RESEARCH REPORT
PORK QUALITY

**Title:** Role of Muscle Cytoskeletal and Intermediate Filament Proteins in the Development of Soft and Exudative Pork - **NPB#02-030**

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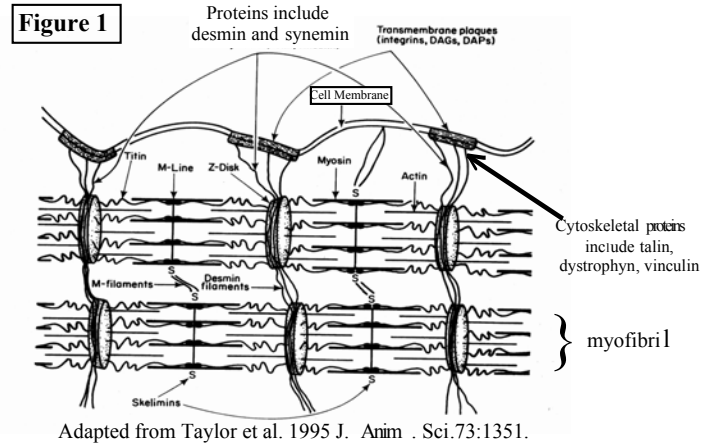
**Abstract:** This project addresses an important topic, the reduction in the amount of pale and exudative pork from non-halothane positive pigs. The central hypothesis for this project is: D*egradation of proteins involved in tying together adjacent myofibrils and the myofibrils to the cell membrane will improve the ability of the muscle cell and ultimately the muscle itself to retain water.* This central hypothesis was tested by accomplishing the following specific research objectives: 1.) Investigate the relationship between early postmortem degradation of intermediate filament (other than desmin) and intermediate filament associated proteins with water-holding capacity of fresh pork. The hypothesis behind this objective is: Specific proteins involved in linking myofibrils to the cell membrane may be degraded earlier than desmin and provide earlier indications of water holding capacity of fresh pork. 2.) Determine the role of intermediate filament protein degradation in water-holding capacity of enhanced pork. The hypothesis behind this objective is: Variation in degradation of intermediate filament and intermediate filament associated proteins is responsible for inconsistent responses to the enhancement process. This study used pork longissimus dorsi from that varied widely in their three hour postmortem pH. Product that had low 3 hour pH had less degradation of the proteins desmin and talin. Furthermore, enhanced product that had more intermediate filament protein degradation had better water-holding capacity. The results of this experiment demonstrate that degradation of intermediate filament proteins such as desmin is at least associated with improved water holding capacity of fresh pork loins. This conclusion supports the hypothesis that proteolysis of specific proteins can improve the water holding capacity of fresh and moisture enhanced pork. Exudative product continues to be a major concern in the pork industry. In order to minimize the incidence of soft, exudative pork, the mechanism underlying the development of this condition must be determined. This research focused on recently discovered relationships between degradation of structural components of the muscle cell and drip loss. Understanding this relationship will provide valuable information regarding direction for research to develop future technologies that will improve pork quality.

**Introduction:** Variation in water-holding capacity is one of the major quality problems that significantly affect the profitability of the pork industry. It has been estimated that as much as 50 % or more of the pork produced has unacceptably high water loss as purge or drip (Kauffman et al., 1992). Valuable water-soluble protein, vitamins, and product weight are lost with this moisture loss. It is clear that early postmortem biochemical and biophysical processes contribute to the development of water-holding capacity. Presently, there is a lack of knowledge regarding specifically how and why many variations in water-holding capacity develop. Rectifying this gap in the knowledge will allow the industry to develop intervention strategies and/or methods to predict quality and will provide the information needed to alleviate unforeseen problems that may develop in the future.

Currently, we do not know the specific biochemical and/or biophysical reasons for differences in meat water-holding capacity. One possible explanation resides in the structure of the muscle cell itself. As the pH of the muscle declines as muscle is converted to meat, the intricate latticework of the myofibril within the muscle cell shrinks, reducing the space within the cell where water can reside. If the proteinacous linkages between the myofibril and the muscle cell membrane are intact, this shrinkage can be translated into constriction of the entire muscle cell, thus creating channels between cells and between bundles of cells that can funnel drip out of the product(Offer and Knight, 1988). These linkages between adjacent myofibrils and myofibrils and the cell membrane are made up of several proteins known as intermediate filament proteins and intermediate filament associated proteins. These intermediate filaments attach to the cell membrane via other cytoskeletal proteins. Proteins that make up, or are associated with the intermediate filaments include desmin, filamin, and synemin. Many proteins are found in the cytoskeleton at the cell membrane, some of the more notable ones are dystrophin, talin and vinculin (Figure 1).

**Figure 1**




Adapted from Taylor et al. 1995 J. Anim . Sci.73:1351.

Research in our lab (Figure 2) and in others has suggested that *reduced* degradation of proteins that tie the myofibril to the cell membrane (such as desmin) results in *increased* shrinking of the muscle cell which is ultimately translated into drip loss (Kristensen and Purslow, 2001; Morrison et al., 1998; Rowe et al., 2001). The strongest relationship is at 5 days postmortem.

This research is important (Rowe et al., 2001) as it shows that there is a relationship between degradation of linkages between myofibrils and the cell membrane. However, other proteins in this structure need to be studied to determine their possible involvement. It is conceivable that

Relationship between desmin degradation and percentage drip loss in porcine longissimus dorsi

| Days Postmortem | % drip lost | | | |
|---|---|---|---|---|
| 2 days | 0 | 0 | 3.14 | 2.45 |
| 4 days | 0 .64 | 1.77 | 5.90 | 6.00 |
| 5 days | 0.92 | 2.47 | 6.86 | 6.92 |

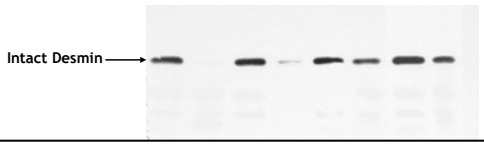



**Figure 2**. Drip loss over the first 5 days postmortem in loins from 4 different animals. Desmin shown at 1 and 5 days postmortem for each of the animals. Lack of a band indicates desmin has been degraded.

degradation of other proteins in this same region of the cell may show a relationship to drip loss at an earlier time postmortem. This information will be important in defining the physical mechanisms that dictate water-holding capacity.
This project addresses one of the resolutions passed by the delegates at the 2001 IPPA Annual Meeting regarding elimination of PSE pork. While this project does not address fresh pork color, this project does seek to identify the mechanism underlying the development of soft and exudative pork.

**Objectives:**
**Project Objectives**
The central hypothesis for this proposed project is: D*egradation of proteins involved in tying together adjacent myofibrils and the myofibrils to the cell membrane will improve the ability of the muscle cell and ultimately the muscle itself to retain water.* This central hypothesis will be tested by accomplishing the following specific research objectives:

**<u>Objective 1.</u> Investigate the relationship between early postmortem degradation of intermediate filament and intermediate filament associated proteins with water-holding capacity of fresh pork.**

**<u>Objective 2.</u> Determine the role of intermediate filament protein degradation in water-holding capacity of enhanced pork**.

**Materials and Methods:** For this project, samples were characterized for rate of pH decline, and drip loss according to the methods described in Melody et al., 2004. Samples were taken from two different lines of pigs that were harvested at a commercial facility on the same day. Samples were all from pigs that do **<u>not</u>** have the halothane gene. 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 3 and one hundred and sixty animals from line 2 measured. To determine which pigs had substantial differences in pH, the 3 h postmortem pH measurements were evaluated. Based on this 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. In addition, immunoblotting analysis of the muscle proteins was done. A standard at-death pork sample was loaded on each gel. Immunoblotting was being done according to standard methods (Huff-Lonergan et al., 1996). Antibodies against proteins desmin and talin were used. Antibody binding was monitored using enhanced chemilumenesnce (ECL-Plus, Amersham Pharmacia Biotech, Piscataway, N.J.) and a 12-bit megapixel CCD camera system (AlphaInnotech ChemiImager 5500). The ChemiImager and the accompanying software will be used for both image capture and one-dimensional image analysis. The intensity of the identified bands will be used to determine the relationship between degradation of these proteins and drip loss.

**Objective 2. Determine the role of intermediate filament protein degradation in water-holding capacity of enhanced pork**. This objective extends the effort to investigate how <u>added water</u> is immobilized in fresh pork. Samples from enhanced pork loins were characterized (Davis et al., 2004). Pork loins were injected on day 1 or day 4

postmortem. Product purge and texture were documented on 192 pork loins 1, 7 and 14 days post processing. Analysis of these samples was done to determine if proteolysis plays a role in binding added water in fresh pork. In addition, these analyses were used to determine if the enhancement process influences proteolysis. Extracts from each sample were prepared for SDS-PAGE and immunoblotting. A reference (at death pork longissimus) sample loaded on each gel. Immunoblotting was done according to standard methods (Huff-Lonergan et al., 1996). Antibodies against proteins in the cytoskeleton, intermediate filaments protein desmin. Antibody binding was monitored using enhanced chemilumenesnce (ECL-Plus, Amersham Pharmacia Biotech, Piscataway, N.J.) and a 12-bit megapixel CCD camera system (AlphaInnotech ChemiImager 5500). The ChemiImager and the accompanying software were used for both image capture and one-dimensional image analysis. The intensity of the identified bands was used to determine the relationship between degradation of these proteins and purge loss.

**Results:**

**<u>Objective 1.</u> Investigate the relationship between early postmortem degradation of intermediate filament and intermediate filament associated proteins with water-holding capacity of fresh pork.**

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 carcasses selected for more detailed evaluation. Additionally, the high and low pH groups were also significantly different ($P < 0.05$) at 45 min and 6 h. At 24 h postmortem, the average pH (5.86) of the longissimus muscles from line 7/high pH group was significantly higher ($P < 0.05$) than all other groups. 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 ($P < 0.05$) (Table 1)

Desmin degradation was expressed as a percentage of an intact desmin standard. Therefore, a larger number indicates lesser degradation (more intact desmin remaining in the sample). Desmin degradation was the least in the samples from the low pH groups at 1 and 2 days postmortem regardless of the line that they came from. In addition, line 7, had greater desmin degradation at 7 days postmortem than did line 6 (Table 2).

The samples that had the lowest three hour pH values also had the least amount of talin degradation. This was seen at all time points (1, 2, 3, and 7 days postmortem) (Table 3).

**<u>Objective 2.</u> Determine the role of intermediate filament protein degradation in water-holding capacity of enhanced pork**.

Little desmin degradation was observed on the first day in loins injected at 1 d postmortem. However, in samples that demonstrated low purge, degradation was observed at 8 and 15 d postmortem. In the low-purge loins injected 4 d postmortem, desmin degradation was observed at 4, 11 and 18 d postmortem. The high purge loins

do not show similar desmin degradation until 15 to 18 d postmortem. Kristensen and Purslow (2001) reported no degradation products of desmin at 1 d postmortem, but at 10 d observed a 29% decrease in desmin (55 kDa band). Kristensen and Purslow (2001) also found that water-holding capacity in pork declined in the first 2-7 d postmortem and increased during aging after 7 d postmortem. The change in water-holding capacity was attributed to desmin degradation. The results of this experiment (Davis et al., 2004) demonstrate that degradation of intermediate filament proteins such as desmin is at least associated with improved water holding capacity of fresh pork loins. This conclusion supports the hypothesis that proteolysis of specific proteins can improve the water holding capacity of fresh and moisture enhanced pork.

**Discussion:** The data in this report indicate that degradation of intermediate filament proteins and intermediate filament-associated proteins is influenced by pH. This is significant because as muscle is converted to meat, the intricate latticework of the myofibril within the muscle cell shrinks, reducing the space within the cell where water can reside. If the proteinacous linkages between the myofibril and the muscle cell membrane are intact, this shrinkage can be translated into constriction of the entire muscle cell, thus creating channels between cells and between bundles of cells that can funnel drip out of the product. This observation extends to moisture enhanced fresh pork. This extension is significant because it provides evidence that the process and ingredients cannot compensate for a poor quality raw material.

**Lay Interpretation:** Exudative product continues to be a major concern in the pork industry. In order to minimize the incidence of soft, exudative pork, the mechanism underlying the development of this condition must be determined. This project on recently discovered relationships between degradation of structural components of the muscle cell and drip loss. This project is unique because many of the proteins discussed in this application have not been examined in regard to this relationship. Understanding this relationship will provide valuable information regarding direction for research to develop future technologies that will improve pork quality.

**References:**

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**List of Tables**

**Table 1.**

Least squares means and standard errors of longissimus muscle pH measurements during the first 24 h postmortem, and drip loss measurements during 24, 48 and 96 h of storage for pigs (n = 40) selected based on rate of pH decline.

| | Line 7/High[1] | Line 7/Low[2] | Line 6/High[3] | Line 6/Low[4] | SEM |
|---|---|---|---|---|---|
| **pH** | | | | | |
| **45 min PM** | 6.21$^{y}$ | 5.91$^{z}$ | 6.27$^{y}$ | 6.02$^{z}$ | 0.046 |
| **3 h PM** | 6.27$^{a}$ | 5.47$^{b}$ | 6.19$^{a}$ | 5.41$^{b}$ | 0.029 |
| **6 h PM** | 5.86$^{a}$ | 5.49$^{b}$ | 5.78$^{a}$ | 5.47$^{b}$ | 0.046 |
| **24 h PM** | 5.86$^{a}$ | 5.65$^{b}$ | 5.66$^{b}$ | 5.54$^{b}$ | 0.042 |
| **% Drip Loss** | | | | | |
| **24 h storage** | 1.31$^{b}$ | 2.98$^{a}$ | 1.92$^{a,b}$ | 3.23$^{a}$ | 0.350 |
| **48 h storage** | 1.20$^{y}$ | 2.89$^{x,z}$ | 2.39$^{y,z}$ | 3.91$^{x}$ | 0.339 |
| **96 h storage** | 1.92$^{z}$ | 4.37$^{y}$ | 3.96$^{y}$ | 5.06$^{y}$ | 0.467 |

**Table 2.**

Desmin Degradation

| | Line | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6 | | 7 | | | | |
| | pH-group | | | | | *P*-value | |
| | H | L | H | L | SEM | Line | pH |
| Days pm | | | | | | | |
| 1 | 92.3 | 108.4 | 80.1 | 110.7 | 6.35 | 0.44 | **< 0.001** |
| | 80.5 | 98.5 | 76.1 | 93.0 | 7.87 | 0.54 | **0.03** |
| | 70.2 | 84.8 | 59.8 | 68.3 | 7.74 | **0.09** | 0.14 |
| | 56.4 | 74.7 | 26.6 | 45.6 | 9.83 | **< 0.01** | **0.06** |

**Table 3.**

Talin Degradation

| | Line | | | | | | |
|---|---|---|---|---|---|---|---|
| | 6 | | 7 | | | | |
| | pH-group | | | | | *P*-value | |
| | H | L | H | L | SEM | Line | pH |
| Days pm | | | | | | | |
| 1 | 56.9 | 109.8 | 46.1 | 152.1 | 19.42 | 0.42 | **< 0.001** |
| | 24.2 | 63.9 | 39.5 | 122.4 | 16.80 | **0.03** | **< 0.001** |
| | 12.8 | 64.0 | 13.1 | 19.5 | 14.39 | **0.07** | **0.03** |
| | 1.4 | 39.2 | 0.6 | 20.8 | 12.09 | 0.44 | **0.02** |