RESEARCHREPORT

pork checkoff

PORK QUALITY

**Title**: Regulation of Pork Water Holding Capacity, Color, and Tenderness by Protein Phosphorylation – **NPB #99-072**

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## I. Abstract

Rapid glycolysis and pH decline in stress-susceptible pigs has recently been associated with increased activity of pyruvate kinase, an enzyme that regulates glycolysis. Pyruvate kinase was found to be more active due to phosphorylation of the enzyme. Additionally, phosphorylation of specific muscle proteins may also reduce protein degradation and consequently reduce meat tenderization caused by calpain proteases. This study examined relationships between glycolytic enzyme activity and pork color and water-holding capacity. Pork tenderness was measured and the associations between tenderness, degradation of muscle proteins and protein phosphorylation were evaluated. Duroc (n=16) or HAL-1843™ free Pietrain (n=16) sired gilts were harvested over a two-week period. Temperature of the *longissimus* muscle (LM) was logged continuously from 45 min to 22 h postmortem at 5 min intervals and LM pH was measured at 20, 45, 180 min and 22 h postmortem. Temperature of LM at 45 min postmortem was negatively correlated with 45 min pH ($P < .05$). Minolta L* values for LM chops at 24 h postmortem ranged from 49.6 to 60.2. Purge, determined as fluid loss from vacuum packaged loin sections, ranged from .79 to 9.91% in loin sections stored at 4°C from d1 to d6 postmortem. After purge determination, two 2.5 cm thick loin chops were cut and allowed to drip in a simulated retail case at 4°C overnight. Drip loss ranged from .3 to 1.8%. Minolta L* values (d1, d2, d6 and d7) were correlated to all measures of fluid loss ($P < .002$). Pyruvate kinase and phosphofructokinase (rate-limiting enzyme in glycolysis) activities were not correlated with LM pH, purge, drip loss, or color ($r<.2$ or $>-.2$; $P > .31$). Tenderness was measured by Warner-Bratzler shear force on chops aged for 7 days at 4°C and cooked to an internal temperature of 71°C. Desmin degradation, determined qualitatively by Western blot analysis, was consistently greater in tender chops compared to less tender chops. However, differences in desmin phosphorylation were not apparent. Collectively, these data indicate that pork color and water holding capacity are not associated with the capacity of enzymes that catalyze the regulated steps of glycolysis. Pork tenderness is associated with rate and extent of desmin degradation.

## II. Introduction

Pork products provide society with a high quality, nutritious and palatable food. The rate of biochemical reactions which occur in skeletal muscle of pigs prior to, and immediately following slaughter have a dramatic impact on the quality of pork produced. Pale, soft and exudative (PSE) pork is a critical quality and economic concern in the pork industry. The Pork Chain Quality Audit found that 10.2% of the pork carcasses surveyed were classified as having PSE meat (Cannon et al., 1996). A mutation of the ryanodine receptor results in abnormal calcium regulation within the muscle cells of stress-susceptible hogs (halothane positive), and can thereby increase the incidence of PSE in these animals (Louis et al., 1993). Although the ability to genetically identify pigs with the ryanodine receptor defect is currently possible, PSE pork characteristics still exist among and within breeds and lines of pigs that do not possess this defect (Pommier and Houde, 1993). Tremendous potential exists to improve pork quality through selective breeding programs, improved handling practices which reduce pig stress, and improved slaughter and carcass handling procedures. An understanding of biochemical events which govern pork quality is essential for development of new strategies to improve the quality and consistency of pork.

Pale, soft, and exudative pork is associated with a rapid postmortem glycolytic rate that causes a rapid pH drop while muscle temperature is still high (Briskey, 1964). The two major regulatory points in glycolysis are the steps catalyzed by the enzymes, phosphofructokinase (PFK) and pyruvate kinase (PK). Both of these proteins have functions regulated by phosphorylation. Schwagele et al. (1996) demonstrated that PK is highly phosphorylated in halothane positive pigs that produce PSE meat when compared to pigs that are halothane negative. Phosphorylation causes pyruvate kinase to retain higher activity under the acidic conditions which occur rapidly in PSE meat from halothane positive pigs (Schwagele et al., 1996). Effects of protein phosphorylation on regulation of PFK and PK in halothane negative pigs have not been determined.

Meat tenderization results from postmortem degradation of myofibrillar proteins by calcium-dependent proteases, or calpains. Phosphorylation of some myofibrillar proteins has been shown to reduce degradation of these proteins by calpains (Di Lisa et al., 1995; Zhang et al., 1988). Phosphorylation of calpastatin (endogenous calpain inhibitor) renders it a more effective inhibitor of m-calpain than unphosphorylated calpastatin (Salamino et al., 1994). Based on these data, we hypothesized that protein phosphorylation may have deleterious effects on pork color, water holding capacity, and tenderness by increasing the rate of muscle glycolysis and decreasing postmortem proteolysis.

## III. Objectives

The objectives were to determine if modification of muscle proteins by phosphorylation 1) increases the rate at which acidic conditions develop in muscle, resulting in pale, soft and exudative (PSE) pork, and 2) reduces the rate of protein degradation which would decrease pork tenderness. To address these objectives, it was necessary to 1) determine if increased *in vitro* glycolytic enzyme activity is associated with rapid decline in postmortem pH that leads to inferior pork color and water holding capacity in HAL-1843™ free pigs, and 2) determine if differences in pork tenderness are related to degradation of specific muscle proteins.

## IV. Procedures

*Animals and meat quality data collection*

Sires from Duroc and HAL-1843™ free Pietrain lines were used to inseminate Yorkshire and $F_1$ Yorkshire-Landrace gilts. Progeny were raised in uniform conditions at the MSU Swine Teaching and Research Farm. Duroc (n = 16) or HAL-1843™ free Pietrain (n = 16) sired gilts were harvested over a two-week period at the MSU Meat Laboratory.

A time line for data collection is shown in Table 1. Loin muscle area, 10$^{th}$ and last rib backfat thickness, and subjective color, firmness and marbling were determined according to current NPPC guidelines (NPPC, 2000). Temperature of the *longissimus* muscle (LM) adjacent to the last rib was measured at 20 min postmortem using a hand held temperature probe. From forty-five min to 22 h postmortem, temperature was logged every five min using DELTA TRAK FlashLink Dataloggers (Pleasanton, CA). *Longissimus* muscle pH was measured at 20, 45, 180 min and 22 h postmortem with a portable pH meter equipped with a puncture-type combination pH electrode (Model 1140, Mettler-Toledo, Woburn, MA). At these times postmortem, samples were also obtained for subsequent measurement of pH using the iodoacetate method (Bendall, 1973). Initial samples (20 min postmortem) were taken midway between the last rib and the cranial edge of the ilium on the left side of the carcass. Subsequent LM samples (45 min, 180 min and 22 h) were taken approximately one inch cranial to the previous sampling site. *Longissimus* samples were cut into 0.5 cm$^3$ pieces, frozen in liquid nitrogen, and stored at -80°C. At 22 h postmortem, a section of loin was removed from the right side of the carcass between the 11$^{th}$ rib and the last lumbar vertebra. From the cranial edge of the loin section, duplicate 10 g samples were obtained and used to determine water-holding capacity by high-speed centrifugation (CWHC) (40,000 x **g** for 30 min; Honikel and Hamm, 1994). Two 2.5 cm thick loin chops were removed from between the 12$^{th}$ and last rib. These chops were used to determine color (L*, a*, b*) at 24 (L1) and 48 h (L2) postmortem with a Minolta chromameter (Ramsey, NJ) and drip loss by the suspension method from 24 to 48 h postmortem (DRIP1) (Honikel and Hamm, 1994). The remaining section was vacuum packaged at 24 h postmortem and held at 4°C until day 6 postmortem. The difference between day 6 and 24 h weight was divided by 24 h weight and expressed as PURGE. After determination of PURGE, two 2.5 cm thick loin chops were cut from the cranial end of the loin section and allowed to bloom before color determination (L6). Chops were allowed to drip for 24 h under simulated retail conditions and drip loss (DRIP6) and color (L7) were recorded at day 7 postmortem. Chops were then cooked to an internal temperature of 71°C on Farberware Open Hearth Grills™ and allowed to cool overnight. Three 1.27 cm diameter cores per chop were taken parallel to the muscle fiber orientation and tenderness was quantified by shearing cores with a Warner-Bratzler shear machine.

*Sample preparation for enzyme assay*

*Longissimus* muscle samples for glycolytic enzyme activity were obtained at 20 min postmortem as described above. Frozen LM samples (1 g) were homogenized using a Polytron (Brinkman, Westbury, NY) for 2 x 20 sec bursts (setting 4) in 10 volumes of ice cold extraction buffer (75 mM KCl, 10 mM $KH_2PO_4$, 2 mM $MgCl_2$, 2 mM EGTA, pH 7.0, containing 50 mM sodium fluoride, 2 mM phenylmethylsulfonyl fluoride, and 6 mg/l of leupeptin). The homogenate was then fractionated into sarcoplasmic and myofibrillar components by centrifugation at 10,000 x **g** for 15 minutes. The supernatant was saved and the pellet was resuspended in 10 ml of extraction buffer and centrifuged again at 10,000 x **g** for 15 minutes. The two supernatants were combined. Protein concentration of supernatants was determined using the bicinchoninic acid protein assay kit (Pierce, Rockford, IL). In preliminary experiments, we found that the sarcoplasmic fraction (supernatant) contained > 95 % of the PK and PFK activity. Thus we used the sarcoplasmic fraction for quantification of PK and PFK activity.


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*Enzyme assay*

Pyruvate kinase activity was measured using a coupled enzyme assay described by Schwagele et al. (1996). The assay was adapted so that it could be performed in a 96-well microtiter plate. The total reaction volume was 210 µl which consisted of 3 mM magnesium acetate, 50 mM KCl, 0.2 mM EDTA, 0.2 mg/ml BSA, 30 mM imidazole (pH 7.0), 1mM ADP, 0.7 mM PEP, 0.1 mM NADH, 2 U/ml LDH and 10 µl of a 1:10 dilution of sarcoplasmic fraction. Activity was measured by following the oxidation of NADH to $NAD^+$ at 340 nm on a VERSAmax plate reader (Molecular Devices, Sunnyvale, CA). Assays performed in the absence of substrate (PEP) contained less than 1% of sample activity, demonstrating that conversion of NADH to $NAD^+$ was coupled to PK activity. Pyruvate kinase activity was calculated from the $V_{max}$ that was determined from the SOFTmax PRO software and expressed as µmole/min·mg$^{-1}$ protein. Samples were simultaneously assayed in triplicate and the intra-assay coefficient of variation was < 4%.

Phosphofructokinase was quantified using a coupled enzyme assay described by Scopes (1977). This reaction was performed in a microtiter plate that contained a total reaction volume of 210 µl of 3 mM magnesium acetate, 50 mM KCl, 0.2 mM EDTA, 0.2 mg/ml BSA, 30 mM Tris base (pH 8.0), 1 mM F-6-P, 0.2 mM NADH, 1 mM ATP, 0.2 mM PEP, 2 units each of PK and LDH/ml and 10 µl of a 1:4 dilution of sarcoplasmic fraction. Assays performed in the absence of substrate (F-6-P) were found to contain approximately 27% of the total activity. This could be attributable to F-6-P or ADP present in the sample. Therefore, negative control assays with no F-6-P were performed for each sample. The $V_{max}$ from control assays were subtracted from the $V_{max}$ for assays performed with F-6-P. The total activity is expressed as µmole/min·mg$^{-1}$ protein. Samples were simultaneously assayed in triplicate and the intra-assay coefficient of variation was < 2%.

*Sample preparation for analysis of protein degradation*

Frozen LM samples (1g) were homogenized using a Polytron (Brinkman, Westbury, NY) for 1 x 20 sec burst (setting 4) in 10 volumes of ice cold 50 mM Tris, 10 mM EDTA pH 8.3, containing 2 mM phenylmethylsulfonyl fluoride, and 6 mg/l of leupeptin. For calpastatin analysis, 2 ml of the crude homogenate were centrifuged at 16,000 x **g** for 30 min. The supernatant was removed and heated at 95°C for 15 min. After disruption of the coagulate, the sample was centrifuged at 16,000 x **g**. Supernatants were mixed with an equal volume of 2x treatment buffer (125mM Tris, 4% SDS, 20% glycerol, 10% mercaptoethanol (MCE) and .08% bromophenol blue, pH 6.8) and heated at 95°C for 3 minutes.

For desmin and filamin analysis, 500 µl of the crude homogenate were mixed with 500 µl of 2x treatment buffer (125mM Tris, 4% SDS, 20% glycerol, pH 6.8) by repeated pipeting and heated at 50°C for 20 min. Samples were mixed again by repeated pipeting, heated for 5 minutes and centrifuged at 16,000 x **g** for 20 min. Samples were mixed with an equal volume of 2x treatment buffer (125mM Tris, 4% SDS, 20% glycerol, 10% MCE and .08% bromophenol blue, pH 6.8) and heated at 95°C for 3 min.

*Sample preparation for phosphorylation of myofibrillar proteins*

Frozen LM samples (1g) were homogenized using a Polytron (Brinkman, Westbury, NY) for 1 x 20 sec burst (setting 4) in 10 volumes of ice cold 50 mM Tris, 10 mM EDTA pH 8.3, containing 2 mM phenylmethylsulfonyl fluoride, and 6 mg/l of leupeptin. Samples were centrifuged for 15 min at 10,000 x **g.** The pellet was washed twice by suspension in 10 ml of the Tris-EDTA buffer and centrifugation. The final pellet

was suspended in 10 ml buffer. One ml of solutions containing myofibrillar proteins were mixed with an equal volume of 2X treatment buffer, without MCE and bromophenol blue. Samples were then heated at 95°C for 3 min and protein concentrations were determined using the BCA assay. Treatment buffer containing MCE and bromophenol blue were added to the samples to adjust protein concentration prior to electrophoresis.

*SDS-PAGE and Immunoblotting*

Calpastatin and desmin were resolved by SDS-PAGE (Laemmli, 1970) on .75-mm-thick 10% (37.5:1) separating gels and filamin was resolved on 8% (50:1) separating gels. In each case, 4% (37.5:1) stacking gels were used. Electrophoresis of proteins on 10% gels was performed at 200 V for approximately 45 min and at 170 V and approximately 5 h for 8% gels. Proteins were electrophoretically transferred to Immobilon-P membrane (Millipore, Bedford, MA) in buffer containing 25 mM Tris, 193 mM glycine, and 15% methanol (Towbin et al., 1979). Lanes containing molecular weight markers were stained with amido black. To prevent non-specific antibody binding, membranes were incubated with blocking buffer (3% BSA in Tris buffered saline [TBS] containing .05% Tween-20, pH 7.4) for 1 h. For detection of calpastatin, membranes were incubated with polyclonal mouse anti-calpastatin antibodies (1:1,000) (generously provided by Dr. M. Koohmaraie) overnight in cold room, followed by an alkaline phosphatase conjugated anti-mouse IgG diluted 1:1,000 (Sigma) for 45 min. For detection of desmin, membranes were incubated in primary D76 hybridoma supernatant followed by an alkaline phosphatase conjugated anti-mouse IgG diluted 1:1,000 (Sigma) for 45 min. The D76 hybridoma, developed by D.A. Fischman, was obtained from the Developmental Studies Hybridoma Bank and developed under the auspices of the NUCHD and maintained by The University of Iowa, Department of Biological Sciences, Iowa City, IA 52242. Filamin was detected by incubation with polyclonal anti-filamin IgG (1:2000, Sigma) for 2 h, secondary anti-goat biotin (1:2000) for 30 min, followed by extravidin-alkaline phosphatase (1:2000). Phosphorylation of desmin was quantified using phosphoserine and phosphothreonine (1:2000), secondary anti-rabbit biotin (1:2000) for 45 min, followed by extravidin-alkaline phosphatase (1:2000) for 30 min. Membranes were washed three times with blocking buffer after each incubation. Antibody binding was visualized by exposure to BCIP/NBT (Bio-Rad).

*Data Analysis*

Least squares means by sire line of meat quality traits were compared using a protected least significant difference test (Freud and Wilson, 1997). Meat quality data were correlated to enzyme activity by analysis of variance using general linear model procedures for a completely randomized design.

## V. Results

Pietrain-sired hogs produced lighter and more heavily muscled carcasses at a constant age (Table 2). Meat quality data are presented by sire line in Tables 3 and 4. No differences in pH, color, water holding capacity, glycolytic enzyme activity or tenderness were found between sire groups ($P > 0.06$). Therefore, relationships between pork quality data and glycolytic enzyme activity or protein degradation are presented and discussed collectively for all pigs.

The portable pH meter and the iodoacetate method of measuring pH produced similar results (e.g. $r = 0.83$ for 32 samples at 3 h postmortem; $P < 0.0001$). Additionally, similar correlations existed between meat quality traits and pH values obtained by either method. Values obtained using the portable pH meter are reported.


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Figure 1 shows the rate and extent of postmortem pH decline. Relatively large standard deviations associated with mean pH values within the first 3 h postmortem reflect differences in the rate of pH decline among longissimus muscles. The range in LM pH was 5.8 - 6.7, 5.6 - 6.6 and 5.3 – 6.4 at 20 min, 45 min and 3 h postmortem. In this study, differences in ultimate pH (22 h postmortem) were relatively small (Figure 1; range 5.4 to 5.6).

Figure 2 depicts the temperature decline in LM from 45 min postmortem to 22 h postmortem. Independent temperature measurements were also taken with a portable temperature probe at 20 min, 45 min, 3 h and 22 h postmortem. Although temperature decline curves among carcasses were generally parallel, differences in muscle temperature were evident (Figure 2). Variation in early postmortem muscle temperature may result from differences in initial body temperature, or metabolic heat production during the conversion of glycogen to lactic acid and/or the hydrolysis of creatine phosphate and ATP. We observed an inverse relationship between temperature at 45 min postmortem and pH at 45 min postmortem (Table 5).

Several measures of color and water holding capacity were made. Day 1 and day 2 measures were performed on different chops than day 6 and day 7. However, the four chops are within approximately a 13 cm region of the same loin section. Although no severe PSE pork was observed among the samples in the current study, the variation in pork color and water holding capacity was of practical importance (Tables 3 and 4). Different measures of water holding capacity (DRIP1, CWHC, PURGE and DRIP6) and color (L1, L2, L3 and L6) were highly correlated to each other (Table 5). Longissimus muscle L* values were positively correlated with water loss. Both water loss and L* value were inversely related to LM pH from 20 to 180 min postmortem (Table 5). Of the pH measures, 45 min pH was most highly correlated with water loss and color (Table 5). Warriss and Brown (1987), demonstrated that ultimate pH was related to reflectance and exudate, but only accounts for about 15% of the variation in these traits. The lack of a relationship between 22 h pH, water holding capacity and L* values at day 1 and 2 may be attributed to the lack of variation in ultimate pH seen in this study. A modest correlation (r~-.4) between 22 h pH and reflectance at day 6 and 7 was observed (Table 5). It is possible that slight differences in ultimate pH may impact long term color stability.

The variation in pork quality observed in this population was expected to be sufficient for determining the relationship between glycolytic enzyme activity and pork color and water holding capacity. Coupled enzyme assays were used to quantify total activity of PFK and PK in the sarcoplasmic fraction of longissimus muscles. Since these assays are conducted under controlled conditions *in vitro*, it should be recognized that activity data reflect the *in vitro* capacity of these enzymes to catalyze their respective reactions, rather than *in vivo* activity. The *in vitro* activity of PFK and PK ranged from .32 to .67 and 3.8 to 6.4 $\mu$mole/min·mg$^{-1}$ protein respectively. No correlation between glycolytic enzyme activity and LM pH, color or water-loss was observed (Table 6). In contrast, Schwagele et al. (1996) demonstrated that muscle from halothane positive pigs had 4 times more total pyruvate kinase activity than control pigs. Additionally, Schwagele et al. (1996) demonstrated that PK isolated from halothane positive pigs lost only 30% of its activity versus a loss of >90% activity in control pig muscle, when the pH was lowered from 7.0 to 5.5. These authors demonstrated that PK in LM of halothane positive pigs was more active and less pH labile due to phosphorylation of the enzyme. Our findings indicate that a similar regulation of glycolysis does not contribute to the differences observed in pork quality in HAL-1843™ free pigs. We also measured activity of PK at pH 5.5 and observed a loss (>88%) of PK activity of all LM samples at this lower pH (data not shown). This provides additional evidence that phosphorylation-

dependent stabilization of enzyme activity, as observed by Schwagele et al. (1996) for halothane positive pigs, is not a key factor regulating variation in pork quality of HAL-1843™ free pigs. Our findings support data previously reported by Scopes et al. (1974), which showed that different concentrations of PFK and PK did not effect the rate at which glycolysis proceeded *in vitro.* We conclude that in HAL-1843™ free pigs, variation in color and water holding capacity is not associated with differences in the capacity of enzymes that catalyze regulated steps of glycolysis.

Meat tenderization has been shown to result from the postmortem degradation of myofibrillar proteins by calcium-dependent proteases, or calpains. Differences in Warner-Bratlzer shear force observed among loin chops cooked at day 7 postmortem were modest (Table 4). Proteolysis of two myofibrillar proteins (desmin and filamin) and the endogenous calpain inhibitor (calpastatin) were monitored by immunoblot analysis of samples at 20 min, 3 h, and day 1, 3, and 7 postmortem. For initial studies, the four most tender and four least tender samples were evaluated. Tender chops exhibited faster and/or more extensive desmin degradation than less tender chops (Figure 3). This relationship was less consistent for calpastatin degradation and only slight filamin degradation was observed. Using specific phospho-amino acid antibodies, we were unable to detect any qualitive differences in phosphorylation state that would explain the variation seen in proteolytic rate of desmin.

In summary, Minolta L* values were positively correlated to all measures of water loss. Of the pH measurements taken, 45 min pH was most highly correlated to water loss and the best predictor of ultimate LM quality. Activity of enzymes that catalyze the regulated steps of glycolysis were not related to pork color or water holding capacity. Thus, differences in glycolytic enzyme capacity (as measured by *in vitro* activity) do not appear to be responsible for the variation observed in pork quality of HAL-1843™ free pigs. Desmin degradation was consistently related to pork tenderness, but differences in desmin phosphorylation were not detected. Additional research is necessary to understand the regulation of pork tenderization and to identify biochemical factors that influence the rate of pH decline and consequently govern pork color and water holding capacity.

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Table 1: Time line for data collection .

| | TIME POSTMORTEM | | | | | | |
|---|---|---|---|---|---|---|---|
| **MEASUREMENTS** | **20MIN** | **45MIN** | **180MIN** | **DAY 1** | **DAY 2** | **DAY 6** | **DAY 7** |
| **pH** | ● | | | ● | | | |
| **TEMP** | ● | | | ● | | | |
| **COLOR** | | | | ● | ● | ● | ● |
| **DRIP LOSS** | | | | ● | ● | ● | ● |
| **CWHC** | | | | ●● | | | |
| **PURGE** | | | | ● | // | | ● |



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Table 2: Mean values and ranges for carcass measurements

| SIRE LINE | N | LIVE WT (kg) | HCW (kg) | 10TH RIB (mm) | LAST RIB (mm) | LEA ($cm^2$) | LENGTH (cm) |
|---|---|---|---|---|---|---|---|
| **Duroc** | 16 | 134 | 103 | 19.8 | 30.7 | 51.5 | 86.2 |
| | ***RANGE*** | ***122-142*** | ***91-108*** | ***12.7-25.4*** | ***17.8-40.6*** | ***41.5-60.6*** | ***83.2-91.2*** |
| **Pietrain** | 16 | 128 | 98 | 16.5 | 27.4 | 61.5 | 84.0 |
| | ***RANGE*** | ***115-141*** | ***86-101*** | ***10.2-25.4*** | ***15.2-38.1*** | ***52.9-73.2*** | ***79.2-86.9*** |


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Table 3: Mean values and ranges for day 1 meat quality measures.

| SIRE LINE | N | DAY 1 L* | DAY 2 L* | LMC | LMF | LMM | DRIP 1 | CWHC |
|---|---|---|---|---|---|---|---|---|
| Duroc | 16 | 53.39 | 53.82 | 2.8 | 2.3 | 1.8 | 1.68 | 15.09 |
| | *RANGE* | *50.32-60.17* | *51.29-61.25* | *2-3* | *1-3* | *1-3* | *0.41-5.20* | *10.31-21.47* |
| Pietrain | 16 | 53.84 | 53.82 | 2.7 | 2.0 | 1.1 | 2.05 | 14.75 |
| | *RANGE* | *49.59-56.58* | *49.58-56.34* | *2-3* | *1-3* | *1-2* | *0.46-3.71* | *8.31-21.05* |


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Table 4: Mean values and ranges for day 6 meat quality meaures.

| SIRE LINE | N | DAY 6 L* | DAY 7 L* | PURGE | DRIP 6 | COOK LOSS | WBS (kg) |
|---|---|---|---|---|---|---|---|
| Duroc | 16 | 53.48 | 54.32 | 3.55 | 0.92 | 31.0 | 3.9 |
| | *RANGE* | *49.74-60.25* | *51.30-60.28* | *0.79-9.91* | *0.32-1.63* | *26.02-40.03* | *3.10-5.12* |
| Pietrain | 16 | 54.04 | 55.22 | 4.20 | 1.15 | 32.3 | 4.2 |
| | *RANGE* | *49.19-57.23* | *50.58-59.04* | *1.82-7.25* | *0.59-1.80* | *24.97-40.19* | *2.98-5.53* |


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Table 5: Correlation coefficients for water-holding capacity and color*

| | pH20min | pH45min | pH180min | pH24h | L1 | L2 | L6 | L7 | DRIP1 | CWHC | PURGE |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pH45min** | 0.781** | | | | | | | | | | |
| **pH180min** | 0.731** | 0.814** | | | | | | | | | |
| **pH24h** | 0.354 | 0.412* | 0.534** | | | | | | | | |
| **L1** | -0.312 | -0.470** | -0.226 | -0.175 | | | | | | | |
| **L2** | -0.425* | -0.576** | -0.292 | -0.145 | 0.921** | | | | | | |
| **L6** | -0.549** | -0.694** | -0.483** | -0.404* | 0.755** | 0.837** | | | | | |
| **L7** | -0.533** | -0.706** | -0.528** | -0.450** | 0.769** | 0.858** | 0.520** | | | | |
| **DRIP1** | -0.625** | -0.633** | -0.482** | -0.313 | 0.733** | 0.828** | 0.783** | 0.772** | | | |
| **CWHC** | -0.559** | -0.614** | -0.428* | -0.348 | 0.672** | 0.739** | 0.720** | 0.748** | 0.874** | | |
| **PURGE** | -0.619** | -0.757** | -0.496** | -0.265 | 0.556** | 0.702** | 0.789** | 0.752** | 0.724** | 0.652** | |
| **DRIP6** | -0.534** | -0.542** | -0.406* | -0.173 | 0.694** | 0.713** | 0.729** | 0.693** | 0.731** | 0.633** | 0.626** |

* Correlation are different from zero P < .05

** Correlations are different from zero P < .01



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Table 6: Correlation Coefficients of Glycolytic Enzyme Activity and Pork Quality Traits*

| | pH45 | L1 | L2 | L6 | L7 | DRIP1 | CWHC | PURGE | DRIP6 |
|---|---|---|---|---|---|---|---|---|---|
| **PFK[a]** | 0.183 | -0.177 | -0.085 | -0.012 | -0.090 | -0.152 | -0.181 | 0.012 | -0.056 |
| **PK[b]** | -0.082 | 0.198 | 0.117 | -0.039 | 0.024 | -0.038 | -0.053 | 0.085 | 0.057 |

*Correlations are not significantly different from zero at the P > .1 level.

[a] PFK value .49 + .06; range = .32 - .67

[b] PK value 5.3 + .6; range = 3.8 - 6.4



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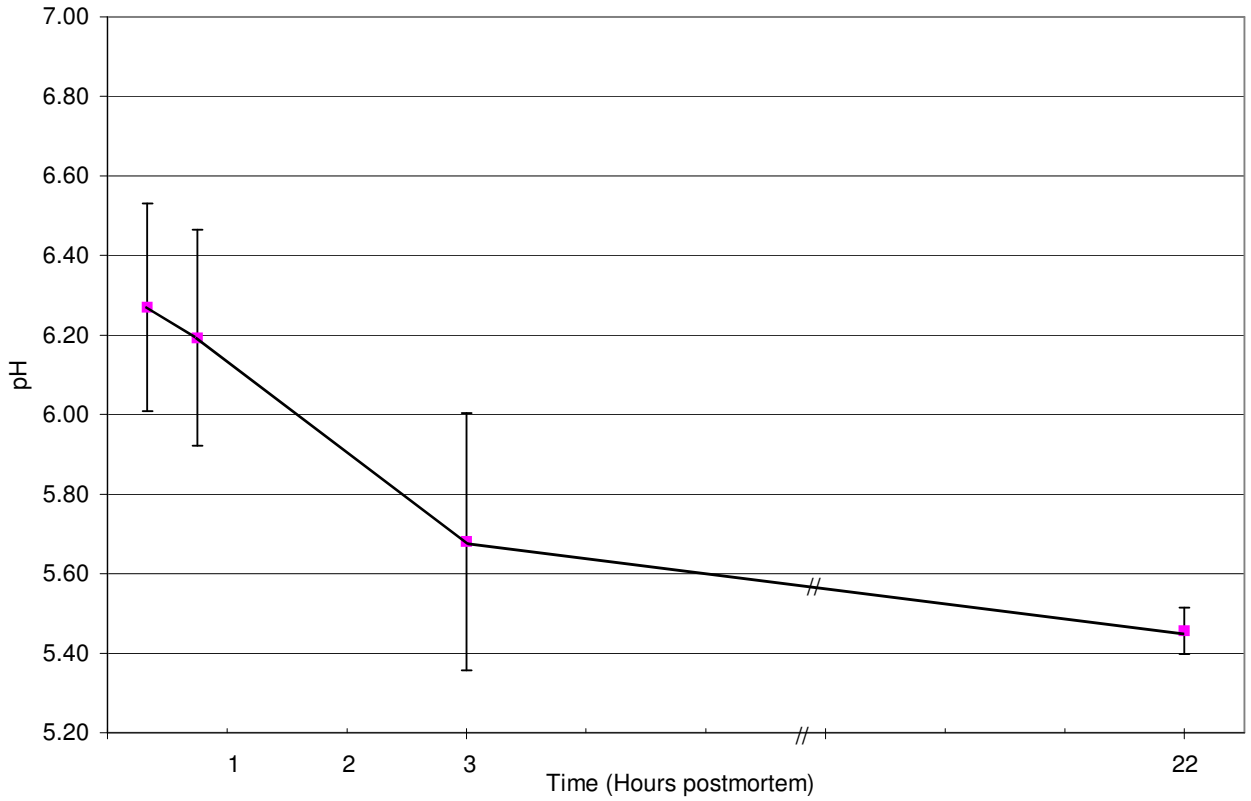


Figure 1. Postmortem pH decline. Measurements were taken with a portable pH meter and puncture type probe adjacent to the last rib. Data points shown represent means $\pm$ S.D.



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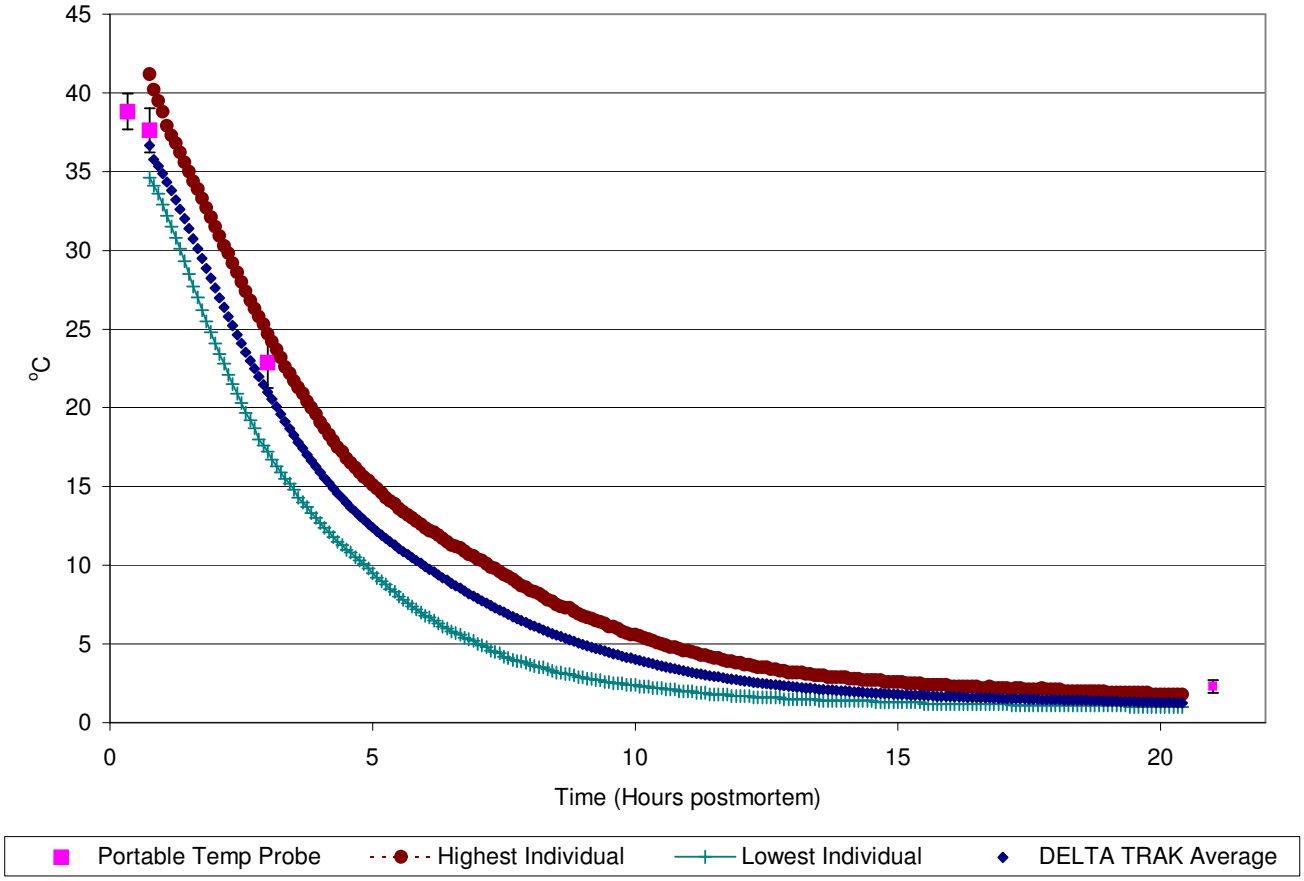


Figure 2. Longissimus muscle temperature decline. FLASHLINK DATALOGGERS were inserted adjacent to the last rib and temperature was logged every five minutes from 45 min to 22 hours postmortem. Means ± standard deviations of longissimus muscle temperature measured using a hand held temperature probe are shown (■). The average temperature measured using the dataloggers is shown for all 32 pigs (◆). Individuals with the highest (●) and lowest (+) temperatures are included to illustrate the range in temperature declines.


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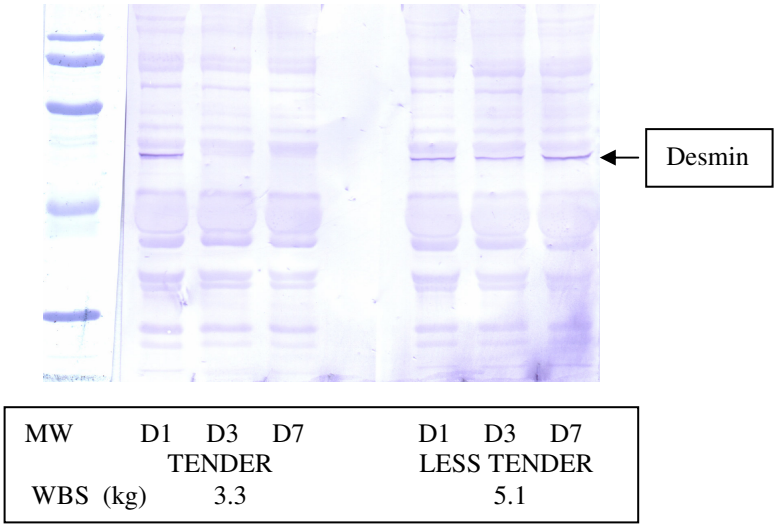


Figure 3. Western immunoblot analysis of desmin degradation. Porcine longissimus samples were aged at 4°C for the indicated times (1, 3 and 7 days postmortem). Samples preparation, electrophoresis and immunoblotting were as described in Procedures. Approximately 15 µg muscle protein per lane were loaded. Molecular weight markers (MW) are shown in the left lane. Location of the desmin band is indicated by the arrow.


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