This is to certify that the
thesis entitled

RELATIONSHIP BETWEEN GLYCOLYTIC OSCILLATIONS AND PORK COLOR AND WATER-HOLDING CAPACITY

presented by

NICHOLAS L. BERRY

has been accepted towards fulfillment
of the requirements for the

M.S. degree in Animal Science

Matthew E. Doumit
Major Professor's Signature

12-9-03
Date

RELATIONSHIP BETWEEN GLYCOLYTIC OSCILLATIONS AND PORK COLOR AND WATER-HOLDING CAPACITY

By

Nicholas L. Berry

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Animal Science

2003

ABSTRACT

RELATIONSHIP BETWEEN GLYCOLYTIC OSCILLATIONS AND PORK COLOR AND WATER-HOLDING CAPACITY

By Nicholas L. Berry

The objective of this study was to determine if differences exist in the pattern of glycolysis in muscle extracts from superior and inferior quality pork loins. Characteristics of superior (n=6) and inferior (n=6) quality loins, respectively, were as follows: 45 min pH (6.40 ± 0.06 vs 5.92 ± 0.10), percent fluid loss after centrifugation (10.22 ± 0.47 vs 20.70 ± 0.32), percent drip-loss by the suspension method (0.66 ± 0.08 vs 3.23 ± 0.45), and day 1 L* (51.37 ± 0.66 vs 56.68 ± 0.86). Longissimus muscle samples were obtained at 20 min postmortem and were immediately frozen in liquid nitrogen and stored at -80° C until analysis. Samples were homogenized in 90 mM potassium phosphate, pH 6.5, and 180 mM potassium chloride. A high-speed supernatant fluid (85,000 x g) was gel-filtered using Sephadex G-25 resin. Gel-filtered muscle proteins were used to determine *in vitro* patterns of glycolysis. Aliquots of glycolytic reaction mixture were removed every 2 min for 46 min and acidified to halt enzyme activity. Enzymatic assays were used to quantify concentrations of ADP, ATP, and lactate. An oscillatory pattern of glycolysis was observed using extracts from both superior and inferior quality pork. No differences in the average ATP:ADP ratio or the overall mean concentrations of lactate or adenine nucleotides were observed in reactions using extracts from superior and inferior quality samples ($P > 0.05$). Thus, sarcoplasmic protein extracts do not appear to produce distinct patterns of glycolysis that are associated with differences in pork quality. However, this system will permit identification of specific metabolites that cause differences in the pattern of postmortem glycolysis.

ACKNOWLEDGEMENTS

I would like to begin my acknowledgements by thanking my friend and major professor, Dr. Matt Doumit. I have truly enjoyed the opportunity to further my education under your guidance at Michigan State. Thank you very much for being patient and for giving me the chance to prove myself as a student, a researcher, and a person.

Thank you Dr. Al Booren, Dr. Gretchen Hill, and Dr. Gale Strasburg for serving on my committee. I greatly appreciate your guidance and support throughout my stay at MSU. I have enjoyed getting to know each of you, not only as researchers, but more importantly as friends.

Thank you Chuck Allison and Emily Helman for not only your time and hard work, but also your friendship. To Chuck, I am greatly appreciative of your efforts to improve my skills as a researcher. You have been a great role model in the lab, as well as an excellent friend to me throughout my time in East Lansing. To Emily, I am greatly indebted to you for your time and patience while developing my skills in the lab. You have been an excellent teacher and friend throughout my stay at MSU.

I would also like to extend my thanks to the undergraduates that have worked in the Doumit lab throughout the completion of my project. I have enjoyed your friendship, and am greatly appreciative of the time and effort you have put forth.

And last, but certainly not least, thanks to all of my friends and family. To my parents, thank you for encouraging me to always pursue my dreams and for instilling in me the value of hard work and persistence. To Kohl Schrader, thank you for putting up with me after long hours in the lab, and for always putting a smile on my face. I'm greatly appreciative of all your support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADP - Adenosine Diphosphate

AMP - Adenosine Monophosphate

APS - Ammonium Persulphate

ATP - Adenosine Triphosphate

CWHC - Centrifugal Water Holding Capacity

DFD - Dark, Firm and Dry

DRIP1 - Suspension Drip Measured from 24 to 48 hours postmortem

EDTA - Ethylenediaminetetraacetic Acid

G-6-P - Glucose-6-Phosphate

GTP - Guanosine Triphosphate

HCl - Hydrochloric Acid

HK - Hexokinase

KOH - Potassium Hydroxide

L1 - Day 1 L*

LM - Longissimus Muscle

MSU - Michigan State University

NAD - Nicotinamide Adenine Dinucleotide

NADH - Nicotinamide Adenine Dinucleotide, reduced form

NPPC - National Pork Producer's Council

P_i - Inorganic Phosphate

PFK - Phosphofructokinase

PK - Pyruvate Kinase

PMSF - Phenylmethylsulfonylfluoride

PSE - Pale, Soft and Exudative

PSS - Porcine Stress Syndrome

RFN - Red, Firm and Non-exudative

RN⁻ - Rendement Napole

RYR1 – Skeletal Muscle Ryanodine Receptor

SDS - Sodium Dodecyl Sulfate

SDS-PAGE - Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

SR - Sarcoplasmic Reticulum

TEMED - N,N,N',N'- Tetramethylenediamine

WHC - Water-holding Capacity

INTRODUCTION

Today's U.S. pork industry is experiencing phenomenal growth as it continues to meet consumer demand. Pork is the most widely eaten meat worldwide. Consumption of pork in the United States has proven to be a valuable protein source as Americans consume 66 lbs/person/year on a carcass disappearance basis (USDA, 2002 Agricultural Statistics). Pork production in the United States is a vital part of the economy. Nearly 19 billion pounds of pork, with a retail value of $38 billion, was processed from about 97 million hogs in 2001. The U.S. pork industry is responsible for over $72 billion in total domestic economic activity. In addition, the pork industry adds over $27 billion of value to basic production inputs such as corn and soybeans (NPPC, 2003).

Currently, the United States is one of the world's leading pork-producing countries and is second to only Canada as the largest exporter in the world. Pork production in the U.S. accounts for about 10% of the world's supply. Additionally, there are approximately 85,760 pork operations today, as compared to nearly three million in the 1950s (NPPC, 2003). The aforementioned statistics mirror the changes seen within the industry, and illustrate the emphasis placed on production and efficiency. Subsequently, the emphasis placed on lean, fast growing pigs has compromised pork quality. Incidence of pale, soft, and exudative (PSE) pork has risen from 10% to 15.5% between 1996 and 2003 (Cannon et al., 1996; Stetzer and McKeith, 2003). Additionally, inferior quality problems cost the industry an estimated $90 million annually (Stetzer and McKeith, 2003).

Developing a thorough understanding of the biochemical processes that are integral in explaining variation observed in ultimate pork quality is of utmost concern.

The objective of this study is to determine if differences exist in the pattern of glycolysis in muscle extracts from superior and inferior quality pork loins. Collectively, these data will provide a better understanding of glycolytic behavior during the postmortem time period. Moreover, this system will allow for identification of specific regulators that cause differences in the pattern of postmortem glycolysis. This knowledge could lead to manipulation of live pigs or carcasses by altering events that influence postmortem energy utilization.

LITERATURE REVIEW

Pork Quality

Red, firm and non-exudative (RFN) pork is considered to be the ideal pork product desired by consumers based on both color and textural attributes. The National Pork Producers Council (NPPC) Pork Quality Solutions Team established pork quality targets in order to define the ideal quality benchmark (NPPC, 1998). The acceptable subjective color score ranges from 3 to 5 on a 6 point scale (1 = pale pinkish gray to white and 6 = dark purplish red). Acceptable lightness (L*) is measured objectively using a Minolta colorimeter and ranges from 37 to 49. Ultimate pH should be between 5.6 and 5.9. Additionally, ideal pork should have less than 2.5% drip loss when measured at 24 hours postmortem (NPPC, 1998). RFN pork exhibits the aforementioned attributes desired by the quality benchmarking system.

A limited pH decline with the onset of rigor mortis at a relatively high pH ($\geq$ 6.0) will produce meat exhibiting a dark red color, a firm texture and a dry appearance. Dark, firm and dry (DFD) pork is often associated with long-term stress (i.e. prolonged injury, illness, or extended hauling) that results in depletion of muscle glycogen prior to harvest. Subsequently, less lactate and H^+ ions accumulate, and the ultimate pH is higher ($\geq$ 6.0). Because of this higher pH, proteins in DFD meat have a relatively high net negative protein charge and undergo minimal protein denaturation (Briskey et al., 1966). These factors result in relatively high water-holding capacity (WHC) due to greater repulsion between myofilaments and greater protein/water interaction compared to RFN or PSE pork. However, the higher ultimate pH of DFD pork makes this product more susceptible to spoilage by bacteria and subsequently shortens shelf life. In addition, many consumers

discriminate against DFD pork due to its unacceptable dark purple color (Kauffman et al., 1992).

Pale, soft and exudative pork is characterized by its pale color and inferior WHC. The product is very watery in appearance and flaccid in texture. As selection for leaner, heavier weight pigs with maximized muscularity has occurred, so has the increased occurrence of PSE pork (Lonergan et al., 2001). A survey consisting of fourteen packing plants revealed that approximately 16% of pork produced exhibited PSE characteristics (Kauffman et al., 1992). The Pork Quality Chain Audit (Cannon et al., 1996) and the National Pork Producers Council (NPPC 1991), also found that greater than 10% of all pork carcasses generated in the U.S. contained PSE meat. A loss of thirty-five cents per carcass is attributable to WHC problems associated with PSE (Cannon et al., 1996). Thus, PSE costs the pork industry millions of dollars every year (Stetzer and McKeith, 2003).

PSE pork characteristics are hard to induce in pigs that are less susceptible to stress suggesting that there is a complex physiological and genetic difference among genetic lines that causes some pigs to have a greater predisposition to develop an abnormal pH decline (Gerrard, 1997). Muscles considered to be PSE generally have significantly lower pH values and higher muscle temperatures at forty minutes postmortem (Briskey et al., 1959). Approximately 20% of the sarcoplasmic and myofibrillar proteins are denatured as a result of rapid pH decline accompanied by high carcass temperatures (Honikel and Kim, 1986). Increased lightness has been associated with reduced sarcoplasmic protein solubility, and to a lesser extent myofibrillar protein solubility (Joo et al., 1999). Phosphorylase, creatine kinase, triose phosphate isomerase,

and myokinase are sarcoplasmic proteins shown to precipitate onto myofibrils in PSE pork (Joo et al., 1999). Reduced WHC has been attributed to denaturation of sarcoplasmic (Joo et al., 1999; Wilson and van Laack, 1999) and myofibrillar proteins (Offer, 1991; Warner et al., 1997). Breaks in the cell membrane through which fluid can quickly exude from the cell are also a characteristic of PSE muscle (Honikel and Kim, 1986).

The most undesirable aspect of PSE pork is the reduced WHC. The inability of meat to bind water greatly reduces the opportunities the packer has to enhance the product and has a detrimental effect on consumer acceptability. For example, during storage PSE hams lose three-fold more moisture than normal hams and seven-fold more moisture than DFD hams (Kauffman et al., 1978). Moisture losses during chilling, as well as during curing and smoking, are higher in PSE hams than in normal or DFD hams. Ham and shoulder cuts that are severely PSE exhibit 33% and 11% lower WHC, respectively, when compared with normal hams and shoulders (Cannon et al., 1995).

Kauffman et al., (1999) subjected eight DFD pork loins and eight PSE pork loins to a series of objective and subjective measures to demonstrate meat quality. When compared to PSE loins, DFD loins averaged 1.5 units higher ultimate pH, 4.7% less drip loss and 13.6% more bound water. Additionally, DFD loins were darker, firmer and more tender. Not all of the previously mentioned attributes allow for optimum quality of pork. DFD meat is a prime target for bacterial problems as many of the species that grow on meat survive within a pH range of 5.0 to 8.0, with optimum growth occurring around pH 7.0 (Cannon et al., 1995). As an industry, our responsibility is to maximize the

quality of the final meat product purchased by the consumer. This goal can be achieved with a further understanding of biochemical events that occur within the muscle tissue.

Genetic Basis for PSE Pork

Porcine stress syndrome (PSS), also referred to as malignant hyperthermia, is a genetic abnormality characterized by the inability of the pig to adapt to stressors. Fujii et al. (1991) demonstrated that PSS was associated with a genetic mutation (HAL-1843) in the muscle ryanodine receptor (RYR1; calcium-release channel), which was associated with abnormal leakage of calcium from the channel. Rapid ATP utilization and glycolysis, such as that induced by excessive calcium release through defective RYR1, are often associated with development of PSE pork (Honikel et al., 1986). Stimulation of muscle contraction, sarcoplasmic reticulum (SR) calcium ATPase (calcium pump) activity, and glycolysis by sarcoplasmic calcium results in heat production and muscle pH decline. Consequently, the combination of low pH and high temperature early postmortem results in a greater occurrence of protein denaturation in pigs with this condition as compared to stress-resistant genotypes (Briskey et al., 1966; Louis et al., 1993).

Based on the findings of Fujii et al. (1991), a DNA-based test was established that could distinguish between homozygous carrier, heterozygous carrier, and homozygous normal pigs with respect to the HAL-1843 mutation. This genetic test largely replaced halothane gas testing of swine, and efforts were made by the National Pork Board to eliminate the HAL-1843 mutation from commercial populations. Nonetheless, Murray and Johnson (1998) reported that of a population of 1006 pigs in two packing plants, 90% of the PSE condition was caused by factors other than the HAL-1843 gene.

The rendement napole (RN-) gene was recently identified as a defect in the AMP-activated protein kinase (Milan et al., 2000). The dominant RN- allele results in higher than normal muscle glycogen stores and an extended postmortem pH decline that leads to pork with a lower than normal ultimate meat pH, higher reflectance (lighter meat color), reduced WHC, and dramatically reduced carcass yield (LeRoy et al., 2000). Monin and Sellier (1985) referred to this condition as the Hampshire effect, due to its prevalence in the Hampshire breed. Other factors, such as enzyme stability at lower pH, must play a vital role in the continuation of glycolytic metabolism in these animals.

Biochemical and Physical Aspects of PSE Pork

Several factors influence the WHC of pork, including myofibrillar lattice spacing, cytoskeletal links, membrane permeability, and the size of fluid channels in the extracellular space (Purslow et al., 2001; Honikel, 2002). Development of PSE pork characteristics can result from a rapid antemortem and/or postmortem pH decline, or an extended postmortem pH decline. The rapid drop in pH in PSE muscles within the first hour after slaughter is influenced by a high ATP turnover. The increased turnover in ATP is attributable to the release of Ca^{2+} ions that induce muscle contraction, and subsequently ATP utilization. Glycogenolysis is also stimulated by conversion of phosphorylase b into phosphorylase a via phosphoylase kinase, which is activated by Ca^{2+} ions (Honikel, et al., 1986). Anaerobic glycolysis results in production of ATP, lactate and hydrogen ions. Hydrogen ion accumulation lowers the muscle pH. Relatively low pH combined with high muscle temperature during the early postmortem period, causes denaturation and reduced solubility of sarcoplasmic proteins (Sayre and Briskey, 1963; Scopes, 1964; Joo et al., 1999) and myosin (Offer, 1991; Warner et al., 1997).

Pale color and reduced WHC associated with PSE pork have been primarily attributed to denaturation of sarcoplasmic and myofibrillar proteins, respectively (Joo et al., 1999). However, Wilson and van Laack (1999) demonstrated that when myofibrils from either PSE or normal pork were combined with sarcoplasmic extract from PSE meat, the water-holding capacity of the myofibrils was lower than when combined with extract from normal pork. These authors concluded that sarcoplasmic proteins also influence WHC, through as yet undefined mechanisms. One possibility is that denatured sarcoplasmic proteins adsorb onto the surface of myofibrils, thereby shielding the charged groups available for fluid binding (Bendall and Wismer-Pedersen, 1962; Boles et al., 1992).

Glycogen Storage in Skeletal Muscle

Glycogen is the storage form of glucose within skeletal muscle. Glycogen is made up of α1-4 and α1-6 linkages of glucose molecules. The synthesis of glycogen involves a protein primer, glycogenin, which acts as a hexosyltransferase (Alonso et al., 1995). Glycogen synthesis begins as the amino acid tyrosine-194 on the glycogenin residue and becomes autocatalytically glucosylated by the addition of UDP-glucose into the system (Lomako et al., 1993). On average, seven other glucose residues are added to tyrosine-194 in the same manner. These eight glucose residues become the primer for the synthesis of proglycogen. Proglycogen is a stable intermediate for both the synthesis and breakdown of glycogen (Alonso et al., 1995). This stable intermediate is formed by the addition of proglycogen synthase and branching enzyme to the fully glycosylated glycogenin molecule. Proglycogen is then converted to macroglycogen, the storage form of glucose, by using macroglycogen synthase and branching enzyme. This reaction is

reversible, allowing for the breakdown of macroglycogen into proglycogen by use of phosphorylase and debranching enzyme (Lomako et al., 1993). In terms of metabolic activity, the larger macroglycogen (10^7 D) is inactive while proglycogen (400,000 D) is an active intermediate (Alonso et al., 1995). In order for glycogen to be used in metabolism, macroglycogen must first be converted into proglycogen.

The implication of this glycogen storage mechanism is that glycogen storage may slow down the rate of glycolysis by storing more glucose as the inactive macroglycogen as opposed to the metabolically active proglycogen. In a study done by Huang and others (1999), it was observed that when rats were exposed to minimal levels of stress, proglycogen was exclusively depleted and was also the only molecule that underwent synthesis and degradation when the rate of glycogen turnover was low. As the stress levels increased, macroglycogen was recruited in glycogen turnover. This study also observed that injecting high levels (greater than 10 mU/min^{-1}) of the hormone insulin promoted the storage of glucose in the form of macroglycogen as opposed to proglycogen.

Anaerobic Metabolism of Glucose

Glycolysis is an important pathway for providing energy from the stepwise degradation of glucose. It is primarily an anaerobic form of metabolism and its use in living animals is to produce short-term energy when oxygen is limiting to the organism. Glycolysis consists of two phases (Garrett and Grisham, 1999). The first phase is a series of five reactions in which glucose is broken down to two molecules of glyceraldehyde-3-phosphate. This phase consumes 2 molecules of ATP, or 1 molecule of ATP if glycogen is utilized as substrate. The second phase converts the two molecules of glyceraldehyde-

3-phosphate into two molecules of pyruvate. The second phase results in a net gain of four ATP, thus glycolysis results in a net gain of two or three ATP, depending on the source of glucose.

The conversion of muscle to meat begins with exsanguination. Biochemical reactions that occur following exsanguination are largely responsible for the ultimate quality of meat. Loss of blood ceases the exchange of oxygen between the circulatory system and the muscle tissue. As oxygen is depleted, a shift from aerobic metabolism to anaerobic metabolism occurs to meet the demands for postmortem ATP utilization. Anaerobic metabolism results in a less efficient production of ATP and accumulation of lactate and hydrogen ions, the end products of anaerobic glycolysis (Goll et al., 1984). In animal tissues that are experiencing anaerobic conditions, the two molecules of pyruvate that are produced by glycolysis are reduced to lactate by lactate dehydrogenase. Accumulation of hydrogen ions results in the reduction in pH of pre-rigor muscle as muscle is converted to meat. The rate of pH decline is related to the temperature of the muscle. A higher muscle temperature will result in a faster rate of pH decline (Sayre and Briskey, 1963), whereas a lower muscle temperature will result in a slower rate of pH decline. Once the ATP is completely consumed, and no more is being produced, permanent crossbridges are formed between myosin and actin to create actomyosin. This condition is termed rigor mortis.

Postmortem ATP-utilization

The major sites of ATP utilization are the myofibrillar (myosin) ATPase, which is activated during muscle contraction, and the calcium ATPase pump that functions to resequester calcium into the sarcoplasmic reticulum (Bechtel and Best, 1985). Creatine

phosphate is readily available for regeneration of ATP via a reaction catalyzed by the enzyme, creatine phosphokinase. Generation of ATP also occurs by a reaction catalyzed by myokinase, which converts two molecules of ADP to ATP and AMP (Bechtel and Best, 1985). These reactions may have a sparing effect on the rate of postmortem glycolysis. However, both myokinase and creatine phosphokinase are susceptible to denaturation as acidic postmortem conditions develop (Joo et al., 1999). Thus, the rate of glycolysis increases in response to increased ATP utilization.

Regulation of Glycolysis

In living skeletal muscle, energy utilization and energy production are highly coordinated events. In fact, Conley et al. (1997) suggested that elevated sarcoplasmic calcium activates muscle contraction, glycogenolysis and glycolysis in parallel, since glycolytic rate is dependent on muscle stimulation frequency and independent of ADP, AMP and P_i concentrations. Once again, this highlights the importance of calcium regulation on muscle metabolism. Control over glycolytic flux, or the flow of intermediates through glycolysis, may also be controlled by covalent modification (enzyme phosphorylation and dephosphorylation), substrate control, and allosteric control mediated by changes in metabolite and co-factor concentrations (reviewed by Connett and Sahlin, 1996). The regulation of glycolytic flux is complex.

Phosphofructokinase (PFK) is generally regarded as the primary regulatory enzyme of glycolysis. It was on this premise that Sayre et al. (1963) investigated PFK activity in porcine muscle extracts. These authors determined that PFK and phosphorylase activities were not associated with the rate of pH decline in longissimus muscle of Hampshire, Poland China and Chester White pigs. Surprisingly, Allison et al.

(2003) found that maximal in vitro PFK activity extracted from longissimus samples frozen at 20 minutes postmortem was inversely correlated with loin chop fluid loss. This likely results from early postmortem inactivation of the acid labile PFK enzyme in muscle undergoing rapid glycolysis.

Although glycolytic enzyme capacity does not appear to explain a large proportion of the variation in pork color and WHC, other levels of glycolytic regulation may contribute to the rapid pH decline often associated with PSE pork. A detailed understanding of factors that regulate postmortem glycolysis in porcine skeletal muscle will improve efforts to control the rate of muscle pH decline.

The reversible binding of enzyme-enzyme and enzyme-contractile protein interactions provide additional possibilities for the regulation of glycolytic flux in skeletal muscle. The proportions of several glycolytic enzymes bound to contractile proteins increase with increased rates of glycolysis, and this may provide a mechanism for enhancing metabolite transfer rates (Parkhouse, 1992). Lee et al. (1989) summarized several studies demonstrating that PFK is phosphorylated in contracting muscle when the need for energy is high. Modification of PFK by phosphorylation favors enzyme binding to actin by increasing its apparent affinity for F-actin. It is unclear if postmortem conditions induce enzyme modifications that are similar to those that occur under normal physiological conditions.

How (or if) the functional coupling of glycolytic enzymes to the major sites of energy utilization (myosin ATPase and calcium ATPase) affect pork quality is currently unknown. However, the effects of this coupling may influence glycolytic rate, ultimate

enzyme location and the degree of denaturation of sarcoplasmic proteins, which in turn, may influence color (Joo et al., 1999) and WHC of pork (Wilson and van Laack, 1999).

Kastenschmidt et al. (1968) quantified levels of glycolytic intermediates and co-factors in longissimus muscles that exhibited fast and slow rates of postmortem glycolysis. Muscles were classified as either "fast-glycolyzing" or "slow-glycolyzing" based on the rate of their post-mortem decline in pH. If muscle pH declined to 5.5 or below at 30 minutes post-mortem, the muscle was termed "fast-glycolyzing." Contrary to this, if muscle pH was 6.0 or higher at 60 minutes post-mortem, the muscle was termed "slow-glycolyzing." "Fast-glycolyzing" muscles are in a highly anaerobic state prior to or simultaneous with death.

Results of this thorough investigation led the authors to conclude that accelerated glycolytic rates appear to result from coordinated stimulation of glycogen phosphorylase, PFK and pyruvate kinase (PK) enzymes. These enzymes have traditionally been considered to catalyze rate-determining steps of glycogenolysis and glycolysis in skeletal muscle. These reactions are far from equilibrium and are catalyzed by PFK and PK and proceed with a large decrease in free energy. However, the precise regulation of these enzymes in postmortem muscle is unclear.

Glycolytic Oscillations

For decades, it has been known that the rate of postmortem glycolysis is variable in the pig muscle (Briskey et al., 1966). Postmortem muscle energy utilization is monitored by measuring pH at specified times. Continuous pH measurement in muscle is difficult to achieve with current instrumentation, yet pH decline data are typically drawn as curvilinear. This measurement undoubtedly reflects the pH of tissue, rather than

individual muscle fibers, and may not accurately reflect the nature of postmortem glycolysis in individual muscle fibers. Additionally, the measurement of early postmortem pH often produces variable results that are not highly correlated with pork quality traits. Oscillatory behavior of the glycolytic pathway has been well documented (reviewed by Smolen, 1995; Tornheim, 1979). In cell-free extracts of skeletal muscle, glycolytic oscillations are generated by repeated bursts of PFK activity. When the [ATP]/[ADP] ratio decreases to a trigger level, this initiates a sudden increase, or burst, in glycolytic flux that restores a high [ATP]/[ADP] ratio. In postmortem tissue, glycolytic bursts may also result in rapid and localized acidification, which could exacerbate protein denaturation.

Under conditions encountered in skeletal muscle, oscillatory behavior of glycolysis has advantages over steady state behavior for regulation of carbohydrate utilization and maintenance of a high [ATP]/[ADP] ratio. It also has greater thermodynamic efficiency (Tornheim et al., 1979, 1991; Termonia et al., 1981). It is not known if postmortem skeletal muscle of the pig exhibits oscillatory patterns of glycolysis and associated bursts in acidification. However, postmortem muscle pH decline may be sporadic in some pigs (R.C. Johnson and C.P. Allison; unpublished data). In whole muscle, glycolytic oscillations may frequently be obscured by cell-to-cell asynchrony (Tornheim et al., 1979). Bursts of acidification resulting from early postmortem glycolytic oscillations would likely cause more extensive protein denaturation than a gradual pH decline. Thus, glycolytic oscillations would be important determinants of pork color and WHC. The potential contribution of these oscillations to the development of PSE pork warrants further investigation.

Regulators of Glycolysis

Oscillatory behavior of glycolysis involves bursts of PFK activity due to autocatalytic activation by fructose 1,6-bisphosphate. Fructose 2,6-bisphosphate is an even more potent activator of PFK and is competitive with fructose 1,6-bisphosphate for the same binding site on PFK. Tornheim et al., (1988) have shown that addition of fructose 2,6-bisphosphate blocked glycolytic oscillations, but did not affect lactate accumulation. Additionally, fructose 2,6-bisphosphate decreased the [ATP]/[ADP] ratio, demonstrating the advantage of the oscillatory behavior in maintaining a high energy state.

Citrate has also been shown to modulate glycolytic oscillations by decreasing their frequency and delaying their on-set (Tornheim et al., 1991). These effects are due to inhibition of PFK, such that a lower trigger value of the [ATP]/[ADP] ratio must be reached before a burst of PFK can be initiated. However, citrate inhibition has little effect on the amplitude of oscillations or the peak [ATP]/[ADP] ratio (Tornheim et al., 1991). In glycolytic oscillations with and without citrate, 50 – 100% more AMP was required to initiate the burst of PFK activity in the presence of citrate. In other words, the [ATP]/[ADP] ratio had to fall to a greater extent in the presence of citrate before PFK was activated (Tornheim et al., 1991).

CHAPTER 1

RELATIONSHIP BETWEEN GLYCOLYTIC OSCILLATIONS AND PORK COLOR AND WATER-HOLDING CAPACITY

ABSTRACT

The objective of this study was to determine if differences exist in the pattern of glycolysis in muscle extracts from superior and inferior quality pork loins. Characteristics of superior (n=6) and inferior (n=6) quality loins, respectively, were as follows: 45 min pH (6.40 ± 0.06 vs 5.92 ± 0.10), percent fluid loss after centrifugation (10.22 ± 0.47 vs 20.70 ± 0.32), percent drip-loss by the suspension method (0.66 ± 0.08 vs 3.23 ± 0.45), and day 1 L* (51.37 ± 0.66 vs 56.68 ± 0.86). Longissimus muscle samples were obtained at 20 min postmortem and were immediately frozen in liquid nitrogen and stored at -80° C until analysis. Samples were homogenized in 90 mM potassium phosphate, pH 6.5, and 180 mM potassium chloride. A high-speed supernatant fluid (85,000 x g) was gel-filtered using Sephadex G-25 resin. Gel-filtered muscle proteins were used to determine *in vitro* patterns of glycolysis. Aliquots of glycolytic reaction mixture were removed every 2 min for 46 min and acidified to halt enzyme activity. Enzymatic assays were used to quantify concentrations of ADP, ATP, and lactate. An oscillatory pattern of glycolysis was observed using extracts from both superior and inferior quality pork. No differences in the average ATP:ADP ratio or the overall mean concentrations of lactate or adenine nucleotides were observed in reactions using extracts from superior and inferior quality samples ($P > 0.05$). Thus, sarcoplasmic protein extracts do not appear to produce distinct patterns of glycolysis that are associated with differences in pork quality. However, this system will permit identification of specific metabolites that cause differences in the pattern of postmortem glycolysis.

INTRODUCTION

The National Pork Benchmarking Audit found that live weight and carcass muscle percentage have increased, and backfat thickness has decreased over the last ten years (Stetzer and McKeith, 2003). Furthermore, as selection for leaner, heavier weight, and more muscular pigs has occurred, so has the increased occurrence of PSE pork (Lonergan et al., 2001). Despite an abundance of research describing PSE pork characteristics, and a reduction in the frequency of major genes with known deleterious effects on pork quality, Cassens (2000) concluded that little progress has been made in reducing the incidence of PSE pork.

Understanding events that dictate superior and inferior quality is essential for development of new strategies to improve the quality and consistency of pork. Briskey et al. (1966) have shown that the rate of postmortem glycolysis is variable in pig muscle. Pale, soft and exudative pork is caused by the denaturation of muscle proteins that result when carcass muscles experience a low pH and high temperature (Briskey and Wismer-Pedersen, 1961). However, low early postmortem pH does not explain all PSE seen in pig muscle. Pork with an apparently normal pH decline may exhibit pale and/or soft and exudative characteristics. Observed inconsistencies in pH decline within a muscle may suggest differences in the pattern of pH decline among muscles or carcasses. In support of this, a recent report suggests that postmortem muscle pH decline may be sporadic in some pigs (Johnson, R.C., 2001), suggesting that differences may exist in the patterns of energy metabolism during the postmortem period. Oscillatory behavior of the glycolytic pathway *in vitro* has been well documented (Tornheim et al. 1971, 1973, 1974, 1991). In whole muscle, glycolytic oscillations may frequently be obscured by cell-to-cell

asynchrony (Tornheim et al., 1979). We hypothesized that inferior pork water-holding capacity and pork color are associated with more pronounced glycolytic oscillations, which exacerbate protein denaturation.

MATERIALS AND METHODS

Animal and Meat Quality Data Collection

Sires from Duroc and HAL-1843-free Pietrain lines were mated to Yorkshire and F1 Yorkshire-Landrace gilts. Crossbred progeny were raised in uniform conditions at the Michigan State University (MSU) Swine Teaching and Research Farm. Four gilts from each sire group were harvested on each of four days within a 2-week period at the MSU Meat Laboratory. Since this project required no experimental manipulation of live animals it was exempt from filing an animal use form based on AUCAUC Policy #9, Policy on Submission of an Animal Use Form, projects involving research on tissues or fluids collected from a food animal killed in a slaughterhouse. Pigs were harvested using standard industry procedures following the Code of Federal Regulations for Humane Slaughter of Livestock.

Muscle tissue and pork quality data were collected as previously described by Allison et al. (2003). Briefly, a longissimus muscle (LM) sample was taken midway between the last rib and the cranial edge of the ilium on the left side of the carcass 20 min postmortem. Each sample (~2.5 cm thick) was cut into 0.5 cm^3 pieces, frozen in liquid nitrogen, and stored at -80° C for later use. Longissimus muscle pH was measured at 20, 45, 180 min, and 22 h postmortem with a portable pH meter (Model 1140, Mettler-Toledo, Woburn, MA) equipped with a puncture-type combination pH electrode (Lot 406-M6-DXK-57/25, Mettler-Toledo, Woburn, MA). At 24 h postmortem, duplicate 10 g samples were obtained from the cranial edge of the loin section to determine WHC by high-speed centrifugation (CWHC) at 40,000 x *g* for 30 min (Honikel and Hamm, 1994). Two 2.5 cm thick loin chops were removed between the 12^{th} and last rib of the section.

These chops were used to determine drip loss (DRIP1) by the suspension method from 24 to 48 h postmortem (Honikel and Hamm, 1994) and color (15 min bloom time; CIE L*, D65, 2 degree standard observer and 50 mm orifice) at 24 h postmortem with a Minolta chromameter (CR-310; Ramsey, NJ).

Based on differences in 45 min pH, CWHC, DRIP1, and L*, samples from three Duroc- and three Pietrain-sired pigs with superior meat quality traits, and from three Duroc- and three Pietrain-sired pigs with inferior meat quality traits were selected. Pigs that were most extreme in their quality differences were selected for the current experiment.

Preparation of muscle protein extracts

Chemicals used in the muscle extraction and assays were purchased from Sigma Chemical Company (St. Louis, MO). Methods used were adapted from those described for rat muscle by Tornheim et al. (1973, 1974, 1979, 1988, and 1991). Frozen LM samples (~7 g) were homogenized in 3 volumes of cold homogenization buffer (90 mM potassium phosphate, pH 6.5, and 180 mM potassium chloride). Homogenization was performed using a Polytron (Brinkman, Westbury, NY) for 3 x 30-sec bursts (setting 4), with 15 sec between bursts. Samples were continuously cooled with ice during homogenization. The homogenate was stirred slowly at 4° C for 1 hour, and then centrifuged at 4° C (SS 34 rotor of a Sorvall RC2-B centrifuge) at 31,000 x g for 10 min to remove insoluble material. A fat cap was carefully removed from the surface with a small spatula and supernatant fluid was decanted and filtered through cheesecloth to remove particulate matter that was not pelleted. Cheesecloth was pre-soaked in homogenization buffer to minimize loss of sample during the filtration process. The

supernatant fluid was then centrifuged at 4° C (rotor 30 of a Spinco model L ultra centrifuge) at 85,000 x g for 30 minutes to remove the membrane fraction. The resulting supernatant fluid obtained was adjusted to contain 5 mM ethylenediaminetetraacetic acid (EDTA) and 0.1 mM dithiothreitol. Then 7 mL of supernatant were placed on a column (Bio-Rad Column 2.5 cm ID x 14 cm length, cat. No. 738-0017) packed with Sephadex G-25 resin (fine; Amersham Pharmacia). Sephadex beads were prepared by soaking in homogenization buffer. They were stirred every h for 3 h, and allowed to swell for a total of 12 h prior to being transferred to the column. The column was flushed with 0.5 L of homogenization buffer to ensure that all Sephadex beads were properly packed and equilibrated prior to sample loading. Protein was eluted from the column with a buffer containing 15 mM potassium phosphate, pH 6.5, 280 mM potassium chloride, 5 mM EDTA, and 0.1 mM dithiothreitol at a rate of 2 mL per minute and collected in 0.5 mL fractions. A Bio-Rad Biologic LP Chromatography System (Cat. No. 731-8300) and Bio-Rad Model 2128 Fraction Collector were used for gel filtration and collection of proteins. Protein concentrations of gel-filtered samples were determined using a commercial kit (BioRad Protein Assay Kit, BioRad Laboratories, Hercules, CA) based on the method of Bradford (1976). Bovine serum albumin was used as a protein standard. Samples with the highest protein concentrations were pooled and frozen in 1 mL aliquots and stored at -80° C for later use. Care was taken to remove all filtrate from the column by flushing the system with an additional 0.5 L of elution buffer, as well as 0.5 L of de-ionized distilled H_2O. The column was prepared for the next sample filtration by flushing with 0.5 L of homogenization buffer.

Polyacrylamide Gel Electrophoresis (PAGE) Analysis of Protein

Separating gels were used to visualize gross differences that may exist in protein profiles of gel-filtered muscle extracts from superior and inferior quality pork samples used in this study. Twenty-eight μg of muscle protein were loaded on discontinuous 12.5% SDS-PAGE separating gels (Laemmli, 1970; 12.5% acrylamide/bis [37.5:1 ratio of acrylamide to N, N'-methylene-bis-acrylamide]; approximately 9.5 cm x 6 cm x 0.75 mm) using a Bio-Rad Mini Protean II apparatus (Bio-Rad, Hercules, CA). Gels were allowed to polymerize for 1 hour before a 4% stacker (4% acrylamide/bis [37.5:1 ratio of acrylamide to N, N'- methylene-bis-acrylamide]) was poured. The composition of the running buffer was 25 mM Tris, 192 mM glycine, and 0.1% SDS, pH 8.3. Gels were run at 200 volts at room temperature for 45 min or until the dye front ran off. After electrophoretic separation, proteins were stained in Coomassie blue R-250 (40% methanol, 7% acetic acid, and 0.0025% coomassie blue) overnight and gels were destained (40% methanol, 10% acetic acid) prior to being dried using the GelAir Cellophane (Bio-Rad cat. No.165-1779) and scanned using a Bio-Rad fluor-s image analysis system (Bio-Rad, Hercules, CA).

Myofibril Preparation

Crude myofibrils were prepared using a protocol modified from Lonergan et al. (1995). Porcine longissimus muscle tissue (1g) obtained at 24 hours postmortem was homogenized in 9 volumes of an extraction buffer containing 10 mM tris hydrochloride, pH 8.3, 10 mM EDTA, and 2mM phenylmethylsulfonylfluoride (PMSF). The sample was then centrifuged at 27,000 x g for 30 min (SS 34 rotor of a Sorvall RC2-B centrifuge). Supernatant fluid was decanted and the pellet was saved. The pellet was

then re-homogenized in 5.0 mL of extraction buffer and centrifuged at 1,500 x g for 10 min. This procedure was repeated three times to further rinse the myofibrils. Following the third centrifugation, the supernatant fluid was decanted and the pellet was re-suspended in 5 volumes of 50% glycerol and stored in 200 uL aliquots at -80° C for later use.

Aliquots (200 uL) were thawed and centrifuged at 1,500 x g for 10 minutes to pellet myofibrils, and glycerol was discarded. Samples were subsequently re-suspended in 50 mM triethanolamine, pH 7.5 prior to use in reaction mixture.

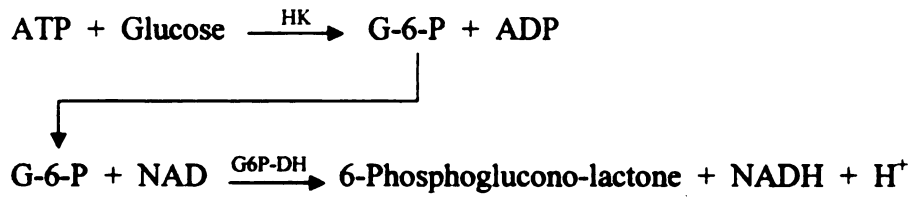
Glycolytic Reaction Conditions

Gel-filtered samples containing muscle protein were exposed to a reaction mixture to initiate glycolysis as described by Tornheim et al. (1991) with minor modifications. The reaction mixture was made in a 10X stock (20 mL), and the final reaction was performed in a volume of 2 mL in a polypropylene microcentrifuge tube that contained 4 mM aspartate, 10 mM glucose, 7.5 mM orthophosphate, 25 mM imidazole HCl, pH 6.9, 8.3 mM magnesium chloride, 0.2 mM calcium chloride, and 1 mM ATP, 0.3 mM GTP, and 0.1 mM NAD, 0.25 mg/mL crude myofibrils, and 1 mg/ml gel-filtered protein. The reaction was started by adding 33.6 µL of a stock yeast hexokinase (5.0 µl of hexokinase in 495 µL of de-ionized distilled H_2O, Sigma cat. No. H-5625) to the 2 mL volume, which provided 0.06 units/mL hexokinase activity in the reaction. Tubes containing reaction mixture were then inverted 3 times to mix the contents. The reaction mixture was maintained in a water bath at 30° C. A portable pH meter with a puncture type probe was used to measure pH at 0, 10, 20, 30, and 40 min following the initiation of the reaction. Samples of 75 µl were removed every 2 minutes from 0 to 46 minutes and

placed in 75 µL of 2 N perchloric acid to deproteinize the sample. Upon completion of the experiment, acidified samples were centrifuged at 1,500 x g for 10 minutes in an Eppendorf 5415 centrifuge. Following centrifugation, perchloric acid in samples was neutralized with 30 uL of 5.4 N KOH. The samples were then re-centrifuged at 1,500 x g for 10 minutes. Supernatant fluid was kept on ice and assays were performed within 2 hours. Unless otherwise indicated, data represent results of an individual experiment.

ATP Assay

Adenosine triphosphate (ATP) was quantified using the enzymatic method of Lamprecht and Trautschold (1972), with the exception that the assay was adapted to a 96-well microtiter plate. The ATP assay reagent consisted of 50 mM triethanolamine buffer, 10 mM NAD, 0.1 M magnesium chloride, and 0.5 M glucose. The total reaction volume was 230 µL and the quantity of ATP was determined by following the conversion of NAD^+ to NADH at 340 nm using a VERSAmax plate reader (Molecular Devices, Sunnyvale, CA). The coupled enzyme reactions that are the basis of this assay are shown below.



Assays were performed in duplicate with 10 µL of sample from the deproteinized reaction mixture followed by 200 µL of assay reagent. An initial absorbance (A1) at 340 nm was recorded. A stock of glucose-6-phosphate dehydrogenase was prepared in H_2O,

and 10 µL (0.46 U/mL in final reaction mixture) was added to each well to start the reaction. The contents of each well were mixed and incubated at 37°C for 15 minutes, and a second A_{340} reading (A2) was taken. This was a modification of the original protocol. It was necessary to run the initial reaction to completion to avoid confounding effects of residual G-6-P produced in the glycolytic reaction. This allowed for the quantification of G-6-P (A2-A1). A stock of hexokinase (Sigma cat. No. H-4502) was also prepared and 10 µL (1.8 U/mL in final reaction mixture) was added to each well. The contents of each well were mixed and incubated at 37°C for 40 minutes. Upon completion of the reaction the absorbance at A_{340} (A3) was again recorded and the change in absorbance (A3-A2) was used for ATP determination. Fresh ATP standards (1 mM, 0.5 mM, 0.25 mM, 0.125 mM, and 0 mM) were prepared daily, and were used to create a linear regression equation ($y = mx + b$) that was implemented to calculate the ATP concentration of sample unknowns. Final concentrations of ATP were multiplied by a dilution factor of 2.4 to account for dilution of sample during acidification and neutralization.

ADP Assay

The enzymatic determination of adenosine diphosphate (ADP) also involved the utilization of methods from Lamprecht and Trautschold (1972). The assay involved an initial creatine kinase reaction to quantitatively convert ADP to ATP. This reaction was performed by adding 30 µl of a solution containing 14 mM creatine phosphate, 0.1 M glycine, 1 mM magnesium sulphate, pH 9.0, and creatine kinase (25 U/mg), and 30 µl of sample to microtubes. Reaction mixtures were incubated for 15 minutes at 37° C and then heated for 3 min in a water bath at 95° C. Samples were subsequently centrifuged at

1,500 x g for 10 min. Then 20 μL of the sample were added to microtiter plate wells, and the aforementioned ATP protocol was utilized to quantify ATP. The concentration of ADP was determined as the difference between the ATP concentration measured before and after conversion of ADP to ATP using the creatine kinase reaction. Final concentrations were derived as previously stated for ATP.

Lactate Assay

Lactate accumulation was measured in samples collected from the glycolytic reaction procedure using methods from Sigma (Procedure No. 826-UV). The assay is based on the reaction shown below.

$$\text{Pyruvate} + \text{NADH} \xrightleftharpoons{\text{LDH}} \text{Lactate} + \text{NAD}$$

The reaction was performed in a 96-well microtiter plate and the final reaction volume was 210 μL. Samples (10 μL) were added in duplicate to each well followed by 200 μL of assay reagent which included 2.47 mM NAD, 0.6 mM glycine, and lactate dehydrogenase (1,000 U/mL). Additionally, elution buffer (10 μL) and assay reagent (200 μL) were used as a blank for this assay. Samples were then mixed and placed in an incubator for 15 min at 37° C.

Upon completion of the reaction, lactate concentration was determined by following the decrease in absorbance at 340 nm due to the oxidation of NADH to NAD^+. Absorbance was measured on a VERSAmax plate reader (Molecular Devices, Sunnyvale, CA), and final concentrations of lactate were calculated using the following equation, where 6.22 represents millimolar absorptivity of NADH at 340 nm.

$$\frac{\Delta A_{340} * \text{Total Volume}}{6.22\ \text{mL}/\mu\text{mol/cm} * \text{Sample Vol.} * \text{Light Path}} = \frac{\Delta A_{340} * 210\ \text{uL}}{6.22\ \text{mL}/\mu\text{mol/cm} * 10\ \text{uL} * 0.65\ \text{cm}}$$

RESULTS AND DISCUSSION

An *in vitro* glycolytic system was used to determine if muscle protein extracts from pigs that produced superior and inferior quality pork (Table 1) exhibited different patterns of glycolysis. Loins from pigs designated superior quality had higher 45 min pH, lower fluid loss in samples subjected to centrifugal force, lower drip loss, and lower Day 1 L* values than loins from pigs designated inferior in quality (Table 1).

Supernatant from longissimus muscle extracts was gel-filtered and the collected fractions (Figure 1) were pooled from pigs representing both types of meat quality. Then fractions were analyzed on an SDS PAGE gel to determine molecular weights and similarities of protein bands (Figure 2, lanes 2 to 7). No apparent differences exist in the protein profiles for the superior and inferior quality samples. In addition to sarcoplasmic proteins (~16.5 to 97 kDa), pig muscle extracts also contain relatively abundant myosin (~200 kDa). The cause for the prominent myosin band could be the relatively high ionic strength of the homogenization buffer used compared to buffers typically used for extraction of sarcoplasmic proteins or myofibrils (Pearson and Young, 1989). The fact that samples were harvested from pre-rigor loin muscle may also contribute to the abundance of myosin in the extract, since myosin is soluble at an ionic strength of 0.23 whereas actomyosin is not (Pearson and Young, 1989). Contrary to the pig muscle, myosin is less evident in the rat hind limb extract (Figure 2, lane 8). This is most likely attributable to the rat muscle tissue being closer to the completion of rigor than the porcine muscle used in this study.

Postmortem Energy Utilization

Oscillatory behavior of glycolysis in skeletal muscle extracts is caused by repeated bursts of PFK activity and associated oscillations in the ATP/ADP ratio. This involves the AMP-dependent activation of PFK by its product, fructose-1,6 diphosphate. When the ATP/ADP ratio lowers to a trigger level (ratio of approximately 2 to 4), this initiates a sudden burst in glycolytic flux that restores a high ATP/ADP ratio (Tornheim et al., 1991). This pattern of glycolytic behavior was observed by Tornheim et al., (1988, 1991) in gel-filtered rat hind limb muscle extracts.

We anticipated that pigs with superior quality traits would exhibit more subtle glycolytic oscillations, while pigs with inferior quality traits would exhibit more pronounced oscillatory patterns of energy production. However, when muscle protein fractions were exposed to glycolytic reaction mixtures, a variety of patterns of glycolysis were observed.

The largest fluctuation in adenine nucleotide concentration occurred from 0 to 20 min following the initiation of the glycolytic reaction in samples of most pigs. Thus, samples were classified as either oscillatory or non-oscillatory based on their pattern of energy generation during the first 20 min. Figures 3.1, 3.2, and 3.3, respectively, depict non-oscillatory patterns of glycolysis. The rate of glycolysis in these samples appears to parallel the rate of ATP utilization, such that the ATP concentration deviates little, while lactate steadily accumulates. This pattern of glycolysis was hypothesized to be associated with superior quality pigs, but was exhibited by both superior and inferior quality pigs. In contrast, figures 3.4, 3.5, and 3.6, respectively, depict an oscillatory pattern of glycolysis. This oscillatory pattern was hypothesized to occur in inferior

quality pigs and be associated with localized acidification that may exacerbate protein denaturation and increase fluid loss from product. However, the oscillatory pattern was observed in both inferior and superior quality pigs.

As expected, ATP and ADP concentrations are inversely related to each other in most samples, regardless of quality designation. There were no differences in the average ATP/ADP ratio or the overall mean concentrations of lactate (Figure 4) or adenine nucleotides in reactions using extracts from either superior or inferior quality loin samples (Table 2). Additionally, sire breed had no effect on the concentrations of adenine nucleotides or lactate. Likewise, no differences were observed in the *in vitro* pH decline between the superior or inferior quality samples (Figure 5). This is in contrast to the more rapid pH decline that resulted in lower 45 min pH of inferior quality loins (Table 1). Although conditions such as temperature and calcium concentration were controlled in the *in vitro* assay, variation in these traits may influence the rate of postmortem glycolysis in carcass muscles. Nonetheless, our data suggest that protein extracts from muscle yielding superior and inferior quality pork do not produce distinct patterns of glycolysis that would explain differences in pork loin quality. Regulation of the glycolytic pattern may be attributed to differences in metabolic effectors or the rate of energy utilization during postmortem glycolysis.

We would expect more pronounced oscillations that occurred from 0 to 20 min to be accompanied by greater H^+ ion accumulation in a localized environment, such as in proximity to the calcium ATPase, where glycolytic enzymes have been shown to be functionally compartmentalized (Xu and Becker, 1998). If this occurred in muscle, one would expect to incur greater changes in color attributes and WHC due to protein

denaturation. However, analysis of the glycolytic pattern throughout the entire time period (0 to 46 min) may be of significance when making implications related to pork quality. All samples exhibited an oscillatory pattern ($\geq$ 2 oscillations) of glycolysis from 20 to 46 min, but the amplitude of these oscillations was lower than those observed from 0 to 20 min. It would appear that reduced amplitude of oscillations occurring from 20 to 46 min may be a result of the system losing finite control over glycolysis due to low pH. At the onset of the reaction, pH is near neutral and substrate is plentiful. As the reaction proceeds and H^+ ions accumulate, the pH is driven downward to ~ 5.7 (Figure 5). As pH continues to fall, the rate of glycolysis slows, ATP production decreases, and lactate accumulates at a reduced rate. Tornheim and Lowenstein (1973) have previously shown that glycolytic oscillations are strongly dependent upon pH. These authors observed that at lower pH, deamination occurred to a greater extent and more time elapsed before reamination.

When compared to rat hind limb muscle extract (Tornheim et al., 1979), pig muscle extract resulted in more rapid *in vitro* glycolysis. A comparison of glycolytic behavior between pig and rat muscle extracts would determine if species or laboratory techniques were responsible for the deviation in energy production. Figure 6 depicts the oscillatory pattern of glycolysis that we achieved using rat hind limb muscle extract. There appears to be a decrease in the rate and extent of lactate accumulation in the rat hind limb muscle when compared to the protein extracts from pig muscle. Our pattern of ocillatory behavior for rat was similar to Tornheim et al. (1974, 1979) where the rate of reaction in extracts from rat hind limb muscle was much slower. Despite the slower rate of lactate accumulation, deamination of adenine nucleotides was evident in reactions

containing rat muscle extract. This was shown in the sharp decline in concentrations of ATP and ADP seen from 20 to 46 min during the reaction period. The decreased lactate accumulation in the rat muscle extract is likely to be attributable to fiber type differences, as rat hind limb would be expected to have a higher proportion of red fibers, less glycolytic activity, and lower ATPase activity than pig longissimus muscle (Pearson and Young, 1989).

Adding an Alternative ATPase

It is well established that the rate of ATP utilization is positively associated with the rate of pH decline. For example, species with a higher proportion of white muscle fibers, such as pigs and poultry, undergo more rapid postmortem ATP utilization and glycolysis than red muscle species (i.e. beef and lamb) due to more active myosin ATPase and more abundant SR calcium ATPase. Increased ATPase activity may reduce the ATP/ADP ratio and induce production of glycolytic "burst" activity. To determine the effect of added ATPase from a myofibrillar source on the pattern of glycolysis, protein extracts from a pig of each quality type (1 mg/mL of gel-filtered protein) were used in combination with 0, 0.25, and 0.50 mg/mL of myofibrils. Although the added ATPase caused increased lactate accumulation over the reaction period of 0 to 46 min, the pattern of glycolysis was not affected by the addition of myofibrils (Fig. 7.1 and 7.2).

How (or if) the functional coupling of glycolytic enzymes to the major sites of energy utilization (myosin ATPase and calcium ATPase) affect pork quality is currently unknown. However, the effects of this coupling may influence glycolytic rate, ultimate enzyme location and the degree of denaturation of sarcoplasmic proteins, which, in turn, may influence the color (Joo et al., 1999) and WHC of pork (Wilson and van Laack,

1999). The functional coupling of glycolytic enzymes to the myofibrillar ATPase was beyond the scope of these experiments.

Effects of Citrate

The experiments previously described demonstrated that pig muscle extracts produce glycolysis with an oscillatory behavior, but no differences were observed in the visual pattern of glycolysis using extracts from superior and inferior pork. Furthermore, no differences were observed in mean concentration of adenine nucleotides or lactate over all time points ($P > 0.05$). Thus, if patterns of glycolysis differ between postmortem muscles that exhibit variation in meat quality, these patterns are not mimicked by extracts containing glycolytic enzymes from those muscles.

Although glycolytic enzyme capacity does not appear to explain a large proportion of the variation in pork color and WHC, other levels of glycolytic regulation may contribute to the rapid pH decline often associated with PSE pork. Citrate is known to be a potent physiological inhibitor of PFK, which is generally regarded as the primary regulatory enzyme of glycolysis. Tornheim et al. (1991) showed that addition of citrate in extracts of rat hind limb muscle decreased the frequency of the oscillations and delayed the first burst of PFK activity. These effects are due to inhibition of PFK, such that a lower trigger value of the [ATP]/[ADP] ratio must be reached before a burst of PFK can be initiated. When 0.15 mM citrate was included in the reaction mixture containing pig muscle extract, the rate of lactate accumulation was decreased (Figure 8). Our results support Tornheim's previous findings, and demonstrate that the *in vitro* system used is responsive to known physiological regulators of glycolysis.

Similar to citrate, fructose 2,6-bisphosphate has a regulatory role in glycolytic behavior. Fructose 2,6-bisphosphate is a potent activator of PFK and is competitive with fructose 1,6-bisphosphate for the same binding site on PFK. Tornheim et al. (1988) have shown that addition of fructose 2,6-bisphosphate will decrease the ATP/ADP ratio, essentially blocking oscillatory patterns of glycolysis. This blockage is the result of autocatalytic activation of fructose 1,6-bisphospate, and thus prevents glycolytic oscillations. With this said, it is apparent that control over glycolytic flux, or the flow of intermediates through glycolysis, as well as regulatory mechanisms influencing postmortem metabolism warrant further research.

IMPLICATIONS

Today's competitive agricultural economy has constricted the profit margins experienced in the swine industry. Consequently, it's not a surprise that costly problems related to inferior meat quality warrant further research. In this study, a system was successfully developed to monitor *in vitro* glycolytic energy metabolism. Oscillatory patterns of glycolysis *in vitro* do not appear to be related to deviations in pork quality measured today. However, this system will permit identification of specific metabolites that cause differences in the pattern of glycolysis. Further research is needed to monitor the influence of individual metabolites on the behavior of glycolytic metabolism.

APPENDICES

Table 1. Means and standard deviations for carcass quality data.[a]

	Superior Quality		Inferior Quality	
	Mean	Stdev	Mean	Stdev
45 min pH	6.40	0.14	5.92	0.24
CWHC, %	10.22	1.15	20.70	0.78
Drip 1, %	0.66	0.19	3.23	1.10
Day 1 L*	51.37	1.61	56.68	2.11

[a] n = 6 pigs in each quality category (superior and inferior).

Table 2. Mean concentrations of adenine nucleotides and lactate observed in glycolytic reactions from 0 to 46 min using protein extracts from either superior or inferior quality pork.[a,b]

	Superior Quality		Inferior Quality	
	Mean	Stdev	Mean	Stdev
ATP, mM	0.539	0.256	0.610	0.268
ADP, mM	0.216	0.137	0.192	0.120
ATP/ADP	4.506	3.390	5.361	3.239
Lactate	1.707	0.790	1.673	0.745

[a] n = 6 pigs in each quality category (superior and inferior).
[b] No differences were observed in reactions using extracts from superior and inferior quality samples ($P > 0.05$).

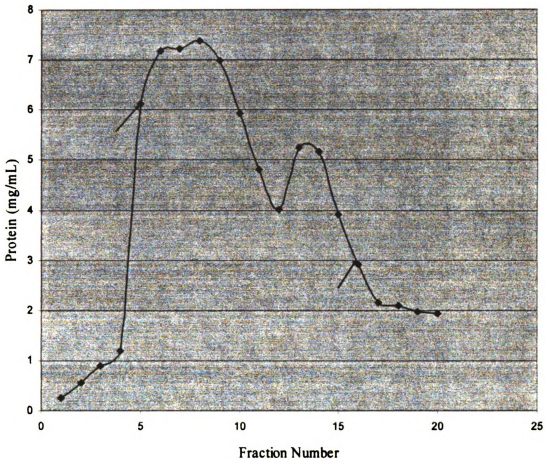


Figure 1. Profile of pig muscle protein eluted from gel filtration column. Fractions of 0.5 mL were collected. Protein eluted between the arrows was pooled and used in reaction mixtures. Gel-filtration was used to remove small molecular weight substances that potentially influence glycolysis.

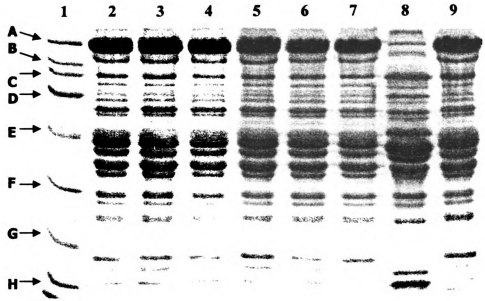


Figure 2. Electrophoretic separation of proteins derived from gel filtration. A 12.5% polyacrylamide separating gel was used to search for crude deviations in the protein profiles of muscle extracts differing in quality. Lane 1 includes molecular weight markers. Lanes 2, 4, and 6 depict protein profiles of pigs designated superior quality. Lanes 3, 5, and 7 depict protein profiles of pigs designated inferior quality. Lane 8 depicts the protein profile of rat hind limb muscle. Lane 9 depicts the protein profile of a HAL-1843 positive pig. Twenty-eight μg of muscle protein were loaded in each lane. Rows A – H represent differing molecular weight markers as follows: A = 200.0 kDa; B = 116.3 kDa; C = 97.4 kDa; D = 66.2 kDa; E = 45.0 kDa; F = 31.0 kDa; G = 21.5 kDa; H = 14.4 kDa.

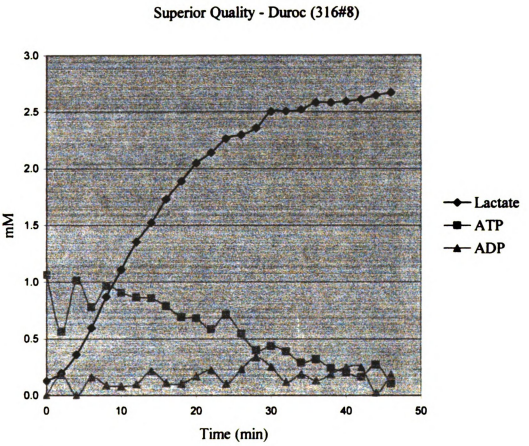


Figure 3.1. Adenine nucleotide (ADP and ATP) and lactate concentrations of a superior quality Duroc pig exhibiting non-oscillatory glycolytic behavior from 0 to 46 min after initiation of glycolytic reaction.

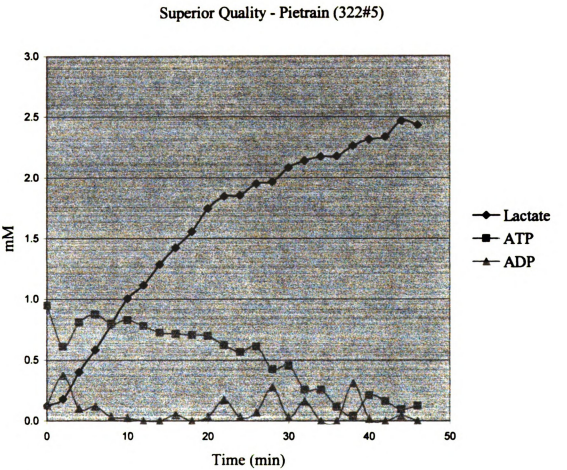


Figure 3.2. Adenine nucleotide (ADP and ATP) and lactate concentrations of a superior quality Pietrain pig exhibiting non-oscillatory glycolytic behavior from 0 to 46 min after initiation of glycolytic reaction.

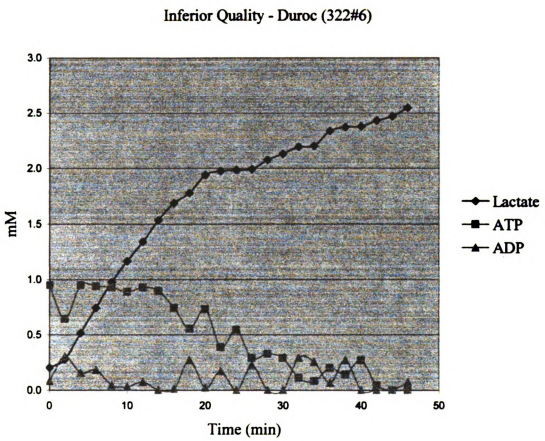


Figure 3.3. Adenine nucleotide (ADP and ATP) and lactate concentrations of an inferior quality Duroc pig exhibiting a non-oscillatory pattern of glycolytic behavior from 0 to 46 min after initiation of glycolytic reaction.

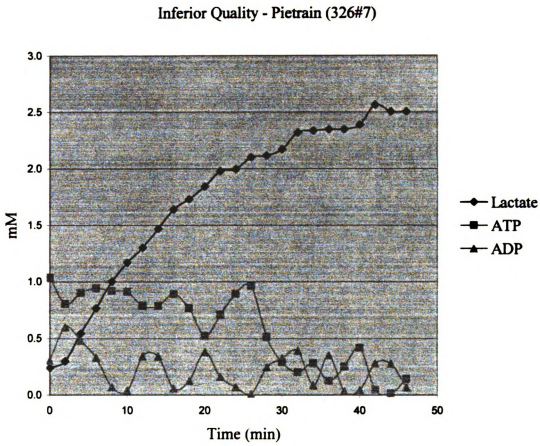


Figure 3.4. Adenine nucleotide (ADP and ATP) and lactate concentrations of an inferior quality Pietrain pig exhibiting oscillatory glycolytic behavior from 0 to 46 min after initiation of glycolytic reaction.

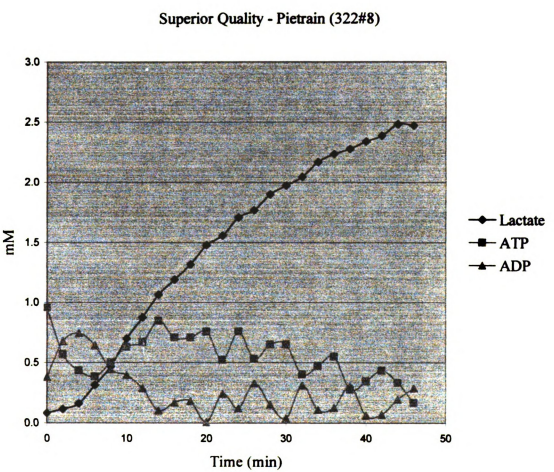


Figure 3.5. Adenine nucleotide (ADP and ATP) and lactate concentrations of a superior quality Pietrain pig exhibiting an oscillatory pattern of glycolytic behavior from 0 to 46 min after initiation of glycolytic reaction.

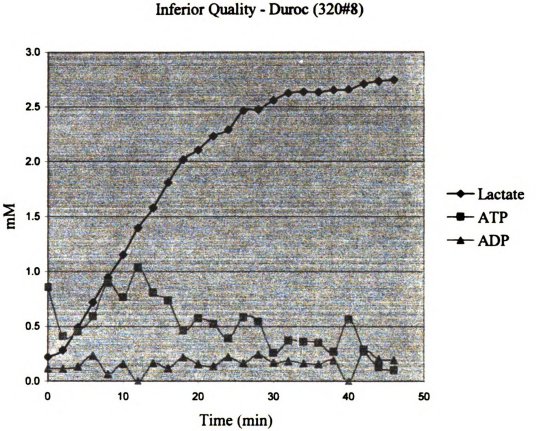


Figure 3.6. Adenine nucleotide (ADP and ATP) and lactate concentrations of an inferior quality Duroc pig exhibiting an oscillatory pattern of glycolytic behavior from 0 to 46 min after initiation of glycolytic reaction.

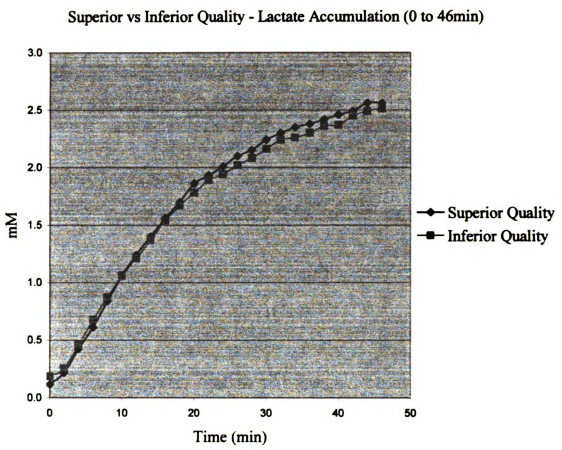


Figure 4. Lactate accumulation from 0 to 46 min after initiation of glycolytic reaction. The data is indicative of the means for each time point of the respective quality groups (superior vs inferior). N = 6 pigs in each quality category (superior and inferior).

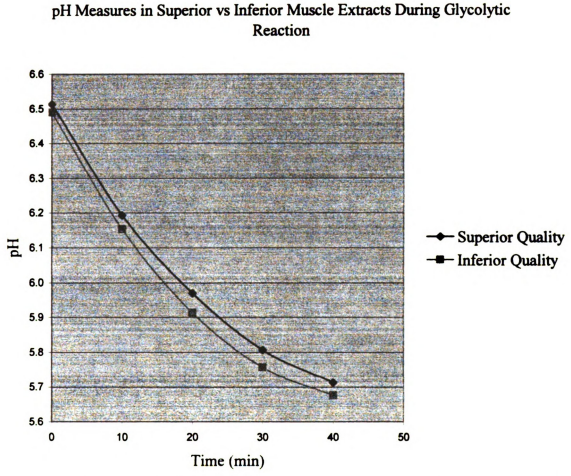


Figure 5. *In vitro* pH decline of superior and inferior quality samples during reactions (0 to 46 min) performed using the glycolytic conditions described in the materials and methods. N = 6 pigs in each quality category (superior and inferior).

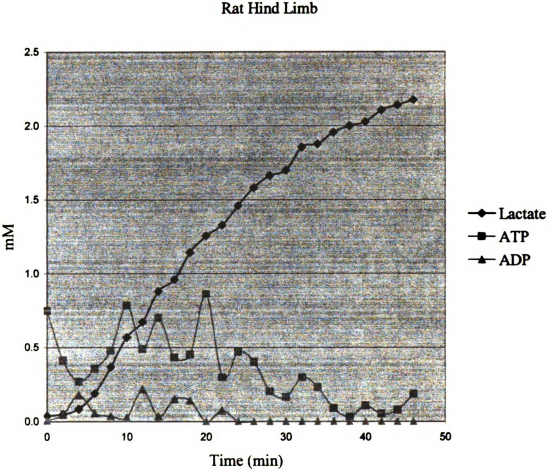


Figure 6. Adenine nucleotide (ADP and ATP) and lactate concentrations of rat hind limb muscle exhibiting an oscillatory pattern of glycolytic behavior from 0 to 46 min after initiation of glycolytic reaction.

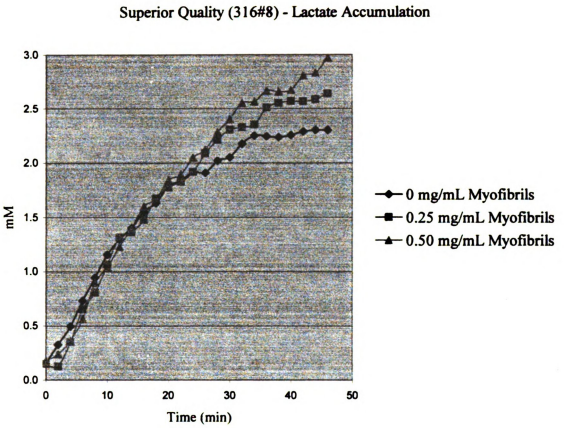


Figure 7.1. Lactate accumulation of a supeior quality Duroc pig with the addition of three differing concentrations of myofibrils from 0 to 46 min after initiation of glycolytic reaction.

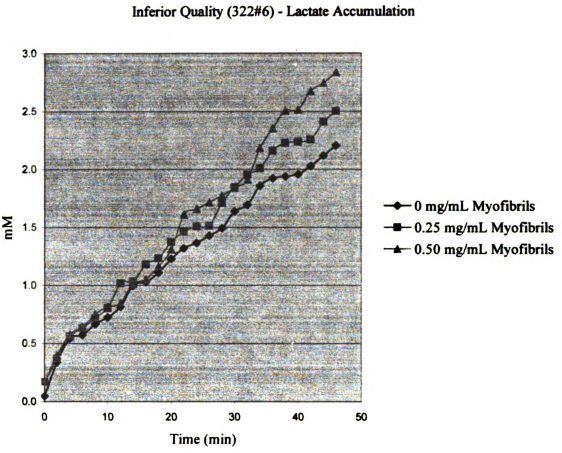


Figure 7.2. Lactate accumulation of an inferior quality Duroc pig with the addition of three differing concentrations of myofibrils from 0 to 46 min after initiation of glycolytic reaction.

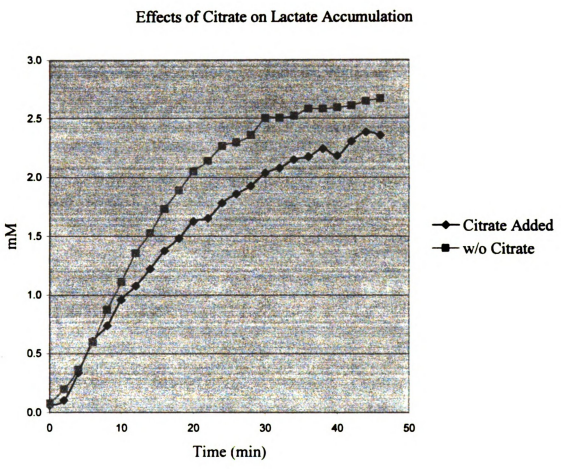


Figure 8. Lactate concentration of pig muscle extract exposed to differing concentrations of citrate from 0 to 46 min after initiation of glycolytic reaction.

LITERATURE CITED

Allison, C.P., R.O. Bates, A.M. Booren, and M.E. Doumit. 2003. Pork quality variation is not explained by glycolytic enzyme capacity. Meat Sci. 63:7-22.

Alonso, M.D., J. Lomako, and W.J. Whelan. 1995. A new look at biosynthesis of glycogen. FASEB J. 9:1126-1137.

Bechtel, P.J. and P.M. Best. 1985. Integration of ATP production and consumption. Rec. Meat Conf. Proc. 38:26-31.

Bendall, J.R., and J. Wismer-Pedersen. 1962. Some properties of the fibrillar proteins of normal and watery pork muscle. J. Food Sci. 27:144-159.

Bertram, H.C., P.P. Purslow, and H.J. Andersen. 2002. Relationship between meat structure, water mobility, and distribution: A low-field nuclear magnetic resonance study. J. Agric. Food Chem. 50:824-829.

Boles, J.A., F.C. Parrish, Jr., T.W. Huiatt, and R.M. Robson. 1992. Effect of porcine stress syndrome on the solubility and degradation of myofibrillar/cytoskeletal proteins. J. Anim. Sci. 70:454-464.

Boles, J.A., J.F. Patience, A.L. Schaefer, and J.L. Aalhus. 1994. Effect of oral loading of acid base on the incidence of pale soft exudative pork (PSE) in stress susceptible pigs. Meat Sci. 37:281-194.

Briskey, E.J. 1964. Etilogical status and associated studies of pale, soft, exudative porcine musculature. Adv. Food Res. 13:89-105.

Briskey, E.J., R.W. Bray, W.G. Hoekstra, P.H. Phillips and R.H. Grummer. 1959. The chemical and physical characteristics of various pork ham muscle classes. J. Anim. Sci. 18:146.

Briskey, E.J., L.L. Kastenschmidt, J.C. Forrest, G.R. Beecher, M.D. Judge, R.G. Cassens, and W.G. Hoekstra. 1966. Biochemical aspects of post-mortem changes in porcine muscle. J. Agric. Food Chem. 14:201-207.

Briskey, E.J. and J. Wismer-Pedersen. 1961. Biochemistry of pork muscle structure. II. Preliminary observations of biopsy samples versus ultimate muscle structure. J. Food Sci. 26:306-313.

Cameron, N.D. 1990. Genetic and phenotypic parameters for carcass traits, meat and eating quality traits in pigs. Livest. Prod. Sci. 26:119-135.

Cannon, J.E., J.B. Morgan, J. Heavner, F.K. McKeith, G.C. Smith and D.L. Meeker. 1995. Pork quality audit: A review of the factors influencing pork quality. J. Musc. Foods. 6:369-402.

Cannon, J.E., J.B. Morgan, J. Heavner, F.K. McKeith, G.C. Smith, and D.L. Meeker. 1996. Pork quality audit survey: Quantification of pork quality characteristics. J. Musc. Foods. 7:29-44.

Cassens, R.G. 2000. Historical perspectives and current aspects of pork meat quality in the USA. Food Chem. 69:357-363.

Cheah, K.S., and A.M. Cheah. 1976. The trigger for PSE condition in stress-susceptible pigs. J. Sci. Food Agric. 27:1137-1144.

Ciobanu, D., J. Bastiaansen, M. Malek, J. Helm, J. Woollard, G. Plastow, and M. Rothschild. 2001. Evidence for new alleles in the protein kinase adenosine monophosphate activated γ_3-subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. Genetics. 159:1151-1162.

Conley, K.E., M.L. Blei, T.L. Richards, M.J. Kushmerick, and S.A. Jubrias. 1997. Activation of glycolysis in human muscle in vivo. Am. J. Physiol. 273:C306-C315.

Connett, R.J., and K. Sahlin. Control of Glycolysis and glycogen metabolism. Handbook of Physiology, Oxford University Press, NY. 1996. P870-911.

Deng, Y., K. Rosenvold, A.H. Karlsson, P. Horn, J. Hedegaard, C.L. Steffensen, and H.J. Andersen. 2002. Relationship between thermal denaturation of porcine muscle proteins and water-holding capacity. J. Food Sci. 67:1642-1647.

Doumit, M.E., C.P. Allison, and M.J. Ritter. 2001. Control of glycolysis in relation to pork quality traits. Reciprocal Meat Conference Proceedings. 54:155-157.

Dutson, T.R. 1983. The measurement of pH in muscle and its importance to meat quality. Reciprocal Meat Conference Proceedings. 36:92-97.

Fujii, J., K. Ostu, F. Zorzato, S.D. Leon, V.K. Khama, J.E. Weiler, P.J. O'Brien, and D.H. MacLennan. 1991. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science. 253:448-451.

Garrett, R.H. and C.M. Grisham. 1999. Chapter 17: Molecular motors in biochemistry. 2nd ed. p 533-564. Saunders College Publishing, Orlando, FL.

Gerrard, D.E. 1997. Pork quality: Beyond the stress gene. National Swine Improvement Federation Proceedings. Vol. 22.

Goll, D.E., R.M. Robson, and M.H. Stromer. 1984. Skeletal Muscle. In:M.J. Swenson (ed.) Duke's physiology of Domestic Animals. 10th ed. p 548-580. Cornell University Press, Ithaca, N.Y.

Goll, D.E., V.F. Thompson, R.G. Taylor, and J.A. Christiansen. 1992. Properties and regulation of the calpain system and its role in muscle growth. Biochimie. 74:225-237.

Honikel, K.O. and C.J. Kim. 1986. Causes of the development of PSE pork. Fleischwirtsch. 66:349-353.

Honikel, K.O. and R. Hamm. 1994. Advances in meat research, Vol. 9 (eds. A.M. Pearson and T.R. Dutson). Chapman and Hall, Glasgow, UK.

Huang, M., C. Lee, R. Lin, and R. Chen. 1997. The exchange between proglycogen and macroglycogen and the metabolic role of the protein rich glycogen in rat skeletal muscle. J. Clin. Invest. 99:501-505.

Huff-Lonergan, E., F.C. Parrish, Jr., and R.M. Robson. 1995. Effects of postmortem aging time, animal age, and sex on degradation of titin and nebulin in bovine longissimus muscle. J. Anim. Sci. 73:1064-1073.

Huff-Lonergan, E., T. Mitsuhashi, D.D. Beekman, F.C. Parrish, D.G. Olson and R.M. Robson. 1996. Proteolysis of specific muscle structural proteins by μ-calpain at low pH and temperature similar to degradation in postmortem bovine muscle. J. Anim. Sci. 74:993-1008.

Ivers, D.J., L.F. Richardson, D.J. Jones, L.E. Watkins, K.D. Miller, J.R. Wagner, R. Seneriz, A.G. Zimmermann, K.A. Bowers, and D.B. Anderson. 2002. Physiological comparison of downer and non-downer pigs following transportation and unloading at a packing plant. Midwest Animal Science Meetings Proceedings. 36:10.

Joo, S.T., R.G. Kauffman, B.C. Kim, and G.B. Park. 1999. The relationship of sarcoplasmic and myofibrillar protein solubility to colour and water-holding capacity in porcine longissimus muscle. Meat Sci. 52:291-297.

Kastenschmidt, L.L., W.G. Hoekstra, and E.J. Briskey. 1968. Glycolytic intermediates and co-factors in "fast-" and "slow-glycolyzing" muscles of the pig. J. Food Sci. 33:151-158.

Kauffman, R.G., D. Waccholz, D. Henderson and J.V. Lochner. 1978. Shrinkage of PSE, normal and DFD hams during transit and processing. J. Anim. Sci. 46:1236-1240.

Kauffman, R.G., R.G. Cassens, A. Scherer, and D.L. Meeker. 1992. Variations in pork quality: history, definition, extent, resolution. National Pork Producers Council Publication. Des Moines, Iowa.

Kauffman, R.G., R.L. Russell, and M.L. Greaser. 1999. Using pork to teach students quality variations and how they are measured. J. Anim. Sci. 77:2574:2577.

Kauffman, R.G., S.T. Joo, C. Schultz, R Warner, and C. Faustman. 1994. Measuring water-holding capacity in post-rigor muscle. Reciprocal Meat Conference Proceedings. 47:70-72.

Koohmaraie, M. 1992. The role of Ca^{2+}-dependent protease (calpains) in postmortem proteolysis and meat tenderness. Biochimie. 74:239-245.

Koohmaraie, M. 1996. Biochemical factors regulating the toughening and tenderization process of meat. Meat Sci. 43:S193-S201.

Koohmaraie, M., G. Whipple, D.H. Kretchmar, J.D. Crouse and H.J. Mersmann. 1991. Postmortem proteolysis in longissimus muscle from beef, lamb and pork carcasses. J. Anim. Sci. 69:617-624.

Krause, U. and G. Wegener. 1996. Control of glycolysis in vertebrate skeletal muscle during exercise. Am. J. Physiol. 270:R821-R829.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.) 227:680-685.

Lee, J.C., L.K. Hesterberg, M.A. Luther, and G-Z. Cai. Allosteric Enzymes. G. Herve (Ed.). CRC Press, Florida. 1989. p 231-254.

Le Roy, P., J.M. Elsen, J.C. Caritez, A. Talmant, H. Juin, P. Sellier, and G. Monin. 2000. Comparison between the three porcine RN genotypes for growth, carcass composition and meat quality traits. Genet. Sel. Evol. 32:165-186.

Lomako, J., M. Lomako, W.J. Whelan, R.S. Dombro, J.T. Neary, and M.D. Norenberg. 1993. Glycogen synthesis in the astrocyte: from glyogenin to proglycogen to glycogen. FASEB J. 7:1386-1393.

Lonergan, S.M., E. Huff-Lonergan, L.J. Rowe, D.L. Kuhlers, and S.B. Jungst. 2001. Selection for lean growth efficiency in Duroc pigs influences pork quality. J. Anim. Sci. 79:2075-2085.

Louis, C.F., W.E. Rempel, and J.R. Mickelson. 1993. Porcine stress syndrome: Biochemical and genetic basis of this inherited syndrome of skeletal muscle. Reciprocal Meat Conference Proceedings. 46:89-96.

Milan, D., J.T. Jeon, C. Looft, V. Amarger, A. Robic, M. Thelander, C. Rogel-Gaillard, S. Paul, N. Iannuccelli, L. Rask, H. Ronne, K. Lundström, N. Reinsch, J. Gellin, E. Kalm, P. Le Roy, P. Chardon, and L. Andersson. 2000. A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. Science. 288:1248-1251.

Monin, G. and P. Sellier. 1985. Pork of low technological quality with a normal rate of muscle pH fall in the immediate post mortem period: The case of the Hampshire breed. Meat Sci. 13:49-63.

Murray, A.C. and C.P. Johnson. 1998. Impact of the halothane gene on muscle quality and pre-slaughter deaths in Western Canadian pigs. Can. J. Anim. Sci. 78:543-548.

National Pork Producers Council (NPPC). 1991. Procedures to evaluate market hogs. Des Moines, Iowa. 3rd ed.

National Pork Producers Council (NPPC). 1998. Pork quality targets. Des Moines, Iowa. www.nppc.org.

National Pork Producers Council (NPPC). 2000. Pork composition and quality assessment procedures. Des Moines, Iowa.

National Pork Producers Council (NPPC). 2003. Pork facts. Des Moines, Iowa. www.nppc.org.

Offer, G. 1991. Modeling of the formation of pale, soft and exudative meat: Effect of chilling regime and rate and extent of glycolysis. Meat Sci. 30:157-184.

Parkhouse, W.S. 1992. Regulation of skeletal muscle metabolism by enzyme binding. Can. J. Physiol. Pharmacol. 70:150-156.

Pate, E. and R. Cooke. 1989. A model of crossbridge action: The effect of ATP, ADP and P_i. J. Muscle Res. Cell Motil. 10:181-196.

Pearson, A.M. and R.B. Young. Muscle and meat biochemistry. Academic Press, Inc., San Diego, CA. 1989. P1-457.

Purslow, P.P., A. Shafer, L. Kristensen, H.C. Bertram, K. Rosenvold, P.R. Henckel, H.J. Andersen, P.J. Knight, T.J. Wess, S. Stoier, and M. Aaslyng. 2001. Water-holding of pork: understanding the mechanisms. Reciprocal Meat Conference Proceedings. 54:134-142.

Sayre, R.N. and E.J. Briskey. 1963. Protein solubility as influenced by physiological conditions in the muscle. J. Food Sci. 28:675-679.

Sayre, R.N., E.J. Briskey, and W.G. Hoekstra. 1963. Comparison of muscle characteristics and post-mortem glycolysis in three breeds of swine. J. Anim. Sci. 22:1012-1020.

Schaefer, A.L., P.L. Dubeski, J.L. Aalhus, and A.K. Tong. 2001. Role of nutrition in reducing antemortem stress and meat quality aberrations. J. Anim. Sci. 79:E91-E101.

Schwägele, F., C. Haschke, K.O. Honikel, and G. Krauss. 1996. Enzymological investigations on the causes for the PSE-syndrome. Comparative studies on pyruvate kinase from PSE- and normal pig muscles. Meat Sci. 44:27-39.

Scopes, R.K. 1964. The influence of post-mortem conditions on the solubilities of muscle proteins. Biochem. J. 91:201-207.

Scopes, R.K. 1977. Purification of glycolytic enzymes by using affinity-elution chromatography. Biochem. J. 161:253-263.

Scopes, R.K. and R.A. Lawrie. 1963. Postmortem lability of skeletal muscle proteins. Nature 197:1202.

Scopes, R.K. 1974. The rate and extent of glycolysis in stimulated post-mortem conditions. Biochem. J. 142:79-86.

Smolen, P. 1995. A model for glycolytic oscillations based on skeletal muscle phosphofructokinase kinetics. J.Theor. Biol. 174:137-148.

Stetzer, A.J., F.K. McKeith. Quantitative strategies and opportunities to improve quality. Final Report, Benchmarking Value in the Pork Supply Chain. AMSA, Savoy, IL. 2003. P1-6.

Suarez, R.K. 1996. Upper limits to mass-specific metabolic rates. Annu. Rev. Physiol. 58:583-605.

Termonia, Y. and J. Ross. 1981. Oscillations and control features in glycolysis: Numerical analysis of a comprehensive model. Proc. Natl. Acad. Sci. U.S.A. 78:2952-2956.

Thomas, S. and D.A. Fell. 1998. A control analysis exploration of the role of ATP utilization in glycolytic-flux control and glycolytic-metabolite-concentration regulation. Eur. J. Biochem. 258:956-967.

Tornheim, K. 1988. Fructose 2,6-bisphosphate and glycolytic oscillations in skeletal muscle extracts. J. Biol. Chem. 263:2619-2624.

Tornheim, K. and J.M. Lowenstein. 1973. Oscillations in metabolite concentrations during the operation of the cycle in muscle extracts. J. Biol. Chem. 248:2670-2677.

Tornheim, K. and J.M. Lowenstein. 1974. Interactions with oscillations of the glycolytic pathway in muscle extracts. J. Biol. Chem. 249:3241-3247.

Tornheim, K., V. Andrés, and V. Schultz. 1991. Modulation by citrate of glycolytic oscillations in skeletal muscle extracts. J. Biol. Chem. 266(24):15675-15678.

Tornheim, K. 1979. Oscillations of the glycolytic pathway and the purine nucleotide cycle. J. Theor. Biol. 79:491-541.

United States Department of Agriculture (USDA). 2002. Agricultural statistics. www.usda.gov/nass/pubs/agstats.htm.

Van Laack, R.L.J.M., S.G. Stevens and K.J. Stalder. 2001. The influence of ultimate pH and intramuscular fat content on pork tenderness and tenderization. J. Anim. Sci. 79:392-397.

Warner, R.D., R.G. Kauffman, and M.L. Greaser. 1997. Muscle protein changes post mortem in relation to pork quality traits. Meat Sci. 45:339-352.

Warriss, P.D. and S.N. Brown. 1987. The relationship between initial pH, reflectance and exudation in pig muscle. Meat Sci. 20:65-74.

Warriss, P.D., E.A. Bevis, and P.J. Ekins. 1989. The relationships between glycogen stores and muscle ultimate pH in commercially slaughtered pigs. Br. Vet. J. 145:378-383.

Wheeler, T.L., S.D. Shackelford, and M. Koohmaraie. 2000. Variation in proteolysis, sacromere length, collagen content and tenderness among major pork muscles. J. Anim. Sci. 78:958-965.

Wilson, G.G., III and R.L. van Laack. 1999. Sarcoplasmic proteins influence water-holding capacity of pork myofibrils. J. Sci. Food Agric. 79:1939-1942.

Wismer-Pedersen, J. and E.J. Briskey. 1961. Rate of anaerobic glycolysis versus structure in pork muscle. Nature 189:318.

Xu, K.Y., J.L. Zweier, J.L., and L.C. Becker. 1995. Functional coupling between glycolysis and sarcoplasmic reticulum Ca^{2+} transport. Circ. Res. 77:88-97.

Xu, K.Y., L.C. Becker. 1998. Ultrastructural localization of glycolytic enzymes on sarcoplasmic reticulum vesicles. J. Histochemistry and Cytochemistry. 46:419-427.