# Final Technical Report

# FCEB Project #22

Florida Cattle Enhancement Fund
Progress Report – Project P0324620 (FCEB #22)
August 15, 2024

# Improving tenderness and consistency of Brahman beef

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## Specific Aims

Our **long-term goal** is to improve the quality and consistency of *Bos indicus* influenced beef. Beef tenderness is a key determinant of product value and consumer acceptability. Yet, *Bos indicus* influenced cattle exhibit greater variability in tenderness than *Bos taurus* breeds, leading to a reputation of being tougher and exclusion from most premium programs. The limited tenderization in *Bos indicus* beef is attributed to the calpain/calpastatin system: calpain is responsible for breaking down proteins in muscle after death, resulting in tenderization; and calpastatin inhibits this process. On average, calpastatin inhibitory activity is greater in *Bos indicus* compared with *Bos taurus*, and greater calpastatin-mediated calpain inhibition blunts proteolysis and tenderization in *Bos indicus*.

While calpastatin is undoubtedly a key factor in tenderization, the regulation of calpastatin inhibitory activity remains poorly understood. In living muscle, the calpain/calpastatin system affects muscle protein degradation, which contributes to normal remodeling necessary for muscle growth and function. There have been many attempts to connect genetic factors or management practices to muscle growth or tenderness through their impact on calpastatin and calpain. However, defining the impact of the factors on calpastatin has been hindered by technical, methodological, and logistical hurdles:

In our efforts to understand beef tenderness development, we have optimized conditions for calpastatin extraction and detection. Our aim was to evaluate calpastatin protein abundance at 1h postmortem as a predictor for proteolysis. Moreover, a unique aspect of this study was that we utilized Brahman steers generated from embryo transfer, which included several full-siblings. The **objectives** were to:

- Evaluate calpastatin protein abundance at 1h postmortem as a predictor for proteolysis and tenderness of 14d aged loin steaks from Brahman steers, and
- Establish a timeline for calpastatin degradation in connection with calpain autolysis and degradation of muscle structural proteins

## Progress and Results (October 2023 – July 2024)

Brahman steers (n=31) were harvested at the UF Meat Processing Center in 2023. Initial postmortem loin samples were collected 1 hour after exsanguination, and additional samples

were collected at 6, 12, 24 and 48h and 14 days postmortem. The frozen muscle samples were processed for quantification of target proteins.

Carcass data for yield and quality grades were collected 48h after slaughter. At this time, an approximately five-inch section of loin was obtained and allocated for further analysis. A ¼" slice was used for proximate analysis of moisture and fat, and the remainder was cut into steaks and allocated to muscle protein analysis, or further aging (14d). Aged steaks were assigned to protein and tenderness analysis (Warner Bratzler shear force and trained sensory panels).

Intramuscular fat percentage ranged from 0.6 to 4.9% (average = 2.2%) and marbling scores ranged from Slight[10] (Low Select) to Modest[10] (Average Choice). There were no differences between treatment groups (P>0.18).

Calpain-1 contributes to protein degradation during meat aging. The intact form of calpain-1 is capable of breaking down proteins, and progression to intermediate and autolyzed forms indicates that calpain-1 has been active. Calpain-1 form was not affected by treatment. On average, 76% of calpain-1 was completely autolyzed by 336h (14d) (Figure 1).

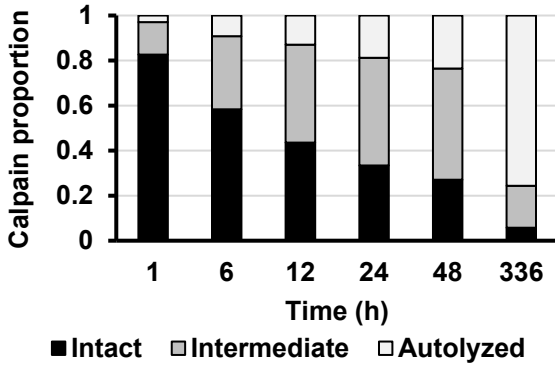


**Figure 1.** Shift in calpain-1 form during aging. Intact calpain exhibits activity toward target proteins, and shift in form (to intermediate and autolyzed) indicates that calpain has been active. Data are means (31 steers).

Troponin-T degradation is considered a good marker for postmortem proteolysis-mediated meat tenderization. Complete degradation of troponin-T at 14d varied considerably -- from ~4 to 47%. However, there was no effect of treatment on proteolysis, and there was also sizable variation between full-siblings. The progression of troponin-T from intact to fully degraded during aging from 1h to 336h (14d) is shown in Figure 2. Calpain-1 autolysis at 336h (14d) was highly associated with degradation of troponin-T ($r^2 = 0.71$, $P<0.001$).

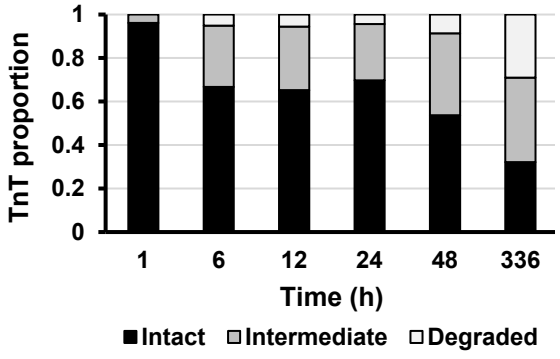


**Figure 2.** Disappearance of intact troponin-T is considered a good marker for protein degradation in meat during aging. Data are means (31 steers).

Calpastatin is the inhibitor for calpain-1, and greater calpastatin inhibition is often cited as the reason for delayed and variable proteolysis in Brahman. Calpastatin abundance was not affected by treatment, and initial (1h) calpastatin abundance had moderate association with degradation of troponin-T at end of the aging period ($r^2 = 0.20$, $P=0.001$). On average, 24% of intact calpastatin was remaining by 6h, and this decreased to <5%

by 12h postmortem. At 12h, calpastatin was noticeable in 6 out of 31 animals. The disappearance of intact calpastatin at 6h and 12h was closely associated with the decrease in intact calpain-1 at the respective times ($r^2$=0.55, $P < 0.0001$ at 6h; $r^2$=0.67, $P < 0.0001$ at 12h) reflective of calpastatin's reduced ability to inhibit calpain-1. Therefore, the abundance of calpastatin serves as an initial barrier to calpain-1 mediated proteolysis and affects the initial rate of tenderization.

Yet, muscle from some animals with slow initial rates of tenderization is able to achieve greater protein degradation, whereas proteolysis stalls in muscle from other animals. Together, this suggests that there are additional "hurdles" in postmortem muscle that influence continued proteolytic activity, or the extent of protein degradation. It is possible that the calpastatin fragments exhibit divergent inhibitory properties, or that variation in muscle/meat properties are modulating calpain activity.

## Next steps

We will perform additional analyses to better understand the basis for variation in proteolysis-mediated tenderization. This includes general characterization of meat properties (pH decline) as well as other analyses associated with calpain activity (calcium, oxidation). We also aim to examine calpain/calpastatin interaction and calpain activity.

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|---|---|
| Invoice Date: | 08/15/2024 |
| Invoice Period: | 05/01/2024 - 07/31/2024 |
| Principal Investigator: | Scheffler,Tracy Leigh |
| Award Begin Date: | 10/30/2023 |
| Award End Date: | 07/31/2024 |
| UF FEIN: | 59-6002052 |

| | |
|---|---|
| Sponsor Award ID: | 22 |
| Award Title: | Improving tenderness and consistency of Brahman beef |
| Award Amount: | $19,912.00 |

| | |
|---|---|
| **Invoice #** | I000130498 |
| **UF Award #** | AWD15804 |
| **Primary Project #** | P0324620 |
| **Primary Department:** | 60090000 |
| **Current Invoice Amount:** | $13,060.16 |

| Description | Current | Cumulative |
|---|---|---|
| Materials and Supplies | $11,660.87 | $12,740.63 |
| Direct Cost | $11,660.87 | $12,740.63 |
| Facilities and Administrative Costs | $1,399.29 | $1,528.86 |
| **Total** | $13,060.16 | $14,269.49 |

**For billing questions, please call 352.392.1235**
**Torres,Kannika S kannika@ufl.edu**
**Please reference the UF Award Number and Invoice Number in all correspondence**

**By signing this report, I certify to the best of my knowledge and belief that the report is true, complete, and accurate, and the expenditures, disbursements and cash receipts are for the purposes and objectives set forth in the terms and conditions of the federal award. I am aware that any false, fictitious, or fraudulent information, or the omission of any material fact, may subject me to criminal, civil, or administrative penalties for fraud, false statements, false claims or otherwise. (U.S Code Title 18, Section 1001 and Title 31, Sections 3729-3730 and 3801-3812).**

Kannika Torres

Certifying Official

| Payment History | |
|---|---|
| Cumulative Invoices: | $14,269.49 |
| Payments Received: | $1,209.33 |
| Outstanding Balance: | $13,060.16 |
| Note: Outstanding balance includes current invoice amount | |

**FOR UF USE ONLY** Additional Projects: N

| Project ID | Deptid | Department Name | Current | Cumulative |
|---|---|---|---|---|
| P0324620 | 60090000 | AG-ANIMAL SCIENCES | $13,060.16 | $14,269.49 |