Introduction

The rate and extent of biochemical reactions that occur in skeletal muscle of pigs prior to, and immediately following slaughter, have a dramatic impact on the quality of pork produced. Despite efforts to understand the causes of pale, soft and exudative (PSE) pork and development of strategies to reduce its incidence, PSE pork remains a critical quality and economic concern (Cannon et al., 1996, Cassens, 2000). Much work has focused on development of PSE pork associated with the stress gene (calcium-release channel defect; Louis et al., 1993) and the Napole gene (RN-; Ellis et al., 1997). Less emphasis has been placed on biochemical characterization of inferior quality pork from pigs that do not possess these gene defects. Additionally, less emphasis has been placed on biochemical examination of superior quality pork. Understanding events that dictate superior and inferior quality is essential for development of new strategies to improve the quality and consistency of pork.

Pale soft and exudative pork is caused by the denaturation of muscle proteins that results when carcass muscles experience a low pH and high temperature (Briskey and Wismer-Pedersen, 1961). These conditions are often associated with a rapid rate of postmortem glycolysis, which generates hydrogen ions, lactate and ATP. Accumulation of hydrogen ions results in a lower pH. A summary of factors that influence postmortem pH decline is illustrated in Figure 1. The rate of glycolysis increases in response to increased 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 (PCr) 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 ADPs 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).

Glycolytic rate and extent is regulated by the activity and stability of enzymes that catalyze multiple steps of glycolysis. Moreover, the extent of pH decline is influenced by glycogen storage, as seen in muscle of RN- pigs that have elevated glycogen stores and consequently produce more hydrogen ions and lactate during postmortem glycolysis (Ellis et al., 1997). This results in reduced water-binding capacity of RN- muscles. In contrast, muscle with a higher ultimate pH generally has better water holding properties. These effects may be due to decreased protein denaturation and/or increased net protein charge, which allow greater myofilament spacing (Offer, 1991). It is not clear if the higher ultimate pH of pork is due to less glycogen storage, improved muscle buffering capacity, less stable glycolytic enzymes, or combinations of these and other mechanisms.

We hypothesized that superior pork water holding capacity and color are associated with
reduced glycolytic enzyme capacity, reduced glycolytic potential, elevated muscle buffering capacity, a lower proportion of fast myosin heavy chain isoforms, increased ability to replenish ATP via non-glycolytic reactions, and more total heme pigment.

**Postmortem pH Decline**

![Diagram](image_url)

Figure 1. Factors expected to influence postmortem pH decline. Traits designated (+) are expected to be positively associated with pH decline, whereas traits designated (-) are expected to be inversely related to pH decline.

**Procedures**

*Hog populations, meat quality and sample collection.* HAL-1843™ free Pietrain or Duroc sires (Experiment 1) and Berkshire or Yorkshire sires (Experiment 2) were used to inseminate Yorkshire and F₁ Yorkshire-Landrace sows. Sires were selected to produce offspring that exhibit contrasting pork quality traits. Progeny were raised in uniform conditions at the MSU Swine and Teaching Research Farm. Sixteen pigs per sire breed were used. Loin quality and biochemical traits were measured on *longissimus* muscle samples removed between the 11th rib and the center of the lumbar region as described by Allison et al. (2002; Experiment 1) and Ritter et al. (2001; Experiment 2). Biochemical characteristics were measured on samples obtained at 20 min postmortem. Samples were frozen in liquid nitrogen and stored at -80°C until analysis.

*Glycolytic Enzyme Analysis.* Phosphofructokinase and pyruvate kinase activities were quantified using coupled-enzyme assays as described by Allison et al. (2002). It should be noted that maximal enzyme activity measured under controlled in vitro conditions reflects capacity to catalyze a specified reaction, rather than in vivo enzyme activity.

*Quantification of glycolytic potential.* Muscle samples obtained at 20 min postmortem were extracted in 0.6 N perchloric acid and glycogen was hydrolyzed with amyloglucosidase as described by Dalrymple and Hamm (1973). Glycogen, glucose, and glucose-6-phosphate were quantified by enzymatic analysis (Sigma, No. 16-UV). Lactate was quantified using a lactate dehydrogenase assay (Sigma, No. 826). Glycolytic potential is expressed as μmol lactate equivalents/g muscle, and is calculated as follows: Glycolytic Potential = 2(μmol glycogen + glucose + glucose-6-phosphate) + lactate.
Quantification of myosin heavy chain isoforms and total heme pigment (fiber type indices). Myosin heavy chain (MHC) isoforms were quantified using the procedure of Talmadge and Roy (13). This electrophoretic procedure produces three MHC bands corresponding to types I, IIa and IIx/IIb isoforms. Total heme pigment was quantified using the method of Warriss (1979).

Determination of buffering capacity. Buffering capacity was determined using a modification of the method described by Puolanne and Kivikari (2000) for postrigor meat. Longissimus samples obtained at 20 minutes postmortem were homogenized in 10 volumes iodoacetate KCl solution (Bendall, 1973). The pH of the homogenate was adjusted to ~7.0 with 0.1 N NaOH and titrations were performed at room temperature using 100 µL aliquots of 0.1 N HCl. Titration curves for the pH range 7.0 to 5.3 were obtained and buffering capacity calculated as $BC = \frac{?A}{?pH}$, where $BC$ = average buffering capacity between the initial and final pH, $?A$ = increment of acid, and $?pH$ = corresponding change in pH.

Quantification of creatine phosphate, ATP and R value. Creatine phosphate and ATP were determined using enzymatic procedures (Lamprecht et al., 1974). R value was determined as described by Honikel and Fischer (1977).

Results and Discussion

Experiment 1 was designed to determine if capacity of pyruvate kinase and phosphofructokinase (important regulatory enzymes of glycolysis) explains variation in meat quality of pigs that are HAL-1843 free. Pietrain-sired hogs produced lighter weight carcasses with larger loin muscle areas than Duroc-sired hogs at a similar age. No differences in pH (20, 45, 180 min or 24 h), color, water-holding capacity or glycolytic enzyme activity were detected between sire groups (P = 0.06 for DRIP6, Pietrain>Duroc; all other traits P > 0.15). Although meat quality traits were highly correlated to each other, the capacity of pyruvate kinase was not correlated with LM pH (20, 45, 180 min or 24 h), purge, drip loss, or CIE L* (P > .2). However, phosphofructokinase capacity was inversely related to fluid loss (P < .05). This finding was unexpected, but may result from PFK becoming partially denatured and inactivated by 20 min postmortem in samples that undergo a rapid pH decline. Capacity of glycolytic enzymes did not differ in loin muscle obtained from Berkshire or Yorkshire sired pigs in experiment 2, although loin chops from Yorkshire progeny were lighter and generally more exudative than loin chops from Berkshire progeny. Collectively, these data indicate that lighter pork color and reduced water-holding capacity are not associated with an increase in the capacity of enzymes that catalyze regulated steps of glycolysis.

In experiment 2, carcasses from Yorkshire-sired pigs were leaner, yielded more pounds of fat-free lean, produced heavier ham and trimmed loins, but lighter bellies than carcasses of similar weight from Berkshire-sired pigs (P<0.5). Loin muscle protein, fat and dry matter content did not differ between breed groups. Berkshire progeny tended to have lower loin muscle glycolytic potential (P<0.06) and had higher loin muscle pH (less acidic) at 180 min and 24 h postmortem (P<.05). Glycolytic potential of loin muscle did not differ between Duroc and Pietrain-sired pigs. R-value is the ratio of inosine monophosphate and inosine to adenine nucleotides (Honikel and Fischer, 1977). A higher R-value is indicative of more rapid energy depletion. Loin muscle from Berkshire-sired pigs had lower R-values at 20 min postmortem than Yorkshire-sired pigs (P<0.05). Lower R-values are consistent with the more gradual pH decline
observed in Berkshire-vs Yorkshire-sired pigs. No differences in R-value were observed between loin muscle from Duroc and Pietrain sired pigs. Additionally, no differences in muscle creatine phosphate, ATP concentrations or buffering capacity were observed between breed groups in either experiment.

Loin chops from Berkshire-sired pigs had higher subjective loin color and marbling scores, and lower (darker) Minolta CIE L* values on d 1 postmortem (P<.05), but did not differ from Yorkshire progeny in total muscle heme pigment concentration. Additionally, myosin heavy chain isoform distribution, which is related to muscle fiber type, did not differ between breed groups. In experiment 2, a harvest day by breed interaction existed for loin muscle temperature at 20 min postmortem, drip loss and fluid loss measured by the filter paper method (P<.05). Yorkshire progeny had higher 20 min loin muscle temperatures and over twice the fluid loss measured by the suspension drip method and the filter paper method on the second harvest day when compared to the first harvest day. It is also interesting to note that on harvest day 2, myosin heavy chain isoforms IIA and IIX/b were correlated to 20 min pH (.61 and -.64, respectively; P<.02), 24 h pH (.61 and -.57, respectively; P<.03) and drip loss (-.51 and .54, respectively; P<.06). Vacuum packaged loin sections from Berkshire-sired pigs had less fluid loss when stored at 4°C for 7 d (P<.003), but both breed groups exhibited similar loin chop tenderness.

In summary, our data suggest that breed differences in color and water-holding capacity are not explained by differences in total heme pigment concentration, glycolytic enzyme capacity, or buffering capacity. Slightly lower glycolytic potential in loin muscle of Berkshire pigs coincided with a slightly higher ultimate pH compared to loin muscle of Yorkshire sired pigs (pH 5.5 vs 5.4; P<.05). However, it seems likely that loin chops from Berkshire-sired pigs had more desirable color and better water-holding capacity due to a more gradual muscle pH decline within the first 180 min postmortem. Yorkshire-sired pigs and/or carcasses responded adversely to conditions on harvest day 2 that resulted in elevated loin muscle temperature and increased fluid loss. Differences in meat quality characteristics between sire-breeds do not generally appear to be associated with the proportion of fast myosin isoforms, although a higher proportion of type IIb/x myosin isoforms may contribute to accelerated pH decline under less favorable antemortem or postmortem conditions. The differences in loin muscle temperature and pH observed between Berkshire- and Yorkshire-sired pigs are likely to be associated with muscle energy consumption. We are currently exploring the relationships between muscle ATPase capacity and pH decline, which influences pork loin color and water-holding characteristics.

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Literature Cited


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Matthew E. Doumit earned a B.S. degree in Animal Science from Washington State University, and M.S. and Ph.D. degrees from South Dakota State University and Michigan State University, respectively. Matt worked for two years as a post-doctoral research associate in the Meats Research Unit at the U.S. Meat Animal Research Center in Clay Center, NE, where he studied factors influencing meat tenderness. In 1996, he joined the faculty at Michigan State University and is currently an Assistant Professor in the Departments of Animal Science and Food Science? Human Nutrition. Dr. Doumit has a 50% teaching and 50% research appointment. He teaches courses in Animal Products, Meat Science and Muscle Biology, and Animal Growth. For his teaching efforts, Dr. Doumit was awarded the Outstanding Young Scientist Award in Teaching by the Midwestern Section of the American Society of Animal Science in 2000. Matt conducts research in the areas of muscle growth and meat quality. His primary focus is on biochemical regulation of meat color, water-holding capacity and tenderness.

BACK TO TABLE OF CONTENTS