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Evaluation of feedlot cattle working chute behavior relative to temperament, tenderness, and postmortem proteolysis

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ABSTRACT

The objective was to investigate if the association between working chute behavior and beef tenderness found in our previous study is related to protein degradation and calpain system activity. Crossbred steers (n = 183) allotted to 16 pens were weighed every 28 d. Temperament was evaluated as exit velocity (EV), chute score (CS), and catch score (CAPS). Between 14 and 16 mo of age (606 ± 52 kg), steers were harvested. Strip steaks were collected and aged for 14 d. Subsamples were collected at 36 h and 7 d postmortem and analyzed for calpastatin activity, μ-calpain autolysis, and troponin-T degradation. Shear force (WBSF) was correlated ($P < 0.05$) with calpastatin activity and measurements of troponin-T. Calpastatin activity, μ-calpain autolysis, and troponin-T measurements did not correlate with the measurements of EV, CS, and CAPS. Therefore, activation of the calpain system or differences in protein degradation did not appear to influence the differences in tenderness that are correlated with working chute behavior.

1. Introduction

Beef tenderness and consumer acceptability are important challenges faced by the beef industry. Beef is more expensive in the retail case compared with other protein sources, and an unsatisfactory eating experience can quickly alter purchasing decisions and consequently, beef demand (Brooks et al., 2000). Research continues to improve methods of evaluating beef tenderness and the use of alternative methods to determine meat quality and palatability before the animal reaches the harvest facility (Core, Miller, Widowski, Mason, and Miller, 2009; Curley, Paschal, Welsh, and Randel, 2006).

Evaluation and quantification of market cattle temperament at the production site have shown promise as a novel, simple, low-cost means to differentiate postmortem beef palatability. Most research has assessed beef cattle temperament by utilizing exit velocity of cattle as they are released from either the head gate restraint or scale (Behrends et al., 2009; Burrow, Seifert, and Corbet, 1988; King et al., 2006). Temperament scores may also be subjectively assigned to specific behaviors exhibited by an animal while it is being held in a chute (Grandin, 1993). Steers subjected to isolation and restrain stress have been shown to have higher longissimus muscle pH and darker colored lean (Apple et al., 2005). Changes in disposition of the animal, whether positive or negative, can lead to changes in feed efficiency, average daily gain, days-to-market, and beef quality (Fordyce, Dodt, and Wythes, 1988; King et al., 2006; Nkrumah et al., 2007; Voisinet, Grandin, O'Connor, Tatum, and Deesing, 1997;). Nkrumah et al. (2007) reported that cattle possessing a calmer disposition have superior nutrient efficiency and generate a more consumer-acceptable retail product, while more excitable animals on average produce tougher beef steaks (King et al., 2006).

Hall et al. (2011) found a correlation ($P = 0.032$; $r = -0.182$) between chute exit velocity (EV) and tenderness as measured by Warner-Bratzler shear force (WBSF). This association is strongest with the first recorded EV ($P = 0.030$; $r = -0.187$) and diminishes over a period of 6 mo with 8 events ($P = 0.087$; $r = -0.150$). We hypothesize that cattle with excitable temperaments have tougher meat due to differences in postmortem proteolysis during aging. The objective of this study was to determine if that inverse association between working chute behavior and beef tenderness is associated with protein degradation during aging and activity of the calpain system.

2. Materials and methods

2.1. Animals

All methods and procedures were reviewed and approved by the North Dakota State University Institutional Animal Care and Use Committee (#A0806). *Bos taurus* crossbred steers 5 to 8 mo of age (n = 183) were consigned to the Carrington Research Extension Center (CREC). Consigned breeds included Angus, Red Angus, Simmental,

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Charolais, Herford, Gelbvieh, South Devon, Chianina, Maine Anjou, and Shorthorn. Upon arrival into the feedlot, steers were blocked into 4 weight groups and sorted into 16 pens based on initial body weight (BW) (275 ± 38 kg) and fed a standard finishing diet (Hall et al., 2011). All pens were identical in size, orientation, drainage, and construction materials. During the finishing phase, 3 steers were removed for reasons independent of treatments: 2 from chronic respiratory conditions and 1 euthanized due to a broken leg. All steers had ad libitum access to fresh water, protection from wind, and bedding during the winter months. Chute behavior data was collected 8 times from October 23 to April 28 with 180 steers harvested on May 5.

2.2. Feedlot data collection

Weights were obtained and measurements of temperament including EV, chute score (CS), and catch score (CAPS) were recorded every 28 d. The steers were moved from their home pens, pen by pen, by the same livestock technicians each time. All groups in one block were moved to the scale house at the same time with one technician moving each pen. The steers were weighed individually beginning with the most distant pens from the weigh barn with pen distances ranging from 150 to 1000 m. Steers were moved through the working chute by the same employees each time using rattle paddles as necessary. As described by Burrow et al. (1988), EV was measured by infrared motion sensors (Farm Tek, Inc., Wylie, TX). The "start" sensor was placed approximately 1/2 m from the end of the working chute (head gate), and the "finish" sensor was placed 1.82 m away. Exit velocity was recorded as the time it took each steer to travel the 1.82-m distance. Chute score was visually observed and scored while steers were on the weigh scale with both entry and exit gates closed. The same technician, with 6 years of experience, assigned CS and CAPS throughout the study from the same vantage point. The CS system was developed by Grandin (1993) where a score of 1 = calm, no movement; 2 = slightly restless; 3 = squirming, occasionally shaking the chute; 4 = continuous, very vigorous movement and shaking of the chute; and 5 = rearing, twisting of the body and struggling violently. Steers were not restrained while on the weigh scale (SenseTek, Saskatoon, SK). Catch scores were recorded utilizing the same numeric scale (1 to 5) as CS; however, this evaluation was recorded based on activity while the steer was captured in the head gate.

2.3. Carcass data

At approximately 14 to 16 mo of age (BW = 606 ± 52 kg), 180 steers were delivered to a commercial packing facility (Dakota City, NE) in 5 drop-center double-decker trailers. Each load held an average of 42 head, and the partial load was filled with other cattle to maintain consistent stocking density. Longissimus muscle pH (Hannah Instruments USA, Woonsocket, RI) between the 12th and 13th ribs was recorded at the packing plant at approximately 45 min postmortem. Carcass measurements including hot carcass weight, ribeye area, 12th rib fat, kidney pelvic and heart fat percentage, marbling score, and final yield grade were obtained at approximately 24 h postmortem. At 24 h postmortem, a 7-cm thick loin muscle sample was obtained caudally to the 12th rib, placed in a labeled Ziploc bag in a cooler on wet ice, and transported to the North Dakota State University (NDSU) Meat Lab (Hall et al., 2011).

Meat samples were unpacked and deboned upon arrival at the NDSU Meat Lab at approximately 36 h postmortem. Intramuscular pH was recorded, and color was measured using a Minolta Chromameter (Konica Minolta, Grand Rapids, MI) to record L*, a*, and b* values from each strip after approximately 15-min bloom time (Wulf and Wise, 1999). A 2.54-cm thick boneless strip steak was cut from each sample, sealed in a vacuum package, and aged for 14 d at 2 °C, and then assessed for tenderness by WBSF (AMSA, 1995) as detailed in Hall et al., 2011. Also at 36-h postmortem, two 100-g subsamples from each meat sample were collected. One sample was immediately frozen, while the second sample was aged in darkness at 4 °C for 7 d in a vacuum sealed bag (7 layer co-ex film: PA 20% + TIE 24% + MPE 56%, 3 mil, 3-sided vacuum pouch; Ultravac Solutions, Kansas City, MO) and then frozen at −80 °C until further analysis.

2.4. Calpastatin activity

Calpastatin activity was determined on samples aged 36 h and 7 d postmortem. Sarcoplasmic proteins were extracted according to Shackelford, Koohmaraie, Wheeler, Cundiff, and Dikeman (1994) with modifications described by Rowe, Maddock, Lonergan, and Huff-Lonergan (2004) and Kristensen, Christensen, and Ertbjerg (2006) who indicated that calpastatin activity can be measured on frozen samples. A 10-g finely diced frozen sample was homogenized in 3 volumes of ice-cold extraction buffer [100 mM Tris, 10 mM ethylenediaminetetraacetic acid (EDTA), pH 8.3; 2 μM E-64; 100 mg/L of trypsin inhibitor; 0.2 mM phenylmethylsulfonylfluoride; 0.1% (vol/vol) 2-mercaptoethanol] using a Polytron PT 10/35 with a PTA-10S aggregate dispensing generator (Brinkman, Westbury, NY). Samples were centrifuged at 21,100 × *g* for 30 min at 4 °C using an Allegra 25R centrifuge (Beckman Coulter, Fullerton, CA). Supernatant fractions were filtered through cheesecloth, and sample volumes were recorded. Protein concentrations of each sample were determined (Quick Start Bradford Protein Assay kit, BioRad Laboratories, Hercules, CA; Bradford, 1976) with bovine serum albumin as the standard.

The sarcoplasmic protein extract was dialyzed (Spectra/Por 7 dialysis membrane MWCO 10,000; Spectrum Laboratories, Inc.; Rancho Dominguez, CA) against 40 volumes of buffer [40 mM Tris, 1 mM EDTA, pH 7.4 with 0.1% (vol/vol) 2-mercaptoethanol] overnight at 4 °C. The dialysate was then heated to 100 °C for 15 min, followed immediately by a 15-min chill on wet ice. Following centrifugation at 2000 × *g* for 30 min at 4 °C and filtration through cheesecloth, calpastatin activity was determined as described by Koohmaraie, Shackelford, Wheeler, Lonergan, and Doumit (1995) and Rowe et al. (2004). One unit of calpastatin activity was defined as having the ability to inhibit one unit of bovine m-calpain activity (Koohmaraie, 1990). Calpastatin activity (total units/g of tissue) and calpastatin specific activity (total units/g of protein in the sarcoplasmic protein extraction) are reported.

2.5. SDS-PAGE and Western blotting

2.5.1. Sample preparation and SDS-PAGE

Whole muscle protein was extracted from 36-h and 7-d aged subsamples following procedures outlined by Huff-Lonergan et al. (1996). Protein concentrations in the extracts were determined (BioRad Laboratories, Lowry, Rosebrough, Farr, and Randall, 1951), and protein gel samples for SDS-PAGE were prepared as described by Huff-Lonergan et al. (1996) and Wang (1982). Protein gel samples were loaded and separated on polyacrylamide gels (60 μg protein load on 8% gel for μ-calpain and 20 μg load on 15% gel for troponin-T) with 5% acrylamide stacking gels.

2.5.2. Western blotting

Western blotting was conducted in accordance with the procedures described by Melody et al. (2004) with minor modifications. Briefly, proteins on gels were wet tank-transferred to polyvinylidene fluoride membranes (Millipore Corporation, Bedford, MA). Membranes were blocked in 5% (wt/vol) nonfat dry milk in 1× phosphate buffered saline with 0.1% (vol/vol) Tween-20 for 1 h at room temperature. Blots were incubated overnight at 4 °C with the troponin-T