RESEARCH REPORT

pork checkoff

PORK QUALITY

**Title:** PSE Development and Detection ***- NPB* # 00-117**

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**I. Abstract:** Wide variation in the color of fresh pork cuts, and the incidence of cuts with high drip loss continue to plague the pork industry worldwide. While the basic causes of these variants have been know for 4 decades, the details needed to detect and prevent these quality problems still elude the industry. This study of PSE development and detection establishes pork carcass electrical stimulation as a means of dropping muscle pH rapidly and raising carcass temperature, which are hallmarks of PSE development. This allows the creation of the condition for research purposes to develop detection and other preventive measures. Additional studies in which ES was applied at different times after exsanguination showed that the susceptibility of a pig carcass to development PSE (via ES) resides within the first 25 min post-exsanguination. Agreement between our biochemical data and those reported by Kastenschmidt et al. (1968) further supports the use of electrical stimulation as a model to study PSE without confounding the study with genotype. These additional data help identify more precisely the rate-limiting steps of glycogenolysis and glycolysis and provide a more comprehensive understanding of how adverse pork quality develops within a genotype as environmental stimuli change. To further understand the development of changes in the ability of muscle to bind the naturally occurring water within it's cells during the changes that occur during PSE development a new technique was developed. Absorptive cotton implants are absorbent and detect the release of water from cells. Utilization of cotton implants may prove valuable in gaining an understanding of water release from pork muscle at the various stages of conversion of muscle to meat and be helpful in elucidating differences in treatments and genotypes with regard to the development of drip loss problems. Previous work has shown that near infrared spectroscopy, may have application in the early detection of drip loss problems, but results have been inconsistent from one data set to another. For the first time, non-ratioed spectra were observed to have predictive value. The implications of being able to utilize non-ratioed spectra are important to the eventual application of NIR to on-line applications because non-ratioed spectra require less probe time to sense the



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required information. This observation is encouraging and appears to merit further study.

**II. Introduction:** Uniform pork quality continues to be one of the most critical issues facing the pork industry. Although food safety is a higher profile issue for the entire meat-producing sector, quality and uniformity influence purchase decisions at the meat counter on a daily basis. Variability in US pork quality has slowed development of markets in Pacific Rim countries, and cause participants in this market to be very selective in the quality of meat marketed in those countries. Since the US industry must continually position itself in the world market, any stagnation in the demand for domestic pork could jeopardize its position in the global market place. One of the keys to a healthy industry resides in improving product quality and consistency.

The most obvious pork quality problems facing the industry are highly variable color of pork cuts and a high incidence of poor water binding properties within meat cuts. This is often lumped under the umbrella term of PSE (pale, soft, exudative pork) when in fact the entire range of color and waterholding capacity can exist in any combination. Much is known about the PSE condition, but the complete picture is less clear when other combinations of color and waterholding capacity develop. Until we understand thoroughly the biochemistry and physiology of how these conditions develop, and have methods to effectively assess quality on-line, development of techniques to retard or modify this condition, or select genotypes resistant to its development will not be possible. Therefore the overall goal of our research program has been to understand the time and molecular basis of color and waterholding capacity in pork so that technologies can be targeted to detect this pork quality problem.

**III. Objectives:**

Determine the changes in muscle metabolites in pigs of different genotypes electrically stimulated at different times post mortem.

Determine the relationships between NIR spectra and muscle pH and temperature declines.

**IV. Procedures:**

*Phase* I A total of sixty-four pigs (average wt 105 kg) were used in the first phase of this study. Pigs of a single genetic line were obtained from the Pig Improvement Company (PIC, Franklin, KY, USA) and fed *ad libitum* prior to slaughter. Pigs were transported to slaughtering facilities approximately sixteen hours before slaughter where they were fed *ad libitum*. Pigs were slaughtered over six days at the Purdue University Meat Science Research and Education Center. Pigs were rendered unconscious by electrical stunning and exsanguinated following standard industry procedures. Exsanguination was considered zero min postmortem. Regardless of treatment, all carcasses were scalded at 5 min postmortem, followed by dehairing. Carcasses were held at room temperature until 60 min postmortem, at which time they were placed in a chill cooler (4°C). Carcasses were split at 24 h. Carcasses were randomly assigned to either electrical stimulation (ES) or no stimulation (NS) treatments. Treated carcasses were subjected to ES (500 V, 26 pulses, 2 s on and 2 s off) at 3, 15, 25, 35, 45 or 55 min

postmortem. Carcass ES was applied according to procedures established by Bowker *et al*. (1999). ES was administered through a 16.5 cm long steel electrode placed in the left shoulder muscles of the carcass with the rail serving as the ground. *Longissimus dorsi* (LD) muscle samples were taken at 10, 20, 30 and 40 min postmortem, snap frozen in liquid nitrogen and stored at $-80^0$C. pH values of the LD were recorded at 5, 10, 20, 30, 40 and 50 postmortem. Near infrared spectroscopy was applied at 30 and 45 minutes postmortem. Half of the carcasses in each treatment cell were scanned for 30 seconds the other half were scanned for 2 minutes. Traditional meat quality characteristics (color, water holding capacity and final pH) were collected at 24 h postmortem. Muscle samples were powdered and analyzed for glycolytic potential by summation of glucose, glycogen, glucose-6-phosphate and lactate levels (Monin et al., 1987). Data were subjected to analysis of variance using the General Linear Models procedure of SAS (SAS, 1986). Student's *t*-test was used to determine significant differences between treatment means. All data were subjected to regression analyses to determine the correlation of various measured parameters to meat quality characteristics.

Phase 2 An initial study was conducted to confirm the consistency and repeatability of utilizing cotton implants to determine the time and amount of water release from postmortem muscle tissues during the conversion of muscle to meat. Data were collected from 5 carcasses harvested utilizing the procedures described above. Temperature probes were used to record the changes in temperature that a carcass experiences throughout the harvesting procedure and during the first 24 hr post exsanguination. Absorption of water in the Loin was measured using cotton implants (o.b. Tampons, regular absorbency). Absorbance measurements were started at 15 minutes post-exsanguination and continued in 15-minute intervals until 180 minutes post-exsanguination. At 180 minutes post-exsanguination, a final pair of cotton implants were positioned in the same locations and allowed to remain in the carcass until 24 hours postmortem. The two slits were made in the LD muscle at the $13^{th}$ rib and approximately 6 inches anterior from the first slit. The area around the initial slits was dried with a brown paper towel and the first pair of cotton implants was inserted into the carcass. At 24 hours postmortem, carcasses were ribbed at the $10^{th}$ rib. Classical carcass data (FT, LMA, HWT) and objective scores for firmness, marbling and NPPC color scores were assessed. Ultimate pH (24 hour) was measured in the same location. A 1-inch thick loin chop was removed from the region between the two slits made for absorption measurements. Driploss was determined in triplicate according to the Danish Drip tube technique. One-inch muscle cores were drilled at three locations within each loin chop. Cored meat samples were placed in driploss tubes and allowed to drip for an additional 24 hrs before the following weights were determined. Also the calculation that was used to determine the individual driploss measurements is included. The average of the three driploss values was used as the reported value.

*Weights*: Empty container
Container with meat and exudate
Container with exudate only

*Calculation for Driploss*

$$\% \text{ DL} = \frac{[\text{container w/exudate wt.} - \text{empty container wt.}]}{[\text{container w/meat and exudate wt.} - \text{empty container wt.}]} \times 100$$

*Phase 3* The final phase of the study was designed to determine the effects of electrical stimulation on pork quality in RN pigs, as, well as to determine the ability of

NIR spectroscopy to predict drip loss in RN genotype pigs. Cotton implants (tampons) were utilized to determine the time and amount of water released from postmortem muscle tissues during the conversion of muscle to meat in stimulated and non-stimulated pork carcasses. 24 hr driploss measurements were also conducted as described above.

In this study, a total of 35 hogs were processed over the span of two days. Slight variations existed between the electrical stimulation process and the number of hogs processed. On the first day, a total number of 14 hogs were slaughtered. Eight of the hogs possessed RN- genetics and six hogs possessed RN+ genetics. Of the 14 hogs, 7 were electrically stimulated at 3 minutes post-exsanguination (13 pulses, 450 volts) to induce PSE conditions. Of the seven, 4 were RN- and 3 were RN+. On the second day, a total number of 21 hogs were slaughtered. Six of the hogs possessed RN- genetics and 15 hogs possessed RN+ genetics. Of the 21 hogs, 11 were electrically stimulated at 6 minutes post-exsanguination (26 pulses, 450 volts) to induce PSE conditions. Of the 11 hogs that were electrically stimulated, 3 were RN- and 8 were RN+.

Hogs were transported to the Purdue Meat Science Research and Education Center the morning they were slaughtered by pen groups to avoid unnecessary stress. Hogs were slaughtered and processed under normal practices. Hogs were scalded, split and held until 60 minutes postmortem before being placed in the chill cooler (4$^{o}$C). Temperature and pH measurements were taken at the last rib at 0, 10, 20, 30, 45, 60, 75, 90, 105 and 120 minutes post-exsanguination (a 15 minute pH measurement was interpolated, not measured on the carcasses). pH probes were cleaned after every fourth hog. In addition to manually taking temperature readings, temperature probes were inserted into the LD muscle after scalding and allowed to record uninterrupted until 120 minutes post-exsanguination. Muscle samples were collected at 0, 30, 60, 90 and 120 minutes post-exsanguination and 24 hr post-exsanguination from the lumbar region (posterior to temperature and pH measurements). The samples were frozen in liquid nitrogen. NIR measurements were taken in 2 intervals at the 12$^{th}$ rib as outlined below. Scans have a 30 second duration each with 6 scans per carcass per time interval meaning that scans last a total of 3 minutes each. NIR measurements were taken at 30 and 45 minutes post-exsanguination. Absorbance data using cotton implants (ob tampon, regular absorbency) was collected on as many carcasses as possible on both days of the study, although an emphasis was placed on stimulated carcasses. Absorbance measurements were started at 15 minutes post exsanguination and continued in 15-minute intervals until 120 minutes post exsanguination. At 120 minutes post-exsanguination, a final pair of cotton implants were positioned in the same locations and allowed to remain in the carcass until 24 hours post-mortem. The two slits were made in the LD muscle at the 11$^{th}$ rib and approximately 4 inches anterior from the first slit. The area around the initial slits was dried with a paper towel and the first pair of cotton implants was inserted into the carcass. Cotton implants were stored in individually labeled plastic bags until they could be weighed. Labeling consisted of a carcass serial number, a cotton implant number and a location number (denoted as 1 or 2). Throughout both days of the study, absorbance data was collected on 20 carcasses, 10 possessed RN- genetics of which 6 had been electrically stimulated. Of the remaining 10 carcasses that were RN+, 6 had been electrically stimulated.

Breakdown of data collection by side:

Left Side of Carcass: Temperature
pH
Muscle samples
Quality Characteristics

Right Side of Carcass: Absorbance
NIR
Temperature probes
Additional muscle samples

On the days following slaughter, 24 hours postmortem, carcasses were ribbed at the $10^{th}$ rib. Classical carcass data (FT, LMA, HWT) and objective scores for firmness, marbling and NPPC color scores were assessed. Ultimate pH (24 hour) was measured in the same location. A 24-hour muscle sample was extracted from the same general location. 24 hour NIR measurements were also collected, again 6 scans at 30 seconds each. The final pair of cotton implants was removed and their weight was determined. Two loin chops, taken from the $10^{th}$/ $11^{th}$ rib region, were used for determining driploss and for assessing Hunter color values. Driploss was determined in triplicate using the Danish drip tube technique. Meat samples were allowed to sit in drip tubes for an additional 24 hours such that the final measurement occurred at 48 hours postmortem. Driploss was then determined according to the calculation previously mentioned in the materials and methods section for Phase 2. The three individual driploss measurements were averaged and used in further data analysis

**V. Results:** *Phase 1* Earlier studies established a means to generate PSE (-like) pork using early postmortem electrical stimulation (ES), (Bowker et al., 1999). There has been, however, some reluctance to accept ES, presumably because enzyme profiles in ES-stimulated PSE may not parallel those found in commercially generated PSE. Application of 26 pulses of 500 V is effective in causing all genotypes to develop characteristics (water-holding capacity, color, firmness) typical of those associated with PSE pork. ES experiments to titrate voltage levels have shown that as little as 100 V (26 pulses) administered 6 min post-exsanguination is sufficient to elicit the same “PSE-like” characteristics in fresh pork. These data support pork carcass electrical stimulation as a means of dropping muscle pH rapidly and raising carcass temperature, which are hallmarks of PSE development. Additional studies in which ES was applied at different times after exsanguination showed that the susceptibility of a pig carcass to develop PSE (via ES) resides within the first 25 min post-exsanguination. (Figures 1 & 2). The exact reason for this is unknown, but may reside in the ability of the tissue to respond to applied voltage, such as diminished sarcoplasmic reticulum sensitivity or saturated enzyme capacity. Changes in concentrations of several key glycolytic metabolites (glucose, glycogen, lactate and glucose 6-phosphate) were evaluated. As expected, glycogen depletion was inversely related to lactate accumulation. Glucose concentration, however, increased with time postmortem and likely represents a by-product of glycogen metabolism, as additional energy would be required to phosphorylate glucose for use in glycolysis. Of particular interest, however, was the change of muscle glucose 6-phosphate during the transformation of muscle to meat. As shown in Figure 3, G6P concentration decreases during early postmortem periods, indicating that glycolysis is proceeding faster than glycogen breakdown and suggesting that glycogen phosphorylase may be rate limiting early. After 60 min, however, G6P

concentration increases suggesting that either glycogen phosphorylase activity is increasing or that glycolysis (those elements downstream of glucose 6-phosphate) is slowing. These data agree with those of Kastenschmidt et al. (1968), who showed that "fast-glycolyzing" pigs, which reflects the genetic component of PSE development follows a similar trend. Agreement between our biochemical data and those reported by Kastenschmidt et al. (1968) further supports the use of electrical stimulation as a model to study PSE without confounding the study with genotype. These additional data help identify more precisely the rate-limiting steps of glycogenolysis and glycolysis and provide a more comprehensive understanding of how adverse pork quality develops within a genotype as environmental stimuli change.

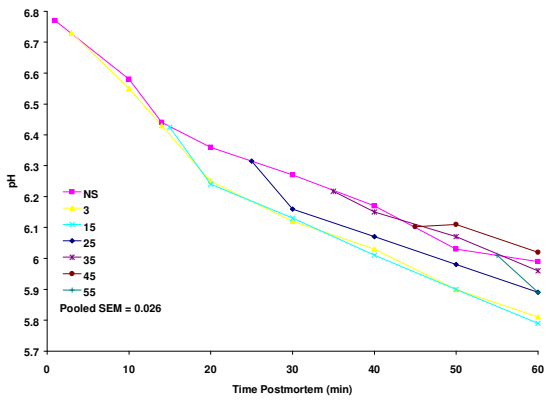



**Figure 1.** Rate of postmortem pH decline is induced by electrical stimulation but is dependent on the time at which carcasses are stimulated.

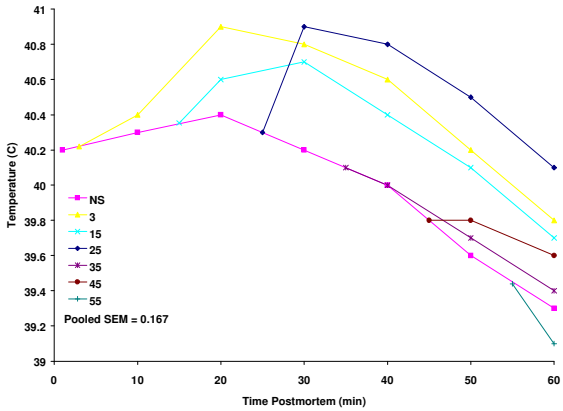



**Figure 2.** Rate of postmortem temperature change is induced by electrical stimulation but is dependent on the time at which carcasses are stimulated.

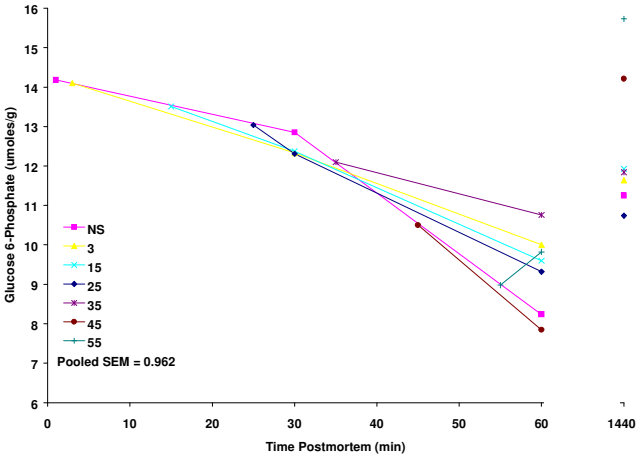



**Figure 3.** Muscle glucose-6-phosphate declines during the first 60 min postmortem then increases afterward.

*Phase 2* The utilization of cotton implants provide a means of tracking changes in the waterholding capacity in fresh muscle tissue over time during the conversion of muscle to meat. We observed that the amount of water absorbed during a 15-minute interval by the implants inserted at 45, 60, 75, 90, 105 minutes post-exsanguination were significantly correlated ($p < .05$) with the average drip loss taken on samples at 24 hr postmortem and allowed to drip for another 24 hours in drip tubes. The highest correlation with average drip loss was observed in a sample from position 1, implant inserted at 90 minutes post-exsanguination $r = .78$ ($p< .0001$). When the water absorbed by the cotton implants were serially added at 15-minute intervals from 15 to 105 minutes the relationship with average drip loss was not as high as when individual measurements were correlated to average drip loss. A relationship between cumulative water absorption and average drip loss was not observed until 60 to 75 minutes post-exsanguination and then at a lower level than observed for individual measurements of absorption. The best cumulative absorption was correlated at time 120 where the implant inserted at 120 minutes post-exsanguination was left in place for the next 22 hours. When all of the water absorbed over the 24 hour period was added together to get a total cumulative absorption the correlation with average drip loss at 24 hours was .74 ($p <.002$). We observed that muscles release small amounts of water even in the very early stages of conversion of muscle to meat. However, larger amounts tend to be released between 60 and 90 minutes in most carcasses. There appeared to be a position affect in that pads inserted in position 1 tended to absorb less water than in position 2. The analysis to determine position affect utilized the MIXED procedure of SAS (version 8). The five covariance structures used included simple, unstructured, compound symmetry, first order autoregressive and first order ante-dependence. Of the five structures, first order ante-dependence yielded the lowest coefficients and was used to determine significance. The position effect was significant ($p < 0.0001$). The least squares mean estimate for Position 2 was higher than the estimate for Position 1 (1.7924 and 1.2594 respectively).

At this point we would conclude that utilization of absorptive pads earlier than 60 minutes post exsanguination provides little information regarding the overall expected drip loss from a muscle.

Table 1. Correlation statistics for models between total absorption after 24 hours for Position 1 and Temperature and pH measured at various times post-exsanguination.

| Time (Minutes PE) | Temperature r | pH r |
|---|---|---|
| 10 | -0.07 | -0.42 |
| 15 | -0.07 | -0.47 |
| 20 | -0.04 | -0.43 |
| 30 | 0.19 | -0.39 |
| 45 | 0.08 | -0.45 |
| 60 | 0.02 | -0.48 |
| 75 | -0.07 | -0.55 |
| 90 | -0.05 | -0.61 |
| 105 | -0.21 | -0.60 |
| 120 | -0.20 | -0.64 |

Table 2. Correlation statistics for models between total absorption after 24 hours for Position 2 and Temperature and pH measured at various times post-exsanguination.

| Time (Minutes PE) | Temperature r | pH r |
|---|---|---|
| 10 | -0.01 | -0.35 |
| 15 | -0.08 | -0.48 |
| 20 | -0.12 | -0.50 |
| 30 | 0.22 | -0.45 |
| 45 | 0.10 | -0.51 |
| 60 | -0.12 | -0.53 |
| 75 | -0.11 | -0.58 |
| 90 | -0.18 | -0.61 |
| 105 | -0.36 | -0.59 |
| 120 | -0.34 | -0.64 |

Table 3. Correlation statistics for models between drip loss measured at 24 hours for and Temperature and pH measured at various times post-exsanguination.

| Time (Minutes PE) | Temperature r | pH r |
|---|---|---|
| 10 | -0.09 | -0.50 |
| 15 | -0.12 | -0.65 |
| 20 | -0.11 | -0.64 |
| 30 | 0.27 | -0.66 |
| 45 | 0.27 | -0.74 |
| 60 | 0.20 | -0.74 |
| 75 | 0.05 | -0.68 |
| 90 | 0.12 | -0.72 |
| 105 | 0.09 | -0.74 |
| 120 | 0.08 | -0.73 |

Tables 1, 2 and 3 show the correlations observed between pH and temperature measured at 10, 15, 20, 30, 45, 60, 75, 90, 105, and 120 minutes post-exsanguination with the total moisture absorbed by the cotton implants after 24 hr at positions 1 and 2, as well as average drip loss at 24 hr, respectively. Temperature was not highly correlated with any of the methods of measuring moisture release at any of the temperature measurement times. Drip loss was highly correlated range (-0.64 to -0.74) with pH at all of the measurement times after 15 minutes post-exsanguination. Lower correlations were observed between pH and the total moisture absorbed by the cotton implants after 24 hours (highest 0.64). As mentioned above the direct correlation between the total moisture absorbed by the cotton implants and average drip loss at 24 hr was 0.74.

**Figure 4. Average Additive Absorbance for Position 1 vs. Time of Insertion 3/01/01-3/20/01 RN Study**

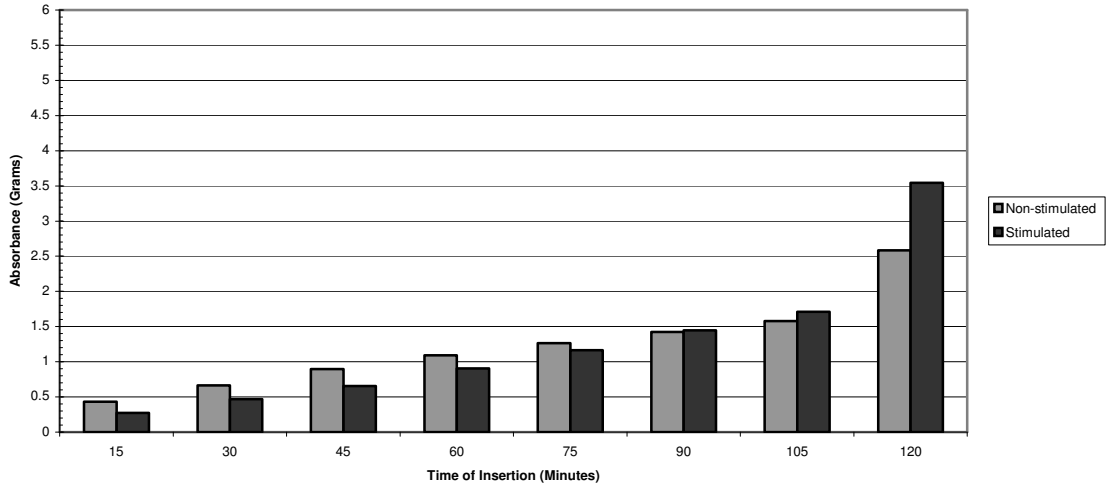
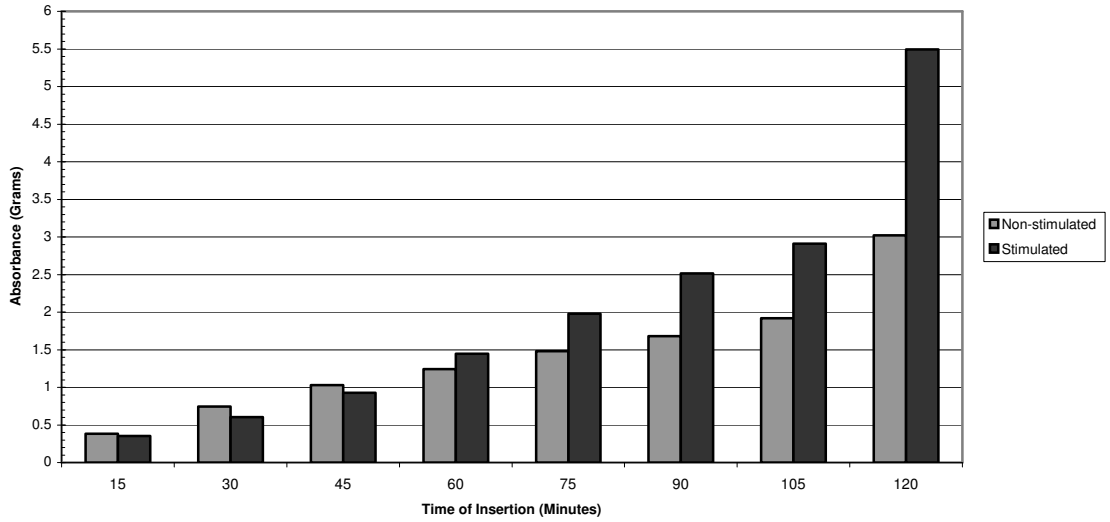



**Figure 5. Average Additive Absorption for Position 2 vs. Time of Insertion 3/01/01-3/20/01 RN Study**




Figures 4 and 5 show the average amount of cumulative water absorption by cotton implants at the 2 positions in the longissimus muscle in stimulated and non-stimulated carcasses. We observed that stimulated muscle tended to release less water into the cotton implants during the first 60 to 90 minutes post-exsanguination than non-stimulated muscle depending upon position. Cotton implants at position 2 tended to absorb increasing amounts of water from the stimulated muscle after 60 minutes post-exsanguination. Utilization of cotton implants may prove valuable in gaining an understanding of water release from pork muscle at the various stages of conversion of muscle to meat and be helpful in elucidating differences in treatments and genotypes with regard to the development of drip loss problems.

*Phase 3* NIR results. We previously reported analysis of data from 116 carcasses using Partial Least Squares Regression (PLSR) resulted in models that predict drip loss with correlation coefficients ranging from 0.14 to 0.77 using reflectance ratios.

In the current data set we observed that average drip loss could be predicted (0.55 to 0.71) with non-ratioed spectra at 30 and 45 min (Table 4). This is the first time that we achieved this result. At this time it is unknown whether this is due to the difference in genotype compared to the previous study or some other factor. The likelihood is high that genotype may play a major role in predicting drip loss in the early stages of conversion of muscle to meat.

Table 4: Regression statistics for predicting WHC using NIR spectra at 30 minutes.

| Model* | Number of PC's** | Calibration r | Validation r *** | RMSEC | RMSEP |
|---|---|---|---|---|---|
| Spectra 1 | 6 | 0.93 | 0.55 | 0.33 | 1.1 |
| Spectra 2 | 6 | 0.94 | 0.45 | 0.30 | 1.3 |
| Spectra 3 | 6 | 0.95 | 0.64 | 0.27 | 1.0 |
| Spectra 4 | 7 | 0.97 | 0.64 | 0.21 | 1.0 |
| Spectra 5 | 4 | 0.92 | 0.56 | 0.40 | 1.1 |
| Spectra 6 | 6 | 0.95 | 0.71 | 0.27 | 0.96 |

* Ratio models are derived from successive spectra divided by the first spectra
** Principle components in the model
*** Validation was performed using full cross validation methods

The implications of being able to utilize non-ratioed spectra are important to the eventual application of NIR to on-line applications because non-ratioed spectra require less probe time to sense the required information. While this observation is encouraging, there is far too little data to determine the reason for the current result.

Figures 6 and 7 show the pH and Temperature changes observed in stimulated and non-stimulated carcasses in the phase 3 study from 10 to 120 minutes post-exsanguination. Temperatures were higher in longissimus muscle of stimulated carcasses compared to non-stimulated carcasses at 30 and 40 minutes post-exsanguination. pH was lower in the longissimus muscles of stimulated carcasses beginning at 10 minutes post exsanguination compared to the non-stimulated carcasses.

Figure 6. Temperature changes in stimulated and non-stimulated carcasses from phase 3 studies.

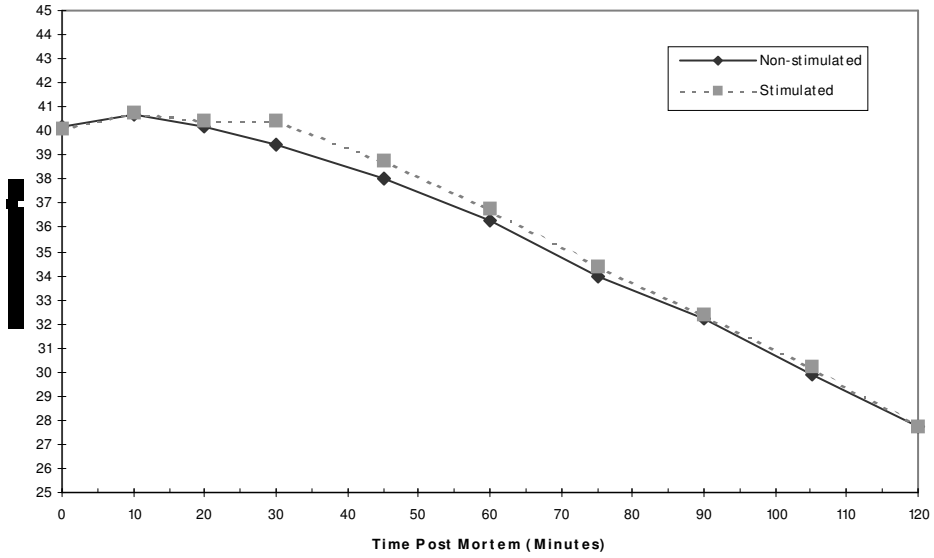



Figure 7. pH decline curves for stimulated and non-stimulated carcasses from phase 3 studies.