1 **Brahman genetics influence muscle fiber properties, protein degradation, and tenderness**

2 **in an Angus-Brahman multibreed herd**

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Abstract

The objective of this study was to determine the influence of Brahman genetics on muscle contractile and metabolic phenotype and postmortem proteolysis. Cattle used in this study represent a continuous spectrum of Angus-Brahman genetic variation. Steers were harvested and *Longissimus* samples were collected at 1.5 h, 24 h, and 14 d postmortem. Proteolysis during the 14d aging period was evaluated, along with Warner-Bratzler shear force (WBSF) and trained sensory panel tenderness. Myosin heavy chain composition and enzymatic activity were used to evaluate fiber type characteristics. As Brahman influence increased, WBSF increased and sensory tenderness decreased. Calpain-1 autolysis decreased as Brahman percentage increased, and corresponded with reduced degradation of troponin-T, desmin, and titin. Increasing Brahman percentage was associated with greater citrate synthase activity and greater cross-sectional area of type IIx fibers. Brahman-influenced cattle produced tougher steaks and exhibited decreased protein degradation. Thus, Brahman genetics impacted not only the calpain-calpastatin system, but also muscle fiber size and metabolic properties.

Keywords: Brahman, Meat quality, Muscle fiber type, Proteolysis, *Bos indicus*

32 **1. Introduction**

33 Brahman and Brahman-influenced cattle are widely utilized in the Southeastern and Gulf
34 Coast regions of the United States. Their heat tolerance and parasite resistance, among other
35 adaptations, make Brahman an integral part of cattle herds in sub-tropical climates (Turner,
36 1980). While Brahman provide beneficial influence to the herd, they exhibit less desirable
37 carcass and palatability traits. When compared to Angus and Angus × Brahman crossbred cattle,
38 Brahman produce carcasses with smaller ribeye areas and their ribeye steaks are tougher than
39 Angus ribeye steaks as measured by Warner-Bratzler shear force (WBSF) values and sensory
40 panelists (Elzo, Johnson, Wasdin, & Driver, 2012; Gonzalez et al., 2014; Huffman, Williams,
41 Hargrove, Johnson, & Marshall, 1990; Whipple et al., 1990).

42 While several factors influence meat tenderness, a primary determinant is the calpain
43 system (Boehm, Kendall, Thompson, & Goll, 1998; Koohmaraie, 1992; Koohmaraie & Geesink,
44 2006). Calpains are calcium-activated cysteine proteases that initiate proteolysis but do not
45 completely degrade their targets. Of the fifteen members within the calpain family, calpain-1 is
46 the most related to meat tenderness; calpain-1 generates myofibrillar degradation products that
47 closely follow the pattern observed during aging of meat (Huff-Lonergan et al., 1996) and
48 calpain-1 knockout mice show limited postmortem proteolysis (Geesink, Kuchay, Chishti, &
49 Koohmaraie, 2006). In the presence of Ca^{2+}, the 80 kDa subunit of calpain-1 autolyzes to a 76
50 kDa subunit which indicates that the calpain has become proteolytically activated. In postmortem
51 muscle, calpain-1 targets several myofibrillar proteins, including titin, nebulin, desmin, and
52 troponin-T (Huff-Lonergan et al., 1996; Huff-Lonergan, Parrish, & Robson, 1995; Koohmaraie,
53 1992). Calpastatin, the endogenous inhibitor specific to calpain, is composed of four domains (I,
54 II, III, and IV) that can each inhibit the proteolytic activity of calpain (Goll, Thompson, Li, Wei,

55 & Cong, 2003; Wendt, Thompson, & Goll, 2004). *Bos indicus* cattle have a higher calpastatin
56 activity when compared to *Bos taurus* cattle, and as Brahman influence increases, the calpastatin:
57 calpain-1 ratio increases. Augmented calpastatin activity in *Bos indicus* cattle decreases
58 postmortem proteolysis, which negatively impacts tenderness (Pringle, Harrelson, West,
59 Williams, & Johnson, 1999; Pringle, Williams, Lamb, Johnson, & West, 1997; Wheeler, Savell,
60 Cross, Lunt, & Smith, 1990; Whipple et al., 1990).
61 During the conversion of muscle to meat, the changing cellular environment also
62 influences proteolysis and tenderization. As homeostatic mechanisms are lost, muscle undergoes
63 energetic, biochemical, and physical changes that dictate meat quality development. Energy
64 metabolism shifts to anaerobic glycolysis, which generates lactate and H^+, and results in a
65 decline in pH. Temperature and pH decline are well-known to affect protein functionality,
66 including calpain-1 activity (Maddock Carlin, Huff-Lonergan, Rowe, & Lonergan, 2009;
67 Mohrhauser, Lonergan, Huff-Lonergan, Underwood, & Weaver, 2014). Postmortem muscle also
68 loses the ability to maintain reducing conditions. Oxidative modifications to proteins increase
69 during meat aging, and even low levels of oxidation reduce tenderness (Harris, Huff-Lonergan,
70 Lonergan, Jones, & Rankins, 2001). The rate of oxidation differs between muscles and may be
71 affected by processing procedures, diet, and the inherent antioxidant systems within muscle
72 (Martinaud et al., 1997; Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004). The intrinsic
73 characteristics of muscle fibers play an important role in shaping these cellular changes during
74 the conversion of muscle to meat.
75 Several factors, including genetics, breed, and body location, influence muscle fiber
76 characteristics; and fibers are also highly adaptable to external cues, such as environment and
77 exercise (Klont, Brocks, & Eikelenboom, 1998; Ozawa et al., 2000; Rosser, Norris, & Nemeth,

78 1992). Muscle fibers are classified based on contractile speed (slow or fast) and predominant
79 type of energy metabolism (oxidative or glycolytic). Myosin heavy chain (MHC) isoform
80 composition is the primary determinant of contractile speed. Cattle express three myosin heavy
81 chain (MHC) isoforms: type I, type IIa, and type IIx (Lefaucheur, 2010). Type I fibers are slow-
82 twitch and rely on oxidative metabolism. In contrast, type II fibers are fast-contracting, but
83 differ in predominant type of metabolism; type IIa fibers are relatively more oxidative while type
84 IIx fibers are more glycolytic.
85 The influence of Brahman genetics on muscle fiber properties is not clear. Fiber area has
86 been shown to differ between *Bos indicus* and *Bos taurus* despite no differences in contractile
87 fiber type (Coles et al., 2014; Seideman, 1985; Waritthitham, Lambertz, Langholz, Wicke, &
88 Gauly, 2010; Whipple et al., 1990). The impact of breed on metabolic characteristics is also
89 uncertain, though it seems logical that Brahman muscle may acquire adaptations that relate to
90 heat tolerance. Muscle fiber properties affect cellular changes in postmortem muscle and thus
91 may be important to understanding meat tenderization in Brahman influenced cattle. Therefore,
92 the objectives of this study are to establish if contractile and metabolic phenotype of muscle may
93 be related to the proteolysis and tenderness differences observed in cattle varying in Brahman
94 composition.
95 **2. Materials and methods**
96 *2.1 Animal breeding and management*
97 Cattle used in this study were part of a long-term genetic study involving Angus,
98 Brahman, and Angus-Brahman crossbreeding. Standards for animal care and use were approved
99 by the University of Florida Institutional Animal Care and Use Committee (IACUC number
100 201003744). Although these cattle represent a continuous spectrum of Angus-Brahman genetic

101 variation (Table 1), they were divided into six breed groups for analysis: 1 = Angus; 2 = ¾
102 Angus, ¼ Brahman; 3 = Brangus; 4 = ½ Angus, ½ Brahman; 5 = ¼ Angus, ¾ Brahman; and 6 =
103 Brahman. A diallel mating system was used in this multibreed herd, where sires from the six
104 breed groups were mated across to dams of the same six breed groups (Elzo & Wakeman, 1998).
105 Calves were born from late November 2013 to early February 2014, castrated at birth, and
106 weaned in August 2014.

107 Preweaning calves were kept with their dams on bahiagrass pastures (*Paspalum notatum*)
108 at the Beef Research Unit of the University of Florida (UFBRU), with free access to a complete
109 mineral supplement (Lakeland Animal Nutrition, Lakeland, FL). Postweaning, calves continued
110 to be kept on bahiagrass pastures at the UFBRU where they received a supplement of bahiagrass
111 hay, concentrate (1.6 to 3.6 kg/d; 14.0% CP, 488 Pellet Medicated Weaning Ration, Lakeland
112 Animal Nutrition, Lakeleand, FL; soy hull pellets), and free access to mineral supplement.
113 Yearling steers were transported to a contract feeder (Suwannee Farms, O Brien, Florida) where
114 they were provided a standard commercial corn-protein diet with vitamins and minerals.
115 Subsequently, steers were selected and sorted into one of three slaughter dates in 2015 based on
116 physiological endpoint; steers were in the finishing phase for 181, 201, and 236 d. The goal was
117 to have cattle finished with 1.27 cm of subcutaneous fat. Finished steers were transported 100
118 km to the University of Florida Meat Laboratory (Gainesville, FL) on the day prior to harvest.

119 *2.2 Sample collection*

120 Steers (n = 12 per day; 2 per breed group) were harvested under USDA-FSIS inspection
121 at the University of Florida Meat Laboratory (Gainesville, FL) on one of three days. Samples
122 from the *Longissimus lumborum* (LL) muscle were collected from the left side of the carcass at
123 1.5 h, 24 h, and 14 d postmortem. At 1.5 h, approximately 50 g muscle was removed 15 to 20 cm

124 caudal to the last costa. A portion of the 1.5 h muscle sample was mounted on a cork and frozen
125 in liquid nitrogen cooled isopentane for histology, and another portion was frozen in liquid
126 nitrogen. At 24 h, roughly 15 g sample was removed from the LL at least 5 cm anterior from the
127 previous location; for the 14 d sample, a 1.0 cm slice was collected and packaged identical to the
128 steaks used for analysis of shear force and sensory evaluation. Muscle samples from all three
129 time points were frozen in liquid nitrogen and stored at -80 °C for subsequent analysis. The pH
130 of the LL was determined at 1.5, 3, 6, 9, and 24 h postmortem with a Hanna HI 99163 meat pH
131 meter (Hanna Instruments, Woonsocket, RI).

132 After initial 1.5 h sampling, hot carcass weight (HCW, kg) was determined, then
133 carcasses received a final wash and were placed in a 0°C cooler. At 48 h postmortem, carcasses
134 were ribbed between the 12th and 13th rib and carcass data was recorded. Dressing percentage
135 (DP, %) was calculated as a percentage of the carcass weight over the live weight. Carcass traits
136 evaluated at the 12th rib were fat over the ribeye (FOE, cm), ribeye area (REA, cm^2), and
137 marbling score (MAB; 100 to 199 = practically devoid, 200 to 299 = traces, 300 to 399 = slight,
138 400 to 499 = small, 500 to 599 = modest, 600 to 699 = moderate, 700 to 799 = slightly abundant,
139 800 to 899 = moderately abundant, 900 to 999 = abundant). Kidney, pelvic, and heart fat (KPH,
140 %) was evaluated as a percentage of the carcass weight. Two 2.54 cm thick steaks were removed
141 from the left side of the carcass posterior to the $12^{th}/13^{th}$ rib interface for subsequent analysis of
142 Warner-Bratzler shear force (WBSF, N) and sensory evaluation. Steaks were individually
143 bagged and then placed in heat shrink vacuum bags (B2570; Cryovac, Duncan, SC) and
144 vacuumed sealed with a Multivac C500 (Multivac Inc., Kansas City, MO). Steaks were aged at 2
145 ± 3°C for 14 d postmortem, then frozen at -40°C until further analysis.

146 *2.3 Warner-Bratzler shear force*

147 Steaks used for WBSF measurements and sensory evaluation were allowed to thaw at 2-
148 5°C for approximately 24 h prior to cooking. Steaks were prepared according to the American
149 Meat Science Association Sensory Guidelines (Belk et al., 2015). Copper-constantan
150 thermocouples (Omega Engineering Inc., Stanford, CT) were placed in the geometric center of
151 each steak to continuously measure internal temperature. Temperatures were monitored using
152 1100 Labtech Notebook Pro Software version 12.1 (Computer Boards Inc., Middleboro, MA).
153 Steaks were cooked on open-hearth grills (Hamilton Beach Brand, Washington, NC) to an
154 internal temperature of 35°C, flipped once and removed when they reached the final internal
155 temperature of 71°C.
156 Cooked steaks used for WBSF were placed on a tray, overwrapped, and chilled at 4 ±
157 2°C for 24 h. After chilling, steaks were trimmed to expose muscle fiber alignment. Six cores
158 (1.27 cm diameter) were removed parallel to the longitudinal axis of the muscle fibers. An
159 Instron Universal Testing Machine (Instron Corporation, Canton, MA) with a Warner-Bratzler
160 shear head (crosshead speed of 200 mm/min) attached to a 490 N load cell was used to measure
161 the force required to shear through the core. Each core was placed so that the sample was sheared
162 through the center of the core, perpendicular to the longitudinal axis of the muscle fibers.
163 Maximum shear force values for each core were recorded and values from all six cores were
164 used to generate a single average shear force value for each steak.
165 *2.4 Sensory evaluation*
166 Steaks used for sensory analysis were removed from the grill and trimmed of any fat and
167 connective tissue. Remaining muscle was sliced on a grid into 1 cm squares that were
168 approximately 2.54 cm thick. Each panelist received two samples per steak and evaluated six
169 steaks in a session. Sessions were held in a positive pressure ventilated room with lighting and

170 cubicles designed for objective sensory analysis. The panel consisted of eight to eleven members
171 trained in accordance with the American Meat Science Association Sensory Guidelines for
172 Sensory Analysis (Belk et al., 2015). The panelists evaluated each sample for 5 attributes:
173 juiciness (1 = extremely dry, 2 = very dry, 3 = moderately dry, 4 = slightly dry, 5 = slightly
174 juicy, 6 = moderately juicy, 7 = very juicy, 8 = extremely juicy), beef flavor intensity (1 =
175 extremely bland, 2 = very bland, 3 = moderately bland, 4 = slightly bland, 5 = slightly intense, 6
176 = moderately intense, 7 = very intense, 8 = extremely intense), overall tenderness (1 = extremely
177 tough, 2 = very tough, 3 = moderately tough, 4 = slightly tough, 5 = slightly tender, 6 =
178 moderately tender, 7 = very tender, 8 = extremely tender), connective tissue (1 = abundant, 2 =
179 moderately abundant, 3 = slightly abundant, 4 = moderate amount, 5 = slight amount, 6 = traces
180 amount, 7 = practically devoid, 8 = none detected), off-flavor (1 = extreme off-flavor, 2 = strong
181 off-flavor, 3 = moderate off-flavor, 4 = slight off-flavor, 5 = barely detected, 6 = none detected).
182 *2.5 Enzyme activity*
183 Citrate synthase (CS) and lactate dehydrogenase (LDH) activities were determined on 1.5
184 h muscle samples. Powdered muscle was diluted 1:20 (w/v) in buffer (0.25 M sucrose, 1mM
185 EDTA, 10mM Tris-HCl pH 7.4) and homogenized at 5,000 rpm for 10s. Muscle homogenates
186 were sonicated and diluted for determining CS and LDH activity. Citrate synthase activity was
187 determined by measuring the reduction of DTNB (5,5'-dithiobis-[2-nitrobenzoic acid]) at 412
188 nm using a microplate spectrophotometer (Biotek; Winooski, VT) according to Scheffler et al.
189 (2014). Lactate dehydrogenase activity was determined in homogenates by the decrease in
190 NADH measured at 340 nm. Diluted muscle homogenate (10 µl) was added to 170 µl reaction
191 media (90 mM sodium phosphate, 4.5 mM EDTA, and 0.6 mM NADH, pH 7.5). After a 2 min
192 background reading, the reaction was initiated by the addition of 30 µl of 8.4 mM sodium

193 pyruvate. Absorbance was measured at 37°C every 20s for 7 min. Enzyme activities were

194 calculated and reported as nmol/min/mg tissue for CS and µmol/min/mg tissue for LDH.

195 *2.6 SDS-PAGE and western blotting*

196 Muscle samples from 1.5 h, 24 h and 14 d were used for SDS-PAGE and western blotting

197 to assess proteolysis during the 14 d aging period. Assessment included the degree of autolysis of

198 the proteolytic enzyme, calpain-1; content of the calpain inhibitor, calpastatin; and the extent of

199 degradation of troponin-T and desmin. Whole muscle samples used for the calpain, troponin-T

200 and desmin analyses were finely powdered with liquid nitrogen and then 100 mg of powdered

201 muscle was diluted with 10 volumes of buffer containing 10 mM sodium phosphate, pH 7.0 and

202 10% (w/v) SDS. For calpastatin content, powdered muscle was diluted 1:50 (w/v) in buffer (0.05

203 M Tris-HCl, pH 6.8) containing 8 M urea, 2 M thiourea, 3% (w/v) SDS, and 75 mM

204 dithiothreitol (DTT). All samples were homogenized at 5,000 rpm for 3 x 10s using a bead-

205 beating homogenizer (Precellys, Bertin Corp.; Rockville, MD), and then centrifuged at 10,000 x

206 g for 10 min at 4°C. The supernatant was transferred to a new tube and protein concentration was

207 determined for all samples using the RC DC protein assay (Bio-Rad 500-0119, Bio-Rad

208 Laboratories, Hercules, CA). Protein samples for calpain, troponin-T and desmin were diluted in

209 Laemmli buffer (final concentration: 40 mM Tris, pH 6.8; 100 mM dithiothreitol, 2% SDS,

210 0.01% bromophenol blue, 10% glycerol) to yield equal protein concentrations and heated to

211 95°C for 5 min. Calpastatin protein samples were diluted with extraction buffer plus 0.03%

212 bromophenol blue to yield equal protein concentrations and heated to 60°C for 10 min. A 7%

213 polyacrylamide separating gel (23% [v/v] 30% acrylamide/0.8% bisacrylamide, 0.37 M Tris, pH

214 8.8, 0.1% [w/v] SDS, 0.13% [w/v] ammonium persulfate, and 0.07% [v/v] TEMED) was used to

215 determine calpain-1 autolysis and calpastatin content, a 15% polyacrylamide separating gel was

216 used to detect the degradation of troponin-T, and a 10% polyacrylamide separating gel was used
217 to determine the degradation of desmin. A 5% polyacrylamide gel (17% [v/v] 30%
218 acrylamide/0.8% bisacrylamide, 0.125 M Tris, pH 6.8, 0.1% [w/v] SDS, 0.15% [w/v]
219 ammonium persulfate, and 0.1% [v/v] TEMED) was used for the stacking gel. Gels were loaded
220 with 15 µg protein for calpain-1, 30 µg protein for calpastatin, 5 µg protein for troponin-T, and
221 10 µg protein for desmin. For each target protein, four gels were needed to accommodate all
222 samples. Within each gel, all breed groups were represented (1-2 steers per breed group on a
223 gel). Reference samples for each time point (1.5 h, 24 h, 14 d) were included on each gel to
224 verify consistency across gels, transfer, and blotting. The reference sample for each time point
225 consisted of pooled protein samples from at least two steers from each breed group. Gels (20 cm
226 wide x 8.5 cm tall x 0.75 mm thick) were run at 60 V for 20 min and then 125 V for 2 h for
227 calpain and desmin and 3 h for calpastatin and troponin-T in a MGV-202-20 electrophoresis unit
228 (C.B.S. Scientific, San Diego, CA).
229 Proteins were transferred from gels to nitrocellulose membranes at 4°C for 60 min at 500
230 mA in EBU-402 transfer tanks (C.B.S. Scientific, San Diego, CA) using transfer buffer (50 mM
231 Tris, 0.38 M glycine, 0.01% [w/v] SDS) with 10% methanol. Membranes were dried and then
232 blocked with Tris-buffered saline, pH 7.5 (TBS) blocking buffer (StartingBlock 37542, Thermo
233 Scientific, Rockford, IL) for 1 h at room temperature. Membranes were incubated with primary
234 antibodies diluted in blocking buffer with 0.2% Tween 20 overnight at 4°C. Primary antibodies
235 were diluted as follows: Calpain-1, 1:10,000 (MA3-940, Thermo Scientific, Rockford, IL);
236 calpastatin, 1:5,000 (MA3-944, Thermo Scientific, Rockford, IL); troponin-T, 1:20,000 (T6277,
237 Sigma, St. Louis, MO); desmin, 1:10,000 (D1033, Sigma, St. Louis, MO). Membranes were
238 washed 3 x 5 min in 1X TBS with 0.1% Tween and then incubated with secondary antibodies

239 diluted in blocking buffer with 0.2% Tween 20 for 1 h at 4°C. Goat anti-mouse secondary
240 antibodies were diluted as follows: Calpain-1, 1:10,000 (IRDye 680RD, LI-COR, Lincoln, NE);
241 calpastatin, 1:10,000 (IRDye 800CW, LI-COR, Lincoln, NE); troponin-T, 1:20,000 (IRDye
242 800CW, LI-COR, Lincoln, NE); desmin, 1:15,000 (IRDye 800CW, LI-COR, Lincoln, NE).
243 Membranes were washed an additional 3 x 5 min in 1X TBS with 0.1% Tween and then rinsed
244 once with 1X TBS. Blots were imaged with an Odyssey CLx (LI-COR, Lincoln, NE) and bands
245 were quantified using Image Studio version 5.2.

246 Total calpain-1 was calculated as a ratio of the total signal at 1.5 h (80 kDa + 78 kDa +
247 76 kDa) to the total signal of a reference sample. The degree of calpain-1 autolysis at 24 h was
248 calculated by determining the ratio of the 76 kDa signal over the total signal (80 kDa + 78 kDa +
249 76 kDa) at 24 h. Calpastatin content at 24 h was calculated as a ratio of the 135 kDa signal at 24
250 h over the 135 kDa signal at 1.5 h. Within each time point (24 h or 14 d), degradation of
251 troponin-T was calculated as a ratio of the degradation products (total signal minus intact
252 troponin-T) over the total signal. Desmin was determined similarly, with 55 kDa considered the
253 'intact' band.

254 *2.7 Agarose gels*

255 Muscle samples from 1.5 h, 24 h and 14 d were used for determining the extent of
256 degradation of titin in agarose gels according to Warren, Krzesinski, and Greaser (2003) with
257 some modifications. Whole muscle samples were finely powdered with liquid nitrogen and
258 diluted in extraction buffer (8M urea, 2 M thiourea, 3% SDS, 75 mM DTT and 0.05 M Tris-Cl,
259 pH 6.8) using a 1:100 sample: buffer ratio (w/v). Samples were homogenized at 5,000 rpm for 3
260 x 10s (Precellys, Bertin Corp.; Rockville, MD), and centrifuged at 13,200 x g for 12 min at 4°C;
261 the supernatant was transferred to a new tube. Sample protein concentration was determined

262 using the RC DC protein assay (Bio-Rad 500-0119, Bio-Rad Laboratories, Hercules, CA).
263 Samples were diluted in extraction buffer with 0.03% bromophenol blue to yield equal final
264 concentrations, and 5 µg of protein was loaded into each well. Gels (20 cm wide x 16 cm tall x
265 1.5 mm thick) were run on a Protean II xi Cell electrophoresis unit (Bio-Rad Laboratories,
266 Hercules, CA) at a constant 30 mA for 5 h. Gels were rinsed with double distilled water for two
267 min prior to staining with coomassie (Imperial Protein Stain, Thermo Scientific, Rockford, IL).
268 Gels were scanned with an Odyssey CLx (LI-COR, Lincoln, NE) and bands were quantified
269 using Image Studio version 5.2. Titin degradation was calculated for each time point as the ratio
270 of the degradation product (T2) to the total signal (T1+T2).

271 *2.8 Immunohistochemistry*

272 Immunohistochemistry was used to detect myosin heavy chain (MHC) types I, IIa and
273 IIx, and determine fiber cross-sectional area (CSA). Cross sections (10 µm) of LL were cut on a
274 cryostat (Microm HM 525, Tritech Inc., Edgewater, MD), placed on silanized slides (Angerer &
275 Angerer, 1991), and stored at -80°C until subsequent analysis. Sections were blocked in 1X PBS
276 containing 5% (v/v) goat serum for 1 h at room temperature. Primary antibodies (Developmental
277 Studies Hybridoma Bank, Iowa City, IA) of unique isotypes were used to detect MHC β/slow
278 (BA-F8; IgG2b), and MHC slow and 2a (BF-32; IgM) fibers. Sections were incubated with
279 primary antibodies (2 µg/ml in blocking solution) for 1 h at 37°C. Then, sections were washed 3
280 x 5 min with 1X PBS and incubated for 1 h at room temperature with goat anti-mouse secondary
281 antibodies conjugated to Alexa Fluor fluorescent dyes (IgG 350 [emission 450, blue]; and IgM
282 594 [emission 620, red]; Thermo Scientific, Rockford, IL) diluted 1:500 in blocking solution.
283 Wheat germ agglutinin tagged with Alexa Fluor 488 (W11261, Thermo Scientific, Rockford, IL)
284 was included (1:200) with the secondary antibodies to label the muscle fiber membrane. After

285 incubation, sections were washed, 3 x 5 min in 1X PBS, and coverslips were mounted (ProLong
286 Gold Antifade, Life Technologies, Eugene, OR) and sealed. Sections were imaged with an
287 EVOS FL fluorescence microscope (Advance Microscopy Group, Bothell, WA). Fibers staining
288 purple (positive for BA-F8 and BF-32) were considered type I, and fibers staining red (positive
289 for BF-32 only) were considered type IIa. Unstained fibers (black) were assumed to be type IIx.
290 Approximately 900 fibers were counted per animal, and MHC fiber type composition was
291 calculated from a ratio of the number of each fiber type over the total number of fibers analyzed.
292 Muscle fiber CSA was obtained using ImageJ 1.46r, and the average CSA of each fiber type was
293 determined for every animal.

294 *2.9 Statistical analysis*

295 Data were analyzed using linear mixed models with SAS-JMP Pro 11. The model for pH
296 decline included postmortem time (1.5h, 3h, 6h, 9h, and 24h) and breed group (1 to 6) as
297 subclass fixed effects and kill day, age at slaughter, and residual as random effects. The model
298 for fiber type composition contained the same effects except for time. The model for all other
299 traits (proteolysis at 24h and 14d, autolysis at 24h, calpastatin content at 24h, fiber area, enzyme
300 activity, sensory, WBSF, and carcass traits) comprised the fixed effect of Brahman percentage
301 (linear covariate), and the random effects of kill day, age at slaughter, and residual. Postmortem
302 time was considered a repeated measure when analyzing pH decline. Breed group least squares
303 means (LSM) and their standard errors were computed for all traits, and compared using
304 Bonferroni's t-tests. A value of $P < 0.05$ was considered statistically significant.

305

306 **3. Results and discussion**

307 *3.1 Carcass and palatability traits*

308 Based on previous data from this multibreed herd, crossbred cattle exhibit heavier HCW,
309 larger REA, and a higher KPH when compared to high percentage Angus and Brahman cattle
310 (Elzo et al., 2012). Of the carcass traits measured (Table 2), there was only a difference between
311 breed compositions when comparing FOE. The FOE decreased as the percentage of Brahman
312 increased ($R^2 = 0.14$, $P = 0.0228$). Cattle were chosen based on finishing at the same
313 compositional endpoint, approximately 1.27 cm of FOE. It is challenging to select animals with
314 the same amount of subcutaneous fat from each breed group to be represented on each slaughter
315 day because these breeds grow at different rates (Turner, 1980). Despite the difference in fat,
316 there was no difference in the age of the animals at slaughter. There were no differences in
317 HCW, REA, and KPH between breeds. Dressing percentage tended to increase as the percentage
318 of Brahman increased ($R^2 = 0.09$, $P = 0.0713$). This trend is consistent with the data presented by
319 Elzo et al. (2012a) and Pringle et al. (1997). Marbling score decreased as Brahman percentage
320 increased ($R^2 = 0.47$, $P < 0.0001$; Figure 1), which is also consistent with previous work (Elzo et
321 al., 2012; Pringle et al., 1997; Wheeler et al., 1990; Whipple et al., 1990).
322 The impact of Brahman composition on palatability attributes was determined using
323 objective tenderness and a trained sensory panel (Figure 2). As Brahman percentage increased,
324 WBSF increased ($R^2 = 0.28$, $P = 0.0009$). According to the USDA Tenderness Program, steaks
325 are considered tender if they have a WBSF value less than or equal to 4.4 kg or 43 N (USDA,
326 2011). While 4 out of 6 Brahman cattle produced “tough” steaks, two Brahman steaks were
327 classified as “tender.” The opposite was true for Angus, with only 33% (2 out of 6) of steaks
328 being considered “tough.” Several researchers have reported increased WBSF values in Brahman
329 or *Bos indicus* cattle compared to Angus or *Bos taurus* cattle (Elzo et al., 2012; Gonzalez et al.,
330 2014; Huffman et al., 1990; Pringle et al., 1997; Whipple et al., 1990). Sensory panelists also

331 detected reduced tenderness as Brahman influence increased (Figure 3; $R^2 = 0.53$, $P < 0.0001$).
332 On average, Brahman and 3/4 Brahman (breed group 5) were considered "slightly tough," and
333 the remaining groups were rated "slightly tender." While two Brahman were classified as tender
334 according to WBSF, only one Brahman steak attained "slightly tender" by panelists, and all
335 Brahman steaks scored below Angus steaks for sensory tenderness. Sensory scores for juiciness
336 and connective tissue also decreased as percentage Brahman influence increased, indicating a
337 less juicy steak with more connective tissue ($R^2 = 0.40$, $P < 0.0001$ and $R^2 = 0.52$, $P < 0.0001$,
338 respectively). Juiciness scores may be partly related to MAB ($R^2 = 0.22$, $P = 0.0041$). The same
339 trends for tenderness, connective tissue, and juiciness were reported by Elzo et al. (2012).
340 According to Gonzalez et al. (2014), Huffman et al. (1990), and Whipple et al. (1990) panelists
341 assigned lower scores for tenderness and connective tissue in Brahman, but found no differences
342 in juiciness. Although panelists perceive differences in connective tissue, there is little evidence
343 that breed composition affects collagen content or solubility (Gonzalez et al., 2014). There were
344 no differences detected in beef flavor intensity or off-flavor between breed compositions.
345 *3.3 Calpain autolysis and calpastatin content*
346 Calpain-1 activity is a major determinant of proteolysis and tenderness. In postmortem
347 muscle, calpain activity is regulated by calcium concentration, calpain autolysis, and calpastatin.
348 In the presence of Ca^{2+}, the 80 kDa catalytic subunit of calpain-1 progressively autolyzes to a 78
349 kDa intermediate, and then an active 76 kDa subunit. The degree of autolysis is used to provide
350 evidence for calpain activity (Cruzen, Paulino, Lonergan, & Huff-Lonergan, 2014; Goll et al.,
351 2003; Lomiwes, Farouk, Wu, & Young, 2014). Breed composition did not affect total calpain
352 content. However, the degree of calpain-1 autolysis at 24 h decreased as Brahman percentage
353 increased ($R^2 = 0.24$, $P = 0.0025$; Figure 3). This is evidenced by a greater percentage of the 78

354 kDa intermediate signal and 80 kDa subunit signal remaining in Brahman at 24 h. Pringle et al.
355 (1997) reported a decrease in calpain-1 activity with an increasing percentage of Brahman.
356 Wheeler et al. (1990) also observed decreased calpain-1 activity at 10 min postmortem in
357 Brahman compared to Hereford, but activity was similar by 24 h. Others have reported no
358 difference in calpain-1 activity when comparing *Bos indicus* and *Bos taurus* (Johnson, Calkins,
359 Huffman, Johnson, & Hargrove, 1990; Pringle et al., 1999; Whipple et al., 1990). Similar
360 calpain-1 activities across breed types within the first hour postmortem is consistent with calpain
361 activity being regulated primarily by cellular conditions (such as pH) and calpastatin, rather than
362 calpain content. However, cellular environment is subject to breed and slaughter conditions,
363 which could impact calpain-1 activity early postmortem. Moreover, conflicting results regarding
364 genetic influence on calpain-1 activity may relate to proportion of *Bos indicus* genetics and
365 specific breeds utilized, as Whipple et al. (1990) and Johnson et al. (1990) used Sahiwal and
366 Brahman influenced animals, respectively, but did not include purebred *Bos indicus*. Markers in
367 the CAPN1 gene are associated with beef tenderness, but specific mechanisms relating to calpain
368 content or activity have not been established.

369 Calpastatin, the inhibitor of calpain-1, consists of four domains that can each inhibit
370 calpain activity. This inhibitor is labile to proteolytic degradation by calpain and other proteases.
371 However, as calpastatin is degraded postmortem, fragments can still retain inhibitory activity
372 (Boehm et al., 1998). Even so, calpastatin loses activity early postmortem and during subsequent
373 aging (Boehm et al., 1998; Koohmaraie, Seideman, Schollmeyer, Dutson, & Crouse, 1987).
374 When calpastatin is present in mammalian skeletal muscle and contains all four domains, it
375 migrates at approximately 135 kDa in SDS-PAGE (Goll et al., 2003). Content of intact
376 calpastatin at 24 h increased as the percentage of Brahman increased ($R^2 = 0.36$, $P = 0.0001$;

377 Figure 4). There was no difference in the calpastatin content at 14 d between breed compositions.
378 Increased calpastatin content at 24h would be expected to reduce calpain-1 autolysis and activity.
379 Consistent with this, greater calpastatin content at 24 h coincided with decreased calpain-1
380 autolysis ($R^2 = 0.33$, $P = 0.0003$). Elevated calpastatin activity is well-known to contribute to
381 reduced tenderness in indicine compared to taurine breeds. As Brahman influence increases, the
382 calpastatin: calpain-1 activity ratio increases (Pringle et al., 1999, 1997; Wheeler et al., 1990;
383 Whipple et al., 1990). Greater calpastatin activity typically persists from pre-rigor (<1 h
384 postmortem) to post-rigor (24h to several days) (Pringle et al., 1999; Wheeler et al., 1990).
385 However, some have reported increased calpastatin activity in postrigor but not prerigor muscle
386 (Whipple et al., 1990), indicating other factors such as Ca^{2+} concentration, may also limit calpain
387 activation in Brahman muscle early postmortem.
388 *3.4 Protein degradation*
389 In postmortem muscle, calpain-1 degrades key myofibrillar and cytoskeletal proteins,
390 which disrupts the structure and integrity of the sarcomere, thereby contributing to tenderness
391 (Huff-Lonergan et al., 1996, 1995; Koohmaraie, 1992; Lomiwes et al., 2014; Whipple et al.,
392 1990). Postmortem degradation of several calpain targets, including troponin-T, desmin, and
393 titin, was evaluated. Troponin-T is part of the troponin complex and interacts with the thin
394 filament during muscle contraction. Because of its location, there is debate whether troponin-T
395 degradation directly contributes to development of tenderness; regardless, troponin-T is a reliable
396 indicator of postmortem proteolysis (Huff-Lonergan et al., 2010). In comparison, desmin and
397 titin play important roles in maintaining muscle structure and organization. Desmin is an
398 intermediate filament that links adjacent myofibrils at their Z-lines, and titin preserves alignment

399 of the sarcomere. Titin spans half the sarcomere from the Z-line to the M-line and is the largest
400 protein (approximately 3000 kDa) found in mammalian tissues.

401 As Brahman influence increased, troponin-T degradation at 24 h and 14 d decreased
402 (Figure 5; $R^2 = 0.16$, $P = 0.0141$ and $R^2 = 0.67$, $P < 0.0001$, respectively). Similarly, desmin
403 degradation at 24 h and 14 d decreased with increasing percentage Brahman (Figure 6; $R^2 = 0.19$,
404 $P = 0.0077$ and $R^2 = 0.42$, $P < 0.0001$, respectively). Whipple et al. (1990) found that desmin
405 degradation was greater at 0, 1, and 14 d postmortem when comparing *Bos taurus* to *Bos indicus*
406 cattle. Titin degradation paralleled the results of troponin-T and desmin; titin degradation at 24h
407 and 14 d decreased with greater Brahman influence (Figure 7; $R^2 = 0.20$, $P = 0.0059$ and $R^2 =$
408 0.19, $P = 0.0331$, respectively). For all three proteins, the degree of calpain-1 autolysis largely
409 explained degradation at 24 h (troponin-T: $R^2 = 0.84$, $P < 0.0001$; desmin: $R^2 = 0.78$, $P < 0.0001$;
410 titin, $R^2 = 0.66$, $P < 0.0001$).

411 Reduced proteolysis contributed to meat toughness, evidenced by increased WBSF with
412 decreased troponin-T degradation at 14 d ($R^2 = 0.32$, $P = 0.0003$). There also was a tendency for
413 titin degradation at 14 d to decrease as WBSF increased ($R^2 = 0.09$, $P = 0.0735$). However, when
414 two samples that exhibited the lowest troponin-T degradation at 24 h were removed,
415 relationships between 14 d protein degradation and WBSF essentially disappeared (troponin T,
416 $R^2 = 0.08$, $P = 0.09$; titin, $R^2 = 0.0003$, $P = 0.92$). This suggests that troponin-T may be more
417 reliable at predicting shear force only when proteolysis is dramatically decreased. In contrast,
418 troponin-T degradation was highly related to sensory tenderness ($R^2 = 0.53$, $P < 0.0001$), even
419 when the two aforementioned samples were removed ($R^2 = 0.50$, $P = 0.0002$). It is not clear why
420 troponin-T degradation is more limited in predicting objective tenderness by WBSF compared to
421 sensory analysis. Objective tenderness varied from ~20 to 55 N, a range that includes steaks that

422 are very tender to tough; and shear force is appropriate for evaluating variation in tenderness of a
423 muscle, but not for comparing different muscles (Belk et al., 2015). For example, longissimus
424 and biceps femoris have similar WBSF, but differ greatly in sensory tenderness (Rhee, Wheeler,
425 Shackelford, & Koohmaraie, 2004; Shackelford, Wheeler, & Koohmaraie, 1995). Therefore, it is
426 possible other factors besides proteolysis are more important to explaining variation in WBSF in
427 Brahman-influenced steaks.

428 *3.5 Muscle fiber characteristics*

429 Muscle fiber type properties represent an important source of variation in meat quality.
430 The cells within a muscle vary in their contractile and metabolic properties, and during the
431 postmortem period, these properties influence pH decline, rigor formation, and the development
432 of meat quality characteristics. Muscle fibers are classified based on contractile speed (slow or
433 fast) and predominant type of energy metabolism (oxidative or glycolytic). Muscle fiber
434 contractile type was determined based on immunofluorescent detection of MHC proteins (Figure
435 8). There were no differences in the percentage of type I fibers between breed groups. The
436 percentage of type IIa fibers was higher in breed group 2 (¾ Angus, ¼ Brahman) when
437 compared to Angus ($P = 0.0434$), and correspondingly, the percentage of type IIx muscle fibers
438 was higher in breed group 1 (Angus) when compared to breed groups 2 (¾ Angus, ¼ Brahman,
439 $P = 0.0067$) and 3 (Brangus, $P = 0.0218$). Others have shown that breed type does not affect
440 fiber type composition when comparing *Bos taurus* to *Bos indicus* cattle (Seideman, 1985;
441 Waritthitham et al., 2010; Whipple et al., 1990). Within the population used in this study, the
442 extreme breed compositions of Angus and Brahman were not different. Therefore, it is unlikely
443 that contractile fiber type directly explains differences in meat aging and tenderness.

444 Mean muscle fiber CSA was also determined for each fiber type for all animals. Breed
445 composition did not affect the mean CSA of types I and IIa fibers. However, the CSA of type IIx
446 muscle fibers increased as the percentage of Brahman increased (Figure 9; $R^2 = 0.32$, $P =$
447 0.0004), resulting in a shift in CSA distribution in Brahman compared to Angus. The effect of
448 breed composition on fiber CSA is conflicting; some reported that breed type does not influence
449 fiber CSA (Waritthitham et al., 2010; Whipple et al., 1990), while others showed mean CSA of
450 IIx or all fiber types is increased *Bos indicus* compared to *Bos taurus* cattle (Coles et al., 2014;
451 Seideman, 1985). Increasing CSA corresponds with greater toughness, assessed by both sensory
452 panelists and WBSF (Chriki et al., 2012; Crouse, Koohmaraie, & Seideman, 1990). Crouse et al.
453 (1990) reported that CSA is associated with WBSF within the first 3 d postmortem, but not at 14
454 d; therefore, as proteolysis increases, CSA of fibers may become less important in modulating
455 tenderness. In contrast, Chriki et al. (2012) found that CSA was related to WBSF of longissimus
456 even at 14 d aging, and CSA was also positively related to total and insoluble collagen content.
457 Considering the reduced proteolysis observed in Brahman, the greater CSA of Brahman IIx
458 fibers could also contribute to reduced tenderness.
459 To assess metabolic characteristics of the LL, activity of key enzymes involved in
460 glycolytic and oxidative metabolism were quantified. Lactate dehydrogenase (LDH) catalyzes
461 the conversion of pyruvate to lactate in anaerobic glycolysis, whereas citrate synthase is a marker
462 of mitochondrial content in muscle. Breed composition did not affect LDH activity (not shown).
463 However, CS activity (Figure 10) increased as the percentage of Brahman increased ($R^2 = 0.20$,
464 $P = 0.0056$). While Brahman exhibited a greater mitochondrial content as evidenced by CS
465 activity, this was not related to MHC composition. Even though a muscle expresses specific
466 contractile proteins, it is not necessarily mirrored in the metabolic phenotype. Shifting metabolic

467 characteristics and expression of protein isoforms besides MHC allows muscle greater flexibility
468 to adapt to specific conditions. Metabolic differences between taurine and indicine cattle are not
469 well understood, but increased mitochondrial content may be an adaptation that allows Brahman
470 muscle to function efficiently in its environment.

471 The energetic properties of muscle may be critical to postmortem energy metabolism and
472 meat aging. The rate of glycolysis dictates pH decline, which may directly impact activity of
473 calpain-1; more rapid postmortem metabolism may also indirectly augment calpain-1 by
474 producing favorable calcium conditions, resulting from reduced ATP available to sequester Ca^{2+}.
475 Postmortem pH decline was monitored (Table 2), but breed composition did not influence pH
476 decline. However, a contrast of Brahman versus Angus at 3 h showed that Brahman exhibited a
477 higher pH compared to Angus (6.30 vs. 6.04, $P = 0.02$). If content of glycolytic enzymes does
478 not differ, post-translational mechanisms may be important for regulating enzymatic activity and
479 glycolytic rate. For instance, phosphorylation sites on the glycolytic enzyme
480 phosphoglucomutase-1 are expected to hasten glycolysis, and these same sites have been
481 identified as potential biomarkers for predicting tenderness in longissimus steaks from British
482 crossbred or Angus and Nellore cattle (Anderson, Lonergan, & Huff-Lonergan, 2014; Rodrigues
483 et al., 2017). In addition, mitochondria may serve key roles in meat aging. Mitochondria
484 contribute to sequestration, transfer, and release of Ca^{2+}, which could delay or accelerate meat
485 aging through regulation of sarcoplasmic Ca^{2+}. In Brahman muscle, greater mitochondria content
486 may increase Ca^{2+} sequestering capacity and prevent increases in free sarcoplasmic Ca^{2+}, thereby
487 delaying activation of calpain-1. Moreover, accumulation of high levels of Ca^{2+} in mitochondria
488 triggers induction of apoptosis and caspase-mediated cell death. Although caspase-mediated
489 proteolysis is controversial, several researchers have provided evidence that greater susceptibility

490 to apoptosis is associated with accelerated rate of beef tenderization (Gagaoua, Claudia Terlouw,
491 Boudjellal, & Picard, 2015; Laville et al., 2009; Rodrigues et al., 2017). Brahman composition
492 influences muscle metabolic properties, but further work is necessary to understand the
493 mechanisms by which glycolytic rate and mitochondria affect tenderization.
494
495 **4. Conclusions**
496 Overall, tenderness is a complex attribute resulting from inherent muscle properties and
497 the cellular and processing milieu during the conversion of muscle to meat. Elevated calpastatin
498 activity is well-known to contribute to reduced tenderness in indicine compared to taurine
499 breeds. Consistent with this, Brahman cattle produced tougher meat based on WBSF and sensory
500 panel scores. Troponin-T degradation, an indicator of proteolysis, explained considerable
501 variation in sensory tenderness, but was more limited in predicting WBSF. Factors other than
502 proteolysis, such as type IIx fiber CSA or connective tissue, may explain variation in objective
503 tenderness. Importantly, despite similar contractile phenotype, Brahman composition affects
504 metabolic phenotype of longissimus. Greater Brahman influence was associated with increased
505 CS activity, a marker of mitochondria content. Shifting muscle metabolic properties may impact
506 events that modulate proteolytic activity, including pH decline, sarcoplasmic Ca^{2+}, and
507 apoptosis. Thus, further work is needed to evaluate impact of muscle metabolic phenotype on
508 postmortem energy metabolism and tenderness development in *Bos taurus* and *Bos indicus*
509 breeds.
510
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515

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669

670

671
672 **Table 1.** Specific composition of Angus and Brahman breed within each breed group

	Breed Group	Angus	Brahman
1	Angus	0.80 - 1.00	0.00 - 0.20
2	¾ Angus, ¼ Brahman	0.60 - 0.79	0.21 - 0.40
3	Brangus	0.625	0.375
4	½ Angus, ½ Brahman	0.40 - 0.59	0.41 - 0.60
5	¼ Angus, ¾ Brahman	0.20 - 0.39	0.61 - 0.80
6	Brahman	0.00 - 0.19	0.81 - 1.00

673

674

675 **Table 2.** Carcass traits and pH decline across all animals

Traits	Mean ± SD
HCW, kg	318 ± 36
DP, %	57.22 ± 2.42
FOE, cm	1.22 ± 0.34
REA, cm^2	73.30 ± 8.74
REA/100 kg HCW	23.18 ± 2.57
KPH, %	2.4 ± 0.5
pH	
1.5 h	6.47 ± 0.07
3 h	6.21 ± 0.09
6 h	5.89 ± 0.07
9 h	5.72 ± 0.06
24 h	5.47 ± 0.03

676

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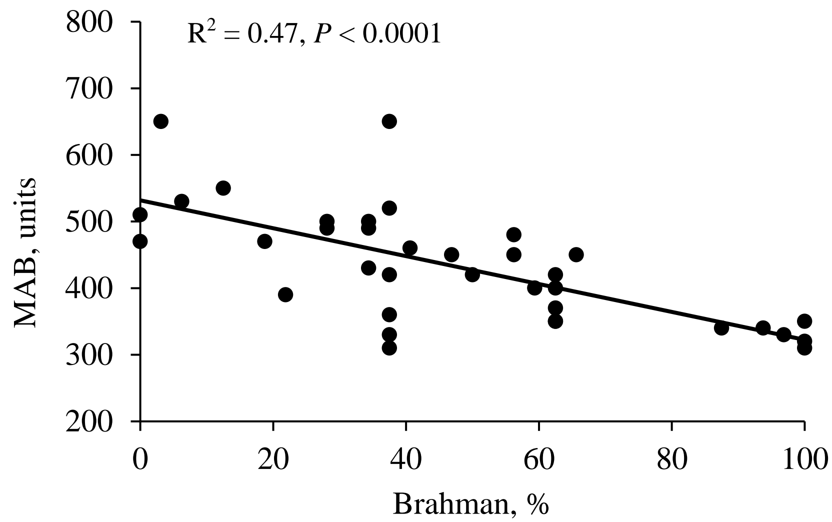
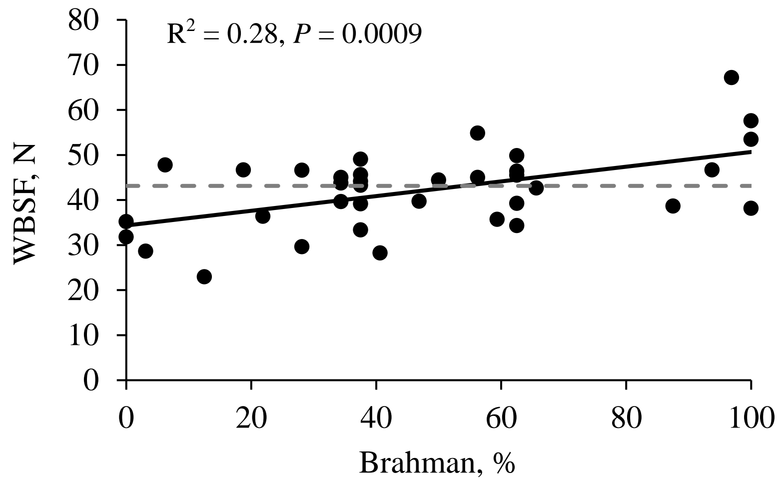


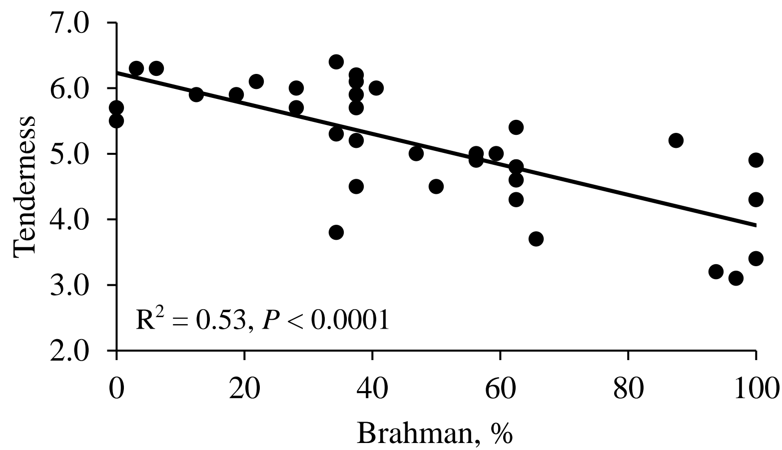
678

679 **Figure 1**. Marbling (MAB) scores in the in the ribeye (longissimus) of steer carcasses ranging in
680 breed composition from 0% Brahman (100% Angus) to 100% Brahman. (MAB: 100 to 199 =
681 practically devoid, 200 to 299 = traces, 300 to 399 = slight, 400 to 499 = small, 500 to 599 =
682 modest, 600 to 699 = moderate, 700 to 799 = slightly abundant, 800 to 899 = moderately
683 abundant, 900 to 999 = abundant)
684

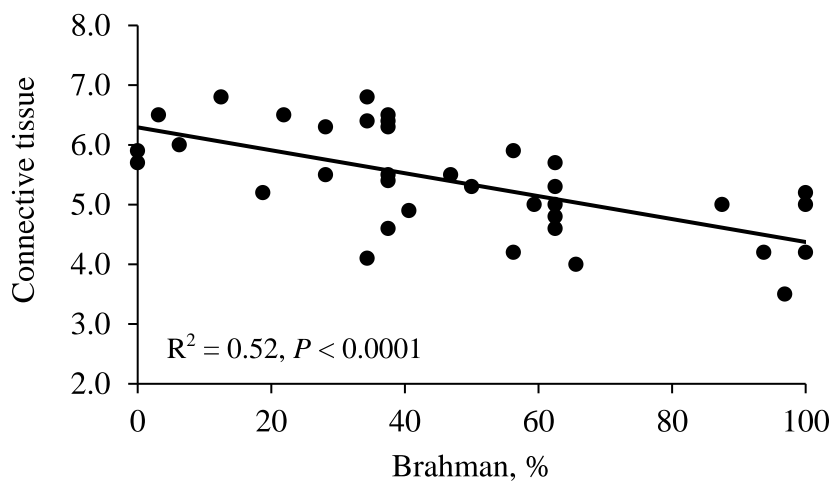
685 **(A)**



686
687 **(B)**



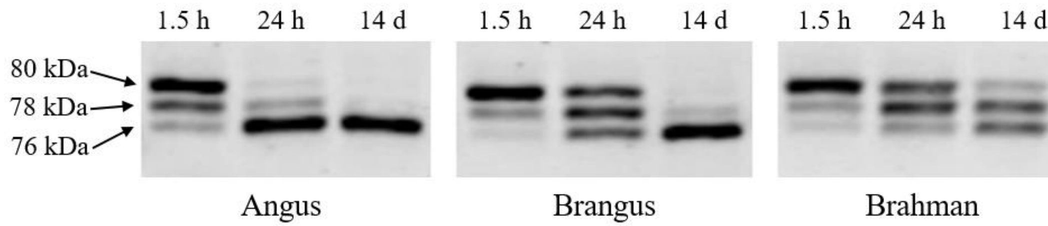
688
689 **(C)**



690
691 **Figure 2.** Palatability attributes of strip loin steaks from steers ranging in breed composition
692 from 0% Brahman (100% Angus) to 100% Brahman. (A) Objective tenderness assessed by

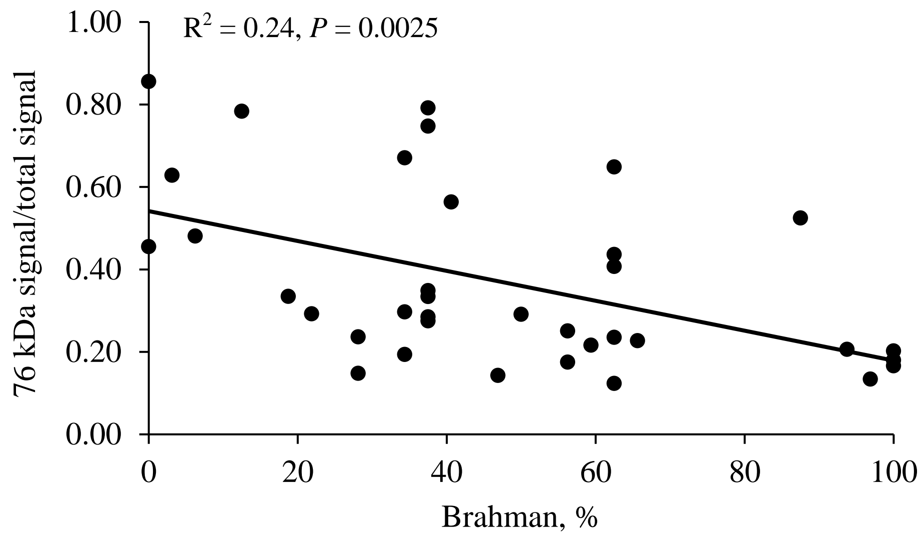
693 Warner Bratzler shear force (WBSF). Dashed line is at 43.1 N (4.4 kg), the boundary between
694 tough and tender as described by USDA (2011). Subjective evaluations by a trained sensory
695 panel for (B) tenderness and (C) connective tissue. (Tenderness: 1 = extremely tough, 2 = very
696 tough, 3 = moderately tough, 4 = slightly tough, 5 = slightly tender, 6 = moderately tender, 7 =
697 very tender, 8 = extremely tender; Connective tissue: 1 = abundant, 2 = moderately abundant, 3 =
698 slightly abundant, 4 = moderate amount, 5 = slight amount, 6 = traces amount, 7 = practically
699 devoid, 8 = none detected)
700

701 (A)



702

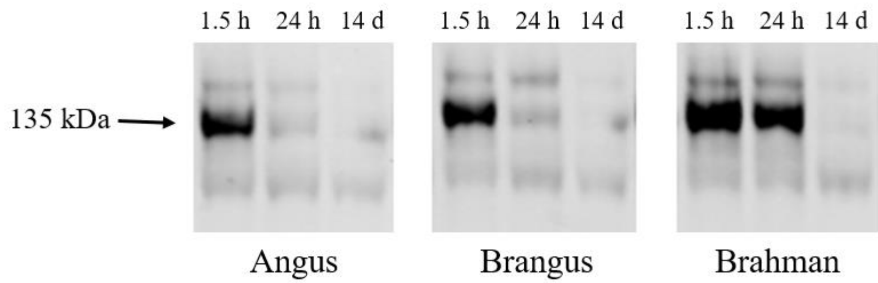
703 (B)



704

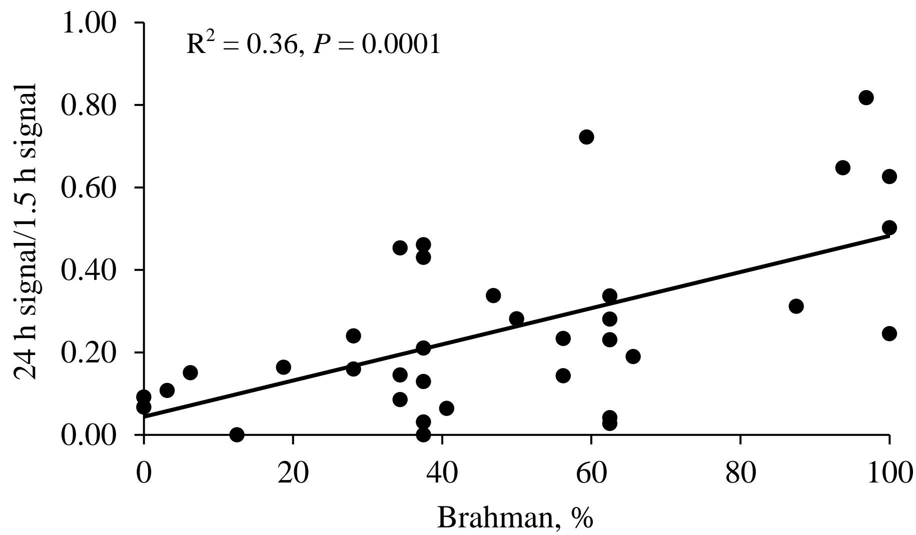
705 **Figure 3**. Effect of breed composition on calpain-1 autolysis in the longissimus. (A) Western
706 blot of Calpain-1 autolysis at 1.5 h, 24 h, and 14 d postmortem in Angus, Brangus, and Brahman
707 breed groups. (B) Degree of calpain-1 autolysis at 24 h ranging in breed composition from 0%
708 Brahman (100% Angus) to 100% Brahman. Autolysis at 24 h was calculated as the ratio of 76
709 kDa signal to total signal (80, 78, and 76 kDa).
710

711 **(A)**



712

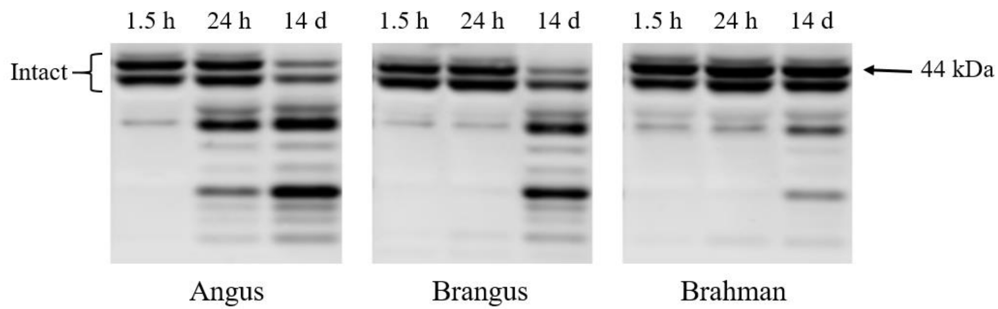
713 **(B)**



714

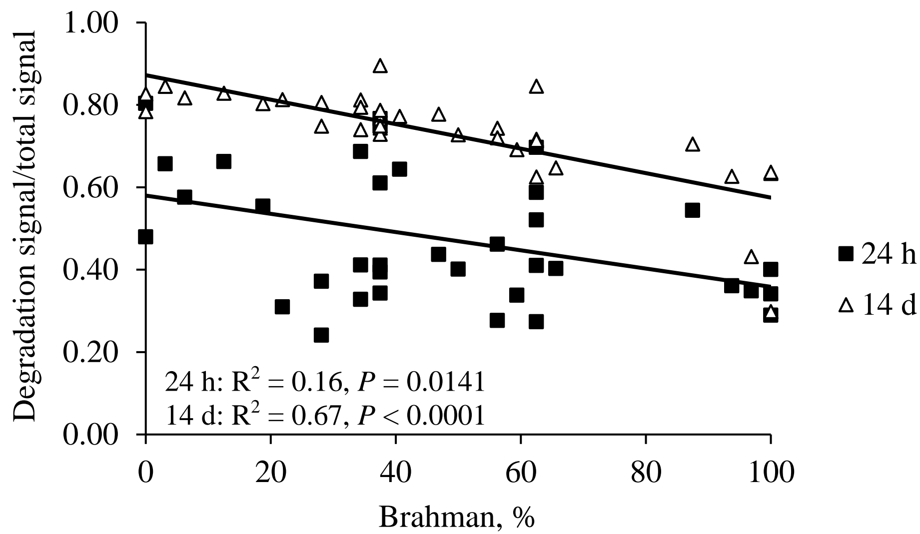
715 **Figure 4**. Effect of breed composition on calpastatin content (135 kDa) in the longissimus. (A)
716 Western blot of calpastatin at 1.5 h, 24 h, and 14 d postmortem in Angus, Brangus, and Brahman
717 breed groups. (B) Calpastatin content at 24 h in longissimus from steer carcasses ranging in
718 breed composition from 0% Brahman (100% Angus) to 100% Brahman. Calpastatin content at
719 24 h was calculated as the ratio of the 135 kDa signal at 24 h relative to the signal at 1.5h.
720

721 **(A)**



722

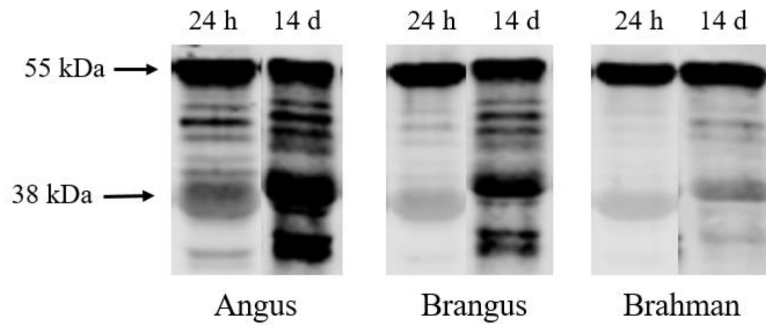
723 **(B)**



724

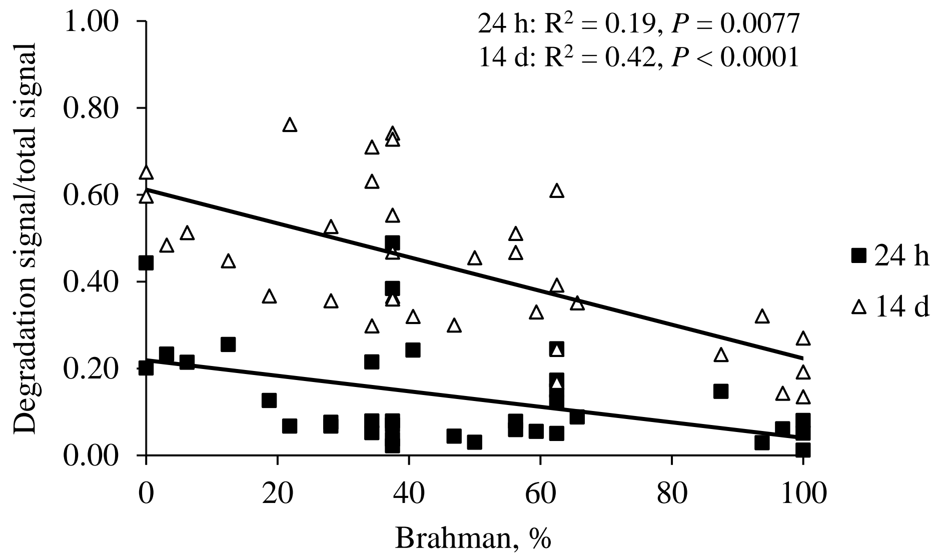
725 **Figure 5**. Effect of breed composition on troponin-T degradation in the longissimus. (A)
726 Western blot of troponin-T degradation at 1.5 h, 24 h, and 14 d postmortem in Angus, Brangus,
727 and Brahman breed groups. (B) Degradation of troponin-T at 24 h and 14 d postmortem ranging
728 in breed composition from 0% Brahman (100% Angus) to 100% Brahman. Troponin-T
729 degradation was determined within each time point, and calculated as a ratio of the degradation
730 products (total signal minus intact signal) over the total signal.
731

732 **(A)**



733

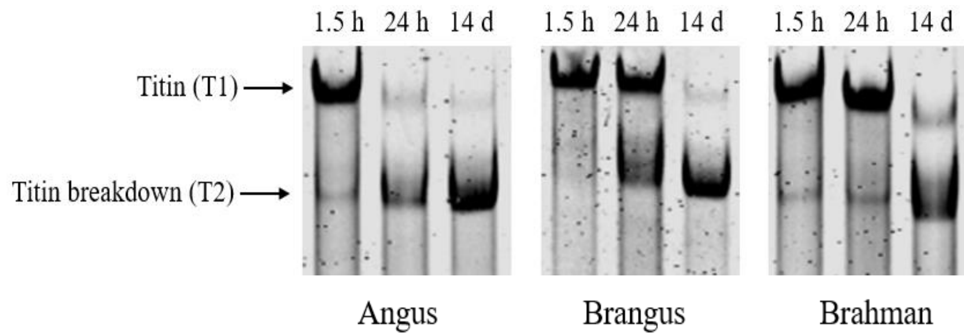
734 **(B)**



735

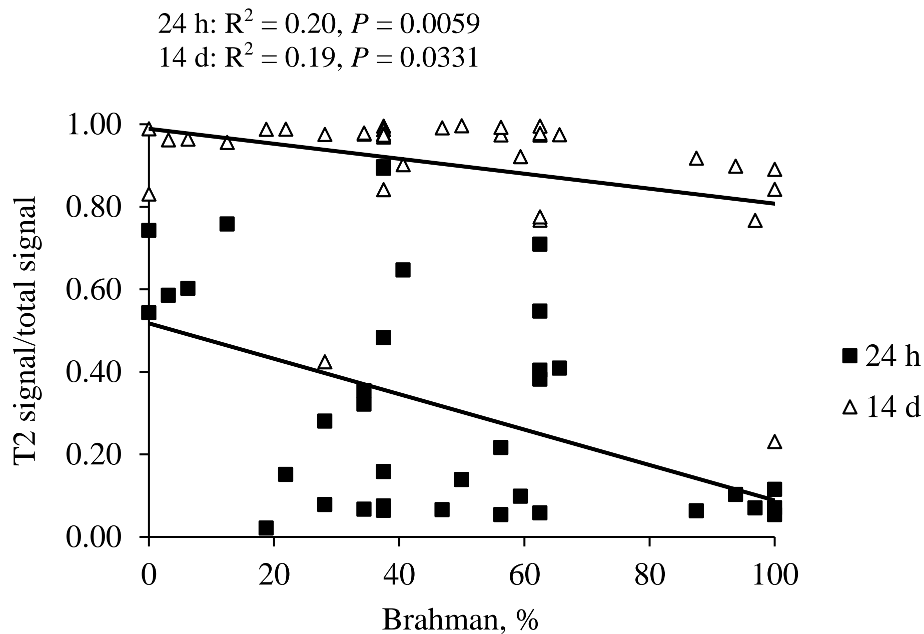
736 **Figure 6**. Effect of breed composition on desmin degradation in the longissimus. (A) Western
737 blot of desmin degradation at 1.5 h, 24 h, and 14 d postmortem in Angus, Brangus, and Brahman
738 breed groups. (B) Degradation of desmin at 24 h and 14 d postmortem ranging in breed
739 composition from 0% Brahman (100% Angus) to 100% Brahman. Desmin degradation was
740 determined within each time point, and calculated as a ratio of the degradation products (total
741 signal minus 55 kDa signal) over the total signal.
742

743 **(A)**



744

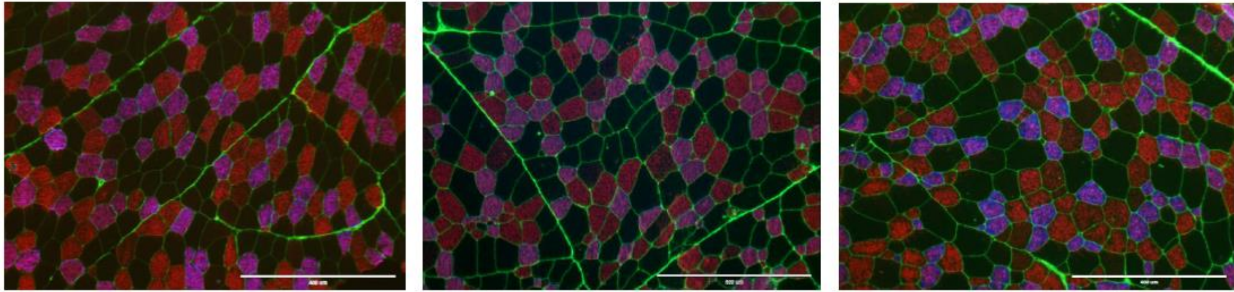
745 **(B)**



746

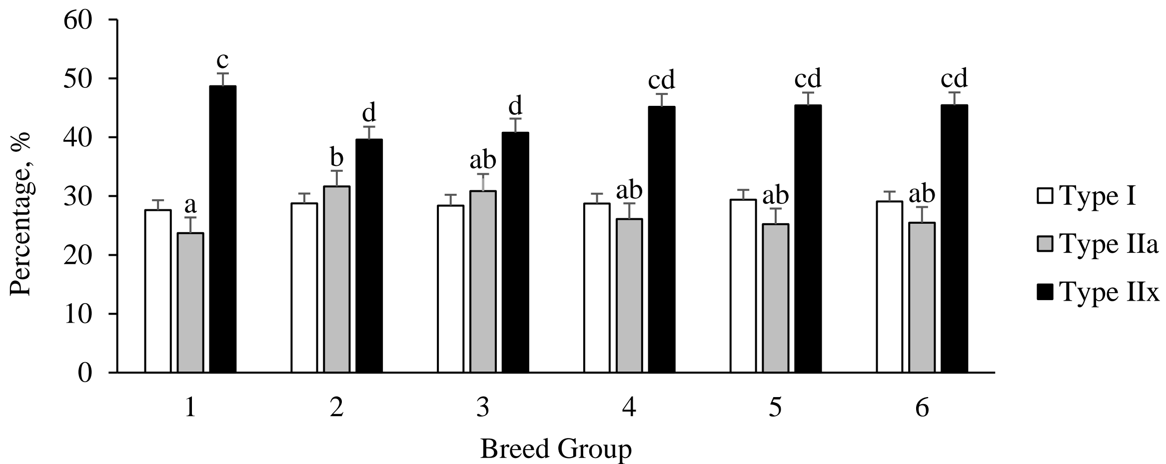
747 **Figure 7.** Effect of breed composition on titin degradation in the longissimus. (A) Agarose gel of
748 titin degradation, stained with coomassie, at 1.5 h, 24 h, and 14 d postmortem in Angus,
749 Brangus, and Brahman breed groups; B) Degradation of titin at 24 h and 14 d postmortem
750 ranging in breed composition from 0% Brahman (100% Angus) to 100% Brahman. Titin
751 degradation was determined within each time point and calculated as a ratio of degradation
752 product (T2) signal to total signal (T1+T2).
753

754 **(A)**



755

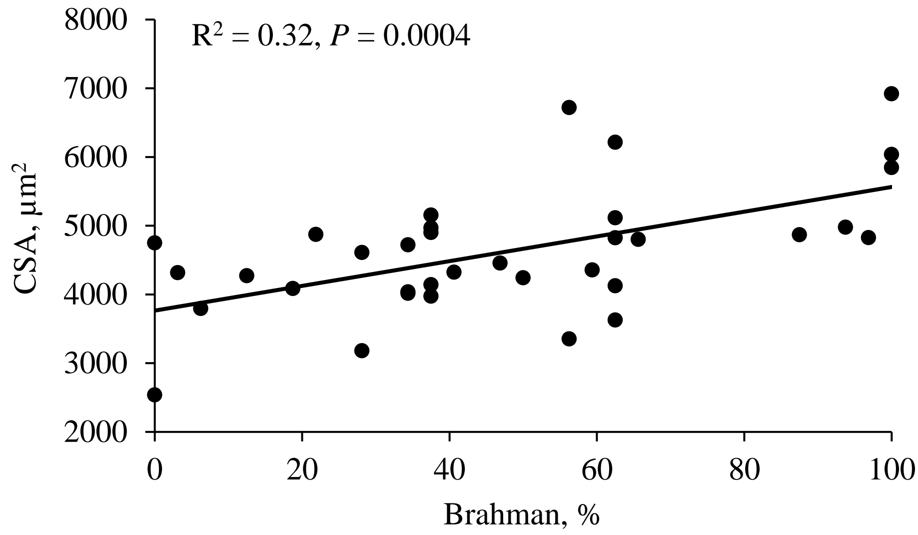
756 **(B)**



757

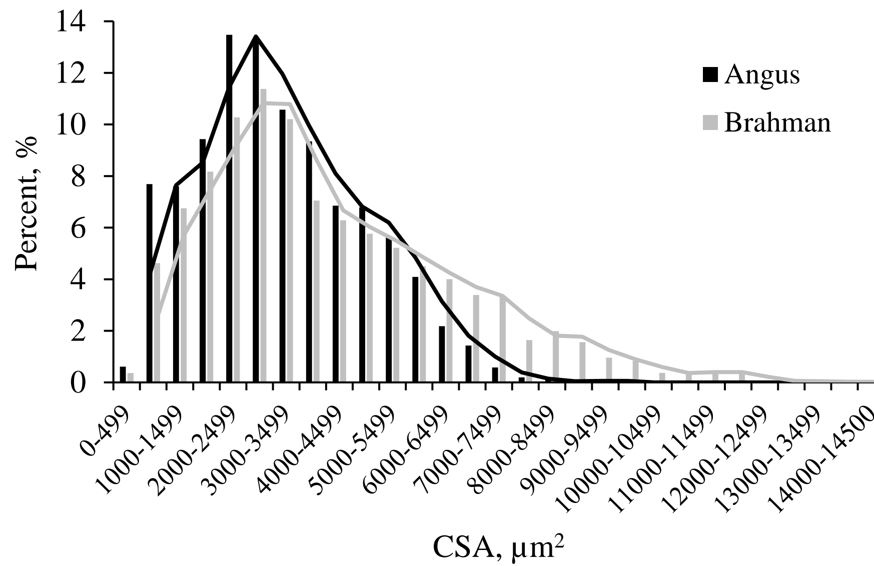
758 **Figure 8**. Myosin heavy chain (MHC) isoform composition of longissimus from steers ranging
759 in breed composition from 0% Brahman (100% Angus) to 100% Brahman. (A)
760 Immunohistochemical staining of MHC isoforms in Angus, Brangus, and Brahman breed groups
761 (type I = purple; type IIa = red; type IIx = black; scale bar = 400 µm). (B) MHC composition
762 (percentage of fibers) for each breed group (LSM ± SE). Within a fiber type, means with
763 different superscripts are significantly different ($P < 0.05$)
764

765 **(A)**



766

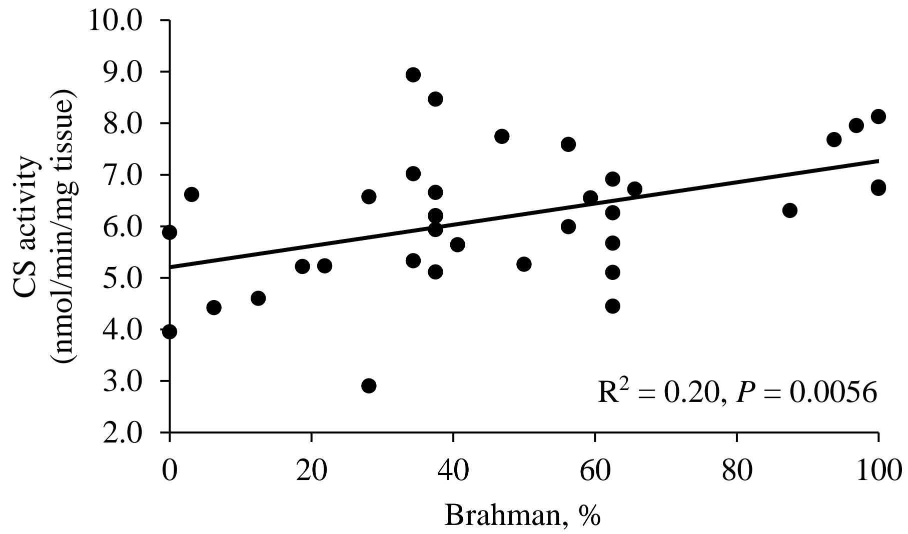
767 **(B)**



768

769 **Figure 9**. Cross-sectional area (CSA) of type IIx fibers in longissimus. (A) CSA of IIx fibers
770 from steers ranging in breed composition from 0% Brahman (100% Angus) to 100% Brahman.
771 (B) Distribution of CSA across all muscle fiber types in Angus (80-100%) and Brahman (80-
772 100%).
773

774



775

776 **Figure 10**. Citrate synthase activity of longissimus from steers ranging in breed composition
777 from 0% Brahman (100% Angus) to 100% Brahman.
778