RESEARCH REPORT
PORK QUALITY

Title: Biochemical Characterization of Pork Quality - **NPB#01-117**

Investigator: Matthew E. Doumit

institution: Michigan State University

Date Received: September 2, 2003

Abstract

Pork loin color and water-holding capacity are influenced by the rate and extent of postmortem muscle acidification (pH decline). The objective of this research was to determine the relationships between pork loin color, water-holding capacity, pH decline and 1) capacity of muscle ATPase activities, 2) buffering capacity, 3) capacity to generate ATP via creatine phosphokinase and myokinase reactions, and 4) protein denaturation. Pietrain or Duroc sires (Experiment 1) and Berkshire or Yorkshire sires (Experiment 2) were used to produce HAL-1843^{TM}-free offspring that exhibit contrasting pork quality traits. 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. R-values determined at 45 and 180 min postmortem, which increase with ATP utilization, were negatively correlated with 45 min and 180 min pH. R-values were also positively associated with 24 hour drip loss from loin chops (P<.02) and purge from vacuum packaged loin sections (P<.01). Although the rate of muscle energy utilization is undoubtedly linked to the activity of ATPases in early postmortem muscle, a faster or slower rate of energy utilization does not appear to be associated with a greater or lesser tissue capacity of myofibrillar or SR calcium-ATPases in pig longissimus muscle. Muscle buffering capacity was positively correlated with glycolytic potential (r = .53; P<.05) and fluid loss (r = .51; P<.05) in experiment two. The latter suggests that elevated buffering capacity may be an adaptation to more frequent or severe acidification of living muscle in some pigs, which also corresponds to production of inferior pork from these animals.

Creatine phosphokinase and myokinase activity at 24 hours postmortem was over 90% and 84%, respectively, of that present at 20 min postmortem. Myokinase activity (24 hours postmortem) was negatively correlated with purge from pork loin sections (P<.03). In Duroc and Pietrain pigs, the activities of both myokinase and creatine kinase at 24 hours postmortem were positively correlated with early postmortem pH (P<.05). The lack of relationship between enzyme activity derived from samples at 20-minute postmortem and early postmortem pH suggests that a greater quantity of these enzymes does not provide a sparing effect on early postmortem glycolysis. Reduced activity of these enzymes and the myofibrillar ATPase, which was also positively correlated with early postmortem pH and negatively correlated with drip loss (P<.05) in experiment one, suggest that denaturation and reduced solubility of both myofibrillar and sarcoplasmic proteins is associated with reduced water-holding capacity. No consistent relationships were observed between denaturation of sarcoplasmic or myofibrillar proteins and pork color. Collectively, these data suggest that inferior pork water-holding capacity is caused by accelerated ATP utilization and pH decline that leads to protein denaturation. Accelerated ATP utilization is not associated with an increased quantity of ATPases or an insufficient quantity of ATP-generating enzymes. Physical or psychological stressors may trigger elevated ATP utilization, or rapid ATP utilization may result from a metabolic disorder similar to that created by the Hal-1843 mutation, which results in abnormal calcium homeostasis.

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 (1, 2). Much of the work in this area has focused on development of PSE pork associated with the stress gene (HAL-1843^{TM}, calcium-release channel defect; 3) and the Napole gene (RN-; 4). 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 (5). These conditions are often associated with a rapid rate of postmortem glycolysis, which generates lactate, hydrogen ions and ATP. 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 sarcoplasmic reticulum (SR) calcium ATPase that functions to resequester calcium into the SR (6). In living muscle, 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 ADPs to ATP and AMP (6). These reactions may have a sparing effect on the rate of postmortem glycolysis. However, both myokinase and creatine phosphokinase appear to be susceptible to denaturation as acidic postmortem conditions develop (7).

The activity and stability of enzymes that catalyze multiple steps of glycolysis potentially regulate the rate and extent of postmortem 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 lactate and hydrogen ions during postmortem glycolysis (4). 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 allows greater myofilament spacing (8). The higher ultimate pH of pork may be 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 ATPase capacity, elevated muscle buffering capacity, increased ability to replenish ATP via non-glycolytic reactions, and reduced denaturation of myosin and sarcoplasmic proteins.

Objectives:

The objective is to determine the relationships between pork loin color, water-holding capacity, postmortem pH decline and 1) capacity of muscle ATPases, 2) muscle buffering capacity, 3) capacity of creatine phosphokinase and myokinase to generate ATP, and 4) protein denaturation as measured by enzyme inactivation. This information will clarify the relative effect that these systems have on the rate and extent of pH decline and subsequent pork quality.

Materials & Methods:

Hog populations, meat quality and sample collection. HAL-1843^{TM} free Pietrain or Duroc sires (Experiment 1) and Berkshire or Yorkshire sires (Experiment 2) were used to inseminate Yorkshire and F_1 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. (9; Experiment 1) and Ritter et al. (10; Experiment 2). At 20, 45,

180 min and 24 h postmortem, samples were removed, cut into 0.5 cm^3 pieces, frozen in liquid nitrogen, and stored at -80°C until analyses were performed. *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 (11). 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([glycogen] + [glucose] + [glucose-6-phosphate]) + [lactate].

Quantification of myosin heavy chain isoforms and total heme pigment (fiber type indices). Myosin heavy chain (MHC) isoforms was quantified using the procedure of Talmadge and Roy (12). 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 (13).

Quantification of ATPase activities. Myofibrillar and sarcoplasmic reticulum calcium-ATPase activities were quantified as previously described (14). ATPase activities were measured by coupled assays in which oxidation of NADH was followed spectrophotometrically.

Determination of buffering capacity. Buffering capacity was determined using a modification of the method described by Puolanne and Kivikari (15) for postrigor meat. Longissimus samples obtained at 20 minutes postmortem were homogenized in 10 volumes iodoacetate KCl solution (16). 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 $BC = \Delta A / \Delta pH$, where BC = average buffering capacity between the initial and final pH, ΔA = increment of acid, and ΔpH = corresponding change in pH.

Quantification of creatine phosphate, ATP, R value, creatine phosphokinase and myokinase activities. Creatine phosphate and ATP were determined using enzymatic procedures (17). R-value was determined as described by Honikel and Fischer (18). R-value is the ratio of inosine monophosphate and inosine to adenine nucleotides. Coupled enzyme assays were used to quantify creatine phosphokinase (19) and myokinase (20) activities.

Results:

Pork loin color, water-holding capacity and postmortem pH decline measurements were completed as part of previous NPB-funded projects and have been included in the final reports for project #99-072 and #00-070. Because Duroc- and Pietrain-sired pigs (experiment one) were raised and harvested prior to Berkshire- and Yorkshire-sired pigs (experiment two), statistical comparisons were made between Berkshire- and Yorkshire-sired pigs or between Duroc- and Pietrain-sired pigs. Pietrain-sired pigs produced lighter weight carcasses with larger loin muscle areas than Duroc-sired pigs at a similar age. No differences in pH (20, 45, 180 min or 22 h), color, water-holding capacity or glycolytic enzyme activity were detected between Pietrain- and Duroc-sired pigs (9). In experiment two, carcass weights did not differ by breed, but carcasses from Yorkshire-sired pigs were leaner, yielded more pounds of fat-free lean, produced heavier hams and trimmed loins, but lighter bellies than carcasses from Berkshire-sired pigs ($P<.05$). Loin muscle protein, fat and dry matter content did not differ between breed groups. Loin chops from Berkshire-sired pigs had higher subjective loin color and lower (darker) Minolta CIE L* values on day 1 postmortem ($P<.05$), but did not differ from Yorkshire progeny in total muscle heme pigment concentration. Berkshire progeny tended to have lower ($P<0.06$) loin muscle glycolytic potential and higher ($P<.05$) muscle pH (less acidic) at 180 min and 24 h postmortem compared with Yorkshire-sired pigs. Glycolytic potential of loin muscle did not differ between Duroc and Pietrain-sired pigs.

The first objective of this study was to determine the relationship between the capacity of muscle ATPases and pork loin muscle pH decline, color or water-holding capacity. R-value, creatine phosphate and ATP were measured to determine the energy status of the longissimus muscle over time postmortem. Creatine phosphate and ATP did not differ between breed groups in either

experiment. R-value is the ratio of inosine monophosphate and inosine to adenine nucleotides (18). A higher R-value is indicative of more rapid energy (ATP) depletion and subsequent deamination of adenosine monophosphate or adenosine to inosine monophosphate or inosine, respectively. Loin muscle from Berkshire-sired pigs tended to have lower early postmortem R-values than Yorkshire-sired pigs. Lower R-values are consistent with the higher ultimate pH and 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. However, R-values obtained at 45 and 180 min were negatively correlated with 45 min and 180 min pH in both experiments (P<.01). These R-values were also associated with 24 hour drip loss from loin chops (r = .63 to .87; P<.02) and purge from vacuum packaged loin sections (r =.60 to .88; P<.01) in both experiments.

Myofibrillar and SR-calcium ATPase did not differ between sire groups and no consistent relationships existed between the *in vitro* activity (capacity) of either ATPase and pork color or water-holding capacity. Additionally, myosin heavy chain isoform distribution did not differ between breed groups. In experiment two, 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 water 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 (intermediate speed of contraction) and IIx/b (fast contracting) were correlated with 20 min pH (r = .61 and -.64, respectively; P<.02), 24 h pH (r = .61 and -.57, respectively; P<.03) and drip loss (r = -.51 and .54, respectively; P<.06).

The second objective of this study was to determine the relationship between muscle buffering capacity and pork loin muscle pH decline, color or water-holding capacity. Buffering capacity did not differ between sire groups in either experiment. Likewise, there was no apparent relationship between buffering capacity and pork quality traits in experiment one. In contrast, buffering capacity of the longissimus was positively correlated with glycolytic potential (r = .53; P<.05), drip loss (r = .51; P<.05) and water loss induced by high speed centrifugation (r = .52; P<.05) in experiment two.

The third objective of this study was to determine the relationships between the ATP generating capacity of creatine phosphokinase or myokinase and pork loin muscle pH decline, color or water-holding capacity. Creatine phosphokinase activity did not differ among sire breeds. However, loin muscle from Duroc-sired pigs had higher (P<.05) myokinase activity than Pietrain-sired pigs. At 24 hours postmortem, creatine phosphokinase and myokinase retained over 90% and 84%, respectively, of the activity present at 20 min postmortem. Myokinase activity (24 hour) was negatively correlated with purge from pork loin sections in both experiment one and two (r = -.61 and -.53, respectively; P<.03). In experiment one, the activities of both myokinase and creatine kinase at 22 hours postmortem were positively correlated with early postmortem pH (20, 45 and 180 minute; P<.05). The lack of relationship between capacity of these enzymes measured on 20 minute postmortem samples and early postmortem pH suggest that a greater quantity of these enzymes does not provide a sparing effect on early postmortem glycolysis.

The fourth objective of this study was to determine the relationships between protein denaturation, evidenced by enzyme inactivation, and pork loin muscle pH decline, color or water-holding capacity. Although differences in myofibrillar protein solubility between pre-rigor and post-rigor muscle samples complicates comparison of myofibrillar ATPase activity over time, post-rigor myofibrillar ATPase activity was positively correlated with early postmortem pH (P<.05) and negatively correlated with drip loss. This is similar to the observations described above for creatine phosphokinase and myokinase. No consistent relationships were observed between denaturation of sarcoplasmic or myofibrillar proteins and pork color.

Discussion:

A more rapid rate of muscle energy utilization is clearly associated with a more rapid pH decline (acidification) leading to inferior pork water-holding capacity. Although the rate of muscle energy utilization is undoubtedly linked to the activity of ATPases in early postmortem muscle, it does not appear to be associated with a greater capacity of myofibrillar or SR calcium-ATPases. In other words, ATPase capacity was not in excess in those longissimus muscles that produce inferior quality pork, nor was it limiting in muscles that produce superior quality pork. Likewise, the capacity of the muscle to generate ATP via non-glycolytic pathways does not appear to be greatly compromised by irreversible protein denaturation. However, the declining pH of postmortem tissue may substantially reduce the actual activity of these enzymes.

In experiment two, 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. Although differences in meat quality characteristics between sire-breeds did not appear to be associated with an increased ATPase capacity or the proportion of fast myosin isoforms, a higher proportion of type IIb/x myosin isoforms appear to have contributed to the accelerated pH decline under less favorable antemortem or postmortem conditions on harvest day 2.

Greater buffering capacity of muscle was expected to be associated with a more gradual pH decline and a higher ultimate pH. On the contrary, buffering capacity was positively associated glycolytic potential and fluid loss in experiment two, indicating that loin muscle with a higher buffering capacity also had greater glycogen stores and became more exudative upon conversion to meat. Elevated buffering capacity may represent an adaptation of the living muscle to more frequent bouts of acidification. This has been demonstrated for rapid glycolyzing, white muscle compared to red muscle (15). Elevated buffering capacity of pork longissimus muscle observed in this study was insufficient to prevent development of inferior water-holding capacity, and may indicate a metabolic adaptation to more frequent or severe acidification of living muscle of some pigs.

Post-rigor activity of both myofibrillar ATPase, myokinase and creatine phosphokinase was positively associated with pre-rigor pH and negatively associated with fluid loss, whereas little association was observed between these traits and enzyme activity measured on early postmortem tissues. Collectively, these data indicate that these enzymes denature under low pH conditions early postmortem. Denaturation of both these enzymes are associated with reduced water-holding capacity of pork loin chops, but have no apparent effect on pork color. Samples with relatively high enzyme activity at 24 hours postmortem are associated with slower apparent pH declines and better water-holding capacity.

Collectively, these data suggest that inferior pork water-holding capacity is caused by accelerated ATP utilization and pH decline that leads to protein denaturation. Accelerated ATP utilization is not associated with an increased quantity of ATPases or an insufficient quantity of ATP-generating enzymes. Physical or psychological stressors may trigger elevated ATP utilization, or rapid ATP utilization may result from a metabolic disorder similar to that created by the Hal-1843 mutation, which results in abnormal calcium homeostasis.

Literature Cited

1. Cannon, J.E., J.B. Morgan, F.K. McKeith, G.C. Smith, S. Sonka, J. Heavner, and D.L. Meeker. 1996. J. Muscle Foods. 7:29.
2. Cassens, R.G. 2000. Food Chem. 69:357-363.
3. Louis, C.F., W.E. Rempel, and J.R. Mickelson. 1993. RMC Proc. 46:89.
4. Ellis, M., F.K. McKeith and D.S. Sutton. 1997. Pork Quality Summit Proceedings p 49.
5. Briskey, E.J. and J. Wismer-Pedersen. 1961. J. Food Sci. 26:297.
6. Bechtel, P.J. and P.M. Best. 1985. Reciprocal Meat Conf. Proc. 48:26-31.
7. Joo, S.T., R.G. Kauffman, B.C. Kim and G.B. Park. 1999. Meat Sci. 52:291-297.
8. Offer, G. 1991. Meat Sci. 30:157-184.
9. Allison, C.P., R.O. Bates, A.M. Booren and M.E. Doumit. 2003. Meat Science. 63:7-22.
10. Ritter, M.J. 2002. MS Thesis. Michigan State University.

11. Dalrymple, R.H. and R. Hamm. 1973. J. Food Technol. 8:439.
12. Talmadge, R.J. and R.R. Roy. 1993. J. Appl. Physiol. 75:2337.
13. Warriss, P.D. 1979. The extraction of haem pigments from fresh meat. J. Food Technol. 14:75-80.
14. Simonides, W.S. and C. van Hardeveld. 1990. Anal. Biochem. 191:321-331.
15. Puolanne, E. and R. Kivikari. 2000. Meat Science. 56:7-13.
16 Bendall, J.R. 1973. In G.H. Bourne (Ed.), The structure and function of muscle (pp. 227-274. New York: Academic Press.
17. Lamprecht, W., P. Stein, F. Heinz, and H. Weisser. 1974. In "Methods of Enzymatic Analysis" Vol. 4, p.1781. Academic Press, Inc., New York, NY.
18. Honikel, K.O. and Fischer, C. 1977. J. Food Sci. 42:1633-1636
19. Szasz, G., W. Gruber annd E. Bernt. 1976. Creatine kinase in serum: 1. Determination of optimum reaction conditions. Clin. Chem. 22:650.
20. S.E. Brolin, 1974. In "Methods of Enzymatic Analysis" Vol. 4, p. 550. Academic Press, Inc., New York, NY.

Lay Interpretation:

Project Title: Biochemical Characterization of Pork Quality - NPB#01-117

Sires from Duroc, Berkshire, Yorkshire and Pietrain breeds were used to produce crossbred progeny, free of the HAL-1843^{TM} gene, that exhibit pork quality differences. Loin muscle temperature, color, firmness/wetness, marbling, and water-holding capacity were determined for sixteen progeny within each sire group (n=64 total). Rapid energy (ATP) consumption in muscle is associated with rapid breakdown of glycogen (glycolysis) and accumulation of hydrogen ions, which cause muscle acidification (pH decline). Rapid muscle acidification early postmortem, while the muscle temperature is still relatively high, causes protein denaturation and reduced protein solubility, which leads to production of pale, soft and exudative products. To better understand the regulation of postmortem energy utilization, muscle acidification and the consequences of these events, estimates of ATP utilization were made, and the capacities of enzymes that regulate energy utilization and production were quantified. Additionally, the ability of muscle to buffer the effects of acidification and the relationship between denaturation of specific proteins and pork quality was determined. Our results confirm that inferior pork water-holding capacity is caused by accelerated ATP utilization and pH decline that leads to denaturation of both contractile and soluble proteins. However, accelerated ATP utilization does not appear to be associated with an increased quantity of ATP-utilizing enzymes or an insufficient quantity of ATP-generating enzymes. Additionally, pork loin muscles with high buffering capacity exhibited a higher degree of fluid loss than those with lower buffering capacity. The latter suggests that elevated buffering capacity may be an adaptation to more frequent or severe acidification of living muscle in some pigs, which also corresponds to production of inferior pork from these animals. Physical or psychological stressors may trigger elevated ATP utilization, or rapid ATP utilization may result from a metabolic disorder similar to that created by the Hal-1843 mutation, which results in abnormal calcium homeostasis. Further elucidation of these mechanisms will lead to strategies that enable consistent production of high quality pork.

Contact Information:

Dr. Matthew E. Doumit, Associate Professor
Departments of Animal Science and Food Science & Human Nutrition
3385B Anthony Hall, Michigan State University
East Lansing, MI 48824
Phone: (517) 355-8452 ext. 203 Fax: (517) 432-0753 e-mail: doumitm@msu.edu