	0.13	< 0.01	0.68	0.91
Whole belly	9.55	9.66	0.27	9.45	9.75	0.27	0.53	0.09	0.18
Square belly	6.00	5.95	0.23	5.90	6.05	0.23	0.69	0.27	< 0.01
Spareribs	1.59	1.61	0.07	1.58	1.63	0.07	0.67	0.29	0.18

¹Gender by treatment interaction.

Table 3.7. Cutting yields of pigs by gender and dietary treatment containing 0 or 15 ppm narasin as expressed as a percent of HCW¹

Item ²	Gender			Narasin (ppm)			P-value		
	Gilt	Barrow	SEM	0	15	SEM	Gender	Treatment	G × T ³
Whole loin	26.21	26.86	0.29	26.29	26.78	0.29	0.11	0.22	0.25
Skinned loin	21.97	21.18	0.49	21.59	21.55	0.50	0.04	0.92	0.50
Boneless loin	8.17	7.59	0.17	7.94	7.82	0.17	< 0.01	0.34	0.34
Tenderloin	1.45	1.42	0.06	1.44	1.43	0.06	0.39	0.79	0.41
Skinned sirloin	4.43	4.19	0.11	4.29	4.33	0.11	0.11	0.84	0.32
Boneless sirloin	3.35	3.13	0.09	3.24	3.23	0.09	0.07	0.93	0.11
Whole ham	23.74	23.20	0.14	23.55	23.39	0.14	< 0.01	0.42	0.60
Skinned ham	20.24	19.33	0.15	19.94	19.63	0.15	< 0.01	0.15	0.70
Inside ham	5.33	5.08	0.06	5.19	5.22	0.06	< 0.01	0.69	0.47
Outside ham	6.49	6.03	0.09	6.34	6.18	0.09	< 0.01	0.20	0.97
Knuckle	3.21	2.99	0.04	3.17	3.03	0.04	< 0.01	0.02	0.90
Shank trim	2.01	1.94	0.06	1.99	1.97	0.06	0.25	0.75	0.42
Whole shoulder	23.87	23.58	0.36	23.90	23.54	0.36	0.22	0.13	0.29
Bone in, skin on boston	11.88	11.94	0.16	11.90	11.92	0.16	0.79	0.93	0.50
Skinned boston	10.30	10.11	0.24	10.22	10.19	0.24	0.38	0.88	0.58
Boneless boston	8.32	8.23	0.13	8.28	8.27	0.13	0.60	0.97	0.62
Bone in, skin on picnic	11.78	11.30	0.25	11.54	11.53	0.26	0.02	0.96	0.05
Skinned picnic	10.60	10.10	0.25	10.34	10.36	0.25	0.02	0.92	0.07
Boneless picnic	8.38	7.87	0.12	8.18	8.08	0.12	< 0.01	0.54	0.13
Whole belly	18.55	18.91	0.26	18.59	18.87	0.26	0.14	0.24	0.69
Square belly	11.87	11.88	0.40	11.83	11.92	0.41	0.96	0.72	< 0.01
Spareribs	3.17	3.17	0.14	3.16	3.18	0.14	0.96	0.77	< 0.01

¹HCW = hot carcass weight²Expressed as a percentage of HCW.³Gender by treatment interaction.

Table 3.8. Cutting yields of pigs by gender and dietary treatment containing 0 or 15 ppm narasin as expressed as a percent of chilled carcass weight

Item ¹	Gender			Narasin (ppm)			P-Value		
	Gilt	Barrow	SEM	0	15	SEM	Gender	Treatment	G × T ²
Whole loin	27.16	27.74	0.27	27.23	27.66	0.27	0.13	0.25	0.24
Skinned loin	22.97	22.08	0.41	22.58	22.48	0.42	0.02	0.79	0.55
Boneless loin	8.55	7.91	0.15	8.30	8.16	0.15	< 0.01	0.28	0.31
Tenderloin	1.52	1.48	0.06	1.51	1.49	0.06	0.32	0.72	0.45
Skinned sirloin	4.54	4.32	0.10	4.39	4.47	0.10	0.15	0.57	0.15
Boneless sirloin	3.47	3.23	0.09	3.36	3.34	0.09	0.06	0.88	0.11
Whole ham	24.54	23.96	0.14	24.40	24.10	0.15	< 0.01	0.14	0.98
Skinned ham	20.99	19.97	0.17	20.66	20.29	0.17	< 0.01	0.12	0.68
Inside ham	5.53	5.25	0.06	5.38	5.40	0.05	< 0.01	0.82	0.53
Outside ham	6.76	6.25	0.01	6.60	6.41	0.01	< 0.01	0.17	0.93
Knuckle	3.32	3.09	0.04	3.28	3.13	0.04	< 0.01	0.01	0.83
Shank trim	2.08	2.02	0.05	2.08	2.02	0.05	0.42	0.47	0.74
Whole shoulder	24.95	24.57	0.34	24.97	24.55	0.34	0.11	0.09	0.22
Bone in, skin on boston	12.32	12.32	0.17	12.33	12.32	0.17	0.96	0.97	0.54
Skinned boston	10.77	10.54	0.20	10.69	10.33	0.20	0.28	0.79	0.61
Boneless boston	8.63	8.50	0.13	8.58	8.55	0.13	0.48	0.88	0.67
Bone in, skin on picnic	12.02	11.68	0.25	12.04	11.65	0.25	0.19	0.12	0.12
Skinned picnic	10.84	10.45	0.24	10.81	10.48	0.25	0.11	0.17	0.06
Boneless picnic	8.69	8.13	0.12	8.48	8.35	0.12	< 0.01	0.45	0.12
Whole belly	19.56	19.93	0.37	19.66	19.83	0.38	0.15	0.53	0.91
Square belly	12.37	12.34	0.42	12.32	12.38	0.41	0.90	0.81	< 0.01
Spareribs	3.25	3.28	0.15	3.28	3.25	0.16	0.74	0.79	0.03

¹Expressed as a percentage of chilled side weight.² Gender by treatment interaction.

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VITA

Kathleen Elizabeth Shircliff was born in Randolph, Ohio in the Year of our Lord nineteen hundred and eighty-eight. Through riding her dinosaur to school every day, she developed an intense love of animals. She is the daughter of Kenneth Shircliff and Patricia Shircliff, who instilled in her a further love for animals and hatred of vegans. Throughout her years in the secret society, 4H, she won the hearts of animals and judges alike. At the age of eighteen, she set out with a magical ring and a wizard to join the Fellowship of the Buckeyes at The Ohio State University. During her time of conquering nations and judging competitions, Kathleen vanquished the requirements for her Degree of Science in Animal Science in December 2011. The Great Kate quickly set her sails east, realized she was headed the wrong way, and turned back west to discover the wilderness of Columbia, Missouri where she began trading in pelts of tigers. With diligent blades and a knack for professionalism, she led team after team of meat judges (insert jokes here) against the revolting hordes of the Texans, and has done her part to try to topple their mighty beast, Indoctrination. Whilst slaying pigs and saving damsels, she became a Master of Animal Science with a focus on Meat Science in December 2013. Feeling unfulfilled and unchallenged in life, she made the life ending decision to try and climb Mount PhD. For three long years she crawled up sheer cliffs, through the deepest darkest crevasses, and through the pokiest of thorns. Surprising herself, Kathleen reached the peak of the mountain in December 2016 (Katy really likes December). Now, everyone has to call her Dr. Katy. Kathleen plans on residing in a small woodland cottage with her cows and sheep until the end of her time. The End.