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Mitochondrial abundance and efficiency contribute to lean color of dark cutting beef

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ABSTRACT

Beef carcasses exhibiting four levels of dark cutting severity (DCS): Severe, Moderate, Mild, and Shady were compared to Control carcasses to investigate biochemical traits contributing to the dark cutting condition. Color attributes of *Longissimus lumborum* (LL) were measured after grading and during simulated retail display. Mitochondrial abundance and efficiency, bloomed oxymyoglobin, reducing ability, glycolytic potential, myoglobin concentration, and protein solubility and oxidation were determined. Glycolytic potential and lactate concentrations decreased ($P < 0.05$) as DCS increased. Residual glycogen was greater ($P < 0.05$) in steaks from Control carcasses compared to DCS classes. Generally, as DCS increased, LL steaks were darker and less red in color ($P < 0.05$). Increased ($P < 0.05$) oxygen consumption and reducing ability coincided with greater myoglobin concentration and greater abundance of less efficient mitochondria as DCS increased ($P < 0.05$). These data suggest the dark cutting condition is associated with greater oxidative metabolism coupled with less efficient mitochondria resulting in depletion of glycogen during stress.

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1. Introduction

The dark lean color, and firm and dry texture of dark cutting beef does not meet expectations for beef product appearance and thus, is discriminated against by consumers. Moreover, dark cutting beef has been associated with increased incidence of off-flavor and greater variability in tenderness compared to normal beef (Calkins & Hodgen, 2007; Wulf, Emnett, Leheska, & Moeller, 2002). Consequently, dark cutting beef is severely discounted by beef packers at a great cost to livestock producers. According to the 2011 National Beef Quality Audit, 3.5% of carcasses presented for grading were affected by the dark cutting condition (Moore et al., 2012).

Dark cutting beef is attributed to depletion of muscle glycogen resulting in a less-than-normal pH decline after slaughter, and consequently, greater-than-normal muscle pH. The depletion of muscle glycogen is thought to be due to long-term stress prior to slaughter. However, the dark cutting condition occurs in a relatively small number of carcasses, and within production lots that are housed and managed together during the weeks leading up to harvest, only a small minority of animals will produce carcasses that display the dark cutting condition. Thus, a greater understanding of the factors predisposing animals to produce carcasses with the dark cutting condition is needed.

Dark cutting beef has been associated with a higher mitochondrial respiration rate, which helps maintain low oxymyoglobin concentrations. Ashmore et al. (1973) and Lawrie (1958) both reported that reduced postmortem muscle pH impairs the overall level of oxygen consumption in normal muscle compared to high pH muscle. These findings would suggest that mitochondrial function may play a role in dark cutting beef. However, it is not clear whether this is a causative relationship or a function of the high pH resulting in conditions more favorable to maintain mitochondrial function postmortem. Therefore, the objectives of the present study were to investigate mitochondrial and glycolytic metabolic traits contributing to lean color and lean color stability in dark cutting and normal beef.

2. Materials and methods

Animal care and use committee approval was not obtained for this study because samples were obtained postmortem from a USDA inspected plant.

2.1. Sample handling and preparation

Beef carcasses (n = 320) were selected from a commercial processing facility that processes young, grain-fed steers and heifers. Carcasses were evaluated as they were presented for grading at approximately

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36 h postmortem. Selection was conducted on 5 selection days over a 2-month-long period. All carcasses selected for inclusion in the present study exhibited A maturity scores (9–30 months of age). Carcasses exhibiting the dark cutting condition (n = 160) were subjectively identified by trained evaluators. When a dark cutter was identified, a cohort exhibiting normal lean color and firmness with a similar marbling score was selected from the same production lot (feedlot pen), so that to the greatest extent possible, factors contributing to the incidence of dark cutting such as breed type, animal age, diet, growth promotants, and exposure to stressful stimuli were balanced across the affected and Control groups. Within each selection day, carcasses were assigned to severity groups based on observed muscle pH: Severe, Moderate, Mild, and Shady. Normal cohorts selected to match each dark cutter were included in a separate Control group. Muscle pH was collected with a Reed SD-230 handheld pH meter (Reed Instruments, Wilmington, NC) on the anterior surface of the *Longissimus lumborum* exposed by ribbing between the 12th and 13th rib on the right side of each carcass. Carcass grade characteristics were collected using an image analysis-based (VBG2000LED) grading system (Shackelford, Wheeler, & Koohmaraie, 2003).

After grading, carcasses were placed on a stationary rail and lean color was assessed with 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 10^0 observer. The CIE L* (lightness), a* (redness), and b* (yellowness) color-space values were reported as the average of duplicate readings taken on the anterior exposed surface of the longissimus thoracis at the 12th–13th rib interface of the left side of each carcass. After fabrication, the beef, loin, strip loin (similar to IMPS #180; (NAMP, 2003; USDA, 1996), which includes the longissimus lumborum, were obtained from the left side of each carcass. Subprimals were transported via refrigerated truck (0 °C) to the U.S. Meat Animal Research Center abattoir and aged until 13 d postmortem.

On day 13 postmortem, the most anterior 13 cm of each subprimal was removed and utilized in a concurrent research project. The remaining portion of the subprimal was cut into steaks of which the longissimus lumborum was used for subsequent measurements. The first steak (2.54 cm) was placed immediately in simulated retail display. The second steak (2.54 cm) was utilized for oxygen consumption and metmyoglobin reducing ability determinations which were completed within 4 h of the steaks being cut on day 13 postmortem. A third steak (1.27 cm) was trimmed free of external fat, and visible connective tissue, diced and frozen in liquid nitrogen, and vacuum packaged for storage at −80 °C for glycolytic potential, myoglobin concentration, sarcoplasmic protein solubility, sarcoplasmic carbonyl formation determination. The fourth steak (1.27 cm) was utilized for mitochondrial isolation and determination of mitochondrial efficiency, which was done immediately (13 d postmortem) on fresh, unfrozen tissue.

2.2. Simulated retail display

Steaks cut on day 13 postmortem, 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 then were placed under continuous fluorescent lighting (color temperature = 3500 K; color rendering index = 86; 32 W; T8 Ecolux bulb, model number F32T8/SPX35, GE, GE Lighting, Cleveland OH) for 11 d. Light intensity at the meat surface was approximately 2000 lx. Retail display was conducted in a refrigerated room (1 °C), and no temperature fluctuations associated with defrost cycles were encountered.

Steaks were allowed to bloom at least 2 h after being packaged 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 settings reported earlier. 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.

2.3. pH and total myoglobin concentration

Steaks designated for determination of total myoglobin concentration were trimmed free of external fat and epimyseal connective tissue, diced, and pulverized in liquid nitrogen to produce a homogenous powder. Myoglobin concentration was determined using a modification of the method reported by Hunt and Hedrick (1977). Duplicate samples (2.5 ± 0.05 g) were homogenized in 25 mL of 800 mM sodium acetate (pH = 4.5). The homogenates were then placed in a shaker at 4 °C for 1 h to allow for pigment extraction before centrifugation (38,000 ×*g*) for 35 min at 4 °C. The supernatant was then poured into a 50 mL conical tube. To ensure complete pigment removal, the pellet was washed two times in 10 mL of cold sodium acetate utilizing vortexing and a glass rod to resuspend the pellet before shaking (4 °C) for 30 min and then centrifuged as described earlier. Supernatant from both washes were combined with the supernatant from the initial extraction. Supernatant was syringed filtered (Nalgene 0.45 μm, surfactant-free cellulose acetate membrane; Thermo Fisher Scientific, Rochester, NY) into 1.5 mL microcentrifuge tube. A 200 μL aliquot of the sample was transferred in triplicate to a 96 well plate and blanked against sodium acetate solution. 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.

2.4. Oxygen consumption and nitric oxide metmyoglobin reducing ability

Steaks designated for oxygen consumption and nitric oxide reducing ability were sampled by removing a 2.54 cm × 2.54 cm × steak thickness cube from the center of the steak, taking care to avoid connective tissue and large pieces of marbling. The cube was divided in half horizontally exposing the interior of the muscle. The top half including the surface previously exposed to light and oxygen was used for nitric oxide reducing ability determination. The bottom half, including the surface never exposed to light or oxygen was used for oxygen consumption.

Oxygen consumption was initially determined by the methods described by King, Shackelford, and Wheeler (2011). The newly exposed surface was allowed to oxygenate at 4 °C for two hours while covered with previously described oxygen permeable film. The sample then was vacuum-packaged and immediately scanned with a Hunter Miniscan colorimeter with settings previously described that had been calibrated through the oxygen impermeable film of a vacuum bag. The vacuum-packaged sample was incubated for 30 min in a 30 °C waterbath, and scanned with the colorimeter to obtain the spectral data. Oxygen consumption was recorded as (% OMb before − % OMb after/% OMb before). Initial analyses indicated that because samples with greater severity of the dark cutting condition had much lower initial (bloomed) levels of oxymyoglobin than Control samples, the percentage of oxymyoglobin deoxygenated was not comparable across dark cutting classes. This difference was likely due to increased oxygen consumption in dark cutting samples limiting the extent of oxygenation