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An evaluation of equipment and procedures for the prediction of intramuscular fat in live swine

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**An evaluation of equipment and procedures for the prediction of intramuscular fat in
live swine**

by

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A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Animal Science

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2010

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CHAPTER 1. INTRODUCTION

Structure of the commercial swine industry is continually changing, and this trend will likely continue. Just as factors associated with swine production change, the type of pig produced is also ever changing. Phenotypically, frame size and the muscle to fat ratio at marketing time have fluctuated the most during the second half of the 20th century.

Advancements in the measurement of fat and muscle in live animals, genetic selection, and the lean value-based marketing system have cumulatively led to a high lean pig population. Consumer demands for leaner pork have undoubtedly been met industry-wide. On the other hand, research has shown that indicators of fresh pork quality and sensory attributes of cooked pork have declined as continued selection for leanness has been practiced over time. Though the healthfulness of cooked pork should remain a priority for the swine industry, issues resulting from intense emphasis being placed on leanness should also be addressed. Production of pork that is safe, healthy, consistent, attractive, and flavorful should be the ultimate goal for the swine industry. Achievement of the aforementioned product should eventually result in continued demand for pork and profitable opportunities for pork producers.

Increasing the lean content of pork carcasses was accomplished relatively quickly. However, it is likely that improvement of overall pork quality will be a long and tedious process due to the following challenges: measuring the traits in live animals, measuring the traits in carcasses, and assigning value to carcasses based at least partially on pork quality traits. Underlying reasons for the decline in pork quality over time are not easily defined. It is likely that multiple factors are responsible. Therefore, improvement of pork quality is a complex issue. From a scientific standpoint, many of the generally accepted indicators of

fresh pork quality are phenotypically related and genetically controlled. In order for genetic selection pressure to be successful, pork quality traits must be measurable, heritable, and have sufficient variation within a population. This thesis will concentrate on the first of these challenges, measurement of pork quality traits.

There has been continuous debate about relationships among pork quality traits and which of the fresh pork quality indicators is the most important determinant of cooked pork sensory attributes. Indicators of fresh pork quality such as pH, water holding capacity, and marbling have traditionally been measured on hanging carcasses or primal cuts. Obviously, genetic selection for these traits would be based upon sibling, relative, or progeny data. To date, the only indicator of fresh pork quality that has been successfully measured in live animals is intramuscular fat (IMF) content of the loin muscle (Ragland, 1998; Newcom et al., 2002). These studies used real-time ultrasound technology and image texture parameters to develop statistical models to predict IMF. Single trait selection for IMF using measurements taken on live animals with real-time ultrasound has been successful, and correlated responses with some indicators of fresh pork quality and sensory attributes have been documented (Schwab et al., 2009). It is evident that if IMF is a trait of interest, real-time ultrasound technology and IMF prediction methodology will allow for effective genetic selection. Furthermore, in relation to pork quality traits limited to postmortem measurements, the opportunity for more rapid genetic progress exists due to accurate measurement of the trait in live animals.

Ultrasound technology has various applications in the livestock industry across meat animal species. Measurements of carcass composition of live animals and reproductive status assessment in beef, sheep, and swine have been the primary applications of ultrasound

technology in livestock for more than 50 years. A common application of ultrasound in the livestock sector is muscle and fat tissue boundary mapping. Measurements of fat depth and muscle thickness or muscle area have generally provided the livestock industry with an accurate method of evaluating carcass composition when carcass data are not easily obtained or desired. These measurements have traditionally been used in genetic selection programs, lean-growth modeling, carcass competitions, prediction of the appropriate endpoint in feedlot cattle, and body condition scoring. The pork packing industry has also utilized ultrasound as a tool for measuring fat and muscle depth of pork carcasses at line speed. More recently, real-time ultrasound technology has been used in conjunction with image analysis software to predict the amount of IMF in the longissimus dorsi muscle of cattle (Herring et al., 1998; Hassen et al., 1999; Hassen et al., 2001) and swine (Newcom et al., 2002).

This thesis is presented as a general introduction, literature review, scientific paper, and a general summary. The literature review is divided into 9 sections that focus on the evolution of the swine industry as it relates to changes in carcass composition, ultrasound technology, genetic selection, pork quality, and the measurement of IMF in live animals using ultrasound technology. Historical and broad issues are covered first and with each subsection, the literature reviewed becomes more detailed and current. The scientific paper compares the accuracy of various systems used for predicting IMF content in the loin of live swine. Different scanners, image capturing devices, image collection methods, and region of interest box options are evaluated. References cited in the literature review can be found following the general summary. All reference citations are in compliance with the Journal of Animal Science, to which the scientific paper will be submitted. The individual paper

consists of an abstract, introduction, materials and methods, results and discussion, and an implication section.

CHAPTER 2. REVIEW OF LITERATURE

Principles of ultrasound

The speed of sound is generally measured in hertz and is commonly referred to as cycles per second. Though variable, the human auditory system can generally detect sound traveling at speeds within a relatively narrow range of 20 to 20,000 hertz. Sound traveling too slowly for humans to detect is known as infrasound. Conversely, sound traveling too quickly for the human ear to detect is defined as ultrasound. The speed of ultrasound commonly used in biological applications travels so quickly it is measured in megahertz (MHz). The standard range of ultrasound transmission for imaging biological tissue is from 2 to 20 MHz. At lower MHz values, the degree of resolution is depressed but the sound waves can penetrate more deeply into biological tissue due to a longer wavelength. Conversely, higher MHz values allow for increased resolution with reduced depth penetration due to a shorter wavelength. Wilson (1994) reported that values of 3 to 3.5 are most common for applications involving livestock.

Ultrasound is used to penetrate a medium and measure the reflection signature or supply focused energy. The general idea behind ultrasound applications centers on the emission, reception, and processing of ultrasonic waves. A critical component to the emission and reception of ultrasonic waves is the ultrasound transducer. Ultrasound transducers produce cyclic sound pressure (pulses of ultrasound) through vibrations of piezoelectric material. Quartz crystals are the commonly found piezoelectric material in ultrasound transducers. Once ultrasound is produced, the transducer emits sound waves in a single beam or linear array, depending on the number of quartz crystals present. At this point, the ultrasound beam or waves can be transmitted through biological tissue.

When ultrasonic waves reach dense tissues or the interface between 2 tissues, a portion of the sound wave continues to penetrate further into the tissue while some of the wave is reflected back to the transducer. This is due to variability of the acoustical properties of different tissues. Examples of dense biological surfaces include skin, membranes between fat layers, membranes between muscle and fat, and membranes between muscle and bone (Boggs and Merkel, 1990). The velocity of sound traveling through biological tissue varies by the type and temperature of the tissue. For example, the speed of sound increases with temperature and travels through fat, muscle, and skin at 1,430, 1,620, and 1,700 meters per second, respectively (Wilson, 1994). The ability to quantify ultrasound wave distance traveled in and rate of return from biological tissue makes ultrasound technology very unique. When sound waves are reflected back from biological tissue, the transducer acts as a receiver as the reflected waves produce mechanical energy, striking the quartz crystals. This energy is then converted to electrical energy, processed by a central processing unit, and displayed in varying formats (Wilson, 1994).

Technological progression has changed the sophistication of ultrasound machines over the years, and there are still many different types of ultrasonic instruments on the market today. Yet, they all operate on the principle of sound waves creating an echo upon contact with a dense surface and reflecting back through the transducer at different rates. The primary differences among types of ultrasound machines are the number of quartz crystals that are used to create the ultrasonic measurement and the sophistication of the information displayed.

Changes in pork carcass composition and the evolution of ultrasound

In the early 1900s and through the end of the second World War, pigs were raised primarily for their fat content. Lard was a cheap, abundant, and important ingredient for numerous goods. However, market signals in the 1950s pointed toward a shift in production to the meat type pig. Though changes in domestic pig type continually occur, this particular shift would forever change the industry. With growing demand for production of leaner pigs, genetic selection programs were developed and called for rapid and accurate measurements of composition in the live pig.

The metal backfat probe, an invasive method for measuring backfat depth, was the first in a series of tools used to make live animal measurements and eventually predict lean content of live pigs. Hazel and Kline (1952) used the metal backfat probe to measure backfat at 4 locations on 96 crossbred pigs weighing an average of 98 kg. Incisions about 0.64 cm deep and 1.27 cm long were made with a scalpel through the skin. A metal ruler with a blunt end was then inserted until it reached muscle tissue.

A correlation of 0.81 between the mean of the 4 live pig measurements and 4 carcass measurements of backfat was reported. A measurement taken directly behind the shoulder produced a correlation with mean carcass backfat measured at 4 locations of 0.79, and was the single most accurate probing site. Correlations between live pig measurements and lean loin area, lean ham area, and percentage primal cuts ranged from -0.32 to -0.56 and yet, were still better indicators of leanness than carcass measurements of backfat.

Though relatively accurate, bacterial infections near the incision and difficulty in animal restraint made the metal probe technique less than adequate. At this point, the swine industry desperately needed a noninvasive tool for measuring carcass composition.

Consequently, numerous animal scientists worked to develop, evaluate, and improve ultrasound technology and measurement techniques in the decades to follow.

Although ultrasound technology has been used in the swine industry for more than a half century, progress and implementation was slow at first due to fragile machines, high investment costs, and a lack of trained technicians (Moeller, 2002). Nonetheless, early researchers in the field worked to find a suitable replacement for the invasive backfat probe. Early machines known as amplitude depth machines (A-Mode) utilized a single quartz crystal transducer to emit and receive a single sound wave. A 1-dimensional display of the amplitude and distance of the returning echo was characterized by vertical peaks along a horizontal axis. The height of the peak corresponded to the amplitude of the echo (Rantanen and Ewing, 1981; Herring and Bjornton, 1985). Using known time and distance relationships, the echoes are converted into signals that are interpreted using an oscilloscope, a series of lights, or light emitting diode readout (Moeller, 2002). Claus (1957) was the first to investigate the feasibility of using ultrasound to evaluate live pig composition. Subsequent reports compared A-mode backfat depth measurements with the metal probe and traditional carcass measurements (Hazel and Kline, 1959; Price et al., 1960a; Price et al., 1960b; Stouffer et al., 1961).

Hazel and Kline (1959) compared 2 ultrasound frequencies and metal probe measurements of backfat on 56 pigs of varying breeds and crosses weighing 86 to 113 kg. Measurements were taken 5.08 cm off midline with a Kelvin and Hughes Mark V flaw detector and metal probe behind the shoulder, at the middle of the back, and at the rear of the loin on each animal.

Means of the 3 locations for ultrasound and metal probe correlated strongly with percentage of lean cuts at -0.90 and -0.89, respectively. However, accuracy of individual measurement locations at predicting percent lean cuts differed greatly for the 2 methods. The metal probe was more accurate at the shoulder while the ultrasonic probe was more accurate over the loin. It is possible that accuracy of the ultrasonic method was reduced at the anterior location due to presence of the spinalis dorsi and trapezius muscles.

In a more expansive study, Price et al. (1960a) also sought to relate ultrasonic measurements with other measurements of leanness and fatness. A Sperry Reflectoscope equipped with a 2.25 MHz crystal was used to measure backfat thickness and loin depth on 158 pigs of several breeds and crosses. Due to differences in animal handling and the measurement procedure, data were divided into 2 subsets. In group 1 (n = 74), animals were restrained in a crate and measured at multiple locations along the dorsal side of the loin. In group 2 (n = 84), animals were allowed to stand naturally and were only measured in the center of the back.

Highly significant correlation coefficients between ultrasonic measurements of backfat and metal probe (0.81), and carcass backfat (0.82) were found for group 1. Ultrasonically-measured backfat on live pigs correlated strongly with measurements taken with the metal probe (0.91) and on the carcass (0.88) for group 2. Conversely, significantly lower relationships were found between live ultrasonically-measured loin depth and other measures of leanness. Loin muscle depth measured ultrasonically over the center of the back was lowly correlated with 10th rib loin muscle area (0.34), and loin muscle depth as determined from a carcass tracing (0.30). The study concluded that ultrasound is a reliable tool for measuring backfat depth on live pigs when compared with the metal probe and

carcass measurements. Additionally, each of the 3 measurements of fat was equally reliable for predicting carcass lean. Although ultrasound measurements of loin depth were related to depth and area determinations taken from a tracing, the measurement did not predict lean content sufficiently.

Nonetheless, other early researchers sought to measure loin muscle area of live pigs. The first 2-dimensional view of the loin muscle was fashioned by recording multiple A-mode measures taken at varying angles. By plotting reflectance peaks at varying angles of incidence, the perimeter of the loin was outlined. Price et al. (1960b) investigated the accuracy of this method using a Sperry Reflectoscope. Loin muscle area was measured on 41 pigs just anterior to the last rib. The region from the center of the back to about 15 cm off midline was marked off in inch long increments. Reflection peaks from each of the measurement locations indicating muscle and fat tissue interfaces were recorded. After measuring the contour of the pig's back at the last rib with a piece of lead wire and transferring this curve to a piece of paper, the depth readings were plotted under the contour line, indicating the dorsal and ventral side of the loin muscle. A polar planimeter was then used to connect the points and calculate the area.

A correlation of 0.74 was reported between last rib carcass loin muscle area as determined by tracings and the ultrasound plot method. The ultrasound method overestimated loin muscle area by 1.29 cm², but this difference was not significant.

Though accuracy of the ultrasound plot method seemed reasonable, several difficulties were cause for concern. Plots of the tissue boundaries between muscle and fat left the medial and lateral ends of the loin unclosed and interpretation required some degree of subjectivity when sketching. The tendency to "round off" the ends could explain the

overestimation in the results. Furthermore, fatter animals produced less consistent plots of depth measurements, possibly due to a significant loss of ultrasound signal. The inability of the reflection peaks to differentiate the third fat layer from the longissimus dorsi muscle was also a common problem. Lastly, the method was tedious and time consuming, making repeated measures impractical. Several challenges associated with animal restraint and the added scanning time required caused this technique to lose attention.

Stouffer et al. (1961) worked to further expedite the aforementioned technique by transferring the multiple depth measurements simultaneously into a cross-sectional photograph using a 35 mm camera. Using a Sperry Reflectoscope equipped with a 1.0 MHz transducer, 9 readings at varying angles over the 12th rib were taken on 42 pigs.

This study reported promising correlations between ultrasound and carcass backfat thickness (0.92) as well as carcass loin muscle area (0.70). Although reasonably accurate, this method was very time consuming. The animal had to remain motionless for 10 seconds to produce a clear image of the loin muscle.

The A-mode machines were demonstrated to be accurate in measuring backfat depth from a single point estimate when operated by trained technicians. However, other sources of variation led to skepticism about the accuracy and repeatability of A-mode ultrasound measures of backfat in pigs. Results varied within and among reports. Pigs with greater amounts of backfat (Claus, 1957; Price et al., 1960b) and measurements taken nearer to the anterior end of the loin muscle (Stouffer et al., 1961; Anderson and Wahlstrom, 1969; Adams et al., 1972; Mersmann, 1982) were found to be less accurate than measurements taken in the center of the back. These findings could be explained by the anterior to posterior fattening pattern in pigs or the presence of adjacent muscle groups near the anterior end of the

longissimus dorsi, decreasing the penetration depth of the ultrasound signal. Sather et al. (1982) and Stouffer et al. (1961) expressed concern about large amounts of variation between operators and machines when measuring backfat depth and stressed the need for competent technicians and further refinements in technique, respectively.

Although A-mode ultrasound was relatively inexpensive, seemingly easy to use, and generally reliable for measuring backfat, it also presented several downfalls. The repeatability of measurement location and variability between operators and machines were significant causes for concern. In general, the accuracy and repeatability of A-mode measurements of muscle depth and area were compromised due to factors associated with anatomical location, angle of the probe, and differences in machines and operator ability.

Technological advances during the 1970s and 1980s greatly improved ultrasound equipment (Houghton and Turlington, 1992) and expanded the capabilities of ultrasound machines. Human medicine along with the livestock industry would forever change with the introduction of brightness mode (B-mode) ultrasound machines in the early 1980s. With this technology came a significant improvement in the ability to measure carcass composition of live pigs. During this era, the swine industry faced continued pressure to produce pigs with less fat and more muscle. The need for improved accuracy and efficiency in measuring the traits on live pigs was ever-present. This trend paralleled the introduction of B-mode ultrasound and a new wave of research by animal scientists.

Unlike the A-mode transducer, a linear array of multiple quartz crystals are arranged inside the B-mode transducer. Sound waves are fired in succession about 30 times per second. Ultrasound frequencies of 3 to 5 MHz provide the optimum combination of resolution and depth penetration and are typically used to create images for composition

assessment. Once inside biological tissue, multiple sound waves interact to form patterns of energy. By varying phases of oscillation of the crystals across the transducer, their energy is steered into a described, constant pattern. Quartz crystals are focused in this way for optimal depth resolution upon reception of the ultrasound waves.

As sound waves are reflected back to the transducer, they are converted to electrical signals which carry time and distance information about the sound waves. A central processing unit then collects and processes the information. Upon processing, changes in sound properties as they pass through and reflect from biological tissue are characterized. Unlike A-mode ultrasound, the central processing unit uses the information to create a 2-dimensional image that can be displayed on a video screen. The 2-dimensional images are displayed as an array of dots known as pixels. The position of each pixel on the video screen is determined by the time it takes for an echo to return to the transducer. The brightness of each pixel is proportional to the amplitude (or strength) of the returning echo (Wilson, 1994). Compared to A-mode, this particular concept was perhaps the most significant advancement to date.

One major downfall to the use of A-mode ultrasound in swine was the questionable repeatability of measurement location on the pig. Genetic progress becomes impeded by this type of variation within a contemporary group. The capability of B-mode ultrasound to image a visible 2-dimensional view of a medium was of particular interest to the swine industry. With the visible imaging capabilities of B-mode ultrasound and knowledge of porcine anatomy, a repeatable location can be established. Technicians were able to refine measurement location based upon the presence or absence of different muscle groups and the shape of the longissimus dorsi muscle. It became possible to visualize each of the 3

individual fat layers to more accurately measure backfat depth and loin muscle area. Still, uncooperative animals posed a problem for technicians because the display was not updated fast enough to keep up with movements of the animal. Thus, the ability to capture clear images at the correct location was still difficult.

Ultrasound units that produce real-time images are now the most commonly accepted ultrasonic instrumentation for use with livestock (Houghton and Turlington, 1992). Real-time ultrasound is the most sophisticated version of B-mode ultrasound. With real-time, the image on the display monitor is updated instantaneously, similar to a live video stream. As a result of continuous transmission and reception of multiple sound waves, visible images are created and displayed at a frame refreshing rate between 8 and 16 frames per second (Moeller, 2002), which is faster than the human eye can detect. Returning echoes are spatially oriented on the display screen with an encoder to depict tissue interfaces. When the image that appears is clearly visible and in the desired anatomical location, it can then be frozen on the display monitor by the technician. Using the distance and area functions on the scanner console, the frozen image can be measured for backfat thickness and loin muscle area at the time of scanning, captured on a VCR, or saved digitally to a personal computer for later interpretation.

Numerous researchers have investigated the sources of variation in accuracy when measuring fat and muscle in live pigs using real-time ultrasound. Different ultrasound systems, probe angles, measurement locations over the loin, and the effects of breed, sex, and live weight on accuracy have all been studied. Christian and Moeller (1990) evaluated the accuracy of an Aloka 633 real-time ultrasound machine fitted with a 12.5 cm, 3.5 MHz transducer. A diverse sample of pigs ($n = 756$) were serially scanned at weights near 68, 86,

and 102 kg at various transverse locations along the back including midline fifth rib, last rib and last lumbar backfat, 10th rib backfat and 10th rib loin muscle area. Shortly following the final scan, pigs were harvested at a nearby commercial packing plant and carcass measures were taken following a 14 to 20 hour chilling period.

Tenth rib backfat measured ultrasonically resulted in the highest correlation with carcass backfat at 0.82 and was within 0.51 cm of the carcass measurement 89% of the time for the final scan. Similarly, the highest correlation between ultrasonic loin muscle area and carcass loin muscle area (0.73) was taken just prior to harvest at the 10th rib. These measurements were within 6.45 cm² of the carcass measurement 92.3% of the time. The study concluded that ultrasonic measurements of backfat and loin muscle area taken just prior to harvest at the 10th rib were the most accurate indicators of carcass composition.

Cisneros et al. (1996) used an Aloka 210 DX scanner fitted with a VST-5021, 3.0 MHz transducer to compare the accuracy of live animal longitudinal and transverse scans on 80 pigs weighing between 108 and 148 kg. Transverse scans were taken at right angles to the midline at the last rib while longitudinal scans were taken 6.35 cm off midline immediately anterior and posterior to the last rib. Measurements of backfat depth, loin muscle depth at the halfway point, and loin muscle area were taken on transverse scans. Measurements of backfat depth and loin muscle depth were taken at the anterior and posterior ends of each of the longitudinal scans.

Correlations for last rib carcass backfat and the ultrasound scans were 0.83 for the transverse and 0.82 for the anterior longitudinal, respectively. Relationships between carcass and ultrasound loin muscle depths were somewhat weaker. Carcass last rib loin depth correlated more strongly with the transverse measurement (0.55) than the anterior

longitudinal measurement (0.42). Nonetheless, both the transverse and anterior longitudinal scans predicted lean cut weight and lean cut percentage equally well when residual standard deviations were compared. Thus, the study concluded that a longitudinal scan taken anterior to the last rib provides accuracy similar to that of a traditional transverse scan for predicting overall carcass composition.

A relatively large amount of the literature investigating the accuracy of ultrasound in measuring live animal composition uses correlations as a primary means of comparison. Houghton and Turlington (1992) brought up several areas of concerns about correlations. Variation within an individual population influences correlations. This alone inhibits making useful comparisons across reports. Larger than normal variation will produce large correlation coefficients and a uniform population will result in much lower correlation coefficients. Correlations also do not reflect the individual measurement bias that a particular scan technician may have. If a technician consistently underestimates or overestimates ultrasonic measurements of fat and muscle, their bias does not inhibit their ability to rank animals correctly for genetic evaluation programs. On the other hand, large, inconsistent bias can influence the genetic merit of animals and result in reduced accuracy of selection (Moeller and Christian, 1998). If measurement bias was calculated in more of the published literature on the use of ultrasound in swine, more effective comparisons and conclusions could be made within and among reports. Another downfall to using correlations for reporting data is that they are typically not well understood by producer groups. Houghton and Turlington (1992) suggested frequency distributions as a data reporting method for researchers in the field.

Regardless of the method used to report accuracy data, it is evident that there is considerable variation between species, technicians, and ultrasonic instrumentation in the ability to predict carcass traits (Houghton and Turlington, 1992). Perhaps a broader view should be taken when evaluating the use of ultrasound in swine. Whether taken ultrasonically or on the carcass, individual measurements are used to predict total carcass muscle in one way or another. With this in mind, it may be more appropriate to correlate ultrasound measurements of backfat and longissimus muscle area to total carcass muscle or lean muscle mass, rather than to the carcass measurements themselves (Houghton and Turlington, 1992).

Moeller and Christian (1998) investigated the effects of breed, sex, and the magnitude of carcass measurements on accuracy of real-time ultrasound backfat and loin muscle area measurements at the 10th rib. Another objective was to compare and evaluate multiple measures of accuracy (i.e. bias, absolute deviation, percentage absolute deviation, and standard error of prediction). A single technician scanned pigs from 2 National Barrow Show Progeny tests (Test 1, n = 630 and Test 2, n = 497) with an Aloka 500V SSD real-time ultrasonic instrument equipped with a 12.5 cm, 3.5 MHz linear array transducer. Pigs were serially scanned at 4 mean body weights with the final scan taking place just prior to slaughter.

Residual correlations accounting for test, sex, and breed effects, among and between scans and carcass measurements were moderate to high for 10th rib backfat ($r = 0.69$ to 0.82) and loin muscle area ($r = 0.57$ to 0.68). The largest correlations with carcass measurements resulted from the final scan. A significant sex difference ($P < 0.001$) was found for loin muscle area bias. Loin muscle area was overestimated in barrows and underestimated in

gilts. Breed differences were significant ($P < 0.001$) for 10th rib backfat and loin muscle area bias. The relative magnitude of carcass measurements also affected the bias and accuracy of real-time ultrasound measurements. Carcasses with < 24.1 mm backfat were overestimated by 0.57 mm. Carcasses with > 30.3 mm backfat were underestimated by 2.81 mm. Similarly, ultrasonic loin muscle area overestimated carcass loin muscle area by 2.34 cm² in carcasses with < 32.5 cm² and underestimated it by 2.29 cm² in carcasses with more than 37.9 cm². It was also determined that the standard error of prediction statistic is the most consistent means of evaluating the accuracy of ultrasonic measurements because it takes bias into account and quantifies the accuracy of ranking animals correctly.

Measurements of 10th rib fat thickness and loin muscle area captured and interpreted by trained technicians using real-time ultrasound technology have proven to be highly effective in ranking animals on lean content. With real-time ultrasonic measurements of 10th rib backfat and loin muscle area becoming the preferred method for estimating body composition and making selection decisions, operator training programs have been implemented to improve the accuracy and consistency of ultrasonic data collected industry-wide. Through the National Swine Improvement Federation (NSIF), the U.S. swine industry initiated an ultrasound training and certification program for composition assessment in 1993. Classroom lectures on porcine anatomy, proper scanning techniques, image quality factors, image interpretation, and accuracy assessment are presented on the first day. On day 2, technicians scan and interpret images on 50 pigs in the morning session. In the afternoon, pigs are renumbered and scanned a second time for repeatability assessment. Upon completion of scanning, animals are harvested at a commercial packing plant. Carcass measurements are taken by 2 experienced meat scientists with a set maximum deviation

between the 2. Standard error of prediction (SEP), standard error of the difference (SED), and bias standards require technicians to measure 10th rib fat thickness and loin muscle area with accuracy and repeatability. Criteria for certification standards are as follows: less than 3.81 mm SEP, SED, and bias for backfat; and less than 3.23 cm² SEP, SED, and bias for loin muscle area.

Using compiled data from 1998 to 2003 of the NSIF certification program, Burkett et al. (2004) investigated the effect of technician, machine, and animal body composition on ultrasonic measures of 10th rib backfat and loin muscle area.

Technician within year accounted for 25.1% of the variation between scan and carcass backfat measures and 52.8% of the variation between scan and carcass loin muscle area. The difference between scan and carcass backfat and the absolute value of that difference was larger for technicians using A-mode machines than those using B-mode. Technician ability, machine type, and animal body composition all impacted the accuracy of backfat and loin muscle area measured on live pigs in this study (Burkett et al., 2004)

Prediction of lean content and carcass merit pricing systems

Despite the shift toward the meat type pig in the 1950s and constant improvements in measuring fat and muscle on live animals, only 3% of pigs marketed in the U.S. were sold on a carcass merit or grade and yield program in 1970 (Ikerd and Cramer, 1970). In this era of swine production, numerous smaller producers typically sold their market pigs to small country buying stations and were paid on a live weight cash market basis. A grade and yield option was eventually presented at the buying stations, but most producers still opted for the live cash price. At this time, carcass pricing was primarily based upon carcass yield (dressing percentage) and USDA muscle score (thin, moderate, or thick). Many producers

did not see an advantage to the idea of “selling on the rail”. Despite this, the percentage of pigs marketed on a carcass merit basis grew to over 10% by 1985 (Hayenga et al., 1985) and to 28% in 1988 (Brorsen et al., 1998). In the years to follow, major changes in carcass evaluation by packers and the pork pricing system took place, driving the percentage of market hogs sold on a carcass merit basis even higher.

In the 1980s, most pork producers considered the lean incentives offered by packers to be fair or poor. The Other White Meat™ national pork advertising campaign was initiated in 1987 and the health conscious consumer was asking for leaner pork at the meat counter. Problems in transmitting carcass value differences accurately to pork producers were evident. To attempt to solve the perceived dilemma between pork producers, packers, and consumers, several researchers in the coming years developed and refined methods of carcass lean estimation and pricing.

Development of regression equations to predict percentage or weight of muscle in pork carcasses dates back to the late 1970s. Fahey et al. (1977) compared the accuracy of several prediction methods and developed linear regression equations to predict total muscle in 41 barrow carcasses. After chilling, measurements of backfat and muscle were made on the carcasses. Measurements included 10th rib loin muscle area and backfat measured 3/4 of the distance from the medial end of the longissimus muscle. Carcasses were broken into primal cuts and carefully dissected into separate bone, fat, and muscle components. Fat-free muscle percentage and total muscle weight were the dependent variables used in the analysis. Carcass weight and 10th rib measures of loin muscle area and backfat depth accounted for the largest amount of variation in fat-free muscle percentage and total muscle weight.

The 3-factor equation developed by Fahey et al. (1977) that included hot carcass weight, 10th rib fat depth, and 10th rib loin muscle area had become the most widely used method of ranking carcasses for lean content in pork carcass contests (Powell et al., 1983). However, the small population used to develop the equation contained only barrows with greater than typical amounts of muscling. Thus, it was hypothesized and tested by later researchers that the equation predicted high percentage lean carcasses more accurately than low percentage lean carcasses. Some researchers suggested that more dissection data was needed to update or improve the accuracy of the formula. Additionally, the USDA pork grading system at this time was based on the estimated yield of the 4 primal cuts and was not applied to the entire hanging carcass.

Powell et al. (1983) looked to validate the equation developed by Fahey et al. (1977) on a different group of carcasses and provide information for possible revisions to the USDA pork grading system. Fifty-two pork carcasses comprising a balance of barrows and gilts and representing a wide range of muscling and fatness were used in this study. After a 24 h chill, carcass length and 10th rib backfat depth and loin muscle area measurements were taken. Percentage of muscle standardized to 10% fat was estimated with the regression equation developed from the data of Fahey et al. (1977). The left side of each carcass was dissected into separable muscle, bone and tendon, fat, and skin. Carcasses were divided into 2 sex groups and 5 percentage lean groups to evaluate variation in the sample population.

Results from this study indicate that as the content of fat increases and muscle decreases, the difference between predicted and actual muscle percentage increases. Tenth rib backfat depth was the single best predictor of percentage muscle while the best 2-trait model also included loin muscle area. The coefficient of determination and standard error

were improved with the addition of hot carcass weight as a third variable. For prediction of total muscle weight, the single best indicator was loin muscle area. Identical to the equation for percentage muscle, the best 3-variable model for total muscle weight included hot carcass weight, 10th rib fat depth, and loin muscle area. There was no additional improvement in accuracy for either equation when a fourth or fifth variable was added. Powell et al. (1983) concluded that the equation developed by Fahey et al. (1977) can accurately rank carcasses on percentage lean, but that it overestimates low percentage lean carcasses. The equations derived from Powell et al. (1983) should more accurately rank carcasses in the packing industry because they were developed using a more diverse and representative population.

A large scale evaluation of procedures to predict fat-free lean in swine carcasses was conducted by Johnson et al. (2004). Fat-free lean was predicted on carcasses of 1,024 pigs that represented a cross section of animals current to the industry. Prediction equations were developed for each of the following 6 measurement techniques: fat and muscle depth using a Fat-O-Meter optical probe, Automated Ultrasonic System, or Ultrafom; carcass 10th rib fat depth and loin muscle area, carcass last rib fat depth, and a live animal scan of fat depth and loin muscle area at the 10th rib. The dissection procedure described by Fahey et al. (1977) was used to establish the amount of fat-free lean in carcasses. This value was used as the dependent variable for each of the analyses.

Tenth rib measurements of backfat and loin muscle area taken on the hanging carcass or via a live animal ultrasonic scan provided the most accurate predictions of percentage fat-free lean. Additionally, measurements of fat and muscle depth with ultrasound or an optical probe were more reliable than a single measurement of fat depth at the last rib.

Stewart and Schinckel (1990) summarized the heritabilities, genetic correlations, and phenotypic correlations among several indicators of swine carcass composition and actual percentage lean. Heritabilities were 0.52, 0.47, and 0.48 for 10th rib backfat, 10th rib longissimus muscle area, and percentage lean, respectively. Genetic correlations with percentage lean were -0.87 and 0.65 for 10th rib backfat and longissimus muscle area, respectively. Additionally, phenotypic correlations with percent lean were -0.81 and 0.62 for 10th rib backfat and longissimus muscle area, respectively.

Wilson (1992) summarized previous reports on the predictive power of loin muscle area and backfat depth and reported that across numerous reports, percentage lean is strongly and positively correlated with loin muscle area and negatively with backfat depth. The magnitudes of heritabilities and trait relationships are encouraging for genetic improvement using live animal measurements for backfat and loin muscle area (Wilson, 1992).

The value of a 10th rib measurement of loin muscle area to predict percent lean in live animals and carcasses has been emphasized (Fahey et al., 1977; Powell et al., 1983; Stewart and Schinckel, 1990; Wilson, 1992; and Johnson et al., 2004). This measurement will undoubtedly remain a core criterion in the ranking of live animals via ultrasonic scans and individual carcasses in contests. However, its adaptability for widespread commercial use in the packing industry is probably unlikely. Unfortunately, the feasibility of routinely ribbing carcasses and breaking wholesale pork loins between the 10th and 11th rib in packing plants has been met with much resistance. Consequently, 10th rib loin muscle area is not used in U.S. carcass pricing systems.

The equation from Powell et al. (1983) was not applicable for the packing industry because the measurements used for predictions could not be made at line speed. In Hayenga

et al. (1985), a diverse group of researchers studied the problems in transmitting value differences accurately to hog producers. Their primary objective was to develop a more standardized objective carcass evaluation system for packers to determine and communicate appropriate carcass merit premiums and discounts. Realizing the impracticality of splitting loins for 10th rib loin muscle area measurements, this study focused on using measurements that could be made effectively at the rapid line speeds of meat packing plants. Hot carcass weight, last rib fat depth, and USDA muscle score were recorded on 185 carcasses. A more comprehensive measure of carcass value was calculated by taking weights of the wholesale cuts, fat, lean trim, and bone, and multiplying by average market prices. After adjusting for estimated packaging costs, the total carcass value was divided by the carcass weight to give a carcass price per hundredweight.

Statistical analysis for the relationships between this measure of carcass value and the easily measured carcass traits was carried out. Hot carcass weight and a single measurement of fat depth at the last rib accounted for 76% of the variation in carcass value. The addition of a muscle score index (thin, moderate, thick) increased R^2 to 0.79 and was included, despite it being a subjective measure. To determine the incremental price adjustments of a packer premium/discount schedule, carcass value per hundredweight was regressed against variables for hot carcass weight in 3.18 kg weight classes, last rib backfat categories in 0.25 cm categories, and a 3-category USDA muscling score. Premiums and discounts for each of the carcass weight classes, last rib backfat classes, and muscling groups were subsequently estimated. Once a base carcass price per hundredweight was established for a specified range within carcass weight class, last rib backfat class, and muscling groups, the associated premiums and discounts were expressed as a percentage of base carcass price per

hundredweight in a matrix. This illustration of pricing structure better communicated consumer demands to pork producers and enhanced the overall acceptance of carcass merit pricing. Packers approved of the matrix because it allowed them to determine base price ranges of carcass weight and fat depth based on their specific retail or wholesale demands.

The idea of a pricing matrix, introduced by Hayenga et al. (1985), was widely adopted across the industry and the percentage of hogs marketed on a carcass basis grew to 78% in 1997 (Brosen et al., 1998) and to 83% in 2002 (Grimes and Plain, 2005). Though on-carcass measurements and premium/discount windows vary across packing companies, all currently use a pricing structure similar to Hayenga et al. (1985) and reward carcasses with greater percentages of lean content. As a result, swine genetics companies and pork producers have capitalized on the carcass pricing system and successfully changed the composition of pork carcasses entering U.S. packing plants in recent decades. Accurate prediction of lean content of live animals through rapid and accurate measurement tools, intense genetic selection programs, and the high heritability of carcass traits have led to incredible success at decreasing fat and increasing muscle content of pork carcasses.

Pork quality deterioration over time

Unfortunately, selection for lean content has resulted in negative impacts on pork quality, and consumer acceptance issues have arisen. The underlying mechanisms responsible for this negatively correlated response are complex, multifaceted, and not easily understood. Thus, this review will simply highlight several unique pieces of literature relating to pork quality changes over time and not attempt to resolve the overwhelming issue.

With the idea of a complex group of factors potentially responsible for pork quality deterioration over time in mind, a closer look into the components of skeletal muscle is

warranted. Skeletal muscle is composed of different types of fibers that are defined by the specific proteins they contain. Fiber types are typically classified by the specific isoforms of myosin heavy chain (a skeletal muscle protein) that are present. Type I, type IIa, and type IIb fibers each exhibit different metabolic processes. For example, type I fibers use slow-oxidative metabolism, type IIa fibers use fast-oxidative-glycolytic metabolism, while type IIb fibers use fast-glycolytic metabolism.

Some studies have indicated that selection for rapid growth rate or less backfat in pigs could alter muscle fiber composition toward a higher percentage of type IIb fibers (Rahelic and Puac, 1981; Brocks et al., 2000). Fast glycolytic metabolism is associated with a more rapid postmortem metabolic rate, lactic acid production, and pH decline. Variations in the rate and extent of postmortem glycolysis have been directly associated with the development of pork quality. The type IIb fibers tend to have less myoglobin and be larger in diameter than other fiber types. It has been hypothesized that a higher proportion of type IIb fibers may result in lighter-colored product and a more rapid postmortem pH decline (Huff-Lonergan et al., 2002). Genetic selection programs focused on leanness may have unknowingly selected for a higher proportion of type IIb muscle fibers over time, and this could partially explain the apparent deterioration of pork quality.

In an effort to establish the relationship between muscle fiber type, postmortem metabolic rate, and pork quality, Ryu and Kim (2005) studied longissimus muscle samples from 231 crossbred animals. Muscle fiber types were identified on glass slides by staining for mATPase activity and visualized using an image analysis system which consisted of a microscope, color camera, and computer. Postmortem metabolic rate was measured with a pH meter at 45 min and 24 h postmortem. Postmortem ATP depletion was quantified as the

adenine/inosine ratio which was referred to as R-value. Drip loss was measured by suspending muscle samples in an inflated plastic bag and by the filter paper method. Meat color was measured with a Minolta chromameter at 45 min and 24 h postmortem. Warner-Bratzler shear force was measured on 6 cores from each sample.

The percentage of type IIb fiber was negatively related to pH 45 min postmortem ($r = -0.33$) and positively to R-value ($r = 0.32$). Drip loss was negatively related to fiber area percentages of type I and IIa ($r = -0.25$ and -0.26 , respectively) and positively related to type IIb percentage ($r = 0.39$). Results show that increasing the percentage of type IIb fibers leads to an accelerated postmortem metabolic rate and a decline in pork quality.

In conjunction with the constant and successful effort to increase the percentage of lean in pork carcasses, the swine industry has also succeeded in decreasing the feed to gain ratio of growing pigs over time. Muscle growth in pigs requires less dietary inputs than fat deposition. This is further explained by the fact that protein synthesis is a more efficient metabolic process than lipogenesis (synthesis and storage of lipids). Therefore, selection for feed efficiency alone has led to correlated responses in compositional traits.

A heritable condition known as porcine stress syndrome (halothane gene) has surfaced in the swine industry. Pigs carrying 1 or 2 copies of a single recessive gene known as the stress gene typically produce carcasses extreme in leanness and muscularity. Along with these perceived advantages is the fact that the gene causes intolerance to stress, accelerated muscle metabolism, and increased heat production in muscles of living animals. Unfortunately, the muscle biochemical conditions of stress positive and carrier animals often leads to the pale, soft, and exudative (PSE) pork condition upon slaughter. The production of lactic acid in postmortem muscle is a normal process when it occurs at a moderate rate over a

prolonged period of time. However, production of lactic acid in the living muscles of stress animals prior to slaughter creates an overly acidic early postmortem environment while carcass temperature is still high. This combination of factors causes denaturation of muscle proteins, excessive water loss from muscles, and the PSE condition.

Another genetic factor known to negatively influence pork quality is the Rendement Napole (RN⁻) gene. The effect of this dominant gene is to reduce the ultimate pH of primarily the loin and ham muscles. The RN⁻ gene is also known for reducing the water holding capacity of meat evidenced by increased cooking loss. Accurate identification of animals with these genetic defects known to negatively influence pork quality has been possible with DNA technology. Genetic screening tools have allowed swine industry personnel to virtually eliminate the halothane and RN⁻ genes from the U.S. breeding herd and moderate improvements in pork quality have been made in recent years. Researchers have speculated however, that the additive effect of a number of other genes may also negatively impact pork quality in the absence of the halothane or RN⁻ gene.

Lonergan et al. (2001) investigated the effects of selection for lean growth efficiency on pork quality in a population negative for the halothane and RN⁻ genotypes. The first objective was to characterize the extent of the impact of selection for lean growth efficiency on fresh pork quality. The second objective was to determine what factors, if any, cause a deterioration in pork quality of animals selected for lean growth efficiency. Two lines of Duroc pigs, consisting of a line selected for lean growth efficiency (Select), and a contemporary line maintained from the foundation herd (Control) were used in this study. Select line pigs (n = 15) and control line pigs (n = 24) from the fifth generation were evaluated. Pigs were harvested in 6 groups at a mean body weight of 105 kg.

Selection for lean growth efficiency resulted in increased lean gain per day, 10th rib loin muscle area, and overall carcass lean. Select line pigs were also leaner, evidenced by less average backfat and backfat at the 10th rib. From a pork quality standpoint, however, the select line had significantly lower subjective firmness scores on longissimus muscle chops and significantly greater drip loss from longissimus, semimembranosus, and semitendinosus muscles. As indicated by higher Warner-Bratzler shear force values, longissimus muscle chops from the select line were less tender. Additionally, less degradation of troponin-T, a structural muscle protein, was detected in samples from the select line longissimus muscle. Although ultimate pH and glycolytic potential was not different between the 2 lines, more lactic acid accumulated by 15 min postmortem for the select line, resulting in a more rapid early postmortem pH decline.

It is possible that the more rapid early postmortem metabolic rate led to decreased water holding capacity observed in the select line. Though the influence of IMF content on pork tenderness is unclear, the lower IMF levels of the select line could partially explain the higher WBS values found. Cumulatively, the results suggest that decreasing the amount of postmortem protein degradation may also decrease the water holding capacity and tenderness of meat. This report illustrates that genetic selection strategies for economically important traits like lean growth efficiency can have negative impacts on pork quality in the absence of known genetic abnormalities.

To better understand the effect of selection for increased leanness on meat and eating quality traits over the long term, Schwab et al. (2006) developed a unique population of purebred Duroc pigs. Littermate and half sib pairs of females were randomly allocated to matings with boars from the current time period (CTP) or old time period (OTP). Old time

period boars were utilized via frozen semen from the mid 1980s, just before carcass merit pricing rapidly gained popularity. A second replication was carried out in which females were mated with a boar opposite the time period used in the first mating to reduce the effect of dam in the analysis. Across the 2 replications, pigs sired by CTP boars (n = 178) and OTP boars (n = 99) were harvested at a commercial packing plant and evaluated for the following meat and eating quality traits: chemical IMF; Minolta reflectance and Hunter L color (24 h); pH (24 h and 7 d); water holding capacity; subjective color, marbling, and firmness (48 h); Instron tenderness; cooking loss; and trained sensory panel evaluations (7 d).

Marked differences in meat and eating quality traits were found between pigs sired by boars from 2 separate time periods. Loin samples from pigs sired by OTP boars had significantly greater IMF content and visual marbling score, required less Instron force, and had darker visual color scores compared to samples from pigs sired by CTP boars. Additionally, loin samples from OTP-sired pigs revealed significantly more pork flavor and less off-flavor as evaluated by a trained sensory panel. The study concluded that over the long term, selection response in carcass composition has been at the expense of some meat and eating quality traits.

Quality characteristics that play an integral role in consumer acceptability, such as tenderness, color, pH, and IMF, have decreased as intense selection emphasis has been placed on carcass lean percentage (Barton-Gade, 1990; Cameron et al., 1990). It has been shown that decreasing overall carcass fatness leads to a simultaneous decrease in IMF or marbling (Barton-Gade, 1990; Cameron et al., 1990).

The importance of pork quality and the role of intramuscular fat

Overall consumer satisfaction should be of utmost importance to all sectors of the swine industry. Interestingly enough, just as consumers initiated the shift toward leaner pork, they have now also called for better pork. The issue of pork quality as it relates to consumers is complicated. A separate set of factors may influence consumer preferences at the meat counter and the subsequent success of their pork eating experience. The visual appraisal of fresh pork cuts may not all be based on pork quality attributes known to positively affect sensory evaluations of cooked meat. The factors most likely to influence a consumer's initial purchasing decision include size, shape, and color of pork cuts, along with a desire to minimize fat intake and ultimately, price (McGill, 1981). Repeat purchasing decisions are largely dictated by the success of previous eating experiences which can be determined by many factors. Sensory attributes play a large role in determining repeat purchases. Other potential effects on the pork eating experience at home such as cooking method, temperature, and time are in the hands of the consumer. More extensive educational programs on this topic may prove beneficial in improving consumer acceptance of pork.

Another viewpoint on the pork quality issue is that different market venues measure quality with different sets of standards. For example, consumers purchasing commodity pork in the grocery store look for a product that is lean, has a consistent color, and holds water. Without realizing it, that same consumer may enjoy a product that is moderately lean and highly marbled at an upper-end restaurant.

Even though some of the factors affecting consumer acceptance of pork cannot be controlled, animal scientists must understand the relationships among measurable pork quality traits if progress is to be made. Unlike carcass composition, meat quality

characteristics are influenced by a variety of factors, making genetic improvement of the traits difficult.

Meat quality is used to describe any trait or group of traits which impact consumer acceptability of fresh meat products. Several generally accepted indicators of fresh pork quality include meat color, firmness, marbling, water holding capacity, and ultimate pH. Indicators of pork quality are measured both objectively and subjectively. Trained sensory panels have also been used to evaluate flavor, off-flavor, juiciness, chewiness, and tenderness of cooked pork. As researchers, we should work to improve all pork characteristics known to increase overall pork demand in the most efficient methods available. If this is achieved, opportunities for producers to add additional value to the pork products they produce will be identified.

To establish relationships between a comprehensive group of pork quality traits, Huff-Lonergan et al. (2002) evaluated product from 525 F2 animals consisting of Yorkshire and Berkshire breeding. Phenotypic correlations among subjective, instrumental, biochemical, and sensory measurements of pork quality were calculated.

Star probe measurements were significantly correlated with total lipid (- 0.14) as determined by the method of Bligh and Dyer (1959). Firmness scores were significantly correlated with marbling (0.37) and total lipid (0.31). Data from this study suggest that changes in some meat quality traits can affect many other meat quality attributes (Huff-Lonergan et al., 2002).

The role of IMF is of particular interest in pigs because selection for carcass lean percentage has significantly decreased marbling fat to levels less than 1% of muscle weight in pork (Wood et al., 2004). This selection emphasis placed on carcass lean percentage has

had a dramatic effect on the overall decrease in IMF concentration and has been associated with lower meat and fat quality (Scott et al., 1981a; Scott et al., 1981b).

Lonergan et al. (2007) studied the influence of lipid content on pork sensory quality within specified pH classifications. A large data set ($n = 1,535$) consisting of 3 years of National Barrow Show Progeny Test animals was used. Animals were comprised of purebred Berkshire, Chester White, Duroc, Hampshire, Landrace, Poland China, Spotted, and Yorkshire barrows and gilts. Loin pH was measured on the carcass at the 10th rib face and recorded at 24 h postmortem. Using a longissimus muscle sample obtained from the 8th to 10th rib, star probe, sensory traits, and lipid content were determined. The following pH classifications were used for statistical analysis: class A, > 5.95 , $n = 186$; class B, > 5.80 to 5.95 , $n = 236$; class C, > 5.65 to 5.80 , $n = 467$; class D, > 5.50 to 5.65 , $n = 441$; class E < 5.50 , $n = 205$.

For star probe values in pH classes C and D and chewiness scores in pH classes B, C, and D; lipid content was a significant source of variation. Within classes C and D, correlations indicated that increasing lipid content is associated with high sensory tenderness, low sensory chewiness, and low star probe values. Lipid content was not a significant source of variation for juiciness scores within any pH class. Taken together, results from this study indicate that the sensory attributes of pork loin are positively affected by intramuscular lipid when the ultimate pH of the fresh product is between 5.5 and 5.8.

Sires from 3 different pure breeds (Berkshire, Duroc, and Hampshire) were mated with Yorkshire x Landrace sows to produce crossbred progeny to be evaluated in a study of the influence of ultimate pH and IMF content on pork tenderness (van Laack et al., 2001). Tenderness and tenderization of meat from the 3 genetic types as well as the relationships

with ultimate pH and IMF were determined. Longissimus samples from crossbred pigs (n = 176) were excised from carcasses following a 24 h postmortem chilling period, packaged, and transported to the University of Tennessee. Ultimate pH and IMF were assessed on a 10th rib longissimus slice at 48 h postmortem. Warner-Bratzler shear force measurements (WBS) were taken 3 times on different loin samples at 2, 7, and 14 d postmortem.

There was a significant correlation ($P < 0.05$) across all genetic backgrounds between IMF and WBS on day 2 ($r = -0.11$), day 7 ($r = -0.21$) and day 14 ($r = -0.19$). Within genotype, however, IMF and WBS were only correlated for the Duroc sired line ($P < 0.05$; day 2, $r = -0.39$; day 7, $r = -0.44$; day 14, $r = -0.46$). A linear relationship was discovered between IMF and WBS for the Duroc line, and IMF accounted for 47% of the difference in WBS. Warner-Bratzler shear force values decreased as IMF increased. Results from this study indicate that the influence of IMF on tenderness of pork measured objectively with shear force is dependent on genetic line. Use of different genetic lines or breeds may result in different relationships between IMF and shear force.

In a similar study by Blanchard et al. (2000), 721 pigs of 3 genotypes (0, 25, and 50% Duroc), were evaluated to determine the influence of carcass backfat and IMF level on pork eating quality. A total of 10 Duroc and 20 Large White boars were mated with 188 sows of differing breed composition to generate the population. Animals were then fed 1 of 7 dietary regimes designed to introduce further variation in carcass fatness at slaughter. At approximately 90 kg body weight, groups of animals consisting of balanced numbers of sex and genotype were harvested weekly for 25 weeks. Fat depth measurements were taken 45 min after slaughter with a Fat-o-Meter at a point 6.5 cm from the dorsal mid-line, level with the last rib. Subjective marbling scores (0 = none to 3 = heavy marbling) were also taken on

the loin at 45 min postmortem. Longissimus muscle samples for shear force measurement, sensory evaluation, and IMF content were removed from carcasses 5 days following slaughter. The shear force measurement was averaged for 4 individual cores. Intramuscular fat content was determined from a 20 mm thick loin chop as the free fat content by the petroleum spirit method. A minimum of 6 trained sensory panelists evaluated juiciness, tenderness, flavor, abnormal flavor, and overall acceptability of 2 samples from each of the animals in the study.

The initial goal of the study to produce great variation in carcass fatness over the entire population was accomplished through genetic makeup, dietary formulation, and feeding level. Carcass backfat thickness (-0.21), marbling score (-0.19), and IMF content (-0.19) were all significantly correlated with shear force values. Backfat depth was significantly correlated with sensory panel juiciness (-0.09). However, neither marbling score nor IMF content was correlated with any of the sensory panel traits measured. The only characteristic to deteriorate significantly with lower fatness levels in this study was shear force. Results do not strongly suggest that fatness as determined by backfat thickness, marbling score, and IMF content influence pork eating quality.

Other research has indicated that pork color and IMF content are 2 of the most important factors involved with determining consumer acceptance of pork products (Faustmen and Cassens, 1990; Fernandez et al., 1999). Research has shown that IMF is influential in determining taste, juiciness, and flavor of the pork loin, and overall consumer acceptance and willingness to purchase pork instead of chicken (NPPC, 1995). Intramuscular fat is influential in determining juiciness and flavor (Hodgson et al., 1991; NPPC, 1995; and Huff-Lonergan et al., 2002), and has become important to the improvement

of pork quality. Since tenderness is not typically a problem when pork is properly prepared, juiciness and flavor should perhaps be emphasized as a priority to the swine industry. Taken comprehensively, the literature measuring relationships between IMF and other pork quality traits provides varying results. Perhaps the results of individual studies are dependent upon the individual populations used. Few researchers have investigated the relationship between IMF and other quality traits in a population with large differences in IMF represented.

Results of studies attempting to quantify the effect of IMF on eating quality are somewhat inconclusive. Rather than a linear relationship with eating quality characteristics, some researchers point toward a threshold level of IMF for acceptable eating quality. Some have demonstrated that a minimum level (2.5 to 3.0%) of IMF is necessary for acceptable tenderness in roasted chops (DeVol et al., 1988). Bejerholm and Barton-Gade (1986) suggested that the IMF content of pork needs to be greater than 2% before any noticeable improvements in sensory attributes of pork can be detected. Conversely, other research has shown that IMF affects the tenderness and sensory traits of pork to a small degree only when pork is categorized by pH, and then only when pH is between 5.5 and 5.8 (Lonergan et al. 2007). Numerous studies have suggested that marbling influences the palatability of cooked meat (Judge et al., 1959; Blumer, 1963; Field et al., 1966; Romans et al., 1965) and the possibility that IMF in pigs has reached or will reach substandard levels has been discussed (Schwörer et al., 1995).

Prediction of intramuscular fat in beef cattle

Measurement of IMF in live animals was first attempted in cattle through the collection of muscle biopsies (Rouse et al., 1989). Fat tissue is a very good reflector of high frequency sound waves and consequently, ultrasound technology has emerged as the tool for

developing objective measurements of marbling (Park, 1991; Whittaker et al., 1992).

Brethour (1990) used an Aloka 210 model ultrasound machine to scan feedlot cattle (n = 619). Using a 7 point scale, subjective “speckle scores” were given to ultrasound images such that lower scores indicated lower levels of marbling.

When compared to carcass marbling scores, speckle scoring correctly classified animals into the proper marbling category 61.8 to 100% of the time. Correlations between speckle and marbling scores ranged from 0.22 to 0.77. Conclusions drawn from one of the first attempts to measure IMF in livestock were that classifying cattle into marbling classes is possible with the method described, but advances in image analysis technology are needed.

Researchers at Iowa State University have successfully enhanced the techniques involved with predicting IMF in live beef animals. Most of the research has focused on the development and validation of prediction models (Wilson et al., 1993; Izquierdo, 1996; Wilson et al., 2001), refinement of image capturing and interpretation systems (Amin et al., 1997a), and development of user-friendly computer software programs (Zhang et al., 1995; Amin et al., 1997b). Other researchers have evaluated the repeatability of predictions (Hassen et al., 1999) and the accuracy of different systems (Herring et al., 1998; Hassen et al., 2001).

The repeatability of ultrasound predictions of IMF and the effect of repeated measures on prediction precision were evaluated by Hassen et al. (1999). One hundred forty-four bulls, heifers, and steers at a mean age of 433 days were scanned by a certified technician with 2 Aloka 500 ultrasound machines. Five to 6 independent images were collected and saved to a personal computer for later processing. Intramuscular fat was predicted from 2 individual region of interest (ROI) boxes on each image. One of the boxes

was centered over the 12th and 13th ribs and the other was positioned in an area with consistent image texture. Variance components and repeatability values were computed by machine, region of interest, sex, and for the overall data. Repeatability was defined as the correlation between repeated measures on the same animal. Precision was quantified as the standard error of the mean (SEM) for an individual animal.

The overall repeatability of ultrasound predicted IMF was 0.63. The repeatability and precision of measurements were statistically similar for the 2 machines and ROI box positions. However, within an animal, variance among images accounted for 70% of the total variance. The SEM was reduced by 29% with the inclusion of a second image and by 50% when 4 images were included. Increasing the number of images beyond 4 showed a diminishing rate of reduction in SEM. Increasing the number of ROI boxes within an image did not reduce SEM as quickly as increasing the number images per animal. This study suggests that a minimum of 4 images per animal with a standard deviation $\leq 1\%$ be used for predicting IMF in feedlot cattle.

Herring et al. (1998) evaluated the accuracy of 4 commercially available real-time ultrasound systems for predicting IMF in feedlot steers. Each ultrasound system company was allowed to send as many technicians as they saw fit to the scanning sessions. Therefore, the systems were represented with anywhere from 1 to 4 technicians. The systems included Animal Ultrasound Services (AUS1 and AUS2), CPEC (CPEC1-4), Critical Vision, Inc. (CVIS), and Classic Ultrasound Equipment (PIE). Images were collected with an Aloka 500 scanner for the AUS1 and CVIS systems. For AUS2 and PIE, images were captured with a PIE Scanner 200. Each of the CPEC technicians used an Aloka 210 ultrasound machine. The image interpretation program for CPEC was developed by Kansas State University,

whereas, the CVIS program was developed by Iowa State University. Image analysis procedures unique to each system were applied to a region of interest within each image captured.

Eighty-one crossbred steers at an average bodyweight of 563 kg and average age of 14.5 months were scanned across the 11th to 13th ribs 8 to 14 d prior to slaughter. After completion of the final scanning session, steers were transported to a packing plant for slaughter and data collection. Marbling scores were assigned by a USDA grader. A longissimus muscle slice was removed from the 12 to 13th rib interface, cold stored, and transported to Kansas State University. Samples were trimmed of external fat, homogenized, and sampled twice for chemical estimation of percentage of ether extractable fat (EE).

Pearson product moment correlations were calculated for system predictions with marbling score and EE. Other simple statistics that were calculated for each system included root mean square error (RMSE), bias, and standard error of prediction (SEP). Before further statistical analysis, system predictions were corrected for each respective system's bias. Both marbling score and percent ether extractable fat were used to generate absolute differences with system predictions. After accounting for other sources of variation, the proficiency of each system was evaluated with linear models using the absolute differences as dependent variables. Steers with USDA marbling scores less than small⁰⁰ were assigned to a low marbling class and all others were assigned to a second class. Data were then analyzed with a model including marbling class.

Correlations for CPEC1 and CVIS were similar and the largest whether using ether extract or marbling score. Across technicians, CVIS and CPEC were the most proficient systems for predicting IMF, especially when marbling score was used as the objective

measurement for comparison. Root mean square error was similar for all systems when compared with ether extract, but larger differences were found when marbling score was used. The CPEC1-3 had the most favorable RMSE, followed by CVIS. The SEP statistic was clearly the lowest for CPEC1-4 and CVIS when using EE or marbling score. Whether evaluated by EE or marbling score, least squares means for CPEC and CVIS systems were more accurate than AUS or PIE and were not different from each other ($P < 0.05$). Cumulatively, results clearly show that the CPEC and CVIS systems were the most accurate for predicting IMF.

Hassen et al. (2001) developed, validated, and compared models for predicting the percentage IMF in feedlot steers using 2 types of ultrasound equipment. Four to 5 longitudinal images across the 11th to 13th ribs were collected on 500 crossbred steers at an average age of 455 d at scanning time. Data from these 500 steers were used for model development. Each steer was scanned with an Aloka 500V and a Classic Scanner 200. At the scanning site, images were digitized using a frame grabber and saved on a computer disk for later processing. Steers were harvested 2 to 5 d after scanning and a longissimus muscle slice was removed from the 12th rib face. Samples were transported to Iowa State University, trimmed of external fat, and chemical IMF was determined using an n-hexane extraction procedure. Ultrasound images were processed using the USOFT software package (Amin et al., 1997). A single 100 x 100 pixel region of interest box was placed between the 12th and 13th ribs on all acceptable images. After processing, texture analysis software produced 10 columns of image parameters. The 4 groups of parameters were Fourier, gradient, histogram, and co-occurrence. Four separate prediction models were developed for both the Aloka 500 and Classic Scanner 200. Models were developed without transformation of chemical IMF

(Model 1), based on logarithmic transformation of chemical IMF (Model II), ridge regression procedure (Model III), and principal component regression procedure (Model IV).

The root mean square error of Aloka 500 models 1, II, III, and IV were 0.84%, 0.85%, 0.91%, and 0.86%, respectively. The corresponding root mean square error values for the Classic 200 were 0.87%, 0.85%, 0.94%, and 0.91%, respectively. The models were initially validated separately on an independent data set from 71 steers. Overall mean bias, standard error of prediction, and rank correlation coefficient across the 4 Aloka 500 models were 0.42%, 0.84%, and 0.88, respectively. For the Classic 200, corresponding values were 0.67%, 0.81%, and 0.91, respectively. After this initial validation, model II was evaluated again on a set of 24 steers for both of the machines. Overall mean bias, absolute difference, and standard error of prediction of Aloka model II were 0.71, 0.92, and 0.98%, respectively. Corresponding values for the Classic 200 model II were 0.59, 0.97, and 1.03%, respectively. Across statistical comparison methods, this study concluded that accuracy for the Aloka 500 and Classic Scanner 200 is similar; and that both types of equipment can be used to capture images for prediction of IMF when prediction models unique to each system are used for interpretation.

Technology associated with prediction of IMF in beef cattle has been largely adopted by the beef industry on a national and global scale. Up to 16 cattle breed associations include or require yearling ultrasonic measurements of IMF in their respective across herd genetic evaluations. Ultrasound field technicians and third party image interpretation personnel are trained at a central site and certified under standardized criteria.

Prediction of intramuscular fat in swine

Fewer reports have been published on the use of ultrasound to predict the amount of IMF in the loin of live swine. A limited amount of literature exists on the accuracy of different ultrasound systems, image collection procedures, and image processing techniques for the prediction of IMF. Perhaps this is due to the fact that pork packers are not measuring IMF and few price incentives are available to pork producers, contrary to the beef industry.

Pork quality, as determined by consumer acceptance and trained sensory panelists, is affected by multiple factors. Many of these determinants of pork quality have been effectively measured using carcasses or pork samples. To date, only IMF percentage of the longissimus dorsi muscle has been successfully measured on live animals. From a genetic progress viewpoint, it would be desirable to measure IMF of potential breeding stock as opposed to using sibling or relative carcass data if IMF is a trait of interest.

Dion et al. (1996) scanned 324 hogs prior to slaughter at an average body weight of 105 kg. A cross sectional image between the third and fourth last ribs and a longitudinal image over the last 4 to 5 ribs were collected with an Aloka 500 ultrasound scanner fitted with a 12.5 cm, 3.5 MHz transducer. Images were stored on a computer and VCR for later analysis using the AUSKey AUTOQ for marbling determination. At the packing plant, carcasses were ribbed between the third and fourth last ribs for visual marbling scoring on the loin face. Using the Agriculture Canada scoring system (1 = trace, 2 = slight, 3 = small, 4 = moderate, and 5 = abundant), a marbling score was assigned to each carcass.

Data were analyzed using the CORR and GLM procedures of SAS. When compared with carcass marbling score, the R^2 of the cross sectional and longitudinal predictions of marbling were 0.001 and 0.01. Though the value of 0.01 for the longitudinal image

prediction was significant ($P < 0.05$), the accuracy of this method of predicting marbling in live swine using real-time ultrasound was essentially zero. The prediction equations severely overestimated the pigs with lower levels of marbling and underestimated those with higher levels.

Ville et al. (1997) measured the longissimus muscle IMF content of pigs at 3 different points during the growing period using various methods. Serial measurements were made just posterior of the last rib at approximately 20, 60, and 100 kg of body weight. Muscle biopsies were collected from pigs at 20 and 60 kg using anesthesia. The final muscle sample was collected from the carcass. Fat was extracted from each longissimus sample to determine IMF content. At 20 kg bodyweight, 3 longitudinal images were collected with a B-mode Toshiba Sonolayer operating at 5 MHz. When pigs weighed 60 and 100 kg, an A-mode device known as the Piglog105 with a 4 MHz transducer was used to measure IMF.

At 20 kg, the only significant correlations between IMF and image parameters were with respect to mean pixel value and its coefficient of variation. At 60 and 100 kg, no significant relationships were found between ultrasound measures collected using the A-mode machine and IMF content. Based upon the manufacturer's calibration equation, mean IMF values predicted from the A-mode machine were 1% greater than biopsy results on average. Neither of the ultrasound methods used to predict IMF in this study was successful.

Ragland (1998) developed a linear regression equation to predict IMF in live swine. When pigs ($n = 300$) reached 109 kg body weight, they were scanned with an Aloka 500V SSD real-time ultrasound machine fitted with a 12.5 cm, 3.5 MHz linear array transducer. A single longitudinal image, parallel to the spine and approximately 5 cm off midline was collected on each animal. The image included the 9th through 11th ribs. Upon completion of

scanning, pigs were harvested at a commercial packing plant. A longissimus muscle slice was excised from the 10th rib interface and IMF content was determined by the total lipid extraction procedure.

Ultrasound images were saved and processed using image interpretation software developed by Iowa State University beef cattle researchers. Images were randomly split into model development (n = 200) and validation (n = 100) groups. Two region of interest boxes were placed on either side of the 10th rib on all acceptable images. Sixteen image texture parameters were calculated from the ROI boxes to be used for model development. Image parameters, ultrasonically measured 10th rib backfat, sex, chemical IMF content, and marbling score were statistically analyzed to select a set of parameters for regression model development. Stepwise regression was used for selection of variables to include in candidate prediction models. Using linear regression, IMF and marbling score were predicted.

Root mean square error ranged from 0.88 to 1.03% for the developmental data and this depended on which variables were included as independent and dependent variables. For the validation data, the mean absolute difference between predicted and carcass IMF ranged from 0.63 to 0.73%. Correlation coefficients between predicted and carcass IMF were between 0.52 and 0.71. For the validation data set, predicted IMF was within 0.50% of carcass IMF for 44 to 51% of the individuals, depending on which parameters were included in the model. Likewise, predicted IMF was within 1% of carcass IMF 72 to 86% of the time. This report was the first to illustrate that objective measurement of IMF on the live pig with real-time ultrasound is feasible.

Building upon concepts used in the technology available for beef cattle and those of Ragland (1998), Newcom et al. (2002) worked to develop and validate a model to predict

loin IMF percentage in live swine. Duroc barrows and gilts ($n = 207$) were used for model development. Pigs were weighed and scanned 5 d prior to slaughter with an Aloka 500V SSD scanner fitted with a 12.5 cm, 3.5 MHz linear array transducer. Using a standoff guide, a cross-sectional image was captured between the 10th and 11th ribs for measurement of loin muscle area and off midline backfat. The standoff guide was removed from the transducer for the collection of at least 4 longitudinal images to be used for IMF prediction. Images were captured 7 cm off midline and contained the 10th to 13th ribs. These images were digitized on site and saved to a computer for later interpretation. Following harvest, a longissimus muscle slice was obtained for determination of carcass IMF percentage. A trained technician placed a region of interest box as close to the 10th to 11th rib interface as possible and used image analysis software to define image texture parameters. Off-test weight, ultrasonic backfat, ultrasonic loin muscle area, and the image parameters (averaged by animal) were initially all included in the model. Using carcass IMF as the dependent variable, stepwise linear regression was used to remove all non significant variables one at a time until only significant ($P < 0.05$) variables remained.

The final model included the ultrasonic measurement of backfat and 5 image parameters. Root mean square error for the prediction model was 1.02%. For model validation, purebred Duroc and Yorkshire pigs ($n = 619$) of mixed sex were used. The same protocol as described for model development was used for scanning, carcass IMF determination, and image processing. Validation data was divided into 3 data sets and included all data (Validation 1), Duroc only data (Validation 2), and Yorkshire only data (Validation 3). The predictive ability of models was determined using the difference between predicted and carcass IMF, absolute value of that difference, standard error of

prediction, and correlations. The model was also tested for its ability to place animals into the correct carcass IMF class. Classes were defined as follows: Class 1 ($\leq 2.0\%$), Class 2 ($> 2.0\%$ and $\leq 3.0\%$), Class 3 ($> 3.0\%$ and $\leq 4.0\%$), Class 4 ($> 4.0\%$ and $\leq 5.0\%$), Class 5 ($> 5.0\%$ and $\leq 6.0\%$), and Class 6 ($> 6.0\%$).

The mean differences between predicted and carcass IMF percentage for validations 1, 2, and 3 were 1.18, 0.75, and 1.68%, respectively. The corresponding mean values for the absolute difference between predicted and carcass IMF percentage for validations 1 through 3 were 1.30, 0.91, and 1.74%, respectively. The standard error of prediction was 0.93, 0.80, and 0.82 for validations 1, 2, and 3, respectively. Correlations between predicted and carcass IMF were moderate for all validation data sets. When displayed in the form of a frequency distribution of the absolute difference between predicted and carcass IMF, the Duroc only validation placed the greatest amount of animals within 0.5, 1.0, 1.5, and 2.0% when compared with validations 1 and 3. Validation 2 was also the most accurate at placing animals into the appropriate carcass IMF class. Across statistical analysis methods, the Duroc only data provided the best overall validation of the model. Perhaps these findings can be explained by the fact that validation 2 contained animals of the same breed as those used for model development. Additionally, carcass IMF values from validation 2 animals were more similar to those of the development group. Results from this study confirm that loin IMF content in live swine can be predicted accurately with the use of real-time ultrasound equipment and image analysis tools. Adoption of the techniques described in this report should allow swine breeders to effectively identify potential breeding stock with greater levels of IMF (Newcom et al., 2002).

Genetic selection for intramuscular fat in the swine industry

In addition to being measurable, a trait of importance must also be heritable and have sufficient variation within the population if selection programs are to be effective. A high heritability does not necessarily mean that selection can be effectively implemented and genetic variation must be large enough to make rapid change (Wilson, 1992). Phenotypic expression of the trait must also be sufficient to allow for detection of differences between animals (Wilson, 1992). Genetic parameters must be estimated to determine the amount of additive genetic variation and heritability for use in a genetic evaluation and selection program (Newcom, 2004). Once superior animals for a trait are identified and retained for breeding purposes, they must be mated in a way that produces the maximum response while limiting any negative impact selection for the trait of interest may have on other economically important correlated traits (Newcom, 2004).

Using data from 2 National Barrow Show Progeny Tests, Newcom et al. (2003) estimated the genetic relationships between IMF and various meat quality traits. Five d prior to harvest, pigs of several pure breeds ($n = 821$) were scanned off test. An Aloka 500V SSD ultrasound machine was used to collect a minimum of 4 longitudinal images and a cross sectional image. Predicted IMF (PIMF) was estimated using a model which included image texture parameters and backfat depth from the longitudinal image. Chemical IMF (CIMF) was determined from a 10th rib longissimus slice. Meat quality traits measured were: Minolta reflectance, Hunter L, pH, water holding capacity, Instron tenderness, cooking loss, trained sensory evaluations, and subjective visual scores for color, marbling, and firmness.

The genetic correlation between CIMF and PIMF was 0.76, and their heritabilities were 0.45 and 0.52, respectively. Genetic correlations for CIMF and PIMF with ultimate pH

were 0.27 and 0.40. The genetic correlations between CIMF and tenderness, juiciness, and flavor were 0.35, 0.53, and 0.54, respectively. The genetic correlations between PIMF and tenderness, juiciness, and flavor were 0.29, 0.67, and 0.66, respectively. The strongest genetic relationships found in this study were between IMF and the sensory panel evaluations of juiciness and flavor. Results indicate that whether IMF is measured chemically or ultrasonically, selection for IMF should result in similar correlated responses in meat quality traits.

Schwab et al. (2009) practiced selection for IMF in Duroc swine using real-time ultrasound for 6 generations. At the initiation of the project, 40 registered Duroc gilts were purchased from 10 Midwestern breeders. Two generations of random mating using semen from regional boar studs was used to establish a base population of 56 litters. Two boars from each litter were randomly selected to remain intact and all others were castrated. Littermate pairs of gilts were randomly selected and assigned to either the control line (CL) or select line (SL) and mated to the same boar to establish genetic ties between lines before selection began.

Off test ultrasonic measurements were collected on all animals ($n = 4,124$) at a mean body weight of 110 kg. A minimum of 4 longitudinal ultrasound images were captured 7 cm off midline across the 10th to 13th ribs using an Aloka 500V SSD ultrasound machine fitted with a 3.5 MHz, 12.5 cm linear array transducer. A regression equation (Newcom et al., 2002) was used to estimate IMF (UIMF) from image parameters that were generated using texture analysis software (Amin et al., 1997b) for the first 3 generations. Updates to the model were made and the resulting model (Schwab and Baas, 2006) was used in generations 4 through 6.

Carcass data and a 10th rib longissimus sample were collected from at least 1 animal in every litter. Carcass IMF (CIMF) was determined from longissimus muscle samples. Breeding values for IMF were estimated for the SL using a 2-trait model that included UIMF and CIMF. Based upon EBV for IMF, the top 10 boars and top 75 gilts in each generation were used to produce the next generation after minimizing inbreeding. To maintain the CL, 1 boar from each sire family and 50 to 60 gilts were randomly selected for breeding in each generation.

Results show an 88% improvement in IMF through 6 generations of selection (4.53% in SL vs. 2.41% in CL). An increase in carcass backfat thickness and decrease in loin muscle area resulted due to selection emphasis being placed on IMF. Longissimus muscle samples from SL animals were significantly more tender (8%) and had more favorable flavor (0.41) and off-flavor (-0.41) scores as evaluated by a sensory panel. Effective selection for IMF using real-time ultrasound is possible but unwanted effects on carcass composition can be expected if intensive selection emphasis is used. Rapid improvement in IMF should not be expected when simultaneous improvement in other trait categories is also pursued (Schwab et al., 2009).

Using data from Schwab et al. (2009), Schwab et al. (2010) estimated genetic parameters associated with economically relevant meat quality, eating quality, and production traits in a population of Duroc swine selected for increased IMF. Over the 6 generations, carcass and ultrasound IMF were moderately heritable (0.31 and 0.38, respectively). Measurements of IMF from chemical analysis and predicted from ultrasound were highly correlated with each other ($r = 0.90$). There was a favorable genetic association of IMF measures with Instron tenderness and sensory panel flavor score. However, selection

for IMF led to a significant decrease in estimated breeding value for loin muscle area and an increase in estimated breeding value for carcass backfat.

Cumulatively, the results confirm the phenotypic correlations observed in Schwab et al. (2009) and show that carcass composition, meat quality, and sensory traits will respond to selection pressure. Estimates from the unique population studied indicate that genetic relationships exist among the different categories of economically important traits and that genetic improvement programs should understand them when implementing IMF into selection criteria.

The genetic correlation between backfat and IMF is low enough that genetic improvement can occur in both simultaneously, and the genetic correlations between IMF and other meat quality traits indicate that when selection for increased IMF occurs, a corresponding improvement in other quality traits is expected (Newcom, 2004). A single genetics company currently influences more than half of the world's commercial swine population. Several others compete for the remainder. Although not as extreme as the poultry industry, a few nucleus animals may eventually impact thousands of commercial market hogs in today's swine industry. If IMF was measured in live animals at the nucleus level and selection emphasis was placed upon IMF, the potential for worldwide improvement of pork quality is tremendous.

Determining the economic value of intramuscular fat to the swine industry

The strength of the export market for U.S. pork is often a major influence on U.S. hog prices. Several of the major importers of U.S. pork have expressed their interest in highly marbled or darker colored pork products. Thus, direct sourcing of pigs from known genetic background for specialty markets is being practiced by some large packing companies.

Sorting product for these traits is done during further processing and not immediately following slaughter when the origin of the carcass is more easily known. However, it is doubtful that producers are realizing the financial benefit of their specialty products. Domestic niche markets or the so called “white tablecloth restaurants” also demand highly marbled pork and are currently relying on small producer groups using alternative genetics or production systems for their supply.

Value of beef carcasses in the United States is determined by yield and quality grades. All beef carcasses are ribbed between the 12th and 13th ribs and assessed by a USDA grader. Yield grade is influenced primarily by the degree of fatness and to a lesser extent, muscularity in relation to carcass weight. Quality grades are determined by visual marbling score and skeletal maturity as assessed by the grader. Carcass price per hundredweight is then calculated based upon the yield and quality grades assigned.

Unlike the beef industry, pork quality is not consistently measured or assigned a value by packing companies. Few incentives are currently available to producers for muscle quality traits, yet surveys have indicated that pork marketers have an opportunity to earn considerable profits by creating differentiated products (See et al., 1995). Effective measurements of pork quality traits at line speed are needed for this to occur. In order to procure a reliable supply of pork with the desired traits, packers will have to offer premiums for them (See et al., 1995). Without the ability to measure pork quality traits shortly following slaughter, there is no opportunity to assign premiums to individual carcasses. Consequently, a current challenge for the swine industry is the lack of an accurate economic value for IMF or any pork quality traits for that matter. Although the importance of pork

quality is known and well understood, commercial market hogs are currently evaluated and priced according to lean content only.

Packer and retailer uncertainty about the volume and amount of potential premiums that could be realized from the sale of differentiated pork products is a current industry roadblock. If an online IMF grading system was developed and implemented by the packing industry, a premium/discount schedule for IMF could be included with carcass weight and carcass composition measurements in a more comprehensive pork pricing matrix. Carcasses containing loins with low, moderate, or high levels of IMF could be identified and sorted at line speed by category, priced accordingly, and loins could eventually be sent to their respective market destination. If the actual economic value of IMF is established, tremendous opportunities for packers and producers will exist.

Knowing that swine producers must select genetic stock that maximizes profits for their particular operation and that profits are determined by many controllable and uncontrollable factors, See et al. (1995) suggested using indexes in selection that incorporate multiple traits with their respective economic values. The economic value for a given genetic trait represents the expected change in producer profit for a unit of change in the trait. See et al. (1995) estimated the economic value of IMF to be \$5.66, which was the highest economic value of all traits listed. Economic values associated with swine traits of interest vary across regions, markets, production systems, and even from farm to farm.

Selection indices ultimately assign a single value to an animal which represents the influence of multiple traits. This allows for effective, cumulative comparisons to be made across animals. If the value of IMF to producers, packers, retailers, and consumers was known and communicated, the IMF trait could be more effectively implemented into

selection indices containing multiple traits of economic importance. Producers would be able to design their genetic selection index based upon available incentives.

Although ultrasound scanning for backfat may have aided in the deterioration of pork quality over time, it is plausible to hypothesize that it ironically may serve as a valuable tool in the improvement of pork quality. If IMF becomes a component in pork carcass pricing in the United States, producers of commercial market pigs may demand terminal line genetics with the ability to generate higher levels of IMF in their commercial progeny. Thus, swine genetics companies will need rapid and accurate methods for predicting IMF content in live animals. It could become imperative for swine breeding companies to actively implement IMF into their genetic selection programs to stay competitive. Genetics companies currently risking capital and investing in the technology may realize a beneficial return in the future.

CHAPTER 3. AN EVALUATION OF EQUIPMENT AND PROCEDURES FOR THE PREDICTION OF INTRAMUSCULAR FAT IN LIVE SWINE

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Abstract

Four hundred fifty-four pigs of 6 pure breeds and crossbreds were used to evaluate the accuracy of 2 commercially available real-time ultrasound scanners, 2 image capturing devices, 2 methods of collecting images, and 3 region of interest box options used to predict intramuscular fat percentage in live pigs. Within 4 d of harvest, longitudinal images encompassing the 10th through 13th ribs were collected approximately 7 cm off-midline on the right side of each animal. Five different combinations of equipment were used for ultrasound image collection. Scanning was accomplished with an Aloka 500V SSD real-time ultrasound scanner fitted with a 3.5 MHz, 12.5 cm linear array transducer and an Aquila Vet real-time ultrasound scanner fitted with a 3.5 MHz, 18 cm linear array transducer. A splitter device attached to the output port of the Aloka 500 allowed for connection of VCE Model B5A01 and Sensoray Model 2255 image capturing devices. A minimum of 6 Aloka 500 images were collected simultaneously with each capturing device. A minimum of 6 Aquila Vet images were collected with the Sensoray. The Sensoray had live video recording capabilities and 2 to 4 s of video (15 frames per s) were recorded using both real-time scanners. Each image capturing device was connected to a portable laptop computer equipped with an image capturing and processing software package. Three region of interest boxes were placed on individually collected images. A single ROI box was placed on images collected via live video recording. Predicted intramuscular fat percentage was compared to

chemical intramuscular fat percentage determined from a 10th rib longissimus muscle slice. Bias and standard error of prediction were calculated for each combination of equipment and procedures. Two linear models were used to evaluate the absolute difference between predicted and chemical intramuscular fat percentage. Results indicate that Aloka 500 and Aquila Vet scanners provide similar accuracy when compared using standard error of prediction. The Aloka 500 was more accurate ($P < 0.0001$) when the absolute value difference between predicted and chemical intramuscular fat was analyzed. Use of Sensoray images gave more accurate predictions of intramuscular fat than VCE images. Further enhancements to the live video image collection process are needed before the concept can be viable for commercial use. The use of multiple region of interest boxes on images improves intramuscular fat prediction accuracy.

Key Words: swine, ultrasound, intramuscular fat, accuracy

Introduction

Driven by consumer health concerns and packer incentives offered through grid-based marketing programs, the swine industry has emphasized carcass leanness in genetic selection programs for several decades. Efforts to change the composition of pork carcasses and produce a leaner end product have been very successful industry-wide. Unfortunately, selection for lean content has resulted in negative impacts on pork quality and consumer acceptance issues have arisen. Intense selection emphasis placed on lean growth efficiency (Lonergan et al., 2001) and carcass lean percentage (Schwab et al., 2006) has had a negative impact on pork quality characteristics that play an important role in consumer acceptability.

Decreasing overall carcass fatness leads to a simultaneous decrease in intramuscular fat or marbling (IMF) (Barton-Gade, 1990; Cameron et al., 1990) and levels less than 1% of

muscle weight have been reported (Wood et al., 2004). Some reports suggest that a decrease in IMF over time may be partially responsible for the overall decline in pork quality that has been well documented. The possibility that IMF in pigs has reached or will reach substandard levels has been discussed (Schwörer et al., 1995).

Research has shown that IMF is influential in determining taste, juiciness, and flavor of the pork loin, and in overall consumer acceptance and willingness to purchase pork instead of chicken (NPPC, 1995). Researchers point toward a threshold level of IMF and have demonstrated that a minimum level (2.0 to 3.0%) is necessary for acceptable eating quality (Bejerholm and Barton-Gade, 1986; DeVol et al., 1988; Barton-Gade, 1990). Others have shown that IMF improves sensory attributes of pork that has a moderate ultimate pH (Lonergan et al., 2007).

Prediction of loin IMF percentage in live swine is possible (Ragland, 1998) and models utilizing parameters derived from image texture analysis software are accurate for predicting IMF (Newcom et al., 2002). Regardless of the role IMF plays in the determination of pork quality, it is the only pork quality trait that has been successfully measured in live animals, allowing for identification of superior animals without sibling or progeny testing. Intramuscular fat has been reported to be moderately heritable and to be genetically associated with other indicators of meat quality (Schwab et al., 2010).

Researchers at Iowa State University have successfully enhanced the techniques involved with predicting IMF in live beef animals. Most of the research has focused on the development and validation of prediction models (Wilson et al., 1993; Izquierdo, 1996; Wilson et al., 2001), refinement of image capturing and interpretation systems (Amin et al., 1997a), and development of user-friendly computer software programs (Zhang et al., 1995;

Amin et al., 1997b). Other researchers in beef have conducted studies more similar to the current study in swine. Hassen et al. (1999) discovered that increasing the number of images per animal improves prediction precision to a greater extent than increasing the number of ROI boxes per image. Herring et al. (1998) compared the accuracy of 4 commercially available software packages for the prediction of IMF in live beef cattle.

Previous research on the prediction of IMF in swine has focused on proof of the concept (Ragland, 1998) and on refinement of prediction models (Newcom et al., 2002). Prior to this study, the accuracy of different ultrasound scanners, image capturing devices, image collection methods, and region of interest box options for the prediction of IMF in swine has not been investigated. With the possibility of IMF becoming a trait of interest in genetic selection programs in the commercial swine industry, there is a need to explore available technologies and procedures for prediction of IMF. The objectives of this study were to compare accuracy of: 1) 2 commercially available ultrasound scanners, 2) 2 image capturing devices, 3) 2 image collection methods, and 4) 3 region of interest box options.

Materials and Methods

Data description

Experimental protocols for this study were approved by the Iowa State University Institutional Animal Care and Use Committee. Animals utilized for this project were from the 2008 National Barrow Show Progeny Test held at the Iowa Swine Testing Station and from the Lauren Christian Swine Research Center, Iowa State University. The population was comprised of barrows and gilts of 6 pure breeds and crossbreds ($n = 454$) that were scanned at a mean live weight (LW) of 115.9 kg. Ultrasound image collection was completed during 8 sessions from June through October of 2008.

Ultrasound image collection procedure

To ensure that images collected across scanning sessions were at consistent pixel value ranges, the image capturing software was calibrated to attain an appropriate pixel value range for each combination of scanner and capturing device prior to the start of each scanning session. Contrast and brightness were adjusted so that the background of the image had a pixel value between 5 and 10 and the brightest point on the grayscale bar had a pixel value between 250 and 254. Animals were restrained in a crate to facilitate image collection and soybean oil was used as a couplant between the ultrasound transducer and the skin. Scanning was accomplished by a National Swine Improvement Federation certified technician that was experienced in collection of longitudinal images. The transducer was positioned on the right side of the animal, parallel to and approximately 7 cm from the dorsal midline. The transducer was positioned by the technician to collect images that included the posterior tip of the trapezius muscle and the 10th through 13th ribs.

Ultrasound scanners

Real-time ultrasound images were collected using 5 different combinations of ultrasound scanner, image capturing device, and image collection method. Pigs were scanned within 4 d of harvest with an Aloka 500V SSD (**AL**) real-time ultrasound scanner fitted with a 3.5 MHz, 12.5 cm linear array transducer (Corometrics Medical Systems, Wallingford, CT) and an Aquila Vet (**AQ**) real-time ultrasound scanner fitted with a 3.5 MHz, 18 cm linear array transducer (Esaote Europe, B.V., The Netherlands). Gain settings were: Overall, 90; Near, -25; Far, 2.1 for the AL and Overall, 255; Near, 80; Far, 1 for the AQ. The AL was set to 1.5x magnification, and focus 1 and 2 were enabled. Magnification for the AQ was set at 26 frames per s.

Image capturing devices and collection methods

A splitter device connected to the output port of the AL allowed for the attachment of 2 image capturing devices. Images were captured with a VCE Model B5A01, Imperx, Inc., Boca Raton, FL (**VCE**) and a Sensoray Model 2255, Sensoray, Inc., Tigard, OR (**SEN**). A laptop computer equipped with an image capturing and processing software package (Biotronics, Inc., Ames, IA) was connected to each capturing device and a minimum of 6 AL images were captured simultaneously with both devices. The SEN and its associated software program had video recording capabilities. After the 6 individual images were captured, the SEN was used to record a live video stream of images for 2 to 4 s at a rate of 15 frames per s. Only the SEN capturing device and a single laptop computer were connected to the AQ scanner. The AQ and SEN combination was also used to collect a minimum of 6 individual images and 2 to 4 s of live video.

For individual image collection, the technician used a freeze switch to momentarily lock the frame on the scanner console monitor to be saved. For live video image collection, the technician maintained the transducer position on the animal during the recording period. The initiation and termination of the recording period was conveyed verbally from the technician to the laptop computer operator.

Meat sample collection and chemical IMF determination

Animals were harvested at Hormel Foods, Austin, MN. At 24 h postmortem, a section of the longissimus muscle containing the 10th through 13th ribs was excised from the right side of carcasses by Iowa State University personnel. Rib sections were identified, wrapped in plastic bags, and packed in ice for transportation to the Iowa State University Meat Laboratory. At 48 h postmortem, the spinal process, ribs, and subcutaneous fat were

removed from the longissimus muscle. A 1.25 cm longissimus slice from the 10th rib end was completely trimmed of subcutaneous fat, vacuum packaged, and frozen, to be used for determination of chemical IMF percentage (**CIMF**).

Longissimus samples were thawed, homogenized with a blender, and sampled in triplicate for the determination of total lipid content. The total lipid extraction procedure was performed on 3 homogenized samples weighing 1.95 to 2.05 g using methanol and chloroform as described by Bligh and Dyer (1959). If the coefficient of variation among the triplicate samples was greater than 10%, the procedure was repeated.

Prediction model development, image processing, and prediction of IMF

Individually collected images from the first 60% of the animals were used to develop IMF prediction models. Image processing for prediction model development was accomplished separately from image processing conducted for the purposes of this study. Image texture parameters were calculated using image analysis software and stepwise linear regression was used to develop prediction models for IMF based on image parameters. Models were developed for the following system combinations: Aloka 500 using individual image collection and VCE (**ALIVCE**), Aloka 500 using individual image collection and SEN (**ALISEN**), and Aquila Vet using individual image collection and SEN (**AQISEN**). The remaining 40% of the animals were used for prediction model validation.

A different interpreter conducted the image processing for this study on a personal computer under dim lighting. The interpreter visually assessed each image individually for image quality. Black images and incomplete frames were deleted from the database and images displaying darkness, blurriness, reflections, inconsistency of texture, or improper anatomical location were marked as rejected images. Rejected images were not included in

IMF predictions. Initially, a single 80 x 80 pixel region of interest box (**ROI**) was placed on all acceptable images. The interpreter positioned the ROI as close to the 10th and 11th rib interface as possible. If a more consistent and desirable image texture was available in the anterior 2/3 of the image, the ROI was placed accordingly. With priority being given to more anterior locations with clear and consistent texture, 2 additional 80 x 80 pixel ROI boxes were placed on individually collected images in the second and third most desirable positions. Boxes were placed individually so as to not overlap and were color coded white, yellow, and green to represent the first, second, and third most desirable locations within an image. To avoid echoes caused by reflections from interfaces between individual fat layers, the ROI boxes were placed to cover more of the ventral portion of the longissimus muscle than the dorsal portion. Region of interest box location was saved automatically in the software program for later batch processing of images for predicted IMF percentage (**PIMF**). Once image processing and ROI placement was complete, applicable prediction models were applied to all animals in the database for prediction of IMF using a batch process function. The ALISEN model was used for Aloka 500 live video images (**ALVSEN**) and the AQISEN model was used for Aquila Vet live video images (**AQVSEN**). The software program produced a PIMF for each individual ROI within treatment by animal. This output was later used to calculate the mean of ROI 1 and 2 in addition to the mean of all 3 ROI boxes for each animal, where applicable.

Statistical analysis

Chemical IMF percentage was used as the objective measurement of IMF to determine accuracy. Systems were evaluated for accuracy using **Bias**, standard error of

prediction (**SEP**), and the absolute difference between predicted and chemical IMF percentage (**ABSDiff**).

Bias for each combination of ultrasound scanner, image capturing device, image collection method, and ROI box option was determined first:

$$Bias = \frac{\sum (P - C)}{n}$$

where P is the predicted percentage of IMF, C is the percentage of IMF determined by chemical extraction, and n is the number of observations. Bias was determined as the mean difference between predicted and chemical IMF percentage for each combination of equipment and procedures. Bias provides information about the average direction and magnitude of error associated with PIMF in relation to CIMF. In genetic improvement programs where ranking of a contemporary group of animals correctly is of primary concern, a small and consistent bias in one direction will not have negative effects on accomplishing this goal. However, prediction accuracy should be questioned in the presence of a large and inconsistent bias.

Bias was then used to calculate the SEP for each combination of scanner, image capturing device, image collection method, and ROI box option:

$$SEP = \sqrt{\frac{\sum (P - C - Bias)^2}{n - 1}}$$

where P is the predicted percentage of IMF, C is the percentage of IMF determined by chemical extraction, and n is the number of observations. The SEP statistic is commonly used in real-time ultrasound technician certification programs in the beef and swine industries (Robinson et al., 1992; NSIF, 1994). The SEP provides information about how

well a system ranks the animals and the degree of accuracy, while accounting for bias. Due to squaring of the bias-corrected difference between predicted and carcass IMF for each observation, a few large errors are magnified and reflected in the SEP to a greater extent than consistent and smaller errors. The SEP indicates that PIMF is within the reported SEP value of CIMF for 67% of the observations. The SEP statistic is more consistent in evaluating accuracy of ultrasonic measurements than bias, absolute deviations, and percentage of absolute deviation (Moeller and Christian, 1998). Because genetic evaluations account for contemporary group effects, SEP would be the most important statistic for evaluating the systems for use in genetic prediction programs (Herring et al., 1998).

In order to evaluate the accuracy of the scanners, image capturing devices, image collection methods, and ROI box options while accounting for other sources of variation, 2 linear models were used to analyze the dependent variable ABSDiff:

$$\text{ABSDiff} = | \text{PIMF} - \text{CIMF} | .$$

The first model was used to compare scanners, image collection methods, ROI box options, and different system combinations that used the SEN image capturing device. Since 3 ROI boxes were used for individual image collection and 1 ROI was used for live video collection, the image collection method and ROI variables were combined to form the variable collection method/ROI. This variable represented the following 4 groups: individual image collection using 1 ROI, individual image collection using 2 ROI, individual image collection using 3 ROI, and live video image collection using 1 ROI. Using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC), least squares means of ABSDiff were estimated with a model that included fixed effects of scanner, collection method/ROI, scanner \times collection method/ROI, and a random effect of pig. Tests of fixed effects revealed

that scanner \times collection method/ROI was significant ($P < 0.0001$), indicating that differences in collection method/ROI are not the same for both scanners. Each pig was included as a random effect to eliminate confounding variables between different pigs, including scan date, scan location, breed, sex, LW, and CIMF. Therefore, a residual that did not contain variability due to individual pigs was used to test differences of ABSDiff. Scanners were compared across image collection methods and ROI box options. Image collection methods were compared across scanners using a single ROI, and ROI box options were compared across scanners using individual image collection. Differences between each individual system combination were also evaluated.

The second model was used to compare image capturing devices, ROI box options, and different system combinations that used the AL scanner and individual image collection. Least squares means of ABSDiff were estimated with a model that included fixed effects of capturing device, ROI, capturing device \times ROI, and a random effect of pig. Tests of fixed effects revealed that capturing device \times ROI was not significant ($P = 0.66$), and the best fitting model did not include capturing device \times ROI. Nonetheless, the interaction was included in the final model to allow for comparisons between all possible combinations of capturing device and ROI. Similar to model 1, each pig was included as a random effect to eliminate confounding variables between different pigs, including scan date, scan location, breed, sex, LW, and CIMF. The image capturing devices, ROI box options, and individual system combinations were evaluated.

Results and Discussion

The frequency and percentage of the population are shown for breed and gender (Table 1) and LW and CIMF by category (Table 2). Most of the animals are normally

distributed around the mean LW of 115.9 kg. Berkshires and Durocs are known to have superior meat quality and higher levels of IMF, which could partially explain the high levels of CIMF present in the current study. Forty-seven percent of the animals had CIMF greater than 3%, and the mean for the population was 3.21%.

Descriptive statistics for the number of images used in prediction of IMF for the different combinations of scanner, image capturing device, and image collection method are presented in Table 3. Across scanner type and image capturing device, the means and standard deviations for both image collection methods are very consistent. It is apparent that differentiation of individual and live video image collection was achieved. The large range of values for live video image collection indicates that extensive rejecting of images was sometimes necessary due to unacceptable image quality, or video containing acceptable image quality was recorded for longer than 4 s. Since poor quality images were marked as being rejected, the number of images used in prediction of IMF was less than the minimum number of images collected. This is more apparent for individual image collection than live video image collection.

Even though the same AL image was captured with the VCE and SEN, the images were not processed side-by-side by the interpreter. Therefore, it is possible that the same image was accepted for one capturing device and rejected for the other. Images captured with the SEN were digitized and displayed larger and with more visual clarity than VCE images. Therefore, it is possible that image quality problems were more readily observed by the interpreter for SEN images. Thus, more images were rejected, which resulted in a lower mean number of images used for prediction of IMF.

Descriptive statistics for CIMF and for PIMF by system combination are presented in Table 4. Across all systems used, mean PIMF was greater than that of CIMF. Based on the standard deviation, there was more variation in CIMF than PIMF for all systems. The AQ consistently overestimated IMF to a greater degree than the AL. Standard error of prediction ranged from 1.07 to 1.33% for the AL and from 1.06 to 1.34% for the AQ. Using individual image collection and 1, 2, or 3 ROI boxes, AL SEP values were 1.24, 1.18, and 1.15% using the VCE, 1.15, 1.08, and 1.07% using the SEN, and AQ SEP values were 1.18, 1.09, and 1.06%, respectively. These values are slightly higher than those of Newcom et al. (2002), who reported SEP ranging from 0.80 to 0.93% using an Aloka 500, individual image collection, and a single 100 x 100 ROI box per image. Herring et al. (1998) reported SEP of 1.79 and 2.35% for 2 technicians using Aloka 500 scanners for the prediction of IMF in live beef cattle. When compared within appropriate image collection methods and ROI box options, the SEP statistic was very similar for both of the real-time ultrasound scanners used in this study. Though the AQ generally exhibited a larger bias, the bias was beneficial to the SEP, meaning that it was consistent in direction. It appears that accuracy of the AL and AQ is similar within image collection methods and ROI box combinations, as evaluated by the SEP statistic.

There was slightly more variation in PIMF for the SEN than the VCE. When compared using 1 or 2 ROI boxes, the SEN overestimated IMF to a greater degree than the VCE, evidenced by a larger bias. However, when 3 ROI boxes were used, bias for the 2 capturing devices was similar. Increasing the number of ROI boxes increased bias for the VCE and decreased bias for the SEN. The SEN had a consistently lower SEP than the VCE, indicating that the bias was consistent in direction and thus, beneficial. Standard error of

prediction ranged from 1.07 to 1.15% for the SEN and from 1.15 to 1.24% for the VCE. Though ALIVCE3 and ALISEN1 had different bias (0.09 and 0.22, respectively), SEP was identical. Across scanners, live video image collection had the largest bias and SEP, and simply overestimated IMF to a greater degree than individual image collection. Within individual image collection, the addition of a second and third ROI box reduced the bias and SEP for both scanners. The addition of a second and third ROI brought the mean PIMF closer to that of CIMF.

Cumulative frequency and cumulative percentage of ABSDiff between PIMF and CIMF for all system combinations is shown in Tables 5 and 6. The cumulative frequency and percentage of animals predicted within 0.50, 1.00, 1.50, and 2.00% of CIMF are listed for each combination of scanner and image collection method (using 1 ROI) in Table 5 and each combination of scanner, image capturing device, and ROI box option (using individual image collection) in Table 6. Using a single ROI, the percentage of pigs for which PIMF was within 0.50% of CIMF was 39% for the AL and 26% for the AQ using individual image collection. Likewise, the corresponding values were 23% for the AL and 15% for the AQ using live video image collection. Across image collection methods, the AL also placed a greater number of animals within 1.00, 1.50, and 2.00% of CIMF than the AQ. Across scanners, individual image collection also placed a greater number of animals within 1.00, 1.50, and 2.00% of CIMF than live video image collection.

At first glance, similar conclusions about the scanners can be drawn from Table 6. The AL had an advantage over the AQ when 1 or 2 ROI boxes were used on individually collected images. The SEN had an advantage over the VCE, regardless of ROI box option. The number of observations in each ABSDiff threshold rises consistently with the number of

ROI boxes used. This effect was the least dramatic for ALIVCE and most dramatic for AQISEN. The individual system placing the highest number of animals within every ABSDiff category was ALISEN2 when either 1 or 2 ROI boxes were used. However, AQISEN3 had the greatest number of animals within 1.00, 1.50, and 2.00% of CIMF. It appears that the addition of more ROI boxes benefits the accuracy of the AQ and SEN to a greater degree than the AL and VCE.

For system combinations using individual image collection, PIMF was within 0.50% of CIMF for 26 to 45% of the observations. This is similar to the findings of Ragland (1998) who reported a range of 44 to 51%, but different from Newcom et al. (2002) who reported 6 to 39%, respectively. Predicted IMF was within 1.0% of CIMF 55 to 75% of the time for system combinations using individual image collection. Again, this is similar to the results of Ragland (1998) who reported a range of 72 to 86%, while Newcom et al. (2002) reported a range of 17 to 65%.

Least squares means of ABSDiff estimated by model 1 are presented by scanner, image collection method, ROI box option, and system combination in Table 7. Across image collection methods and ROI box options, the AL was more accurate than the AQ ($P < 0.0001$). Using a single ROI box per image and comparing across scanners, a large difference between image collection methods was found ($P < 0.0001$). Using individual image collection and comparing across scanners, accuracy improved with the addition of a second ($P < 0.0001$) and third ($P = 0.0021$) ROI box. Least squares means for ALISEN2, ALISEN3 and AQISEN3 were similar and the most accurate. Accuracy of ALISEN1 and AQISEN2 was similar and intermediate. The remaining systems including AQISEN1, ALVSEN1, and AQVSEN1 were the least accurate. The AQISEN1, ALVSEN1, and

AQVSEN1 systems were different from each other and all other system combinations ($P < 0.05$).

Broad conclusions about the scanners drawn from Tables 5, 6, and 7 agree with the fact that the AQ had a consistently larger bias than the AL. It is important to remember that PIMF values were not corrected for bias prior to calculating ABSDiff. If the scanners were evaluated solely on least squares means across image collection method and ROI box option, the AL has an advantage. In genetic improvement programs emphasizing PIMF, an equipment bias would be captured in the contemporary group effect, and would not reduce the ability of the AQ to rank animals correctly. Furthermore, least squares means of ABSDiff were similar for the AL and AQ when 3 ROI boxes were used. Both scanners also had similar SEP statistics, confirming that the AQ should not be overlooked as an equipment option for prediction of IMF in live swine.

Least squares means of ABSDiff estimated with model 2 are shown in Table 8. Across ROI box options, the SEN was more accurate than the VCE ($P = 0.0004$). Across capturing devices, the addition of a second ROI box increased prediction accuracy ($P < 0.0001$) beyond that of a single ROI box. Although 3 ROI boxes were more accurate than a single ROI ($P < 0.0001$), there was not a significant benefit to accuracy when box number was increased from 2 to 3 ($P = 0.1726$). This is different from the results estimated by model 1 in Table 7, where the 3 ROI option was different from the 2 ROI option. When individual system combinations were compared, no differences were observed. However, ALISEN2 and ALISEN3 were similar and had the most accurate estimates of ABSDiff. The ALIVCE1 and ALISEN1 systems were similar and had the least accurate estimates of ABSDiff.

It should be mentioned that the same Aloka 500 and Aquila Vet scanners were used throughout the trial. It will remain unknown whether the specific scanners used contributed to the results that were observed. The effects of factors such as equipment age, maintenance, and the extent of prior usage on prediction accuracy are also unknown. Likewise, the same transducer for each scanner was also used for the entire trial. All of the quartz crystals were fully functioning for both of the transducers throughout the trial. The transducer used with the AQ was 5.5 cm longer than the AL transducer, so placement of multiple ROI boxes was more easily accomplished on AQ images. Aquila Vet images were larger and had more visual clarity than Aloka 500 images. Despite the fact that a 100 x 100 pixel ROI box would have typically fit in the loin region of AQ images, box size was standardized at the 80 x 80 pixel size because this size worked for the vast majority of images and the effect of ROI box size on prediction accuracy was not evaluated. Since the AQ SEP and ABSDiff were reduced the most from the addition of a third ROI, it is possible that this scanner may also have benefited from the use of a 100 x 100 pixel ROI.

Across scanners, individual image collection had a lower SEP than live video image collection. There was a large and significant difference between least squares means of ABSDiff for the image collection methods. Results provide evidence that further enhancements to the live video image collection process are needed before the concept can be viable for industry use. With live video image collection, the ability of the technician to control image quality was reduced significantly, which could explain the accuracy disadvantages that were found. Based on visual assessment and the results of this study, image quality is more important than quantity for predicting IMF in swine using real-time ultrasound. Live video images were generally consistent within an animal, yet of lower

overall quality than individually collected images. An immobilization device to effectively restrain animals during scanning would likely allow the technician to have greater control of image quality. The manual processing of images collected via live video in this study was also very time consuming and thus, impractical for commercial application. The length of live video image collection time required to optimize prediction accuracy should be of interest in future research if image quality can be controlled in a way similar to individual image collection. If image quality can be improved and the optimum length of collection time is determined, the technique may prove to be less time consuming and more accurate than the traditional individual image collection method.

Regardless of the ultrasound scanner or capturing device used, addition of a second and third ROI box resulted in incremental reductions in SEP. In the model 1 analysis where SEN was the only capturing device used, ABSDiff across scanners was reduced significantly with the addition of a second and third ROI. Results from Table 7 show that prediction accuracy is optimized for the AL when 2 ROI boxes are used. Likewise, prediction accuracy for the AQ is optimized when 3 ROI boxes are used. In the model 2 analysis where AL images were captured simultaneously with the VCE and SEN, the addition of a second ROI improved accuracy significantly for both devices. Hassen et al. (1999) proposed that increasing the number of observations per animal will increase IMF prediction precision in beef cattle. In that study, increasing the number of images per animal was a more effective method of improving the precision of IMF predictions than increasing the number of ROI boxes per image. In the current study, where the number of images used was higher than Hassen et al. (1999) and was held constant within individual image collection, increasing the number of ROI boxes used improved prediction accuracy. Textural properties can vary

within an image. Increasing the amount of information gathered per image by increasing the number of ROI boxes was a benefit to prediction accuracy in this study. Future research might also investigate the effects of ROI box size and image quality on IMF prediction accuracy. Development of an image quality scoring system will be necessary in order for this to occur.

Implications

Novel equipment and procedures for the prediction of IMF in live swine were introduced and evaluated in this study. The Aquila Vet scanner compares favorably with the Aloka 500 and is a viable option for commercial use. Further development and research is needed before the live video image collection method will produce accuracy similar to that of the traditional individual image collection method. The Sensoray image capturing device was more accurate and offers more versatility than the VCE. Prediction accuracy is improved by increasing the number of ROI boxes per image.

Real-time ultrasound has been shown to be a valuable tool in the improvement of pork quality. If IMF becomes a component in pork carcass pricing in the United States, producers of commercial market pigs may demand terminal line genetics with the ability to generate higher levels of IMF in their commercial progeny. Thus, swine genetics companies will need to utilize the most rapid and accurate methods available for predicting IMF content in live animals to stay competitive. Results from this study can be used as a decision making guide for equipment and procedures used for the prediction of IMF. Useful information is provided for those interested in enhancing their IMF prediction techniques.

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Table 1. Distribution of animals by sex and breed for a population used to evaluate accuracy of equipment and procedures for the prediction of intramuscular fat percentage in live swine (N = 454)

	N	%
<i>Sex</i>		
Barrow	248	55
Gilt	206	45
<i>Breed</i>		
Berkshire	161	35
Chester White	37	8
Crossbred	95	21
Duroc	96	21
Landrace	28	6
Spotted	7	2
Yorkshire	30	7

Table 2. Distribution of animals by live weight and chemical intramuscular fat category for a population used to evaluate accuracy of equipment and procedures for the prediction of intramuscular fat percentage in live swine (N = 454)

	N	%
<i>LW</i> ¹		
98 - 109	53	12
110 - 113	137	30
114 - 118	152	33
119 - 122	47	10
123 - 127	30	7
128 - 125	35	8
<i>CIMF</i> ²		
0.99 - 2.00	84	18
2.01 - 3.00	161	35
3.01 - 4.00	112	25
4.01 - 5.00	53	12
5.01 - 6.00	23	5
6.01 - 11.83	21	5

¹ LW = Kilograms of live weight at the time of scanning.

² CIMF = Percentage of intramuscular fat determined by chemical analysis of muscle sample.

Table 3. Descriptive statistics for the number of images used in prediction of intramuscular fat percentage by 5 combinations of scanner, image capturing device, and image collection method

System	Mean	SD	Minimum	Maximum
ALIVCE ¹	5.43	1.03	2	8
ALISEN ²	5.04	1.35	1	8
AQISEN ³	5.30	1.06	2	8
ALVSEN ⁴	45.94	14.37	11	113
AQVSEN ⁵	42.77	14.63	3	111

¹ ALIVCE = Aloka 500 using individual image collection and a VCE capturing device.

² ALISEN = Aloka 500 using individual image collection and a Sensoray capturing device.

³ AQISEN = Aquila Vet using individual image collection and a Sensoray capturing device.

⁴ ALVSEN = Aloka 500 using live video image collection and a Sensoray capturing device.

⁵ AQVSEN = Aquila Vet using live video image collection and a Sensoray capturing device.

Table 4. Descriptive statistics for intramuscular fat percentage measured by chemical extraction and predicted by different combinations of scanner, image capturing device, image collection method, and region of interest box option (N = 454)

Measurement	Mean	SD	Minimum	Maximum	Bias	SEP
CIMF ¹	3.21	1.49	0.99	11.83		
ALIVCE1 ²	3.23	0.89	1.16	5.83	0.02	1.24
ALIVCE2 ³	3.23	0.90	0.95	6.23	0.03	1.18
ALIVCE3 ⁴	3.30	0.87	0.85	6.28	0.09	1.15
ALISEN1 ⁵	3.43	1.04	0.88	6.99	0.22	1.15
ALISEN2 ⁶	3.34	1.02	0.62	6.74	0.14	1.08
ALISEN3 ⁷	3.25	1.02	0.63	7.20	0.04	1.07
AQISEN1 ⁸	3.75	0.91	1.26	7.52	0.54	1.18
AQISEN2 ⁹	3.58	0.89	1.19	6.96	0.37	1.09
AQISEN3 ¹⁰	3.38	0.91	1.19	6.55	0.18	1.06
ALVSEN1 ¹¹	4.06	1.26	0.93	8.44	0.86	1.33
AQVSEN1 ¹²	4.37	1.17	-1.90	8.67	1.17	1.34

¹ CIMF = Percentage of intramuscular fat determined by chemical analysis of muscle sample.

² ALIVCE1 = Aloka 500, individual image collection, VCE, and 1 region of interest box.

³ ALIVCE2 = Aloka 500, individual image collection, VCE, and 2 region of interest boxes.

⁴ ALIVCE3 = Aloka 500, individual image collection, VCE, and 3 region of interest boxes.

⁵ ALISEN1 = Aloka 500, individual image collection, Sensoray, and 1 region of interest box.

⁶ ALISEN2 = Aloka 500, individual image collection, Sensoray, and 2 region of interest boxes.

⁷ ALISEN3 = Aloka 500, individual image collection, Sensoray, and 3 region of interest boxes.

⁸ AQISEN1 = Aquila Vet, individual image collection, Sensoray, and 1 region of interest box.

⁹ AQISEN2 = Aquila Vet, individual image collection, Sensoray, and 2 region of interest boxes.

¹⁰ AQISEN3 = Aquila Vet, individual image collection, Sensoray, and 3 region of interest boxes.

¹¹ ALVSEN1 = Aloka 500, live video image collection, Sensoray, and 1 region of interest box.

¹² AQVSEN1 = Aquila Vet, live video image collection, Sensoray, and 1 region of interest box.

Table 5. Cumulative frequency (cumulative percentage) of absolute difference between predicted and chemical intramuscular fat percentage for different combinations of scanner and image collection method using a single region of interest box per image (N = 454)

Scanner	Aloka 500		Aquila Vet	
Collection Method	Individual	Live video	Individual	Live video
± 0.50 %	179 (39)	104 (23)	119 (26)	67 (15)
± 1.00 %	308 (68)	209 (46)	250 (55)	175 (39)
± 1.50 %	384 (85)	291 (64)	370 (82)	251 (55)
± 2.00 %	427 (94)	365 (80)	423 (93)	328 (72)

Table 6. Cumulative frequency (cumulative percentage) of absolute difference between predicted and chemical intramuscular fat percentage for different combinations of scanner, image capturing device, and region of interest box option using individual image collection (N = 454)

Scanner/Device # of ROI boxes	Aloka 500/VCE			Aloka 500/Sensoray			Aquila Vet/Sensoray		
	1	2	3	1	2	3	1	2	3
± 0.50%	173 (38)	180 (40)	177 (39)	179 (39)	193 (43)	203 (45)	119 (26)	153 (34)	199 (44)
± 1.00%	310 (68)	322 (71)	326 (72)	308 (68)	329 (73)	341 (75)	250 (55)	308 (68)	350 (77)
± 1.50%	386 (85)	398 (88)	405 (89)	384 (85)	400 (88)	407 (90)	370 (82)	402 (89)	418 (92)
± 2.00%	419 (92)	429 (95)	429 (95)	427 (94)	432 (95)	436 (96)	423 (93)	433 (95)	437 (96)

Table 7. Least squares means (\pm SE) of absolute difference between predicted and chemical intramuscular fat percentage by scanner, image collection method, region of interest box option, and system combination

Effect	Least Squares Means
<i>Scanner</i> ¹	
Aloka 500	0.92 \pm 0.032 ^a
Aquila Vet	1.03 \pm 0.032 ^b
<i>Image Collection Method</i> ²	
Individual	0.94 \pm 0.034 ^a
Live Video	1.38 \pm 0.034 ^b
<i>ROI Box Option</i> ³	
1	0.94 \pm 0.034 ^c
2	0.82 \pm 0.034 ^b
3	0.75 \pm 0.034 ^a
<i>System Combination</i> ⁴	
ALISEN1	0.85 \pm 0.039 ^b
ALISEN2	0.78 \pm 0.039 ^a
ALISEN3	0.75 \pm 0.039 ^a
AQISEN1	1.03 \pm 0.039 ^c
AQISEN2	0.86 \pm 0.039 ^b
AQISEN3	0.74 \pm 0.039 ^a
ALVSEN1	1.28 \pm 0.039 ^d
AQVSEN1	1.48 \pm 0.039 ^e

Least squares means within a column and within scanner, image collection method, ROI box option, and system combination without a common superscript differ ($P < 0.05$).

¹ Comparisons were made across image collection methods and ROI box options.

² Comparisons were made across scanners using a single region of interest box per image.

³ Comparisons were made across scanners using individual image collection.

⁴ Refer to Table 4 for system combination abbreviations.

Table 8. Least squares means (\pm SE) of absolute difference between predicted and chemical intramuscular fat percentage by image capturing device, region of interest box option, and system combination

Effect	Least Squares Means
<i>Image Capturing Device</i> ¹	
VCE	0.84 \pm 0.036 ^b
Sensoray	0.80 \pm 0.036 ^a
<i>ROI Box Option</i> ²	
1	0.87 \pm 0.036 ^b
2	0.81 \pm 0.036 ^a
3	0.78 \pm 0.036 ^a
<i>System Combination</i> ³	
ALIVCE1	0.89 \pm 0.038 ^d
ALIVCE2	0.83 \pm 0.038 ^{bc}
ALIVCE3	0.82 \pm 0.038 ^{bc}
ALISEN1	0.85 \pm 0.038 ^{cd}
ALISEN2	0.79 \pm 0.038 ^{ab}
ALISEN3	0.75 \pm 0.038 ^a

Least squares means within a column and within image capturing device, ROI box option, and system combination without a common superscript differ ($P < 0.01$).

¹ Comparisons were made across region of interest box options.

² Comparisons were made across image capturing devices.

³ Refer to Table 4 for system combination abbreviations.

CHAPTER 4. GENERAL SUMMARY

The primary objective of this thesis was to evaluate equipment and procedures used for the prediction of IMF in live swine. The results are intended to first serve the needs of the pork industry, and second, to open the door for future research in the field. Pertinent related literature was reviewed in Chapter 2. The scientific research conducted in this project was the first of its kind and the most similar studies were conducted in beef cattle. Therefore, a broad base of literature was reviewed. The primary objectives of the thesis were accomplished in Chapter 3. In the process of reviewing literature and accomplishing the primary objectives, ideas for future research and challenges associated with large scale commercial industry adoption of routine prediction of IMF in swine surfaced.

Chapter 3 showed that the traditional method of collecting ultrasound images individually was vastly more accurate than the continuous video recording method introduced through this study. Animal restraint issues led to the inability of the technician to control image quality during live video image collection. An animal immobilization crate designed specifically for swine scanning should be developed to more effectively compare the individual and live video image collection methods. An image quality scoring system should be developed to determine the effect of image quality on IMF prediction accuracy. Chapter 3 also concluded that increasing the number of ROI boxes on images improves IMF prediction accuracy. In this study, ROI boxes were standard in size and placed independently in the first, second, and third most desirable locations as visually assessed by the interpreter. Future ROI-related research should investigate the effects of ROI size, ROI independence, and ROI location. Whether or not the human eye can detect image quality differences within an image remains unknown.

Collection of high quality ultrasound images to be used for the prediction of IMF is not an easy task for a field technician. However, it is achievable through proper training and continued exposure. Swine breeding companies implementing IMF prediction technology will need to have their technicians professionally trained if accurate prediction of IMF is desired. If image processing is to be completed properly, technicians will also need to be trained in image interpretation and ROI box placement for the technology to be effective. If a company is interested in measuring IMF but unwilling to invest in their own equipment, the other option would be more similar to the beef industry, where independent certified technicians offer their services to beef producers. The idea of centralized or third party image processing also comes to mind with regard to the beef industry. Current swine industry structure may not be conducive to the use of independent technicians for hire by swine breeding companies. However, centralized processing of images may be a possibility.

As stated previously in Chapter 2, pork producing individuals and companies will not realize the true benefit of using IMF prediction technology until the IMF trait is measured and rewarded effectively by pork packing companies. Once an economic value for IMF is established, the technologies discussed in this thesis and genetic selection principles can be set into motion in the swine industry. We must remember that regardless of the effectiveness level associated with live animal measurement and genetic improvement of the trait, a financial incentive will need to be provided before widespread measurement of IMF in live swine is practiced.

CHAPTER 5. LITERATURE CITED

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