THE BIOLOGICAL BASIS FOR ANIMAL VARIATION IN BEEF LEAN COLOR STABILITY

A Thesis

by

RUSSELL OWEN MCKEITH

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Jeffrey W. Savell
Co-Chair of Committee,	Kerri B. Harris
Committee Members,	Rhonda K. Miller
	Davey B. Griffin
	D. Andy King
Head of Department,	H. Russell Cross

May 2014

Major Subject: Animal Science

ABSTRACT

Two experiments were conducted to investigate biochemical factors that influence beef lean color and beef lean color stability. The first study was conducted to investigate biochemical traits contributing to lean color and lean color stability in dark cutting beef. The second study was conducted to evaluate the effects of mitochondrial functionality during electron transport on beef lean color stability in beef (n = 160). Beef carcasses exhibiting various levels of dark cutting were selected based on pH: (severe dark cutter **SEDC**; mean pH = 6.50, n = 40), moderate dark cutter (**MODC**; mean pH = 6.33, n = 40), mild dark cutter (**MIDC**; mean pH = 6.09, n = 40), shady dark cutter (**SHDC**; mean pH = 5.92, n = 40), and normal cohorts (mean pH = 5.53; n = 160) from the same production lot and similar marbling. For both studies, strip loin subprimals were aged until 13 d postmortem, when longissimus lumborum steaks were cut for simulated retail display. Instrumental color attributes [lightness (L*), redness (a*), yellowness (b*), chroma and hue angle] were determined on d 0, 1, 4, 7, and 11 of simulated retail display. Overall color change from d 0 was calculated for d 1, 4, 7, and 11 of simulated retail display. Additional steaks were used for determination of electron loss from the electron transport chain utilizing succinate and glutamate as substrates, oxygen consumption, nitric oxide metmyoglobin reducing ability, glycolytic potential, mitochondrial abundance, myoglobin concentration and protein oxidation. The results from the first experiment suggested that greater electron loss is associated with decreased metmyoglobin reducing ability and, consequently, reduced beef lean color stability. The results from the second experiment suggested that dark cutting beef

carcasses favored oxidative metabolism, and overall color stability was greater in dark cutting carcasses than cohort carcasses. Data from these experiments indicate that biochemical factors affect initial lean color attributes, as well as lean color stability attributes.

DEDICATION

I dedicate this work to my wife and family. Amanda, you are the greatest thing to ever happen to me, and I am so lucky to call you my wife. While in graduate school, you have been there for me in the good and bad times. Again, without your support, I would not be where I am today and I love you very much. To my parents, you have always been there to support me in my endeavors, and I am forever grateful. Dad, I would personally like to thank you for stimulating the interest for me to pursue a career in meat science. You have been a wonderful mentor and thanks for always being there, whenever I needed you. Without everyone's support I would not be where I am today. Again, I love you all very much and dedicate this work to all of you.

ACKNOWLEDGEMENTS

There are several individuals to whom I need to express my sincere gratitude for contributing to my success while here at Texas A&M University and the US Meat Animal Research Center. First and foremost, I thank Dr. Jeff Savell and Dr. Kerri Harris for being the co-chairs and their mentorship during my graduate program. Moreover, I express appreciation to my other committee members, Dr. Andy King, Dr. Davey Griffin, and Dr. Rhonda Miller, who have contributed significantly to my graduate program while here at Texas A&M University. Also, I sincerely appreciate the guidance and mentoring I receive from Dr. Andy King while at the US Meat Animal Research Center. Furthermore, I thank the scientists and technicians at the US Meat Animal Research Center with whom I worked and collaborated with during my time there. I have learned a great deal of knowledge from this experience and plan to take it with me and use it in my future career.

I thank all of my family and friends because this would not have been possible without your support during my time here at Texas A&M University. Through teaching, research, or extension activities I have worked with numerous people during my time here at Texas A&M University, and these people have helped me become the person I am today. With that said, I am a very lucky person to have a close nit group of family and friends that have supported me with their advice, encouragement and knowledge, so thank you very much.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

Increasing shelf-life of case-ready meat products has been a focus of the retail sector of the meat industry as retailers try and mitigate the amount of product that gets devalued due to insufficient color stability. There are several different biochemical factors such as pigment concentration, mitochondrial oxygen consumption and mitochondrial reducing capacity that have been attributed to influencing color stability (Faustman and Cassens, 1990; Bekhit and Faustman, 2005; Mancini and Hunt, 2005). Metmyoglobin reducing activity and oxygen consumption have impacted meat color stability to the greatest extent (Hood, 1980; O'Keeffe and Hood, 1982; Ledward, 1985). Additionally, (McKenna et al., 2005) reported that discoloration differences between muscles were related to the amount of reducing activity relative to oxygen consumption rate.

The electron transport chain has been shown to be a primary source of reactive oxygen species production, resulting in increased electron loss due to site specific defects in the electron transport chain (Bottje and Carstens, 2009). Researchers have revealed a link between mitochondrial function, respiratory chain activity, and electron leak to feed efficiency in broilers (Bottje et al., 2002), rats (Lutz and Stahly, 2003), and cattle (Kolath et al., 2006). Moreover, Bottje et al. (2006) determined that there were site-specific defects in electron transport in muscle from low-feed efficient broilers within complex I and complex III in the electron transport chain. Perhaps inefficiency in respiration is a source of variation in color stability in meat products, but limited

research has been conducted to understand the impact of the loss of electrons in the electron transport chain on variation in lean color stability.

A quality defect that impacts meat color and color stability is dark-cutting beef. The 2011 National Beef Quality Audit Moore et al. (2012) reported that the incidence of dark cutters was 3.5%. The dark cutting condition has generally been attributed to high muscle pH due to antemortem depletion of glycogen in the muscle. It has been reported that the overall darker color of dark cutting beef is directly related to a higher mitochondrial respiration rate, which helped maintain the low oxymyoglobin concentrations (Ashmore et al., 1973). This causes increased light absorption and water binding, resulting in dark, firm, and dry appearance to the muscle (Lister, 1988). Moreover, Ashmore et al. (1973) identified that the reduction of postmortem muscle pH through glycolysis impaired the overall level of oxygen the mitochondria consumed in normal muscle. Lawrie (1958) determined that mitochondrial cytochrome oxidase was more active at pH values above 6.0, and thus concluded that increased oxygen consumption of dark cutting meat increased the concentration of deoxymyoglobin.

Two studies were conducted to investigate biochemical factors that influence beef lean color and beef lean color stability. The objective of the first study was to compare biochemical traits contributing to lean color and lean color stability in dark cutting and normal beef. The objective of the second study was to assess the effects of electron loss in the electron transport chain on the different biochemical factors that impact beef lean color stability.

CHAPTER II

LITERATURE REVIEW

2.1. Overview

Color is a very important component of consumer acceptability in regards to meat products (Brewer, 1998). Specifically, color is produced when energy in the visible (400-700 nm) light range is perceived by the human eye (Brewer, 1998). Moreover, the energy that produced the color is contained in the light, and the pigments where the molecules that absorbed wavelengths from the light, which illuminated the object. Brewer (1998) stated that wavelengths of light that are absorbed by an object are not seen, whereas the light that is reflected by an object produces the light observed (Brewer, 1998). Associated with color are three attributes known as hue, chroma, and value. Hue describes the wavelength of light radiation, chroma describes the intensity of a color with respect to the amount of white light that is mixed with it, and value is used as an indication of overall light reflectance of a color (AMSA, 2012).

The quality factor that influenced a consumer's decision the most when red meat products are purchased is color because the product is visually assessed (Faustman and Cassens, 1990). Consumers evaluate how much of the product is visually discolored because it is used as an indicator of product freshness and wholesomeness (Faustman and Cassens, 1990; Faustman, 1991). Due to this type of decision making, approximately 15% of retail beef is discounted in price due to surface discoloration resulting in annual revenue loss of $1 billion (Smith et al., 2000). Over the years, meat color and meat color stability have been very important areas of research, and several

researchers have identified several biochemical and physical factors (oxygen consumption, metmyoglobin reducing activity, mitochondrial activity, lipid oxidation, light, and temperature) that have the potential to affect color and color stability of fresh meat (Faustman and Cassens, 1990; McKenna, 2003). These different biochemical and physical factors do not act independently of each other. As a result, these factors interact, and there has been a general lack of knowledge in the scientific community about how these factors have interacted and affected meat color, which have contributed for the need of further understanding (Faustman, 1991). This review has attempted to address the importance of color and some of the biochemical factors that impact meat color stability.

Variation in lean color stability has been an area of increased interest in the last decade. Research has revealed that genetic influences have contributed to lean color stability (King et al., 2010). King et al. (2011b) determined that animal variation contributed to variation in beef muscle color stability; however, it was a smaller source of variation than the type of muscle affected within the carcass. Moreover, evidence has revealed that color stability has been genetically regulated. With this said, genetic effects seem to have a greater influence on the maintenance of lean color stability than initial color (King et al., 2010). King et al. (2010) reported that meat from different breeds of cattle have different lengths of color life, and this could potentially be due to greater amounts of key metabolic intermediates needed for metmyoglobin reduction.

2.2. Meat Color Chemistry

In meat, the primary sarcoplasmic heme protein responsible for color is myoglobin (Mancini and Hunt, 2005). There are other sarcoplasmic proteins such as hemoglobin and cytochrome C that contribute to the overall color of meat (Mancini and Hunt, 2005). Myoglobin is classified as a water soluble globular protein made up of eight right-handed α helices named A through H, and a prosthetic heme group containing a centrally located iron atom positioned in the protein's hydrophobic core (Mancini and Hunt, 2005). In myoglobin, there is a heme ring that forms six bonds. Of these six ligands associated with this iron atom, four of them attach iron to the heme ring, and the fifth ligand is bound to the proximal histidine-93 molecule (AMSA, 2012). The sixth ligand has the ability to reversibly bind ligands and determines the color of meat. Also, the distal histidine-64 influences the color dynamics through affecting space relations within the hydrophobic heme pocket (Mancini and Hunt, 2005).

The different forms of myoglobin --deoxymyoglobin, oxymyoglobin, carboxymyoglobin, and metmyoglobin-- are determined based on the valence electron state of iron and what is bound at the sixth ligand (Mancini and Hunt, 2005). The deoxymyoglobin state occurs when water, or no ligand is present and heme is in the ferrous state (Mancini and Hunt, 2005). The resulting color state of the meat is deep dark purplish-red, and is commonly seen in meat products that have been vacuum-packaged (the partial pressure of oxygen is extremely low). When meat that is in the deoxymyoglobin state is exposed to oxygen, it enters the oxymyoglobin state. In this state, the heme iron has to be in the ferrous state. Meat can be readily converted from

the deoxymyoglobin state to the oxymyoglobin state. Meat that is in the oxymyoglobin state appears bright cherry red in color, and the longer that it is exposed to oxygen, the deeper oxymyoglobin penetrates into the interior of the meat. Unlike deoxymyoglobin, oxymyoglobin has a high partial pressure of oxygen (40 torr) and is very unstable, so its stability is dependent on the amount of oxygen that is continually available in order for it to remain in this state (Mancini and Hunt, 2005).

When oxymyoglobin is oxidized from the ferrous state to the ferric state, myoglobin enters the metmyoglobin state (Wallace et al., 1982). As a result, when the predominant color of metmyoglobin is present, meat appears brown on the surface as well as the subsurface. The subsurface metmyoglobin is located between the superficial oxymyoglobin and the interior deoxymyoglobin, and over time this subsurface layer thickens and moves toward the surface (Mancini and Hunt, 2005). The formation of metmyoglobin is the result of the low partial pressure of oxygen (5-10 torr).

When hydrogen sulfide reacts with myoglobin in the ferrous state of iron, it produces a green pigment known as sulfmyoglobin (Cornforth and Jayasingh, 2004). These bacteria required low oxygen tension conditions as well as a pH less than 5.9 for hydrogen sulfide from sulfur-containing amino acids to be produced (Cornforth and Jayasingh, 2004). Meat should be vacuum-packaged or modified-atmosphere packaged for this to be prevented (Cornforth and Jayasingh, 2004).

Myoglobin and hemoglobin have a much greater affinity to carbon monoxide than oxygen (Cornforth and Jayasingh, 2004). When myoglobin is bound to carbon monoxide it produces carboxymyoglobin, which is bright red in appearance. Moreover,

the visible light absorption of carboxymyoglobin is virtually the same when compared to oxymyoglobin (Cornforth and Jayasingh, 2004). Carboxymyoglobin is more resistant to oxidation than oxymyoglobin due to the stronger binding of carbon monoxide to the iron-porphyrin binding site on myoglobin (Cornforth and Jayasingh, 2004).

2.3. Methods for Determining Meat Color

Meat color can be measured either objectively or subjectively. Objective color measurements are used to confirm or correlate with human perception (subjective measurements). Two common instrumental color methods include extraction and reflectance. Extraction instrumental color is used to describe and estimate the total pigment of the object being measured. Collecting these measurements has been known to be laborious, destructive, and unrepeatable. A positive aspect to this method has been that total pigment can be quantified (Mancini and Hunt, 2005).

Reflectance color measures the color that is visualized on the surface of meat products (Hunt et al., 1991). This method utilized an x and y-axis, which represents wavelength (nm) and percent reflectance, respectively. There are two different tristimulus systems commonly used, CIE (Commission International d'Eclairage) or Hunter Lab; both systems have implemented several different illuminants (A, C, or D65) to obtain color data (L*, a*, b* or L, a, b). Also, spectral color has the ability to quantify myoglobin and/or measure the surface pigments in the 400-730 nm range. Reflectance measurements have typically been superior to extraction due to their ability of being rapidly measured, non-destructive, repeatable, and color descriptive. When measuring changes in meat color over time, CIE L*, a*, and b* values have been typically utilized.

The *L* or L* values are used to determine change in lightness with 0 equal to black and 100 equal to white. The a* or a are used to represent measurements from red (+) to green (-), whereas b* or b are used to represent measurements from yellow (+) to blue (-) (Mancini and Hunt, 2005).

Subjective color measurements have been referred to as the "fundamental standard" of color measurements because they most accurately evaluate consumers' perceptions, as well as set benchmarks for instrumental measurements comparisons (AMSA, 2012). Visual panels traditionally have been very difficult to conduct as multiple factors can influence repeatability (AMSA, 2012). When measuring subjective color, trained visual color or consumer panels have been the most commonly used panels in meat science research. With trained visual color panels, panelists undergo very rigorous screening and training in order to produce quantitative data on anchored scales (AMSA, 2012). Consumer panels have typically been used for providing information based on their preferences and acceptability of the product (AMSA, 2012). The type of panel chosen for subjective color measurements needs to be determined based on the type of research question being asked (AMSA, 2012).

2.4. Metmyoglobin Reducing Activity

Metmyoglobin reducing activity has been extensively studied, and has been associated with color stability of muscles through the reduction of metmyoglobin to deoxymyoglobin. The first observations of metmyoglobin studied in meat products were conducted by Dean and Ball (1960) through the reduction of surface metmyoglobin in vacuum-packaged meat. Metmyoglobin reduction requires NADH and this has been

evaluated and questioned to some extent in postmortem muscle because NADH was only available in small amounts (Mancini and Hunt, 2005). Therefore, the mechanisms in which the pooled NADH regeneration has not been entirely understood and different mechanisms have been suggested. Research conducted by Bekhit et al. (2003) suggested that the amount of NADH present impacted beef color stability greater than metmyoglobin reducing activity. Mancini et al. (2004) investigated the role of NADH in an enhanced beef model. They reported that lactate dehydrogenase was involved in regeneration of NADH and metmyoglobin reduction. From these results, they proposed that lactate dehydrogenase was converted postmortem when the lactate was injected into the brine, and then was converted to pyruvate and NADH; this replenished the reducing equivalent pool within postmortem muscle, and chemically reduced metmyoglobin due to greater metmyoglobin reducing activity (Mancini and Hunt, 2005).

Researchers also have investigated different enzymes capable of reducing metmyoglobin. One enzyme that has been extensively studied has been NADH-cytochrome b_5 reductase, which is found in a variety of tissues, and has been reported to reduce metmyoglobin (Bekhit and Faustman, 2005). Arikara et al. (1995) demonstrated the presence of NADH-cytochrome b_5 reductase in bovine muscle, which had an absorption spectrum assembly identical to purified erythrocyte cytochrome b_5 reductase found in bovine red blood cells. They localized NADH-cytochrome b_5 reductase to the mitochondrial fraction, and also at a lower level within the mitochondrial fraction. Arikara et al. (1997) estimated that bovine skeletal muscle contained approximately 13.8 $\pm$ 2.6 μg NADH-cytochrome b_5 reductase/ g tissue in the biceps femoris muscle. In a

subsequent study, they quantified cytochrome b_5 concentration in the biceps femoris as 59.0 ± 20.9 μg/g muscle tissue. From these two studies, NADH-cytochrome b_5 reductase was capable of reducing electron acceptors like cytochrome b_5, and had a very high affinity for cytochrome b_5.

Several scientists have developed different assays to measure metmyoglobin reducing activity. These different methods used exogenous NADH along with different substrates. The first method developed was metmyoglobin reducing activity published in 1965 (Stewart et al., 1965). This methods was highly criticized because it used ferricyanide as an oxidant. Another negative about the metmyoglobin reducing activity assay developed by Stewart et al. (1965) was that it has to be used in real-time. This procedure was later modified by using sodium nitrite solution instead of ferricyanide as an oxidant (Watts et al., 1966). The advantage of nitrite over ferricyanide was that it oxidized pigments without oxidizing the constituent sulfhydryl groups in the protein, or caused the proteins to denature (Watts et al., 1966). Sammel (2002) further modified this procedure who used meat cubes, increased the concentration of sodium nitrite, vacuum packaged samples and incubated sample at 30° C.

Research conducted by Giddings (1974) suggested that mitochondria or sub-mitochondrial particles could potentially play a role in metmyoglobin reduction in vacuum-packaged meat cuts by having the ability to scavenge residual oxygen. This eliminated the potential for low-oxygen-mediated myoglobin oxidation, or the potential reversal of electron transport. In a subsequent study, Giddings (1977) hypothesized that mitochondria were involved with metmyoglobin reduction because the mitochondria

were supplying meat with NADH, which were generated by the reversal of electron transport. Furthermore, the mitochondria could have served as a source of reducing equivalents for extra mitochondrial pyridine nucleotide reduction, which would have provided the NADH necessary for metmyoglobin reductase to function properly (Bodwell et al., 1965; Giddings, 1977) . Echevarne et al. (1990) determined that the highest levels of metmyoglobin reductase activity were primarily located in the fraction composed of microsomes as well as the intact mitochondria.

2.5. Oxygen Consumption Rate

By definition, oxygen consumption is where a series of enzyme reactions in the Krebs Cycle consume oxygen in meat (AMSA, 2012). It has been shown that oxygen consumption rate is associated with residual mitochondrial respiration found within postmortem muscle (Bendall and Taylor, 1972). The oxygen consumed by the meat affects the myoglobin oxygenation as a result of mitochondrial enzymes and myoglobin competing for available oxygen. It has been shown that as muscles increased in postmortem age, mitochondrial activity decreased, thus allowing for increased myoglobin oxygenation (AMSA, 2012).

Lanari and Cassens (1991) determined that differences in oxygen consumption rate can be due to differences in muscle and breed type in terms of beef discoloration rate. Other researchers have determined that it is due to differences in muscle (O'Keeffe and Hood, 1982; Renerre and Labas, 1987). Also, oxygen consumption rate has been associated with the depth of oxygen penetration (McKenna, 2003). It has been known that lower oxygen consumption rates have allowed for greater penetration of oxygen in

muscle, which has been associated with muscles that have greater color stability (McKenna, 2003).

Oxygen consumption rate is greatly affected by the rate of metmyoglobin accumulation on the meats surface (O'Keeffe and Hood, 1982). McKenna et al. (2005) reported that discoloration differences between muscles are related to the amount of reducing activity relative to oxygen consumption rate. They determined that muscles superior in color stability have high or low oxygen consumption rates, but the reducing activity proportionately exceeded the oxygen consumption rate. Muscles inferior in color stability have high or low oxygen consumption rates, but the reducing activity is proportionately lower compared to the oxygen consumption rate (McKenna et al., 2005). As a result, myoglobin is more oxidized, and thus increased levels of metmyoglobin, which consequently decreased product shelf-life (Lanari and Zaritzky, 1988).

Oxygen consumption rate has been associated with oxygen penetration depth because as oxygen consumption rate decreases the oxygen penetration depth increases (McKenna, 2003). Oxygen penetration is determined by the rate of oxygen diffusion into, and the oxygen consumption of the tissue due to the pressure of oxygen at the surface of the meat (O'Keeffe and Hood, 1982). O'Keeffe and Hood (1982) and Hood (1980) determined that oxygen consumption rate had an influential effect on the oxymyoglobin depth formation because three-day-old meat had a higher consumption rate than seven-day-old meat that had more rapid discoloration. Morley (1971) reported that oxygen penetration increased linearly with time and was greatly influenced by muscle type, as well as the species of the animal.

2.6. Glycolytic Potential

The major source of carbohydrate found within animal muscle is glycogen (McKeith et al., 1998). Chemically, glycogen is a polymer of glucose units that are linked via α1-4 and α1-6 linkages formed around P-glycogenin (Brooks et al., 1996). When energy is required for muscle, glycogen is hydrolyzed to glucose via glycogen phosphorylase where α1-4 linkages are cleaved, a de-branching enzyme is needed to cleave α1-6 linkages (McKeith et al., 1998). The end product of this metabolic pathway is lactate. In antemortem metabolism, lactate is shuttled to the liver where it is reconverted to pyruvic acid and then utilized as an energy substrate (McKeith et al., 1998). During postmortem metabolism, glycogen is depleted and the accumulation of lactate is observed. Ultimate muscle pH is established because all the glycogen is depleted and no longer converted to lactate. The ultimate pH of beef is 5.5-5.7 for beef, but in beef carcasses classified as dark cutters their ultimate pH is above 6.0 (Lawrie, 1998). The reason dark cutting carcasses have higher ultimate pH values is due to lower initial glycogen concentrations, which has been associated with long-term pre-harvest stress (Aberle et al., 2001).

In order to evaluate the amount of glycogen present in postmortem muscle that was converted to lactate, glycolytic potential was calculated. Also, this procedure is used when researchers want to explain differences in meat quality as it is related to glycogen content present in the animal prior to slaughter (Maribo et al., 1999). By utilizing glycolytic potential as a measurement, it evaluates whether the experimental units had the same muscle energy prior to slaughter. This measurement has been

calculated as [2 × (glycogen+glucose+glucose-6-phosphate) + lactate] (Monin and Sellier, 1985). In this process, glucose and glucose-6-phosphate are intermediates produced during the transformation of glycogen to lactate (Maribo et al., 1999). Hanson et al. (2001) suggested glycolytic potential will be underestimated if muscle samples were taken at 45 min postmortem. This is likely due to the fact that glycogen was caught in various stages of glycolysis, and the intermediate products of glycolysis were not measured by the glycolytic potential assay (Hanson et al., 2001).

There have been different methods developed to measure glycolytic potential. When researchers first utilized this process, muscle biopsies from live animals or samples directly after exsanguination were taken. Research has shown that there was no difference in the amount of glycolytic potential in the *M. Longissimus dorsi* when taken immediately following exsanguination and up to 30 h postmortem (Maribo et al., 1999). A later study conducted by Hansen et al. (2001) reported that glycolytic potential measurements prior to rigor mortis underestimated glycogen by approximately 10-15%, so they recommended that this measurement be taken after rigor was completed.

Most research conducted utilizing glycolytic potential has focused on pork, resulting in limited research on beef. Wulf et al. (2002) investigated the relationship between glycolytic potential in the *M. Longissimus lumborum* muscle of dark cutting beef carcasses. They determined that there was a curvilinear relationship between glycolytic potential and ultimate pH. They reported a threshold of approximately 100 µmol/g, below this threshold, glycolytic potential was associated with high ultimate pH, but above this threshold, glycolytic potential did not impact ultimate pH. Hanson et al.

(2001) reported that dark cutting conditions were more likely to occur when muscle glycogen concentrations were less than 80 mmol/kg.

2.7. Lipid Oxidation

Lipid oxidation has been known to impact meat color. Oxidation affects both the lipid and protein components of meat, thus reducing the quality and shelf-life of the meat products (Morrissey et al., 1998). During lipid oxidation, iron is released from high molecular weight sources like hemoglobin and myoglobin. Next, the released iron is made available to lower weight molecular compounds such as amino acids and nucleotides, which have been thought to result in the production of chelates. These chelating compounds then catalyze lipid oxidation in muscle tissue (Morrissey et al., 1998).

Reactive oxygen species and lipid peroxides in meat have resulted in the oxidation of the ferrous iron in myoglobin and oxymyoglobin to the ferric iron in metmyoglobin. In meat and other food products, iron has been known to be a pro-oxidant through the breakdown of lipid oxidation products via free radical formation of hydrogen peroxide (Brewer, 2008). There are three different steps that occur during lipid oxidation: initiation, propagation, and termination. During initiation, a hydrogen atom is cleaved from an unsaturated fatty acid to form an alkyl radical (Fennema, 1996). This reaction then undergoes propagation from one fatty acid to another, which then leads to the formation of lipid hydroxyperoxides. This process is terminated when two radicals combine (Brewer, 2008).

In the meat industry, antioxidants are readily used to delay oxidation in meat by inhibiting initial free radical formation, or preventing free radicals from producing more free radicals (Fennema, 1996). Antioxidants have the ability to bind metals or scavenge oxygen, so that they cannot initiate or perpetuate oxidation. There are many compounds that have exhibited antioxidant properties such as vitamins, herbs, spices, synthetic antioxidant compounds, and chelating agents (Brewer, 2008). The most effective antioxidants are capable of interrupting the free radical chain reaction since they structurally possess aromatic rings in which they donate a hydrogen radical to free radicals formed during lipid oxidation. In essence, the antioxidant compound is sacrificed by giving up a hydrogen atom, and then rearranges the compound to a stable form (Brewer, 2008).

Researchers have conducted studies to determine efficacy of different antioxidant compounds and their impact on meat color. Faustman et al. (1989b) reported that increased lipid oxidation was related to the same type of increase in myoglobin oxidation. This is why there has been extensive research conducted investigating different antioxidant compounds in meat to try and mitigate lipid oxidation. The antioxidant vitamin E has been investigated to see whether or not it improved color stability. Faustman et al. (1989a) reported that meat from cattle fed vitamin E had greater color stability than meat from cattle not fed vitamin E. Other studies reported that feeding vitamin E improved color stability and lipid stability in beef (Phillips et al., 2001), whereas when implemented in pork diets, lipid oxidation was minimized, but a color-stabilizing effect was not observed (Phillips et al., 2001). Further research

conducted by Suman et al. (2006) reported that there were four histidine residues (positions 36, 81, 88, and 152) adducted in beef oxymyoglobin, whereas there were only two histidine residues (24 and 36) adducted in pork oxymyoglobin. Moreover, beef myoglobin contains 13 histidines where pork myoglobin only contains 9, which indicated that lipid-oxidation-induced oxymyoglobin oxidation was more critical to color quality in beef than in pork. Suman et al. (2007) also found that there was preferential adduction at the proximal 93 histidine in beef and not in pork. This helped explain why lipid oxidation induced myoglobin oxidation appeared in beef and not in pork, as well as why vitamin E supplementation has had a limited effect on pork color.

2.8. pH

Another factor that impacts all of the factors previously mentioned is muscle pH, and this factor is fundamentally linked to meat color. Depending on the ultimate pH of the muscle, it can greatly impact the metabolism of the muscle, which in turn can directly impact other biochemical factors previously explained. Normal ultimate pH of postmortem muscle (beef) is approximately 5.4-5.7. Two important meat quality anomalies have occurred due to pH. These quality anomalies are dark, firm, and dry (DFD) meat and pale, soft, and exudative (PSE) meat.

Pale soft and exudative pork has a very fast pH decline during post-mortem glycolysis with either a normal ultimate pH of 5.5, or an unusually low ultimate pH of 4.8 (Lawrie, 1958). The PSE condition has been characterized as pale in color, soft in texture, and exudative in appearance (Lee and Choi, 1999). With PSE conditions, approximately 20% of the sarcoplasmic and myofibrillar proteins have been denatured,

resulting in increased water expelled from the muscle due to reduced myosin head length (Honikel and Kim, 1986; Offer et al., 1989). Denaturation of the sarcoplasmic proteins were the primary reason for pork to be pale in color, whereas denaturation of myofibrillar proteins were the leading contributor to decreased water holding capacity (Joo et al., 1999). The dark cutting beef phenomenon has been attributed to antemortem depletion of muscle glycogen reservoirs, which prevented lactic acid accumulation in postmortem muscle tissue. Dark cutting beef has been characterized as having a high ultimate pH (greater than 6.0), high water-holding capacity, a dry, firm, and sticky lean muscle tissue, and very dark-red to black lean color (Apple et al., 2011).

Myoglobin can be greatly affected by pH. Ledward (1970) reported that reduced metmyoglobin reductase activity was associated with lower ultimate pH values. Moreover, Ledward et al. (1986) reported that beef with an ultimate pH greater than 5.8 had greater color stability than meat with an ultimate pH of 5.6. Also, a low pH environment has been shown to accelerate protonation of bound oxygen and favored the release of pro-oxidant species (Livingston and Brown, 1981). In precooked meat products, Trout (1989) showed that heat denaturation was affected by pH. When pH is increased, less denaturation of proteins occur.

2.9. Influence of Mitochondria on Meat Color

Mitochondria are vital to the cell and generate approximately 90% of cellular ATP (Lehninger et al., 1993). There are different components to mitochondria. The outer mitochondrial membrane consists of a phospholipid bilayer and the inner mitochondrial membrane contains many cristae, with extensive surface area. In regards

to cellular metabolism, the electron transport chain resides within the inner mitochondrial membrane (Lehninger et al., 1993). The mitochondrial electron transport chain has five different multi-protein enzyme complexes. These complexes include: complex I (NADH: ubiquinone oxidoreductase), complex II (succinate: ubiquinone reductase), complex III (ubiquinol: cytochrome c oxidoreductase), complex IV (cytochrome c oxidase), and complex V (F_1F_0 ATP synthase) (Lehninger et al., 1993).

Evidence has shown that mitochondrial respiration impacts beef color (Ashmore et al., 1972; Egbert and Cornforth, 1986). These researchers determined that mitochondrial oxygen metabolism influenced meat color through two different mechanisms. The first mechanism was that mitochondria reduces the oxygen partial pressure, which minimizes the formation of oxymyoglobin from existing myoglobin. Secondly, mitochondria interacted with oxymyoglobin, which causes a transfer of oxygen from myoglobin to mitochondria.

Extensive research has been conducted to investigate substrates that have been used in the electron transport chain, and inhibitors to determine their effects on the NADH pool and color stability. Tang et al. (2005) determined that when succinate was added to isolated mitochondria, there was increased oxygen consumption. They thought that the increased oxygen consumption was due to the increased number of available electrons that could be transferred to metmyoglobin. Ramanathan and Mancini (2010) investigated the effects of pyruvate on metmyoglobin reduction utilizing bovine heart mitochondria. They determined that pyruvate could be used as a substrate to regenerate reducing equivalents that were used for mitochondria mediated metmyoglobin reduction.

In addition to isolating mitochondria, scientists also have investigated the use of mitochondrial substrates in striated muscle tissue. Ramanathan et al. (2011) determined that succinate, lactate, and pyruvate could be used in enhancement technology for fresh beef to improve color stability during storage, but darkening of the product would occur. Similarly, Kim et al. (2009) reported that variation in color stability could be regulated by differences in NADH supply through different fluxes of lactate dehydrogenase found in different muscles. Kim et al. (2009) concluded that lactate dehydrogenase played a specific role in metmyoglobin reduction through regeneration of NADH, which directly affected the color stability of beef muscles. Utilizing muscle homogenates, Mohan et al. (2010) reported that when lactate and or malate was added at two percent it was more effective at improving color stability during retail display than pyruvate. They showed that adding lactate, malate, and pyruvate at one percent was not as effective as when it was added at two percent. From these data, they showed that enhanced beef with metabolites such as lactate, malate, and pyruvate extended post-rigor shelf-life through providing ample reducing conditions for myoglobin.

2.10. Dark Cutting Beef

Dark cutting beef is typically discounted in the retail store for consumers due to the unappealing dark color. Viljoen et al. (2002) determined that consumers preferred the general appearance, color, and acceptability of normal beef steaks when compared to dark cutting beef steaks. They reported that when consumers evaluated cooked steaks, there were no significant differences between normal and dark cutting steaks in regards to odor, appearance, color, taste, texture, juiciness, and overall acceptability. On the

other hand, Wulf et al. (2002) reported that dark cutter muscles (longissimus, gluteus medius, and semimembranosus) were less tender than normal muscles. Moreover, they determined that dark cutting beef carcasses produced a higher percentage of tough longissimus steaks, and a lower percentage of very tender steaks compared to their counterparts.

Several different stress factors have been attributed to the incidence of dark cutting beef. Studies conducted by Grandin (1992) observed that dark, firm, and dry beef had the highest incidence rate during very cold weather combined with precipitation that caused the animal's rate of body heat loss to increase. These researchers also noted that DFD beef was high during warm weather conditions, or when the weather underwent large fluctuations over a short time period. Kreikemeir et al. (1998) reported that the highest incidence of dark cutting beef over twelve months occurred during August, September, and October varying from 1.1–1.4 percent, whereas the incidence rate ranged 0.4 – 0.7 percent in other months. To reduce dark cutting beef, several researchers have recommended that control over antemortem stress management would be effective (Grandin, 1992; Shackelford et al., 1994).

Research studies have been conducted to try to understand the biochemical factors that influence dark cutting beef. It has been reported that the overall darker color of dark cutting beef was directly related to a higher mitochondrial respiration rate, which helped maintain low oxymyoglobin concentrations. Moreover, Ashmore et al. (1973) identified that the reduction of postmortem muscle pH through glycolysis impaired the overall level of oxygen the mitochondria consumed in normal muscle. Lawrie (1958)

determined that mitochondrial cytochrome oxidase was more active at pH values above 6.0, and concluded that increased oxygen consumption of dark cutting meat increased the concentration of deoxymyoglobin. As a result, the characteristic dark meat color was produced.

CHAPTER III

DIFFERENCES IN BIOCHEMICAL TRAITS CONTRIBUTING TO LEAN COLOR AND LEAN COLOR STABILITY IN DARK CUTTING VERSUS NORMAL BEEF

3.1. Materials and Methods

Animal care and use committee approval was not obtained for this study because samples were obtained from federally inspected plants.

3.1.1 Sample Handling and Preparation

Beef carcasses (n = 320) were selected on 5 d (selection days were approximately 2 wk apart) from a commercial processing facility as they were presented for grading. All carcasses had been chilled for approximately 36 h before grading. Carcasses exhibiting various levels of dark cutting beef (n = 160), severe dark cutter (**SEDC**; mean pH = 6.50, n = 40), moderate dark cutter (**MODC**; mean pH = 6.33, n = 40), mild dark cutter (**MIDC**; mean pH = 6.09, n = 40), shady dark cutter (**SHDC**; mean pH = 5.92, n = 40) were selected. Each time a dark cutting carcass was selected, a cohort exhibiting normal lean color and similar marbling level (**NC**; mean pH = 5.53; n = 160) from the same production lot was selected. On-line Longissimus lumborum muscle pH was collected with a Reed SD-230 handheld pH meter (Reed Instruments, Wilmington, NC) on the anterior surface of the interface between the 12^{th} and 13^{th} rib on the right side of each carcass, which was used to classify the dark cutter carcasses. An image analysis-based instrument (VBG 2000; Shackelford et al., 2003) assessed marbling, adjusted backfat thickness, ribeye area, and calculated yield grade. During fabrication, the strip

loin from the left sides of the carcasses [similar to Institutional Meat Purchase Specification (**IMPS**) #180; (NAMP, 2005)] were retrieved and vacuum-packaged then transported under refrigeration to USMARC and aged (1°C) until 13 d postmortem.

After aging, subprimals were cut into steaks. The first steak (1.27 cm) was cut, trimmed free of external fat, and visible connective tissue, diced and frozen in liquid nitrogen, and stored at -80°C for protein carbonyl determination and mitochondrial abundance. The second steak (2.54 cm) was collected and placed in simulated retail display. The third steak (2.54 cm) was collected for oxygen consumption and metmyoglobin reducing ability. From this steak a cube was removed from the center portion of the steak avoiding connective tissue and or large pieces of marbling. The cube (2.54-cm-thick) was then divided in half with the top portion designated for metmyoglobin reducing ability, and the bottom portion designated for oxygen consumption analysis. A fourth steak (1.27 cm) was collected, trimmed free of external fat, and visible connective tissue, diced and frozen in liquid nitrogen and stored at -80°C for glycolytic potential, myoglobin concentration, and pH determination. The fifth steak (1.27 cm) was collected and retained for mitochondrial isolation and determination of mitochondrial efficiency.

For simulated retail display, steaks were placed on polystyrene trays with soaker pads and overwrapped with oxygen-permeable polyvinylchloride (**PVC**) film [stretchable meat film 55003815; Prime Source, St. Louis, MO; oxygen transmission rate = 1.4 mL/ ($cm^2 \cdot 24$ h) at 23°C]. Steaks were then placed under continuous fluorescent lighting (color temperature = 3,500 K; color rendering index = 86; 32 W; T8

Ecolux bulb, model number F32T8/SPX35, GE, GE Lighting, Cleveland OH) for 13 d. Light intensity at the meat surface was approximately 2,000 lx. Retail display was conducted in a refrigerated room (1°C), and no temperature fluctuations associated with defrost cycles were encountered.

The steaks were allowed to bloom at least 2 h after being packaged in retail display before color measurements were collected. Instrumental color readings were taken on each steak on d 0, 1, 4, 7, and 11 of display using a Hunter Miniscan XE Plus Colorimeter (Hunter-Lab, Reston, VA) with a 25-mm port. The colorimeter was set to collect spectral data with Illuminant A and a 10° observer. The CIE L* (lightness), a* (redness), and b* (yellowness) color-space values were reported as the average of duplicate readings taken on each steak. Greater L*, a*, and b* values signify increased lightness, redness, and yellowness, respectively. Color intensity (also referred to as chroma or saturation index) was calculated using the following formula: $[(a^{*2} + b^{*2})^{0.5}]$. Hue angle (redness) was calculated using the formula: [(arctangent (b*/a*) × 180/3.142]. Overall color change during retail display (referred to as ΔE) was calculated using the following formula: $[(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{0.5}]$, where ΔL*, Δa*, and Δb* was the difference between d 0 and d 1, 4, 7, and 11 values for L*, a*, and b*, respectively.

3.1.2 pH and Myoglobin Concentration

Steaks designated for determination of laboratory pH and myoglobin concentration were trimmed free of external fat and epimyseal connective tissue, diced, and pulverized in liquid nitrogen to produce a homogenous powder. Muscle pH was determined following the procedure by Bendall (1973). Duplicate samples (2.5 g ± 0.05

g) were homogenized in 10 volumes of a solution containing 5 m*M* iodoacetate and 150 m*M* KCL (pH = 7.0). The homogenates were allowed to rest at room temperature for a minimum of 1 h. Prior to measuring pH, samples were mixed via vortexing and pH was then measured in duplicate using a Reed SD-230 handheld pH meter with a pH probe (Omega PHE 2385 pH probe, Omega Engineering INC., Stamford, CT). The pH meter was calibrated with standardized pH solutions (pH = 4 and pH = 7), and calibration was conducted approximately every 10 samples.

Myoglobin was extracted and quantified following the method described by Warriss (1979) as modified by Hunt et al. (1999) with modifications. For myoglobin concentration duplicate samples (2.5 ± 0.05 g) were homogenized in 25 mL of 800 m*M* sodium acetate (pH = 4.5). The homogenates were then placed in a shaker at 4ºC for 1 h to allow for pigment extraction before centrifugation (38,000 × *g*) for 35 min at 4ºC. The supernatant was then poured into a disposable 50 mL conical tube, and 10 mL of cold sodium acetate was added to the pellet. The pellet was then washed two times via vortexing to resuspend the pellet in 10 mL of sodium acetate and placed in a shaker 4ºC for 30 min and then centrifuged. Washing of the sample was repeated again and then placed in a shaker 4ºC for 15 min followed by centrifugation. Supernatant from both washes were combined with the supernatant from the initial extraction. The supernatant was then syringed filtered (Nalgene 0.45 µm, surfactant-free cellulose acetate membrane; Thermo Fisher Scientific, Rochester, NY) into 1.5 mL micro-centrifuge tubes. A 200 µL aliquot of the sample was transferred in triplicate to a 96 well plate and blanked against a standard solution of sodium acetate. Absorbance spectra at 525 nm and

700 nm were collected using a Spectramax plus 96-well plate reader (Molecular Devices, Sunnydale CA). Extracted myoglobin pigment concentration (mg/g meat) was calculated taking the difference between the absorbance at 525 nm and 700 nm, a millimolar extinction coefficient of 7.6 mM^{-1} cm^{-1}, the molecular weight of myoglobin (17,000), and the appropriate dilution factor.

3.1.3 Oxygen Consumption

Oxygen consumption (**OC**) was determined by the method utilized by King et al. (2011b). A 3 cm × 3 cm ×2 cm sample with minimal visible fat or connective tissue was removed from a steak. The freshly cut surface of the sample was then covered with a small piece of oxygen permeable film to avoid drying and allowed to bloom for 2 hr at 1°C. The sample was then vacuum-packaged, and immediately scanned with a Hunter Miniscan colorimeter with settings described that had been calibrated through the oxygen impermeable film of a vacuum bag (3-Mil vacuum bags, Prime Source, Kansas City, MO; oxygen transmission rate = 0 cc · cm−2 · 24 h−1 at 23°C). The vacuum-packaged bag was incubated for 30 min at 30°C, and then scanned with the colorimeter to obtain the spectral data. The proportion of oxymyoglobin was calculated on the oxygenated and deoxygenated samples previously described by the AMSA (2012). Oxygen consumption was reported as (% OMb before - % OMb after / % OMb before). The bloom level of OMb for the dark cutters was much lower than the controls, so when the absolute difference was calculated, the cohorts had much greater oxygen consumption. When the percentage change was calculated the data revealed a different result. Within dark cutter classes, both methods seemed to sort variation adequately.

The method of using initial bloom level was an accurate measurement to compare across dark cutting severity classes and cohorts. Theoretically, this measurement represents oxygen consumption because competition for O_2 is limiting oxygenation. Bloom was calculated as the % OMb before.

3.1.4 Nitric Oxide Metmyoglobin Reducing Ability

Metmyoglobin reducing ability was measured as described by Sammel et al. (2002). A 3 cm × 3 cm × 2 cm sample with minimal visible fat or connective tissue was removed from a steak. It was then submerged in 0.3% $NaNO_2$ solution for 20 min at approximately 20°C to induce metmyoglobin formation. The sample was then removed from the solution, blotted, and then vacuum-packaged. Immediately after packaging, the sample was scanned in duplicate with a Hunter Miniscan colorimeter with the previous settings already reported. The sample was then allowed to be reduced in a water bath (30°C) for 2 h, and then scanned again in duplicate. Surface metmyoglobin was quantified using the equations defined AMSA (2012). The proportion of surface metmyoglobin that was initially recorded after oxidation with nitrite and was reported as initial metmyoglobin formation (**IMF**). The proportion of surface metmyoglobin recorded after the 2 h reduction period was reported as the post-reduction metmyoglobin (**PRM**). Nitric oxide metmyoglobin reducing ability (**NORA**) was reported as the absolute difference in surface metmyoglobin between initial (oxidized) readings and the final (reduced readings). The nitric oxide metmyoglobin reducing ability values for the dark cutters were much lower than the controls, so when the absolute difference was calculated, the cohorts had much greater nitric oxide metmyoglobin reducing ability.

When the percentage change was calculated the data revealed a different result. Within dark cutter classes, both methods seemed to sort variation adequately. The method of using initial metmyoglobin formation level was an accurate measurement to compare across dark cutting severity classes and cohorts. Theoretically, this measurement represents nitric oxide metmyoglobin reducing ability because competition for O_2 is limiting oxygenation.

3.1.5 Glycolytic Potential

Glycolytic Potential (**GP**) determination was conducted following a modified procedure prescribed by Miller et al. (2000) with additional modifications (Souza et al., 2011). A 3.00 ± 0.05 g sample of homogenous powder was homogenized in 15.0 mL of 0.6 *N* perchloric acid, and 200 µL of this homogenate was then transferred in duplicate into 1.5 mL microcentrifuge tubes. This aliquot was digested with 1.0 mL of cold amyloglycosidase solution (pH = 4.8; AGS aliquot; Sigma Aldrich, St. Louis, MO) and 20 µL 5.4 *N* potassium hydroxide. The amyloglucosidase cleaved α(1-4) glycosidic linkages and α(1-6) glycosidic linkages in glycogen to yield glucose. Another set of microcentrifuge tubes were prepared to ensure that sample digestion was effective. In these tubes were 200 µL of homogenate, 1 mL of 0.2 M acetate buffer (pH = 4.8), and 20 µL 5.4 *N* potassium hydroxide.

The AGS and non-AGS aliquots were then incubated at 37ºC for 3 hr with inverting the samples every 20 min to thoroughly mix. After incubation, AGS and non-AGS aliquots were placed on ice and 100 µL of cold 3 *N* perchloric acid was added, and then centrifuged (10,000 × *g*) at 4ºC for 5 min to allow for pellet precipitation. The

amounts of glycogen, glucose, and glucose-6-phosphate were determined using a coupled enzymatic assay kit (hexokinase and glucose-6-phosphate dehydrogenase, Sigma-Aldrich, St. Louis, MO), and read at 340 nm using a Spectramax plus 96-well plate reader along with a standard curve prepared using the glucose that was supplied in the kit. Lactate content was measured using an enzyme assay including lactate dehydrogenase and read at 340 nm against a lactate standard curve. Glycolytic potential was calculated utilizing the following formula: GP = 2[(glycogen) + (glucose) + (glucose-6-phosphate)] + [lactate], and was expressed as lactate equivalents per g muscle (Monin and Sellier, 1985).

3.1.6 Mitochondrial Isolation and Mitochondrial H_2O_2 Production

Beef longissimus muscle mitochondria were isolated according to Cawthon et al. (1999) and Iqbal et al. (2005) with modifications. Preliminary data suggested that assays conducted more than 2 hr after extraction produced spurious results. Moreover, assays that exhibited a loss of fluorescence during incubation were removed from the dataset. Thus a subset of the carcasses used in this study were selected for mitochondrial isolation and mitochondrial H_2O_2 production to determine mitochondrial efficiency. The number of samples with data for H_2O_2 production with succinate and glutamate as energy sources were 177 and 211, respectively. In a given day approximately 40 samples could be run, so these samples were randomly selected from the steaks collected. A 10.0 ± 0.10 g of finely minced beef longissimus muscle was added to 40 mL of isolation buffer (220 m*M* D-Mannitol, 70 m*M* sucrose, 2 m*M* HEPES, 0.5 mg/mL BSA, 1m*M* EGTA, pH 7.4) in a 50 mL conical tube, and then homogenized in an

Eberbock blender on low for 10 s, followed by further homogenization in a Potter-Elvenhjem vessel with a Teflon pestle of 0.15 mm clearance (Thomas Scientific, Swedesboro, NJ). A 500 µL aliquot of trypsin (40 mg/mL) was then added to the homogenized sample and vortexed for 10 s, followed by centrifugation (1,000 × *g*) at 4ºC for 11 min. The supernatant then was filtered through glass wool sandwiched between two layers of cheese cloth into another 50 mL conical tube. Again, the supernatant was centrifuged (1,000 × *g*) for 11 min and then filtered through two layers of cheese cloth with glass wool in between into a 35 mL conical tube. The supernatant then was centrifuged (10,000 × *g*) at 4ºC for 15 min and the resulting pellet was washed twice with wash buffer (220 m*M* D-Mannitol, 70 m*M* sucrose, 2 m*M* HEPES, 0.5 mg/mL BSA, pH 7.4). Following centrifugation (10,000 × *g* at 4ºC for 15 min) the pellet was resuspended in 500 µL of wash buffer. Protein determination was conducted using a BCA reaction with a standard BSA curve.

Mitochondrial H_2O_2 production determination was conducted following the method of Bottje et al. (2002) with modifications. The generation of H_2O_2 was measured fluorometrically in 96-well black microplates at an excitation/emission wavelength at 480/530 nm using a Spectramax plus 96-well plate reader (Molecular Devices, Sunnydale CA). The reaction conditions for H_2O_2 measurement included the addition of 90 µg of mitochondrial protein, 52 µ*M* 2'-7' dichlorfluorescin diacetate, 50 µ*L* energy substrate buffer containing 145 m*M* KCL, 30 m*M* HEPES, 5m*M* KH_2PO_4, 3m*M* $MgCl_2$, and 0.1 m*M* EGTA. Superoxide dismutase (20 units per well) was added to each well on the microplate to ensure that all $O^{2\bullet-}$ was converted to H_2O_2. The mitochondria were

provided separate assays with glutamate (8m*M*) and succinate (8m*M*) as energy substrates to provide the reducing equivalents needed for the electron transport chain. The total volume for each well was 95µL, and the microplate was incubated at 37ºC then read by a Spectramax plus 96-well plate reader at 10 and 30 min. Electron loss was determined as the percentage increase in fluorescence units.

3.1.7 Mitochondrial Abundance

Relative mitochondrial abundance was determined by quantifying the relative abundance of mitochondrial and genomic DNA. Following the protocol outlined by the manufacturer, DNA was extracted from muscle samples using Promega Wizard SV 96 Genomic DNA Purfication System (Promega Corp, Madison, WI). Special care was taken to ensure complete digestion of the muscle sample before isolating DNA. DNA was diluted to a final concentration of 50 ng/µL. Mitochrondrial DNA was quantified by real-time RT-PCR using previously published primers for cattle (Iwata et al., 2011) and genomic DNA was quantified using previously published primers for the follicle stimulating hormone receptor (FSHR) (Marson et al., 2008). Twenty five µL reactions were run using 8.5 µL of water, 12.5 µL of Master Mix containing SYBR Green (Bio-Rad catalog # 170- 8893), 1.5 µL of the forward primer, 1.5 µL of reverse primer, and 1 µL of DNA. The PCR was performed as described by Iwata et al. (2011). Initial denaturation was for 5 min at 95°C followed by 40 cycles at 95°C, 58°C, and 72°C for 30 s each. A melting curve was performed to check the specificity of the products. Relative mount of mitochondrial DNA was expressed as the ratio of the threshold cycle of mitochondrial DNA to the ratio of the threshold cycle for FSHR (genomic DNA).

3.1.8 Protein Solubility and Measurement of Carbonyl Content in Sarcoplasmic Fraction

Carbonyl content (protein oxidation) was spectrophotometrically determined to indicate the amount of oxidative damage to the sarcoplasmic protein fraction following the protocol of Keller et al. (1993) and Reznick and Packer (1994) with modifications. A 5 ± 0.1 g sample of frozen minced beef longissimus muscle was homogenized in 15 mL of post rigor extraction buffer (100 m*M* Tris, 10 m*M* EDTA, 0.05% 2-mercaptoethanol [MCE], adjusted with HCl to pH 8.3) in a 35 mL conical tube. Samples were then centrifuged (27,000 x *g*) for 30 min at 4°C and filtered through cheesecloth. Protein determination was conducted with a BCA reaction using a BSA standard curve. Protein solubility was reported as mg of soluble protein per g of wet tissue. Sarcoplasmic fraction samples were adjusted to 1 ml of 6 mg/mL solution and then placed in 15 mL conical tubes in duplicate. To one tube, 4 mL of 10 m*M* 2, 4-dinitrophenylhydrazine (**DNPH**; Sigma Aldrich Inc., St. Louis, MO) in 2.5 *M* HCl was added while in the other tube only 4 mL of 2.5 *M* HCl solution was added. Samples were incubated for 1 h at room temperature in the dark and were vortexed every 15 min during incubation. After incubation, 5 mL of 20% trichloroacetic acid (**TCA**) was added to both DNPH and HCl samples and incubated on ice for 10 min. After incubation, samples were centrifuged (1000 x *g*) at room temperature for 7 min and then the supernatant was discarded. Next, 4 mL of 10% TCA was added to the sample, and the pellet was sonicated to ensure that the pellet was completely solubilized. Following sonication, samples were centrifuged (1000 x *g*) at room temperature for 7 min and then

the supernatant was discarded. For both tubes, the sample pellet was then washed in a mixture of ethanol/ethyl acetate (1:1) followed by sonication and then centrifuged (1000 x *g*) for 7 min at room temperature. This series of steps was repeated two more times. The precipitated protein pellet was then re-dissolved in 2 mL of 6 *M* guanidine hydrochloride (Sigma Aldrich Inc., St. Louis, MO) and incubated at 37°C for 10 min.

Protein determination was conducted with a BCA reaction using a BSA standard curve. Carbonyl content was calculated by obtaining a reading at 360 nm for the DNPH-treated samples and these samples were scanned against the samples treated with 2.5 *M* HCl on a DU640 spectrophotometer (Beckman Coulter Inc., Fullerton, CA). The protein carbonyl content (nmol/mg protein) was calculated using the molar extinction coefficient for DNPH (22,000 M^{-1} cm^{-1}) and the following formula: protein carbonyl content = (absorbance x 106/22,000) / mg protein.

3.1.9 Statistical Analysis

Data were analyzed as a completely randomized block design using the PROC Glimmix procedure of SAS (SAS Inst., Inc., Cary, NC) comparing dark cutting severity classes to normal cohorts. Initial analysis indicated that the individual cohort groups were similar with regard to all of the traits measured in the experiment. Thus, were pooled together for comparisons with the various dark cutter classes. Least squares means for dark cutter classes and the cohort groups were generated with the LS means option and contrasts statements, respectively. Comparisons between the dark cutter classes and cohorts were done with orthogonal contrasts. A significance level of 0.05 was used to determine significance between dark cutter classes and the pooled normal

cohort group. Additionally, least squares means of color attributes from dark cutting carcass classes and cohorts and were generated for each color trait.

3.2. Results and Discussion

Figure 1 presents the regression lines for the color traits collected, which describe the changes in these traits during simulated retail display. The shape of the regression lines for measurements of darkness (L*) indicated not much change in dark cutting classes and cohorts throughout simulated retail display (Figure 1A). All dark cutting carcasses were darker in appearance (lower L* values) throughout simulated retail display (Figure 1A). The regression curve for redness (a*) decreased from the beginning to the end of simulated retail display for cohort carcasses (Figure 1B). For the dark cutting carcasses, redness (a*) did not change much throughout simulated retail display (Figure 1B). By d 9 of simulated retail display, cohort carcasses were less red (a*) than dark cutter carcasses through the end of simulated retail display (Figure 1B). The regression curve for yellowness (b*) decreases from the beginning to the end of simulated retail display for cohort carcasses (1C). For dark cutting carcasses, yellowness (b*) increased during the beginning of simulated retail display (d 0 to d 3), and from d 3 through the end of simulated display did not change (1C).

The regression curve for hue angle remained relatively constant through simulated retail display for dark cutting carcasses (1D). For cohort carcasses, there was not much change in hue angle from d 0 to d 6 of simulated retail display, but increased from d 7 to d 11 of simulated retail display (1D). In regards to chroma, cohort carcasses decreased from the beginning to the end of simulated retail display (1E). For cohort

carcasses, there was an increase in chroma from d 0 to d 5 of simulated retail display, but slightly decreased from d 6 to d 11 of simulated retail display (1E). For overall color stability (ΔE), dark cutting and cohort carcasses were similar from the beginning to d 5 of simulated retail display (Figure 1F). Dark cutting carcasses decreased in overall color change from d 5 to d 9, and increased from d 9 to d 11 of simulated retail display (Figure 1F). Cohort carcasses increased in overall color change from d through the end of simulated retail display (Figure 1F).

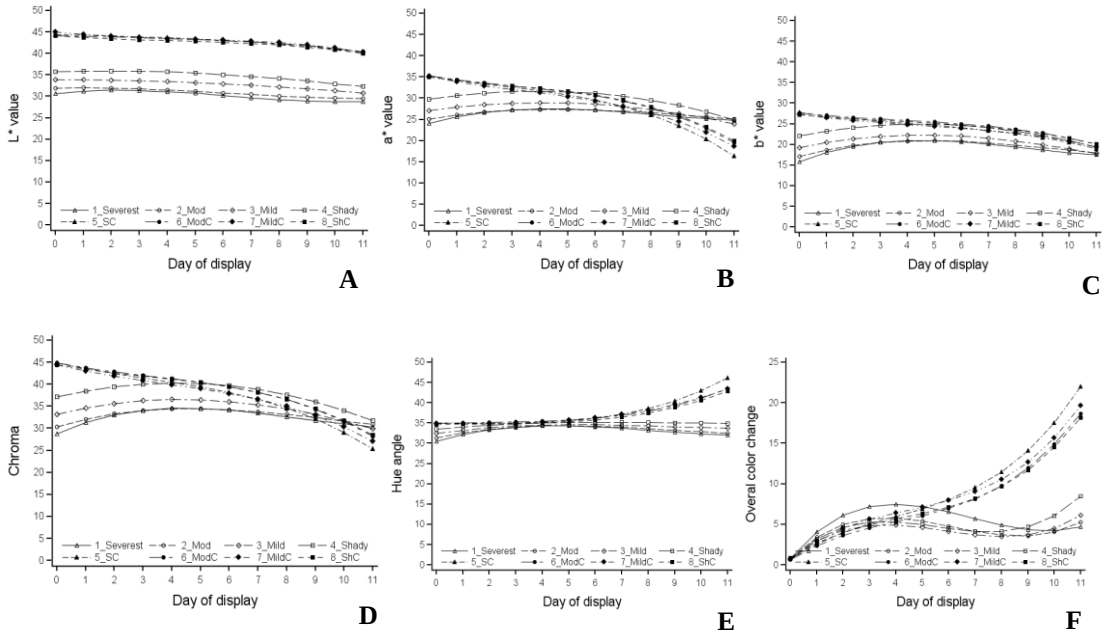

Figure 1. Least square means of color attributes from dark cutting and cohort LM steaks during 11 d of simulated retail display at 14 d postmortem.

All dark cutting carcass severity classes (SHDC, MIDC, MODC, and SEDC) had higher ($P < 0.01$) muscle pH values than the cohorts (Table 1). Within the dark cutting classes, pH numerically increased as dark cutting severity increased (Table 1). Page et

al. (2001) evaluated 1,000 beef carcasses, 80% of carcasses evaluated were within the pH range of 5.40 to 5.59, which is deemed a normal pH for the beef longissimus muscle undergoing postmortem metabolism (Lawrie, 1958; Tarrant and Mothersill, 1977). Additionally, Page et al. (2001) reported that dark cutting carcasses had a pH range of 5.87 to 6.89, which is similar to data from the current study.

Table 1. Least-squares means for muscle pH, glycolytic potential, and protein solubility values from dark cutting and cohort LM samples.

			Dark Cutting Class			
Item	N	Cohort[1]	Shady	Mild	Moderate	Severe
pH	320	5.53	5.92	6.09	6.33	6.50
P > F[2]			<0.01	<0.01	<0.01	<0.01
SEM			0.03	0.03	0.03	0.03
Glycolytic Potential	320	147.70	80.84	72.30	65.49	46.32
P > F[2]			<0.01	<0.01	<0.01	<0.01
Protein Solubility	320	21.12	22.77	22.43	22.90	22.94
P > F[2]			<0.01	<0.01	<0.01	<0.01
SEM			0.38	0.38	0.38	0.38

[1] Pooled averaged across cohort groups
[2]P value denotes contrasts between dark cutter classes and cohort

All dark cutting severity classes (SHDC, MIDC, MODC, and SEDC) had greater ($P < 0.05$) protein solubility than the cohort carcasses (Table 1). The amount of soluble protein in the sarcoplasmic fraction than can be extracted can be used as an indication of the amount of denaturation that has occurred in muscle tissue. Joo et al. (1999) reported in pork that protein solubility increases with increasing ultimate pH, which is in agreement with the present study. Silva et al. (1999) suggested that higher protein solubility at a pH of 7.0 in DFD meat could potentially be due to increased proteolysis.

The normal cohort carcasses had greater ($P < 0.05$) glycolytic potential values (µmol/g) than all dark cutting severity classes (SHDC, MIDC, MODC, and SEDC) (Table 1). Across dark cutting classes, glycolytic potential values (µmol/g) numerically decreases as dark cutting severity increases. Dark cutting carcasses (SHDC, MIDC, MODC, and SEDC) had greater ($P < 0.05$) protein solubility than the cohort carcasses (Table 1). Glycolytic potential is a measurement that was developed to evaluate the amount of glycogen present in postmortem muscle that is converted to lactate. Glycogen level or concentration is the amount of glycogen that is actually present in the muscle. Wulf et al. (2002) stated that carcasses with glycolytic potential values below approximately 100 µmol/g had higher muscle pH values because the glycogen stores found in the muscle tissue were depleted. In regards to muscle glycogen levels, Hanson et al. (2001) reported that dark cutting conditions are more likely to occur in carcasses when muscle glycogen concentrations are less than 80 mmol/kg.

Dark cutting carcasses (SHDC, MIDC, and MODC) had greater ($P < 0.01$) myoglobin concentration than cohort carcassses, but SEDC were not different ($P = 0.43$) from cohorts (Table 2). Moreover, within the dark cutting classes, myoglobin concentration numerically decreased as dark cutting severity increased (Table 2). Hunt and Hedrick (1977) reported that dark cutting carcasses had greater myoglobin concentrations than normal carcasses. Moreover, Hunt and Hedrick (1977) suggested that this could be a result of uncontrolled vasodilation when animals are undergoing stressful conditions, thus resulting in blood pooling within the muscle.

Table 2. Least-squares means for myoglobin concentration, mitochondrial abundance, protein carbonyl values, and measurements of electron loss utilizing glutamate and succinate from dark cutting and cohort LM samples.

			Dark Cutting Class			
Item	N	Cohort[1]	Shady	Mild	Moderate	Severe
Myoglobin Concentration	320	4.36	4.78	4.91	4.68	4.46
P > F[2]			<0.01	<0.01	<0.01	0.43
SEM			0.13	0.13	0.13	0.13
Mitochondrial Abundance	320	0.596	0.597	0.609	0.626	0.643
P > F[2]			0.83	0.02	<0.01	<0.01
SEM			0.01	0.01	0.01	0.01
Protein Carbonyl	320	2.18	2.30	2.20	2.21	2.60
P > F[2]			0.56	0.93	0.88	0.05
SEM			0.21	0.21	0.21	0.21
Electron loss glutamate	211	10.95	18.19	30.94	17.91	9.13
P > F[2]			0.28	<0.01	0.27	0.78
SEM			6.73	6.52	6.33	6.43
Electron loss succinate	177	10.76	19.70	25.03	17.72	18.75
P > F[2]			0.03	<0.01	0.08	<0.05
SEM			3.88	4.00	4.16	4.17

[1] Pooled averaged across cohort groups

[2]P value denotes contrasts between dark cutter classes and cohorts

For mitochondrial abundance, MIDC, MODC, and SEDC had greater amounts of mitochondria ($P < 0.05$) than cohorts, but SHDC were not different ($P = 0.83$) from cohorts (Table 2). Scientists have conducted research to understand how mitochondria from normal animals and animals induced with epinephrine differ (Ashmore et al., 1971). These conditions were induced as opposed to naturally occurring. Ashmore et al. (1971) determined that there were no significant differences in mitochondrial protein recovered, specific gravity of mitochondrial succinate dehydrogenase, respiratory control ratios between mitochondria from control muscles and muscles injected with

epinephrine. Moreover, Ashmore et al. (1972) reported that mitochondria in normal muscle tissue rapidly lost capacity for respiration due to a decreased muscle pH and respiration was maintained at higher levels and for longer time periods in dark cutting beef. A major difference between Ashmore et al. (1971) and the current study is induced versus natural conditions. In the present study, dark cutting carcasses exhibited these conditions within the muscle tissue. Both determined that there were differences in mitochondria.

In regards to protein oxidation (carbonyl formation), SEDC had greater (P = 0.05) protein oxidation than cohorts (Table 2), but SHDC, MIDC, and MODC were not different ($P > 0.05$) than cohorts. It has been reported that biochemical metabolic factors associated with muscle tissue give rise to the formation of reactive oxygen species, thus resulting in carbonyl formation, and decreased sulfhydryl content of the proteins (Hoffman and Hamm, 1978; Martinaud et al., 1997; Xiong, 2000). King et al. (2010) suggested that muscles possessing greater oxidative metabolism would have a greater amount of mitochondria and mitochondrial enzymes that compete with myoglobin for available oxygen. Dark cutting beef has greater amounts of mitochondria utilizing the available oxygen in the muscle, so a greater portion of myoglobin is in the deoxygenated form, thus consequently could be more susceptible to oxidation. This could help explain the difference in protein oxidation between severe dark cutting carcasses and the cohort carcasses.

Measurements of electron loss utilizing glutamate as a substrate were greater ($P < 0.05$) for MIDC than cohorts, but SHDC, MODC, and SEDC were not different ($P >$

0.05) than cohorts (Table 2). Measurements of electron loss utilizing succinate as a substrate were greater ($P < 0.05$) for SHDC, MIDC, and SEDC than cohort carcasses (Table 2). Also, there was a trend ($P = 0.08$) for MODC to have greater measurements of electron loss utilizing succinate as a substrate than cohorts (Table 2). Ramanathan et al. (2009, 2010) and Tang et al. (2005) reported that when pyruvate and succinate are used as substrates for mitochondrial oxygen consumption, myoglobin oxygenation decreases, resulting in darker lean muscle color.

All dark cutting severity carcasses (SHDC, MIDC, MODC, and SEDC) had less ($P < 0.01$) bloom (measurement of oxygen consumption) than the cohort carcasses (Table 3). Across the dark cutting classes, bloom (measurement of oxygen consumption) numerically decreased as dark cutting severity class increased (Table 3). Bendall (1972) reported that mitochondrial oxidation is 50-75% higher at a pH of 7.2 than at a pH of 5.8. As a result, high mitochondrial concentrations maintained a lower concentration of oxymyoglobin. Lawrie (1958) concluded that increased concentration of dark-red deoxymyoglobin was due to increased oxygen consumption and decreased availability of oxygen in meat.

Table 3. Least-squares means for bloom (oxygen consumption) and initial metmyoglobin formation (nitric oxide metmyoglobin reducing ability) values from dark cutting and cohort LM samples.

			Dark Cutting Class			
Item	N	Cohort[1]	Shady	Mild	Moderate	Severe
Bloom	320	91.54	79.56	72.97	67.07	63.25
P > F[2]			<0.01	<0.01	<0.01	<0.01
SEM			1.15	1.15	1.15	1.15
Initial Metmyoglobin formation	320	70.60	59.47	55.78	51.25	49.34
P> F[2]			<0.01	<0.01	<0.01	<0.01
SEM			0.69	0.69	0.69	0.69

[1] Pooled averaged across cohort groups
[2]P value denotes contrasts between dark cutter classes and cohorts

Nitric oxide metmyoglobin reducing ability utilizing IMF, all dark cutting severity classes (SHDC, MIDC, MODC, and SEDC) had lower ($P < 0.01$) IMF (measurement of NORA) than the cohorts (Table 3). Across dark cutting classes, IMF (measurement of NORA) decreased as dark cutting severity class increased (Table 3). Initial metmyoglobin formation could be less in dark cutting carcasses than cohort carcasses due to increased concentration of muscle in the deoxymyoglobin state. Giddings (1974) suggested that mitochondria or sub mitochondrial particles could potentially play a role in metmyoglobin reduction in vacuum packed meat cuts by having the ability to scavenge residual oxygen, thus eliminating the potential for low oxygen mediated myoglobin oxidation. Additionally, the mitochondria could serve as a source of reducing equivalents for extra mitochondrial pyridine nucleotide reduction, which would provide the NADH necessary for metmyoglobin reductase to function properly (Bodwell et al., 1965; Giddings, 1977)

CHAPTER IV

INFLUENCE OF MITOCHONDRIAL EFFICIENCY ON BEEF LEAN COLOR STABILITY

4.1 Materials and Methods

Animal care and use committee approval was not obtained for this study because samples were obtained from federally inspected plants.

4.1.1 Sample Handling and Preparation

Beef carcasses (n = 160) were selected on 5 d (selection days were approximately 2 wk apart) from a commercial processing facility as they were presented for grading. All carcasses had been chilled for approximately 36 h before grading. Carcasses exhibiting normal lean color were selected across numerous production lots and grades. Carcasses were ribbed between the 12^{th} and 13^{th} ribs, and an image analysis-based (VBG2000) grading system (Shackelford et al., 2003) assessed adjusted backfat thickness, ribeye area, and calculated yield grade. During fabrication, the strip loin from the left sides of the carcasses [Institutional Meat Purchase Specification (**IMPS**) #180; (NAMP, 2005)] were retrieved and vacuum packaged then transported under refrigeration to USMARC and aged (1°C) until 13 d postmortem.

After aging, subprimals were cut into steaks. The first steak (1.27 cm) was cut, trimmed free of external fat, and visible connective tissue, diced and frozen in liquid nitrogen, and stored at -80°C for protein carbonyl determination and mitochondrial abundance. The second steak (2.54 cm) was collected and placed in simulated retail display. The third steak (2.54 cm) was collected for oxygen consumption and

metmyoglobin reducing ability. From this steak a cube was removed from the center portion of the steak avoiding connective tissue and or large pieces of marbling. The cube (2.54-cm-thick) was then divided in half with the top portion designated for metmyoglobin reducing ability, and the bottom portion designated for oxygen consumption analysis. A fourth steak (1.27 cm) was collected, trimmed free of external fat, and visible connective tissue, diced and frozen in liquid nitrogen and stored at -80°C for glycolytic potential, myoglobin concentration, and pH determination. The fifth steak (1.27 cm) was collected and retained for mitochondrial isolation and determination of mitochondrial efficiency.

For simulated retail display, steaks were placed on polystyrene trays with soaker pads and overwrapped with oxygen-permeable polyvinylchloride (**PVC**) film [stretchable meat film 55003815; Prime Source, St. Louis, MO; oxygen transmission rate = 1.4 mL/ (cm^2·24 h) at 23°C]. Steaks were then placed under continuous fluorescent lighting (color temperature = 3,500 K; color rendering index = 86; 32 W; T8 Ecolux bulb, model number F32T8/SPX35, GE, GE Lighting, Cleveland OH) for 13 d. Light intensity at the meat surface was approximately 2,000 lx. Retail display was conducted in a refrigerated room (1°C), and no temperature fluctuations associated with defrost cycles were encountered.

The steaks were allowed to bloom at least 2 h after being packaged in retail display before color measurements were collected. Instrumental color readings were taken on each steak on d 0, 1, 4, 7, and 11 of display using a Hunter Miniscan XE Plus Colorimeter (Hunter-Lab, Reston, VA) with a 25-mm port. The colorimeter was set to

collect spectral data with Illuminant A and a 10° observer. The CIE L* (lightness), a* (redness), and b* (yellowness) color-space values were reported as the average of duplicate readings taken on each steak. Greater L*, a*, and b* values signify increased lightness, redness, and yellowness, respectively. Color intensity (also referred to as chroma or saturation index) was calculated using the following formula: $[(a^{*2} + b^{*2})^{0.5}]$. Hue angle (redness) was calculated using the formula: [(arctangent (b*/a*) × 180/3.142]. Overall color change during retail display (referred to as ΔE) was calculated using the following formula: $[(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{0.5}]$, where ΔL*, Δa*, and Δb* was the difference between d 0 and d 1, 4, 7, and 11 values for L*, a*, and b*, respectively.

4.1.2 pH and Myoglobin Concentration

Steaks designated for determination of laboratory pH and myoglobin concentration were trimmed free of external fat and epimyseal connective tissue, diced, and pulverized in liquid nitrogen to produce a homogenous powder. Muscle pH was determined following the procedure by Bendall (1973). Duplicate samples (2.5 g ± 0.05 g) were homogenized in 10 volumes of a solution containing 5 m*M* iodoacetate and 150 m*M* KCL (pH = 7.0). The homogenates were allowed to rest at room temperature for a minimum of 1 h. Prior to measuring pH, samples were mixed via vortexing and pH was then measured in duplicate using a Reed SD-230 handheld pH meter with a pH probe (Omega PHE 2385 pH probe, Omega Engineering INC., Stamford, CT). The pH meter was calibrated with standardized pH solutions (pH = 4 and pH = 7), and calibration was conducted approximately every 10 samples.

Myoglobin was extracted and quantified following the method described by Warriss (1979) as modified by Hunt et al. (1999) with modifications. For myoglobin concentration, duplicate samples (2.5 ± 0.05 g) were homogenized in 25 mL of 800 m*M* sodium acetate (pH = 4.5). The homogenates were then placed in a shaker at 4ºC for 1 h to allow for pigment extraction before centrifugation (38,000 × *g*) for 35 min at 4ºC. The supernatant was then poured into a disposable 50 mL conical tube, and 10 mL of cold sodium acetate was added to the pellet. The pellet was then washed two times via vortexing to resuspend the pellet in 10 mL of sodium acetate and placed in a shaker 4ºC for 30 min and then centrifuged. Washing of the sample was repeated again and then placed in a shaker 4ºC for 15 min followed by centrifugation. Supernatant from both washes were combined with the supernatant from the initial extraction. The supernatant was then syringed filtered (Nalgene 0.45 µm, surfactant-free cellulose acetate membrane; Thermo Fisher Scientific, Rochester, NY) into 1.5 mL micro-centrifuge tubes. A 200 µL aliquot of the sample was transferred in triplicate to a 96 well plate and blanked against a standard solution of sodium acetate. Absorbance spectra at 525 nm and 700 nm were collected using a Spectramax plus 96-well plate reader (Molecular Devices, Sunnydale CA). Extracted myoglobin pigment concentration (mg/g meat) was calculated taking the difference between the absorbance at 525 nm and 700 nm, a millimolar extinction coefficient of 7.6 mM^{-1} cm^{-1}, the molecular weight of myoglobin (17,000), and the appropriate dilution factor.

4.1.3 Oxygen Consumption

Oxygen consumption (**OC**) was determined by the method utilized by King et al. (2011b). A 3 cm × 3 cm ×2 cm sample with minimal visible fat or connective tissue was removed from a steak. The freshly cut surface of the sample was then covered with a small piece of oxygen permeable film to avoid drying and allowed to bloom for 2 hr at 1°C. The sample was then vacuum-packaged, and immediately scanned with a Hunter Miniscan colorimeter with settings described that had been calibrated through the oxygen impermeable film of a vacuum bag (3-Mil vacuum bags, Prime Source, Kansas City, MO; oxygen transmission rate = 0 cc · cm−2 · 24 h−1 at 23°C). The vacuum-packaged bag was incubated for 30 min at 30°C, and then scanned with the colorimeter to obtain the spectral data. The proportion of oxymyoglobin was calculated on the oxygenated and deoxygenated samples previously described by the AMSA (2012). Oxygen consumption was reported as (% OMb before - % OMb after / % OMb before).

4.1.4 Nitric Oxide Metmyoglobin Reducing Ability

Metmyoglobin reducing ability was measured and described by Sammel et al. (2002). A 3 cm × 3 cm × 2 cm sample with minimal visible fat or connective tissue was removed from a steak. It was then submerged in 0.3% $NaNO_2$ solution for 20 min at approximately 20°C to induce metmyoglobin formation. The sample was then removed from the solution, blotted, and then vacuum-packaged. Immediately after packaging, the sample was scanned in duplicate with a Hunter Miniscan colorimeter with the previous settings already reported. The sample was then allowed to be reduced in a water bath (30°C) for 2 h, and then scanned again in duplicate. Surface metmyoglobin was

quantified using the equations defined AMSA (2012). The proportion of surface metmyoglobin that was initially recorded after oxidation with nitrite and was reported as initial metmyoglobin formation (**IMF**). The proportion of surface metmyoglobin recorded after the 2 h reduction period was reported as the post-reduction metmyoglobin (**PRM**). Nitric oxide metmyoglobin reducing ability (**NORA**) was reported as the absolute difference in surface metmyoglobin between initial (oxidized) readings and the final (reduced readings).

4.1.5 Glycolytic Potential

Glycolytic Potential (**GP**) determination was conducted following a modified procedure prescribed by Miller et al. (2000) with additional modifications (Souza et al., 2011). A 3.00 ± 0.05 g sample of homogenous powder was homogenized in 15.0 mL of 0.6 *N* perchloric acid, and 200 µL of this homogenate was then transferred in duplicate into 1.5 mL microcentrifuge tubes. This aliquot was digested with 1.0 mL of cold amyloglycosidase solution (pH = 4.8; AGS aliquot; Sigma Aldrich, St. Louis, MO) and 20 µL 5.4 *N* potassium hydroxide. The amyloglucosidase cleaved α(1-4) glycosidic linkages and α(1-6) glycosidic linkages in glycogen to yield glucose. Another set of microcentrifuge tubes were prepared to ensure that sample digestion was effective. In these tubes were 200 µL of homogenate, 1 mL of 0.2 M acetate buffer (pH = 4.8), and 20 µL 5.4 *N* potassium hydroxide.

The AGS and non-AGS aliquots were then incubated at 37ºC for 3 hr with inverting the samples every 20 min to thoroughly mix. After incubation, AGS and non-AGS aliquots were placed on ice and 100 µL of cold 3 *N* perchloric acid was added, and

then centrifuged (10,000 × *g*) at 4ºC for 5 min to allow for pellet precipitation. The amounts of glycogen, glucose, and glucose-6-phosphate were determined using a coupled enzymatic assay kit (hexokinase and glucose-6-phosphate dehydrogenase, Sigma-Aldrich, St. Louis, MO), and read at 340 nm using a Spectramax plus 96-well plate reader along with a standard curve prepared using the glucose that was supplied in the kit. Lactate content was measured using an enzyme assay including lactate dehydrogenase and read at 340 nm against a lactate standard curve. Glycolytic potential was calculated utilizing the following formula: GP = 2[(glycogen) + (glucose) + (glucose-6-phosphate)] + [lactate], and was expressed as lactate equivalents per g muscle (Monin and Sellier, 1985).

4.1.6 Mitochondrial Isolation and Mitochondrial H_2O_2 Production

Beef longissimus muscle mitochondria were isolated according to Cawthon et al. (1999) and Iqbal et al. (2005) with modifications. Preliminary data suggested that assays conducted more than 2 hr after extraction produced spurious results. Moreover, assays that exhibited a loss of fluorescence during incubation were removed from the dataset. Thus, a subset of the carcasses used in this study were selected for mitochondrial isolation and mitochondrial H_2O_2 production to determine mitochondrial efficiency. The number of samples with data for H_2O_2 production with succinate and glutamate as energy sources were 177 and 211, respectively. In a given day approximately 40 samples could be run, so these samples were randomly selected from the steaks collected. A 10.0 ± 0.10 g of finely minced beef longissimus muscle was added to 40 mL of isolation buffer (220 m*M* D-Mannitol, 70 m*M* sucrose, 2 m*M* HEPES, 0.5 mg/mL

BSA, 1m*M* EGTA, pH 7.4) in a 50 mL conical tube, and then homogenized in an Eberbock blender on low for 10 s, followed by further homogenization in a Potter-Elvenhjem vessel with a Teflon pestle of 0.15 mm clearance (Thomas Scientific, Swedesboro, NJ). A 500 µL aliquot of trypsin (40 mg/mL) was then added to the homogenized sample and vortexed for 10 s, followed by centrifugation (1,000 × *g*) at 4ºC for 11 min. The supernatant then was filtered through glass wool sandwiched between two layers of cheese cloth into another 50 mL conical tube. Again, the supernatant was centrifuged (1,000 × *g*) for 11 min and then filtered through two layers of cheese cloth with glass wool in between into a 35 mL conical tube. The supernatant then was centrifuged (10,000 × *g*) at 4ºC for 15 min and the resulting pellet was washed twice with wash buffer (220 m*M* D-Mannitol, 70 m*M* sucrose, 2 m*M* HEPES, 0.5 mg/mL BSA, pH 7.4). Following centrifugation (10,000 × *g* at 4ºC for 15 min) the pellet was resuspended in 500 µL of wash buffer. Protein determination was conducted using a BCA reaction with a standard BSA curve.

Mitochondrial H_2O_2 production determination was conducted following the method of Bottje et al. (2002) with modifications. The generation of H_2O_2 was measured fluorometrically in 96-well black microplates at an excitation/emission wavelength at 480/530 nm using a Spectramax plus 96-well plate reader (Molecular Devices, Sunnydale CA). The reaction conditions for H_2O_2 measurement included the addition of 90 µg of mitochondrial protein, 52 µ*M* 2'-7' dichlorfluorescin diacetate, 50 µ*L* energy substrate buffer containing 145 m*M* KCL, 30 m*M* HEPES, 5m*M* KH_2PO_4, 3m*M* $MgCl_2$, and 0.1 m*M* EGTA. Superoxide dismutase (20 units per well) was added to each well of

the microplate to ensure that all $O^{2\bullet-}$ was converted to H_2O_2. The mitochondria were provided separate assays with glutamate (8m*M*) and succinate (8m*M*) as energy substrates to provide the reducing equivalents needed for the electron transport chain. The total volume for each well was 95µL, and the microplate was incubated at 37ºC then read by a Spectramax plus 96-well plate reader at 10 and 30 min. Electron loss was determined as the percentage increase in fluorescence units.

4.1.7 Mitochondrial Abundance

Relative mitochondrial abundance was determined by quantifying the relative abundance of mitochondrial and genomic DNA. Following the protocol outlined by the manufacturer, DNA was extracted from muscle samples using Promega Wizard SV 96 Genomic DNA Purfication System (Promega Corp, Madison, WI). Special care was taken to ensure complete digestion of the muscle sample before isolating DNA. DNA was diluted to a final concentration of 50 ng/µL. Mitochrondrial DNA was quantified by real-time RT-PCR using previously published primers for cattle (Iwata et al., 2011) and genomic DNA was quantified using previously published primers for the follicle stimulating hormone receptor (FSHR) (Marson et al., 2008). Twenty five µL reactions were run using 8.5 µL of water, 12.5 µL of Master Mix containing SYBR Green (Bio-Rad catalog # 170- 8893), 1.5 µL of the forward primer, 1.5 µL of reverse primer, and 1 µL of DNA. The PCR was performed as described by Iwata et al. (2011). Initial denaturation was for 5 min at 95°C followed by 40 cycles at 95°C, 58°C, and 72°C for 30 s each. A melting curve was performed to check the specificity of the products. Relative mount of mitochondrial DNA was expressed as the ratio of the threshold cycle of mitochondrial DNA to the ratio of the threshold cycle for FSHR (genomic DNA).

4.1.8 Protein Solubility and Measurement of Carbonyl Content in Sarcoplasmic Fraction

Carbonyl content (protein oxidation) was spectrophotometrically determined to indicate the amount of oxidative damage to the sarcoplasmic protein fraction following the protocol of Keller et al. (1993) and Reznick and Packer (1994) with modifications. A 5 ± 0.1 g sample of frozen minced beef longissimus muscle was homogenized in 15 mL of post rigor extraction buffer (100 m*M* Tris, 10 m*M* EDTA, 0.05% 2-mercaptoethanol [MCE], adjusted with HCl to pH 8.3) in a 35 mL conical tube. Samples were then centrifuged (27,000 x *g*) for 30 min at 4°C and filtered through cheesecloth. Protein determination was conducted with a BCA reaction using a BSA standard curve. Protein solubility was reported as mg of soluble protein per g of wet tissue. Sarcoplasmic fraction samples were adjusted to 1 ml of 6 mg/mL solution and then placed in 15 mL conical tubes in duplicate. To one tube, 4 mL of 10 m*M* 2, 4-dinitrophenylhydrazine (**DNPH**; Sigma Aldrich Inc., St. Louis, MO) in 2.5 *M* HCl was added while in the other tube only 4 mL of 2.5 *M* HCl solution was added. Samples were incubated for 1 h at room temperature in the dark and were vortexed every 15 min during incubation. After incubation, 5 mL of 20% trichloroacetic acid (**TCA**) was added to both DNPH and HCl samples and incubated on ice for 10 min. After incubation, samples were centrifuged (1000 x *g*) at room temperature for 7 min and then the supernatant was discarded. Next, 4 mL of 10% TCA was added to the sample, and the pellet was sonicated to ensure that the pellet was completely solubilized. Following sonication, samples were centrifuged (1000 x *g*) at room temperature for 7 min and then

the supernatant was discarded. For both tubes, the sample pellet was then washed in a mixture of ethanol/ethyl acetate (1:1) followed by sonication and then centrifuged (1000 x *g*) for 7 min at room temperature. This series of steps was repeated two more times. The precipitated protein pellet was then re-dissolved in 2 mL of 6 *M* guanidine hydrochloride (Sigma Aldrich Inc., St. Louis, MO) and incubated at 37°C for 10 min.

Protein determination was conducted with a BCA reaction using a BSA standard curve. Carbonyl content was calculated by obtaining a reading at 360 nm for the DNPH-treated samples and these samples were scanned against the samples treated with 2.5 *M* HCl on a DU640 spectrophotometer (Beckman Coulter Inc., Fullerton, CA). The protein carbonyl content (nmol/mg protein) was calculated using the molar extinction coefficient for DNPH (22,000 M^{-1} cm^{-1}) and the following formula: protein carbonyl content = (absorbance x 106/22,000) / mg protein.

4.1.9 Statistical Analysis

Simple statistics, mean, standard error, minimum and maximum values, and coefficient of variation, were generated for each trait on d 0, 1, 4, 7, and 11 as well as change in color during display using the PROC MEANS procedure of SAS (v.9.3, SAS Institute, Cary, NC). Correlation analysis (PROC CORR) was conducted to analyze the relationships between important color variables measured on d 0, 1, 4, 7 and d 11 of retail display, as well as change in color during display. The color variables included marbling score, pH, GP, myoglobin concentration, muscle pH, OC, NORA, IMF, PRM, protein solubility, mitochondrial abundance, protein oxidation, and electron loss utilizing

glutamate and succinate as substrates. Significance for correlations were set at $P < 0.05$ and $P < 0.10$.

4.2. Results

Simple statistics of lean color measurements (Table 4) showed that there was variation in initial lean color and lean color stability through simulated retail display.

Table 4. Simple statistics for color parameters, oxygen consumption, and nitric oxide metmyoglobin reducing ability on d 0 and d 11 of simulated retail display.

Variable	Mean	Standard deviation	Minimum	Maximum	Coefficient of variation
Myoglobin concentration, (mg/g)	4.36	0.66	2.84	6.27	15.14
d 0 L*	44.59	3.05	37.33	55.43	6.84
d11 L*	40.36	3.45	32.16	49.79	8.55
d 0 a*	35.57	1.66	31.09	40.04	4.67
d 11 a*	19.17	6.63	8.70	35.88	34.59
d 0 b*	28.00	1.97	22.68	32.53	7.04
d 11 b*	19.81	2.97	11.80	29.48	14.99
d 0 Chroma	45.28	2.50	38.77	51.45	5.52
d 11 Chroma	27.78	6.49	14.76	46.44	23.36
d 0 Hue Angle	35.13	0.74	32.86	36.82	2.11
d 11 Hue Angle	43.61	6.34	33.92	56.15	14.54
Δ E[1]	19.09	7.21	0.67	33.76	37.77
OC, (%)[2]	35.09	8.92	11.22	64.08	25.42
NORA, (%)[3]	65.25	14.10	10.73	99.10	21.61
IMF, (%)[4]	70.59	3.04	62.46	79.63	4.31
PRM, (%)[5]	24.73	10.66	0.60	68.77	43.11

[1]Overall color change $[(L^{*2} + a^{*2} + b^{*2})^{0.5}]$ calculated using the change in instrumental color space variables between d 0 and d 11 of retail display.
[2]Oxygen consumption
[3]Nitric oxide metmyoglobin reducing activity; Initial metmyoglobin formed – post-reduction metmyoglobin.
[4]Initial metmyoglobin formed during 30 min incubation with 0.3% sodium nitrite.
[5]Post-reduction metmyoglobin; nitric oxide metmyoglobin remaining after 2 h in vacuum package.

Lean color was more variable relative to the means on d 11 than d 0 of simulated retail display as seen in the CV values. The simple statistics for the plant and laboratory

parameters measured on d 0 were reported in Table 5. The pH values for these carcasses were representative of what was expected for carcasses exhibiting normal beef color.

Table 5. Simple statistics for plant parameters and laboratory parameters on d 0 for beef longissimus lumborum steaks aged for 13 d.

Variable	Mean	Standard deviation	Minimum	Maximum	Coefficient of variation
Marbling score[1]	428	78.74	284	704	18.37
pH	5.54	0.05	5.43	5.76	0.90
Protein solubility, (%)	21.10	2.38	15.25	27.70	11.28
Protein oxidation, (nmol/mg protein)	2.17	1.37	0.01	7.84	63.13
Mitochondrial abundance, (%)	0.60	0.03	0.53	0.71	5.00
Glycolytic Potential[2]	146.25	29.20	36.46	221.22	19.97
EL Glutamate[3]	10.18	12.67	-0.38	85.11	124.46
EL Succinate[3]	11.97	11.62	0.00	54.89	97.08

[1]100 = Devoid^{00}; 200 = Practically Devoid^{00}; 300 = Traces^{00}; 400 = Slight^{00}; 500 = Small^{00}; 600 = Modest^{00}; 700 = Moderate^{0}; 800 = Slightly Abundant^{00}.

[2]Glycolytic potential calculated {2[(glycogen) + (glucose) + (glucose-6-phosphate)] + [lactate]}, and was expressed as lactate equivalents per g muscle.

[3] Electron loss (EL) was determined as the percentage increase in fluorescence units resulting from incubating (37°C for 20 minutes) isolated mitochondria in the presence of 2'-7' dichlorfluorescin diacetate with succinate and glutamate as a substrate for electron transport.

4.2.1 Correlations of LL Muscle Color Measurements with Parameter Measurements

Correlation coefficients between longissimus lumborum L* values measured on d 0, 1, 4, 7, and 11 of simulated retail display to biochemical traits measured on the longissimus lumborum steaks are reported in Table 6. Through the 11 d simulated retail display, marbling score was moderately correlated ($P < 0.05$) with L* values on d 0, 1, 4, 7 and 11. Measurements of pH were moderately negatively correlated ($P < 0.05$) with L* values taken on all days. Moreover, myoglobin concentration, oxygen consumption, and nitric oxide metmyoglobin reducing ability were moderately negatively correlated ($P < 0.05$) to L* values, whereas initial metmyoglobin formation was moderately positively correlated ($P < 0.05$). Protein oxidation was positively correlated ($P < 0.05$)

with L* values for all days of simulated retail display. Mitochondrial abundance was lowly correlated on d 1, 4, and 7 of simulated retail display. Measures of electron loss were weakly correlated ($P < 0.10$) to L* values on d 0 (electron loss glutamate), and d 1 and d 4 (electron loss succinate), but moderately correlated ($P < 0.05$) on d 0 (electron loss succinate) and d 11 (electron loss succinate and glutamate).

Table 6. Pearson correlation coefficients between lightness (L*) values of longissimus lumborum steaks measured on d 0, 1, 4, 7 and 11 of simulated retail display and the parameters measured on the steaks.

	Longissimus Lumborum L* values				
Parameters	Day 0	Day 1	Day 4	Day 7	Day 11
Marbling score	0.42**	0.34**	0.36**	0.41**	0.43**
pH	-0.34**	-0.27**	-0.27**	-0.21**	-0.25**
Myoglobin concentration	-0.39**	-0.39**	-0.47**	-0.49**	-0.44**
Oxygen consumption	-0.34**	-0.17**	-0.29**	-0.16**	-0.16**
Nitric oxide metmyoglobin reducing activity	-0.39**	-0.27**	-0.30**	-0.21**	-0.26**
Initial metmyoglobin formation	0.42**	0.26**	0.41**	0.32**	0.30**
Protein solubility	-0.10	-0.14*	0.02	0.03	-0.08
Protein oxidation	0.25**	0.22**	0.22**	0.22**	0.20**
Mitochondrial abundance	-0.03	-0.14*	0.15*	0.14*	0.01
Glycolytic potential	0.17**	0.13	0.12	0.08	0.15*
Electron loss glutamate	0.17*	0.15	0.12	0.04	0.20**
Electron loss succinate	0.28**	0.19*	0.18*	-0.02	0.26**

*$P < 0.10$; ** $P < 0.05$

Correlation coefficients between a* values measured on longissimus lumborum steaks on d 0, 1, 4, 7, and 11 of display to parameters measured on the longissimus lumborum steaks are reported in Table 7. Marbling score was moderately correlated ($P < 0.05$) to a* color values on d 4, 7, and 11 of simulated retail display. Measurements of pH and myoglobin concentration was moderately correlated ($P < 0.05$) to a* values measured on d 0, 1, 4, 7, and 11 of simulated retail display. Oxygen consumption was moderately correlated to a* values at the beginning simulated retail display (d 0), and

weakly correlated at the end of display (d 7 and d 11). Nitric oxide metmyoglobin reducing ability was strongly correlated to a* values on d 4 and d 7, moderately correlated ($P < 0.05$) at the beginning and end of simulated retail display, and weakly correlated ($P < 0.10$) on d 1. Initial metmyoglobin formation was moderately negatively ($P < 0.05$) correlated to a* values measure on d 1, 4, and 7 of simulated retail display. Protein solubility was moderately correlated ($P < 0.05$) to a* color space values at the beginning (d 0 and d 1) of retail display, whereas mitochondrial abundance was strongly correlated ($P < 0.05$) to a* color space values at the beginning (d 0 and d 1) of retail display. Glycolytic potential values were moderately correlated ($P < 0.05$) to a* values on d 4 and d 7 of simulated retail display. Measurements of electron loss utilizing succinate as a substrate were strongly correlated ($P < 0.05$) to a* color value measurements on d 4, 7, and 11 of retail display, whereas measurements of electron loss utilizing glutamate as a substrate were moderately correlated ($P < 0.05$) to a* color value measurements on d 4, 7, and 11 of retail display.

Table 7. Pearson correlation coefficients between redness (a*) values of longissimus lumborum steaks measured on d 0, 1, 4, 7 and 11 of simulated retail display and the parameters measured on the steaks.

	Longissimus lumborum a* values				
Parameters	Day 0	Day 1	Day 4	Day 7	Day 11
Marbling score	0.09	0.03	-0.21**	-0.21**	-0.21**
pH	-0.34**	0.00	0.25**	0.26**	0.16**
Myoglobin concentration	0.17**	-0.12	-0.28**	-0.27**	-0.24**
Oxygen consumption	-0.24**	0.08	0.11	0.14*	0.16**
Nitric oxide metmyoglobin reducing activity	-0.19**	0.14*	0.47**	0.37**	0.19**
Initial metmyoglobin formation	0.05	-0.26**	-0.28**	-0.23**	-0.10
Protein solubility	-0.25**	-0.25**	0.12	0.04	0.01
Protein oxidation	0.00	0.04	-0.03	0.00	-0.01
Mitochondrial abundance	-0.30**	-0.43**	0.00	0.00	0.04
Glycolytic potential	0.12	0.02	-0.27**	-0.21**	-0.10
Electron loss glutamate	-0.07	-0.05	-0.25**	-0.23**	-0.27**
Electron loss succinate	0.15	-0.02	-0.45**	-0.36**	-0.33**

*$P < 0.10$; **$P < 0.05$

Correlations generated for b* instrumental color values measured on d 0, 1, 4, 7, and 11 of simulated retail display and the parameters measured are reported in Table 8. Instrumental b* color values were moderately correlated ($P < 0.05$) to marbling score on d 4, 7, and 11 of simulated retail display. Measurements of pH were moderately correlated ($P < 0.05$) to b* color values on d 0, 4, and 7 of retail display. Myoglobin concentration was weakly correlated ($P < 0.10$) to b* values on d 1, but moderately correlated to b* values on d 4, 7, and 11 of retail display. Oxygen consumption was weakly correlated ($P < 0.10$) to b* color values on d 0 and d 7 of retail display. Measurements of nitric oxide metmyoglobin reducing ability were weakly correlated to b* values at the beginning (d 0) and end (d 11) of retail display, but strongly correlated ($P < 0.10$) on d 4 and d 7. Protein solubility was moderately correlated to b* color space values at the beginning (d 0 and d 1) of retail display, whereas mitochondrial abundance was strongly correlated ($P < 0.05$) to b* color space values at the beginning (d 0 and d 1)

of retail display. Glycolytic potential values were moderately correlated ($P < 0.05$) to b* color space values on d 4 and d 7 of simulated retail display. Measurements of electron loss utilizing succinate as a substrate were strongly correlated ($P < 0.05$) to a* color value measurements on d 4 and d 7, and moderately correlated ($P < 0.05$) on d 11 of retail display. Additionally, measurements of electron loss utilizing glutamate as a substrate were moderately correlated ($P < 0.05$) to a* color value measurements on d 4, 7, and 11 of retail display.

Table 8. Pearson correlation coefficients between yellowness (b*) values of longissimus lumborum steaks measured on d 0, 1, 4, 7 and 11 of simulated retail display and the parameters measured on the steaks.

	Longissimus lumborum b* values				
Parameters	Day 0	Day 1	Day 4	Day 7	Day 11
Marbling score	0.10	0.05	-0.18**	-0.25**	-0.20**
pH	-0.28**	-0.01	0.20**	0.25**	0.09
Myoglobin concentration	0.02	-0.15*	-0.28**	-0.24**	-0.19**
Oxygen consumption	-0.15*	0.08	0.08	0.15*	0.08
Nitric oxide metmyoglobin reducing activity	-0.14*	0.10	0.40**	0.38**	0.15*
Initial metmyoglobin formation	0.01	-0.24**	-0.28**	-0.24**	-0.05
Protein solubility	-0.23**	-0.26**	0.03	-0.01	-0.06
Protein oxidation	0.01	0.06	-0.04	-0.05	-0.05
Mitochondrial abundance	-0.30**	-0.44**	-0.09	-0.11	-0.05
Glycolytic potential	0.07	0.02	-0.22**	-0.21**	-0.09
Electron loss glutamate	-0.10	-0.04	-0.24**	-0.28**	-0.25**
Electron loss succinate	0.10	0.02	-0.37**	-0.38**	-0.27**

*$P < 0.10$; **$P < 0.05$

The relationships among chroma color values on longissimus lumborum steaks on d 0, 1, 4, 7, and 11 of simulated retail display and the parameters of interest measured were similar to those found for a* and b* color space values (Table 9). Correlation coefficients between chroma values and marbling score were moderately

correlated ($P < 0.05$) on d 4, 7, and 11 of display. Measurements of pH was moderately correlated ($P < 0.05$) to chroma values measured on d 0, 4, and 7 of retail display, but weakly correlated ($P < 0.10$) on d 11 retail display. Myoglobin concentration was moderately correlated ($P < 0.05$) to chroma values from d 4 to d 11 of retail display, but weakly correlated on d 1 of retail display. Oxygen consumption was moderately correlated ($P < 0.05$) to chroma values at the beginning simulated retail display (d 0), but weakly correlated ($P < 0.10$) at the end of display (d 7 and 11). Nitric oxide metmyoglobin reducing ability was strongly correlated ($P < 0.05$) to chroma values on d 4 and 7, respectively, and moderately correlated ($P < 0.05$) at the beginning (d 0) and end (d 11) of simulated retail display, but only weakly correlated on d 1 of display. Initial metmyoglobin formation was moderately negatively correlated ($P < 0.05$) to chroma values from d 1 through d 7 of simulated retail display. Protein solubility was moderately correlated ($P < 0.05$) to chroma color values at the beginning (d 0 and d 1) of retail display, whereas mitochondrial abundance was strongly correlated ($P < 0.05$) to chroma color values at the beginning (d 0 and d 1) of retail display. Glycolytic potential values were moderately correlated ($P < 0.05$) to chroma values on d 4 and d 7 of simulated retail display. Measurements of electron loss utilizing succinate as a substrate were strongly correlated ($P < 0.05$) to chroma color value measurements on d 4, 7, and 11 of retail display, whereas measurements of electron loss utilizing glutamate as a substrate were moderately correlated ($P < 0.05$) to chroma color value measurements on d 4, 7, and 11 of retail display.

Table 9. Pearson correlation coefficients between chroma values of longissimus lumborum steaks measured on d 0, 1, 4, 7 and 11 of retail display and the parameters measured on the steaks.

	Longissimus lumborum chroma values				
Parameters	Day 0	Day 1	Day 4	Day 7	Day 11
Marbling score	0.10	0.04	-0.20**	-0.23**	-0.23**
pH	-0.31*	0.00	0.24*	0.27*	0.14*
Myoglobin concentration	0.10	-0.13*	-0.28**	-0.26**	-0.23**
Oxygen consumption	-0.20**	0.08	0.10	0.15*	0.14*
Nitric oxide metmyoglobin reducing activity	-0.17**	0.12	0.45**	0.38**	0.18**
Initial metmyoglobin formation	0.03	-0.25**	-0.29**	-0.24**	-0.09
Protein solubility	-0.24**	-0.26**	0.09	0.02	-0.01
Protein oxidation	0.00	0.05	-0.04	-0.02	-0.03
Mitochondrial abundance	-0.30**	-0.44**	-0.04	-0.04	0.01
Glycolytic potential	0.10	0.02	-0.26**	-0.21**	-0.10
Electron loss glutamate	-0.08	-0.05	-0.25**	-0.25**	-0.27**
Electron loss succinate	0.13	-0.00	-0.43**	-0.38**	-0.32**

*$P < 0.10$; **$P < 0.05$

The correlation for hue angle measurements taken on steaks at d 0, 1, 4, 7, and 11 of simulated retail display and parameters measured on the longissimus lumborum steaks are reported in Table 10. Marbling score was moderately correlated ($P < 0.05$) to hue angle measurements at the end of retail display (d 11). Correlation coefficients between pH and hue angle measurements were weakly negatively ($P < 0.05$) correlated on d 0, 7, and 11 of retail display. Myoglobin concentration was moderately ($P < 0.05$) correlated to hue angle measurements on d 0, 1, 7, and 11 of retail display. Measurements of oxygen consumption and hue angle values were weakly correlated only at the end (d 11) of display. Nitric oxide metmyoglobin reducing ability was moderately correlated ($P < 0.05$) to hue angle values at the end (d 7 and d 11) of retail display. Measurements of initial metmyoglobin formation were weakly negatively correlated to hue angle values on d 1 of retail display. Protein solubility was weakly correlated to hue angle color values initially (d 0) of retail display, but moderately correlated to hue angle values on d

1 and d 4 of retail display. Mitochondrial abundance was moderately negatively correlated ($P < 0.05$) to hue angle measurements on d 0, 4, and 7 of retail display, but strongly negatively correlated ($P < 0.05$) on d 1 of retail display. Glycolytic potential measurements were weakly correlated ($P < 0.10$) to hue angle values on d 4 and d 7 of retail display. Measurements of electron loss utilizing both glutamate and succinate were moderately correlated ($P < 0.05$) to hue angle values only at the end (d 11) of retail display.

Table 10. Pearson correlation coefficients between hue angle values of longissimus lumborum steaks measured on d 0, 1, 4, 7 and 11 of simulated retail display and the parameters measured on the steaks.

	Longissimus lumborum hue angle values				
Parameters	Day 0	Day 1	Day 4	Day 7	Day 11
Marbling score	0.11	0.11	0.06	0.03	0.18**
pH	-0.14*	-0.01	-0.12	-0.17**	-0.16**
Myoglobin concentration	-0.24**	-0.20**	0.00	0.22**	0.23**
Oxygen consumption	0.04	0.10	-0.04	-0.02	-0.16**
Nitric oxide metmyoglobin reducing activity	-0.03	0.01	-0.15	-0.19**	-0.18**
Initial metmyoglobin formation	-0.07	-0.17**	0.00	0.10	0.12
Protein solubility	-0.16**	-0.27**	-0.25**	-0.11	-0.07
Protein oxidation	0.04	0.08	-0.01	-0.11	-0.03
Mitochondrial abundance	-0.25**	-0.43**	-0.28**	-0.18**	-0.07
Glycolytic potential	0.00	0.04	0.15*	0.14*	0.09
Electron loss Glutamate	-0.12	0.00	0.04	0.02	0.25**
Electron loss Succinate	0.03	0.11	0.19*	0.12	0.30**

*$P < 0.10$; **$P < 0.05$

The relationships among overall color change (Δ E) values on longissimus lumborum steaks taken on d 0, 1, 4, 7, and 11 of simulated retail display and the parameters of interest measured are reported in Table 11. Marbling score was weakly associated ($P < 0.10$) to overall color change on d 4 of retail display, whereas on d 7 and d 11 of retail display marbling score and Δ E were moderately associated ($P < 0.05$).

Measurements of pH were moderately negatively correlated ($P < 0.05$) to Δ E on d 4, 7, and 11 of retail display. Oxygen consumption was moderately associated ($P < 0.05$) to Δ E initially (d 1) as well as d 7 and d 11 of retail display. Measurements of nitric oxide metmyoglobin reducing ability were weakly correlated ($P < 0.10$) with overall color change initially (d 1) in color display, and during the middle of display (d 4 and d 7) nitric oxide metmyoglobin reducing ability and overall color change were strongly correlated ($P < 0.05$), but by the end of retail display (d 11) nitric oxide metmyoglobin reducing ability and overall color change were only moderately correlated ($P < 0.05$). Initial metmyoglobin formation was moderately associated ($P < 0.05$) to Δ E from d 1 through d 7 of retail display. Protein solubility was moderately associated ($P < 0.05$) to overall color change on d 1 and d 4 of color display, whereas mitochondrial abundance was strongly associated ($P < 0.05$) with overall color change initially (d 0), but on d 4 mitochondrial abundance and Δ E were only moderately associated ($P < 0.05$). Glycolytic potential measurements were moderately correlated ($P < 0.05$) to Δ E values on d 4 and d 7 of retail display. Measurements of electron loss utilizing succinate as a substrate were strongly correlated ($P < 0.05$) to Δ E values on d 4, 7, and 11 of retail display, whereas measurements of electron loss utilizing glutamate as a substrate were only moderately correlated ($P < 0.05$) to Δ E value measurements on d 7 and d 11 of retail display.

Table 11. Pearson correlation coefficients between overall color change (Δ E) of longissimus lumborum steaks measured on d 1, 4, 7 and 11 of simulated retail display and the parameters measured on the steaks.

	Longissimus lumborum overall color change (Δ E)			
Parameters	Day 1	Day 4	Day 7	Day 11
Marbling score	-0.06	0.15*	0.21**	0.23**
pH	-0.09	-0.20**	-0.20**	-0.24**
Myoglobin concentration	0.19*	0.24**	0.28**	0.27**
Oxygen consumption	-0.17**	-0.12	-0.18**	-0.22**
Nitric oxide metmyoglobin reducing activity	-0.15*	-0.45**	-0.42**	-0.24**
Initial metmyoglobin formation	0.28**	0.23**	0.22**	0.11
Protein solubility	0.18**	-0.25**	-0.12	-0.07
Protein oxidation	-0.05	0.04	0.01	0.01
Mitochondrial abundance	0.40**	-0.16**	-0.10	-0.11
Glycolytic potential	0.00	0.18**	0.20**	0.13
Electron loss glutamate	0.02	0.14	0.19*	0.23**
Electron loss succinate	0.00	0.41**	0.40**	0.35**

*$P < 0.10$; **$P < 0.05$

Pearson correlation coefficients between the parameters measured are presented in table 12. Oxygen consumption was strongly correlated ($P < 0.05$) to nitric oxide metmyoglobin reducing ability, initial metmyoglobin formation, and pH, moderately correlated ($P < 0.05$) to mitochondrial abundance, and weakly correlated ($P < 0.10$) to myoglobin concentration and glycolytic potential. Measurements of nitric oxide metmyoglobin reducing ability were strongly correlated ($P < 0.05$) to initial

metmyoglobin formation and pH, moderately associated to glycolytic potential, and weakly associated ($P < 0.05$) to protein solubility and myoglobin concentration. Initial metmyoglobin formation was moderately associated ($P < 0.05$) to pH, but weakly associated ($P < 0.10$) to mitochondrial abundance. Measurements of pH were strongly correlated ($P < 0.05$) to glycolytic potential, and only weakly correlated ($P < 0.05$) to protein oxidation. Protein oxidation was weakly negatively correlated ($P < 0.10$) to myoglobin concentration and moderately positively correlated ($P < 0.05$) to mitochondrial abundance. Measurements of electron loss utilizing glutamate were only moderately positively correlated ($P < 0.05$) to protein oxidation. Measurements of electron loss utilizing succinate were moderately positively correlated ($P < 0.05$) to initial metmyoglobin formation and glycolytic potential, negatively moderately negatively correlated ($P < 0.05$) to nitric oxide metmyoglobin reducing ability, and strongly positively correlated ($P < 0.05$) to protein oxidation.

Table 12. Pearson correlation coefficients between parameters measured in the laboratory.

Variable	Oxygen consumption	Nitric oxide metmyoglobin reducing activity	Initial metmyoglobin formation	pH	Protein solubility	Protein oxidation	Myoglobin concentration	Mitochondrial abundance	Glycolytic potential	Electron loss glutamate
Oxygen consumption										
Nitric oxide metmyoglobin reducing ability	0.40**									
Initial metmyoglobin formation	-0.42**	-0.47**								
pH	0.44**	0.36**	-0.29**							
Protein solubility	0.09	0.16**	-0.09	0.02						
Protein oxidation	-0.04	-0.01	0.11	-0.16**	0.06					
Myoglobin concentration	-0.14*	-0.17**	0.02	-0.09	0.02	-0.16**				
Mitochondrial abundance	-0.18**	-0.05	0.14*	0.00	0.18**	0.18**	0.00			
Glycolytic potential	-0.14*	-0.26**	0.11	-0.46**	-0.02	-0.01	0.11	-0.17**		
Electron loss glutamate	-0.04	-0.10	0.13	0.01	-0.02	0.21**	0.02	0.09	0.10	
Electron loss succinate	-0.08	-0.23**	0.27**	-0.12	0.10	0.32**	0.03	-0.06	0.25**	0.73**

*$P < 0.10$; **$P < 0.05$

4.3. Discussion

Previous research has shown that there is substantial variation across animals in regard to color stability (King et al., 2010). There are several different biochemical factors in which contribute to variation in beef lean color stability. This study is an attempt trying to further understand what contributes to variation in lean color stability by focusing on biochemical factors with particular interest on whether color stability is more dependent on production of NADH to supply the NADH pool or whether increased lactate concentrations enable greater myoglobin stability.

Instrumental L* values were lowly to moderately correlated to pH, nitric oxide metmyoglobin reducing ability, initial metmyoglobin formation and protein oxidation from d 0 to d 11 of retail display. In regards to pH, as pH decreases L* values increase, which is in agreement with Page et al. (2001). Protein oxidation, which is an indicator of oxidative damage to the sarcoplasmic protein fraction was positively correlated with L* values. Moreover, measurements of electron loss utilizing succinate and were weakly to moderately positively correlated with L* values at the beginning and end of simulated retail display. Ramanathan et al. (2009, 2010) and Tang et al. (2005) reported that when pyruvate and succinate are used as substrates for mitochondrial oxygen consumption, myoglobin oxygenation consequently decreases, resulting in darker lean muscle color.

Correlation coefficients from d 0 and d 11 for the parameters of interest measured do not indicate much change. Typically, there is not much change in L* values during simulated retail display in beef. Therefore, L* values are an indicator of

overall protein functionality. Initially, (d 0) protein oxidation was occurring and persisted through d 11 of simulated retail display, but the correlation coefficients remained relatively the same. This could have potentially contributed to an increase of electrons lost during the beginning and at the end of simulated retail display.

Measurements of pH was moderately positively correlated from d 4 to 11 of retail display with a* and b* measurements, which is in agreement with Page et al. (2001). Moreover, Page et al. (2001) reported that a* and b* were more strongly correlated with muscle pH than L* values, and the present data disagrees with this because L*, a*, and b* values are moderately correlated to the three pH measurements taken. Hood et al. (1980) and McKenna et al. (2005) reported that color changes are not greatly affected by pH, but rather changes in OC in muscle; whereas, Joo et al. (1999) reported that pH impacts color changes, which also affects mitochondrial activity and protein denaturation.

Initially (d 0 and d 1) in simulated retail display, protein solubility and mitochondrial abundance were moderately to strongly negatively correlated to a* and b* color measurements. At the beginning of display, there were less mitochondria and less sarcoplasmic protein present, thus appearing less red in color. This increased lightness initially (d 0 and d 1) in retail display, could be due to a denaturation during pH and temperature decline in postmortem muscle. The ultimate pH of normal beef is in the range of 5.4-5.8, and low pH values favor the oxidation of myoglobin. Ledward et al. (1986) reported that beef having an ultimate pH 5.8 or greater, was more color-stabile than similar meat with an ultimate pH of 5.6. Furthermore, Livingston and Brown

(1981) reported that a low pH environment accelerates the protonation of bound oxygen and favors the release of a superoxide anion. In regards to temperature, myoglobin oxidation is greatly accelerated with increased temperature. More specifically, increased temperature accelerates pigment oxidation by increasing the rate of any pro-oxidant reactions within the muscle tissue (Faustman and Cassens, 1990).

Nitric oxide metmyoglobin reducing ability was weakly to strongly positively correlated to a* and b* color measurements from d 4 to d 11 of simulated retail display. Moreover, measurements of electron loss utilizing succinate and were strongly to moderately negatively correlated to a* and b* measurements from d 4 to d 11 of simulated retail display. In terms of redness, electron loss was mitigated from d 4 to d 7 of retail display. This increased redness later in simulated retail display could be due to greater supplies and/or replenishment of the NADH pool. Echevarne et al. (1990) reported that the NADH pool and metmyoglobin reductase activity was the limiting factor dictating color-life, which is in agreement with the present study. King et al. (2010) proposed that genetic influences in color stability can be mediated through metabolic differences due to increased availability or regeneration of NADH.

In regards to overall color stability (Δ E), biochemical NORA was weakly to strongly negatively correlated to Δ E throughout simulated retail display. Moreover, measurements of electron loss utilizing succinate and were strongly positively correlated to overall color change from d 4 to d 11 of retail display, and measurements of electron loss utilizing glutamate were moderately positively correlated to overall color change from d 7 to d 11 of retail display. With the increase of electron loss towards the end of

retail display there could be due to the increase in oxidative stress within the muscle system. This data is in disagreement with Echevarne et al. (1990) who reported that muscles with increased oxidative metabolism would have greater metmyoglobin reducing activity. Moreover, the muscle may not be able to maintain color stability towards the end of retail display due to the depleted amount of reducing capacity in the muscle.

King et al. (2010) mentioned that muscles possessing greater oxidative metabolism would have a greater amount of mitochondria and mitochondrial enzymes that compete with myoglobin for available oxygen. This agrees with data from the current study because from d 7 to d 11 of retail display oxygen consumption is moderately negatively correlated to overall color because as overall color change increased, oxygen consumption decreased from d 7 to 11 of retail display. King et al. (2010) thought that a greater proportion of myoglobin was in the deoxygenated form, which consequently would be more susceptible to oxidation.

Oxygen consumption and nitric oxide metmyoglobin reducing ability were both correlated to most of the biochemical traits of interest measured on d 0 of retail display. More specifically, oxygen consumption was lowly correlated to glycolytic potential ($r = -0.14$) and myoglobin concentration ($r = -0.14$), moderately correlated to mitochondrial abundance ($r= -0.18$), and strongly correlated to IMF ($r = -0.42$), nitric oxide metmyoglobin reducing ability ($r = 0.40$), and pH ($r = 0.44$). Similarly nitric oxide metmyoglobin reducing ability was weakly correlated to myoglobin concentration ($r = -0.17$), protein solubility ($r = 0.16$), moderately correlated to electron loss succinate ($r = -$

0.23), glycolytic potential (r = -0.26), and strongly correlated to initial metmyoglobin formation (r = -0.47) and pH (r = 0.36).

With many of these biochemical traits strongly associated with oxygen consumption and nitric oxide metmyoglobin reducing ability, are these biochemical traits working partially through oxygen consumption and nitric oxide metmyoglobin reducing ability, or independently? Studies conducted by King et al. (2011a) and Mancini et al. (2008) investigated the relationships between NORA, IMF, and PRM to color change during simulated display. Mancini et al. (2008) reported that IMF was the most strongly related to color stability, and further reported that NORA values were not significantly correlated to color stability in any of the muscles evaluated, which was in disagreement with current study. (King et al., 2011a) reported that NORA and PRM values were more strongly correlated to color stability than IMF values, which was in agreement with data from the current study. Additionally, McKenna et al. (2005) reported that color stability was not determined by NORA or OCR, but rather determined by the proportion of these two components, which was supported by the current study. Therefore, perhaps these other biochemical traits could be contributing through OC and NORA.

It has been reported that biochemical metabolic factors associated with muscle tissue give rise to the formation of reactive oxygen species, thus resulting in carbonyl formation and decreased sulfhydryl content of the proteins (Hoffman and Hamm, 1978; Martinaud et al., 1997; Xiong, 2000). For this experiment, protein oxidation was moderately positively correlated to glutamate and highly positively correlated succinate

(r = 0.32); thus, as protein oxidation increased, succinate and glutamate increased. This further suggested that as muscle protein oxidation increased that mitochondrial electron loss increased, which could then negatively impact beef lean color stability.

CHAPTER V

CONCLUSIONS

The meats industry has heavily focused on increasing the shelf-life of case-ready meat products by trying to reduce the amount of product that gets devalued as a result of insufficient color stability or color life. Several different biochemical factors and/or traits greatly influence beef lean color stability. The two different experiments conducted, investigated biochemical factors that influence lean color and lean color stability in beef.

The first experiment compared biochemical factors that impact beef lean and beef lean color stability in dark cutting and normal beef. This experiment suggested that dark cutting carcasses favored oxidative metabolism. Severe dark cutting carcasses had greater protein oxidation than the cohorts. Also, dark cutting carcasses had a greater percentage of myoglobin in the deoxymyoglobin state. Dark cutting carcasses could potentially be favoring oxidative metabolism due to decreased mitochondrial efficiency. Redness was maintained throughout simulated retail display for dark cutting carcasses. This could potentially be due to a greater percentage of deoxymyoglobin concentration, and greater mitochondrial abundance in dark cutting carcasses.

In regards to color stability throughout simulated retail display, dark cutting carcasses were darker than the cohorts. This was attributed to the dark cutting carcasses having higher pH values, greater protein solubility, decreased glycolytic potential, and a higher percentage of myoglobin in the deoxymyoglobin state than the cohort carcasses. In regards to overall color change, dark cutting carcasses had decreased overall color

change from the middle through the end of simulated retail display than the cohort carcasses. This was a result of dark cutting carcasses having a greater percentage of deoxymyoglobin concentration, lower percentage of initial metmyoglobin formation, and increased mitochondrial abundance.

The second experiment assess the effects of electron loss in the electron transport chain on the different biochemical factors that impact beef lean color stability. This experiment suggested that both oxidative and glycolytic metabolism impact and contribute to beef lean color stability. Furthermore, these data suggested that biochemical traits measured contribute to initial beef color as well as overall color stability. Oxidative stress resulting in increased protein oxidation and electron loss was associated with increased lightness at the beginning of simulated retail display. This could potentially be due to oxidative damage within the sarcoplasmic fraction of the muscle proteins. Moreover, oxidative stress was associated with decreased redness and increased color change at the end of display, which could result from the depletion of reducing capacity of the muscle. Increased glycolytic metabolism (pH decreased, glycolytic potential and protein solubility increased) was associated with increased lightness at the beginning of simulated retail display. This could potentially be due to increased protein denaturation during pH and temperature decline in postmortem muscle. Additionally, increased glycolytic metabolism was associated with increased redness and decreased color change late in simulated retail display. This possibly suggests that there were greater supplies or replenishment, or both in combination of the NADH pool towards the end of simulated retail display.

REFERENCES

Aberle, E. D., J. C. Forrest, D. E. Gerrard, and E. W. Mills. 2001. Principles of meat science 4th edition. Kendall/Hunt Publishing Co., Dubuque, IA.

AMSA. 2012. AMSA Meat Color Measurement Guidelines. In: M. C. Hunt and D. A. King (eds.) American Meat Science Association. p 1-80, Champaign, IL.

Apple, J. K., J. T. Sawyer, J.-F. Mueullenet, J. W. S. Yancey, and W. M. D. 2011. Lactic acid enhancement can improve the fresh and cooked color of dark-cutting beef. J. Anim. Sci. 89: 4207-4220.

Arikara, K., R. G. Cassens, M. L. Greaser, J. B. Luchansky, and P. E. Mozdiak. 1995. Localization of metmyoglobin-reducing enzyme (NADH-cytochrome b5 reductase) system components in bovine skeletal muscle. Meat Sci. 39: 205-213.

Arikara, K., M. Itoh, and Y. Kondo. 1997. Quantification of NADH-cytochrome b5 reductase (Metmyoglobin-reducing enzyme) in bovine skeletal muscle by an immunoblotting assay. Anim. Sci. Technol. 68: 29-33.

Ashmore, C. R., F. Carroll, L. Doerr, G. Tompkins, H. Stokes et al. 1973. Experimental prevention of dark-cutting meat. J. Anim. Sci. 36: 33-36.

Ashmore, C. R., L. Doerr, G. Foster, and F. Carrol. 1971. Respiration of mitochondria isolated from dark-cutting beef. J. Anim. Sci. 33: 574-577.

Ashmore, C. R., L. Parker, and L. Doerr. 1972. Respiration of mitochondria isolated from dark cutting beef: Postmortem changes. J. Anim. Sci. 34: 46-48.

Bekhit, A. E. D., and C. Faustman. 2005. Metmyoglobin reducing acitivity. Meat Sci. 71: 407-439.

Bekhit, A. E. D., G. H. Geesink, M. A. Ilian, J. D. Morton, R. Sedcole et al. 2003. Particulate metmyoglobin reducing activity and its relationship with meat color. J. Agri. Food Chem. 51: 6026-6035.

Bendall, J. R. 1972. Consumption of oxygen by muscles of beef animals and related species, and its effect on color of meat. I. Oxygen-consumption in pre-rigor muscle. J. Food Sci. Agric. 23: 61-72.

Bendall, J. R. 1973. Postmortem changes in muscle. In: G. H. Bourne (ed.) Structure and function of muscle. p 243-309. Academic Press, New York, NY.

Bendall, J. R., and A. A. Taylor. 1972. Consumption of oxygen by the muscles of beef animals and related species, and its effect on the color of meat. II. Consumption of oxygen by post-rigor muscle. J. Food Sci. Agric. 23: 707-719.

Bodwell, C. F., A. M. Pearson, and R. A. Fennel. 1965. Post-mortem changes in muscle. III. Histochemical observations in beef and pork. J. Food Sci. 30: 944-954.

Bottje, W., M. Iqbal, Z. X. Tang, D. Cawthon, R. Okimoto et al. 2002. Association of mitochondrial function with feed efficiency within a single genetic line of broiler. Poultry Sci. 81: 546-555.

Bottje, W., N. R. Pumford, C. Ojano-Dirain, M. Iqbal, and K. Lassiter. 2006. Feed efficiency and mitochondrial function. Poultry Sci. 85: 8-14.

Bottje, W. J., and G. E. Carstens. 2009. Association of mitochondrial function and feed efficiency in poultry and livestock species. J. Anim. Sci. 87: E48-E63.

Brewer, M. S. 1998. Consumer attitudes towards color and marbling of fresh pork. National Pork Board, Des Moines, IA.

Brewer, M. S. 2008. Preserving beef quality with natural antioxidants. Fact sheet prepared by the National Cattlemen's Beef Association. http://www.beefresearch.org/factsheets1.aspx. Accessed 3 March 2013.

Brooks, A. B., T. D. Fahey, and T. P. White. 1996. Exercise physiology: Human bioenergetics and its applications. Mayfield Publishing Co., Mountain View, CA.

Cawthon, D., R. McNew, K. Beers, and W. Bottje. 1999. Evidence of mitochondrial dysfunction in broilers with pulmonary hypertension syndrome (Ascites): Effect of t-butyl hydroperoxide on hepatic mitochondrial function, glutathione, and related thiols. Poultry Sci. 78: 114-124.

Cornforth, D. P., and P. Jayasingh. 2004. Colour and pigment. In: C. D. W. K. Jensen, and M. E. Dikeman (ed.) Encyclopedia of meat sciences. Elsevier LTD, Oxford, UK.

Dean, R. W., and C. O. Ball. 1960. Analysis of myoglobin fractions on the surfaces of beef cuts. Food Technol. 14: 271-285.

Echevarne, C., M. Renerre, and R. Labus. 1990. Analysis of myoglobin fractions on the surfaces of beef cuts. Meat Sci. 27: 161-172.

Egbert, W. E., and D. P. Cornforth. 1986. Factors influencing color of dark cutting beef muscle. J. Food Sci. 51: 57-65.

Faustman, C. 1991. Meat color stability. J. Food Sci. 56: R4-R4.

Faustman, C., and R. G. Cassens. 1990. The biochemical basis for discoloration in fresh meat: A review. J. Muscle Foods 1: 217-243.

Faustman, C. C., R. G. Cassens, D. M. Schaefer, D. R. Buege, S. N. Williams et al. 1989a. Improvement of pigment and lipid stability in Holstein steer beef by dietary supplementation with vitamin E. J. Food Sci. 54: 858-862.

Faustman, C., R. G. Cassens, D. M. Schaefer, D. R. Buege, and K. K. Scheller. 1989b. Vitamin E supplementation of Holstein steer diets improves sirloin steak color. J. Food Sci. 54: 485-486.

Fennema, O. R. 1996. Food chemistry 3rd edition. Marcel Dekker, Inc., New York, NY.

Giddings, G. G. 1974. Reduction of ferrimyoglobin in meat. CRC Cri. Rev. Food Technol. 5: 143-173.

Giddings, G. G. 1977. The basis of color in muscle foods. CRC Cri. Rev. Food Sci. Nutr. 9: 81-114.

Grandin, T. 1992. Problems with bruises and dark cutters in harvest steers/heifers. In: Improving the consistency and competitiveness of beef-A blueprint for total quality management in the fed-beef industry. The final report for the National Beef Quality Audit-1991, Colorado State University, Fort Collins; Texas A&M University, College Station.

Hanson, D. J., K. Kirchofer, and C. R. Calkins. 2001. The role of muscle glycogen in dark cutting beef. http://digitalcommons.unl.edu/animalscinbcr/298. Accessed February 10, 2013.

Hoffman, K., and R. Hamm. 1978. Sulfhydryl and disulfide groups in meats. Adv. Food Res. 24: 1-111.

Honikel, K. O., and C. J. Kim. 1986. Causes of the development of PSE pork. Fleischwirtsch 66: 349-353.

Hood, D. E. 1980. Factors affecting the rate of metmyoglobin accumulation in pre-packaged beef. Meat Sci. 4: 247-265.

Hunt, M. C., J. C. Acton, R. C. Benedict, C. R. Calkins, D. P. Cornforth et al. 1991. Guidelines for meat color evaluation. In: 44th Annual Reciprocal Meat Conference, Manhattan, Kansas. p 1-17.

Hunt, M. C., and H. B. Hedrick. 1977. Chemical, physical, and sensory characteristics of bovine muscle from four different quality groups. J. Food Sci. 42: 716-720.

Hunt, M. C., O. Sorheim, and E. Slinde. 1999. Color and heat denaturation of myoglobin forms in ground beef. J. Food Sci. 64: 847-851.

Iqbal, M., N. R. Pumford, Z. X. Tang, K. Lassiter, C. Ojano-Dirain et al. 2005. Compromised liver mitochondrial function and complex activity in low feed efficient broilers are associated with higher oxidative stress and differential protein expression. Poultry Sci. 84: 933-941.

Iwata, H., H. Goto, H. Tanaka, Y. Sakaguchi, K. Kimura et al. 2011. Effect of maternal age on mitochondrial DNA copy number, ATP content and IVF outcome of bovine oocytes. Reprod. Fertil. Dev. 23: 424-432.

Joo, S. T., R. G. Kauffman, B. C. Kim, and G. B. Park. 1999. The relationship of sarcoplasmic and myofibrillar protein solubility to colour and water-holding capacity in porcine longissimus muscle. Meat Sci. 52: 291-297.

Keller, R. J., N. C. Halmes, J. A. Hinson, and N. R. Pumford. 1993. Immunochemical detection of oxidized proteins. Chem. Res. Toxicol. 6: 430-433.

Kim, Y. H., J. T. Keeton, S. B. Smith, L. R. Berghman, and J. W. Savell. 2009. Role of lactate dehydrogenase in metmyoglobin reduction and color stability of different bovine muscles. Meat Sci. 83: 376-382.

King, D. A., S. D. Shackelford, L. A. Kuehn, C. M. Kemp, A. B. Rodriguez et al. 2010. Contribution of genetic influences to animal-to-animal variation in myoglobin content and beef lean color stability. J. Anim. Sci. 88: 1160-1167.

King, D. A., S. D. Shackelford, A. B. Rodriguez, and T. L. Wheeler. 2011a. Effect of time of measurement on the relationship between metmyoglobin reducing activity and oxygen consumption to instrumental measures of beef longissimus color stability Meat Sci. 87: 26-32.

King, D. A., S. D. Shackelford, and T. L. Wheeler. 2011b. Relative contributions of animal and muscle effects to variation in beef lean color stability. J. Anim. Sci. 89: 1434-1451.

Kolath, W. H., M. S. Kerley, J. W. Golden, S. A. Shahid, and G. S. Johnson. 2006. The relationships among mitochondrial function and residual feed intake in steers. J. Anim. Sci. 84: 861-865.

Kreikemeier, K. K., J. A. Unruh, and T. P. Eck. 1998. Factors affecring the occurence of dark-cutting beef and selected carcass traits in finished beef cattle. J. Anim. Sci. 76: 388-395.

Lanari, M. C., and R. G. Cassens. 1991. Mitochondrial activity and beef color stability. J. Food Sci. 56: 1476-1479.

Lanari, M. C., and N. E. Zaritzky. 1988. Potassium sorbate effect on pigment concentration of refrigerated beef. J. Food Sci. 53: 1621-1627.

Lawrie, R. A. 1958. Physiological stress in relation to dark cutting beef. J. Sci. Food Agric. 11: 721-727.

Lawrie, R. A. 1998. Lawrie's meat science. Woodhead, Cambridge, UK.

Ledward, D. 1970. Metmyoglobin formation in beef stored in carbon dioxide enriched and oxygen depleted atmospheres. J. Food Sci. 35: 33-37.

Ledward, D., R. Dickinson, V. Powell, and W. Shorthose. 1986. The colour and colour stability of beef longissimus dorsi and semimembranosus muscles after effective electrical stimulation. Meat Sci. 16: 244-265.

Ledward, D. A. 1985. Post-slaughter influence on the formation of metmyoglobin in beef muscles. Meat Sci. 15: 149-171.

Lee, Y. B., and Y. I. Choi. 1999. PSE (pale, soft, exudative) pork, the causes and solutions - Review. Asian-Australasian J. Anim. Sci. 12: 244-252.

Lehninger, A. L., D. L. Nelson, and M. M. Cox. 1993. Principles of biochemistry 2nd edition. Worth Publishers, New York, NY.

Lister, D. 1988. Muscle Metabolism and amimal physiology in the dark cutting condition. In: Dark-cutting in cattle and sheep of an Australian workshop., Sydney South, NSW, Australia.

Livingston, D., and W. Brown. 1981. The chemistry of myoglobin and its reactions. Food Technol. 35: 244-252.

Lutz, T. R., and T. S. Stahly. 2003. Quantitative relationship between mitochondrial bioenergetics and efficiency of animal growth. J. Anim. Sci. 81 (Suppl. 1): 141 (Abstr.).

Mancini, R. A., and M. C. Hunt. 2005. Review: Current research in meat color. Meat Sci. 71: 120-121.

Mancini, R. A., M. C. Hunt, Y. B. Kim, and T. E. Lawrence. 2004. How does lactacte enhancement stabilize beef color? In: Proceedings 50th International Congress of Meat Science and Technology, 8-13 August 2004, Helsinki Finland.

Mancini, R. A., M. Seyfert, and M. C. Hunt. 2008. Effects of data expression, sample location, and oxygen partial pressure on initial nitric oxide metmyoglobin formation and metmyoglobin-reducing-activity measurement in beef muscle. Meat Sci. 79: 244-251.

Maribo, H., S. Stoier, and P. F. Jorgensen. 1999. Procedure for determination of glycolytic potential in porcine *M. Longissimus dorsi*. Meat Sci. 51: 191-193.

Marson, E. P., J. B. S. Ferraz, F. V. Meirelles, J. C. C. Baliero, and J. P. Eler. 2008. Effects of polymorphisms of LHR and FSHR genes on sexual precocity in *Bos taurus* x *Bos indicus* beef composite beef population. Genet. Mol. Res. 7: 243-251.

Martinaud, A., Y. Mercier, P. Marinova, C. Tassy, P. Gatellier et al. 1997. Comparison of oxidative processes on myofibrillar proteins from beef during maturation and by different model oxidation systems. J. Agric. Food Chem. 45: 2481-2487.

McKeith, F. K., M. Ellis, K. D. Miller, and D. S. Sutton. 1998. The effect of RN genotype on pork quality. In: 51st Annual Reciprocal Meat Conference, Storrs, Connecticut. p 119-125.

McKenna, D. R. 2003. Biochemical, physical, and color characteristics of selected bovine muscle. Ph.D. Dissertation, Texas A&M University, College Station.

McKenna, D. R. et al. 2005. Biochemical and physical factors affecting discoloration characteristics of 19 bovine muscles. Meat Sci. 70: 665-682.

Miller, K. D., M. Ellis, D. S. Sutton, F. K. McKeith, and E. R. Wilson. 2000. Effects of live animal sampling procedures and sample storage on the glycolytic potential of porcine longissimus muscle samples. J. Muscle Foods 11: 61-67.

Mohan, A., M. C. Hunt, T. J. Barstow, T. A. Houser, and S. Muthukrishnan. 2010. Effects of malate, lactate, and pyruvate on myoglobin redox stability on homogenates of three bovine muscles. Meat Sci. 86: 304-310.

Monin, G., and P. Sellier. 1985. Pork of low technological quality with a normal rate of muscle pH in the immediate post-mortem period: The case of the Hampshire breed. Meat Sci. 13: 49-63.

Moore, M. C., G. D. Gray, D. S. Hale, C. R. Kerth, D. B. Griffin et al. 2012. National Beef Quality Audit–2011: In-plant survey of targeted carcass characteristics related to quality, quantity, value, and marketing of fed steers and heifers. J. Anim. Sci. 90: 5143-5191.

Morley, M. J. 1971. Measurement of oxygen penetraion into meat using an oxygen micro-electrode. Food Technol. 6: 371-381.

Morrissey, P. A., P. J. A. Sheehy, K. Galvin, and J. P. Kerry. 1998. Lipid stability in meat and meat products. Meat Sci. 49: 73-86.

NAMP. 2005. The meat buyers guide. North American Meat Processors Association, Reston, VA.

O'Keeffe, M., and D. E. Hood. 1982. Biochemical factors influencing metmyoglobin formation on beef from muscles differing colour stability. Meat Sci. 7: 209-228.

Offer, G., P. Knight, R. Jeacocke, R. Almond, T. Cousins et al. 1989. The structural basis of the water-holding, appearance and toughness of meat and meat products. Food Microstruct. 8: 151-170.

Page, J. K., D. M. Wulf, and T. R. Schwotzer. 2001. A survey of beef muscle color and pH. J. Anim. Sci. 79: 678-687.

Phillips, A. L., C. Faustman, M. P. Lynch, K. E. Govoni, T. A. Hoagland et al. 2001. Effect of dietary α-tocopherol supplementation on color and lipid stability in pork. Meat Sci. 58: 389-393.

Ramanathan, R., and R. A. Mancini. 2010. Effects of pyruvate on bovine heart mitochondria-mediated metmyoglobin reduction. Meat Sci. 86: 738-741.

Ramanathan, R., R. A. Mancini, and G. A. Dady. 2011. Effects of pyruvate, succinate, and lactate on beef *longissimus* raw color. Meat Sci. 88: 424-428.

Ramanathan, R., R. A. Mancini, and M. K. Konda. 2009. Effects of lactate on beef heart mitochondrial oxygen consumption and muscle darkening. J. Agric. Food Chem. 57: 1550-1555.

Ramanathan, R., R. A. Mancini, and M. K. Konda. 2010. Effect of lactate enhancement on myoglobin oxygenation of beef *longissimus* steaks overwrapped in PVC and stored at 4C. J. Muscle Foods 21: 669-684.

Renerre, M., and R. Labas. 1987. Biochemical factors influencing metmyoglobin formation in beef muscles. Meat Sci. 19: 151-165.

Reznick, A. Z., and L. Packer. 1994. Oxidative damage to proteins: Spectrophotometric method for carbonyl assay. Method Enzymol. 233: 357-363.

Sammel, L. M., M. C. Hunt, D. H. Kropf, K. A. Hachmeister, and D. E. Johnson. 2002. Comparison of assays for metmyoglobin reducing ability in beef inside and outside semimembranosus muscle. J. Food Sci. 67: 978-984.

Shackelford, S. D., M. Koohmaraie, T. L. Wheeler, L. V. Cundiff, and M. E. Dikeman. 1994. Effect of biological type of cattle on the incidence of the dark, firm, and dry condition in the longissimus muscle. J. Anim. Sci. 72: 337-343.

Shackelford, S. D., T. L. Wheeler, and M. Koohmaraie. 2003. On-line prediction of yield grade, longissimus muscle area, preliminary yield grade, adjusted yield grade, and marbling score ising the MARC beef carcass image analysis system. J. Anim. Sci. 81: 150-155.

Silva, J. A., L. Patarata, and C. Martins. 1999. Influence of ultimate pH on bovine meat tenderness during ageing. Meat Sci. 52: 453-459.

Smith, G. C., K. E. Belk, J. N. Sofos, J. D. Tatum, and S. E. Williams. 2000. Antioxidants in muscle foods: Nutritional strategies to improve quality. In: E. Decker, C. Faustman and C. J. Lopez-Bote (eds.) Economic implications of improved color stability in beef. p 397-426. J. Wiley & Sons, New York, NY.

Souza, C. M., D. D. Boler, D. L. Clark, L. W. Kutzler, S. F. Holmer et al. 2011. The effects of high pressure processing on pork quality, palatability, and further processed products. Meat Sci. 87: 419-427.

Stewart, M. R., B. K. Hutchins, M. W. Zipser, and M. B. Watts. 1965. Enzymatic reduction of metmyoglobin by ground beef. J. Food Sci. 30: 487-491.

Suman, S. P., C. Faustman, S. L. Stamer, and D. C. Liebler. 2006. Redox instability induced by 4-hydroxy-2-nonenal in porcine and bovine myoglobins at pH 5.6 and 4°C. J. Agric. Food Chem. 54: 3402-3408.

Suman, S. P., C. Faustman, S. L. Stamer, and D. C. Liebler. 2007. Proteomics of lipid oxidation-induced oxidation in porcine and bovine oxymyoglobins. Proteomics 7: 628-640.

Tang, J., C. Faustman, T. A. Hoagland, R. A. Mancini, M. Seyfert et al. 2005. Postmortem oxygen consumption by mitochondria and its effects on myoglobin form and stability. J. Agric. Food Chem. 53: 1223-1230.

Tarrant, P. V., and C. Mothersill. 1977. Glycolysis and associated changes in beef carcasses. J. Sci. Food Agric. 28: 739-749.

Trout, G. R. 1989. Variation in Myoglobin denaturation and color of cooked beef, pork, and turkey meat as influenced by pH, sodium-chloride, sodium tripolyphosphate, and cooking temperature. J. Food Sci. 54: 536-540.

Viljoen, M., H. L. de Lock, and E. C. Webb. 2002. Consumer acceptability of dark, firm and dry (DFD) and normal pH beef steaks. Meat Sci. 61: 181-185.

Wallace, W. J., R. A. Houtchens, J. C. Maxwell, and W. S. Caughey. 1982. Mechanism of autooxidation for hemoglobins and myoglobins. J. Biol. Chem. 257: 4966-4977.

Warriss, P. D. 1979. The extraction of haem pigments from fresh meat. J. Food Tech. 14: 75-80.

Watts, B. M., J. Kendrick, M. W. Zipser, B. Hutchins, and B. Saleh. 1966. Enzymatic reducing pathways in meat. J. Food Sci. 31: 855-862.

Wulf, D. M., R. S. Emnett, J. M. Leheska, and S. J. Moeller. 2002. Relationships among glycolytic potential, dark cutting (dark, firm, and dry) beef, and cooked beef palatability. J. Anim. Sci. 80: 1895-1903.

Xiong, Y. L. 2000. Protein oxidation and implications for muscle food quality. In: E. A. Decker, C. Faustman and C. J. Lopez-Bote (eds.) Antioxidants in muscle foods. p 85-111. Wiley, New York, NY.