RESEARCH REPORT
PORK QUALITY

Title: Impact of Proteolysis on pH Decline and Water Holding Capacity of Fresh Pork - **NPB #02-034**

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Abstract: The objective of this study was to determine the relationship between pH decline, µ-calpain autolysis, and degradation of membrane proteins involved in calcium homeostasis. Commercial hybrid pigs (n = 309) from two different genetic lines (lines 1 and 2) were harvested. Longissimus dorsi (LD) pH measurements were taken at 45 min, 3, 6, and 24 h postmortem (PM). In order to obtain animals with variability in pH measurements, two groups (high and low pH at 3 h PM) were selected within each line. Percent drip loss after 24, 48, and 96 h storage was determined. Ten animals were selected within each group to determine autolysis of µ-calpain and extent of proteolysis of the ryanodine receptor (RyR1), and the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2) at 1 d, 48, 72, and 120 h PM. The line 1/high pH group had a higher ($P < 0.05$) pH than all other groups at 24 h PM and the least ($P < 0.05$) drip after 96 h storage. The line 2/low pH group had the highest ($P < 0.05$) L* value than all other groups. At 1 d postmortem, the high pH groups had increased ($P < 0.05$) µ-calpain autolysis compared to the low groups. Increased autolysis of µ-calpain was correlated ($P <0.05$) to higher pH and decreased drip. Line 2 had more ($P < 0.05$) RyR1 degradation (band 2) than line 1 at 48 h PM. An increase in RyR1 degradation (band 2) was related to lower 6 h ($P < 0.05$) and 24 h ($P < 0.01$) pH and increased drip loss at all times measured ($P < 0.01$). Increased proteolysis of intact RyR1 at 1 d and 48 h was correlated ($P < 0.01$) to increased µ-calpain autolysis. Increased proteolysis of SERCA2 was correlated ($P < 0.05$) with lower pH and increased drip loss. Decreased degradation ($P < 0.05$) of vinculin was found in line 1/low pH compared to both line 2 groups. These results show that µ-calpain autolysis and proteolysis may be related to Ca^{2+} regulation and may affect pork quality attributes such as water-holding capacity.

Introduction: One of the most prevalent pork quality issues in the industry today is unacceptably high purge loss in fresh and minimally processed products. Purge results in economic losses in numerous ways including reduction of salable product weight and the loss of export customers who demand high quality product with a minimum amount of purge upon delivery.

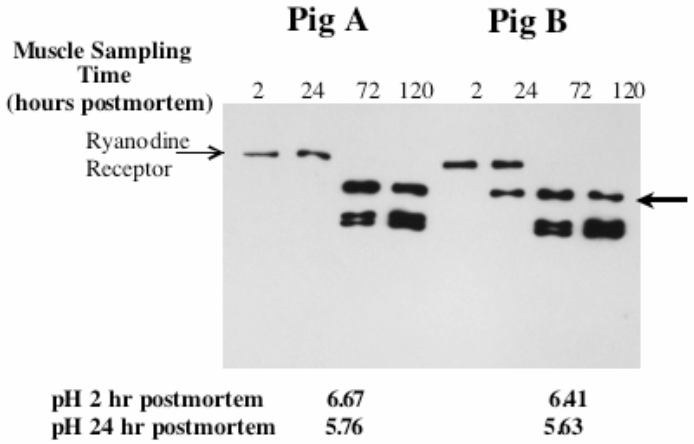
Accelerated pH decline and low ultimate pH are related to the development of low water-holding capacity and unacceptably high purge loss. Rapid pH decline resulting in ultimate or near ultimate pH while the muscle is still warm causes the denaturation of many proteins, including those involved in binding water. On the other hand, a normal rate of pH decline that results in a very low ultimate pH also negatively influences the water-holding capacity of the product (Lonergan et al., 2001) Factors controlling the rate and the extent of pH decline need to be fully explored if researchers are to ever identify ways to predict and control water-holding capacity.

The most severe purge or drip loss is often found in PSE product from pigs that have inherited a mutation in the ryanodine receptor/calcium release channel (halothane gene) in the sarcoplasmic reticulum. This mutation results in impairment of the ability of this channel to control calcium release into the sarcoplasm of the muscle cell, particularly under periods of physical stress. Accelerated release of calcium causes rapid contraction and an increase in the rate of muscle metabolism and in the rate of pH decline. This particular mutation in the halothane gene can be identified in parent stock. Because a commercial test for this mutation exists, the industry is making great strides in reducing its incidence. However, poor water-holding capacity can be found in lines of pigs that have been tested and found to be free of this genetic defect (Lonergan et al., 2001). This points to the fact that other factors in muscle need to be examined to continue to make progress in improving water-holding capacity of fresh pork. Because calcium is an important mediator of muscle metabolism, it is highly likely that other perturbations in calcium regulation may be at the root of other unexplained variations in the rate of pH decline.

Another important calcium regulatory protein in muscle is the sarcoplasmic reticulum Ca^{2+}-ATPase pump (SERCA). This pump removes calcium from the sarcoplasm and returns it to the sarcoplasmic recticulum. Calcium sequestered in the sarcoplasmic reticulum is typically not available to participate in metabolic processes in the sarcoplasm until released again by the ryanodine receptor. This calcium pump functions as long as ATP is available during the early postmortem period, a time period when it is critical to maintain low levels of calcium in the cell.

Both the ryanodine receptor and the sarcoplasmic reticulum Ca^{2+}-ATPase pump have been suggested to be substrates of the endogenous calcium dependent μ- and m-calpain enzymes (Iino et al., 1992; Purintrapiban et al., 2001; Shevchenko et al., 1998; Wingertzahn and Ochs, 1997). It is possible that degradation of the sarcoplasmic reticulum Ca^{2+}-ATPase impairs its ability to remove calcium from the sarcoplasm. This in turn could lead to accelerated metabolism and accelerated pH decline.

The ryanodine receptor has been shown to be cleaved by the proteolytic enzyme μ-calpain. This cleavage stimulates Ca^{2+} entry into the cell (Shoshan-Barmatz et al., 1994). This fact may be very important in postmortem tissue. Our lab has novel data indicating that in product with an accelerated rate of pH decline, the ryanodine receptor appears to be degraded more quickly (Figure 1). While this result needs to be further explored in a larger population, it provides an intriguing possibility; the possibility that earlier postmortem cleavage of the ryanodine receptor could be associated with accelerated pH decline. This current project was based on these results.



Immunoblot of degradation of the ryanodine receptor. The heavy arrow shows a major degradation product that appears early. This band appears more quickly in products with a lower pH.

Figure 1.

One of the features of the enzyme µ-calpain is its propensity to autolyze (undergo specific cleavage) when exposed to sufficient amounts of calcium. While complete autolysis inactivates µ-calpain, initial autolysis actually lowers the amount of calcium µ-calpain needs to be active. Autolysis of the catalytic subunit of µ-calpain can be followed using highly sensitive immunoblotting techniques (Figure 2). During autolysis, the 80 kDa catalytic subunit is progressively degraded to a 78 kDa and then to a 76 kDa peptide. Results of a project in our lab that was funded by NPB (2000) showed that in product with an accelerated pH decline (but not PSE), µ-calpain was autolyzed more rapidly (Rowe et al., 2001a, b). This same project has shown that calpastatin, the endogenous inhibitor of µ- and m-calpains, loses activity more quickly in samples with accelerated pH decline (Melody et al., 2004). Interestingly, in the preliminary studies mentioned above, samples with earlier degradation of the ryanodine receptor also had more rapid pH decline and more rapid autolysis of µ-calpain (Figures 1 and 2). These results indicate the importance of further characterizing the relationship between degradation of proteins involved in muscle cell calcium regulation (the ryanodine receptor and the sarcoplasmic reticulum Ca^{2+}-ATPase) and the calpain system, particularly µ-calpain and calpastatin.

Description of the Research Project: This project is testing new hypothesis governing the mechanism behind pH decline. The PI's had novel preliminary data regarding the potential interaction between the ryanodine receptor (calcium release

channels in muscle) and components of the calpain system. This project is examined these relationships in lines of pigs from a commercial company. This project provided relevant information that will aid in allowing producers and processors to improve pork quality.

Objectives: The **central hypothesis** for the proposed research was: *Protelolytic processes in early postmortem pork influence pH decline and water-holding capacity.* We plan to test this central hypothesis by accomplishing the following specific research objectives.

Objective 1. Determine the relationship between pH decline, μ-calpain autolysis and degradation of membrane proteins involved in calcium homeostasis. The hypothesis behind this objective is: *Earlier postmortem degradation of the ryanodine receptor and/or the sarcoplasmic Ca^{2+}-ATPase pump results in more rapid pH declin*e.

Objective 2. Determine the relationship between pH decline and alterations of key muscle proteins. The hypothesis behind this objective is: *Early postmortem pH decline and water-holding capacity are influenced by degradation or post-translational modifications of specific muscle proteins involved in regulating postmortem processes.*

Materials and Methods:

Animals

Three-hundred and nine commercial hybrid pigs from two different genetic lines (Line 1 and Line 2) were used in this study. Both lines are free of the halothane and RN^- gene.

The pigs were harvested using humane procedures at a commercial slaughter facility. Measurements for pH (Mettler-Toledo glass tipped probe, Mettler-Toledo Process Analytical Inc., Wilmington, MA) were taken at 45 min, 3 h, and 6 h postmortem in the longissimus dorsi (LD) on the right side of the carcass at the last rib. The 24 h pH was taken in the center of the longissimus dorsi after its removal from the carcass. At 45 min and 3 h postmortem, pH was measured on three hundred and nine pigs, with one hundred and forty-nine animals from line 2 and one hundred and sixty animals from line 1 measured. To determine which pigs had substantial differences in pH, the 3 h postmortem pH measurements were evaluated. Based on these data, groups of pigs with longissimus muscles that had relatively high and low early pH measurements were defined within each genetic line. The two groups were classified into low (pH < 5.7 at 3 h) and high (pH > 6.0 at 3 h) pH groups by using the 3 h postmortem pH data. From this data, twenty-seven animals in the low pH group and twenty-six animals from the high pH group for line 2 were selected and twenty-seven pigs in the low pH group and twenty-seven pigs in the high pH group for line 1 were selected. The 6 h and 24 h pH data were taken on the one hundred and seven selected animals from both genetic lines.

At 24 h postmortem, the loins were removed from the left side of each carcass and color (L* - muscle lightness; a* - muscle redness; b* - muscle yellowness) data was collected using a Minolta Colorimeter (CR-310, Minolta Camera Co., Ramsey, NJ), which was calibrated according to manufacturer's directions (settings: D65 illuminate, 10˚ observer). Measurements for 24 h pH were also taken at this time. The boneless loins were then vacuum packaged and immediately transported to Iowa State University, Ames, Iowa.

Drip Loss Analysis

After transport to the Iowa State University Meat Laboratory, nine 2.54-cm thick boneless loin chops were removed from each loin at 1 d postmortem, external adipose tissue was trimmed, and each chop was weighed. Three chops per animal were stored in a sealed plastic bag under atmospheric pressure at 4˚C (Lonergan et al, 2001) for an additional 24 h, 48 h, and 96 h for drip loss evaluation (nine chops total). The chops were removed from each bag and weighed after 24 h, 48 h and 96 h storage (48 h, 72 h and 120 h postmortem). Drip loss was recorded as a percentage of weight lost during storage. Chops were then vacuum packaged and stored at -20˚C until further biochemical analysis.

Whole Muscle Sample Preparation

Ten pigs from each group (line 1/high pH, line 1/low pH, line 2/high pH, and line 2/low pH) were selected for western blotting, thus whole muscle samples were made for forty animals per postmortem time point (1 d, 48 h, 72 h, and 120 h). Whole muscle samples were prepared (Huff-Lonergan et al., 1996) from previously frozen 1 d, 48 h, 72 h and 120 h postmortem samples. In order to prepare whole muscle samples, a frozen portion (0.2 g) was removed from the center of the longissimus, finely chopped, added to 5 mL whole muscle protein extraction buffer (2% SDS, 10 mM sodium phosphate, pH 7.0), and homogenized. The samples were clarified by centrifugation (1500 x g) at 20˚C for 20 min. Samples were prepared for SDS page according to Huff-Lonergan et al., 1996 and were stored at -80˚C until further analysis of μ-calpain, sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2), and ryanodine receptor (RyR1).

SDS-PAGE Electrophoresis Gels

Whole muscle samples were run on polyacrylamide gels) as described by Lonergan et al. (2001). A 9% acrylamide separating gel was used for μ-calpain analysis, an 8% acrylamide separating gel was used for SERCA2 analysis and a 6% acrylamide separating gel was used for RYR1 analysis.

SDS-PAGE of μ-calpain and RYR1 was done using Hoefer SE280 (10 cm wide x 12 cm tall x 1.5 cm thick) Tall Mighty Small gel units (Hoefer Scientific Instruments, San Francisco, CA). For SDS-PAGE of SERCA2 and vinculin, Hoefer SE260 (10 cm wide x 10 cm tall x 1.5 cm thick) Mighty Small gel units were used (Hoefer Scientific Instruments, San Francisco, CA). For SDS PAGE of μ-calpain, 120 μg of whole muscle sample was loaded per lane and gels were run at 120 V at room temperature with a molecular weight standard (Kaleidoscope Prestained Standard, Cat # 161-0324; BioRad, Richmond, CA). For analysis of RYR1, 160 μg of whole muscle sample was loaded per lane and the gel was run at room temperature overnight at a constant amperage of 3 mA. For SDS-PAGE of SERCA2, 60 μg of whole muscle sample was loaded per lane and was run at a constant voltage of 30 V overnight at room temperature. An at-death standard (whole muscle sample made from porcine longissimus dorsi immediately post-exsanguination) was loaded on all gels in all systems.

Transfer Conditions

After electrophoresis, all gels were blotted onto Poly Screen polyvinylidene difluoride (PVDF) transfer membrane (NEN Life Science Products, Inc., Boston, MA). For μ-calpain, SERCA2, a Hoefer TE22 Mighty Small Transphor unit was used to transfer proteins to the PVDF membrane. A constant voltage setting of 90 V for 90 min and a transfer buffer containing 25 mM Tris, 192 mM glycine, and 15% methanol [vol/vol] were used. A refrigerated Lauda RE106 circulating water bath (Brinkman Instruments Inc, Westbury, NY) set at 0.4˚C was used to maintain the temperature of the transfer buffer at refrigerated temperatures (for μ-calpain, SERCA2, and vinculin). Gels for RyR1 were transferred to PVDF membranes using a TE62 Transphor Cooling Unit (Hoefer Scientific Instruments, San Francisco, CA) at a constant amperage of 400

mA for 2 h followed by a constant amperage of 1000 mA for 3 h. The transfer buffer used for this system contained 25 mM Tris, 192 mM glycine, and 10% methanol [vol/vol]. The transfer buffer was kept cold using a Lauda RE106 circulating water bath set at -5.0˚C for 2.5 h followed by -10˚C for an additional 2.5 h.

Western Blotting

Western blotting was done as described by Huff-Lonergan et al. (1996). After blocking, membranes were placed in a primary antibody specific to the protein desired. Primary antibodies used in the western blotting procedure included monoclonal (mouse) anti-μ-calpain (Cat # MA3-940; Affinity BioReagents, Golden, CO) incubated for a minimum of 12 h (4˚C) at a 1:10,000 dilution in PBS-Tween; monoclonal (mouse) anti-SERCA-2 ATPase (Cat # MA3-919; Affinity BioReagents, Golden, CO) for 2 h (room temperature) at a dilution of 1:20,000 in PBS-Tween; monoclonal (mouse) anti RyR1 (Cat # MA3-925; Affinity BioReagents, Golden, CO) for a minimum of 12 h (4˚C) at a 1:40,000 dilution in PBS-Tween. Secondary antibody for all proteins was sheep-anti-mouse horseradish peroxidase (HRP) (Cat # NA931; Amersham Biosciences, Piscataway, NJ) at a 1:5000 dilution in PBS-Tween. A chemiluminescent system was used to detect the labeled protein bands. The detection reagents used were ECL Plus kit (Amersham Pharmacia Biotech, Piscataway, NJ) for μ-calpain, SERCA2 while ECL-Advance kit (Amersham Pharmacia Biotech, Piscataway, NJ) was used for RYR1. Both kits were used according to manufacturer's instructions. A 16 bit megapixel charge coupled device (CCD) camera FluorChem 8800 (Alpha Innotech Corporation, San Leandro, CA) and FlourChem IS-8800 software (Alpha Innotech, San Leandro, CA) were used to detect chemiluminescence. Densitometric measurements were determined using AlphaEase FC Version 3.1.2 (Alpha Innotech, San Leandro, CA). For analysis of μ-calpain, the 80 kDa subunit was measured using densitometry while for SERCA2 the intact band was evaluated. Densitometric measurements for RyR1 were determined on the intact band as well as two degradation products termed in this study as band 2 and the doublet band. The final values for the 80 kDa subunit of μ-calpain, intact SERCA, intact RyR1 as well as the RyR1 degradation products (band 2 and doublet) were determined as a ratio of protein in the sample compared to an at-death standard.

Statistical Analysis

Data were analyzed using the general linear models procedure of SAS (version 8.2, SAS Institute, Cary, NC). Least squares means were computed and means were separated using Tukey's test. Each of the pigs selected within group served as replicates for pH (45 min, 3 h, 6 h, and 24 h postmortem), drip loss analysis (24 h, 48 h, and 96 h storage), and analysis of μ-calpain, RYR1, SERCA2, and vinculin. Significance was determined at the $P < 0.05$ level. Main effects were separated using PDIFF when least squares means were determined significant ($P < 0.05$) in the analysis of variance table. Data was additionally analyzed using the PROC CORR procedure of SAS. Significance of the correlation was determined at the $P < 0.05$ level.

Results:

Objective 1 Determine the relationship between pH decline, μ-calpain autolysis and degradation of membrane proteins involved in calcium homeostasis

pH and drip loss data

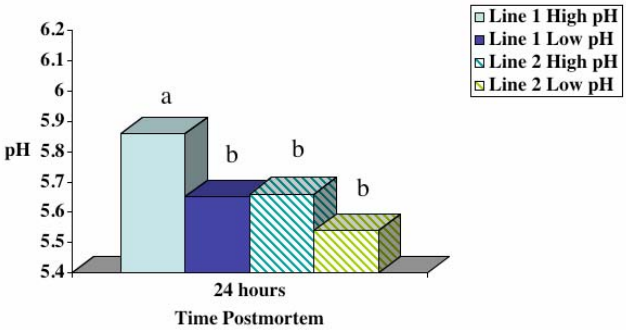
The rate and extent of pH decline have long been known to influence meat color and water-holding capacity (Briskey, 1964). In the current study, no significant differences were observed between lines at 45 min postmortem for the initial 309 pigs measured. The average pH for both lines at this time was 6.00 ± 0.02. At 3 h

postmortem, the average pH (6.00 ± 0.02) measured in line 1 was significantly higher (P < 0.01) than the average pH (5.86 ± 0.02) in line 2.

Pigs were selected according to high and low pH measurements at 3 h postmortem in order to obtain groups with different early postmortem pH measurements; thus, as expected, there were significant differences (P < 0.01) within lines between high and low pH measurements at 3 h postmortem for the 40 pigs selected for more detailed evaluation (Figure 2). Additionally, the high and low pH groups were also significantly different (P < 0.05) at 45 min and 6 h (Figure 1). At 24 h postmortem, the average pH (5.86) of the longissimus muscles from line 1/high pH group was significantly higher (P < 0.05) than all other groups (Figure 3).

a, b Means lacking a common letter within a time point differ P < 0.01
x, y, z Means lacking a common letter within a time point differ P < 0.05

Figure 2.



a, b Means lacking a common letter within a time point differ P < 0.01
x, y, z Means lacking a common letter within a time point differ P < 0.05

Figure 3.

Drip loss in the longissimus dorsi was affected by pH. The current study showed that the line 1/high pH group had the least amount of drip loss ($P < 0.01$) after 24 h of storage in comparison to line 1/low and line 2/low pH groups, but was not different than line 2/high pH group. After 48 h of storage, the high and low pH groups were significantly different ($P < 0.05$) within both lines. Additionally, the line 1/high pH group had significantly less ($P < 0.05$) drip loss at 48 h storage than the line 2/low pH group but was not different from the line 2/high group (Table 2). After 96 h of storage, the line 1/high pH group exhibited the least amount of drip loss of all groups ($P < 0.05$) (Figure 4).

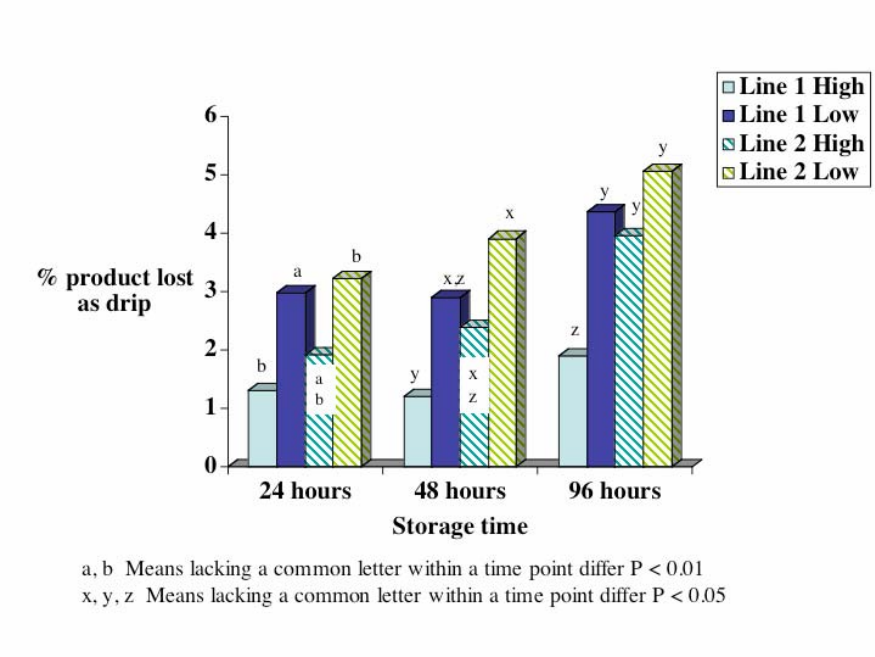


Figure 4.

A significant ($P < 0.01$) negative correlation existed between pH at 45 min, 3 h, 6 h, and 24 h postmortem and drip loss at 24 h, 48 h, and 96 h of storage (Table 3). This indicates that the lower pH values at all measured time points are highly related to lower water-holding capacity of pork LD muscle.

Color Analysis

Results of the Minolta color analysis at 24 h postmortem revealed differences in the LD muscles from pigs evaluated in this study (Table 1). At 24 h postmortem, the LD muscles from the line 2/low pH group had a higher ($P < 0.05$) mean reflectance value ($L^* = 49.49$) than the other 3 groups. No significant differences between groups were observed for a^* or b^* values. The samples from line 1/low pH group also had a lower pH at 45 min, 3 h, and 6 h postmortem than the line 2/high pH group and were different from the line 1/high pH group at all measured time points. This data suggests that LD from pigs that had a lower early postmortem pH also had a lighter fresh pork color.

Table 1.

Least squares means and standard errors of longissimus muscle L*, a* and b* values at 24 h postmortem for loins (n = 40) selected based on rate of pH decline.

	Line 1/High[1]	Line 1/Low[2]	Line 2/High[3]	Line 2/Low[4]	SEM
Minolta					
L*	41.46[b]	44.32[b]	44.67[b]	49.49[a]	0.984
a*	0.0815[a]	0.7720[a]	-0.3111[a]	0.7514[a]	0.359
b*	8.889[a]	9.730[a]	9.157[a]	10.524[a]	0.446

[1] Longissimus dorsi from line 1 pigs with a high pH at 3 hrs postmortem; n = 10.
[2] Longissimus dorsi from line 1 pigs with a low pH at 3 hrs postmortem; n = 10.
[3] Longissimus dorsi from line 2 pigs with a high pH at 3 hrs postmortem; n = 10.
[4] Longissimus dorsi from line 2 pigs with a low pH at 3 hrs postmortem; n = 10.

[a,b,c] Means within rows lacking a common letter differ P < 0.01

μ-Calpain

Differences in the level of autolysis were observed at 1 d postmortem in the LD. More autolysis, suggested by the loss of the 80 kDa subunit, occurred in the line 1/high pH group than the line 2/low pH group at 1 d postmortem (P < 0.05). Additionally, more autolysis was observed in the line 2/high pH group than the line 1/low pH group at 1 d postmortem (P < 0.05; Figure 1). This suggests that samples with a higher early postmortem pH had increased autolysis at 1 d postmortem (Figure 5).

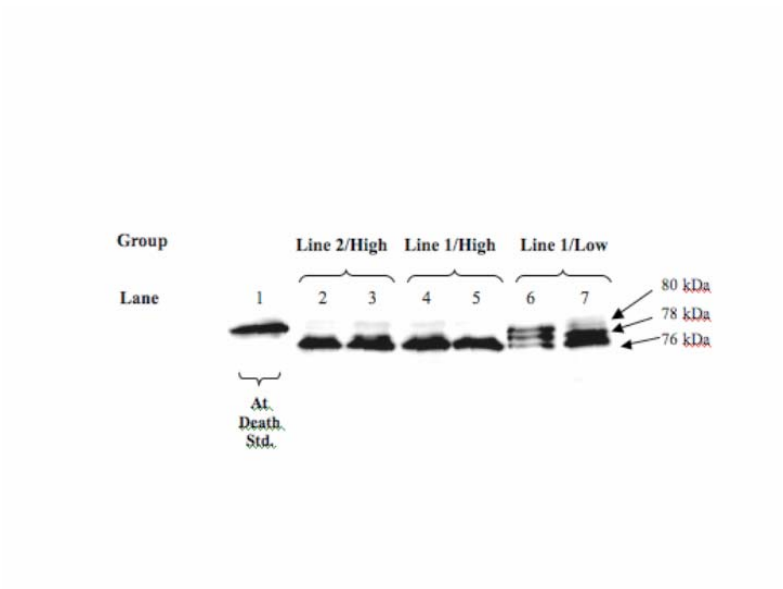


Figure 5.

Ryanodine Receptor (RyR1) analysis

Our analysis revealed no significant differences in degradation of the intact band of RyR1 between the four groups. However, significant differences ($P < 0.05$) as determined by densitometry were noted at 48 h postmortem between the line 1/low pH group and the line 2/high pH group for degradation of RyR1 immunoreactive bands that migrated as a doublet. The doublet was comprised of two lower molecular weight bands that were immunoreactive with the monoclonal antibody specific for RyR1 and that appeared in samples with increased proteolysis of the intact RyR1 band, thus the doublet is most likely a degradation product of this protein. This RyR1 degradation product was more prominent in the line 2/high pH group than the line 1/low pH group at 48 h postmortem. Additionally, less intensity of a higher migrating degradation product (band 2) was observed in line 1 than line 2 at 48 h postmortem (Figure 6). When evaluating band 2 in our study, it was observed that there was more degradation in line 2 than line 1 at 48 h postmortem ($P < 0.01$).

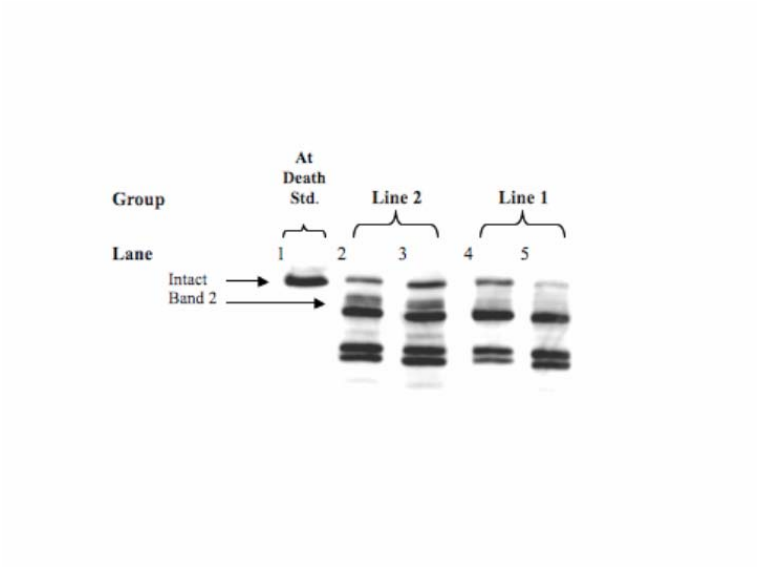


Figure 6.

Sarcoplasmic Reticulum Ca^{2+}-ATPase analysis
This study found no significant differences in degradation of intact SERCA2 between or within groups.

Objective 2 Determine the relationship between pH decline and alterations of key muscle proteins.

μ-Calpain
Increased autolysis of calpain was associated with lower pH (Table 2; Table 4). The presence of the intact 80 kDa subunit of μ-calpain at 1 d postmortem was negatively correlated to pH at 3 h ($P < 0.01$) and 6 h ($P < 0.05$) postmortem (Table 5). At 48 h postmortem, degradation of the 80 kDa subunit was negatively correlated with 45 min ($P < 0.05$), 3 h ($P < 0.01$), 6 h ($P < 0.05$), and 24 h ($P < 0.05$) pH values (Table 5). These correlations suggest that longissimus muscles with higher pH have increased autolysis of the 80 kDa subunit of μ-calpain at 1 d and 48 h postmortem.

Ryanodine Receptor

Degradation of the RyR1 (band 2) and pH decline were found to be related. The intermediate degradation product (band 2) of RyR1 was negatively correlated to pH at 6 h ($P < 0.05$) and 24 h ($P < 0.01$) postmortem. This indicates that increased proteolysis of RyR1 is associated with lower pH. Additionally, degradation of RyR1 (band 2) was correlated to drip loss in this study. Band 2 of RyR1 at 48 h PM was strongly positively correlated ($P < 0.01$) with drip loss at 24 h, 48 h, and 96 h storage. This indicates that samples with a higher amount of degradation of RyR1 also had decreased water-holding capacity as indicated by increased drip loss. Thus, it can be suggested by our data that increased proteolysis of RyR1 was found in pork LD samples that had a lower pH, which subsequently may have caused poorer water-holding capacity.

Sarcoplasmic Reticulum Ca^{2+}-ATPase analysis

This study found no significant differences in degradation of intact SERCA2 between or within groups. This study found no significant differences in degradation of intact SERCA2 between or within groups. However, degradation of intact SERCA2 was related to pH. The presence of intact SERCA2 at 1 d and 72 h postmortem was positively correlated ($P<0.05$) with 24 h pH measurements. These correlations suggest in products with a high early postmortem and/or high ultimate pH, there is more intact SERCA2.

Significant correlations also existed between intact SERCA2 and drip loss. A negative correlation ($P < 0.05$) is shown between intact SERCA at 120 h postmortem and drip loss at 24 h storage. Therefore, less intact SERCA2 is associated with increased drip loss after 24 h of storage.

Discussion: Drip loss in longissimus dorsi chops was affected by pH. The current study showed that the line 1/high pH group had the least amount of drip loss ($P < 0.01$) after 24 h of storage in comparison to line 1/low and line 2/low pH groups, but was not different than line 2/high pH group (Table 2). After 48 h of storage, the high and low pH groups were significantly different ($P < 0.05$) within both lines (Table 2). Additionally, the line 1/high pH group had significantly less ($P < 0.05$) drip loss at 48 h storage than the line 2/low pH group but was not different from the line 2/high group (Table 2). After 96 h of storage, the line 1/high pH group exhibited the least amount of drip loss of all groups ($P < 0.05$; Table 2).

A significant ($P < 0.01$) negative correlation existed between pH at 45 min, 3 h, 6 h, and 24 h postmortem and drip loss at 24 h, 48 h, and 96 h of storage (Table 3). This indicates that the lower pH values at all measured time points are highly related to lower water-holding capacity of pork LD muscle.

The observations between drip loss and pH in this study are in agreement with Warriss and Brown (Warriss and Brown, 1987) who found significant negative correlations between 45 min pH and drip loss at 48 h. Similarly, van Laack et al. (van Laack et al., 1994) observed significant negative correlations between 45 min pH and drip loss at 72 h. Bowker, Wynveen, Grant and Gerrard (Bowker et al., 1999) observed a similar relationship between pH and drip loss in pork carcasses. The authors saw that the loins from electrically stimulated pork carcasses (lower early postmortem pH) had a greater percent drip loss ($P < 0.05$) than the carcasses that had not been treated with electrical stimulation. This coincides with our results that carcasses with a lower early postmortem pH and/or a lower ultimate pH also had a higher amount of drip loss.

Other studies have shown relationships between lower early postmortem pH and/or a lower ultimate pH and increased drip loss, which coincide with our results.

Joo, Kauffman, Kim and Park (Joo et al., 1999) showed that loins with a lower ultimate pH had increased drip loss. Lonergan et al. (2001) found that pigs selected for lean growth efficiency, but that were free of the halothane and RN^- gene, had a significantly faster rate of pH decline than the control line. This study also found that the animals with the faster rate of pH decline also had a greater amount of drip loss in the longissimus dorsi, semimembranous, and semitendinous (Lonergan et al., 2001).

As a low early postmortem pH may indicate an increased rate of pH decline, the relationship between low early pH and increased drip loss may be attributed to higher rate of protein denaturation. Offer and Knight (Offer and Knight, 1988)showed that a faster rate of pH decline while the carcass temperature was still warm could result in more protein denaturation. This can be detrimental to water-holding capacity as contractile proteins such as myosin may undergo extreme denaturation, which would alter the crossbridges between actin and myosin and cause the distance between the two proteins to shrink (van Laack, 1999). This lateral shrinkage would subsequently result in more myofibrillar water to be expelled into the extracellular spaces and thus decrease water-holding capacity.

Color

Our findings are in agreement with Enfält et al. (Enfalt et al., 1993) who found that loins exhibiting a paler meat color also had a greater amount of drip. Claeys, De Smet, Demeyer, Geers and Buys (Claeys et al., 2001) also found that loins with a faster rate of pH decline had a higher CIE L* value ($P < 0.05$). It is possible that protein denaturation that occurs as a result of a faster rate of pH decline may be likely to induce a higher L* value (Rees et al., 2003). Alternatively, the lighter appearance (higher L* value) may be due to the acceleration of post slaughter metabolism combined with a lower muscle pH at rigor (Rees et al., 2003). The lower pH may cause an increase in free water at the cut surface of the cell which would result in an increased reflectance and subsequently paler meat (Pearson and Dutson, 1985)

μ-Calpain

Our findings concerning the relationship between high pH and increased μ-calpain autolysis have been shown by several other authors. The optimum pH for calpain activity is 7.4 - 7.6 (Edmunds et al., 1991) However, postmortem conditions of meat are at a pH of approximately 5.5. Thus, it would be expected that the calpains would less active or undergo autolysis more slowly with the lower pH conditions found in postmortem meat. This is supported by our study as we found that samples with a higher early postmortem pH had increased autolysis. Koohmaraie, Schollmeyer and Dutson (Koohmaraie et al., 1986)showed that in conditions similar to postmortem aging with a pH 5.5 to 5.8 and at 5˚C, μ-calpain retained 24-28% of its maximum activity at pH 7.5 and at 25˚C, which suggests that a sample with a higher pH would have greater calpain activity. Claeys et al. (2001) supported these findings when they observed a lower μ-calpain activity in the genetic line with a faster rate of pH decline than the genetic line with a slower rate of pH decline.

Proteolysis of myofibrillar proteins by the calpains can affect water-holding capacity. In the current study, μ-calpain autolysis was also related to drip loss. The presence of the 80 kDa subunit of μ-calpain at both 1 d and 48 h postmortem was positively correlated ($P < 0.05$) to drip loss after 24 h, 48 h and 96 h of storage (Table 5). These correlations suggest that samples that exhibited less intact 80 kDa μ-calpain also exhibited a lower percent of drip loss. This could indicate that increased autolysis may be occurring, and as autolysis has been shown to parallel activation (Baki et al.,

1996), increased substrate proteolysis by calpain may also result, particularly of those proteins involved in water-holding capacity.

Ryanodine Receptor

The RyR1 is a substrate of μ-calpain (Gilchrist et al., 1992; Iino et al., 1992; Shoshan-Barmatz et al., 1994). Gilchrist et al. (1992) found that when RyR1 was exposed to μ- and m-calpain, a 410 kDa and 150 kDa degradation product was produced. In our study, autolysis of the 80 kDa subunit of μ-calpain at 1 d postmortem was positively correlated with degradation of the intact RyR1 at 1 d and 48 h (Table 6). Autolysis of the 80 kDa μ-calpain subunit at 48 h postmortem was also positively correlated with proteolysis of intact RyR1 at 1 d and 48 h (Table 6). The 80 kDa subunit at 1 d and 48 h was negatively correlated to degradation of the RyR1 doublet at 48 h postmortem (Table 6). These correlations suggest that in samples with more degradation of RyR1 a greater degree of autolysis of the 80 kDa subunit had occurred.

The current study observed relationships between increased μ-calpain autolysis and increased degradation of RyR1. Additionally, we found that increased RyR1 proteolysis and increased drip loss were positively correlated. It can be hypothesized that proteolysis of the RyR1 by calpain may promote the release of Ca^{2+} during the postmortem time period, which could cause the level of sarcoplasmic Ca^{2+} to rise. The increased concentration of Ca^{2+} may promote the further activation of μ-calpain which may result in degradation of proteins that affect water-holding capacity.

Our hypothesis can be supported by the work of other authors. Shoshan-Barmatz et al. (1994) showed that cleavage of the RyR1 by calpain stimulated Ca^{2+} release. Thus, this degradation could allow Ca^{2+} leakage which may elevate the Ca^{2+} ion concentration enough to further activate the calpains. Iino et al. (1992) reported that the RyR1 released Ca^{2+} at an increased maximum rate when degraded by m-calpain. It is possible that degradation of the RyR1 by calpain may alter the channel's ability to function and could stimulate a large influx of Ca^{2+} into the cell sarcoplasm (Iino et al., 1992). This loss of function could be detrimental to pork quality by causing an increased metabolism and thus an accelerated rate of pH decline and/or a lower ultimate pH, which could result in poor water-holding capacity. However, a slight alteration to the RyR1 and its function could result in a sufficient localized sarcoplasmic Ca^{2+} increase that may activate μ-calpain, and possibly m-calpain, which could subsequently promote the further degradation of RyR1 and other muscle proteins associated with water-holding capacity. More research is needed to explore the relationship between degradation of the skeletal muscle RyR1 and the calpain system.

SERCA2

A decrease in activity of the SERCA pump could be detrimental to pork quality early postmortem by limiting the amount of Ca^{2+} that could be removed from the sarcoplasm back to the sarcoplasmic reticulum. The subsequent higher concentration of Ca^{2+} in the sarcoplasm could promote increased muscle contraction and a faster rate of anaerobic metabolism that could ultimately lead to a faster rate of pH decline. This is in agreement with our study as we observed that increased degradation of intact SERCA2 was closely associated with decreased pH. It may be suggested that degradation of the pump is causing the Ca^{2+} concentration to rise intracellularly, which would result in a faster rate of muscle metabolism and acidic conditions. Additionally, the current study also showed that increased degradation of SERCA2 was related to more drip loss after 24 h of storage.

Lay Interpretation: Degradation two of the major components of the calcium regulating system in skeletal muscle is associated with differences in pH decline and drip loss. This study indicates that there may be heretofore unobserved differences in these proteins and/or their associated regulatory proteins. This study provides critical evidence that may lead to producing pigs that will more consistently produce high quality pork.

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