Determining the underlying factors of fresh ham color variation

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ABSTRACT

Consumers associate meat color with quality. In some cases, especially in fresh and cured hams, the surface of a ham, whole, boneless or sectioned and formed displays a color gradient, which is unsightly and generally is considered of lower quality and must be discounted or processed different where color is less critical to the ultimate value of the resulting product. This disparity in color uniformity across fresh and cured products is sometimes known as two-toning and is most often found in the semimembranosus (SM) and associated muscles of fresh hams and is exacerbated with curing. The underlying color of fresh meat may be a function of postmortem metabolism or the underlying characteristics of those muscles involved. Therefore, the objective of this study is to determine the changes in underlying muscle type and postmortem metabolism in those muscles responsible for fresh ham color variation. Semimembranosus (SM) muscles of 15 mixed bred pigs were collected at 30 min and 1440 min postmortem, and muscle color was determined and muscles were collected and snap frozen for various energy metabolism analyses. Differences in color (L*, a* and b*) were noted across the face of the muscle by zone and time (P < 0.0001) but no differences were detected in pH and lactate, glucose, glucose-6-phosphate, and glycogen metabolisms. Glycolytic potential was also measured on a lactate basis and showed no differences across zone (P = 0.0746) but increased over time (P < 0.006). Lactate and pH were plotted and showed a linear relationship (R^2 = 0.928337) at 30 min (P < 0.0001) and at 1440 min (R^2 = 0.161412; P < 0.0015). Muscle type characteristics showed no difference between zones and time. Buffering capacity showed a significant difference at pH 6 (P < 0.0359) and with time across all pH measured (P < 0.0001). These data
suggest inherent differences, such as location and function, in the semimembranosus muscle may be more critical in developing fresh color than aberrations in postmortem metabolism.
DEDICATION

I dedicate this thesis to all who have helped me along the way. I am eternally grateful for the support, patience, and kind words of reassurance my family and friends have given me. I mostly thank Jesus Christ for being with me every step of the way. I hope to one day give back to the community and those who have given so much to help me achieve my goals that shaped me into the person I am today.
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CHAPTER 1. Literature Review

Postmortem meat color development in hams

Overview

Producing a quality ham with consistent color has been a challenge to the pork industry recent past. Meat color is one of the most important quality characteristics, according to Troy and Kerry (2010) and is the first perception of quality perceived by the consumer and determines whether a purchase will be made. Meat color is predominately determined by the amount of myoglobin in the tissue. The pork industry currently experiences great color disparities across fresh and cured hams. This variation in color is described as a “halo” or “two-toning” effect (Wilson et al., 1959). Before conducting research in this area, factors influencing fresh pork color must be fully understood.

A number of factors influence fresh meat color and include but are not limited to: location of the muscle, muscle fiber type composition, postmortem metabolism, pre-slaughter stress, handling, and processing (Joo et al., 2013). Muscle fibers can be classified into four different types in adult pig muscle using a variety of experimental approaches: slow-oxidative (Type I), fast oxidative-glycolytic (Type IIA), and fast glycolytic (Type IIX and IIB) (Shiaffino & Reggiani, 1996). As there are different functions for muscles, the relative composition of these muscle fibers within a muscle influences the overall metabolic and functional properties of the muscle. In addition to location, muscle fibers vary with breed, sex, growth and level of nutrition (Joo et al., 2013).

Handling of animals pre-slaughter is important for both animal welfare and product quality as pre-slaughter stress can adversely affect the meat quality development. Long-term stress such as handling on the farm, loading and transportation can cause dark, firm and dry
(DFD) meat while short-term stress prior to slaughter can cause pale, soft and exudative (PSE) meat. Pigs exposed to long-term stress have greater glycogen depletion, which results in a higher ultimate pH of the meat (Faucitano, 1998; Warris & Brown, 1985; Warris et al., 1998). In addition, short-term stress results in lower muscle pH when carcass temperatures are elevated and thus results in greater protein denaturation during the early postmortem period and a corresponding loss of water and changes in the way light interacts with the tissue (Brown, Warris, Nute, Edwards & Knowles, 1998; D’Souza, Dunshea, Warner, & Leury, 1998; D’Souza et al., 1998, Gariepy, Amiot, & Nadia, 1989, Henckel, 2001; van der Wal et al., 1997, van der Wal et al., 1999).

Conversion of Muscle to Meat and Postmortem Metabolism

For muscle to function properly, ATP must be readily available to fuel the myosin molecule and the calcium pump on the sarcoplasmic reticulum (Endo, 1985). When a muscle contracts, the sarcoplasmic reticulum releases calcium which binds to troponin C. During this binding, a conformational change takes place and allows the myosin binding site on actin to become uncovered. Once myosin and actin bind, the myosin head then hydrolyzes ATP and once ADP and Pi is released from the actin site, then the head moves to the next actin available (Weber & Murray, 1973). After an animal has been sacrificed, muscle continues to use energy throughout the postmortem period. From an energy standpoint, the muscle is supplied energy by ATP and creatine phosphate, an immediate reserve for the rephosphorylation of ATP. This phosphocreatine system phosphorylates ADP using creatine kinase. Using myokinase, two ADP molecules are combined with one AMP to create one ATP molecule (Scheffler & Gerrard, 2007).

In living animals, energy is produced in two main pathways, glycolysis and oxidative phosphorylation (Pösö & Puolanne, 2005). Oxidative phosphorylation is the most efficient
pathway for generating ATP and requires oxygen. When muscles are rapidly using ATP and there is not enough oxygen supplied to the muscle and anaerobic glycolysis upregulates to metabolize energy reserves in the tissues (Scheffler & Gerrard, 2007). When an animal is exsanguinated, muscles rapidly becomes anaerobic and glycogen within the muscle is metabolized to generate more ATP; however, because anaerobic metabolism is less efficient, the ATP levels drop more rapidly compared to that of living tissues (Bowker, Grant, Forrest, & Gerrard, 2000). Glycogen phosphorylase and the glycogen debranching enzyme catalyze the degradation of glycogen (Scheffler & Gerrard, 2007). The addition of inorganic phosphate at the α-1, 4 linkage sites allows glycogen phosphorylase to cleave its substrates creating glucose 1-phosphate. Phosphoglucomutase then catalyzes the isomerization transforming glucose 1-phosphate to glucose 6-phosphate which then continues through glycolysis. When phosphorylase gets to the fourth glucose from a branch point, a transferase then shifts residues to the main glycogen chain and a free glucose is released by the glycogen debranching enzyme breaking the linkages at α-1, 6.

If free glucose is used, it first must be phosphorylated to glucose-6-phosphate by hexokinase and a phosphate ion using ATP (Burleigh & Schimke, 1969). From fructose 1,6-bisphosphate, aldolase forms dihydroxyacetone phosphate and glyceraldehyde phosphate. Dihydroxyacetone phosphate is turned into glyceraldehyde phosphate using triose phosphate isomerase, yielding two glyceraldehyde phosphates. In the presence of triose phosphate dehydrogenase, two glyceraldehyde phosphates donate a hydrogen ion to transform NAD+ to NADH. This creates two 1,3-bisphosphoglycerate molecules and an additional phosphate group. One phosphate group from both 1,3-bisphosphoglycerate is removed by phosphoglycerokinase and added to one ADP, creating two molecules of ATP and two 3-phosphoglycerate molecules.
and then moves from the third carbon to the second, creating two 2-phosphoglycerate molecules (Morgan & Parmeggiani, 1964). Enolase then removes one molecule of water from each of the 2-phosphoglycerate molecules to form phosphoenolpyruvic acid (PEP). A phosphate group is removed from each PEP by pyruvate kinase (PK) and transforms ADP to two ATP molecules and two phosphate molecules. At the end of glycolysis, there is a total of 4 ATP (but as two ATP are being expended during glycolysis the net yield is 2 ATP), 2 NADH, and 2 pyruvates, but if glucose is used from glycogenolysis then one less ATP is utilized creating a total of 3 ATP (Morgan & Parmeggiani, 1964).

There are certain cases where PK and PFK change during the postmortem period. If the pH drops below 6.0, PFK begins to lose function. Studies have shown that PFK becomes completely inactive at a pH of 5.5 (England et al., 2014). PK activity can be changed depending on the amount of ATP available (England, Scheffler, Kasten, Matarneh, & Gerrard, 2013). In the absence of oxygen, each pyruvate is converted to lactate and a hydrogen ion by lactate dehydrogenase and NADH. The hydrogen comes off NADH creating NAD+ (Scheffler, Park, & Gerrard, 2011). As a result, hydrogen ions accumulate causing the pH to fall from 7.5 to 5.6 in 24 hours (Bowker et al., 2000). Once ATP is completely utilized, rigor mortis is completed (Scheffler & Gerrard, 2007).

**Factors that affect postmortem metabolism**

*ATP availability*

Flux through glycolysis is influenced by the amount of ADP in the muscle (Scheffler et al., 2011). When ATP is being consumed at higher rates, greater amounts of ADP are formed and the pH declines at a faster rate. Bowker et al. (2004) stated that at 24 hours postmortem there was a significant decrease in ATPase activity when using poor quality pork because of the hastened
pH decline most likely by altering ATPase perhaps through direct denaturation or indirectly through denaturation of a cytoplasmic protein. Warner et al. (1997) conducted research with PSE pork that resulted in significantly lower ATPase activity when compared to other qualities of pork, even though PSE pork is related to glycolytic muscles.

**Muscle Fiber Type**

Muscles contain different types of muscle fibers and each fiber varies in speed of contraction and the primary type of energy metabolism used. Speed of contraction is highly predicated on myosin heavy chain (MyHC) isoform composition, after all its ability to hydrolyze ATP forms the basis for contraction. As such, adult muscle fiber types can be defined as the predominate MyHC in the fiber and are categorized into: Type I, IIA, IIX, and IIB (Schiaffino et al., 1989). Type I fibers are generally considered slower contracting fibers; contain mainly type I MyHC compared to their faster contracting counter parts. These fibers are also smaller in diameter and more red in color. Type II, on the other hand, contain predominately type IIA, IIB or IIX MyHC isoforms and are faster contracting than type I fibers (Stufft et al., 2017). Muscle fiber type also plays a role in the glycolytic capacity and energy flux. Type I and type IIA fibers have a more oxidative capacity and have a decreased glycogen catabolism postmortem. The conversion of glycogen to lactate in these fibers occur at much slower rates (VØllestad, Tabata, & MedbØ, 1992) because of their inherent differences in glycogenolytic and glycolytic enzyme concentrations and activity (Burleigh & Schimke, 1969). Their lower glycolytic capacity causes less lactate production (Karlsson et al., 1999) and thus a higher ultimate pH (Scheffler & Gerrard, 2007). Fiber types that have a higher glycolytic capacity, types IIX and IIB, have significantly faster glycolytic rates. An increased rate of glycolysis allows more substrate
breakdown to occur, dropping pH faster from the increased production of lactate (Karlsson et al., 1999).

Muscle fiber types vary not only between muscles, but within the muscle as well (Armstrong et al., 1987). For example, the longissimus dorsi contains more type IIB fibers whereas the semimembranosus is more oxidative (Karlsson et al., 1999). There are numerous factors that can influence fiber type such as, location and function, sex, age, and genetics. In reference to location and function, muscles that are associated with posture have more oxidative fibers that are able to resist fatigue over extended periods of time (Choi & Kim, 2009). Through research, Totland and Kryvi (1991) found that the increase in glycolytic fibers distal to the bone creates mechanical benefits to animals because muscles more distal to bone allow for more force to be applied on the bone to acquire more power.

Within the meat industry older pigs are used for further processing such as sausage or ground pork. When born, piglets have more oxidative fibers and these decrease with age and become more glycolytic (Karlsson et al., 1999). Research based on muscle fiber type in correlation to sex has presented conflicting findings. Miller et al. (1975) found no significant differences between muscle fiber compositions across sexes. Likewise, Karlsson et al. (1993) found no significant difference in muscle fiber type; however, small but significant differences were noted for cross sectional areas of muscle fiber type composition. In yet another study conducted by Latorre et al. (2004) no sex effects were noted.

As the swine industry demands have changed over time, genetic programs have selected for leaner animals with greater growth rates. This selection process has led to greater contributions of type IIB fibers (Weiler, et al., 1995). Overtime as the industry has become more
advanced and the domestication of hogs have increased, muscle fiber types have become more
glycolytic compared to wild hogs and have more type IIB fibers (Ryu & Kim, 2005).

Initial Glycogen Concentration

Glycogen concentration prior to slaughter influences the extent of glycolysis postmortem. Muscles that contract at a faster speed generally have higher amounts of glycogen. Muscles that have a higher glycogen concentrations at slaughter tend to have lower overall extent postmortem pH (Wittman et al., 1994). Stress prior to slaughter also influences the amount of glycogen available in the muscle during slaughter. As stress occurs, more energy is needed for muscles to contract while increasing glycogenolysis (Richter et al., 1982).

Glycolytic Flux

Oxidative fibers have lower amounts of glycogen when compared to glycolytic fibers, which means the conversion of glycogen to lactate occurs at a slower rate in oxidative fibers resulting in a higher pH (Burleigh & Schimke, 1969). As glycolytic fibers have more glycogen, glycolysis occurs at a faster rate allowing for an increase rate of substrate breakdown creating a faster pH decline and more lactate accumulation (Karlsson et al., 1999).

pH and Temperature

As muscle is converted to meat, the rate and extent of pH decline impacts the quality of meat tremendously (Scheffler & Gerrard, 2007). The normal pH of muscle in a living animal is around 7.4 but reaches an ultimate pH of around 5.5 postmortem (Briskey & Wisemer-Penderson, 1961). If the pH drops dramatically or does not reach the normal ultimate pH the quality of meat can be significantly lowered (Bendal & Swatland, 1988). Temperature is also an important factor when pertaining to the quality of meat. When initial pH is low, paired with high
temperatures, meat proteins are denatured. In addition, if there is high initial temperatures with low pH postmortem the chances of inducing PSE are greater (Harmoen et al., 2013; Thompson, 2002).

**Myoglobin**

Myoglobin is the protein containing iron that gives meat its color. Myoglobin allows muscles to store oxygen just as hemoglobin allows blood to store oxygen. The more oxygen meat retains the deeper red the color of meat will appear. In relation to the following livestock animals, beef has the highest myoglobin content, pork has a moderate amount, and chicken contains the least amount of myoglobin out of the three species. Other than species, the age and location of muscle affects the amount of myoglobin present. Older animals contain more myoglobin and muscles used more for locomotion are generally more red in color (Mancini & Hunt, 2005). Myoglobin is water-soluble and may leak from the muscle during the postmortem period and is often referred to as “purge.” Purge is the combination of water and other soluble proteins, including myoglobin. As purge increases, myoglobin leaves the tissues possibly impacting the overall color and making it appear lighter in color (Seideman et al., 1984).

**Factors affecting myoglobin**

*Fiber type*

Different fiber types vary in myoglobin concentrations. More oxidative fibers have higher amounts of myoglobin than glycolytic fibers (Klont et al., 1998) and are more red in appearance, as noted by lower meat color lightness (L* values) and greater redness (a*) values (Klont et al., 1998). Muscles with greater glycolytic flux likewise have lighter meat (Choe et al., 2008) while muscles with more type I fibers have significantly lower L* values (Ryu et al., 2008).
Muscle Structure

Pigment in meat can be perceived based on the integrity of its structure and is measured by the amount of light being reflected or absorbed by the meat (Young & West, 2001). Muscle structure is disrupted during the transformation of muscle to meat mainly due to denaturation of proteins and their precipitation on to various entities in the structure. As a result, the amount of light being reflected or even scatter by the tissues changes (Bendall & Swatland, 1988). This is likely due to greater amounts of liquid (water) being on the surface and a scattering of light, which is then not detected.

Denaturation of proteins

Initial high temperatures coupled with low ultimate pH can change the quality of meat and affect the characteristics of the muscle fibers. Sarcoplasmic and myofibrillar proteins and ATPases have a higher rate of protein denaturation. Myosin denatures through a series of 10 unfolding sequences that have their own temperature requirements needed to unfold that varies with pH (Vega-Warner & Smith, 2001). An increase in glycolysis would change the overall pH, influencing the temperature requirements for unfolding to occur, resulting in a change in the function of properties. Kim et al. (2014) also provided evidence that ATPase decreases with exposure to higher ambient temperatures.

A study conducted by Scopes (1964) found that precipitation of sarcoplasmic proteins were maximized at the isoelectric point with a temperature of 40 °C pre rigor. Glycolytic enzymes represent the biggest portion of sarcoplasmic proteins (Clarke et al., 1980). Myoglobin is responsible for meat color but only accounts for a small portion of sarcoplasmic proteins but classifies as one of the more stable proteins (Rhee & Zirpin, 1987). During the postmortem
period, there can be water loss from purging which is primarily sarcoplasmic proteins (Savage et al., 1990). When rigor begins to occur, myofibrils begin to shrink pushing water into perimysial and endomysial space (Offer & Cousins, 1992). As pre rigor temperatures rise, the amount of purging also increases, consequently increasing the amount of sarcoplasmic protein being lost (Farouk & Swan, 1998). When purging increases so does the amount of native myoglobin that is being lost.

Metabolites

There are two extreme cases of less desirable quality traits in pork: pale, soft, and exudative (PSE) and dark, firm, and dry (DFD). DFD meat occurs when an animal is exposed to long-term stress prior to slaughter creating a higher than normal pH which creates a darker color (Adzitey & Nurul, 2011). When muscle temperatures are high and the pH is low muscle proteins denature and decrease the water holding capacity (WHC) of the meat while increasing oxymyoglobin (Kapper et al., 2014). Through these changes, a pale color is observed as PSE meat. The extent and rate of pH decline follow postmortem glycolysis, as glycogen converts to lactate and hydrogen ions build up (Kastenschmidt et al., 1968). PSE meat is caused by rapid glycolysis as there is a dramatic drop in pH to less than 6.0 during the first hour after slaughter and reaching an ultimate pH of 5.3-5.7 (Scheffler & Gerrard, 2007). This combination results in the denaturation of sarcoplasmic and myofibrillar proteins which creates a low water holding capacity. As there is a greater amount of purge and myoglobin loss, the meat appears paler.

Summary

Quality of meat is influenced by numerous factors. The rate and extent of pH decline is a major driver of pork quality. Glycogen concentrations, the amount of myoglobin, and muscle
fiber type are also important factors that affect quality. As location of the muscle and function changes, muscle fiber type composition is altered. Inherent myoglobin concentrations correlate to differences in fiber type, which vary not only between muscles, but within the muscle as well. This variation in color is unsavory to consumers as even a difference of five L* units can deter away a potential costumer (Norman et al., 2003). Any color variation in fresh meat is extended during the curing process, therefore; thoroughly assessing the factors that influence color in fresh products can help better understand the abnormalities observed within a muscle to create a uniform product overall.
References


*Determining the underlying factors of fresh ham color variation*
Introduction

Meat color is an important quality attribute for consumers making purchasing decisions (Troy & Kerry, 2010). In recent years, producers have focused on producing carcasses with increased lean deposition rates and higher meat yields. However, variations in pork quality, specifically pale, soft, and exudative (PSE) meat have become more prevalent (Scheffler & Gerrard, 2007). In 2003, 15.5% of the United States pork products had PSE abnormalities (Stetzer & McKeith, 2003). As PSE represents a discolored product, consumers are reluctant to purchase such products that are not uniform in color, costing the industry money.

Meat color is impacted by numerous attributes, most often mediated through aberrant fluctuations in pH development postmortem. The pH of a living muscle is around 7.4 dropping between 5.8 and 5.5 over the 24 h postmortem period. The rate and extent of postmortem pH decline dictate water-holding capacity, which influences the overall meat color (Bendall & Swatland, 1988). An attenuated pH decline, with high ultimate pH, leads to increased water-holding capacity generating a darker color. Muscles having a higher prevalence of PSE or DFD characteristics are the longissimus lumborum, semimembranosus, biceps femoris, gluteus medius, and the lateral section of the semitendinosus (Warner, Kauffman, & Russel, 1993). Additionally, meat color is impacted by species, age, and muscle fiber type composition. The color differences observed between these are factors associated with variation in myoglobin (Joo et al., 2013), a heme pigment responsible for giving meat its color (Rhee & Zirpin, 1987). In meat, there are numerous factors that affect myoglobin content, including but not limited to breed, sex, pH, and muscle (Lindahl et al., 2001); however, muscle fiber type can be the most significant (Stufft, 2017). Each fiber has different characteristics such as speed of contraction, oxidative and glycolytic capacity, glycogen levels, and myoglobin concentration (Schiaffino &
Reggiani, 1994; Schiaffino & Reggiani, 1996). Oxidative fibers, associated with type I fibers, tend to have a higher concentration of myoglobin when compared to glycolytic fibers, those associated with type II fibers, portraying a more red color (Klont et al., 1998). This variation in muscle fiber type can be seen not only between muscles, but also within the same muscle based on location and function. Armstrong et al. (1987) stated that redder, more oxidative fibers are more abundant near the skeleton in order to maintain stability. Changes in muscle fiber type across the face of hams may be related to what Wilson et al. (1959) referred to as “two-toning”.

Difference in muscle fiber type can influence the rate and extent of postmortem metabolism, affecting the overall quality during the muscle to meat conversion (Fernandez, Lefaucheur, & Candek, 1995). After an animal has been exsanguinated, oxidative metabolism ceases and glycolysis becomes the predominate type of metabolism postmortem (Bowker et al., 2000). Starting substrate amounts and flux through glycolysis influence the end products of glycolysis, hydrogen ions and lactate. As the ability to carry metabolites away from the tissues is lost, hydrogen ions build up in muscle tissues and lower the pH. Both the rate and extent of pH decline influences the overall quality of meat (Scheffler & Gerrard, 2007).

One important quality factor associated with meat is color. Color is considered one of the first factors when a consumer is making a purchasing decision. Any product that has color variation is discriminated against, costing the industry money by offering the product at a discounted price. In hopes to create a more uniform product for industry and consumers, understanding the underlying causes of color variation is the primary goal of this study. This study focused on identifying the causes of “two-toning” or the discoloration across the semimembranosus muscle in fresh hams by determining the proportional contribution of postmortem metabolism and the inherent differences in muscle type.
Materials and Methods

Muscle sampling

Fifteen market weight mixed commercial bred pigs, negative for the halothane and rendement napole genes were harvested at the Virginia Tech Meat Science Center. Semimembranosus (SM) muscles were collected at 30 min and 24 h (1440 min) postmortem. Muscle samples were trimmed and divided into four zones: A, B, C, and D. The SM muscle was separated into equal thirds from cranial to caudal end labeled respectively as A, B, and C. From the most caudal portion of zone C, the muscle was labeled as zone D. Once fresh color was determined, samples were snap frozen in liquid nitrogen and stored at -80 °C. Samples for exudate determination were collected at 30 min from each zone and placed in water-holding capacity tubes. Samples were weighed and allowed to purge overnight, when they were reweighed at 1440 min postmortem. Resulting purge was then transferred to clean tubes and stored at -20 °C until analysis.

Color Analysis

Color measurements were taken at a 0 ° angle using a Konica Minolta CR300 colorimeter (Ramsey, NJ, USA), Illuminant D. Measurements were collected in triplicates across each zone that had no perceptible color irregularities. Color data (L*, lightness; a*, redness) for each zone were averaged.

Sample Preparation and protein determination

Frozen muscle samples were ground using liquid nitrogen and a prechilled mortar and pestle. Aliquots of 100 mg samples were lysed in 1 ml of titin buffer containing 8 M urea, 2 M thiourea, 3% SDS (wt/vol), 75 mM dithiothreitol, and 50 mM Tris-HCL (pH 6.8) (Warren et al.,
Once titin buffer was added, samples were homogenized for 2 min and heated at 95 °C for 10 min. Samples were then diluted 1:20 with water. Reducing Agent and Detergent Compatible (RCDC) Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA) was used to determine the protein concentrations of each sample using bovine serum albumin as a standard curve (Fisher Scientific, Pittsburg, PA, USA). Concentration was measured spectrophotometrically at 750 nm. RCDC was also used to measure myoglobin in the exudate. Samples were directly diluted 1:10 in titin buffer and heated at 90 °C for 10 min. Samples were then diluted 1:20 with water and protein concentrations were measured as outlined above. Ground tissue for myoglobin were also homogenized in one ml of myoglobin extraction buffer containing 20 mM KH₂PO₄ (pH 5.6), 20 Mm Tris Buffer (pH 7.5), and a protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN, USA). Samples were then vortexed vigorously for one minute and incubated overnight at 4 °C. The following day, samples were centrifuged at 10,000 x g for 10 min at 4 °C and the supernatant was then transferred to a labeled tube. Samples were then diluted 1:10 with water and protein concentrations were measured as outlined above.

\[ pH \]

Iodoacetic acid was used to prepare muscle samples for pH according to Bendall (1973) with some modifications. Powdered muscle samples (100 mg) were lysed with 0.8 ml of 25 mM iodoacetic acid at 4 °C (pH 7 using 750 mM KCl buffer). Samples were then centrifuged at 13,000 x g for 5 min and then placed at 25 °C for an additional 5 min before pH was measured using Orion Ross Ultra pH electrode (Thermo Scientific, Pittsburg, PA).

\[ Glycolytic\ Intermediates \]

Muscle glycogen was measured from a separate aliquot of powdered 100 mg tissue to which one ml of 1.25 M HCl was added. Samples were then homogenized and placed at 90 °C.
for 2 h and centrifuged at 13,000 x g for 5 min. Supernatants were then transferred to a new tube and equal parts of 1.25 M KOH (Bergmeyer, 1984) were added and stored at -80 °C until further analysis. To measure glycogen, triethanolamine (TEA) buffer was added to the supernatant as described by Hammelman et al. (2003) and aliquoted into a 96 well plate and read for the Optical Density (OD) 1. To measure OD2, glucose-6-phosphate dehydrogenase, hexokinase, and ATP were added to the 96 well plate and allowed to incubate in solution for 15 min before reading. The total amount of glycogen was then measured by subtracting OD1 from OD2 values and compared to a standard curve.

Glucose-6-phosphate, and lactate were also measured using separate aliquots of 100 mg powdered tissue. Samples were homogenized in 1 M 4 °C perchloric acid and quickly placed on ice for 20 min and then centrifuged at 13,000 x g for 5 min. Supernatants were then carefully transferred to a new tube. Equal parts of 2 M KOH were added (Bergmeyer, 1984) to neutralize the samples and then were stored at -80 °C until further analysis. Buffer containing 200 mM Tris-hydrazine and 0.4 mM nicotinamide adenine dinucleotide was added and samples were pipetted on a 96 well plate and OD1 was recorded. Lactate dehydrogenase was added to each sample and allowed to incubate at 25 °C for 90 min before measuring OD 2. Lactate values were calculated by subtracting OD1 from OD2 and comparison to a standard curve (Bergmeyer, 1984; Hammelman et al., 2003). TEA buffer was added to the original supernatant and then pipetted into a 96 well plate to measure OD1. Glucose-6-phosphate dehydrogenase was then added to the samples and allowed to incubate at 25 °C for 15 min before reading OD2. To measure the final (OD3) reading, hexokinase and ATP was added and incubated for 15 min prior to measurement. Glucose-6-phosphate was calculated by subtracting OD2 from OD1 while glucose was calculated by subtracting OD2 from OD3 (Bergmeyer, 1984; Hammelman et al., 2003).
**Buffering Capacity**

Muscle buffering capacity was determined as described by Puolanne & Kivikari (2000) and Henckel, Jorgensen, & Jensen (1992) with slight modifications. Muscle samples (2 g) were ground using liquid nitrogen in a mortar and a pestle. Samples were homogenized at 1:10 (wt/vol) in a 5 mM iodoacetic acid at 4 °C and 150 Mm potassium chloride buffer (pH 7.0). Samples were equilibrated at 25 °C for 5 min. All samples were brought to a pH of 7.0 with the use of 5 M NaOH. All samples were titrated to pH 5.3 by adding 100 µl aliquots of 0.1 M HCl. Immediately following the addition of HCl samples were vortexed for two min and the pH was measured. Sample pH was evaluated using an Orion Ross Ultra pH glass electrode (Thermo Scientific, Pittsburg, PA, USA). Buffering capacity was calculated using the formula $BC = \frac{\Delta B}{\Delta \text{pH}}$ where $\Delta B$ is a constant expressed as µmol of HCl/g of tissue (for example, 100 µl x 0.1 M HCl = 10 µmol) and $\Delta \text{pH}$ is the change in pH from each measurement. Titration curves were displayed for every zone of each animal by plotting BC values against $\Delta \text{pH}$ using a third order polynomial.

**SDS-PAGE and Western Blotting**

All samples were prepared for western blotting by conducting protein analysis. After determining the protein content, samples were diluted with a solubilization buffer (Warren et al., 2003) and 0.05% bromophenol blue for a final concentration of 3 µg/ µl and 6 µg/ µl and placed at 90 °C for 5 min. Samples were diluted with water and Laemmlie buffer containing 0.5 M Tris HCL (pH 6.8), 0.5 M of DTT, 10% SDS (wt/vol), 0.5% Bromophenol blue (wt/vol), 50% glycerol (wt/vol) for a final concentration of 1 µg/ µl and placed at 90 °C for 5 min. Samples were then kept at – 80 °C until needed for western blotting, where they were thawed and placed at 90 °C for 5 min.
Muscle samples for gel electrophoresis were loaded into individual lanes using concentrations of 3 µg, 6 µg, 24 µg, and 36 µg protein for measurements of myoglobin ground tissue, lactate dehydrogenase (LDH), myoglobin in exudate, and succinate dehydrogenase subunit a (SDHa), respectively. Beta actin was used as a loading control for both forms of myoglobin and LDH, while alpha tubulin was used as a loading control for SDHa. Using methods adapted from Warren et al. (2003), SDS-PAGE was performed. Myoglobin in exudate was evaluated using a 15% acrylamide gels, while myoglobin determination in ground samples was performed using 12% gels. Finally, LDH and SDHa were determined using a 10% gel. Myoglobin in exudate, LDH, and SDHa were electrophoretically separated for 20 min at 60 V and 120 V for 2 h, myoglobin on ground samples were electrophoretically separated for 20 min at 60 V and 200 V for 1 h. After separation, proteins were transferred to nitrocellulose membranes using 45 V for 1 h. Myoglobin determination in the exudate, LDH, and SDHa were blocked in Prometheus™ OneBlock™ Blocking buffer for 1 h while myoglobin blots containing samples from ground tissue were blocked in the aforementioned buffer 2 h at room temperature. Blots for determining myoglobin in both the exudate and ground muscle were incubated with rabbit anti-myoglobin while blots for LDH were incubated with rabbit anti-LDH. LDH blots were incubated with primary antibodies for 1 h at room temperature while all other blots were incubated overnight at 4 °C. Blots were also incubated with anti β-actin and anti α- tubulin overnight. All blots were then washed three times with Tris Buffered Saline containing 0.1% tween-20 (1X TBS-T): 20mM Tris Base, 140mM sodium chloride. Primary antibody dilutions were: anti-myoglobin [(ground) 1:2000 and (exudate) 1:1000 ab77232, Abcam, Cambridge, MA, USA], anti-LDH (1:2000 NBP1-48336, Novus Biologicals LLC Littleton, CO, USA), and anti-SDHa (1:1000, ab14715, Abcam, Cambridge, MA, USA). Loading control antibodies were β-
actin (1:5000, NB600-503, Novus Biologicals LLC Littleton, CO, USA) and α- tubulin (1:5000, ab7291, Abcam, Cambridge, MA, USA). Finally, blots were then incubated with the appropriate IRDye 680- or 800-conjugated anti IgG or anti IgM secondary antibody (LICOR Biosciences, Lincoln, NE) for 1 h at room temperature. Final washes were conducted using 1X TBS-T. All blots were imaged and band intensity was determined using Odyssey Infrared Scanner (Li-Cor, Inc., Lincoln, NE, USA).

Statistical Analysis

Data were analyzed using the Fit Model in JMP (SAS Institute, Cary, NC, USA). The model was a two x four factorial design using the semimembranosus as a random effect but separated by the four zones and two time points. The least standard mean (LSM) of zone and time were determined using the Student’s t test with significant differences being that of P < 0.05. Buffering capacity graphs were plotted as a titration curve and calculated for each increment of acid. The values were plotted against the midpoint of each respective pair of pH values measured.

Results and Discussion

Color Analysis

Color data (Figure 2) reveal the more caudal end of the SM muscle (zones C and D) have higher L* values (P < 0.0001) and lower a* values (Figure 3; P < 0.0001) than the cranial portions (zones A and B) with no interaction between time and zone. Color data also show an increase in both L* and a* values with time postmortem. These findings are consistent with that reported by Stufft et al. (2017) and Armstrong et al. (1987) who also found that the muscles further away from the bone tend to be lighter in color compared to muscles that are redder
located next to the bone. Muscles that are associated with posture are more oxidative than those involved in motion (Totland and Kryvi, 1991; Henckel, 1992). In pigs, deeper muscles contain the highest amount of slow oxidative fibers compared to the outer muscles that tend to have higher glycolytic fibers (Armstrong et al. 1987). Similar results were found in the semitendinosus muscle in beef (Brandstetter et al., 1997). In this study of the pork SM muscle, these findings support that inherently the more distal the muscle is from the bone, the lighter the muscle appears. Not only are there differences in color between muscles, but also differences within the same muscle exist as well. Wilson et al. (1959) stated that color variation within a muscle is called “two-toning” and creates a nonuniform product that consumers find unsavory.

**Buffering Capacity**

To understand better the differences in color across the ham, buffering capacity was examined. Buffering capacity is defined as the ability of the muscle to resist a change in pH (Kivikari, 1996). The most important factors for determining buffering capacity at a pH range of 5.5-7.0 are phosphate compounds, dipeptides carnosine and anserine, and histidylimidazole from myofibrillar proteins (Kivikari, 1996). Lighter, more glycolytic muscles generally have greater buffering capacities than darker more oxidative muscles as more hydrogen ions are produced at the end of glycolysis ultimately lowering the pH, this white fibers need a more effective buffering capacity (Puolanne & Kivikari, 2000).

At a pH of 5.5, (Figure 4) zone D had a higher buffering capacity than the other zones and 1440 min had a greater buffering capacity than at 30 min (P < 0.0004). In regards to a pH of 6.0, similar results were observed (Figure 5). Zone D had the highest buffering capacity at 1440 min (P < 0.0001). Interestingly, at a pH 7, (Figure 6) zone D was second to that of zone B with the highest buffering capacity. Again, 1440 min had significantly higher values for buffering
capacity (P < 0.0001). When examining the buffering capacity across the pH of 5.5-7.0, (Figure 7) zone D had the greatest values and 1440 min had a significantly higher buffering capacity than 30 min (P < 0.0001). Overall, the titration curves showed zone D had higher numerical buffering capacities at both 30 min and 1440 min, though not significant (Figure 8).

In regards to the analysis of buffering capacity at a pH 7, this could be due to the method of raising the starting pH to 7.0 for all zones to calculate the buffering capacity. Data represented by zone supports previous findings of Puolanne and Kivikari (2000) and Kylä-Puhju et al. (2004) that more glycolytic muscles have higher buffering capacities compared to their oxidative counterparts. Puolanne and Kivikari (2000) collected m. longissimus (LD) and m. triceps brachii (TB) muscles from ten porcine and ten bovine carcasses one day after slaughter. Experiments were based on varying levels of titration amounts and discovered that mainly the dilution amounts had significant effects on the shape of the curve. From their findings, they stated that light muscles have better buffering capacities than dark muscles as they are composed of primarily white muscle fibers that contain glycolytic enzymes. The end product of glycolysis is lactate and hydrogen ions which ultimately lowers the pH allowing white fibers to have a more efficient buffering mechanism than that of red muscle fibers. In another study conducted by Kylä-Puhju et al. (2004), they collected muscles from Finnish Landrace and Large White pigs. Samples were collected from light gluteus superficialis, longissimus dorsi, semimembranosus muscles and from dark infraspinitus and masseter muscles. Samples were taken within 40 min after slaughter and then again at 24 h postmortem. To measure buffering capacity, each muscle’s buffering capacity was calculated based on lactate concentrations between the first and second sampling periods. Results showed similar values of the light muscles with those seen in Puolanne & Kivikari (2000). In comparison to the light and dark muscles, even though small, there was a
noticeable difference in buffering capacity. There has been studies on buffering capacity that tested varying levels of titration and dilution amounts, but few studies have compared changes in buffering capacity from early postmortem (30 min) to that of 1440 min within the same muscle.

Muscle pH

As muscle converts to meat, anaerobic glycolysis creates H\(^+\) ions. Postmortem metabolism fails to remove of waste products so the H\(^+\) accumulate in the muscle tissue and lowers the overall pH. The rate and extent of pH decline greatly influences the quality of meat (Sheffler & Gerrard, 2007). In normal cases, pH of a live animal is around 7.4 and gradually declines postmortem to a pH of 5.6-5.7 within the first 8 h. At 1440 min or 24 h, the ultimate pH is attained, usually ranging from 5.3-5.7 (Briskey & Wisemer-Penderson, 1961). The quality of meat is significantly altered if the pH declines very dramatically or slowly or if the ultimate pH is very low or high (Bendal & Swatland, 1988).

During this study, the pH was not different across zones (Figure 9), at a pH of about 6.0, even though there were significant differences in color. Obviously, there was a difference when comparing 30 min samples to that of 1440 min (P < 0.0001) as 30 min had a pH of about 6.3 and declined to an ultimate pH of about 5.7. Because the pH values did not differ, values did not correlate with differences in tissue or meat color. As there is a difference in color, the assumption could be made that one would expect to see a change in pH amongst zones. This phenomenon could be related to the buffering capacity measured on each zone and time. The buffering capacity for each zone remained constant for the majority of pH measured. As buffering capacity is the ability of meat to resist a change in pH, and buffering capacity was the same amongst zones, which could mean that each zone was able to resist the changes in pH during the early
stage of postmortem. In addition, having the same pH amongst zones can lead to the assumption that each zone can have similar metabolic rates as well muscle fiber type capabilities.

**Metabolites**

Creation of energy in living muscle is derived through two major types of metabolisms: oxidative and glycolytic (Poso & Puolanne, 2005). Oxidative-based metabolism utilizes oxygen and is the most efficient means of powering muscles, at least in slow-contracting, myoglobin reach muscles. Glycolytic metabolism, on the other hand, is better designed for generating high levels of ATP over a short period of time and is generally found in faster contracting, powerful muscles lacking excessive oxygen and myoglobin (Scheffler & Gerrard, 2007). Through the process of glycolysis, glycogen, which is a major energy source in muscle and a polymer of glucose, is converted into lactate and a hydrogen ion. In a living animals, lactate is then converted back to glucose in the liver or pyruvate is oxidized in the tricarboxylate acid cycle (Poso & Puolanne, 2005). After an animal is exsanguinated, the muscle lacks the oxygen that is needed for oxidation and must go through glycolysis. Furthermore, circulatory failure destroys the ability of the muscle to remove lactate and hydrogen from the tissue and pH declines. Moreover, heat accumulates in the tissue, delaying the decrease in temperature decline of the tissues postmortem (Ryu, Choi, & Kim, 2005). Thus, the rate and extent of pH decline has major influences on quality, in addition, glycogen and lactate concentrations also have an influence on quality (Scheffler & Gerrard, 2007).

Glycogen, glucose, glucose-6-phosphate, and lactate were measured on all zones at both time points to measure the ability of the muscle to go through glycolysis. Based on color, more glycolytic fibers are subject to have higher concentrations of initial glycogen. Glycogen showed an ascending trend from the cranial to caudal end but the significant differences were not evident
(Figure 10), though 1440 min had significantly lower amounts of glycogen when compared to that of initial sampling (P < 0.0001). There was no significant difference when measuring glucose concentrations in either zone or time (Figure 11). Glucose-6-phosphate showed a similar trend across the SM (Figure 12) but only differences in time were evident. Lactate production, an indicator of glycolytic flux, should correlate with the amount of pH produced (Scheffler & Gerrard, 2007). As there was no significant differences in pH between zones, there were no differences in lactate production (Figure 13). As there is no difference in lactate and pH, it is interesting to see trends in glycogen production and significant differences in color.

In an attempt to understand the aforementioned disparity in color, pH and metabolites, we correlated pH and lactate (Figure 14). Using this approach, these data showed a linear relationship exists between pH at 30 min and tissue lactate abundance; however, at 1440 min was quite different. We have observed this type of relationship in past studies (Matarneh et al., 2015). What is unique about this finding is that there is a more linear relationship when comparing lactate and pH during early postmortem periods, whereas the relationship weakens in the latter stages postmortem. Buffering capacity, glycogen, glucose, and color data show that zone D is lighter in color and more glycolytic. As there is no differences in lactate and pH data, there are differences in buffering capacity, color, and intermediates of glycolysis. In normal cases, it is safe to assume that a color difference can be caused by changes in pH, lactate, muscle fiber type, and varying amounts of substrates in glycolysis; however, in this study, we see a color difference but no significant differences elsewhere. From this finding, we can conclude that during the early stages of postmortem glycolysis, lactate and pH are very closely correlated but as postmortem time increases that relationship becomes weak. At some point during postmortem metabolism, the relationship of pH becomes disrupted and weakened possibly from lactate or hydrogen ions.
coming from somewhere other than anaerobic metabolism. To try and better understand this phenomenon, glycolytic potential was measured on each zone by time.

**Glycolytic Potential**

Glycolytic potential is an estimate of the total flux possible for glycolysis during the postmortem period. This estimate measures glycolytic substrates, intermediates and final lactate concentrations (Monin & Sellier, 1985). This equation is based on the assumption that all glycogen and intermediates in glycolysis are converted to the end substrate of lactate. Glycolytic potential is useful means of normalize muscle tissues for sampling times postmortem. However, studying the initial starting glycogen amounts against the final 24 h (1440 min samples) lactate accumulations may lead to a better understanding of glycolytic flux. By taking 30 min and 1440 min samples in this study, the amount of glycogen and lactate at both early and final stages of postmortem can calculated and compared to the glycolytic potential for accuracy of the estimate.

When calculating glycolytic potential on a lactate basis (Figure 15), there is a notable numeric trend from the redder portion, zone A, to the whiter portion of the muscle, zone D. This follows the same trend observed in glycogen and glucose-6-phosphate measurements. When comparing 30 min and 1440 min glycolytic potential on a lactate basis, 1440 min had significantly higher glycolytic potential than that of 30 min (P < 0.0006). Copenhafer et al. (2006) stated that glycolytic potential is used an indicator for the potential of muscle to go through glycolysis. More oxidative fibers convert glycogen to lactate at slower rates than glycolytic fibers (VØlllestad, Tabata, & MedbØ, 1992) ultimately increasing the ultimate pH (Scheffler & Gerrard, 2007). Glycolytic fibers have faster glycolytic rates, which increases the rate at which substrates are broken down increasing the accumulation of lactate that drops the pH at a faster rate (Karlsson et al., 1999). Lactate and pH directly reflect one another in this study, as
they should (Scheffler & Gerrard, 2007). Again, glycolytic potential is based solely on the assumption that all glycogen and intermediates are converted to lactate, which is not always the case. Scheffler et al. (2015) stated that when glycogen is not limited pH values may plateau even though there are available substrates, influencing the amount of lactate accumulation. From the data collected at both 30 min and 1440 min one can conclude that the equation used for glycolytic potential is not completely accurate but strictly used as an estimated predicator for glycolytic potential.

**Myoglobin**

Meat color can be influenced by many factors, including age and muscle type and both are influenced by the amount of myoglobin present. Myoglobin’s function is to store and deliver oxygen in the muscle (Joo et al., 2013). Other factors that are important in fresh meat color is the amount of drip loss and purge, which is lost during further manipulations and storage. Both of these are influenced by the water holding capacity and water holding capacity is related to color by its loss in myoglobin and reflectance on the face of the meat (Joo et al., 1995). Myoglobin concentrations from both the exudate and ground samples from all zones were quantified using western blotting techniques. The exudate and ground muscle samples that were analyzed for myoglobin concentrations showed similar trends as that of color values. The more cranial portion of the SM muscle had higher concentrations of myoglobin and descended when moving towards the more caudal portion of the muscle (Figure 16). Myoglobin concentrations on ground tissue were also examined over time and showed a slight decrease in concentration (Figure 17). The myoglobin concentrations across zones are not significant; however, they follow the same trends presented in the color data showing that zone A was darker in color and had more myoglobin and
descended moving caudally towards zone D having a lighter color and less myoglobin present in both the exudate and ground tissue.

Oxidative Composition

In this study, there were significant differences in color but interestingly while examining muscle fiber types there were no differences between zones with SDH or LDH. This suggests that the metabolic capacity for each zone and abundance of muscle fiber type are the same, which correlates with the results found in metabolites and glycolytic potential. To explain muscle fiber types further, studies have shown that variation in meat quality is not only seen between animals but within the same muscle as well (Karlsson et al., 1999). Skeletal muscle characteristics can strongly influence energy metabolism in both living and postmortem tissue (Monin & Quali, 1991). Studies have shown that there are no identical skeletal muscles. Within the same muscle, there can be differences in muscle fibers between species and strains (Karlsson et al., 1999). Muscle fibers are classified by speed of contraction and primary type energy metabolism (Schiaffino et al., 1989). One oxidative enzyme that is more abundant in slower contracting red muscles is succinate dehydrogenase (SDH) (Klont, Brocks, & Eikelenboom, 1998). SDH is a citric acid cycle enzyme that is membrane bound iron-sulfur flavoprotein (Hederstedt, 1986). SDH catalyzes the oxidation of succinate to fumarate with aerobic cells that are directly related to the respiratory chain. SDH was quantified using western blotting techniques (Figure 19). Despite the significant differences observed in color data, SDH had no significant differences between zones or time points.

One glycolytic enzyme is lactate dehydrogenase (LDH). LDH is water soluble and converts pyruvate to lactate at the end of glycolysis (Savage et al., 1990). LDH has the ability to increase its relationship with the actin filament during such cases of extreme glycolysis (Clarke...
et al., 1980). As a predictor that zone D would have more glycolytic tendencies, zone D should have a higher amount of LDH. To determine the proportions of LDH in each zone, proteins were separated and subjected to western blotting techniques (Figure 18). Again, despite the differences in color, there was no significant differences between zones or time points measured for LDH. Stufft et al (2017) also examined LDH concentrations on the porcine semimembranosus muscle at 24 h on cases of normal, PSE, and DFD. Their findings showed no significant differences amongst zones as well. Leading the assumption that as there were no significant differences across the face of the muscle, meaning that there is no difference in metabolic capacity between zones or time, indicating that the each zone has the same potential to reach the same ultimate pH.

**Conclusion**

The SM muscle in pork hams varies in color. Specifically, the more cranial aspects of the muscle are darker than the more caudal regions. Postmortem metabolism has a profound impact on fresh pork quality development, yet the underlying metabolism does not explain differences in color across this muscle. Underlying muscle characteristics also impact meat quality, after all myoglobin is the major pigment responsible for meat color. Myoglobin, though not significant, descended from the cranial portion of zone A to the caudal portion of zone D. This finding supports the more caudal zone has more glycolytic characteristics than that of the cranial zone. From collecting samples at 30 min and that of 1440 min, one can conclude that color differences observed across the semimembranosus muscle is not solely caused by postmortem metabolism but relies heavily on inherent characteristics of the muscle itself.
Figure 1: Sampling locations – taken from the cut surface of the porcine *semimembranosus* muscle. The dotted lines separate zones A, B, and C, while the sampling took place in the gray area of each zone.
Figure 2: Lightness ($L^*$) Minolta Colorimeter Data – separated by (A) representing zone ($P < 0.0001$) and (B) representing time ($P < 0.0001$) from the cut surface of the SM muscle. Bars with letters or asterisk represent significant differences.
Figure 3: Redness (a*) Minolta Colorimeter Data – separated by (A) representing zone (P < 0.0001) and (B) representing time (P < 0.0001) from the cut surface of the SM muscle. Bars with letters or asterisk represent significant differences.
Figure 4: Buffering Capacity pH 5.5 – separated by (A) representing zone (P = 0.7450) and (B) representing time (P < 0.0004) from the cut surface of the SM muscle. Bars with letters or asterisk represent significant differences.
Figure 5: Buffering Capacity pH 6.0 – separated by (A) representing zone (P < 0.0359) and (B) representing time (P < 0.0001) from the cut surface of the SM muscle. Bars with letters or asterisk represent significant differences.
Figure 6: Buffering Capacity pH 7.0 – separated by (A) representing zone (P = 0.9798) and (B) representing time (P < 0.0001) from the cut surface of the SM muscle. Bars with letters or asterisk represent significant differences.
Figure 7: Buffering Capacity pH 5.5 – 7.0 – separated by (A) representing zone (P = 0.5034) and (B) representing time (P < 0.0001) from the cut surface of the SM muscle. Bars with letters or asterisk represent significant differences.
Figure 8: Buffering Capacity Titration Curve – separated by (A) representing 30 min and (B) representing 1440 min. Titration curves represents each zone based on the legend.
Figure 9: Ultimate pH – separated by (A) representing zone (P = 0.9777) and (B) representing time (P < 0.0001) from the cut surface of the SM muscle. Bars with letters or asterisk represent significant differences.
Figure 10: Glycogen – separated by (A) representing zone ($P = 0.081$) and (B) representing time ($P < 0.0001$) from the cut surface of the SM muscle. Bars with letters or asterisk represent significant differences.
Figure 11: Glucose – separated by (A) representing zone ($P = 0.7858$) and (B) representing time ($P = 0.6152$) from the cut surface of the SM muscle. Bars with letters or asterisk represent significant differences.
Figure 12: Glucose-6-phosphate – separated by (A) representing zone (P = 0.1657) and (B) representing time (P < 0.0001) from the cut surface of the SM muscle. Bars with letters or asterisk represent significant differences.
Figure 13: Lactate – separated by (A) representing zone (P = 0.9884) and (B) representing time (P < 0.0001) from the cut surface of the SM muscle. Bars with letters or asterisk represent significant differences.
Figure 14: Linear Relationship between pH and Lactate – separated by (A) representing 30 min ($P < 0.0001$) and (B) representing 1440 min ($P < 0.0015$).
Figure 15: Glycolytic Potential – separated by (A) representing zone ($P = 0.0746$) and (B) representing time ($P < 0.0006$) from the cut surface of the SM muscle. Bars with letters or asterisk represent significant differences.
Figure 16: Exudate Percentage and Myoglobin Concentrations – separated by (A) representing the amount of exudate as a percentage by zone (P = 0.6076) and (B) representing the amount of myoglobin in exudate by zone (P = 0.8251) from the cut surface of the SM muscle using Western Blot techniques. Blot (C) shows the intensity of bands. Lanes are marked with their zone they represent. Bars with letters or asterisks represent significant differences.
Figure 17: Ground Tissue Myoglobin Concentrations – separated by (A) representing zone ($P = 0.0737$) and (B) representing time ($P = 0.9247$) from the cut surface of the SM muscle using Western Blot techniques. Blot (C) shows the intensity of bands. Lanes are marked with their zone and time they represent. Bars with letters or asterisk represent significant differences.
**Figure 18: Lactate Dehydrogenase Concentrations** – separated by (A) representing zone (P = 0.9288) and (B) representing time (P = 0.6914) from the cut surface of the SM muscle using Western Blot techniques. Blot (C) shows the intensity of bands. Lanes are marked with their zone and time they represent. Bars with letters or asterisk represent significant differences.
**Figure 19: Succinate Dehydrogenase Concentrations** – separated by (A) representing zone (P = 0.9973) and (B) representing time (P = 0.1774) from the cut surface of the SM muscle using Western Blot techniques. Blot (C) shows the intensity of bands. Lanes are marked with their zone and time they represent. Bars with letters or asterisk represent significant differences.
References


