CHARACTERIZING THE AMOUNT AND VARIABILITY OF INTRAMUSCULAR FAT DEPOSITION OF PORK LOINS USING BARROWS AND GILTS FROM TWO SIRE LINES

BY

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THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Animal Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2020

Urbana, Illinois

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ABSTRACT

Two subsequent studies were conducted to characterize marbling variability in pigs. A total of 196 pigs (at 10 weeks of age) were raised in a 98 d growth study. Pigs were fed in a university finisher barn in pens of 4 pigs each. Equal numbers of barrows and gilts sired by boars targeting meat quality or lean growth were used. All pigs were fed the same corn-soybean diet formulated to meet or exceed nutrient requirements based on the 2012 National Research Council requirements of swine, for a 3-phase feeding program. All pigs were ultrasonically scanned at wk 10, 15, 20, and 24 of age to estimate marbling, back fat, and loin depth. Two or four contemporary pigs were scanned at each of these time points and then slaughtered on the following day to validate the ultrasound procedure. All trial pigs were slaughtered at 24 weeks of age, and loins were collected. Bone-in loins were sliced into 2.54 cm thick chops. Chops from the 6th rib, 10th rib, last rib and 4th lumbar vertebrae were analyzed for visual color and visual marbling, subjective firmness, extractable lipid (IMF) and moisture content, and Warner-Bratzler shear force (WBSF). Determination of marbling from ultrasound images in this trial was not an accurate indicator of marbling deposition throughout the growing phase. Ultrasonic values overestimated marbling in young pigs and reported a lesser range of values than was actually observed in market weight pigs. This could be due to technician error or use of the technology outside of the recommended scope of use. Data from the serial slaughter of young pigs did not validate ultrasonic marbling estimates, but it did provide valuable information about marbling deposition. The pattern of marbling with maximum values and minimum values observed along the length of the loin at early ages mimicked the pattern observed in market weight pigs. Even though intramuscular fat is often thought of as a tissue that develops later in life, the differences in marbling deposition along a loin were present beginning at the conclusion of the nursery phase. The applied meat quality portion of the study characterized variability of marbling

throughout the loin. Intramuscular fat was the greatest at the anterior and posterior chop locations, intermediate at the 10^{th} rib, and the least at the last rib. Visual marbling score was the least at the anterior location and the greatest at the posterior location. Chops from the 6^{th} rib location were the most tender and chops from the last rib area were the least tender. There were also differences in color score, firmness, moisture, and cook loss across the different locations. Therefore, marbling and tenderness varied among locations in a pork loin. Sex, sire line and anatomical location accounted for 47% of the variability in IMF. However, these factors only explained 14% of the total variability in tenderness. From these results the following conclusions can be made. Intramuscular fat is present and variable in pigs from a very young age, however quantification of marbling deposition throughout the growing period is difficult to estimate. Ultrasound imaging of a young pig is not ideal to identify pigs with a potential to be highly marbled. Mean differences for marbling and WBSF existed throughout the loin of market weight pigs, however marbling exhibited differences in variability within each of the annotated groups but WBSF did not. Therefore, marbling is variable, but how this might contribute to variability in eating experience warrants more investigation. As overall quality of a loin improves its variability also increases. As a producer improves meat quality, they will also increase the variability of that quality. This is not necessarily a bad thing if there is incentive for improved meat quality, but is important to be aware of when selecting for increased marbling.

ACKNOWLEDGEMENTS

The writing of this thesis and the work that it details would not have been possible without the help of a large group of people. I cannot begin to name everyone that has had a positive impact and assisted me over the last two years but would like to recognize a few. First off, I would like to thank Drs. Anna Dilger, Bailey Harsh, and Dustin Boler for their guidance, teaching, and friendship as my advisors. Drs. Chad Stahl and Jon Beever provided knowledge, encouragement, and humor that was vital as I worked through the scientific process. Without these five none of my education or research would have been possible. I would like to thank all my colleagues in the laboratory, undergraduate assistants, and Ben Peterson for the help in completing this project. I would like to thank God and my parents for the guidance and support that has put me on this path through life. Thanks to my older brother for providing me a role model and someone always willing to discuss research. On the other hand, I would like to thank my best friend Brayden and my girlfriend DeLaney for the daily conversations that covered everything in life, except research! In my time at the University of Illinois I have grown as a scientist, but more importantly as a person. I will always be thankful for the people that made that possible.

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CHAPTER 1: REVIEW OF THE LITERATURE

INTRODUCTION

Pork has historically been the most consumed meat in the world with a 40.1% market share in global consumption in 2018 (USDA, 2020). However, consumers' preference for the appearance of pork varies widely among countries and even from person to person within a contemporary group (Brewer et al., 2001; Ngapo et al., 2018). Ultimately it is the visual appeal of the fresh chop and sensory satisfaction of the eating experience that drives purchasing decisions and encourages consumers' repeat purchases of pork (Cannata et al., 2010). Domestically, pork consumption lags behind beef and chicken, ranking third among meat options. Due to this competition, and pork consumption declining as a function of total meat consumed, the U.S. pork industry must continue striving to produce a desired product.

One trait that plays a role in pork selection at the retail level is marbling. Marbling or intramuscular fat (IMF) is a variable trait in pigs that plays a role in consumers' acceptability of pork products, but the magnitude of its role has been different from study to study. Marbling is the fat deposited within the perimysial connective tissues around and between muscle bundles (Gerrard and Grant, 2006). It is important in determining texture and flavor in most meat products (Nishimura, 2010), and is commonly associated with the desirability of pork chops (Brewer et al., 2001), both positively and negatively. The USDA has emphasized marbling and color as the primary traits for categorizing pork (USDA, 2017). However, neither of these traits have demonstrated predictive ability of eating quality independently (Wilson et al., 2017), but characterization of both traits in conjunction with each other represents potential in ensuring a pleasant eating experience.

Existing literature tells a conflicted story about marbling's effect in pork. In one study, chops with high (3.46%) marbling were less likely to be selected by consumers but also were rated as more desirable by those same consumers in a cooked evaluation (Brewer et al., 2001). There are more studies that confirm the positive influence of marbling on chop tenderness and juiciness (DeVol et al., 1988; Cannata et al., 2010; Font-i-Furnols et al., 2012), however there are also studies that reported no effect of IMF level on sensory characteristics or instrumental tenderness (Rincker et al., 2008; Wilson et al., 2017). These conflicting results could be attributable to differences in genetics, harvest processes, taste panel sensitivity, or other unknown factors, but they provide little guidance in the search for a consensus "ideal chop". Regardless of consumers' preference for an optimal IMF content, it remains important to characterize the differences that do exist.

Within a population of pigs raised and processed under similar conditions, marbling can be highly variable from pig to pig due to differences within sex and sire line (Overholt et al., 2016; Arkfeld et al., 2017). Another challenge of using intramuscular fat in the evaluation of loin quality is that the amount of IMF within a loin is known to vary considerably from the anterior end to the posterior end of the loin (Faucitano et al., 2004; Homm et al., 2006). It is also known that 10^{th} rib fat thickness is correlated (0.38; $P < 0.01$) with visual marbling scores (Huff-Lonergan et al., 2002). This could indicate that targeting the greatest amount of marbling by selective breeding and pushing pigs to fatter levels will also create the greatest amount of variability of marbling in those pigs. Most previous work has focused on a single anatomical location or ribbed chop face of a contemporary population, rather than characterizing the variability of marbling throughout the entire loin from different types of pigs. Another area of interest is to determine when marbling is developed. Technologies exist that are designed to predict the amount of IMF and other carcass characteristics in growing and finishing livestock. These tools have been tested and used in scientific literature with varied success.

Knowing how the amount of marbling varies in different types of pigs and when it develops in the growing pig will allow for future decisions within the swine and pork industries. This review will discuss the information that is known from current literature centered on what marbling is, how it develops, factors affecting its deposition, ways to predict it, and the effect that it has on eating experience.

PIG GROWTH PATTERNS AND MARBLING DEPOSITION

The three primary carcass tissues in livestock are deposited at different rates and at different times throughout the growth period. Bone, muscle, and fat, respectively, follow a sequential hierarchy of development in growing animals. The body's general prioritization of energy use dictates that calories first go towards the needs of bone formation, then muscle growth, then fat development. The rate of bone and muscle development starts faster and peaks earlier in the growth curve then plateaus towards physiological maturity, while fat accumulation accelerates during later growth stages (McMeekan, 1940). As an animal's live weight increases through the feeding period, the relative amount of muscle and bone in a carcass decreases while fat levels increase (Richmond & Berg, 1972; Bruns et al., 2004).

The growth process sets a relatively predetermined structure for the developmental timing of adipose tissue, although external influences have demonstrated some ability to alter this pattern. Fat development is further organized into specific fat depots which are filled in a somewhat pre-determined order; visceral, subcutaneous, intermuscular, and then intramuscular fat, which is the last to begin developing in livestock species (Gerrard and Grant, 2006).

Intramuscular fat is adipose deposited within the perimysium in the muscle (Nishimura, 2010), and is composed of phospholipids, triglycerides, and cholesterol. The triglycerides are primarily (~80%) stored in the muscle adipocytes found between fiber bundles and individual fibers, while a minor proportion is stored as lipid droplets within myofibers in the cytoplasm. Between muscles, the phospholipid content is relatively constant (approximately 1% of fresh muscle in pigs), whereas the muscle triglyceride content is variable between muscles and species (Fernandez et al., 1999). Increased intramuscular fat typically reflects an increase in total carcass fat (Kauffman et al., 1964). In a population of purebred Duroc barrows selected for marbling and raised past a typical slaughter point to 32 weeks of age, longitudinal data demonstrated that IMF content increased linearly with age at a rate of 0.05% per day from 160 to 220 days of age (Bosch et al., 2012). Back fat deposition followed a negative quadratic trend, but IMF remained linear. Authors of the study stated that a delay in age at slaughter may enhance IMF content, as results demonstrated that growing pigs to an older age could help to break the linear relationship between IMF and back fat. In another group of Duroc pigs, serial ultrasound displayed a linear relationship between back fat and body weight at 73 kg – 118 kg, however a curvilinear deposition pattern was observed for loin muscle area and IMF (Schwab et al., 2007). The greater marbled group of pigs exhibited a greater daily accretion pattern of IMF early in the test period (73 kg – 86 kg) compared to the less marbled pigs which deposited IMF in a more linear fashion throughout, although both groups demonstrated that IMF increased as a component of growth throughout the entire period. In beef, a 5% increases in carcass fat generally corresponds to a 1% increase in IMF content (Hocquette et al., 2010). A serial slaughter study conducted on Angus beef steers demonstrated that marbling was developed at a greater rate than subcutaneous fat early in the finishing phase (HCW < 300 kg), but by the end of the finishing phase (HCW > 300 kg) back fat was increasing at a faster rate than marbling (Bruns et al., 2004). Therefore, IMF content steadily increased with carcass weight, however the rate at which IMF was deposited actually decreased over time, concluding that IMF can begin to develop earlier in a typical high-energy feeding program. These trials suggest that marbling was typically a late developing tissue in the studied livestock, but a specific timeline for the amount of IMF deposited can vary due to species, breed, and other factors.

Substantial evidence is still lacking for the biological mechanisms behind intramuscular fat content differences in pigs. Most studies that have investigated adipocyte development involved diet manipulation, which undoubtedly confounds the conclusions. Even once treatment effects have been accounted for there have been multiple explanations for what is causing the differences in marbling development. Variability in IMF is linked to the number and size of intramuscular adipocytes developed through the growing and finishing periods. However, the relative contributions of adipocyte hyperplasia and hypertrophy to increased IMF deposition is unclear. In a group of lean type crossbred pigs, increased IMF was associated with greater intramuscular adipose hypertrophy rather than an increase in adipocyte number (Barnes et al., 2012). In contrast, a study using Large White x Duroc barrows attributes the differences in IMF to variation in adipocyte hyperplasia with fewer differences noted in adipocyte size (Damon et al., 2006). Listrat et al., (2016) reported that in pigs and cattle, variation in IMF content of a certain muscle between animals is associated with variation in hyperplasia, but when that marbling change comes from a diet manipulation the resulting variation in IMF is credited to hypertrophy. In a group of Iberian barrows, a large number of preadipocytes were observed in muscle tissue during early growing (35.8 kg) but that number actually decreased through finishing (158 kg) (Ayuso et al., 2015). This could be an illusion of proportions, as it has been demonstrated that increasing total muscle mass can decrease perception of IMF percentage, as it dilutes fat content relative to muscle volume (Kauffman et al., 1968; Hocquette et al., 2010). Many congenital differences in the populations used could have led to the disparity in these results. Early events that influence adipogenesis in the muscle such as proliferation and differentiation of adipose cells, and the connective tissues embedding adipocytes, likely led to differences in IMF content in the pig at slaughter age.

There is evidence that IMF deposition is related to the proportion of fiber types in a muscle. Fiber types are classified by a combination of metabolic, contractile, and physiological characteristics. They are commonly referred to as red (type I; slow, oxidative), intermediate (type IIA; fast, oxidative and glycolytic), or white (type IIX and IIB; fast, glycolytic) fibers (Gerrard & Grant, 2006; Lee et al., 2010). Typically, marbling is associated with more oxidative muscle fibers as lipids are mainly stored around type I and some type IIA fibers (Essén-Gustavsson et al., 1994; Aberle et al., 2012). Red muscle better promotes IMF development because nearby blood vessels aid in intramuscular adipocyte growth (Hocquette et al., 2010). Microscopically, marbling is a specific adipose depot with adipocytes embedded in a connective tissue matrix in close proximity to a blood capillary (Hocquette et al., 2010). In highly marbled loins it becomes apparent that the intramuscular adipose tissue is formed between muscle fiber bundles, where it disrupts the honeycomb structure of the endomysia and separates the perimysium into thinner collagen fibers (Nishimura, 2010). The accretion of IMF has also been inversely linked to muscle growth rate, which means typically a muscular animal with a greater proportion of glycolytic fibers has reduced IMF development (Hocquette et al., 2010). In another study, no correlation ($r = -0.05$ to 0.04; $P > 0.05$) was found between IMF and any fiber type percentage (Larzul et al., 1997). These pigs, however, were French large white pigs slaughtered at 100 kg BW. The biochemical and structural foundation of muscle fibers, intramuscular connective tissue, and intramuscular fat do appear to have somewhat independent functions and developmental patterns (Listrat et al., 2016), which suggests that the properties of these various muscle components can possibly be manipulated and improved by genetic selection independent of one another, to improve meat quality.

EFFECTS OF BREED, SIRE LINE, AND SEX

Pork quality is heavily influenced by the differences between sexes, breeds, and specific sires, and has been well documented in literature (Martel et al., 1988; Ellis et al., 1996; Klont et al., 1998; Overholt et al., 2016). The proliferation, differentiation, and lipogenesis of primary cultured porcine pre-adipocytes from different depots of different pig breeds are distinct (Zhang et al., 2014). Breed of sire has been proven significant in its influence on muscle type and IMF level (Judge et al., 1959; Ellis et al., 1996), and IMF has been found to be moderate to highly heritable ($h^2 = 0.61 – 0.79$) in pigs (Hovenier et al., 1992; Ros-Freixedes et al., 2014; Cabling et al., 2015). Of all the pig breeds used in commercial production, Durocs are considered to provide producers with the desired economically relevant traits of growth performance and feed efficiency, and still maintain a respectable level of carcass quality (Kauffman et al., 1968; Ellis et al., 1996). Duroc and Berkshire sires have been used to increase marbling in an effort to increase eating quality of the offspring. Tests designed to compare the effects of Duroc and Berkshire breeds to crossbred lines demonstrated that Duroc and Berkshire sired pigs have greater marbling, and that IMF was associated with improved tenderness and juiciness scores in the longissimus muscle (Wood et al., 2004). In a direct comparison between these two breeds, Duroc and Duroc-cross progeny had greater IMF content than Berkshire and Berkshire-cross pigs (Suzuki et al., 2003). When bred to lean lines in a crossbreeding system, the Duroc breed increased IMF (Ellis et al., 1996). When tested against a Pietrain × Large White cross, Duroc sired progeny had increased IMF (3.4% vs. 2.7%; $P < 0.01$) while maintaining similar levels of back fat ($P > 0.10$), and Duroc sired pork chops were instrumentally more tender ($P < 0.05$) (Latorre et al., 2003). Duroc based pigs also produced loins that were visually darker and more marbled when compared to Pietrain type pigs (Ellis et al., 1996; Edwards et al., 2003). Even in papers demonstrating the positive effects of saturated fat diets on IMF levels (D'souza et al., 2003; Olivares et al., 2009), the authors stated that genetics played a greater role in IMF level than the diet.

Selective pressure has identified and built upon more specialized genotypes of sire lines with specific purposes, in an effort to add value (Schwab et al., 2007). This creates another factor to consider within breed, such as boar lines bred for exceptional meat quality or selected for maximum growth and carcass leanness. The polarity of the different markets that receive U.S. pork creates a need for both lean growth and meat quality focused hog production, and Duroc based pork is often used to supply the needs of a quality focused market like Japan (Ngapo et al., 2012). A study using Duroc boars from two different time periods found that progeny from the (then) current time period (2000s; aimed at lean growth) boars deposited more loin muscle depth and less IMF per kg of BW gain than pigs sired by old time period (1980s; aimed at meat quality) boars (Schwab et al., 2007). These authors concluded that increased carcass leanness accomplished over time was not accompanied by equivalent improvements in growth rate, and actually came at the expense of meat quality traits, especially IMF. From a research standpoint, the genetic makeup of the pigs in discussion must be known and understood in order to make sound comparisons and draw proper conclusions.

Barrows and gilts make up over 96% of federally inspected hog slaughter in the U.S. (USDA, 2020), and these two have consistently exhibited differences in carcass characteristics and IMF deposition in previous literature (Martel et al., 1988; Ellis et al., 1996; Overholt et al., 2016). Barrows are typically fatter than gilts, and therefore the traits associated with fatness, such as IMF, often differs between the sexes (Latorre et al., 2003). Sex has an effect on composition of the carcass which then influences fresh pork products such as loin chops. Loin muscle from barrow carcasses have repeatedly had more visible marbling and greater IMF content than gilt carcasses (Judge et al., 1959; Unruh et al., 1996; Latorre et al., 2003; Overholt et al., 2016). It is generally accepted that the differences in IMF between sexes is attributed to the rate of maturity, as barrows tend to grow to physiological maturity at a faster pace than gilts and therefore begin to deposit intramuscular fat at a younger age than gilts (Lee et al., 2013).

Although not reviewed here, there are many other factors that influence pork quality, and a few that could affect IMF specifically, including diet, age, weight, etc. Ante-mortem handling and post-mortem processes are well known to have an impact on pork quality due to the issues that can arise in the conversion of muscle to meat, mainly deviations in pH and temperature (Aberle et al., 2012), however it is important to note that they will not affect intramuscular fat content and variability (Witte et al., 2000).

ANATOMICAL LOCATION AND LOIN VARIATION

The pork industry and the utility of the pork loin depends on consistency of product and experience. However, it is known that the meat quality parameters of one animal does not represent every animal within its contemporary group, and the loin quality of an animal may not represent the quality of all muscles within that single carcass (Arkfeld et al., 2017). Previous literature even reports that objective measures of the chop face designated to represent an animal could vary considerably from other chop locations within that same loin on that same animal (Faucitano et al., 2004; Homm et al., 2006). Although the chemical components of meat remain relatively constant (75% water, 19%-20% protein, 1%-2% mineral), the lipidic part is highly variable among species, between individuals of the same species, and among muscles and cuts (Hocquette et al., 2010).

The first source of variation to address is the deviation among individuals within a contemporary group. Marbling can be highly variable from pig to pig, and the differences between animals raised under similar conditions is likely caused by differences in various intrinsic (genetic) and extrinsic (environmental) factors (Klont et al., 1998). In a population of over 7000 pigs (Overholt et al., 2016) barrows had greater variances in marbling score than gilts and pigs bred for meat quality had greater variances for marbling than pigs selected for lean growth. Animals with greater marbling are generally going to be more variable in terms of that marbling (Overholt et al., 2016). It has been previously reported that variability of visual marbling on the ventral surface of the loin could be attributed to sex (12%), production focus (39%), and biological variation (49%) among individual pigs. The greatest percentage of variation in carcass quality was due to factors not accounted for in normal marketing practices, here called biological variation. Seasonality and marketing groups did not contribute to visual marbling variability (Arkfeld et al., 2017). Variation in IMF and other meat quality characteristics increases the number of carcasses within a single marketing group that fall outside of premium qualifications and miss fresh quality specifications (Arkfeld et al., 2017).

Within a carcass there can be a substantial amount of variation in IMF found among different muscles. Marbling can be costly and destructive to measure on a carcass depending on which muscle and sampling location is chosen. Standard ribbing of a carcass (cutting between ribs to expose the longissimus) lessens the value of an entire primal, so there is interest in using a different area of the loin or a different muscle all together for quality measurement. Knowing how each muscle correlates with one another is vital to enable optimization of sampling techniques. Sampling the pork longissimus can be difficult to obtain and costly, whereas a large sample of gluteus medius can be more easily obtained in a commercial setting. Selection for IMF in the gluteus medius led to positive results in the longissimus and vice versa (Ros-Freixedes et al., 2014). These two muscles can be used alternatively for selection purposes, however neither of them produced strong phenotypic correlations (r = 0.21, longissimus; r = 0.15, gluteus medius) with the semimembranosus. Work in beef carcasses also reported that there was a wide range of IMF among muscles (4.4%-16.1%), but that the intramuscular fat content of all muscles was related linearly with the IMF of the longissimus, reporting a strong coefficient of determination (R^2 values ranged from 0.67 to 0.84) (Brackebusch et al., 1991). Therefore, authors stated that the loin can be used to predict the relative IMF content of other muscles with confidence (Brackebusch et al., 1991). Klont et al. (1998) confirms that considerable variation in meat quality characteristics between various muscles within a carcass exists, and this inter-muscle variation is often directly related to the metabolic and contractile properties, as determined by their muscle fiber type distribution. However, Arkfeld et al. (2017) reported that loin quality is not necessarily indicative of ham or belly quality on the same carcass. This suggests that there may be value in a system such as the Australian Meat Standards, that gives eating grades on an individual cut basis (Konarska et al., 2017; MSA, 2020).

Subcutaneous fat thickness has correlated with IMF in the longissimus (r = 0.32) and gluteus medius (r = 0.29), and is easily determined on a hanging carcass (Ros-Freixedes et al., 2014). Traits associated with fatness differ in variability between sexes due to inherent sex effects on fat deposition. Carcass fat depth is known to be correlated with marbling score, but only 1.6% of the variation in marbling score was explained by fat depth (Jones et al., 1994). Another variable to consider is the side of the carcass in which a muscle or sample is obtained, and whether that also introduces variation. Breidenstein et al. (1964) reported no significant differences in loin muscle area or IMF content between opposite pairs of any muscles sampled. They did confirm large differences in fat content between muscles on a single side, as discussed above, however they conclude that bilateral asymmetry does not exist and variation between sides can be attributed to experimental error.

Specifically focusing on the longissimus, many studies have reported variations in chop size, shape, and quality when observed at different loci within a single loin. In a study consisting of 50 commercial pork loins sliced every 2 cm from the 5th thoracic vertebrae to 3rd from last lumbar vertebrae, each chop was analyzed for fat content in order to understand IMF distribution and to identify the best predictive site on the loin. The greatest IMF content and marbling scores were observed in the middle section of the thoracic region and the middle caudal portion of the lumbar region. The poorest IMF content was found in the middle of the sample region around the thoracic lumbar junction (last rib). Repeated measure analysis demonstrated that IMF content varied significantly along the longissimus. Specific location did not yield significant interaction effects with sex of pig (Faucitano et al., 2004). Another study reported that the longissimus thoracis (anterior) was the juiciest and most tender, while the lumborum (posterior) portion had intermediate quality, and the mid-loin exhibited the poorest quality (Van Oeckel et al., 2003). Authors stated that a low IMF content throughout the entire loin yielded no differences ($P \geq 0.05$) in IMF by location but noted a slight tendency for less fat content in the middle portion when compared to both ends. A study examining the 7th rib, 13th rib, and 6th lumbar vertebrae locations of pork loins for IMF followed a similar pattern, finding that the 6th lumbar chops contained a significantly greater fat content than the other two, while the 13th rib chops were the lowest, and 7th rib chops were intermediate (Carpenter et al., 1961). Homm et al., (2006) evaluated location effects on both 8-rib and 11-rib center cut boneless loins. On the 8-rib loins (approximately 7th rib to the hip bone), visual marbling was the greatest on both ends, and the least marbled at the intermediate locations. Similarly, IMF was the greatest in the most anterior chops, and the least in the middle chops. The 11-rib boneless loins (approximately 5th rib to the hip bone) yielded comparable results, with the greatest marbling scores coming from the posterior end of the loin (chops collected 2.5 cm and 7.6 cm from the break point). Chops from both the anterior and posterior ends of the loins had greater percentages of extractable lipid than chops from the middle. Lowe et al., (2011) reported analogous results to these above, as loins became lighter in color, firmer, and contained more marbling moving from anterior (5th/6th rib interface) to posterior (loin/sirloin junction). The authors also characterized changes in chop shape throughout the loin, reporting that the posterior portion had the largest loin muscle area and greatest loin width, while the anterior portion had a greater ratio of depth to width ratio and depth to depth ratio, indicating a smaller, rounder chop face. In closure, there are many changes in the longissimus that occur along the length of the loin that stand to compromise the uniformity of all boneless pork chops derived from a single loin.

A strong implication of these studies is: picking an appropriate site for grading and evaluation may be a challenge yet is vital to accurate characterization of pork quality. The work mentioned earlier by Faucitano et al., (2004) found that thoracic vertebrae 10-12 demonstrated the greatest coefficient of determination (R^2 = 0.94-0.95) with the entire longissimus average IMF content, meaning that somewhere in this area would best represent the composite quality of the entire loin. Carpenter et al. (1961) confirmed that the 7th rib and 13th rib locations are not different than the composite average of the whole loin, therefore somewhere between the 7th and 13th ribs should be used to best represent the entire loin. Van Oeckel et al. (2003) maintained that that the mid-loin is the best reference place for evaluation, however in this case that location is chosen because it was found to have the least marbling. A slightly different report stated that much like different muscles, there is substantial variation among different locations within a loin, but they demonstrate strong correlation with one another (Carpenter et al., 1961). The authors conclude that the specific sampling location chosen may not be as important as strictly following a standardized sampling procedure.

A certain chop may be the best representative of the average fat content of the entire loin, but knowing the previous literature discussed at the top of the section tells us that the average of the loin is not representative of each chop that loin will produce. Knowing how mean differences and variability changes throughout the loin should allow for a more thorough inference of whole loin characteristics based off a single location. The next step to developing this knowledge would be to characterize variation within the loin of pigs from different genotypes and of different sexes.

PREDICTIVE TECHNOLOGY

The importance of marbling discussed throughout this review has created an equally valuable need for technology that can predict the amount of IMF in a live animal. When searching for a tool to use for this purpose, the technology must be accurate and efficient, and it has to be an affordable system that is practical within a production setting. I will discuss the efficacy of some of the technologies that have been tested in academic research, and in some cases implemented in commercial production. Ultrasound is the most common on-farm technology, and has been reported to be an accurate method for estimating loin measurements and back fat, and more recently IMF in live pigs (Ragland et al., 1998; Newcom et al., 2002). Ultrasound is sound waves travelling too fast for the human ear to detect. There are different types of ultrasound machines, but they all operate with sound waves bouncing off different tissues at different rates. Ultrasound has long been used in the medical field, but the introduction of brightness mode (b-mode) ultrasound machines with real-time imaging created a practical use for this technology in livestock industries (Schulte et al., 2011). When ultrasonic waves hit dense carcass tissues or the interface between two tissues, some of the wave lengths will reflect back to the transducer while others penetrate further. Different structures in the soft tissues such as IMF causes ultrasonic waves to scatter which produces a graininess on the image in b-mode ultrasound images (Kim et al., 1997). This texture allows a technician to decipher different tissues from the ultrasonic image. Fat tissue is a good reflector of high frequency sound waves, so ultrasound has become the primary tool (other than visual appraisal) for estimation of IMF in a live animal (Schulte et al., 2011). Marbling gives a distinct pattern in ultrasonic images, so it can be objectively estimated by texture analysis algorithms. (Kim et al., 1997). Through the use and development of texture analysis software, selection for IMF using real-time ultrasound can be used in pigs (Schwab et al., 2009).

Common practice for drawing an accurate IMF estimation from ultrasonic imaging includes capturing a minimum of four longitudinal images, with the transducer placed approximately 7 cm from the midline across the 10^{th} to 13^{th} ribs. A trained technician uses image analysis software to interpret the images. For validation of this process, a chop from the area of the 10^{th} rib of previously scanned pigs was collected post-mortem for chemical IMF determination. When compared to Yorkshires, Duroc pigs provided the best validation results for the model. Durocs produced a relatively strong ($r = 0.60$) Pearson correlation coefficient between estimated IMF and chemical IMF. Authors concluded that real-time ultrasound can be used to estimate IMF in swine (Newcom et al., 2002). Bahelka et al. (2009) further tested the use of ultrasound for IMF determination by testing the accuracy of prediction at different intensity levels. This was performed on cross-sectional images at the last rib. Five intensity levels were tested, ranging from 70% to 90% of total amplifying of sonograph, using an Aloka ultrasound machine. Although not unanimous in favor of one, 75% and 80% intensity was proven most suitable, as the higher intensity levels overestimated IMF. Authors recommended grouping values into bins of marbling scores, rather than analyzing an absolute value. This method of using groups or bins is supported by Newcom et al. (2002), who reported that ultrasound IMF values did not reach the extremes actually seen in the pigs, maintaining a smaller range of IMF values than the subsequent proximate analysis realized. It could be argued that ultrasound is not proficient in doing what breeders expect of it: to identify population outliers to use for genetic progression. Still, correlations ($r \geq 0.60$) have been demonstrated between chemical (CIMF) and ultrasound (UIMF) determined IMF, and UIMF was highly heritable ($h^2 = 0.48$), so authors confirm that UIMF can be used in place of CIMF, and ultrasound is a reliable process to improve pork quality (Newcom et al., 2002; Jung et al., 2015). Another method of predicting IMF in live pigs that has been performed in previous literature is biopsy sampling. A study collected 1-3 biopsies from the longissimus dorsi of live pigs and determined IMF content via quantitative gas chromatography after direct transesterification. This method was performed due to small tissue sample size (< 1g) (Bosch et al., 2009). Biopsy sampling overestimated IMF with decreasing numbers of samples. This is most likely due to the possible contamination of samples with subcutaneous fat. Repeated biopsy to track IMF development with age is limited by high sampling variance and potential bias, but this method could still hold an advantage over serial slaughter, especially from financial and animal welfare standpoints. Computed tomography (CT) is another technology developed in the medical field that has been adapted and introduced into livestock selection systems. Computed tomography provides the best results for estimation of fat and lean in pigs, but accuracy for IMF is not acceptable at this point according to Carabús et al., (2016) and Font-i-Furnols et al., (2019). Additional downfalls of this technology include a high cost, lengthy process, and the need for anesthetics, all of which detract from its on-farm practicality. There is clear value in a non-invasive and cost effective way to accurately estimate IMF in swine. Ultrasound is currently the most reliable and accessible method, and therefore the most commonly utilized in the industry. The importance of prediction ability for genetic selection and slaughter optimization should push the industry to continue refining current technologies and developing new technologies to accurately and affordably estimate the composition of live pigs.

There is also work towards developing technologies to predict the IMF content throughout an entire intact loin after fabrication of a pork carcass. Once again, marbling can be non-invasively analyzed by computed tomography on the whole loin. In this case, the use of ordinary linear regression with data from tomograms demonstrated success in prediction of IMF (R^2 = 0.83) (Font-i-Furnols et al., 2013). Near-infrared spectroscopy (NIR) performed on a loin has also been successful in predicting marbling scores along the entire longissimus. Using a NIR image of the rib end has demonstrated strong prediction ability (R_p = 0.89) (Huang et al., 2017), which would allow for a study of the distribution of IMF throughout the loin without sampling individual chops. If the desired objective is evaluation of a whole boneless loin to predict retail chop quality, previous studies have demonstrated that a subjective evaluation of the ventral surface of fresh boneless loins is successful in determining aged chop quality. Lowell et al., (2017) reported that early ventral marbling correlated (r = 0.57, barrows; r = 0.59, gilts) with aged (14 d) chop marbling. Subsequent work confirmed that early visual marbling is strongly correlated (r = 0.84) with aged chop marbling in Duroc pigs (Lowell et al., 2018). A limitation of this study considering the IMF variation discussion in the previous section, is that ventral quality was only compared to a single location of approximately the 10^{th} rib chop face. A transitive conclusion of this work in conjunction with studies centered on IMF differences by location, may allow for inference into the relationship between ventral marbling and the marbling of each chop within that loin.

EATING EXPERIENCE

The pork industry places emphasis on increasing palatability, which is driven by three sensory traits: tenderness, juiciness, and flavor. Each contributes differently to how a consumer evaluates pork, and the amount of emphasis placed on each varies based on individual preference (Cannata et al., 2010; Murphy et al., 2015). Palatability is very important because it influences a consumer’s choice of protein and impacts repeat purchase decisions. In the United States, color and marbling are the visual cues most determining of purchasing decisions, however tenderness is the most important attribute for a satisfactory eating experience (Cannata et al., 2010). Consumers use fresh color and marbling as indicators for tenderness and juiciness (Wood et al., 2004; Lonergan et al., 2007), so the pork industry must continue to value those traits in an effort to please consumers. Consumer preferences for pork varies in different countries, and due to the U.S. export capacity, it’s pork industry must facilitate preferences for all who consume its pork. The U.S. is a primary supplier of pork to Japan, who greatly values eating quality and product specifications (marbling and color) when selecting pork, while Mexican consumers prefer very lean pork (Murphy et al., 2015; Ngapo et al., 2018). The role of marbling specifically, in consumer preference and eating quality is a topic of much debate. There is no “typical” consumer (McGill, 1983), which leaves a task of targeting standards that do not really exist. Visible IMF in meat is considered both as a positive or a negative quality criterion depending on the country and specific consumer in question (Hocquette et al., 2010). Marbling is a visual factor that is pleasing to some consumers who associate it with flavor and juiciness, while others may health-consciously object to any visual fat in the meat they buy (Aberle et al., 2012). Studies have identified some consumers that select purposely for marbling due to its perceived benefit to eating quality, while many consumers consistently select against any IMF because of perceived health consequences (Cannata et al., 2010; Font-i-Furnols et al., 2012). Historically, tenderness has been associated with the amount of IMF in a product. A pork loin or chop with a greater amount of IMF is expected to be more tender and provide a better eating experience (Brewer et al., 2001; Wood et al., 2004). This relationship is disputed though, as much literature has reported no association between IMF and any improvement in eating quality, while some suggests there is a certain threshold that must be met and after that improvement plateaus.

Meisinger (1996) reported that consumers can discern differences between levels of pork loin quality. The amount of marbling is a factor consumers regularly use to assess the value and quality of a product (Brewer et al., 2001). One study split consumers into ‘lean loin lovers’ or ‘marbled loin lovers’ based on their visual preferences of chop quality. However in a cooked evaluation all consumers gave greater scores for overall acceptability to group 4 chops (5.78% ± 0.19 IMF) over group 3 (3.72% ± 0.26), both of which scored more acceptable than groups 2 and 1 (2.11% ± 0.07 and 0.96% ± 0.30, respectively) (Font-i-Furnols et al., 2012). In a study using trained panelists, chops with the greatest IMF level (3.56%) scored the highest for sensory tenderness and juiciness over chops containing low IMF levels (1.96%), while chops with medium IMF levels (2.50%) were intermediate (Cannata et al., 2010). Marbling demonstrated a positive correlation (r = 0.49; $P < 0.05$) with sensory tenderness but was not correlated (r = -0.19; $P > 0.05$) with slice shear force in this data. Another study, however, demonstrated that more visible marbling was associated with juicier and more acceptable meat with a lesser numerical shear force (Ellis et al., 1996). This association has been explained by intramuscular lipid's arrangement around muscle bundles , which causes the weakening of cross linkages between collagen fibers where the IMF infiltrates (Fortin et al., 2005). Intramuscular fat serves as a lubricant between the muscle fibers and may consequently improve juiciness. This intramuscular fat disruption could improve tenderness and flavor directly, or those traits may simply be rated higher psychologically just because the product is more juicy (Kauffman et al., 1964).

Other sources realize there is little evidence to demonstrate a strong and direct positive influence of marbling on instrumental tenderness, believing it likely just acts as a lubricant in chewing of less tender meat and improves the perception (Aberle et al., 2012). In response to the previously discussed experiments, recent work has reported no causal association between marbling and tenderness (Rincker et al., 2008). In that study, marbling did not influence eating quality or shear force within a single genetic line, even when cooked to a well-done degree of doneness. Chops for the study include an IMF range of 0.76% to 8.09%. Likewise, Wilson et al., (2017) reported no effect of IMF level on instrumental tenderness, and trained panelists could not detect a difference in tenderness. Chop IMF from 0.80% to 5.52% was used, and it was determined that marbling explained less than 1% of the variation in sensory tenderness of pork loins cooked to a medium-rare degree of doneness (63°C). An inconsistent effect of marbling score on sensory characteristics has been reported within pH classes. Increasing lipid tended to increase sensory tenderness of loins with an ultimate pH between 5.50 and 5.80 but did not affect sensory tenderness in loins with lesser or greater ultimate pH values (Lonergan et al., 2007). Marbling was not a significant source of variation in juiciness within any pH class. Authors concluded that IMF was a partial source of variation in texture and tenderness of pork with an intermediate pH level, but not at a greater or lesser pH range. Lipid content influences pork sensory quality only at intermediate pH and increasing IMF will not consistently improve the eating quality of low pH pork which has poor eating quality or high pH pork which has superior eating quality.

Another set of work has established a threshold or minimum level of IMF that must be met for acceptable quality and then declared diminishing returns beyond that point. There is also the challenge to find an optimal level that balances perception of a good eating experience with alleviating any perceived health concerns. The lower marbling scores affected tenderness more than the higher scores, indicating a plateau effect of marbling on tenderness (Ramsey et al., 1990). In a study correlating IMF with eating quality parameters, only IMF correlated with tenderness and WBSF (0.32 and -0.29, respectively; $P < 0.01$), although these correlations were low in reality (DeVol et al., 1988). Authors state that the reason for low correlation may be because there is a threshold value for IMF rather than a simple linear relationship between IMF and tenderness, although it is also important to note that these pigs were randomly selected in a plant with no background knowledge. The recommended level of IMF to target from this report is between 2.5 and 3.0%. Another report indicated that increasing IMF past 1.5% did not change panelist's scores for initial tenderness (Fortin et al., 2005). Instrumental tenderness was poorer when IMF was below 1% but did not improve when IMF was greater than 1%. Fernandez et al.,

(1999) determined that flavor and juiciness were significantly enhanced with IMF levels greater than 2.5%, and Font-i-Furnols et al., (2012) concluded that a minimum IMF to ensure palatability is between 2.2 and 3.4%. These results suggest that an effect of IMF on the sensory qualities of pork is not systematic.

The opportunity to choose among displayed packages is important to shoppers (Aberle et al., 2012). However, consumers unknowingly create a dilemma between what they select and what they actually prefer to eat. Several previous studies were designed to determine both the consumer perception of marbling on pork quality based on visual appearance and its actual influence on palatability and eating experience. In evaluating purchase intent, chops with the greatest level of marbling (3.46%) actually scored the poorest for visual acceptability compared to chops with low and medium IMF levels (1.05% and 2.33%, respectively) by pork consumers (Brewer et al., 2001). Lean and medium (<2.5%) marbling were chosen by 80% of consumers surveyed. These results were attributed to health concerns over fat levels. When these same consumers were blindly given chops with the same levels of IMF in a cooked evaluation, the highly marbled chops were rated highest for juiciness, tenderness, and overall flavor. When evaluated in a cooked evaluation at home, however, consumers rated the chop they originally chose as more tender, juicy, and flavorful than when they evaluated them blind. There is clearly a disparity between purchase intent from visual evaluation and quality attributes of cooked product. Another study that offered consumers four classes of pork chops with varying levels of IMF found that of the four groups, both extremes were typically chosen as the last choice. People were aware of their reasoning, purposefully selecting for or against marbling in the chops. In this study the Spanish consumers used were very polarized in their opinion for or against marbling, but the general population may not be that segmented in reality (Font-i-Furnols et al., 2012).

Literature is not conclusive on the effect of IMF on palatability traits, specifically tenderness. The most likely explanation is that marbling plays a small role in a large group of factors ranging from congenital characteristics to animal handling to product preparation and everything in between.

OBJECTIVES

The literature discussed above provides a wealth of knowledge on what marbling is and why it is important, as well as the physiology behind marbling development and how that is affected by sex and genetic factors, and then how that IMF can be estimated in a live animal. The focal point of this review as well as the focus of the subsequent studies is the variability of IMF in a live animal and in a pork loin product from different types of pigs. The ultrasound technology discussed above will be used to track IMF deposition and variability throughout the live phase. The subsequent work will build on Homm et al., (2006) who discovered differences in various portions of a boneless pork loin and suggested sectioning and sorting loins and chops to provide a more uniform purchasing unit. This leads to the objectives of the current work:

1) **Determine at what stage of development during the growing and finishing period of production intramuscular fat deposition occurs**
2) **Characterize differences in the amount and variability of intramuscular fat throughout pork loins**

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CHAPTER 2: CHARACTERIZING INTRAMUSCULAR FAT DEPOSITION IN PIGS THROUGHOUT THE GROWING PERIOD USING ULTRASOUND

ABSTRACT

The objective was to characterize the development of intramuscular fat (IMF) deposition throughout the growing-finishing period of pigs. The population of pigs evaluated were sired by commercial Duroc boars selected for meat quality (MQ; n = 96) or lean growth (LG; n = 96) and equally split between barrows and gilts. Pigs were scanned with a real-time ultrasound machine at four time points following the nursery phase starting at growing and finishing just before slaughter (10, 15, 20, 24 weeks). A minimum of five longitudinal scan images were collected on each pig at each time point and texture analysis software was used to determine back fat thickness, loin depth, and IMF. Two or four contemporaries to the population on study were slaughtered and fabricated after each scan day, and extractable lipid was determined for each chop from those pigs to validate the ultrasound process. Data were analyzed in Microsoft Excel. Means were calculated for ultrasound scan results at each time point for each group. Individual values were reported for serial slaughter IMF. No further statistical analysis was performed, because the data suggest that ultrasound is likely not a viable tool for distinguishing differences in IMF among young pigs. From wk 15 through the end of the trial period, barrows were fatter with a greater loin depth than gilts. At all scan points, pigs bred for meat quality were generally fatter with a lesser loin depth than those bred for lean growth. A comparison of 24 wk ultrasound IMF to 10th rib IMF content after slaughter illustrates the inconsistencies between the scan estimation and actual IMF. Serial slaughter confirmed inconsistencies between scan estimations and actual IMF values. In conclusion, there are differences in composition development between sexes and sire lines throughout the growing period, however the ultrasound technology

performed under current settings was not accurate enough to characterize IMF deposition in a growing pig.

INTRODUCTION

Marbling or IMF, is commonly associated with pork quality and is used by consumers to assess the value of fresh pork chops and determine purchasing decisions (Brewer et al., 2001; Cannata et al., 2010). It is one factor used by some consumers as an indicator of tenderness and juiciness in the cooked product (Wood et al., 2004; Lonergan et al., 2007), so the pork industry must continue to characterize and value this trait in ever-evolving pig populations. The amount of chop marbling preferred by consumers is highly variable (Cannata et al., 2010; Murphy et al., 2015), however the opportunity to choose is important to consumers, and many of them are polarized in their opinion for or against marbling (Font-i-Furnols et al., 2012).

The body's prioritization of energy usage determines that calories first go towards the needs of bone formation, then muscle growth, then fat development (McMeekan, 1940). As live weight increases throughout the growing period, the relative proportion of muscle and bone will decrease while fat increases (Richmond and Berg, 1972). Generally, fat is first deposited in visceral areas, then subcutaneous, then intermuscular, and the latest depot to begin developing is the intramuscular fat (Gerrard and Grant, 2006) which is the adipose depot of interest in this study. Previous work using longitudinal data to characterize IMF deposition in Duroc barrows determined that intramuscular fat (IMF) content increased linearly with age at a rate of 0.05%/day from 160 to 220 days of age (Bosch et al., 2012). In another study, there was a linear relationship between subcutaneous fat and body weight of Duroc pigs as they grew from 73 kg to 118 kg BW. The deposition curve was more curvilinear for loin muscle area and IMF in the same pigs (Schwab et al., 2007). Variability of marbling in market weight pigs differ between barrows and gilts and among sire lines (Overholt et al., 2016; Arkfeld et al., 2017). What has not been established is when deviation occurs between sexes and sire lines.

The importance of marbling creates a need for a technology that can quantify marbling in a live animal. Ultrasound is the most practical technology for on-farm use in a commercial setting, and it has been determined to be capable of estimating muscle, fat, and IMF content in live pigs (Ragland, 1998; Newcom et al., 2002). When ultrasonic waves hit dense tissue or the interface between two tissues, some of the wave lengths will reflect back to the transducer while others penetrate further. Different structures in the soft tissues cause ultrasonic waves to scatter which produces a graininess on the image in b-mode ultrasound images (Kim et al., 1997). This texture allows a technician to differentiate tissues from the ultrasonic image. Fat tissue is a good reflector of high frequency sound waves, so ultrasound has become the primary tool for measurement of IMF in a live animal (Schulte et al., 2011). Marbling gives a distinct pattern in ultrasonic images, so it can be objectively estimated by texture analysis algorithms. (Kim et al., 1997). Through the use and development of texture analysis software, selection for IMF using real-time ultrasound can be performed on pigs (Schwab et al., 2009).

As breeding goals change and evolve, selective genetic pressure quickly follows. This creates a need for technology that can determine characteristics in live animals so boars can be selected and mating decisions can be made. Characterizing the deposition of marbling to know how and when it is developed throughout the growing period would allow for a better understanding of its variability. The aim of this study was to use real-time ultrasound and serial slaughter to determine IMF content and loin variation of pigs at different points throughout the growing-finishing period.

MATERIALS AND METHODS

Experimental procedures for this study were approved and monitored by the University of Illinois Institutional Animal Care and Use Committee.

Pig Background

Pigs (192 total) used for the experiment were sired by commercial Duroc boars (Choice Genetics, West Des Moines, IA) from 2 distinct sire lines selected for either meat quality (MQ; *n* = 96) or lean growth (LG; *n* = 96). Boars were mated to Camborough (Pig Improvement Company, Hendersonville, TN) sows and parity was balanced between sire lines. Of the resulting progeny, 48 barrows and 48 gilts were selected from each sire line to fill a 2 x 2 factorial arrangement of treatments in a randomized complete block design. Block was used to define farrowing group, with pigs in block 1 (*n* = 96) being 2 weeks older than pigs in block 2 (*n* = 96). Pigs chosen for the experiment were selected to minimize variation in initial body weight within block. Pigs were housed in pens of 4 pigs of the same sex and sire line. A total of 48 pens of pigs were used for the experiment with 24 pens used in each block.

Pigs were raised at the University of Illinois Swine Research Center. At 10 weeks of age, pigs were allocated to research pens and moved from the nursery to the grower/finisher facility and began a 3 phase, 98 d grow-finish feeding program. All pigs were fed the same corn-soybean meal based diet that was formulated to meet or exceeded nutrient requirements for growing-finishing pigs based on the recommendations of the 2012 NRC of swine. Day 98 for each block was the end of the feeding portion of the trial. All pigs were scanned with real-time ultrasound technology at allocation and at each subsequent dietary phase change (10, 15, 20, 24 weeks of age) for determination of composition characteristics throughout the growing period.

Ultrasonic Imaging and Image Analysis

Pigs were scanned for back fat (BF), loin muscle depth (LD) and intramuscular fat percentage (IMF). An Aloka 500V SSD ultrasound machine was fitted with a 3.5-MHz 12.5cm linear array transducer. Pigs were restricted to a small area in a scale box for ultrasound image collection. The transducer was placed approximately 5 cm from the midline of the pig to represent the midpoint of loin where a back fat thickness would be measured on a ribbed chop face. A minimum of 5 longitudinal images were captured targeting the area of the 9-12 ribs. Commercial vegetable oil was used as a couplant between transducer and skin, no standoff guide was needed on the probe because cross-sectional images were not collected. All images were collected and analyzed by a single trained technician. System settings were adjusted to the recommended software settings. Gain settings for the Aloka ultrasound machine were: Overall, 90; Near, 25; and Far, 2.1. Images were captured and saved at the farm using a laptop computer with processing software connected to the ultrasound machine, then analyzed later. A freeze button was used to momentarily lock the frame on the machine interface until captured on the laptop by the laptop computer operator. Ultrasound image capturing and processing software was used to measure scan images for determination of backfat, loin depth, and percent intramuscular fat. Back fat and loin depth were measured at the 10^{th} rib location. The posterior tip of the trapezius muscle was used to locate the 10^{th} rib. Region of interest (ROI) boxes used for IMF determination were optimally placed across the interface of the longissimus muscle. Pixel (size) of the ROI varied based on pig size to optimize the amount of muscle texture that was analyzed. The technician made a subjective visual determination on whether each image was acceptable for analysis.

Serial Slaughter

After allocation, farrowing group contemporaries remained on feed alongside the pigs chosen for the experiment. Two or four non-trial contemporaries from the second block were slaughtered at each time point that scanning occurred, to verify ultrasound results. Entire loins (IMPS Item No. 410) from these pigs were sliced on a band saw into 2.54 cm thick chops. Extractable lipid (IMF) was determined on all chops. Chops from the area that the probe was placed were compared to the ultrasound report to test accuracy of the ultrasound procedure.

Statistical Analysis

Main effect means for ultrasound-determined carcass measurements were calculated in Microsoft Excel. Serial slaughter IMF was reported as individual values with no statistical analysis. An average IMF value of the four or five chops that would be evaluated by the ultrasound probe was used to compare to chemically determined IMF. After slaughter of the trial population, a sample of longissimus was collected from the area of the 10th rib and used for proximate analysis to determine the percent fat, and this was used for a regression analysis to determine the efficacy of the ultrasound technique. Regression was performed in SAS 9.4 with chemically determined IMF as the independent factor and ultrasound IMF as the dependent factor.

RESULTS

Ultrasonic Imaging and Image Analysis

Ultrasound image analysis results are displayed in Table 2.1 and 2.2. Barrows were fatter and heavier muscled than gilts at every time point after allocation (Table 2.1). Pigs sired for meat quality were fatter than pigs bred for lean growth and generally lighter muscled (Table 2.2). These tables also include main effects of sex and sire line on ultrasound IMF at each time point. A comparison of the range of values between ultrasound determined IMF the day before slaughter and chemically determined IMF from the 10th rib chop after fabrication can be found in Figure 2.1. The coefficient of determination explaining the efficacy of using ultrasound to predict IMF was R^2 = 0.20. No further statistical analysis was performed because the above analysis of chemically determined IMF proved that scan results were not accurate enough to analyze mean differences throughout the growing period.

Serial Slaughter

Serial slaughter was performed on a small set of contemporaries of the pigs on trial in order to validate the ultrasound procedure. This supplementary study confirmed that ultrasound analysis was not accurately estimating IMF. Table 2.3 contains individual observations – not means – for each chop from each pig that was fabricated for supplementary information. The extractable lipid of the set of chops that was scanned and analyzed did not align with the subsequent scan IMF values as observed in Table 2.4. Figure 2.2 illustrates average IMF by chop from the serial slaughter pigs that were slaughtered at each time point. The variable IMF pattern of maximums on both ends and a minimum point in the middle that is similarly displayed in each of these sample groups provided insight that helped to design the loin variability portion of the study that followed.

DISCUSSION

Ultrasound imaging was not successful in predicting the amount of marbling deposition throughout the growing period. The first scan yielded much greater IMF means than any subsequent scans which suggests inadequacy of the technology for use in young pigs. A look at the overall range of IMF values from the final ultrasound scan against chemically determined IMF values

(Figure 2.1) illustrates that the distribution of values from ultrasound does not reflect actual IMF. Newcom et al. (2002) recommended analyzing data in groups or bins because ultrasound IMF values did not reach the extremes observed in the pigs, maintaining a lesser range of IMF values than the subsequent proximate analysis realized. The present study encountered the same limitation of ultrasound values not accurately estimating the extreme observations of the population and estimating a disproportionate number of loins near the median. From this, it could be argued that ultrasound is not proficient in doing what breeders expect of it, identifying population outliers to use for genetic improvement. The study did, however, elucidate useful information about loin variation that helped plan the subsequent meat quality work, as well as providing practical insight into the limitations of ultrasound technology.

Small (young) pigs are difficult to restrain and position for scanning, which at times caused poor connection between probe and pig. Poor connection can result in inaccurate or unclear images. Even if quality images were collected, many of the pigs at the first time point of scanning did not have enough loin depth to adequately position ROI boxes for IMF determination. If any part of the ROI contained bone or any tissue other than longissimus muscle, the texture analysis for IMF determination was skewed. There are three different pixel (size) choices for the box and up to three boxes can be placed per image. The maximum box size and number were used in each case to optimize the amount of loin that was being analyzed.

Loin depth and back fat thickness would seem to be easier to obtain a more accurate measurement from ultrasound images than IMF, however pig positioning can have large impacts on accuracy and repeatability of all measurements. Head and neck position, probe angle, flexing or arching of the back, as well as relaxing or sinking of the back can all have drastic effects on probe connection and subsequent carcass measurements. One pig had a spinal injury that rendered all scan images negligible. On a single image, fat and muscle measurements can be measured at several different anatomical locations. In the present study loin depth was measured from the epimysium to the most dorsal point of the 10th rib, which does not replicate the measurement that would be collected on a ribbed carcass (split between ribs). However, this was the most easily anatomical identifiable location and least susceptible to variation due to animal positioning, so it provided the greatest opportunity for repeatable results. Fat depth measurements were drawn from the top of the image to the top of the longissimus. Fat thickness was measured at the center of the ultrasound image because that is where the probe had the best contact with the skin.

Literature states that a creamy texture to the loin represents a greater IMF content (Biotronics Inc., 2008), however poor image quality also causes a grainy or blurry texture, which can appear quite similar to the prototypical “high marbling” texture. There is not a relationship between loin texture and IMF that can be subjectively validated by the technician, so the software must be completely trusted or totally ignored. Loins from the first ultrasound point that provided clear streaking in the scans had estimated IMF values of approximately 2%, likely those were the most accurate, and the high IMF images were not actually analyzing marbling. It is hard to tell whether the texture analysis is actually picking up on differences in marbling, or simply random texture differences and differences in image quality. Thought should be given to various ways of analysis if this experiment were to be replicated and results were trusted. Bins or grouping may be used to more accurately represent the ultrasound results. Early scans in younger pigs could provide satisfactory results with a relative ranking, but once pigs approach market weight a more exact value should be found for this technology to have value and usefulness.

CONCLUSION

The current practice performed with the same technician and software used in this study is not accurate enough for scientific interpretation. Experience and attention to details learned in this study, along with some improvements in the setup and implementation of the technology may allow for successful future work in this area. This work confirmed known confirmation differences throughout the growing period between sexes and sire lines. The marbling variation found in the loins of pigs slaughtered for ultrasound validation aligned with the work of Faucitano et al., (2004) and helped strengthen the meat quality component performed in the next chapter.

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FIGURES

Figure 2.1. Intramuscular fat content of market weight (24 weeks) pigs determined by chemical extraction (A) and estimated by ultrasound (B).

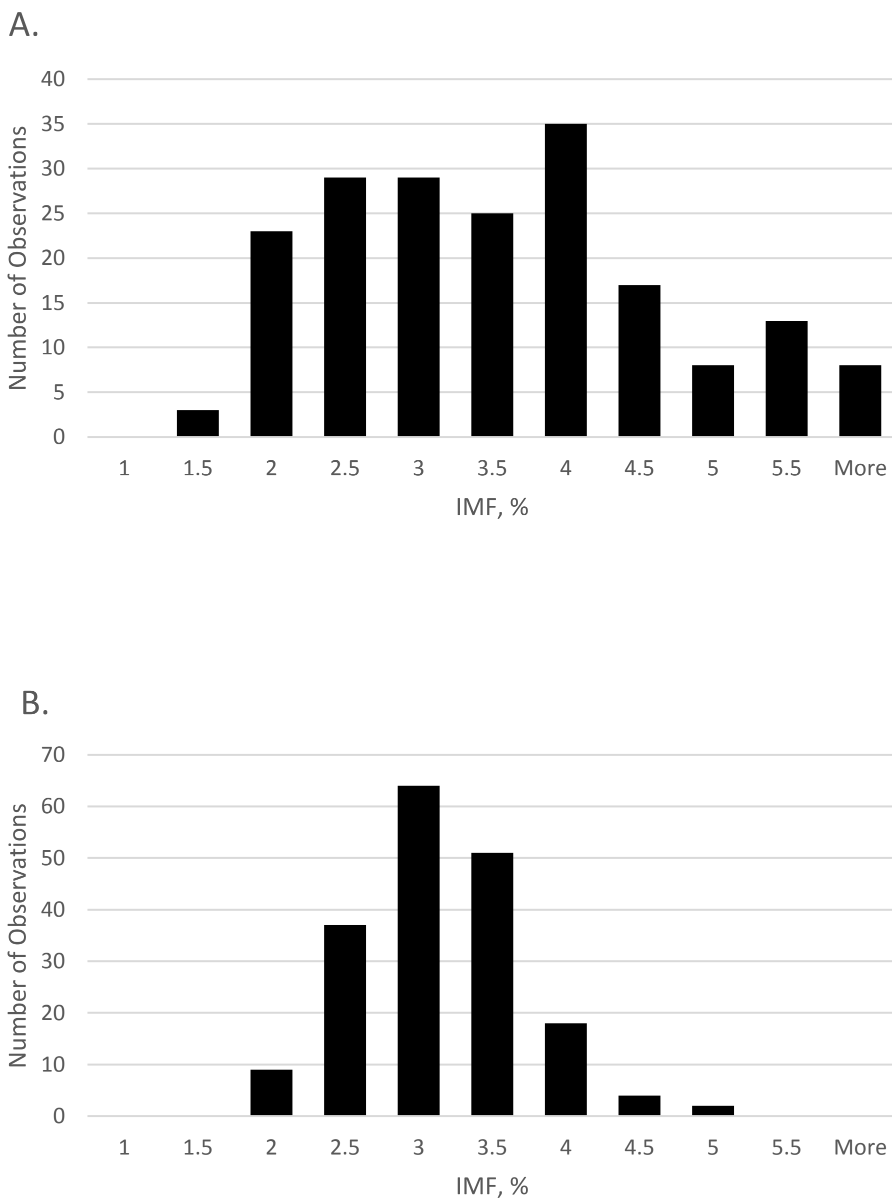
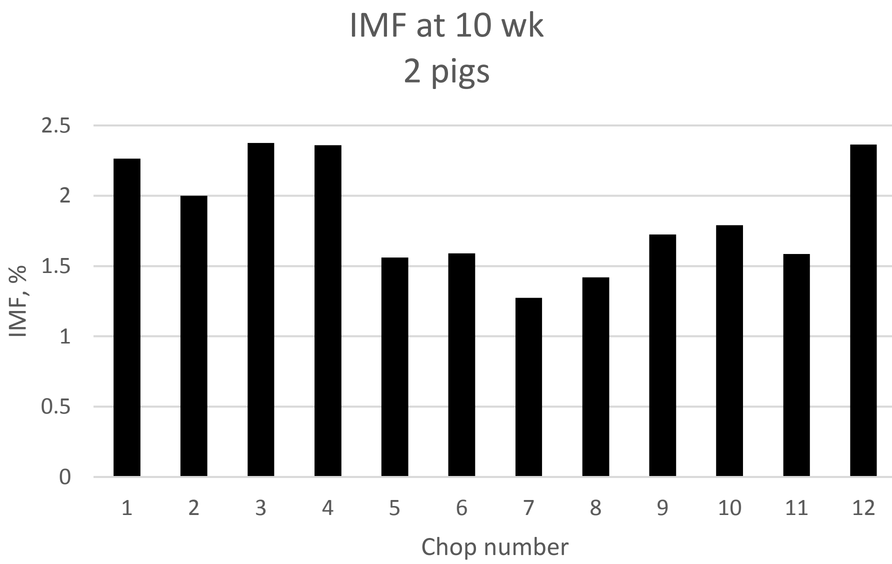


Figure 2.2. Intramuscular fat content of serial chops from pork loins (numerically from anterior to posterior) at 10, 15 and 20 weeks of age.



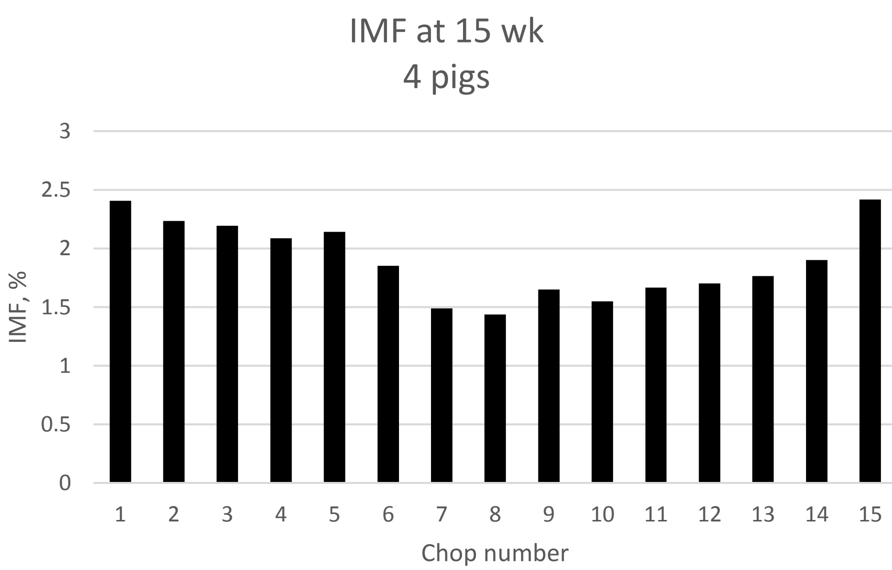
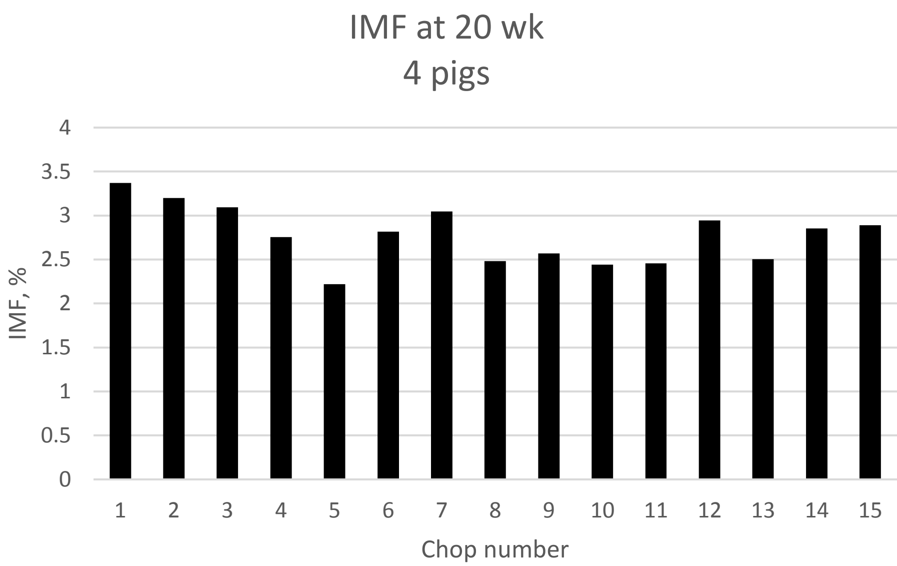


Figure 2.2. (cont.)



TABLES

Table 2.1. Ultrasound means by sex and age

	Barrows			Gilts		
Age, wk	Fat Depth, cm	Loin Depth, cm	IMF, %	Fat Depth, cm	Loin Depth, cm	IMF, %
10	0.43	2.57	3.22	0.43	2.57	3.27
15	0.91	3.94	2.41	0.81	3.84	2.59
20	1.60	5.44	2.89	1.30	5.13	2.84
24	2.16	5.87	2.99	1.68	5.66	2.87

Table 2.2. Ultrasound means by sire line and age

	Meat Quality			Lean Growth		
Age, wk	Fat Depth, cm	Loin Depth, cm	IMF, %	Fat Depth, cm	Loin Depth, cm	IMF, %
10	0.46	2.51	3.51	0.41	2.64	3.06
15	0.91	3.81	2.61	0.79	3.96	2.38
20	1.55	5.13	2.94	1.35	5.41	2.79
24	2.08	5.66	3.02	1.75	5.84	2.84

Table 2.3. Serial slaughter percentage extractable lipid by age and chop (numerically from anterior to posterior)

ID	Age, wk	Sex	Sire	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
545	10	Gilt	LG	1.67	1.45	1.61	1.56	0.75	0.94	1.05	1.12	1.27	1.44	1.28	2.31				
633	10	Barrow	MQ	2.86	2.55	3.14	3.16	2.37	2.24	1.50	1.72	2.18	2.14	1.89	2.42				
535	15	Barrow	MQ	1.56	1.70	1.49	1.30	1.25	0.56	0.72	0.45	1.06	0.98	0.55	1.09	1.13	1.10	1.92	
571	15	Gilt	LG	3.37	2.66	3.16	3.06	3.07	2.74	2.41	2.69	1.73	2.21	2.28	2.52	2.46	2.53	2.81	3.46
646	15	Barrow	MQ	2.35	2.52	1.62	1.98	1.96	1.99	1.23	1.20	1.95	1.39	1.87	1.39	1.81	1.80	2.19	
681	15	Barrow	LG	2.34	2.05	2.50	2.00	2.28	2.12	1.59	1.40	1.86	1.61	1.96	1.80	1.66	2.17	2.75	
540	20	Barrow	MQ	3.54	3.42	3.58	2.30	2.49	2.44	2.07	2.33	2.07	2.46	2.77	2.11	2.24	2.58		
546	20	Gilt	LG	2.17	2.11	2.15	1.74	1.69	1.46	1.31	1.62	1.39	1.31	1.24	1.77	1.71	1.58	1.75	2.19
600	20	Barrow	MQ	4.83	3.99	3.88	4.11	4.05	3.65	3.62	3.64	3.35	3.44	3.87	3.5	4.14	3.25		
712	20	Gilt	LG	2.94	3.28	2.76	2.87	2.48	2.94	2.93	2.68	2.96	2.61	3.89	2.64	3.32	3.54		

Table 2.4. Comparison of scan values and extractable lipid

ID	Scan	Sex	Sire Line	Scan IMF	Chemical IMF[1]
545	1	Gilt	LG	2.4	1.10
633	1	Barrow	MQ	4.9	1.91
535	2	Barrow	MQ	2.4	0.80
571	2	Gilt	LG	2.1	2.26
646	2	Barrow	MQ	1.7	1.44
681	2	Barrow	LG	2.0	1.62
540	3	Barrow	MQ	2.2	2.23
546	3	Gilt	LG	1.7	1.41
600	3	Barrow	MQ	2.9	3.51
712	3	Gilt	LG	2.7	2.80

[1]Average IMF content of chops that would be included in the ultrasound image

CHAPTER 3: CHARACTERIZING THE AMOUNT AND VARIABILITY OF INTRAMUSCULAR FAT DEPOSITION THROUGHOUT PORK LOINS USING BARROWS AND GILTS FROM TWO SIRE LINES

ABSTRACT

The objective was to determine the amount and variability of intramuscular fat in a pork loin attributable to anatomical chop location, sex, and sire line. The population of pigs evaluated were sired by commercial Duroc boars selected for meat quality (MQ; $n = 96$) or lean growth (LG; $n = 96$) and equally split between barrows and gilts. After slaughter and fabrication, bone-in chops were removed from four locations of a loin (A = 6th rib, B = 10th rib, C = last rib, D = 4th lumbar vertebrae). A consecutive pair of chops from each location was evaluated for visual color, visual marbling, subjective firmness, Warner-Bratzler shear force, and determination of moisture and extractable lipid (IMF). Data were analyzed using the MIXED procedure of SAS as a 3-way ANOVA. Farrowing group (block) was used as a random effect. Homogeneity of variances was tested on raw data using Levene's test of the GLM procedure, and found to be heterogenous, so a 2-variance model was fit using the REPEATED statement of the MIXED procedure, grouped by sex, sire line, or chop for analysis of least squares means. The mivque(0) option of the VARCOMP procedure was used to calculate the proportion of variability each individual factor contributed to total variance. Barrows (3.64%) produced chops with greater ($P < 0.01$) IMF content than gilts (3.20%) and barrows (2.14) had greater ($P < 0.01$) IMF variability than gilts (1.23). Chops from MQ pigs (4.02%) exhibited greater ($P < 0.01$) IMF content than LG (2.82%) and barrows (1.97) had greater IMF variability ($P < 0.01$) than LG pigs (0.97). Chops from location A (3.80%) and D (3.77%) had greater IMF than B (3.34%; $P < 0.01$), and A, B, and D had greater IMF than C (2.77%; $P < 0.01$). Variances of IMF also differed (A = 1.44, B = 1.59, C = 1.05, D = 2.18; $P = 0.01$) across chop locations. Of the variability in IMF,

33.0% was attributed to sire line, 10.16% to chop location and 4.01% to sex, with 52.83% not accounted for by these three factors. Location A chops were the most tender (2.57 kg; $P < 0.01$) and C chops the least tender (2.93 kg; $P < 0.01$). No differences in variability ($P = 0.40$) of tenderness were observed among chop locations (A = 0.31, kg B = 0.24 kg, C = 0.24 kg, D = 0.23 kg). These results demonstrated that variability in tenderness values did not reflect the variability of IMF present in tested chops. In conclusion, chop location, sex, and sire line all contribute to the amount and variability of pork loin marbling.

INTRODUCTION

When consumers evaluate marbling in loins at retail, this most often occurs within chops individually or as an assorted package. However, when pork quality is evaluated by commercial processors, evaluations are conducted on the ventral surface of a whole boneless loin, and general estimations about the amount of marbling of that loin are assigned to the entire loin without regard to any differences that may exist in chop marbling throughout the length of the loin (King et al., 2011). It is possible that the sorting criteria assigned to an entire loin based on the color and marbling appraisal of the ventral surface of the loin or of a single ribbed (cutting between ribs to expose the longissimus) chop face may not be representative of all the chops produced from that loin. Due to anatomical differences, loins classified as "high marbling" at the processing facility may not result in uniformly highly marbled chops at retail or produce a consistently high quality eating experience.

In addition to absolute differences in the amount of marbling produced by barrows and gilts or by pigs from two different sire lines, the variability of that marbling differs between sexes and sire lines (Overholt et al. 2016; Arkfeld et al., 2017). There are differences in the amount of marbling in chops from different locations along the loin (Faucitano et al., 2004;

Homm et al., 2006). Marbling on the ventral surface of fresh loins correlates (r = 0.84) with marbling of a chop collected at the 10th rib (Lowell, et al. 2018), but that relationship has not been defined with chops collected at other anatomical locations throughout the loin. Therefore, a critical need exists to characterize differences in the amount of marbling and the variability of marbling throughout the loin of different types of pigs so that packers can provide consumers with chops that are more uniform at the retail level to provide greater consumer satisfaction. A loin evaluation system based on the visual appraisal of the ventral surface, without an understanding of the variability in marbling distribution of that loin at retail directly compromises the ability to please the consumer and encourage pork purchases.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment.

Experimental Design

Pigs (192 total) used for the experiment were sired by commercial Duroc boars (Choice Genetics, West Des Moines, IA) from 2 distinct sire lines selected for either meat quality (MQ; *n* = 96) or lean growth (LG; *n* = 96). Boars were mated to Camborough (Pig Improvement Company, Hendersonville, TN) sows and parity was balanced between sire lines. Of the resulting progeny, 48 barrows and 48 gilts were selected from each sire line to fill a 2 × 2 factorial arrangement of treatments in a randomized complete block design. Block was used to define farrowing group, with pigs in block 1 (*n* = 96) being 2 weeks older than pigs in block 2 (*n* = 96). Within the contemporary age groups, pigs chosen for the experiment were selected to minimize variation in initial body weight. Pigs were housed in pens of 4 pigs of the same sex and sire line. A total of 48 pens of pigs were used for the experiment with 24 pens used in each block.

Pigs were raised at the University of Illinois Swine Research Center. At approximately 10 weeks of age, pigs were allocated and moved from the nursery to the grower/finisher facility and began a 3 phase, 98 d grow-finish feeding program. All pigs were fed the same corn-soybean meal based diet that was formulated to meet or exceed nutrient requirements for growing-finishing pigs based on the recommendations of the 2012 National Research Council (NRC) of swine. Synthetic lysine was included, and the finishing diet (phase 3) had a calculated SID lysine value of 0.68%. Day 98 for each block was the end of the feeding portion of the trial. Pigs were weighed, and this was recorded as final trial weight. On d 98, 32 pigs per block were selected and transported to the University of Illinois Meat Science Laboratory (Urbana, IL) for slaughter on d 99. These 32 pigs were selected for tissue collection to fulfill a subsequent experiment. On d 99, all of the remaining pigs (block 1 = 63, block 2 = 64) were transported approximately 50 kilometers to a federally inspected abattoir and slaughtered. All pigs were tattooed on the ham with an individual identification number matching their identification tag number prior to transport.

Slaughter, Carcass Characteristics, and Loin Collection

Pigs slaughtered at the commercial abattoir were held in lairage for a minimum of 3 hours prior to slaughter. Pigs were immobilized via carbon dioxide gas and terminated via exsanguination. A sequential identification number was written on the shoulder of each carcass with a food safe crayon after evisceration to help align carcass data. Hot carcass weight (HCW) was recorded immediately before each carcass entered the blast chiller. After the approximately

90 minute blast-chill, carcasses were moved to an equilibration cooler where they remained for approximately 20 h until fabrication. Estimates of carcass composition were determined on the left side of each carcass. Midline back fat thickness at the area of the last rib was recorded. Bone-in skinned left side loins (modified IMPS Item No. 410, skin-on) from the left side were collected, boxed, and promptly transported to the University of Illinois Meat Science Laboratory.

Pigs harvested at the University of Illinois were held in lairage for approximately 16 h prior to slaughter. They were provided access to water but had no access to feed during this time. Pigs were slaughtered under the supervision of the Food Safety and Inspection Service of the U.S. Department of Agriculture (USDA). Pigs were immobilized via head-to-heart electrical stunning and terminated via exsanguination. Carcasses were weighed approximately 30 min postmortem to determine HCW. Carcasses were chilled at 4°C for a minimum of 20 h. During fabrication on d 1 postmortem, bone-in skin-on left side loins (IMPS Item No. 410) were collected and tagged with the tattoo identification number from the ham.

Loin Quality Evaluation

Loins from both the commercial facility and the University meat lab were collected to be evaluated simultaneously. On the evening of d 1 postmortem, the tenderloin (IMPS Item No. 415) and sirloin (modified IMPS Item No. 413D, bone-in pork sirloin split at the anatomical landmark of the last sacral vertebrae) were removed from all loins, then loins were laid out on tables in the cooler, covered, and allowed approximately 12 h to acclimate. At d 2 postmortem, all bone-in loins were sliced into 2.54 cm thick chops on a band saw. The number of chops produced by each loin was recorded. Chops were collected in order on a tray, then moved to a separate location where desired chops were identified and tagged. Pairs of chops were collected from 4 locations throughout the loin (A = 6th rib, B = 10th rib, C = last rib, D = 4th lumbar; Figure 3.1). Of each pair, the anterior chop was designated for proximate composition analysis (PA) and the posterior chop was assigned to Warner-Bratzler shear force (WBSF) determination. The anterior chop from the 10th rib location from each loin was traced on to acetate paper for loin eye area (LEA) determination and those with skin-on (pigs harvested at the university meat science laboratory) were measured for back fat thickness. Chops chosen for WBSF were trimmed of subcutaneous fat leaving the epimysium intact, then vacuum packaged, boxed, aged for 7 days, and then frozen at less than -29°C.

Chops chosen for PA were used first to score visual color (NPPC, 1999), visual marbling (NPPC, 1999), and subjective firmness (NPPC, 1991). After receiving a tag, chops were allowed a minimum of 20 min for oxygenation of myoglobin, and then scores were assigned in batches of approximately 40 chops at a time, randomized for anatomical location and pig. After this, PA chops were closely trimmed to remove all peripheral fat and connective tissue, placed into a whirl-pak bag (Nasco, Gurnee, IL) and immediately frozen at less than -29°C.

Proximate Composition Analysis

Chops frozen for analysis of moisture and extractable lipid content were allowed to partially thaw at approximately 22°C with careful attention to prevent loss of exudate and then homogenized in a Cuisinart food processor (East Windsor, NJ). Duplicate 10 g samples of the homogenate were weighed and placed in an oven for drying at 110°C for at least 24 h. Dried samples were weighed to determine percent moisture. Samples were then placed in extractor columns and washed in a mixture of chloroform and methanol for at least 8 h as described by

Novakofski et al. (1989). After extraction, samples were re-dried for at least 24 hours and then weighed to determine lipid percentage.

Warner-Bratzler Shear Force

Chops designated for WBSF were removed from frozen storage and allowed to thaw for at least 24 h at approximately 4°C prior to analysis. Shear force was performed on all chops from an individual pig on the same day to eliminate variation in instrumental tenderness due to shear day. Chops were individually weighed and then cooked on a Farberware Open Hearth grill (model 455N, Walter Kidde, Bronx, NY). Chops were cooked on one side to an internal temperature of 31°C, then flipped and cooked until they reached an internal temperature of 63°C, at which point they were removed. While cooking, internal temperature was monitored using copper constantan thermocouples (Type T, Omega Engineering, Stamford, CT) placed in the geometric center of each chop and connected to a digital scanning thermometer (model 92000-00, Barnat Co., Barrington, IL). Chops were allowed to cool and then weighed again to determine percent cook loss. A target of five 1.25 cm diameter cores (some location A chops were too small in diameter to collect 5 cores) were removed from each chop parallel to the orientation of the muscle fibers and sheared using a Texture Analyzer TA.HD Plus (Texture Technologies Corp., Scarsdale, NY/Stable Microsystems, Goldalming, UK) with a blade speed of 3.33 mm/s and a load cell capacity of 100 kg. The shear force values for all cores from a single chop were averaged and the average was reported in kg of force as WBSF.

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC) as a 3-way ANOVA in a randomized complete block design. Farrowing group (block) was a random variable and fixed effects were sex, sire line, and chop location. The UNIVARIATE procedure was used to test normality among residuals. Carcass was the experimental unit for all carcass characteristics and chop was the experimental unit for all chop quality evaluations. Homogeneity of variance of the residuals was tested using Levene's test of the GLM procedure, which revealed that the variance of residuals of severable variables were unequal. Variances were considered different at $P \leq 0.05$. Therefore, a 2-variance model was fit using the REPEATED statement of the MIXED procedure, grouped by chop. Mean, sample variance, and CV of variables were calculated using the MEANS procedure.

Differences in variances of selected traits are illustrated by box and whisker plots. The bottom line of the box indicates quartile 1 (Q1; 25th percentile); the middle line is the median (50th percentile); and the top line represents quartile 3 (Q3; 75th percentile). Interquartile range (IQR) was calculated as Q3 – Q1. An upper fence was calculated as Q3 + (1.5 x IQR) and a lower fence was calculated as Q1 – (1.5 x IQR). Whiskers represent the maximum and minimum observations of data within the respective fences that are not outliers. Any observation greater than the upper fence or less than the lower fence was considered an outlier. Although outliers were factored into variance calculations, they are not graphically illustrated on box and whisker plots for clarity purposes. The *P* – values displayed on the box plots represent differences in variance determined by Levene's test. Coefficient of variation has been included as an additional measure of variability that takes into account the magnitude of differences in means.

The mivque(0) option of PROC VARCOMP was used to evaluate the percentage of the total variation each variable (sex, sire line, chop location) contributed to total variance. Variation that could not be attributed to one of these variables was attributed to biological differences between pigs (error) and other variation not accounted for by sex, sire line, or chop anatomical location. Computed negative estimates were treated as contributing zero variation to the population. Due to the nature of the statistical analysis used, variation percentages should total to 100%.

RESULTS

Carcass Characteristics

Main effects on carcass characteristics are in Table 3.1. Differing numbers of observations reflect the two locations in which these pigs were slaughtered. No significant sex by sire line interactions existed for these carcass characteristics. Carcasses from barrows had 7.44 kg greater HCW (P < 0.01) than carcasses from gilts, resulting from 5.50 kg greater live weights (P < 0.01). Barrows were fatter than gilts both at the 10^{th} rib (3.17 cm vs. 2.39 cm; P < 0.01) and the last rib (3.39 cm vs. 2.96 cm; P < 0.01) but had similar (P = 0.37) loin eye areas. Barrows produced loins with approximately 0.6 greater (P < 0.01) chops per loin. Carcasses from MQ sired pigs had 2.92 kg greater HCW (P < 0.05) than those sired by LG boars, resulting from 9.95 kg greater live weights (P < 0.01). Carcasses from MQ pigs were 0.59 cm fatter at the 10^{th} rib (P < 0.01) than LG pigs, with 5.16 cm^2 less loin eye area (P < 0.01) and had approximately 0.5 greater (P < 0.05) chops per loin.

Loin Quality Evaluation

Effects of sex, sire line, and chop location on chop quality are in Table 3.2. No sex by location or 3-way interactions were significant (P > 0.05) for any of the observed parameters. Visual color score demonstrated an interaction of sire line and sex (P < 0.01). Barrows were no different than gilts within the MQ group, but LG gilts scored 0.12 units greater than LG barrows (data not displayed). Combining all chop locations and both sire lines, no differences in visual color (*P* = 0.18) or subjective firmness (*P* = 0.06) existed between sexes. Also, no differences in visual color (*P* = 0.64) or subjective firmness (*P* = 0.06) were observed between sire lines. Within loin, visual color score differed (*P* < 0.01) with A chops scoring the darkest (4.46) and location B chops the lightest (4.06) of all locations. Subjective firmness scores increased from anterior to posterior (A = 2.06; B = 2.21; C = 2.85; D = 3.22; *P* < 0.01), with all means differing.

Visual marbling score exhibited an interaction of sire line and sex (*P* < 0.01). Barrows scored 0.24 units greater than gilts within the MQ group but no difference between sexes was found within LG pigs (data not displayed). Barrows had a significantly greater visual marbling score (3.12) than gilts (3.02; *P* < 0.05) regardless of sire line. Chops from MQ sired pigs had a greater visual marbling score (3.28) than LG (2.86; *P* < 0.01). Differences in visual marbling existed among all chops (*P* < 0.01) with location D chops (3.50) being greater than C chops (3.11), which were greater than B chops (2.92) which all scored greater for marbling than A chops (2.76).

There was an interaction in IMF between sex and sire line (*P* < 0.01) and location and sire line (*P* = 0.01). Barrows had 0.69% greater IMF than gilts within the MQ sire group but no sex difference was found within LG pigs (data not shown). A large difference of 1.69% IMF (*P* < 0.01) can be observed at the D chop location between MQ and LG pigs while the other 3 locations remain in similar proportions within sire line. Barrows (3.64%) produced chops with greater (*P* < 0.01) IMF than gilts (3.20%) regardless of sire lines. Chops from barrows (73.68%) contained less moisture (*P* < 0.01) than chops from gilts (74.06%). Chops from MQ pigs (4.02%) exhibited greater (*P* < 0.01) IMF than chops from LG pigs (2.82%). Chops from MQ pigs (73.40%) contained a lower (*P* < 0.01) moisture content than chops from LG pigs (74.33%). Differences in IMF by chop location existed (*P* < 0.01), with chops from location A (3.80%) and

D (3.77%) greater than B chops (3.34%), and all three locations greater than C chops (2.77%). Mean differences for moisture content existed across chop locations ($P < 0.01$).

Variability of IMF Percentage

Chops from barrows were more variable in IMF content (s^2: 2.14% vs. 1.23%; $P < 0.01$; CV: 40.32 vs. 34.61) than gilts (Figure 3.3.A). Chop IMF from MQ pigs had greater variance than LG (1.76% vs. 0.97%; $P < 0.01$), but with the other measure of variability the treatments were reversed (CV: 33.06 vs. 35.00, respectively) (Figure 3.3.B). Variance of IMF also differed (A = 1.44%, B = 1.59%, C = 1.05%, D = 2.18%; $P < 0.05$) across chop locations (Figure 3.3.C). The CV of chops B and C were similar, but within the two locations with the greatest means, A chops are the least variable while D chops remain the most variable in both CV and variance. Due to the nature of its calculation, a greater mean typically equates to a greater variance. Coefficient of variation accounts for differences in magnitudes of means. Of the variability in IMF, 33.0% was attributed to variation in sire line, 10.16% to chop location and 4.01% to sex, with 52.83% unaccounted for by factors in this model (Figure 3.4.A).

Warner-Bratzler Shear Force

Barrows and gilts did not differ in shear force ($P = 0.06$) or in cooking loss ($P = 0.94$). Chops from MQ sired pigs (2.66 kg) were more tender ($P < 0.01$) than LG chops (2.85 kg), but even with a 6.7% difference, chops from both sire lines should be considered very tender. Cooking loss did not differ between sire lines ($P = 0.08$). Shear force differed by location ($P < 0.01$) as location A chops were the most tender (2.57 kg) and C chops the least tender (2.93 kg), with D and B chops intermediate (2.75 kg and 2.79 kg, respectively). Differences existed among all chops in cooking loss ($P < 0.01$) with location A chops (15.33%) having the least and D chops (18.74%) with the greatest cooking loss, while B and C were intermediate (16.65% and 18.01%, respectively). Variance of WBSF did not differ (P = 0.31) between sexes (Figure 3.5.A). No differences in variance of WBSF (P = 0.11) was observed between sire lines (Figure 3.5.B). No differences in variability (P = 0.40) of WBSF were observed between chop locations (A = 0.31, kg B = 0.24, kg C = 0.24, kg D = 0.23 kg) (Figure 3.5.C). Of the variability in WBSF, 6.04% was attributed to sire line, 7.47% to chop location and 0.52% to sex, with 85.97% unaccounted for by factors in this model (Figure 3.4.B).

DISCUSSION

Variation in quality exists in the pork industry (Arkfeld et al., 2017), and management of this variability is a vital step to add value to pork products. The opportunity to choose among displayed packages is important to shoppers (Aberle et al., 2012), and every consumer has a different perspective of the ideal characteristics of a pork chop (Brewer et al., 2001; Cannata et al., 2010; Murphy et al., 2015), so rather than attempting to eliminate all variation in quality, that variability must instead be characterized in an effort to leverage those differences for a positive benefit. Variability within a package is selected against by consumers, while variation within a retail case allows consumers to choose the product that they prefer. Differences in composition and IMF means between sex and sire lines in the present study were aligned with previous literature (Martel et al., 1988; Ellis et al., 1996; Lee et al., 2013). In agreement with Overholt et al., (2016) and Arkfeld et al., (2017) who reported differing variability due to sex and sire line, the present study confirmed that IMF in barrows is greater and more variable than gilts, and that MQ sired pigs have greater IMF and are more variable in that marbling than LG sired pigs. Sire lines used in this study were not replicates of those used in the previously mentioned literature, but in both cases pigs were sired by boars selectively bred for either improved meat quality or increased lean gain.

In the current study, intramuscular fat content is increased at both the anterior and posterior chop locations, with an intermediate IMF content at the area of the 10^{th} rib and the least amount at the area of the last rib. This phenomenon was demonstrated repeatedly across all examined treatment groups (Figure 3.2). This is similar to results found by Faucitano et al. (2004) who reported the greatest IMF values in the middle thoracic (6^{th} rib) and the caudal lumbar (4^{th} lumbar) portions of pork loin, and the least amount located where the thoracic region meets the lumbar region (last rib), with the area between the 10^{th} and 12^{th} ribs best representing the average of the whole loin. Similarly, Homm et al. (2006) reported that posterior chops were the most marbled and tender in 8-rib boneless loins, and the posterior and anterior ends of 11-rib boneless loins had increased marbling content with the least amount in the middle. Those chops were collected from a boneless loin, so exact connections cannot be made with the rib locations from the current study, however posterior chops and middle chops from Homm et al. (2006) should align with D and C chops (respectively) in the current data.

Beyond simple mean differences in IMF by location, the present study demonstrates significant differences in the variability of that level of IMF between locations. In addition to the sex and sire line variability discussed above, the location within a loin from which a chop is selected adds more variability. The most posterior chops have as much IMF content as any, but they also have the greatest amount of variability in that marbling. With these data, it is evident that combining factors to increase the average marbling of a set of pigs will increase the variability as well. A chop selected from the posterior end of the loin of a barrow from a meat quality sire line within the present population has a mean of 4.97% but also an s^2 of 2.60% for

IMF. Chops from location A had the same amount of IMF as those posterior chops from location D, however A chops were much less variable in relation to that increased IMF, exhibiting the second lowest s^2 and the lowest CV of all four locations (Figure 3.3.C).

Meat quality differences stemming from changes in anatomical location within a loin is an additional variable to be aware of. A stark difference may be realized between a pair of chops selected from the 4th lumbar vertebrae and a pair of chops selected just anterior to that at the area of the last rib. Two locations are typically just 3 or 4 chops apart on a sliced boneless loin contrast each other with the greatest and least total amount and variability of IMF observed out of all locations sampled in the current work. From this evidence, it could be suggested that when chops are sliced consecutively from a boneless loin, they will not have same composition. If packaged together for consumer display, this may appear as inconsistent product. Additionally, chop selection, specifically in regards to location within the loin, is of vital importance and should be strategically considered and reported to ensure efficacy and repeatability of research experiments. Rincker et al., (2008) reported that IMF had little influence on the eating quality of pork chops, however in that study a chop taken adjacent to the 10th rib face was used for determination of extractable lipid and that value was assigned to the entire loin, while the chops given to trained panelists for evaluation came from a location 3-5 chops posterior to the chop that was extracted. This places the chops that were actually eaten in the area of the last rib, which this study, along with many others (Carpenter et al., 1961; Van Oeckel et al., 2003; Faucitano et al., 2004; Homm et al., 2006;) deem to have the poorest quality of the entire loin. Inconsistencies in the effect of IMF on eating quality has been previously credited to genetics, processing conditions, degree of doneness, etc. (Koohmaraie et al., 2002), but with current knowledge it can be assumed that differences in the amount and variability of IMF between different regions of the loin could have been another factor that affected those outcomes.

Visual marbling score did not align with extractable lipid in the present study. Specifically, chops from location A exhibited the greatest lipid content in chemical determination yet scored the lowest for visual marbling. A similar discrepancy was also reported by Homm et al., (2006) who reported extractable lipid peaking on both ends of the loin while visual marbling increased from anterior to posterior. Pigs have a varying number of ribs and the Homm study used both 8 and 11 rib boneless loins, so anterior chops from that study may not replicate location A chops from the current study. The inconsistency in marbling quantification found in both studies is most likely due to the appearance of the graded surface of chops from various locations in the loin differing greatly. Location A chops typically had a much smaller diameter and had other muscles exposed on the cut surface, which could have ultimately led to misestimates of true longissimus marbling in subjective scoring. It is also known that chop color can have an effect on visual marbling scores and vice versa. In retail, chops used for center cut boneless loin packages (IMPS Item No. 1414) could exclude the most anterior chop from the present study, depending on the number of ribs the pig has and whether an 8-rib (IMPS Item No. 412) or 11-rib (IMPS Item No. 412C) boneless pork loin is being fabricated, so nonconforming chops from location A may not adversely affect consumer’s visual acceptance of center cut boneless pork chops. In this study subjective color and firmness scores also exhibited significant differences due to the location of where they were removed from the loin. There were clear differences in chop shape among the various locations selected, and although not characterized in this study it is yet another factor that could come into play as having adverse effects on chop uniformity. Lowe et al., (2011) reported that anterior portions of the loin measured the widest and had the largest loin eye area, but more anterior chops had greater depth to width and width to width ratios indicating a rounder shape. Real-time subjective analysis by packers and consumers of the marbling, color, and overall appearance of pork products will determine sorting and selection in real world applications.

Almost half of the variability in IMF was explained by the three factors in the model, with the greatest of those attributed to variability within sire line. As was expected, the difference in the two Duroc sire lines selected played a role in variability. There was still, however, a majority of that variability that was unaccounted for and not explained. On the other hand, all together, the factors in the model explained only 14% of variability in WBSF. The current data reported mean differences as well as differences in variability of IMF within each of the annotated groups. There were mean differences for WBSF within the groups, however no differences in variability existed within any of the groups used in this study. Many other factors are evidently at play for explaining the variation in chop tenderness.

CONCLUSION

Results from this study confirms the hypothesis that differing variability does exist in pork loin marbling and can be attributed to sex, sire line, and chop location. These findings have relevance to producers that are attempting to target a particular amount of marbling to reach a desired product line specification. As expected, barrows from genetic lines selected for meat quality give the breeder the best chance to meet this goal, but these animals also have the most variability of marbling, now knowing that even different locations within their loin will fluctuate in IMF content. Therefore pigs with the best chance of hitting the highest mark for meat quality also bring about a greater likelihood of missing that target, although it should be noted that they will still meet high standards for meat quality much more consistently than lower quality, less variable pigs, such as the LG pigs. For the packer, as loins approach high quality product line qualifications based on visual appraisal of the ventral surface, the likelihood of making inaccurate predictions about the quality of individual chops derived from those loins also increases. The current study confirms that the 10^{th} rib is indeed intermediate in terms of quality, and therefore the best representative of the average of the entire loin, but it is clear that the composite is not representative of every individual part. Current speculators of changing the standard location for quality measurements should be aware that a different chop face would greatly skew quality measurements in favor of the producer (moving anterior) or the packer (moving posterior; ribbing at last rib). Possibly the biggest implication is on retail selection for packaging, now knowing that a continuous selection of loin chops from a single loin may not lay out next to each other and appear congruent. Thought should be given to whether a group of chops from the same anatomical location on multiple loins or chops from multiple locations on the same loin are actually perceived better by consumers. It is unclear what the ideal chop is or which combination of quality traits provides the best eating experience, so the astute action to ensure a pleasant retail experience is to allow each consumer to pick a package with all included pork chops meeting the criteria specific to their liking.

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FIGURES

Figure 3.1. Illustration of chops selected for meat quality measurements.

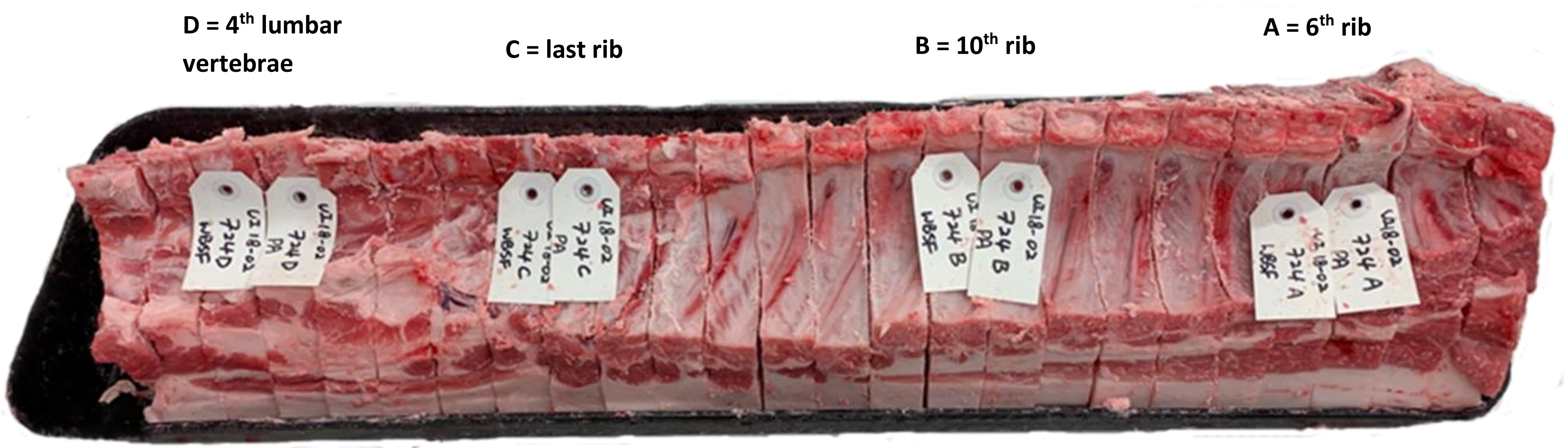


Figure 3.2. Differences in intramuscular fat (IMF) percentage by treatment group. Chops were collected from four locations; A = 6th rib, B = 10th rib, C = last rib, D = 4th lumbar vertebrae. Pigs were sired by boars selected for either meat quality (MQ) or lean growth (LG). Means within a comparison (total, sex, sire line) that do not share a common superscript are different ($P < 0.05$).

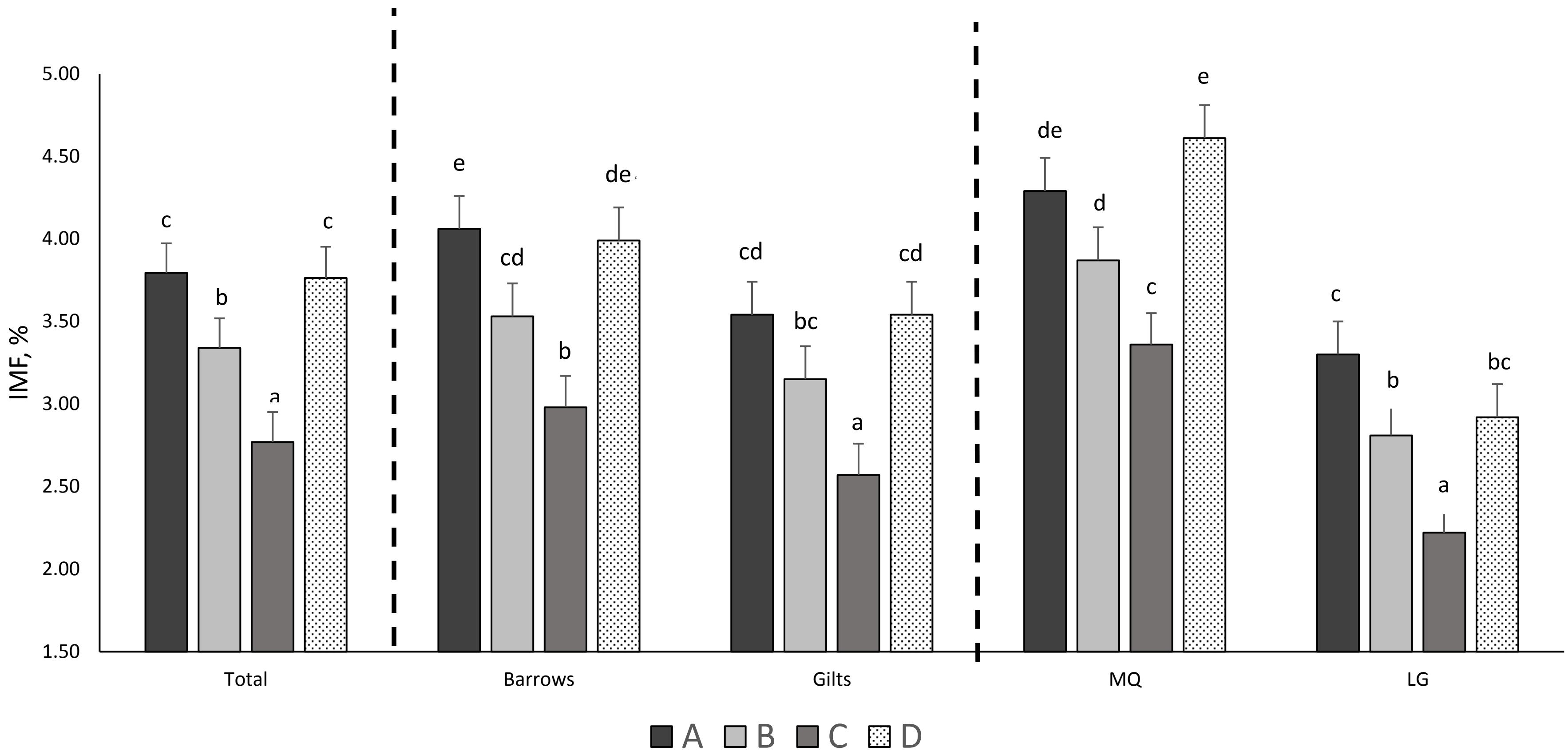


Figure 3.3. A-C Variability of intramuscular fat (IMF) percentage by A) sex; B) sire line; C) chop location. Chops were collected from four locations; A = 6th rib, B = 10th rib, C = last rib, D = 4th lumbar vertebrae. Pigs were sired by boars selected for either meat quality (MQ) or lean growth (LG).

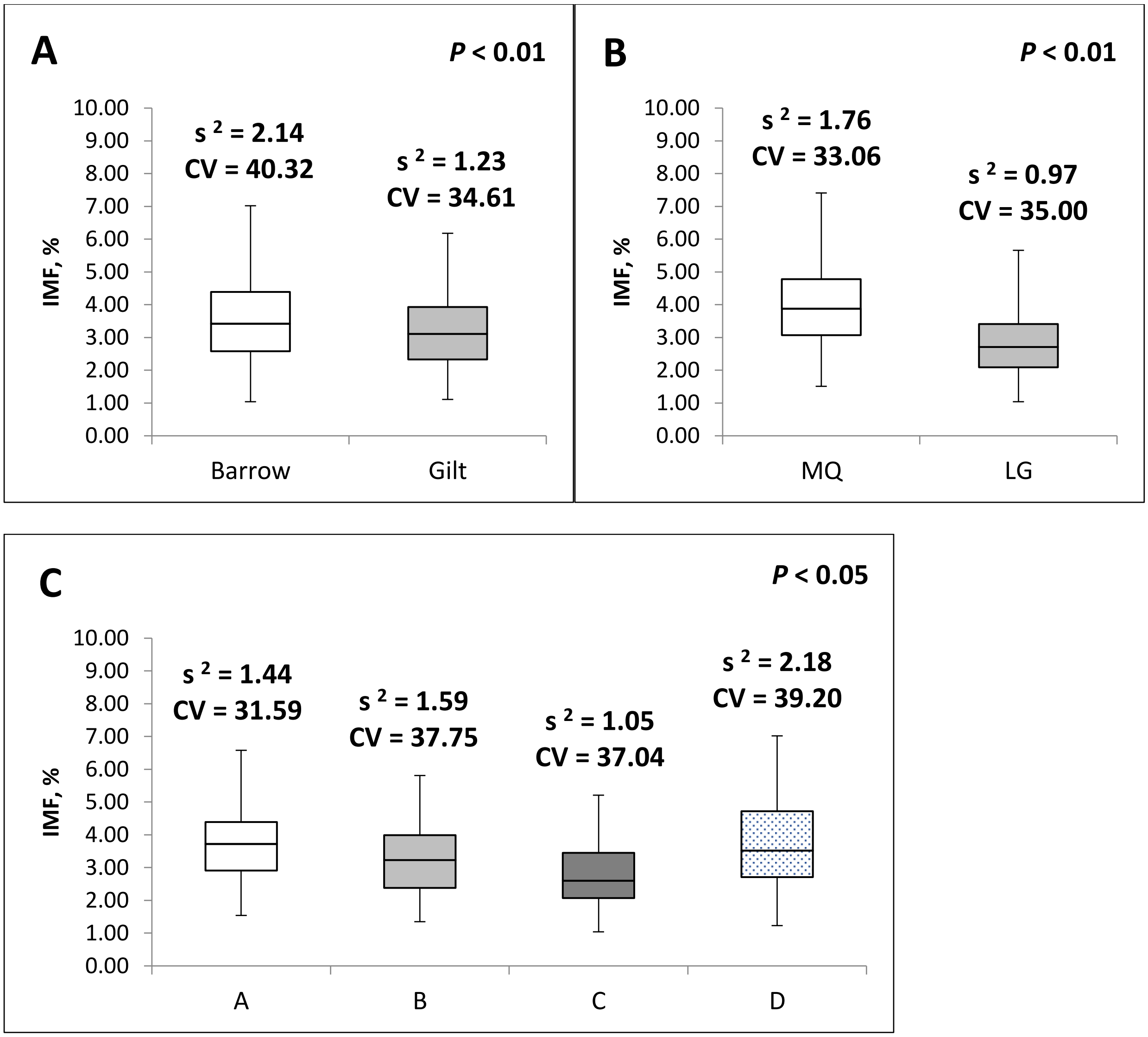


Figure 3.4. Percent of total variation that sex, sire line, chop location and pig (unaccounted for error) contributed to A) intramuscular fat percentage; B) Warner-Bratzler shear force.

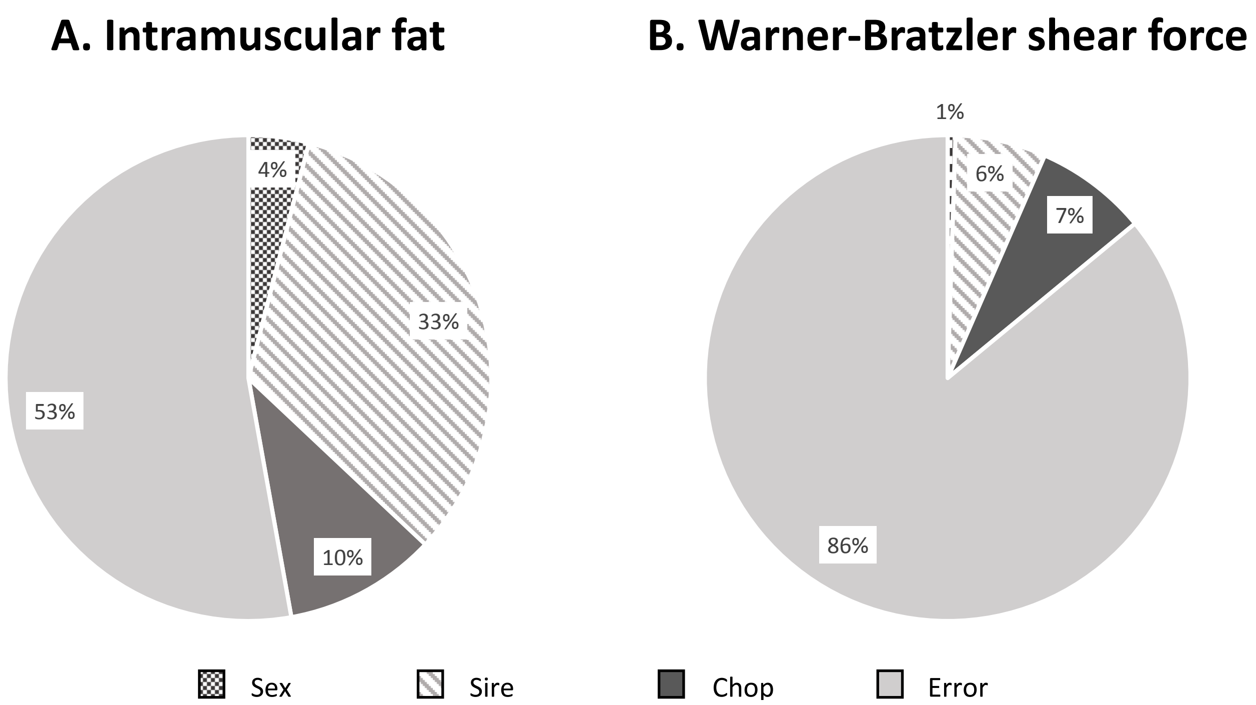
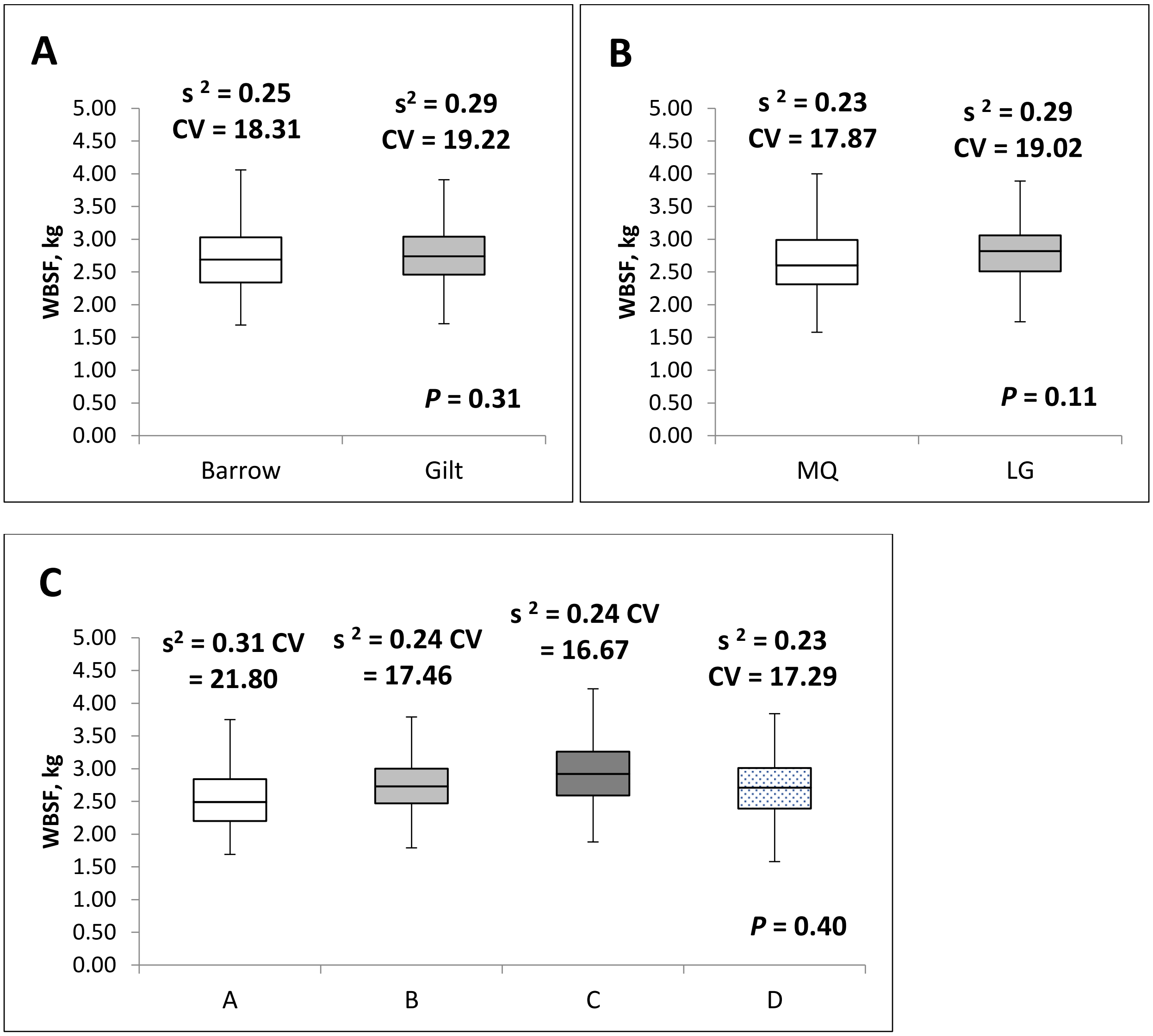


Figure 3.5. A-C Variability of Warner-Bratzler shear force by A) sex; B) sire line; C) chop location. Chops were collected from four locations; A = 6th rib, B = 10th rib, C = last rib, D = 4th lumbar vertebrae. Pigs were sired by boars selected for either meat quality (MQ) or lean growth (LG).



TABLES

Table 3.1. Main effects of sire line and sex on carcass characteristics and chop number per loin

Item	Total Observations	Sex		Sire Line[1]		SEM[2]	*P*-value		
		Barrows	Gilts	MQ	LG		Sex	Sire Line	Sire Line × Sex
Final trial wt, kg	191	131.72	126.22	133.94	123.99	1.36	< 0.01	< 0.01	0.40
Hot carcass wt, kg	188	102.30	94.86	100.04	97.12	0.87	< 0.01	0.02	0.43
Loin eye area, 10^{th} rib, cm^2	189	47.00	47.67	44.75	49.91	0.59	0.37	< 0.01	0.66
Back fat, 10th rib, cm	64	3.17	2.39	3.07	2.48	0.12	< 0.01	< 0.01	0.35
Back fat, last rib, cm	126	3.39	2.96	3.27	3.08	0.16	< 0.01	0.12	0.80
Chop count[3]	190	23.92	23.37	23.89	23.40	0.11	< 0.01	< 0.01	0.46

[1]Pigs were sired by boars selected for either meat quality (MQ) or lean growth (LG)

[2]Standard error of least squares means

[3]Number of chops (2.54 cm) cut from whole bone-in loins

Table 3.2. Main effects of sex, sire line, and chop location on quality parameters of loins

	Sex		Sire Line[2]		Chop Location[3,5]					*P*-value				
Item	Barrows	Gilts	MQ	LG	A	B	C	D	SEM[4]	Sex	Sire Line	Location	Sex × Sire line	Sire Line × Location
Visual Color Score [1]	4.22	4.26	4.24	4.25	4.46[c]	4.06[a]	4.23[b]	4.22[b]	0.05	0.18	0.64	< 0.01	< 0.01	0.13
Visual Marbling Score [1]	3.12	3.02	3.28	2.86	2.76[a]	2.92[b]	3.11[c]	3.50[d]	0.16	0.02	< 0.01	< 0.01	< 0.01	0.12
Firmness Score [1]	2.62	2.56	2.62	2.56	2.06[a]	2.21[b]	2.85[c]	3.22[d]	0.08	0.06	0.06	< 0.01	0.83	0.59
Lipid, % (IMF)	3.64	3.20	4.02	2.81	3.80[c]	3.34[b]	2.77[a]	3.77[c]	0.19	< 0.01	< 0.01	< 0.01	< 0.01	0.01
Moisture, %	73.68	74.06	73.40	74.33	73.92[b]	73.93[b]	74.12[c]	73.49[a]	0.18	< 0.01	< 0.01	< 0.01	0.30	0.14
WBSF[6], kg	2.73	2.79	2.66	2.85	2.56[a]	2.79[b]	2.93[c]	2.75[b]	0.04	0.06	< 0.01	< 0.01	0.79	0.41
Cook Loss, %	17.17	17.19	16.97	17.40	15.33[a]	16.65[b]	18.01[c]	18.74[d]	0.45	0.94	0.08	< 0.01	0.65	0.01

[1]National Pork Producers Council subjective scoring system for color (1999), marbling (1999), and firmness (1991)

[2]Pigs were sired by boars selected for either meat quality (MQ) or lean growth (LG)

[3]Chops were collected from four locations; A = 6th rib, B = 10th rib, C = last rib, D = 4th lumbar vertebrae

[4]Standard error of least squares means
[5]Within a main effect, means without a common superscript letter differ ($P < 0.05$)
[6]Warner-Bratzler Shear Force determination on chops cooked to 63°C
[7]No significant ($P < 0.05$) Sex by Location or 3-way interactions existed