Early postmortem harvest practices influence pork color

Rachel Page Daniels

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David E. Gerrard, Committee Chair
Hao Shi
Samer El-Kadi
Doug Sutton

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Abstract

Consumers are often confused and reluctant to purchase products from fresh retail meat counters that exhibit significant color variations. This hesitation to purchase results in annual profit loss to the pork industry. Processes to minimize color variation remain a major focus of the industry and many meat science programs across the globe. Previously, we found inherent muscle characteristics contribute to variations in pork quality but these characteristics fail to explain the high frequency of two-toning and other pork quality defects routinely occurring in many hog processing facilities. Therefore, we hypothesized harvesting practices, such as scald alter color across muscles of the ham. Scald time was initially investigated using 32 carcasses subjected to either a 4 (n=16) or 8 (n=16) min scald time. Samples were collected before or after scalding and at 24 hrs. A 50% reduction in scald time resulted in ($p < 0.0001$) lighter muscle color (L*) early postmortem, although the 8 min scald treatment was lighter ($p < 0.005$) at 24 hrs. Although differences in pH ($p < 0.0001$) and color were noted, ultimate carcass temperature was not affected. To that end, we moved to validate our hypothesis in an industrial plant setting. Carcasses (n=200) were assigned treatments of 6.5 or 7.5 min scald times, and SM muscle samples were collected at 24 hrs. Surprisingly, the shorter scald time resulted in ($p < 0.05$) a lighter color, contradicting our first study. To explore this color issue further, we uncoupled scald from the dehairing process. To achieve this goal, carcasses (n=24) were assigned to either an 8 or 16 min time to dehair, with or without scalding. Protracted time to dehair resulted in higher ultimate pH ($p < 0.005$) and less color variation across the muscle ($p < 0.05$). Though a color gradient remained, the variation across the muscle was reduced by increasing time to dehair. These data show time to dehair affects pork quality development and suggest that delaying time to

physical manipulation of the carcass may improve pork color, thus increasing consumer acceptance.

Discoloration, or variation of color, in meat has been heavily investigated. Though much progress has been made in ensuring color development and stability, inconsistencies in muscles still exist. Recently, we have investigated the two-toning phenomenon in the semimembranosus muscle (SM) of pork and found that inherent characteristics of the SM only partially explain the color disparities within the muscle. Therefore, we hypothesized harvesting practices such as scald and dehairing time accelerated postmortem metabolism resulting in a color gradient across the muscle. This study consists of two experiments; testing the scald time and then testing the time to dehair. First, 32 carcasses received either a 4 min or 8 min scald treatment and samples we taken pre scald or post scald and 24 hrs. By decreasing scald time by 50% we observed lighter color (L*) across the muscle at 0 min ($p < 0.0001$), but at 24 hrs, the 8 min scald treatment was lighter ($p < 0.005$). Although differences in pH ($p < 0.0001$) and color were noted, ultimate carcass temperature was not affected. We moved to validate our hypothesis in an industrial plant setting, where longer scald times showed decreased color variation across the muscle with lighter muscle observed in the shorter scald treatment ($p < 0.05$). In order to better understand the effects of harvesting practices on this color variation phenomenon, a second study was conducted to determine the impact of increasing time to dehairing on ultimate color. Animals (n=24) were assigned to either an 8 or 16 min time to dehair, with or without scalding. Higher ultimate pH was observed in the 16 min treatment ($p < 0.005$), as well as, decreased color variation (L*) across the SM muscle. Though a color gradient remained across the muscle, color variation was reduced by increasing the time from stunning to dehairer. These data suggest that delaying the time to physical manipulation of a carcass may improve consistency in pork.

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Review of Literature

Introduction

Meat appearance, primarily lean color is recognized as the leading factor driving consumer purchasing decisions (Hutchings, 1977; Troy & Kerry, 2010). Consumers perceive meat color as an indicator of freshness and eating quality (Carpenter, Cornforth, & Whittier, 2001), and though there is little evidence to support that meat color correlates to overall freshness and palatability (Taylor & Shaw, 1977), consumers still demand uniform color and consistency throughout the meat case (Cassens, 2000). However, color and quality inconsistencies have plagued the pork industry for over 60 years (Briskey, Bray, Hoekstra, Phillips, & Grummer, 1959; Henry, Billon, & Haouza, 1955; Ludvigsen, 1954). Though efforts have been made to improve product consistency, variations in pork color remain.

Pale, Soft, and Exudative (PSE)

Arguably, the biggest meat color aberration facing the industry is a condition known as pale, soft, and exudative (PSE) pork. PSE normally occurs when muscle experiences an accelerated rate in pH decline due to an early postmortem glycolysis (Bendall & Swatland, 1988; Briskey, 1964). This low pH (5.4 - 4.8), coupled with elevated carcass temperature, results in denatured sarcoplasmic and myofibrillar proteins, ensuing in excessive purge and scattered light reflectance, attributing to the pale appearance. Typically, pigs harboring the halothane gene are at higher risk of PSE (Bendall & Swatland, 1988) because they release Ca^{2+} from the sarcoplasmic reticulum (SR) at a faster rate than normal pork muscle (Barbut, Sosnicki, Lonergan, Knapp, Ciobanu, Gatcliffe, Huff-Lonergan Wilson, 2008). The release of Ca^{2+} is double that of normal pork muscle

(Cheah & Cheah, 1976; Mickelson et al., 1988), resulting in an accelerated muscle metabolism (Barbut et al., 2008) and ultimately inferior meat quality. However, non-halothane positive pigs can also fall susceptible to PSE when animals experience short-term stress, such as antemortem handling. Due to selective breeding and improvements in antemortem handling, PSE occurrences have decreased from over 15% in 2003 to less than 2% in 2017 (National Pork Board, 2017). However, PSE is not just one condition, but rather a set of characteristics influenced by mainly by genetics and handling. These pale, soft, and exudative characteristics together cause undesirable products throughout the industry negatively impact the economic health of the pork industry. While frequency of PSE has generally decreased within the pork industry, other meat quality defects still exist; one being two-toning.

Two-Toning

Two-toning, or Halo Effect, are terms used to describe the inconsistency or color gradient found in meat between muscles, or sometimes within the same muscle. Typically, a muscle is classified as two-toned when color variation is observed between adjacent muscles. For example, the *longissimus dorsi* and *psoas major* have been suggested to differ in muscle color due to differing muscle metabolism and myoglobin concentrations (Wilson, Ginger, Schweigert, & Aunan, 1959). However, two-toning can also occur within a muscle and customarily results in a darker color towards the inner portion of muscle, while the outer superficial muscles tend to possess a lighter color, thus, giving the product a halo appearance (King, Shackelford, Schnell, Pierce, & Wheeler, 2018). This color defect is primarily observed in fresh ham and loin muscles

(King et al., 2018), and becomes exacerbated in cured products (McDonagh, Troy, Kerry, & Mullen, 2005) resulting in an undesirable product.

Though some associate quality defects with genetic lines (Wilson et al., 1959) or ante-mortem handling stresses (Moss, 1984) much like PSE, King and Pierce (2015), found two-toning within pork muscles exist even within in varying genetic lines and production settings. Additionally, research suggests the halo condition is present in muscle immediately excised post exsanguination and may be manifested in living animals (King et al., 2018). However, Stufft et al. (2017), found no difference in myosin heavy chain type I, or myoglobin across the muscle of the *semimembranosus* muscle, suggesting other factors may play a role in two-toning.

Despite extensive research, nearly 15% of hams and 12% of loins are still impacted by this two-toning phenomenon (Cannon, Morgan, McKeith, Smith, Sonka, Heavner & Meeker 1996). With today's consumer becoming increasingly sensitive to not only where their food comes from, but also the sudden shifts in quality, it is of the utmost importance to continue to investigate the underlying factors that impact pork color, and furthermore, two-toning.

Muscle Metabolism and Myoglobin

Factors such as species, age, sex, and nutrition influence meat color (Suman & Joseph, 2013), and though extrinsic factors affect the ultimate color of a product, inherent muscle characteristics lay the foundation for meat pigment (Hunt & Hedrick, 1977). Meat color is dependent on the abundance, or concentration, of the pigment protein myoglobin (Wittenberg & Wittenberg, 2007). Myoglobin is a water-soluble protein responsible for transporting and storing oxygen from the blood to the muscle (Wittenberg, Wittenberg, &

Caldwell, 1975). Myoglobin abundance varies depending on muscle energy demands (Wittenberg, 1970). Skeletal muscles are composed of a heterogeneous population of muscle cells, with distinct metabolic characteristics relying on varying combinations of aerobic (oxidative) and anaerobic (glycolytic) metabolism. Endurance muscles and muscles that are more fatigue resistant, such as muscles located near the bone, need oxygen, as they tend to be rich in mitochondria and utilize oxidative metabolism as a source for energy production. Due to the extensive need for oxygen, myoglobin is in high abundances and causes the muscle to have a deeper red color (Seideman, Cross, Smith, & Durland, 1984). Glycolytic muscles are typically muscles used for quick burst of energy, and because aerobic metabolism is less important in fast twitch muscles, the relative abundance of myoglobin is less (England, Matarneh, Oliver, Apaoblaza, Scheffler, Shi, & Gerrard, 2016) giving the muscle a lighter, or paler, appearance.

Postmortem Metabolism

Though myoglobin and fiber type establish meat color, postmortem metabolism and pH decline ultimately drive color development (Briskey Kastenchmidt, Forrest, Beecher, Judge, Cassens, & Hoekstra, 1966). Postmortem muscle metabolism and pH decline are a result of muscle cells attempting to maintain homoeostasis (Warner, 2016). In a living muscle cell, neural impulses stimulate the release of calcium from the SR flooding the cell. Calcium then binds with troponin C causing a conformational change to the troponin, tropomyosin complex, allowing for the formation of the actin myosin cross-bridge. However, in order for the muscle to contract, energy is needed. The hydrolyzation of adenosine triphosphate (ATP) generates energy in order to create a powerstroke. The powerstroke ultimately hydrolyzes the ATP into ADP (adenosine diphosphate) and an

inorganic phosphate (P_i). This energy conversion causes the sliding in filaments, while the rest of the energy is released as heat. ADP is then released after the tilt of the head allowing another ATP molecule to bind, detaching the cross-bridge.

During the transformation of muscle to meat, muscles labors to maintain homeostasis. In order for the muscle to remain within homeostatic set points, the muscle must maintain ATP levels. ATP is critical to cellular muscle function and when ATP is hydrolyzed during muscle contraction postmortem, a cellular buffering response is stimulated to prevent the loss of ATP. Muscles function to buffer the loss of ATP through three mechanisms: the phosphagen system, mitochondrial respiration, and glycolysis, all which occur at different times during the conversion of muscle to meat.

Initially, post exsanguination, the phosphagen system is activated in attempts to maintain ATP. Much like in living tissue, muscle will proceed to contract and relax early postmortem, converting ATP to ADP by the utilization of phosphocreatine. However, creatine reservoirs are rapidly depleted postmortem, requiring muscles to shift to an alternative metabolism.

Under aerobic conditions, mitochondrial respiration functions to produce ATP through the tricarboxylic acid (TCA) cycle. Briefly, glycogen undergoes glycogenolysis (the breakdown of glycogen), causing a glucose residues to be released as glucose-1-phosphate. Following glycolytic reactions, glucose-1-phosphate is shuttled into mitochondria in the form of two pyruvates. Once taken up in the mitochondria, TCA reactions generate 35 molecules of ATP. Additionally, each molecule of pyruvate will also yield 10 H^+ when metabolized through the TCA cycle. However, following exsanguination, mitochondria and the TCA cycle can no longer fully metabolize pyruvate

due to the absence of oxygen. Although there are data suggesting mitochondria still respire postmortem (England, Matarneh, Mitacek, Abraham, Ramanathan, Wicks, Shi, Scheffler, Oliver, Helm, Gerrard, 2018), as well as, strong evidence supporting the role of mitchondria in pH decline and the conversion of muscle to meat (Matarneh, England, Scheffler, Yen, Wicks, Shi & Gerrard, 2017), muscle is forced to function under anaerobic metabolism postmortem in order to maintain homeostasis and ATP production.

Anaerobically, muscle will metabolize energy through glycolysis. Glycolysis, though less efficient than the TCA cycle, will rapidly convert glucose to pyruvate, and furthermore lactate. This change of reactions ultimately yields three ATP, and three H^+ ions. This process is tightly regulated by the enzyme phosphofructokinase (PFK), and is fully activated by several substrates and metabolites, including ADP and calcium. It is important to note that, in living tissue, lactate would be transported to the liver and re-synthesized into glucose and glycogen; however, lactate and H^+ accumulate in the muscle postmortem and lower the pH.

During postmortem glycolysis, ATP is consumed faster than it can be produced, leading to an increase in glycolytic flux and accelerated glycolysis. This acceleration increases not only the abundance of lactate, but also the accumulation of H^+. While H^+ are buffered in an effort to maintain homeostasis early postmortem, concentrations eventually exceed buffering capacity and the pH of the muscle begins to decline.

A normal rate and extent of pH decline generates a gradual decline from approximately 7.0 to 5.7 within 6 to 8 hours in porcine muscle. This tissue acidity is impacted by many factors. Both an abbreviated and extended pH can occur, as well as increase rate of decline. Typically, an abbreviated pH decline is associated with limited

glycogen in the muscle prior to harvest, and results in a darker color lean due to the increase in bound water absorbing the light source. Alternatively, when glycolytic flux increases, rate and extent of pH can be increased resulting in a lower than normal pH. Furthermore, this increase metabolism will elevate muscle temperature, contributing to protein denaturation and structural loss. This structural loss of proteins creates an inability for muscle fibers to retain moisture in the tissues. Ultimately this causes meat quality defects of low water-holding capacity (WHC), as well as, an increase in light refraction, giving the muscle a lighter color.

Harvesting techniques effect on pork quality

It has been well established that pH and temperature have profound impacts on meat quality development (Briskey et al. 1966; Fernandez, Forslid, & Tornberg, 1994; Gardner, Huff-Lonergan, Rowe, Schultz-Kaster, & Lonergan, 2006; Sebranek & Judge, 1990). Additionally, the mechanism is well-understood (Bendall, 1973; Sayre, Briskey, & Hoekstra, 1963; Warriss, Bevis, & Ekins, 1989). Simply, when metabolism is accelerated due to a number of factors that increase glycolytic flux, pH declines because of ATP consumption, resulting in an accumulation of lactate and H^+, and furthermore, an increase in muscle temperature due to increased metabolism in the tissues. This forces muscle pH to reach levels near the isoelectric point creating a neutral charge resulting in the inability for water to remain bound to the muscle cell (Berg, 2002). This inability to maintain protein structure, as well as WHC, ultimately leads to overall poor meat quality. Therefore, industry practices have worked hard to alleviate these challenges over the years by improvement in genetic selection, antemortem handling, and the implementation

of blast chilling. However, pork processing, specifically during time of harvest, has still shown to influence overall pork quality.

Stunning Methods

Three different stunning methods are accepted in the pork industry: chemical, mechanical, and electrical. Each stunning method causes variations in pork quality. Due to these differences, studies have been conducted to determine which stunning method leads to the best ultimate pork quality. Hambrecht et al. (2003), evaluated carcass characteristics of pigs stunned by either CO_2 or electricity at varying line speeds and varying chilling methods. A higher pH at 30 mins was observed for carcasses stunned by an electrical method at a slower time to chill at 45 mins, than that of the pigs passing through a chilling tunnel prior to cold storage. It was concluded that CO_2 stunning produced inferior quality pork compared to carcasses that were stunned using electricity. CO_2 stunning resulted in pork with increased drip loss and lighter color lean. Furthermore, the authors concluded that, regardless of stunning method, carcasses with elevated temperature coupled with low pH postmortem resulted in overall poor meat quality. Although, pH was higher in the group of electrically stunned pigs, it is worth noting, that the authors found more confirmed PSE carcasses in the group of electrically stunned pigs than that of CO_2 stunning method.

CO_2 stunning allows pigs to move in groups and is thought to result in a better pork quality due to a decrease in stress of animals (Channon, Payne, & Warner, 2002). Although CO_2 gas stunning has become prevalent in industry, likely due to improvement in antemortem handling (Sante-Lhoutellier & Monin, 2014), the use of electrical stunning

is still used in many smaller plants and as a backup stunning method in larger harvesting facilities.

Electrical stunning provides a higher risk of quality defects, such as, blood spots and blood splash due to severe muscle contractions (Sante-Lhoutellier & Monin, 2014) post stunning. Methods of electric stunning include head-only, head-to-back, and head-to-chest. Head-to-back and head-to-chest methods limit the amount of back leg kicking (Wotton, Anil, Whittington, & McKinstry, 1992), which results in better meat quality due to decreased muscle contraction.

Additionally, Channon et al. (2002) found differences in muscle pH when comparing electrical and chemical stunning methods. Specifically, these researchers observed a quicker rate of pH decline in carcasses electrically stunned. However, Hambrecht et al. (2003), acknowledged that stunning methods, coupled with varying other processing factors, can attribute to quality variations across plants, and may not function independently in overall pork quality.

Scalding and Dehairing Methods

Following stunning and exsanguination, carcasses are subjected to a dehairing or dehiding process. Although dehiding is widely accepted amongst small meat processors, large industrial facilities prefer the dehairing method in order to capture a higher yield. Dehairing is typically a multi-step process, which includes scalding of the carcass prior to undergoing a vigorous tumbling, or paddling, to remove hair from the follicle. Remaining hair is removed by brief singeing.

Maribo et al. (1998) found dehided (skinned) carcasses have lower muscle temperatures during about one hour. Additionally, scalded carcasses have higher initial

muscle temperatures and an accelerated pH decline compared to skinned carcasses. These carcasses also produce lean that is paler and have greater drip loss. Furthermore, as expected, skinned carcasses had higher glycogen, glucose-6-phosphate, and lower lactate levels 24 hr postmortem, all contributing to a slower rate of pH decline and higher ultimate pH.

Maribo et al. (1998) suggested that scalding adds energy to the carcass and contributes to increased carcass temperatures early postmortem. However, others have argued that notion. Van der Wal et al. (1993), showed no significant effect of scald on the temperature of the muscle, and the ultimate quality of fresh pork. Monin et al. (1995) extended these results and showed no differences exist between carcasses dehaired through singeing alone, or a scald and singe method, suggesting carcass temperature is not affected significantly during scalding of carcasses. Moreover, van der Wal et al. (1993) showed no adverse quality effects were noted even if carcasses scalded for as long as 9 mins at 60℃.

In contrast, Mowafy & Cassens (1975) found carcasses scalded at a higher temperature for an extended amount of time leads to significant meat quality defects, possibly due to a delay in carcass chilling. Similarly, Marbio et al. (1998), found the rate of pH decline of scalded carcasses is faster than skinned carcasses over a 6 hr period. At 6 hrs, scalded carcasses were already in rigor suggesting protein denaturation is more prevalent in scalded carcasses compared to those dehided. Although scalding as a dehairing method may not influence muscle temperature or contribute directly to ultimate pork quality, acceleration of biochemical reactions has been observed in scalded and dehaired carcasses, causing a more rapid pH decline (Troeger & Woltersdorf, 1986).

During postmortem muscle contraction, ATP is hydrolyzed and eventually depleted, leading to protein denaturation and the inability of actomyosin crossbridges to breakdown. When scalded carcasses experience rigor at an accelerated rate, it will result in a paler color and lower WHC.

Though data outlined above convey a strong argument for dehiding carcasses in order to achieve better pork quality, this approach could lead to financial losses due to decreased yields. Therefore, if scalding is preferred method of dehairing, time and temperature must be carefully considered studied to create a quality pork product. Consequently, scalding is preferred by most hog processors given the financial benefits.

Carcass Chilling

During the harvesting operation, carcasses need to reach the chiller as quickly as possible in order to bring the temperature of the carcass down and eliminate any food safety concerns. In addition, data suggest that carcass quality is improved by shortening the time from stick to chill, preferably under 45 mins (Maynard & Warner, 1996). Gardner et al. (2006) reports that decrease dwell, the time from exsanguination to scald, and scald time decreases time from stun to chill and results in a more consistent, higher quality pork carcass. The faster a carcass can be chilled is thought to lead to a reduction in postmortem acidification of pork muscle, and improvements in water holding capacity and meat color (Channon et al., 2002; Offer, Knight, Jeacocke, Almond, Cousins, Elsey, Purslow, 1989). However, Honkavaara (1989) found only minor differences in temperature of carcasses being processed at different speeds.

While early pH decline can be used as an indicator of ultimate pork quality, there is also strong evidence suggesting rapid chilling approaches improve pork quality

(Cassens, 2000). Rapid chilling methods, such as the use of liquid nitrogen, have been widely accepted, and have been found to greatly reduce protein denaturation and thus, the presence of PSE in pork carcasses (Briskey et al., 1966; Cassens, 2000). Though this method reduces PSE prevalence, rapid chilling methods can lead to other quality concerns, such as, cold shortening and less tender meat (Barton-Gade, 1987; Sante-Lhoutellier & Monin, 2014). Although many of these processing practices do not work independently to drive meat quality develop, when coupled together correctly, they can significantly improve pork quality (Maribo et al., 1998).

Conclusion

Though the pork industry has developed processes to mitigate quality issues, color remains a major influence in consumer choices. The interaction between rate of temperature and pH decline and ultimate meat quality has been established, independent from scald. However, data suggest there may be a relationship between dwell time of scald and dehairing on temperature, and furthermore, rate and extent of postmortem pH decline. While breeding, nutrition, handling, and processing have been optimized for the pork industry (Barbut et al., 2008), there has been little research to examine how harvesting may improve meat quality. Much of the research shows the influences of scald temperatures, dehairing methods, and chilling time on the rate and extent of factors influencing postmortem metabolism; however, these data have made no advances in improving pork quality. Further research on harvest processing is needed in order to improve quality defects in pork muscle in order to decrease the financial impact on the pork industry.

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Chapter 2

Abstract

Color development is largely driven by pH and temperature declines postmortem. Accelerated postmortem metabolism results in rapid pH decline at a high carcass temperature. Together, this denatures muscle proteins, decreasing water holding capacity, which alters light refraction, thus, producing an undesirable meat color. We hypothesized changes in harvesting practices, such as, shortening scald time to improve color development by reducing carcass temperature earlier postmortem. Length of scald was investigated by thirty-two carcasses undergoing either a 4 (n=16) or 8 min (n=16) scald time at 60°C. Samples were collected before or after scalding and at 24 hrs. Interestingly, muscle temperature decreased after scalding regardless of scald time ($p < 0.05$). Although no changes in muscle temperature were detected, a 50% reduction in scald time resulted in lighter color (L*) muscle early post-mortem ($p < 0.0001$), while the 8 min scald treatment was darker ($p < 0.005$) at 24 hrs. Although differences in pH ($p < 0.0001$) and color were observed, carcasses undergo changes that cannot be attributed to carcass temperature as a result of scald time. To that end, we tested this hypothesis in a processing plant setting to further understand the effects of scald time on ultimate color. Two-hundred carcasses were assigned treatments of 6.5 (n=100) or 7.5 (n=100) min scald times, and SM muscle samples were collected at 24 hrs. A shorter scald time resulted in ($p < 0.05$) a lighter color, contradicting our first study. To study this issue further, we uncoupled scald from the dehairing process. To achieve this goal, twenty-four carcasses were assigned to either an 8 or 16 min time to dehair, with or without scalding (n=6 per treatment). Protracted time to dehair resulted in a higher ultimate pH ($p < 0.005$) and less color variation across the muscle ($p < 0.05$). Though a color gradient remains, the

variation across the muscle was reduced by increasing time to dehair. These data show that time to dehair impacts pork quality development and suggest that delaying time to physical manipulation of the carcass may improve pork color.

Introduction

In addition to price, consumer purchases are largely driven by meat appearance with an emphasis on product color and consistency (Hutchings, 1977; Troy & Kerry, 2010). As consumers often associate consistency and color with freshness and palatability, color variations both within and between products deter consumers, costing the pork industry billions annually (Van Laack, Kauffman, & Polidori, 1995). Changes in practices and operations to alleviate meat color defects have been the focus of meat science research for several decades. While the prevalence of some defects such as PSE have been decreased from over 15% in 2003 to less than 2% in 2017 (National Pork Board, 2017), variations in fresh lean color still exist. Of particular concern, the phenomenon of two-toning results in variable color both between adjacent muscles and within the same muscle, giving the product a halo appearance (King, Shackelford, Schnell, Pierce, & Wheeler, 2018). Though less frequent in the loin, ham muscles are more susceptible to the condition (King et al., 2018) and this defect becomes more exacerbated in cured products (McDonagh, Troy, Kerry, & Mullen, 2005). Therefore, understanding the factors that influence this color phenomenon are needed.

Two-toning and color variations can be partially attributed to products being derived from muscles that have inherently different muscle characteristics, which is a result of functional disparities. Muscles closer to the bone, such as those towards the center of hams, are fatigue resistant and function to stabilize joints, whereas, superficial muscles,

those found on the outside of the ham, are purposed for generating quick, powerful movements. These functional differences, coupled with underlying biochemistry during the conversion of muscle to meat, contribute to color defects; however, other practices can alleviate or exacerbate pork color defects.

While two-toning was once thought to be associated with specific genetic lines (Wilson, Ginger, Schweigert, & Aunan, 1959) or ante-mortem handling (Moss, 1984), King and Pierce (2015) reported two-toning in pork muscles from various suppliers and genetic lines. Additionally, muscles excised immediately post-exsanguination display two-toning, suggesting the defect is present in live muscle (King, Shackelford, Schnell, Pierce, & Wheeler, 2018). Although a color and pH gradient exist across the semimembranosus muscle at 24 hrs postmortem, the abundance of myoglobin or myosin heavy change type 1 isoform did not vary (Stufft, Elgin, Patterson, Matarneh, Preisser, Shi, England, Scheffler, Mills, Gerrard, 2017). Collectively, this suggests that while muscle characteristics may contribute to ham two-toning, other factors are at play early postmortem.

Different stages of harvesting have been heavily studied in our attempts to optimize meat quality. Carbon dioxide stunning has become increasingly prevalent in industry replacing electrical stimulation, to reduce animal stress (Channon, Payne, & Warner, 2002) and muscle contractions that would otherwise increase glycolytic flux early postmortem and increase the rate of pH decline (Sante-Lhoutellier & Monin, 2014). Further, dehairing methods have come into question for the potential to elevate or elongate periods of high carcass temperature. In animals exposed to ante-mortem stressors or genetic mutations, the rise in metabolic rate increases carcass temperature and accelerates glycolytic flux and pH decline. This combination of high temperature and rapid pH decline denatures muscle

proteins, which disrupts water holding capacity (WHC). Reductions in WHC decrease moisture retention, producing a meat surface that refracts rather than absorbs light. Improvements in WHC and meat color are observed when the carcass is cooled quickly to avoid situations of high temperature and rapid pH decline. It was thought longer scald times would negatively impact meat quality as a result of prolonged high carcass temperatures. However, longer scald time resulted in only minor changes on muscle temperature decline and meat quality (Van der Wal, Van Beek, Veerkamp, & Wijngaards, 1993), whereas, carcasses that alternatively had the hide removed showed an improvement in WHC and darker meat color (Maribo, Olsen, Barton-Gade, & Møller, 1998). Skinned carcasses have a lower temperature for the first hour postmortem compared to scalded carcasses (Maribo et al., 1998) and demonstrates the importance of heat dissipation early post mortem.

Although hide removal may improve meat quality attributes, this method is not used often because of the economic loss to the industry. Nonetheless, regulation of carcass temperature has been exploited in industry to improve quality, specifically the implementation of blast chillers. This improved rate of temperature decline influences glycolytic flux with a faster rate of temperature decline resulting in a higher pH, less protein denaturation, and ultimately improves meat color (D'souza, Dunshea, Warner, & Leury, 1998; Gardner, Huff-Lonergan, Rowe, Schultz-Kaster, & Lonergan, 2006; Goldspink & McLoughlin, 1964). Though carcass temperature does not begin to significantly drop until evisceration (Maribo, Olsen, Barton-Gade, Møller, & Karlsson, 1998), the defect is detected early postmortem (King et al., 2018) suggesting a process early in harvesting is the primary driver of pork qualty. Therefore, we investigated how changes in carcass temperature, as mediated by scald can impact fresh pork color in the SM muscle.

Materials and Methods

Exp. 1 Scald time affects two-toning independent of temperature

Thirty-two market weight pigs were raised at a commercial farm, transported to the Virginia Tech Meat Center, and harvested under normal operating procedures with the following exceptions. Pigs were randomly assigned to either a 4 (n=16) or 8 min (n=16) scald time. Animals were humanely harvested by electrical stunning (Model ES Best and Donovan, Blue As, OH, USA), exsanguinated, and samples were collected before scalding (pre-scald) or immediately after scalding (post-scald) from the *semimembranosus muscle* (SM). Temperature of the scald tank (Boss Machinery, Cincinnati, OH) remained constant at 60°C throughout process for all treatments. Either pre- or post-scald, the entire SM was excised from the carcass. A center slice of the SM was cut, approximately 5 cm thick, and was then separated into four zones (A, B, C, D) from the most cranial part of the muscle being zone A and the most caudal part being zone D (Figure 1). Zones A-C were sectioned in equal parts, while the most caudal tip contained zone D. Muscle samples from each zone were diced, snap frozen in liquid nitrogen, and stored in at -80°C until further analysis. Additionally, 24 hr SM muscle samples were then taken using the opposite ham of each carcass as described for initial sample collection and stored at -80°C until further analysis.

Color Analysis

The SM muscle was collected as previously mentioned, and color measurements were taken from each zone (A, B, C, D) in triplicates, averaged, and recorded. The Minolta CR300 colorimeter (Ramsey, NJ, USA), Illuminant D, 0° observer angle was used and color measurements were taken in areas where there were no visible color

defects. Colors were expressed as Commission Internationale de l'Éclairage (CIE) L* (lightness).

Temperature

Temperature measurements were collected using a ThermoPro Digital Food Thermometer (Model No.: TP-16, Dultuh, GA). Measurements were taken from the SM muscle, while still in the carcass, before scald (pre-scald), after scald (post-scald), 45 min, 180 min, and at 24 hrs. Three temperature measurements were then taken from the center slice, excised muscle at Zone A, Middle (Zone B and C), and Zone D to determine temperature changes across muscle.

Statistical Analysis

Continuous variables, including temperature, color, pH, lactate, and glycogen were all analyzed by ANOVA using PROC MIXED procedure using SAS version 9.3 (SAS Institute Inc., Cary, NC). Carcass was the experimental unit and the model included the scalding treatment, zones and time of sampling as fixed effects. Means were compared using Tukey-Kramer Multiple Comparison Test if a significant effect was detected. Data on graphs are least square means ±standard error means (SEM), and differences were considered significant at $p < 0.05$.

Exp. 2 In-plant study

To validate the findings of the pilot plant, two hundred market weight pigs were raised and harvested by an industry partner. Carcasses received either a 6.5 (n=100) or 7.5 (n=100) min scald treatments. Animals were humanely harvested under normal processing procedures with the exception of length of scald. Color was then taken at 24 hrs using a Minolta Colorimeter using the same technique as the initial study.

Exp. 3 Adjusting time to dehairer decreases two-toning in selected ham muscle

Twenty-four market weight pigs of similar genetics of experiments outlined above were raised at a commercial farm and transported to the Virginia Tech Meat Center for harvesting. Pigs were randomly assigned to either an 8 (n=12) or 16 (n=12) min time to dehair, with (n = 6) or without (n = 6) scalding. Pigs were harvested as outlined in Exp 1 except that all carcasses were then subjected to 1 min of dehairing then dehided. Samples were collected from the *semimembranosus* muscle (SM). Muscle samples were collected and stored in the same manner as the previous experiment.

Temperature measurements were taken using the same thermometer as the previous experiment. Measurements were taken from the SM muscle, while still in the carcass, before scald, after dehairing, 45 min, 180 min, and at 24 hrs. Temperature was collected in the same locations as outlined above. Color, pH, and lactate were collected and analyzed using the same techniques as in experiment 1.

Results and Discussion

Scald time affects two-toning independent of temperature

In attempts to reduce carcass temperature early postmortem and improve ultimate meat color, we reduced the scald time to 50% of industry standards. We predicted the shorter scald time would result in less heat (energy) input that would reduce protein denaturation and pH decline, and ultimately mitigate color defects. Carcasses were assigned to either a 4 or 8 min scald time. Temperatures of the SM were collected before scald (pre-scald), after scald and dehairing (post-scald), 45 min, 180 min, and at 24 hrs. Although carcasses were exposed to scald tank temperature, SM muscle temperature significantly decreased ($p < 0.05$) immediately after scald (Figure 2). No significant temperature differences were detected from a 50% reduction in scald time suggesting a

shorter scald simply does not affect heat dissipation or retention. These findings correspond to those reported by van der Wal et al. (1993) that showed longer scald times had minimal effects on temperature decline and meat quality; though differences in some meat quality attributes were noted. Though no differences were seen between treatments, the rate of temperature decline across zones varied ($p < 0.0005$), with zone A showing a significantly lower temperature at 180 mins compared to all other zones (Figure 3). The variation in temperature decline may be a result of zone A being the most caudal zone, closest to the bone. Zone A would be the least susceptible to any temperature effects from scald, and a lower muscle temperature while pH is also declining can result in better muscle color.

Color variation was evident ($p < 0.0001$) across zones already at 0 min for both treatments (Figure 4a). The magnitude across zones A and D was 8.7042, which shows a visible color difference across the muscle at 0 min. After 24 hrs, L* values increase ($p < 0.0001$) in each zone, resulting in a higher magnitude (9.5007) between zones A and D (Figure 4b). While each zone increases over time postmortem, figure 4 shows the caudal zones increasing at a quicker rate causing a lighter outer color. Though no treatment interaction was observed between scald time and zone, we can observe that there is a significant gradient across the muscle, with zones A and B having no differences.

Regardless of muscle temperature, pork from 4 min scalded carcasses had higher lightness values (L*) compared to those scalded for 8 min ($p < 0.0001$) early postmortem (Figure 5a). Curiously, a shorter scald time resulted in the opposite color change, independent of muscle temperature. While the exact reason for this change in L* value is not known, it suggests some other perturbation may adversely alter pork quality rather than temperature. Unfortunately, in the case of our studies, shortening scald time also shortened

the time to dehairing paddles, confounding scald time with time to paddles. Surprisingly; however, at 24 hrs, we observed ($p < 0.005$) significantly different L* values between treatments (Figure 5b). These data agree with Eldridge, Ball, & Knowles (1993) who found that by delaying the time to evisceration, muscles would have lower pH and lighter lean color, and Gardner et al. (2006) found decreased loin quality as a result of increasing time spent in a scald tank. Higher 24 hr L* values can be a result of prolonged heat exposure or slowed heat dissipation, causing muscle temperature to decline at a slower rate. When muscle temperature is high and pH is low, protein denaturation occurs (Honikel & Kim, 1986; Sayre, Briskey, & Hoekstra, 1963), causing increase drip loss and paler meat (D'souza et al., 1998; Eldridge et al., 1993), and in extreme cases PSE meat. pH is a good early indicator of color development (D'souza et al., 1998); however, no differences in pH at 0 min were observed ($p > 0.05$) between zones or treatments, suggesting glycolytic flux is unaffected by scald. Even so, however, ultimate pH declined ($p < 0.0001$) with zone (A to D) (Figure 6). Data in figure 4 are pooled across both scald times indicating that a color gradient exists in the 24 hrs muscle samples; however, no interaction between treatment and zone was evident. The variation in ultimate pH across zone is in contrast to initial pH values and illustrates the inherent variation in rate or extent of pH decline in the ham muscle. Because rate and extent of pH decline influence meat quality (Briskey, 1964), we can predict that pH declines at a faster rate, as a result of increasing scald time, and causes paler meat. Stufft et al. (2017) found an accelerated pH decline in the most superficial part of the SM muscle (Zone D) in varying ham colors suggesting varying rates in postmortem metabolism across the SM muscle.

Lactate and glycogen were measured across zones at both 0 min and 24 hrs; however, lactate showed no differences across zone either initially or at 24 hrs. Differences in glycogen were noted ($p > 0.05$) among zones, and though not significant at 0 min, higher glycogen levels were found in zones C and D (Figure 7a). Figure 7b shows the glycogen levels differed ($p < 0.001$) across zones at 24 hrs, with higher glycogen levels in zones C and D. Higher levels of glycogen could be a result of slower rates of glycogen degradation in the outer zones. Muscle fiber type has been shown to influence the rate of glycolysis, suggesting more oxidative fibers have a slower rate of glycogen degradation (VØIllestad, Tabata, & MedbØ, 1992). More superficial zones (C & D) may have lower glycolytic capacity, slowing down the rate of glycolysis and decreasing lactate production (Karlsson, Klont, & Fernandez, 1999). Slower rates of glycogen degradation have been shown to result in higher pH (Scheffler & Gerrard, 2007) and subsequently darker meat color; however, these data show a lower muscle pH in zones with increased glycogen content at 24 hrs. Honkavaara (1989) found an increased rate of glycogen breakdown in carcasses scalded for longer periods of time, and while not significant, we also observed increased ($p < 0.05$) in glycogen breakdown (Figure 7) coincided with decreases ($p < 0.05$) in lactate accumulation (Figure 8b) as a result of longer scalding periods. Figure 7a shows a decrease ($p < 0.05$) in glycogen while figure 7a shows an increase ($p < 0.005$) in lactate post scald at 0 min, supporting previous knowledge that postmortem metabolism causes an increase in lactate accumulation and decrease in glycogen. When an animal is harvested, anaerobic glycolysis takes place and breaks down glycogen in order to produce enough ATP to remain in homeostasis. As a result, lactate and H^+ ions accumulate, and heat is produced

(Scheffler & Gerrard, 2007). Higher lactate accumulation results in a lower muscle pH, which can also lead to the paler meat.

Exp. 2 In-plant study

In order to validate these findings, we tested the aforementioned findings in a large-scale commercial hog processing facility. Two-hundred (200) commercially raised hogs with similar genetics to those used in the aforementioned study were harvested and were scalded for 6.5 or 7.5 min. In-plant color (L*) variation data were collected at 24 hr (Figure 9). Curiously, we noted that a longer scald resulted in lower L* or more favorable color values in zones C and D, or less total color variation across the muscle compared to those carcasses scalded for a shorter time ($p < 0.05$). From this study, we observed an opposite effect of scald on ultimate color from the initial study in a pilot plant. These data contradict previous data claiming that a longer scald time causes poor meat quality (D'souza et al., 1998; Eldridge et al., 1993). While the exact reason for this disparity is not known, they could be a result of a number different factors, such as, stunning methods (CO_2 vs. electrical), lairage time, line speeds, etc. Though these may influence muscle color, we observed that color in Zones A and B were similar across treatments, and zones C and D differed significantly.

After investigating scalding impacts, further investigation was needed to determine the impacts of harvest processes on meat color. In reviewing our protocol, we noticed that scald time was always confounded with the time carcasses were mechanically dehaired. On further review of the literature, this is often, if not always the case (Gardner, Huff-Lonergan, Rowe, Schultz-Kaster, & Lonergan, 2006; Mowafy & Cassens, 1975; Van der Wal et al., 1993). In addition, Hammelman, Bowker, Grant, Forrest, Schinckel, & Gerrard

(2003) demonstrated muscle reacts negatively to physical disturbances early postmortem suggesting a critical window exists in muscle whereby metabolism may be negatively affected. Therefore, we hypothesized that dehairing or the mechanical perturbations associated with that process may be responsible, in part, for changes in meat quality development.

Adjusting time to dehairer decreases two-toning in selected ham muscle

To test the contribution of time between exsanguination and dehair on fresh pork color development in the ham, carcasses we assigned to either and 8 or 16 min dwell, with or without scald. Differences in carcass (lean) temperatures immediately after hide removal (0 min) were detected ($p < 0.05$) between scald and non-scald treatments (Figure 10a). This is in conflict with previous research suggesting scalding does not impact carcass temperature (Honkavaara, 1989; Van der Wal et al., 1993). While we do not argue scald affects temperature, our data suggest scald may limit the rate of heat released from the carcass rather than raising the temperature. Additionally, an interaction between scald and time to dehair was noted ($p < 0.05$) for carcass temperature at 24 hrs (Figure 10b). The reason for this interaction is difficult to understand because differences in muscle temperature are observed between scald and no scald, if time to dehair is 8 min versus 16 min. Yet, no difference in temperature is noted if time to dehair is lengthened by 8 min. Though significant, this difference may be due to difference of cooling rate throughout chilling coolers. However, the temperature differences between treatments could also be influenced by varying cooling rates as a result of the prevention of heat loss from scalding in combination with earlier dehairing causes elevated temperatures early postmortem. Furthermore, difference in ultimate color is heavily associated with the

decline of temperature rather than the individual 24 hr temperature, as the rate of decline must agree with the rate of pH decline to ensure a quality product.

Significant differences in initial pH of muscle were observed ($p < 0.05$) between 8 and 16 min treatments at 0 min. Specifically, 16 min time to dehair had higher ($p < 0.005$) initial pH values compared to those dehaired sooner in the harvesting process (Figure 11a). A similar response was noted ($p < 0.005$) in the ultimate pH (Figure 11b). These data support our hypothesis and are in agreement with Hammelmann et al. (2003), who reported physical disruptions to the muscle early postmortem increase the rate of pH decline. Figure 10c shows a significant difference ($p < 0.001$) exists in pH across zones at 24 hrs, consistent with our 24 hr L* data (Figure 12a). Stufft, Elgin, Patterson, Matarneh, Preisser, Shi, Gerrard (2017) also produced similar results across zones attributing differences in pH to glycolytic flux, while Huff-Lonergan, Baas, Malek, Dekkers, Prusa, & Rothschild (2002) suggest differences in ability to utilize glycogen postmortem. Furthermore, figure 9d illustrates a difference ($p < 0.005$) in pH at 24 hrs between scald and no scald treatments with a higher pH occurring in the no scald treated carcasses. Maribo et al. (1998) suggest scalding can add energy to the carcass, resulting in a generation of heat, or perhaps inhibits the release of heat, early postmortem. Even this small flux in temperature could contribute to pH, especially rate early postmortem.

Figure 12a shows lightness (L*) values across zones. Understanding that muscles near the bone tend to be less manipulated by postmortem metabolism (Kim, Yang, & Jeong, 2018), it is no surprise there was no difference observed between A and B zones. However significant difference was observed in both C and D zones ($p < 0.0001$) Additionally, neither A or B zones differ regardless of time to dehair; however, C and D

zones differ ($p < 0.0001$) across zones within allotted treatment (Figure 12b). These data are consistent with Stufft et al. (2017) and King et al. (2018) who also found increases L* values on the outer most regions of the muscle. Though a color gradient still exists across the SM muscle, regardless of time, the magnitude of 16 min treatment is less severe than that of the early dehairing time of 8 min (9.36 vs. 13.43). It is worth noting, a shift in 2 L* values can be seen by the naked eye (Brewer & Zhu, 1999), making these data quite intriguing and translatable. However, these data differ to those of D'souza, Dunshea, Warner, & Leury, (1998) where L* values were higher in carcasses undergoing delayed processing. The exact reason for this discrepancy remains somewhat unclear, but may be due to the steps at which dwell times are taking place prior to evisceration. D'Souza et al. (1998) recommends that the harvest process should be completed in under 45 min in order to ensure meat quality. Many processing plants are able to complete harvest before 45 min (Maynard & Warner, 1996), allowing carcasses to enter the cooler faster, resulting in quicker temperature decline. However, these data suggest extending the time to the dehairer may result in improved overall color and color variation across the SM muscle.

Finally, lactate was analyzed in order to understand better the biochemistry underlying postmortem metabolism (Figure 13). Only zones A and D were analyzed given that A and B, and C and D tend to behave similarly. Lactate was used because of its relationship to glycolytic flux in the tissue. Postmortem, glycogen decreases as lactate increases, however alterations in glycolytic flux cause varying levels of lactate accumulation. ATP consumption causes glycolysis to accelerate, forcing an accumulation of lactate and H+. Lactate accumulation was higher ($p < 0.05$) in both 8

and 16 min treatments in zone D at both 0 min and 24 hrs, which corresponded to lower pH values (Figure 11c). Though not significant, 16 min treatment resulted in higher ($p > 0.05$) lactate accumulation compared to the 8 min treatment in zone A at both 0 min and 24 hrs, yet zone D of the 8 min treatment had higher lactate accumulation at 24 hrs. Zone D, being the most superficial of the SM muscle, experiences the most manipulation and is most susceptible to muscle changes. These data suggest that a delay in time from stun to dehair will improve pH and color in zone D, by decreasing glycolytic flux early postmortem.

Conclusion

These data show that scald time affects fresh ham color, as documented by objective L* values, independent of changes in temperature. Previous research (Sosnicki, Wilson, Sheiss, & de Vries, 1998) suggests shortening the exsanguination process in order to reach the scald tank and the cooler quicker improves carcass quality; however, our data suggest the opposite. This study suggests delaying time to dehair may decrease glycolytic flux early postmortem and alter the rate and extent of pH decline, resulting in improved meat color development or a reduced magnitude of two-toning across fresh hams.

The pork industry has always sought to make the harvest process as fast as possible for both food safety and financial reasons. However, through previous (Hammelman et al., 2003) and current research, the data shows that any physical manipulation prior to 30 mins postmortem will have a negative impact on pork quality. Therefore, suggesting that by limiting the amount of physical manipulation to the carcass prior to 30 mins postmortem, the pork industry should see overall improved products

consistency and increased consumer acceptance. . Carcasses should still reach the chiller within 45 minutes, but steps early postmortem should allow for a substantial amount of time prior to any physical manipulation (scald/dehair). The transition to an increased time to dehair will require changes in the harvest process in order to not slow down production in consecutive steps prior to chilling. To that end, with more consumer acceptance the profits associated with improving quality should also increase

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Chapter 3
Future Implications

Variation in pork quality impacts consumer purchasing decisions, and ultimately costs the industry in value. Much effort has been made over the years to improve pork quality; however, additional work is still needed. Collectively, our data suggest a critical "window" exists in muscle early postmortem that when mechanically disturbed, can lead to altered pH decline, and result in poor meat quality. Therefore, further investigation the size of this "window" is necessary in order to identify best operating procedures to improve overall pork quality. Research should be conducted in a commercial processing plant in order to validate our findings in relation to time to dehair. Furthermore, additional steps should be taken to gain better understanding of potential to increase yield through ultimately increasing pH and; furthermore, WHC. Finally, understanding that 75% of pork is furthered processed annually, testing the performance of these muscles under curing conditions to determine if functionality is improved is needed.

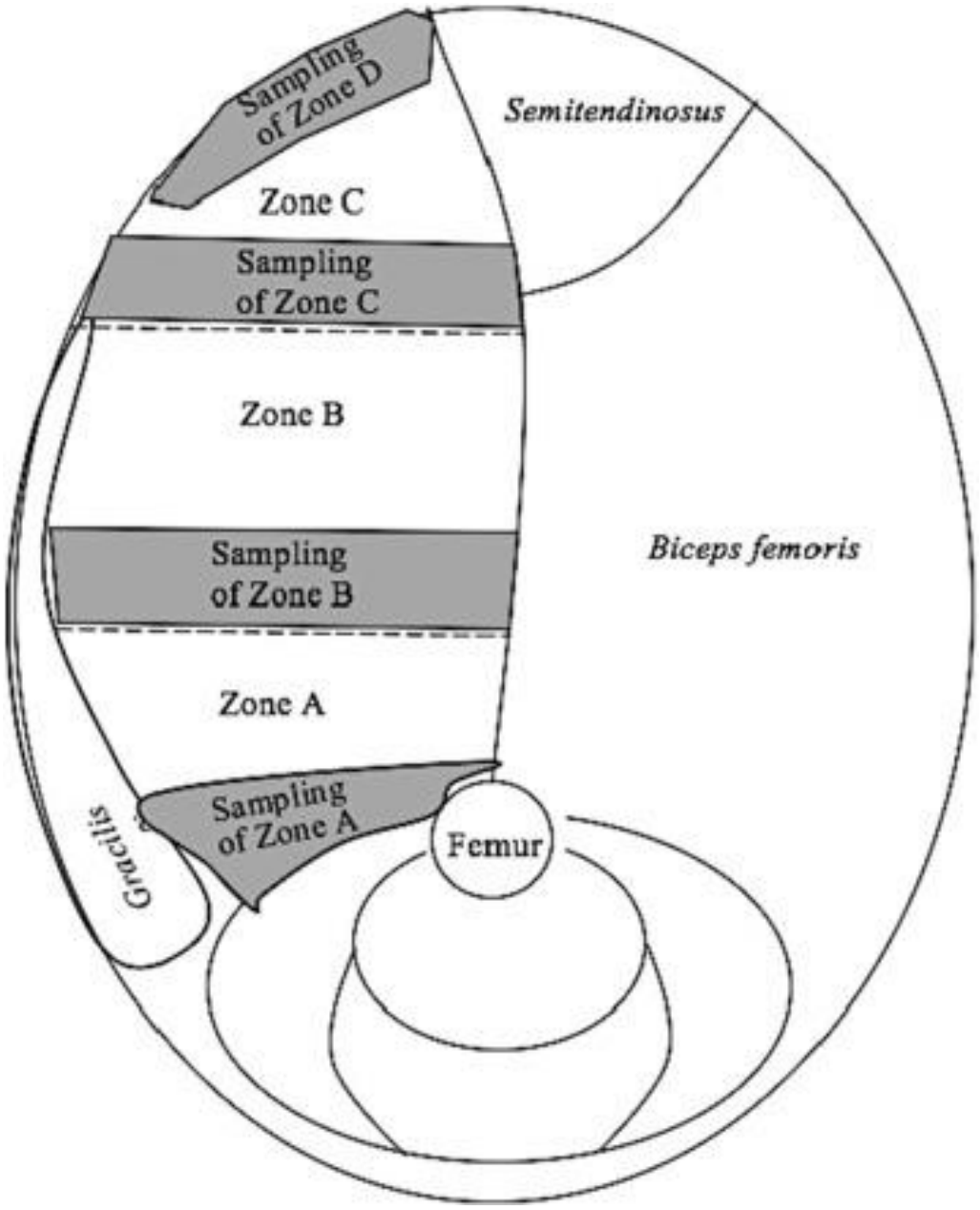

Figure 1. *Semimembranosus* sampling locations. Cross-section of porcine ham, designating sampling zones (A, B, C, D) in the semimembranosus muscle. Samples were taken from the shaded gray portion of each zone.

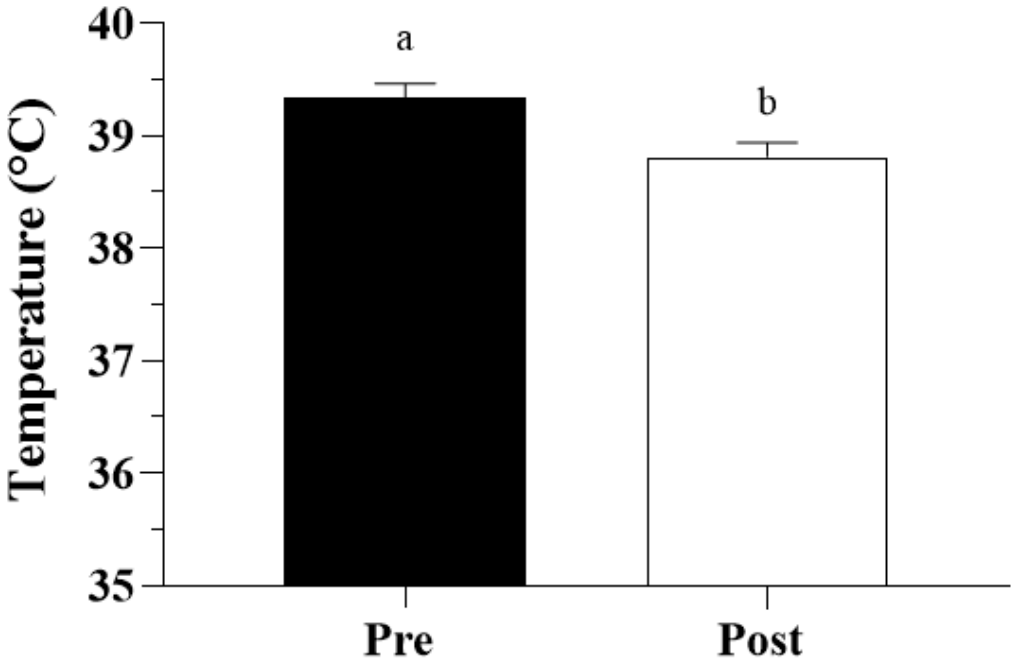

Figure 2. Mean temperature (°C) of the *semimembranosus* muscle pooled across zones immediately prior to (Pre) and after (Post) scald. Data are LS means ± SE. Means bearing different letters differ ($p < 0.05$).

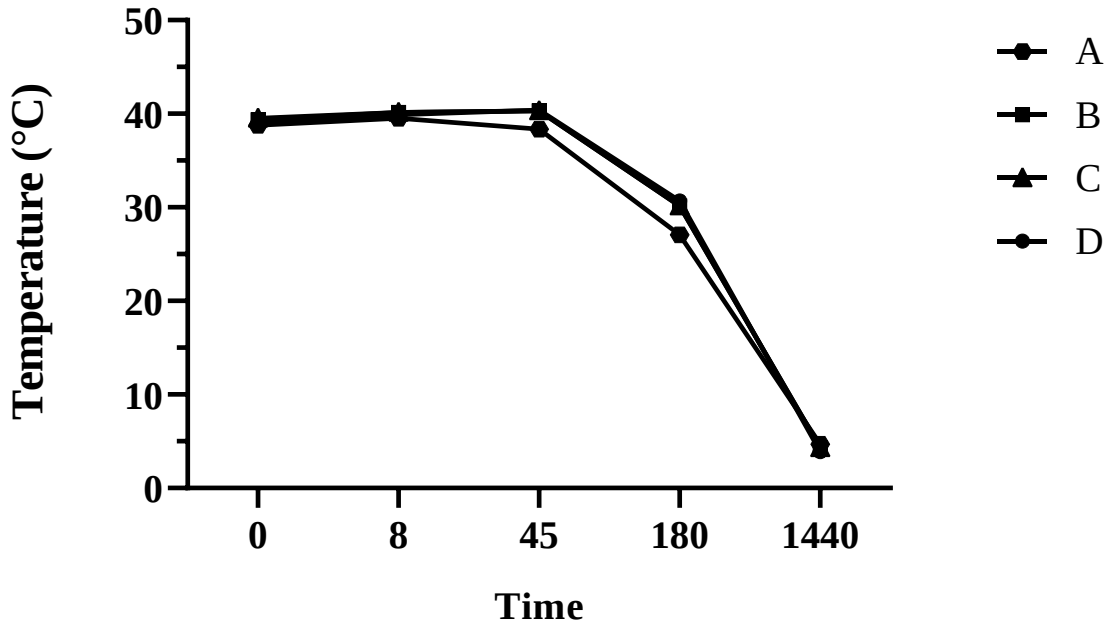

Figure 3: Temperature decline at time points pre (0), post (8), 45 min, 180 min, and 24 hrs across all zones.

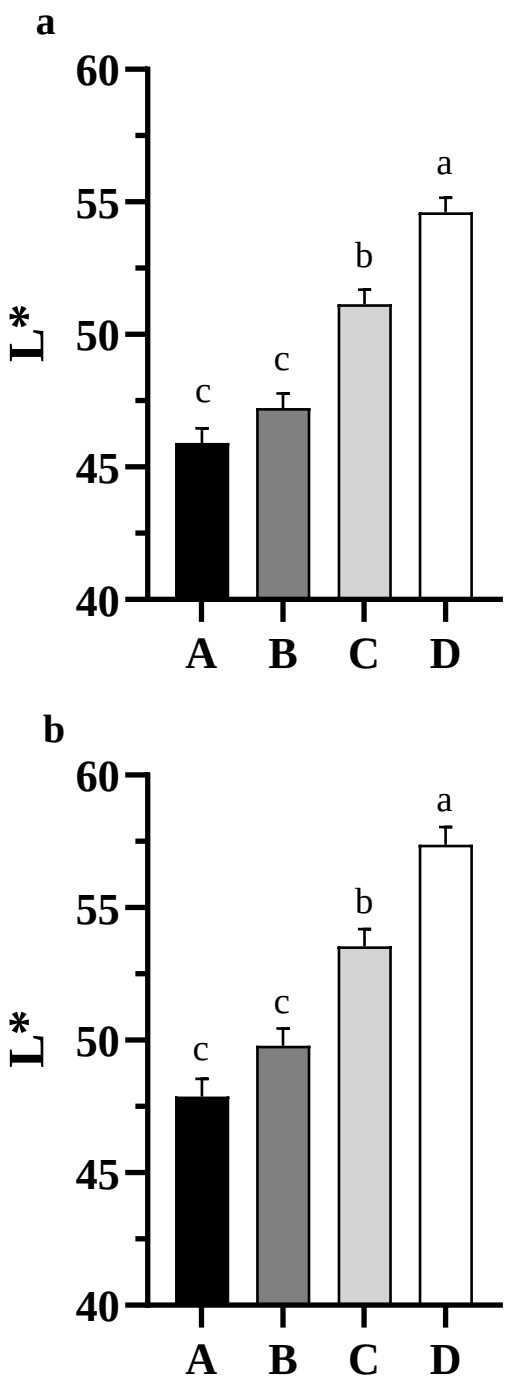

Figure 4: Mean lightness (L*) color values for zones of *semimembranosus* muscle at 0 mins (a), and at 24 hrs postmortem (b). Means bearing different letters differ ($p < 0.05$).

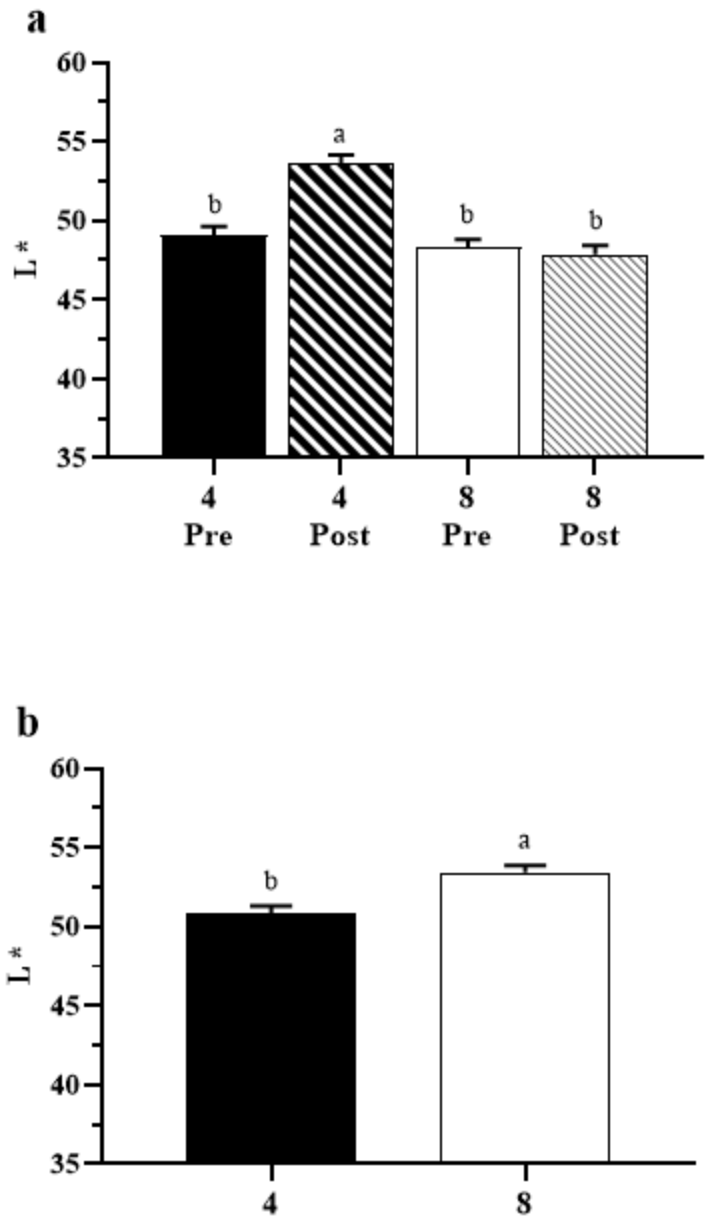

Figure 5: Mean lightness (L*) color values for *semimembranosus* muscle immediately prior to (Pre) and after (Post) a 4 and 8 min scald (a), and at 24 hrs postmortem (b). Means bearing different letters differ ($p < 0.05$).

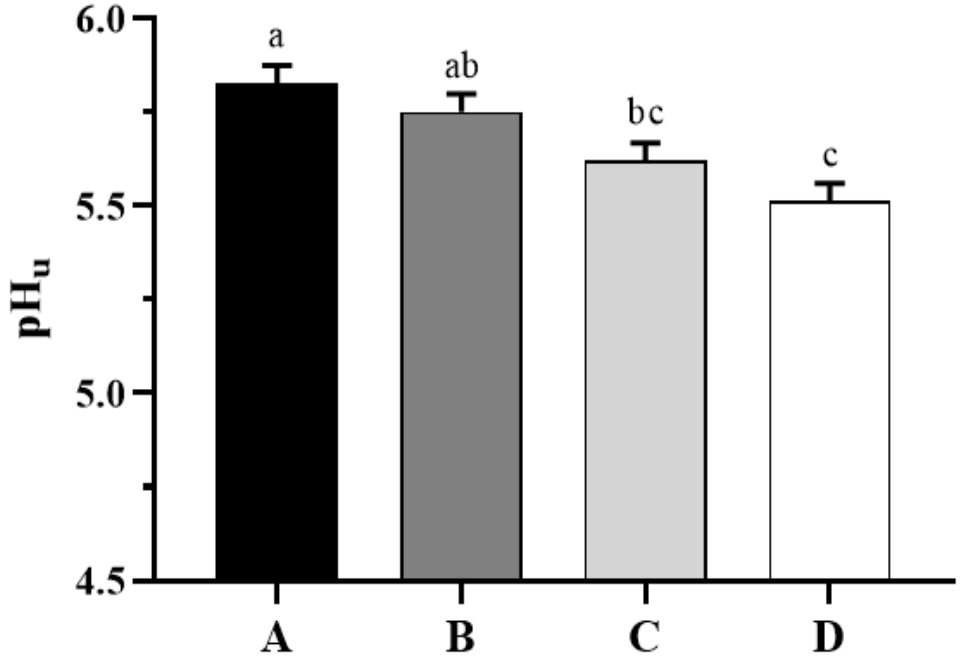

Figure 6: Ultimate pH (pHu) by zone across the *semimembranosus* at 24 hrs postmortem. Means bearing different letters differ ($p < 0.05$).

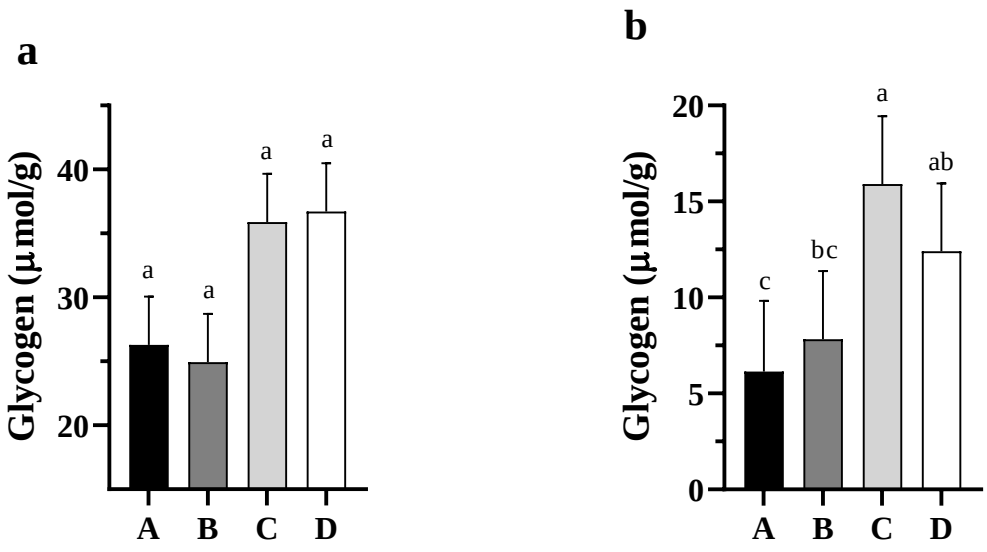

Figure 7: Mean glycogen (µmol/g) content by zone across the semimembranosus muscle at 0 minutes (a) and 24 hrs (b). Means bearing different letters differ ($p < 0.05$).

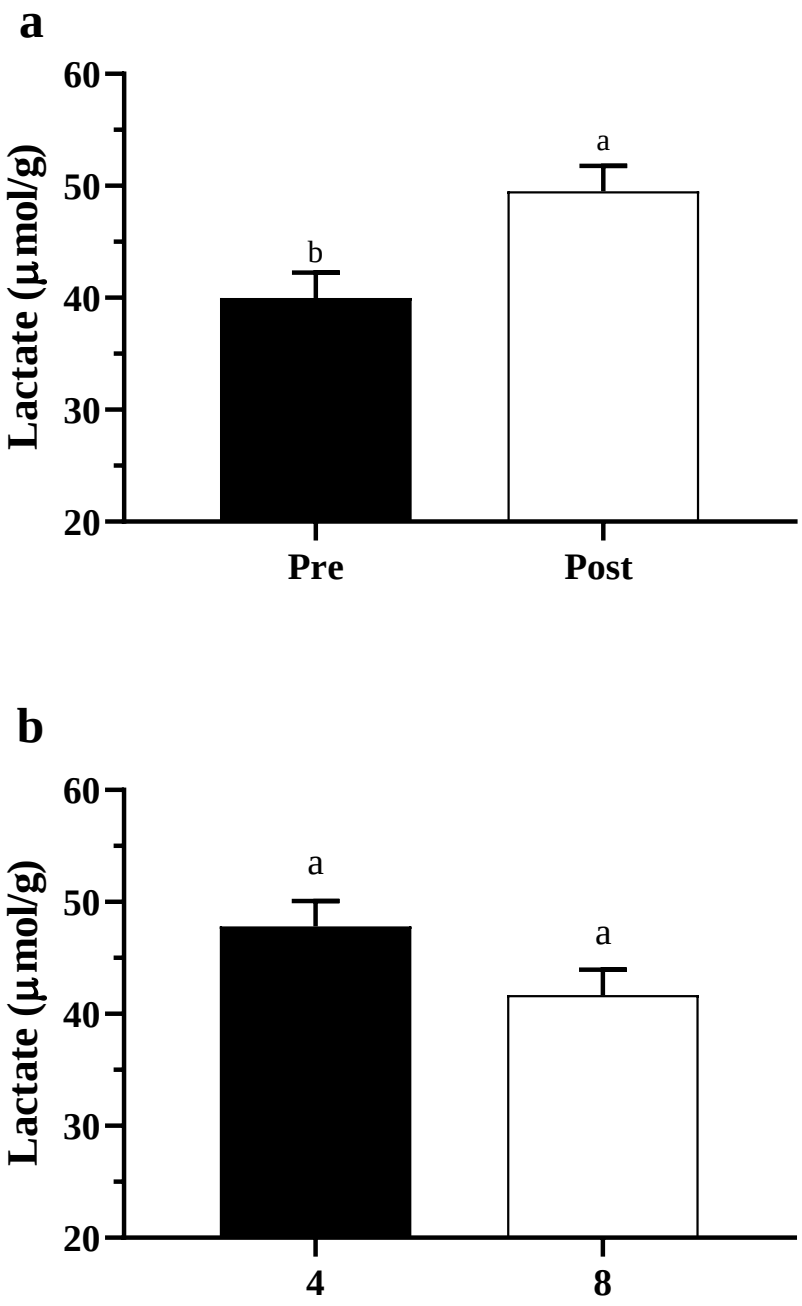

Figure 8: Mean lactate (µmol/g) of the *semimembranosus* muscle at 0 min postmortem measured (a) immediately prior (Pre) and after (Post) scalding and (b) immediately prior to (Pre) and after (Post) a 4 and 8 min scald Means bearing different letters differ ($p < 0.05$)

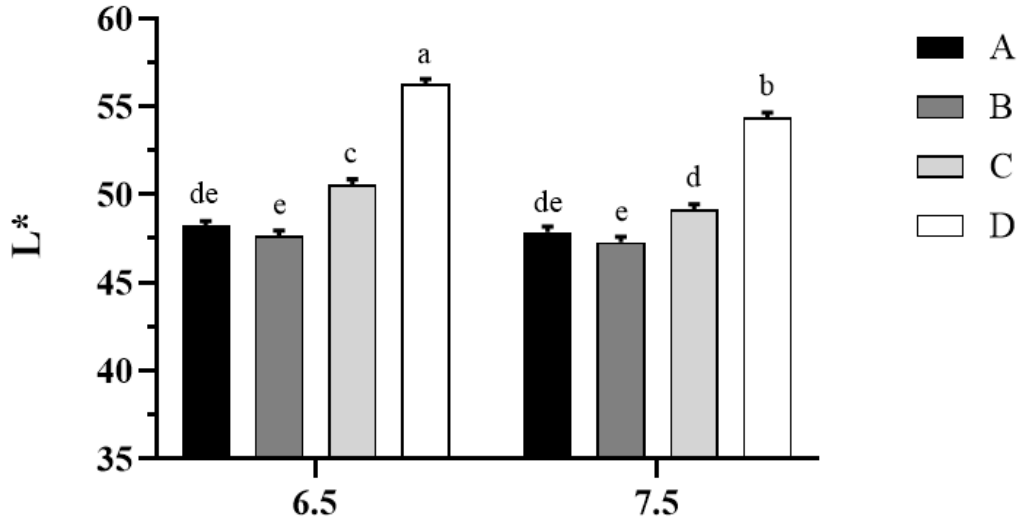

Figure 9: Mean lightness (L*) color values for *semimembranosus* muscle at 24 hours postmortem in a commercial processing facility after a 6.5 and 7.5 min scald. Means bearing different letters differ ($p < 0.05$).

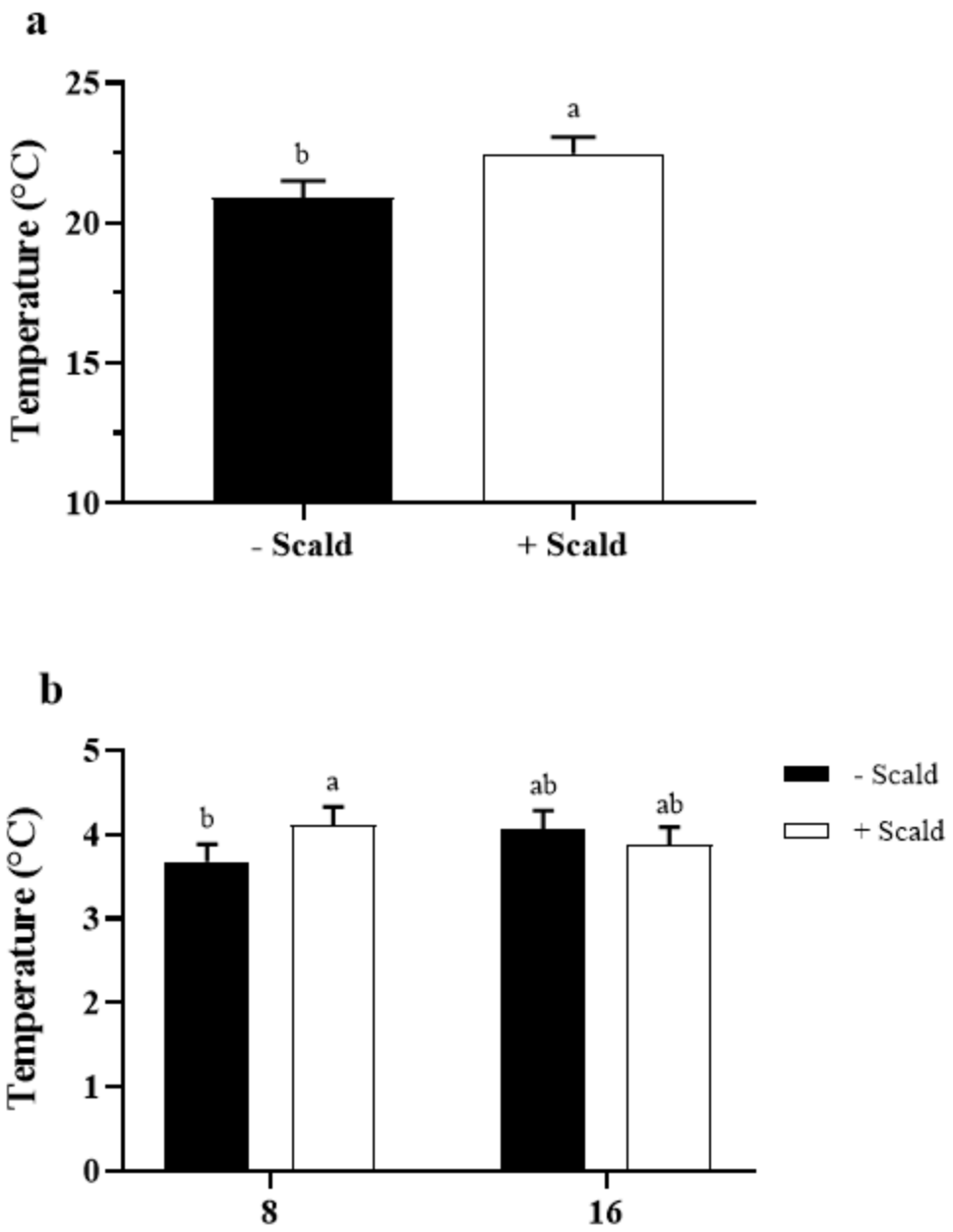

Figure 10: Mean temperature (°C) of *semimembranosus* muscle of (a) unscalded (-) and scalded (+) carcasses at 0 mins, and (b) scalded and unscalded carcasses allowed to dwell for 8 or 16 min prior to dehair at 24 hrs. Means bearing different letters differ ($p < 0.05$).

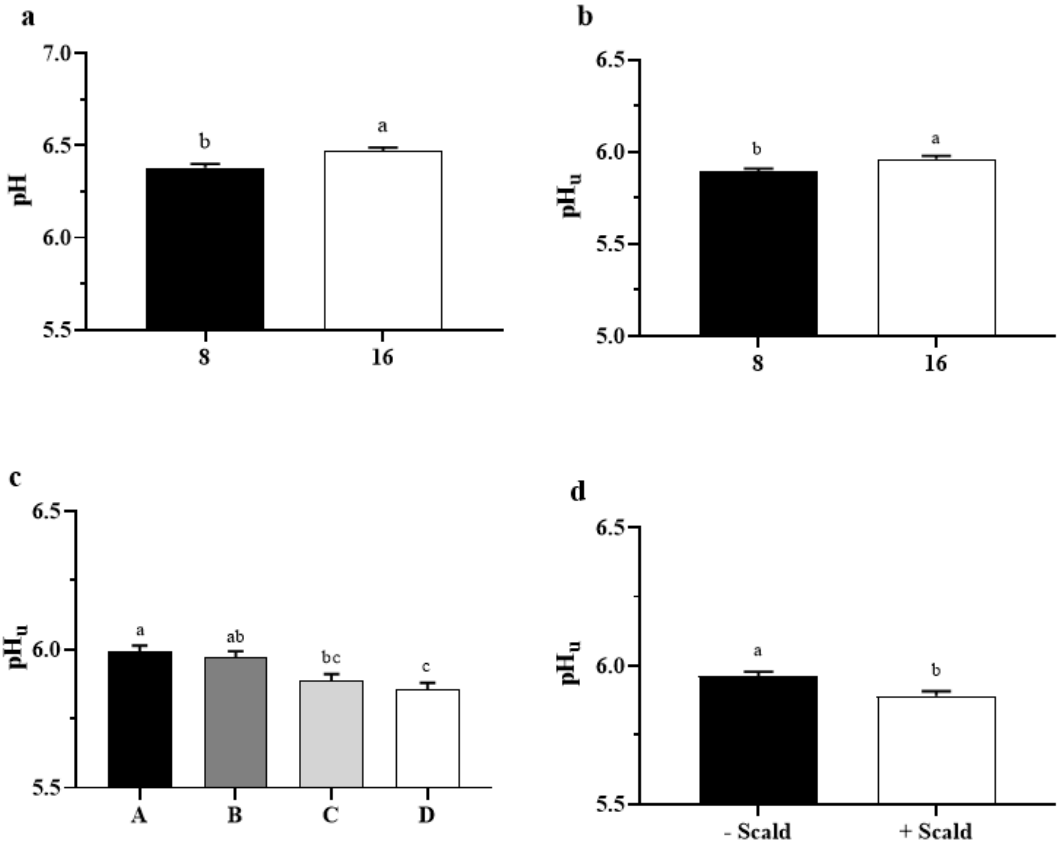

Figure 11: Mean pH of *semimembranosus* muscle samples (a) immediately after a 8 and 16 min time to dehair (b) at 24 hrs (pHu) after a 8 and 16 min time to dehair, (c) across zones at 24 hrs (pHu) and (d) from scalded (+) and unscalded (-) carcasses at 24 hrs. Means bearing different letters differ. ($p < 0.05$).

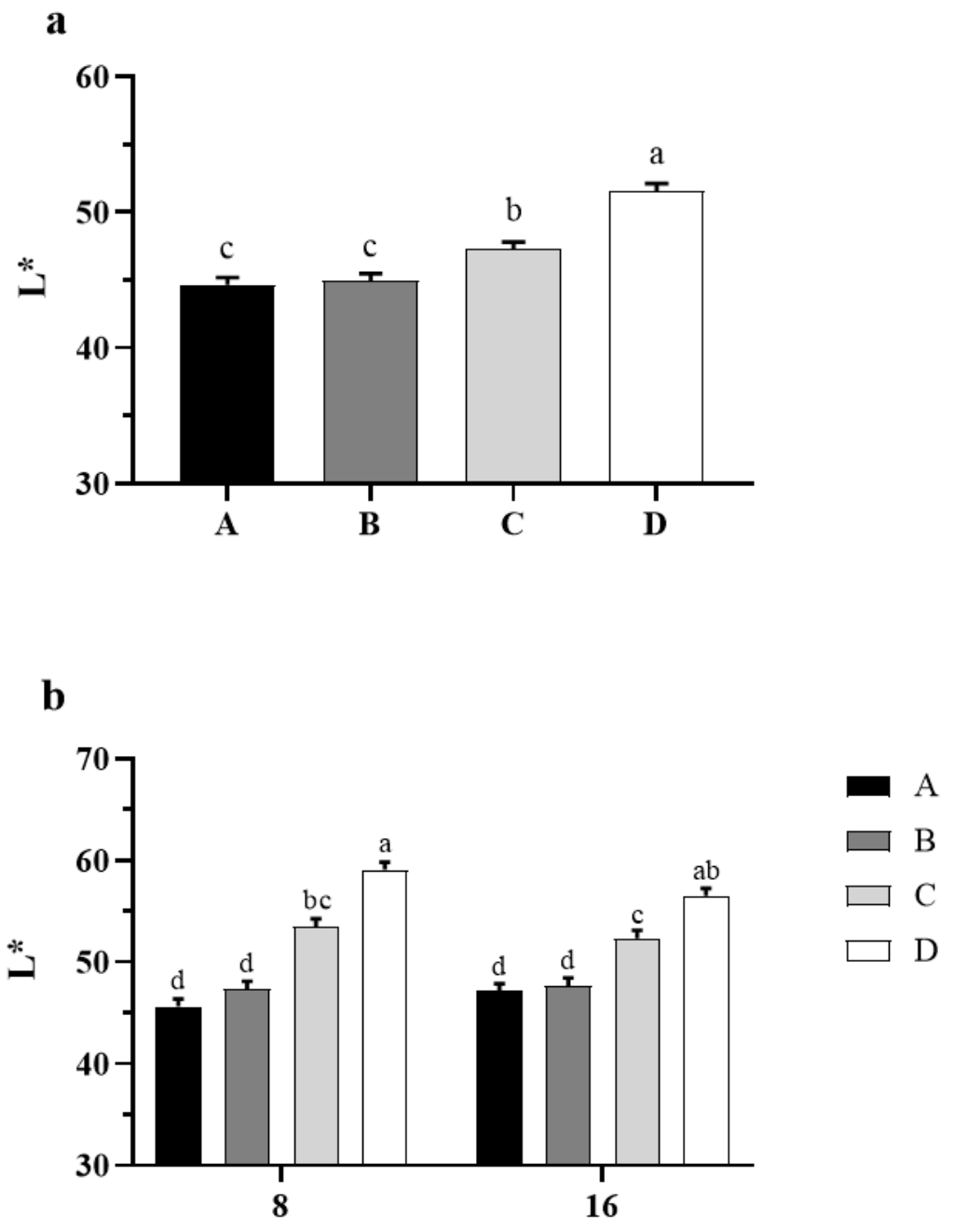

Figure 12: Mean lightness (L*) of the *semimembranosus* muscle at (a) 0 mins postmortem across zones and (b) at 24 hrs across zone by time to dehair. Means bearing different letters differ ($p < 0.05$).

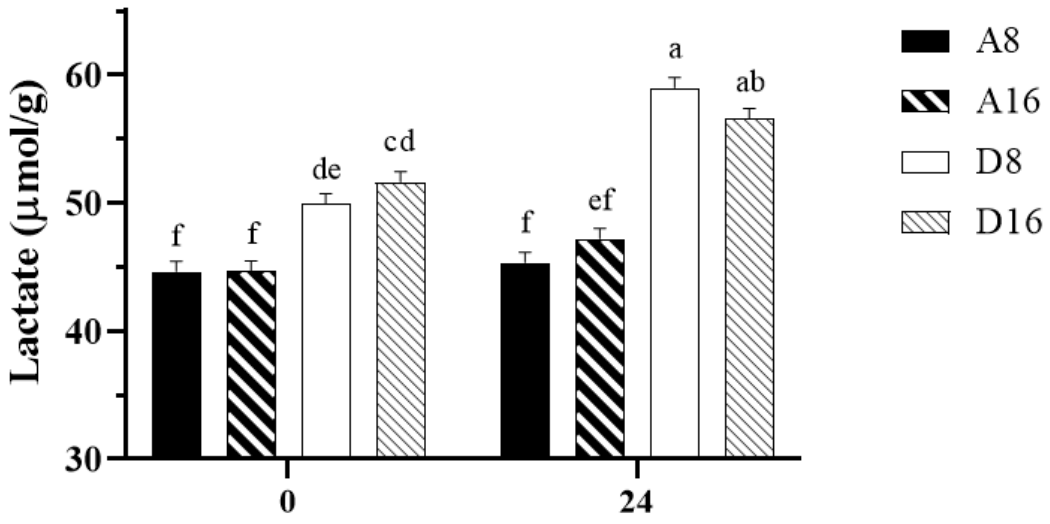

Figure 13: Mean lactate (µmol/g) in the semimembranosus muscle in zone A and D (bold) and time (min) to dehair (8 and 16) immediately after and 24 hrs postmortem. Means bearing different letter differ ($p < 0.05$).