Title: Modeling Postmortem Physical and Chemical Changes in Muscle to Predict Water Holding Capacity and Color in Fresh Pork – **NPB# 99-121**

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I. Introduction

This research project focused on several physical and chemical changes in porcine muscle that occur early postmortem and are related to ultimate meat quality, in particular water-holding capacity (WHC) or drip loss. The principle investigators are currently evaluating several measurement techniques including near infrared reflectance (NIR) and tetrapolar impedance to determine their potential for identifying poor quality carcasses early in the slaughter process. However, since the development of water-holding capacity is a very dynamic process in the first 24 h after slaughter, many interacting events may lead to low water-holding capacity. Previous projects using technologies such as NIR and tetrapolar impedance and have shown promise for predicting WHC but yielded mixed results. Based on these previous results, it was evident that a more precise understanding of the changes in muscle properties that affect the measurement methods and/or the ultimate meat quality was needed. Identifying methods for monitoring the development of WHC during the first few hours after slaughter was the emphasis of this project.

II. Proposal Objectives

The main objective of this project was to improve understanding of the development of water-holding capacity (defined as drip loss) early postmortem. More specifically, 1) changes in water-holding capacity were to be modeled as a function of temperature, pH, and rigor development between zero and 24 h postmortem, 2) the resulting model would then be used to improve testing of quality measurement probes in packing plant environments.

The main goal of the project was to better understand the rates of change of quality-related properties in the muscle tissue during the first few hours postmortem. This information was determined to be critical to the successful development of future sensors and their use in predicting water-holding capacity in processing plants during this early post mortem timeframe. In order to create higher drip losses in carcasses, electrical stimulation was applied to the carcass as described by Bowker et al. (1999).

Since this method of creating higher drip losses may affect the applicability of the results, these methods may need to be further validated in a packing plant.

III. Procedures

The procedures for this project are divided into two sections. The first section describes data collected on carcasses from a study to model pH and temperature changes versus time. These pigs were used to evaluate the NIR probe for predicting WHC within the first hour postmortem. The second section describes procedures used to evaluate methods of measuring changes in WHC during the first 3 h postmortem.

NIR Probe Study

One hundred and twenty gilts (85-125 kg) from three genetic lines were utilized in this study. The pigs, obtained from Pig Improvement Co., Franklin, KY, were also used in a study described by Bowker et al. (1999). In that study, a randomly selected portion of the pigs were electrically-stimulated (26 pulses, 60 Hz, 500 V, 1 s on and 2 s off) at 3 min postmortem using a 16.5 cm long steel electrode placed in the left shoulder muscles of the carcass. All pigs were scalded at 7 min postmortem and processed according to normal slaughter procedures. Carcasses were held at room temperature until 60 min postmortem and then placed in a chill cooler (4 C) for 24 h.

Data was collected on changes in temperature and pH between zero and 24-hr postmortem. All pH measurements were taken in the right *longissimus dorsi* (LD) muscle at the last rib using a Beckman 110 ISFET pH meter with a spear-tipped KCl^- gel probe (Fullerton, CA). The probe was inserted approximately 5 cm into the LD muscle through the skin and fat approximately 5 cm lateral to the midline of the carcass. In the same location, temperature was measured using a VWR brand Traceable Digital Thermometer (Friendswood, TX). Temperature and pH measurements were recorded at approximately every six minutes from 1 to 60 minutes after slaughter and then at 24 h.

A fiber-optic, near reflectance probe was inserted into the LD muscle opposite the 3^rd^ lumbar vertebrae. The NIR spectra ranging from 900 to 1800 nm was recorded for 6 min beginning at 30 min postmortem. A total of 12 reflectance spectra were recorded from each carcass. Data from spectra 2-12 were divided by the first spectra to create ratioed spectra as described by Forrest et al. (2000) and jointly patented by Purdue Research Foundation and Danish Meat Research Institute (2000).

At 24 h, color and firmness scores of the LD between the 10th and 11th rib and Hunter colorimeter measurements were made on each of the carcasses (Bowker et al., 1999). Water-holding capacity was also determined using the drip loss method following procedures described by Rasmussen and Andersen (1998), Rasmussen and Stouffer (1996).

Early WHC Measurement Study

New methods were evaluated to rapidly measure the WHC of the tissue from 0-24 h postmortem. The goal was to be able to measure WHC changes during at least the first 3 h postmortem. Of the methods proposed, two were evaluated during the project. The first method was based on centrifuging excised core samples for 2-5 min periods of time at speeds ranging from 1000 to 2000 rpm to accelerate the separation of "free" water from the samples. As expected, speeds too slow did not provide enough force to expel the water and speeds too fast may have artificially changed the WHC of the tissue. One of the main concerns with this method was that the samples changed temperature rapidly when removed from the carcass. This change in temperature is suspected to affect the muscles' WHC but how much is still unknown.

The second method that was studied involved the insertion of water absorption implants into carcasses for short time intervals. Sixteen pigs were slaughtered in the Purdue University Meat Science Teaching and Research Laboratory using standard procedures. At approximately 15 min postmortem, half of the carcasses (randomly selected) were electrically stimulated to increase the drip loss. At 20 minutes postmortem, slits were made at 4 locations within the loin between the third rib and the fourth lumbar vertebra using a knife. Then, cotton implants (O.B. Tampons, regular absorbancy) were inserted into the slit at each of the 4 locations, beginning at 20 min postmortem. Each of the implants was removed and replaced with a new one every 15 minutes until ~180 min postmortem. In some preliminary tests, implants were removed every 30 min. Once each implant was removed, it was weighed to determine how much fluid it had absorbed from the tissue. At 24 h postmortem, WHC and pH were measured using the same equipment and procedures described above.

IV. Results

NIR Probe Study

Figure 1 shows an example of the ratioed NIR data collected on each of the carcasses between 30 and 40 min after sticking. The graph shows the first 3 spectral reflectance curves that were divided by the first curve. These ratioed curves indicate changes in the reflectance over 30 second intervals. These differences in NIR reflectance (ratios) are thought to be caused by changes in carcass' properties during rigor.

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. Table 1 describes the models and their correlation statistics.

Table 1: Regression statistics for predicting WHC using NIR spectra at 30 minutes.

Model*	Number of PC's**	Calibration r	Validation r ***	RMSEC	RMSEP
Spectra 1	3	0.44	0.28	3.3	3.6
Ratio 2	1	0.23	0.14	3.6	3.7
Ratio 3	1	0.43	0.39	3.3	3.4
Ratio 4	5	0.74	0.53	2.4	3.2
Ratio 5	5	0.75	0.59	2.4	3.0
Ratio 6	5	0.77	0.64	2.3	2.9
Ratio 7	4	0.72	0.63	2.5	2.9
Ratio 8	5	0.75	0.62	2.4	2.9
Ratio 9	5	0.76	0.65	2.3	2.9
Ratio 10	1	0.59	0.57	3.0	3.0
Ratio 11	1	0.61	0.59	2.9	3.0
Ratio 12	1	0.62	0.60	2.9	3.0

* 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

From Table 1, correlation coefficients (r) increase up to Ratio 6 model and then decrease. Also, the root mean square error of prediction (RMSEP) for each model is close to 3.0 % drip loss. One would expect an increase in correlation as more time passes between successive spectra since this gives the muscle more time to go through changes and gives more time to assess the rate of post-mortem change. For example, between spectra 1 and spectra 2, on average only 30 seconds has passed, whereas between spectra 1 and spectra 6, 3 min has passed. The slight decrease in correlation from Ratio 6 to Ratio 12 can't be explained at this time.

Similar analysis was performed to predict pHu from NIR spectra. However, the largest correlation coefficient was 0.32 indicating that there was not much correlation between NIR measurements at 30 min postmortem and pH at 24 h.

Figures 2 & 3 illustrate the effects of the electrical stimulation on rates of temperature and pH change. The electrical stimulation was successfully used to generate differences in WHC of carcasses since the mean drip loss for nonstimulated and stimulated carcasses was 3.59 $\pm$ 0.25 (%) and 10.13 $\pm$ 0.22 (%) respectively. Models where created to predict WHC from pH and temperature measured during the first 60 min postmortem. The statistics for the resulting models are listed in Table 2.

Table 2: Correlation statistics for models between WHC measured at 24 h and Temperature and pH measured at various times postmortem.

Model* Time pm	Temperature r	Temperature RMSEP	pH r	pH RMSEP	Temperature and pH R	Temperature and pH RMSEP
1 min	0.16	3.63	-0.02	3.66	0.12	3.66
7 min	0.63	2.86	-0.34	3.48	0.64	2.80
20 min	0.61	2.90	-0.60	2.95	0.69	2.65
26 min	0.65	2.77	-0.67	2.74	0.75	2.43
32 min	0.67	2.71	-0.68	2.70	0.76	2.40
38 min	0.68	2.67	-0.71	2.61	0.78	2.30
44 min	0.70	2.62	-0.74	2.50	0.80	2.20
50 min	0.67	2.73	-0.78	2.27	0.82	2.09
56 min	0.69	2.64	-0.77	2.36	0.82	2.10
24 hr	-	-	-0.55	3.08	-	-

* Model predicting WHC measured at 24 h and temperature and/or pH measured at respective times postmortem.

The correlation coefficients in Table 2 indicate that both pH and temperature are correlated to WHC as early as 7 min after exsanguination. The highest correlation between WHC and temperature occurs at 44 min, r = 0.70. The highest correlation between WHC and pH occurs at 50 min, r = 0.78. The best correlation between WHC and temperature and pH combined occurs at 50 and 56 min, r = 0.82. All of these models suggest WHC of the meat at 24 h is greatly affected by properties of the muscle between 44 and 56 min postmortem.

Early WHC measurement results

Figure 4 shows some results of centrifuging samples excised from a carcass every hour from 30 min to 4 h 30 min postmortem. One half the carcass was stimulated to increase the 24 h drip loss while the other side was treated normally. The results show that significantly more water could be removed from the stimulated half as early 1 h 30 min postmortem. This time of peak water loss from centrifuged samples is later than the 44-56 min time period that best correlated with 24 h drip loss. This result suggests that the mobility of water, or WHC, of the muscle may lag behind the temperature and pH changes in the muscle. In order to better define this relationship, nuclear magnetic resonance (NMR) methods would be required to rapidly measure the states of water mobility and changes during this time period.

Figure 5 illustrates the differences in water absorbed by the cotton implants inserted into the loin muscle and removed every 30 min. Again the stimulated side shows significantly more water loss than the non-stimulated side of the same carcass, as expected. Results of this method compare well to the centrifuge method in Figure 4 since the peak water loss occurs around 1 h 50 min postmortem. Since the cotton implant method gave similar results and involved less time and labor, it was chosen as the preferred method for estimating WHC early postmortem.

Figure 6 shows the average absorption results from using the cotton implants on 16 carcasses. The drip losses for the carcasses ranged from 0.43 % to 4.6 %. Two groups of carcasses were created. One group had an average drip loss of 1.2 % and the other had an average of 3.2 %. The higher drip loss group resulted in significantly higher water absorption by the cotton implants for 9 out of the 12 measurement times. The pattern of these curves seems to be fairly repeatable. That is, there is usually a peak of water absorption between 60 and 120 minutes postmortem on those carcasses with higher drip loss. This procedure is being further developed to determine if WHC at 24 h can be predicted early postmortem.

One challenge with research on predicting WHC (or 24 h drip loss) using any of these methods is the low repeatability of the reference method for measuring drip loss. For example, Figure 7 shows the correlation between drip loss measurements made on the same carcasses at two different locations (3rd lumbar vertebrae and 9th rib) by two different graduate students. From this Figure one can see that the correlation between the two measurements is only 0.77. The sources of this type of variation in drip loss must be determined in order to improve the predictability of any early postmortem methods.

V. References

Bowker, B.C., E.J. Wynveen, A.L. Grant, and D.E. Gerrard. 1999. Effects of electrical stimulation on early postmortem muscle pH and temperature declines in pigs from different genetic lines and halothane genotypes. Meat Science 53:125-133.

Forrest, J.C., M.T. Morgan, J.R. Andersen, C. Borggaard, A.J. Rasmussen and B.L. Jespersen. 2000. Development of technology for the early post mortem prediction of water holding capacity and drip loss in fresh pork. Journal of Meat Sciences. 55(1):115-122.

Rasmussen, A. J. and M. Anderson. 1998. Danish Meat Research Institute, maglegaardsvej 2, DK-400 Roskilde, Denmark

Rasmussen, A.J. and J.R. Stouffer. 1996. New method for determination of drip loss in pork muscles. In Poster proceedings of the 42nd International Congress of Meat Science and Technology, Norway, CPP, 286-287.

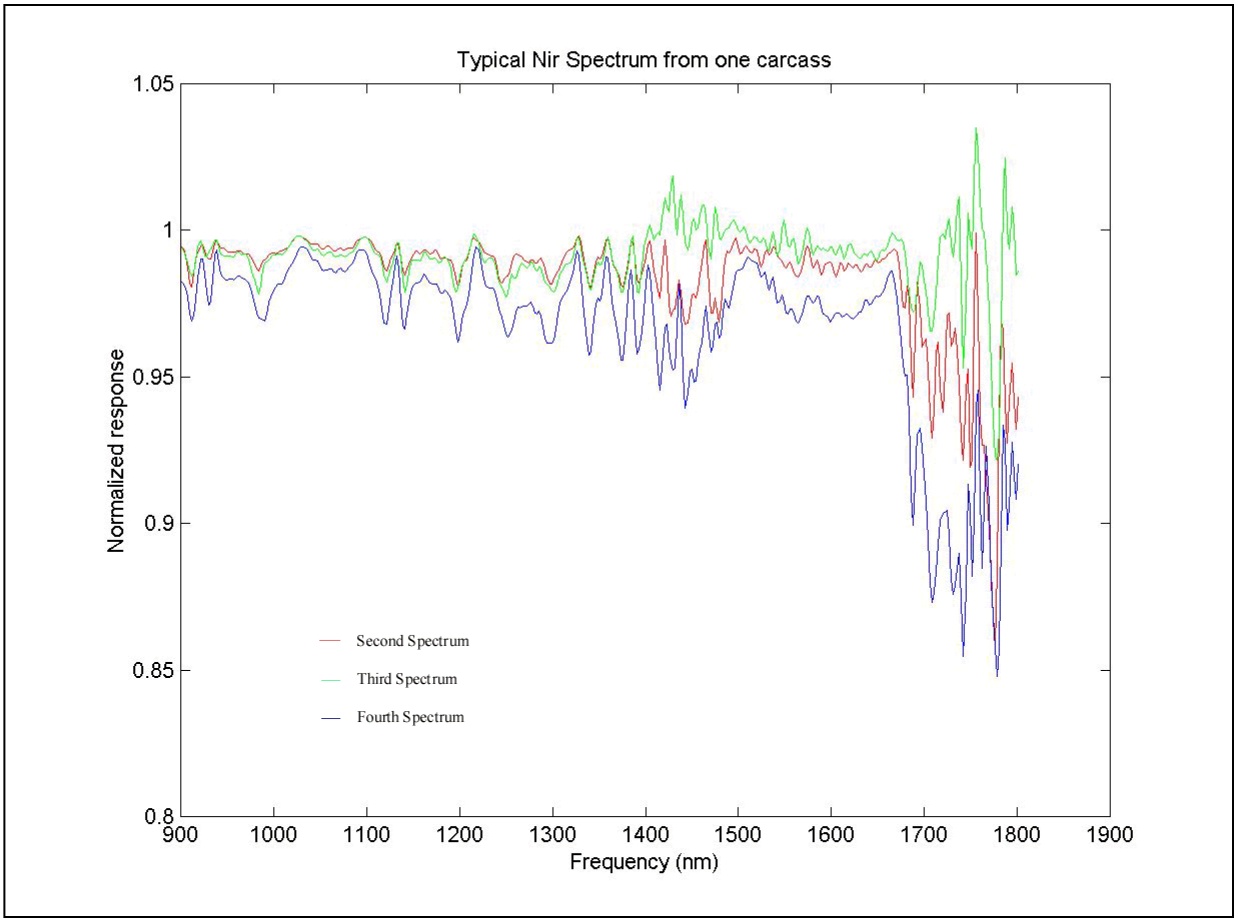

Figure 1: Typical NIR ratio spectra from 30 min to 32 min postmortem.

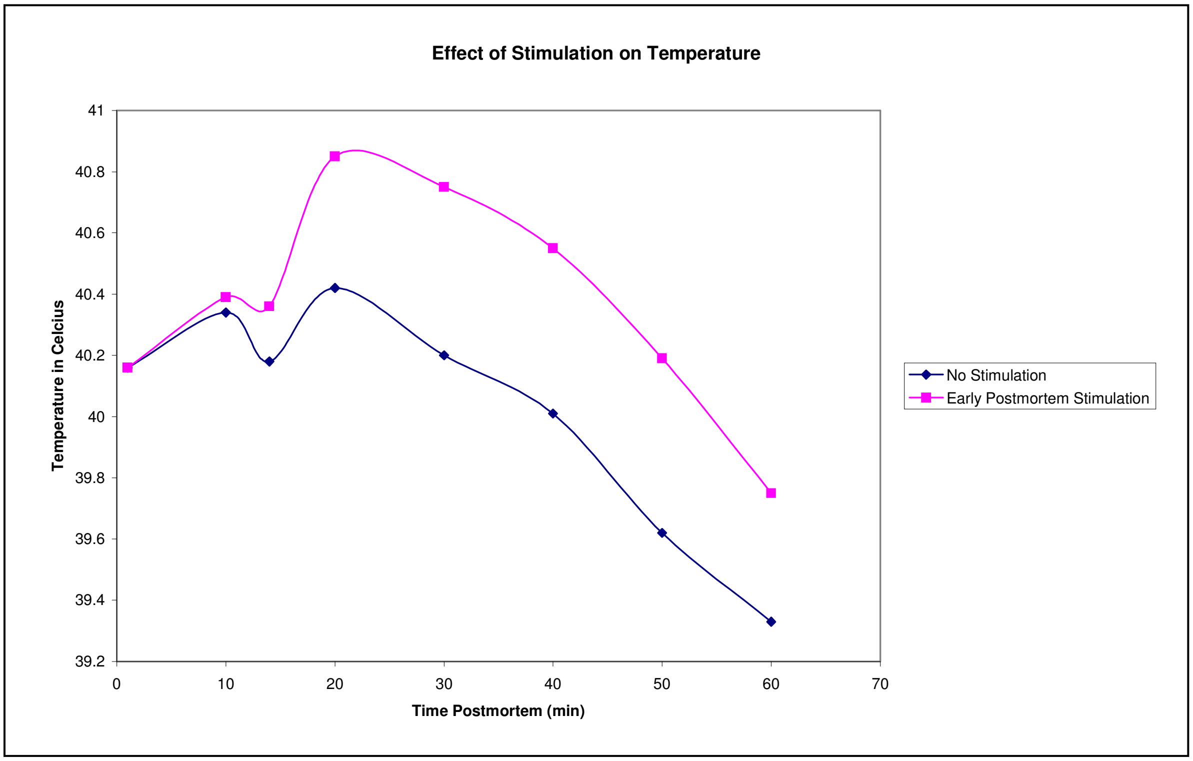

Figure 2: Temperatures for electrically stimulated vs non-stimulated carcasses.

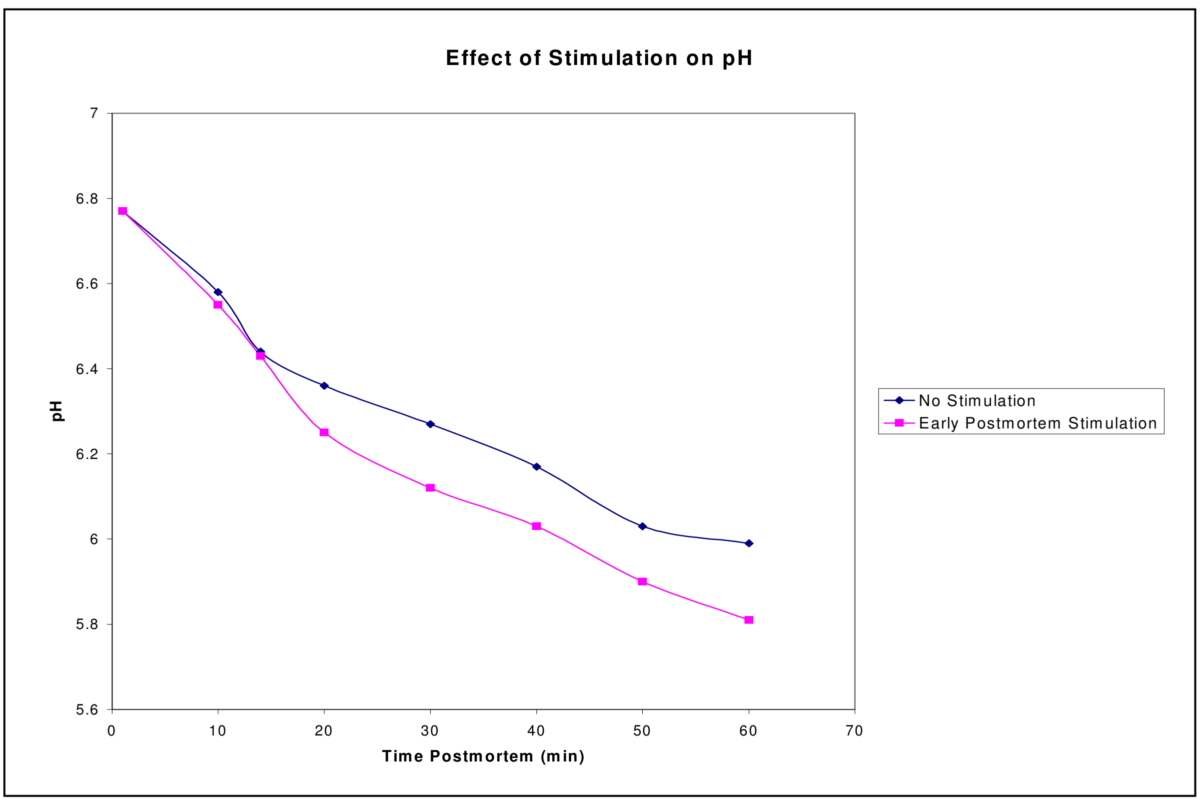

Figure 3: pH profiles for stimulated and no-stimulated carcass

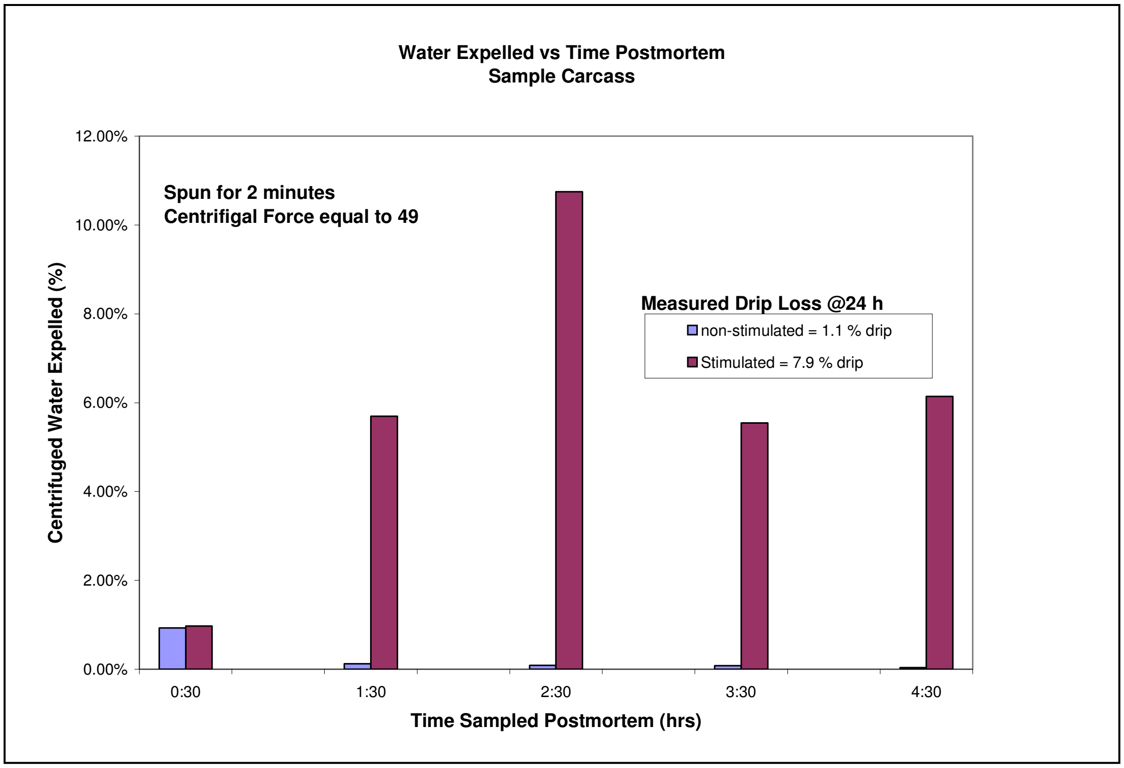

Figure 4: Water expelled from carcass samples during centrifuge method.

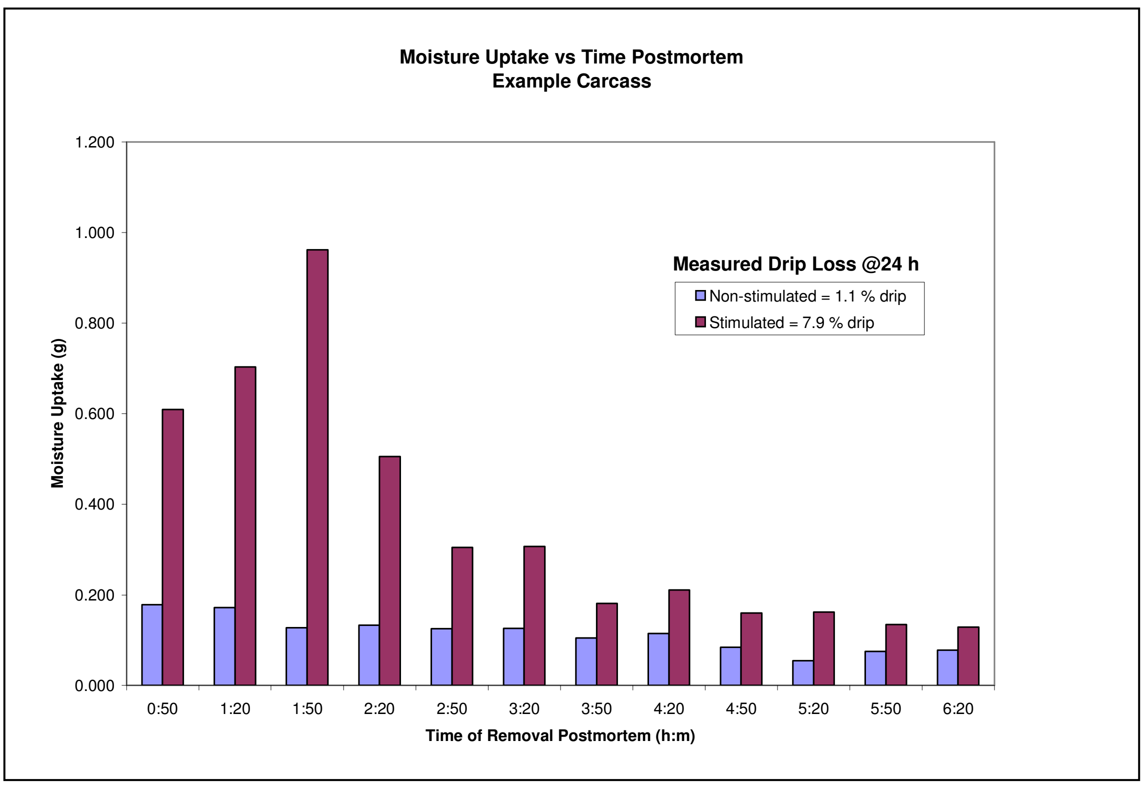

Figure 5: Water absorbed from a carcass using cotton implants removed every 30 minutes.

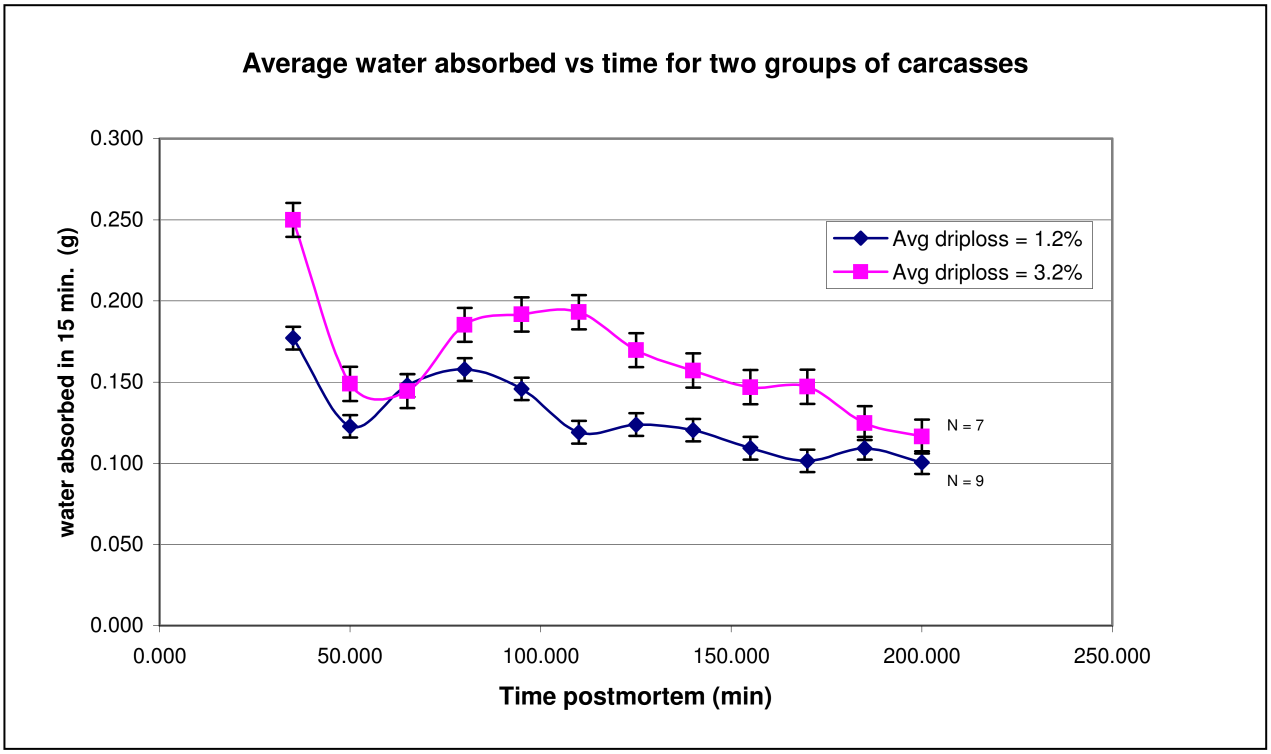

Figure 6: Average water absorbed by cotton implants for two groups of carcasses during first 3 h postmortem

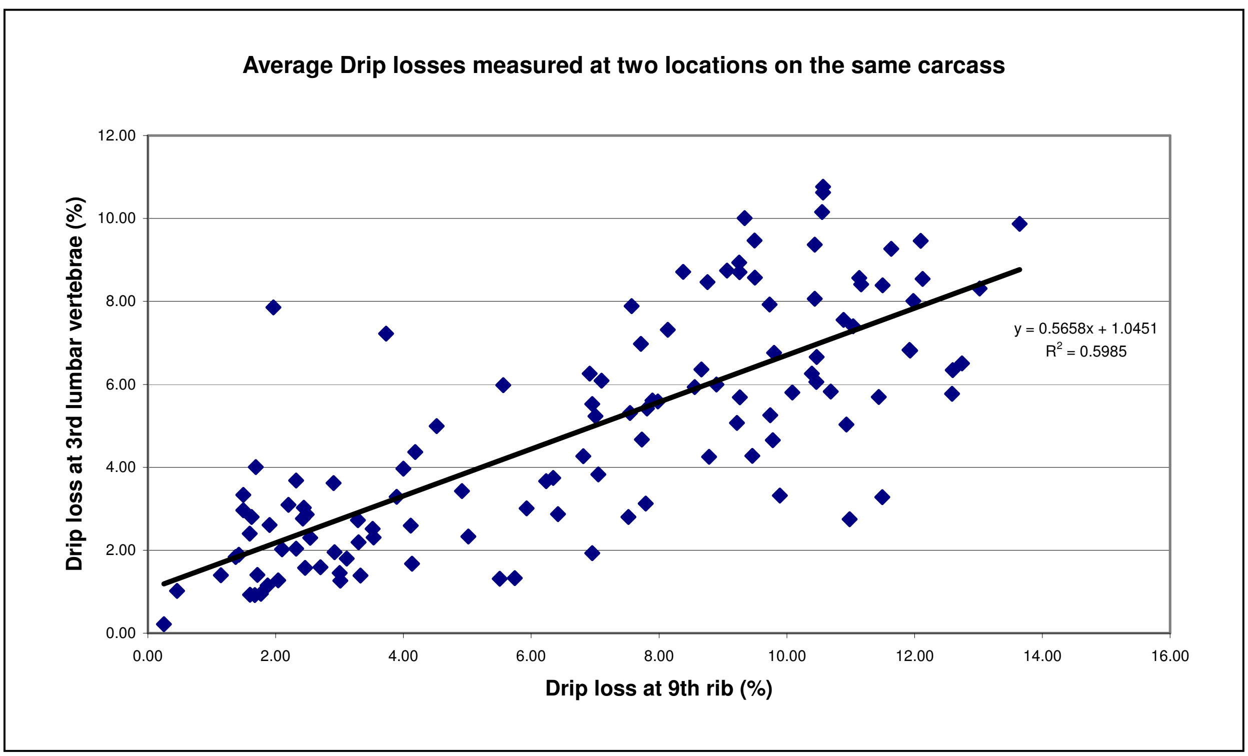

Figure 7: Comparison of drip loss measurements on the same carcass at two different locations.