

AN EVALUATION OF TENDERNESS OF THE LONGISSIMUS MUSCLE OF ANGUS BY HEREFORD VERSUS BRAHMAN CROSSBRED HEIFERS¹

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ABSTRACT

Postmortem aging of carcasses obtained from Angus-Hereford (n = 8) and 5/8 Brahman crossbred (n = 8) heifers was investigated to determine the cause of variation in meat tenderness. Raw longissimus muscle (LM) myofibril fragmentation index was lower and cooked LM Warner-Bratzler shear force was greater for the 5/8 Brahman crossbreds ($P < .05$). The activities of calcium-dependent protease (CDP) -I and -II were not affected ($P > .05$) by breed; however, CDP inhibitor activity was higher ($P < .05$) in the 5/8 Brahman carcasses. The activities of cathepsins B and B + L were not affected by breed or postmortem storage time (0, 1, 3, 7 or 14 d). Hereford-Angus carcasses were fatter opposite the 12th rib and had higher USDA yield grades and marbling scores ($P < .05$). Hereford-Angus crossbreds had less dark, coarse band formation around the exterior of the LM and lighter, finer-textured lean ($P < .05$). Cooking loss (%) and cooking rate (g/min) were not affected by breed or postmortem aging ($P > .05$). The increased toughness in the 5/8 Brahman carcasses may be due to increased CDP inhibitor activity.

Key Words: Brahman, Crossbreds, Tenderness, Aging, Proteolysis

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Introduction

Brahman cattle, and other *Bos indicus* breeds, have been utilized in crossbreeding programs in attempts to improve the efficiency of beef production. The use of Brahman cattle in crossbreeding programs in tropical and semitropical climates has been proven to be of

economic value (Carroll et al., 1955; Cole et al., 1963; Crockett et al., 1979).

Tenderness has been reported to be the primary palatability attribute affecting consumer acceptability of meat (Rhodes et al., 1955; Van Syckle and Brough, 1958). Meat from *Bos indicus* breed crosses of cattle was less tender than meat from *Bos taurus* breed crosses (Ramsey et al., 1963; Koch et al., 1982; Crouse et al., 1987, 1989). The variation in tenderness between *Bos indicus* breed crosses and *Bos taurus* breed crosses was greater than the variation in tenderness among *Bos taurus* breed crosses (Koch et al., 1976, 1979, 1982). Whipple et al. (1990) identified calcium-dependent protease inhibitor as one possible cause for the difference in tenderness between *Bos indicus* and *Bos taurus* breed crosses.

The objectives of this study were 1) to determine what factors cause meat from Brahman-cross cattle to be tougher than that from *Bos taurus* cattle and 2) to determine the

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effect of postmortem aging on the tenderness of meat from Brahman cattle.

Materials and Methods

Angus-Hereford (AH, $n = 8$) and 5/8 Brahman \times AH ($n = 8$) crossbred heifers were weaned at 6 to 8 mo of age, fed an alfalfa haylage (48.6% as fed) -corn silage (49.6% as fed) diet for 4 mo, and then were fed a corn (53.5% as fed) -corn silage (43.1% as fed) diet until slaughter at 15 to 17 mo of age. Heifers within breed groups were assigned randomly to one of two slaughter groups (four per breed per slaughter group) that were slaughtered 2 wk apart to facilitate collection of data. Carcasses were chilled for 24 h at -1.5°C and then maintained at 2°C for the remainder of the study. Carcasses were not electrically stimulated. Temperature and pH of the longissimus muscle (LM) were determined at 0, 3, 6, 9, 12 and 24 h postmortem. Temperature and pH were determined at stratified points along the length of the LM (left side of the carcass) to eliminate location effects on the measurements taken at the various times postmortem. Within each breed, each postmortem measurement occurred the same number of times at any location. Additionally, temperature values were obtained at three different depths of the LM and averaged to further eliminate location effects using an electronic digital probe⁶. To determine pH, 2.5 g of LM was homogenized in 10 volumes of 5 mM iodoacetate containing 150 mM KCl as described by Bendall (1973). The pH of the homogenate was determined with an electronic pH meter⁷.

Fifty-gram samples were taken from the 12th rib region at 0 and 24 h postmortem for determination of calcium-dependent protease (CDP) -I, -II and inhibitor activities. Those activities were determined on fresh samples according to Koohmaraie (1990). Activities were expressed as the amount of CDP caseino-

lytic activity in 50 g of muscle. One unit of CDP-I and -II activity was defined as the amount of enzyme that catalyzed an increase of 1.0 absorbance unit at 278 nm in 1 h at 25°C . One unit of inhibitor activity was defined as the amount that inhibited one unit of CDP-II activity.

At 24 h postmortem, the right side of each carcass was ribbed between the 12th and 13th ribs for determination of USDA quality and yield grade characteristics (USDA, 1989). Live weight, hot carcass weight, dressing percentage, actual fat thickness, adjusted fat thickness, rib-eye area, percentage kidney, pelvic and heart fat, USDA yield grade, skeletal maturity, lean maturity, overall maturity and marbling score were recorded. Dark, coarse band formation (heat ring), lean color, lean firmness and lean texture were scored on an 8-point scale (8 = undetectable, bleached, firm or fine and 1 = severe, dark, soft or coarse).

At 24 h postmortem, the portion of the LM (right side) from the 7th thoracic vertebra to the 5th lumbar vertebra was cut into steaks 2.5 cm thick and vacuum packaged. Steaks were assigned to 1, 3, 7 or 14 d postmortem vacuum aging (2°C) by stratifying storage time along the length of the LM such that each postmortem aging period was assigned to each location the same number of times within each breed. Within each stratum (days postmortem), steaks were further stratified to be used for Warner-Bratzler shear force, myofibril fragmentation index and lysosomal enzyme activity determinations.

Steaks were broiled to an internal temperature of 40°C , turned, and broiled to an internal temperature of 70°C on electric broilers⁸ for determination of Warner-Bratzler shear force at 1, 3, 7 or 14 d postmortem. Internal temperature was monitored by an iron/constantan thermocouple probe attached to a potentiometer⁹. Weights were recorded before and after cooking for determination of cooking loss (%) and cooking rate (g/min). Steaks were allowed to cool for 24 h at 4°C and six 1.3-cm cores were removed from each steak parallel to the longitudinal orientation of the muscle fibers. Cores were sheared with a Warner-Bratzler shear device attached to an universal testing machine¹⁰ equipped with a Microcon computer. The crosshead speed was 5 cm/min and the fail criterion was 75%.

At 1, 3, 7 or 14 d postmortem, myofibril fragmentation indices (MFI) were determined

⁶Digital Multimeter, Model 8020A, John Fluke Mfg. Co., Inc., Mountlake Terrace, WA.

⁷PHM62 Standard pH Meter, Radiometer America, Inc., Cleveland, OH.

⁸Farberware Open-hearth Broilers, Model 450N, Kidde, Inc., Bronx, NY.

⁹Honeywell Potentiometer Multipoint Recorder, Model 112.

¹⁰Model 1122, Instron Universal Testing Machine, Instron Corp., Canton, MA.

TABLE 1. CARCASS CHARACTERISTICS OF ANGUS-HEREFORD AND 5/8 BRAHMAN CROSSBRED HEIFERS

Trait	Angus-Hereford	5/8 Brahman	SEM
Live wt, kg	459.9	457.4	12.2
Hot carcass wt, kg	292.9	291.2	8.0
Dressing percentage	63.7	63.7	.6
Measured fat thickness, mm	15.1 ^b	11.4 ^c	1.0
Adjusted fat thickness, mm	15.2 ^b	11.9 ^c	.8
Ribeye area, cm ²	71.1	72.4	2.5
Percentage kidney, pelvic and heart fat	2.9	2.7	.2
USDA yield grade	3.5 ^b	3.1 ^c	.1
Skeletal maturity	A71	A63	2.9
Lean maturity	A36 ^c	A45 ^b	2.4
Overall maturity	A54	A54	1.8
Marbling	Small25 ^b	Slight85 ^c	13.2
Heat ring ^a	7.6 ^b	6.6 ^c	.2
Lean color	4.9 ^b	4.3 ^c	.1
Lean firmness	6.0	6.3	.3
Lean texture	6.8 ^b	6.1 ^c	.2

^aHeat ring, lean color, lean firmness and lean texture were scored on an 8-point scale (8 = Undetectable, bleached, firm or fine and 1 = severe, dark, soft or coarse).

^{b,c}Means in the same row with different superscripts differ ($P < .05$).

on fresh muscle samples according to Culler et al. (1978).

Samples for determination of lysosomal enzyme activities were frozen in liquid nitrogen after 0, 1, 3, 7 or 14 d postmortem aging and stored at -70°C until extraction. Muscle extracts were prepared from 5 g of LM according to Etherington et al. (1987). The homogenate was allowed to stand for 1 h before centrifugation at $25,000 \times g$ for 30 min to remove debris. The supernatant fluid was filtered through glass wool, and 2 ml of the supernatant fluid was allowed to react (end-over-end mixing) for 2 h with 2 ml of S-carboxymethylated-papain-Sepharose (Koochmariaie and Kretchmar, 1990) in a mini-column¹¹. The resin was prepared by coupling CNBr-activated Sepharose¹² to papain¹³ according to Anastasi et al. (1983). The sample was eluted and the resin was washed with 8 ml of buffer according to Koochmariaie and Kretchmar (1990). Protein concentration of the pre- and post-column supernatant fluids was deter-

mined spectrophotometrically with Bicinchoninic Acid (BCA) protein assay reagent¹⁴ according to Smith et al. (1985). Activities of cathepsins B and B + L were determined according to Kirschke et al. (1983) as modified by Koochmariaie and Kretchmar (1990) using amino-methyl coumarin as a fluorescent tag on the substrates, Z-Arg-Arg-NMec and Z-Phe-Arg-NMec (where Z = benzyloxycarbonyl and NMec = 4-methyl-7-coumarylamide) with a 15-min incubation at 37°C . Activities were expressed as $\text{nmol}\cdot\text{min}^{-1}\cdot\text{g}$ of muscle⁻¹.

An analysis of variance (Steel and Torrie, 1980) for a 2 (breed cross) \times 2 (slaughter group) randomized complete block design was used to analyze all carcass data according to procedures outlined by SAS (1985). Time postmortem was included in the model as a sub-plot for all postmortem aging traits. When the main effect or interaction was significant, means were separated using least squares procedures (Montgomery, 1984). The predetermined level of significance was $P < .05$.

Results and Discussion

There were no differences ($P > .05$) in live weight, hot carcass weight, dressing percentage, ribeye area, percentage kidney, pelvic and heart fat, skeletal maturity, overall maturity and lean firmness between breed crosses (Table 1). Crouse et al. (1989) observed

¹¹Bio-Rad Econo-column, Bio-Rad Laboratories, Richmond, CA.

¹²Pharmacia LKB, 800 Centennial Ave., Piscataway, NJ.

¹³Sigma Chemical Co., P. O. Box 14508, St. Louis, MO.

¹⁴Product 23225, Pierce, Rockford, IL.

decreased final weights in cattle possessing greater than 25% *Bos indicus* inheritance, although Koch et al. (1982) observed heavier weights in F1 Brahman crossbreds relative to AH crossbreds at constant ages. The AH crossbred carcasses had higher measured and adjusted fat thicknesses, USDA yield grades and marbling scores ($P < .05$). In similar findings, Crouse et al. (1989) reported that Angus-Hereford crossbreds had higher adjusted fat thicknesses and marbling scores than Brahman crosses. Whipple et al. (1990) found no difference in marbling scores between 5/8 Sahiwal crossbreds and AH crossbreds. Brahman crossbred carcasses had darker, coarser-textured lean, more dark, coarse band formation and older lean maturity scores ($P < .05$). Previous research (Campion et al., 1975; Crouse et al., 1978, 1989) indicated that differences in lean color and texture within

youthful cattle did not affect palatability characteristics. The presence of slightly detectable dark, coarse bands around the exterior of the LM implies that cold shortening could have occurred. However, these cattle possessed 1) enough fat cover (> 7.6 mm) to prevent cold-induced toughening (Dolezal et al., 1982) and 2) a pH/temperature relationship (pH was below 6.0 well before the muscle temperature was below 10°C) such that cold shortening is doubtful (Lochner et al., 1980).

Temperature and pH of the LM were affected by breed cross and time postmortem (Table 2). Temperature was higher and pH was lower in the AH crossbred carcasses ($P < .05$). Because breed cross and time postmortem interacted to effect pH ($P < .05$), these main effects are meaningless. Although pH was not different between breeds at 0 h postmortem, at 3 and 6 h postmortem, pH was higher in the Brahman crossbred carcasses. At 9, 12 and 24 h postmortem, there again was no difference in pH between breed crosses ($P > .05$). The AH crossbred carcasses reached their ultimate pH by 6 h postmortem, whereas the Brahman crossbred carcasses did not reach their ultimate pH until 9 h postmortem. These differences in pH might have been due to differences in metabolic rates between the two breed types. Catheptic and CDP systems have been shown to be greatly influenced by pH by numerous researchers. Thus, these differences in pH might have been partially responsible for differences in tenderness.

There were no differences between breed crosses in the activities of CDP-I and -II (Table 3). However, CDP inhibitor activity was higher in 5/8 Brahman crossbred carcasses ($P < .05$). Whipple et al. (1990) reported that the activity of CDP inhibitor was higher at 24 h postmortem in 5/8 Sahiwal and 3/8 Sahiwal crossbreds than in cattle possessing only *Bos taurus* inheritance. In the same study, no differences were seen in CDP-I, -II and inhibitor activities at 0 h postmortem and in CDP-I and -II activities at 24 h postmortem. Wheeler et al. (1990) showed that CDP-I activity was greater and CDP inhibitor activity was less in H than in Brahman Cattle. In the present study, CDP-I activity might have been significantly greater in 5/8 Brahman than AH cattle if a greater number of animals had been used. There was no decline in CDP-II activity from 0 to 24 h postmortem ($P > .05$), although CDP-I and inhibitor activities both declined

TABLE 2. TEMPERATURE AND pH OF THE LONGISSIMUS MUSCLE OF ANGUS-HEREFORD AND 5/8 BRAHMAN CROSSBRED HEIFERS AT 0, 3, 6, 9, 12 AND 24 HOURS POSTMORTEM

Item	pH	Temperature, $^{\circ}\text{C}$
Breed cross		
Angus-Hereford	5.71 ^b	22.7 ^a
5/8 Brahman	5.80 ^a	21.6 ^b
SEM	.02	.2
Hour postmortem		
0	6.68 ^a	39.7 ^a
3	5.82 ^b	32.2 ^b
6	5.63 ^c	22.4 ^c
9	5.51 ^d	17.5 ^d
12	5.42 ^d	16.1 ^e
24	5.45 ^d	4.9 ^f
SEM	.03	.4
Interaction		
Probability level	.01	.95
Angus-Hereford, h 0	6.63 ^a	40.1
Angus-Hereford, h 3	5.72 ^c	32.9
Angus-Hereford, h 6	5.48 ^d	23.1
Angus-Hereford, h 9	5.52 ^d	17.9
Angus-Hereford, h 12	5.42 ^d	16.8
Angus-Hereford, h 24	5.45 ^d	5.2
5/8 Brahman, h 0	6.72 ^a	39.3
5/8 Brahman, h 3	5.91 ^b	31.6
5/8 Brahman, h 6	5.79 ^{bc}	21.7
5/8 Brahman, h 9	5.50 ^d	17.1
5/8 Brahman, h 12	5.42 ^d	15.4
5/8 Brahman, h 24	5.44 ^d	4.6
SEM	.05	.5

a,b,c,d,e,f Means in the same column, within a main effect or interaction, with superscripts that do not have a common superscript letter differ ($P < .05$).

TABLE 3. CALCIUM-DEPENDENT PROTEASE (CDP) LEVELS OF THE LONGISSIMUS MUSCLE OF ANGUS-HEREFORD AND 5/8 BRAHMAN CROSSBRED HEIFERS AT 0 AND 24 HOURS POSTMORTEM^a

Item	CDP-I	CDP-II	CDP Inhibitor
Breed cross			
Angus-Hereford	42.3	53.3	131.5 ^c
5/8 Brahman	51.3	57.7	158.1 ^b
SEM	3.5	2.5	8.7
Hour postmortem			
0	64.2 ^c	54.8	181.3 ^b
24	29.4 ^d	56.3	107.4 ^c
SEM	3.5	2.5	8.7
Interaction			
Probability level	.12	.87	.62
Angus-Hereford, h 0	59.2	52.3	170.5
Angus-Hereford, h 24	25.4	54.4	90.5
5/8 Brahman, h 0	69.1	57.2	192.0
5/8 Brahman, h 24	33.4	58.2	124.3
SEM	4.9	3.6	12.3

^aValues expressed are units of enzyme activity per 50 g of muscle.

^{b,c}Means in the same column, within a main effect or interaction, with superscripts that do not have a common superscript letter differ ($P < .05$).

TABLE 4. LYSOSOMAL ENZYME ACTIVITIES OF THE LONGISSIMUS MUSCLE OF ANGUS-HEREFORD AND 5/8 BRAHMAN CROSSBRED HEIFERS AT 0, 1, 3, 7 AND 14 DAYS POSTMORTEM^a

Item	Pre-column cathepsin B	Pre-column cathepsin B + L	Post-column cathepsin B	Post-column cathepsin B + L
Breed cross				
Angus-Hereford	42.7	70.5	41.9	144.7
5/8 Brahman	44.9	66.9	41.7	140.0
SEM	2.2	4.0	1.3	4.7
Day postmortem				
0	42.1	68.8	45.5	144.3
1	38.1	65.9	43.4	147.4
3	41.7	66.3	40.0	138.3
7	48.3	69.9	40.6	140.1
14	48.7	72.6	39.3	141.5
SEM	3.6	6.3	2.1	7.4
Interaction				
Probability level	.77	.93	.98	.72
Angus-Hereford, d 0	41.0	72.5	45.4	150.4
Angus-Hereford, d 1	36.4	67.3	43.6	148.3
Angus-Hereford, d 3	42.1	70.1	40.9	139.0
Angus-Hereford, d 7	43.5	68.5	39.1	135.2
Angus-Hereford, d 14	50.2	74.3	40.2	150.4
5/8 Brahman, d 0	43.2	65.2	45.6	138.3
5/8 Brahman, d 1	39.8	64.5	43.1	146.6
5/8 Brahman, d 3	41.3	62.6	39.2	137.6
5/8 Brahman, d 7	53.1	71.4	42.1	144.9
5/8 Brahman, d 14	47.1	70.9	38.4	132.6
SEM	5.0	9.0	3.0	10.4

^aThere were no significant differences ($P < .05$). Values expressed are nmole amino-methylcoumarin-min⁻¹.g of muscle⁻¹.

from 0 to 24 h postmortem ($P < .05$). There was no significant interaction between breed cross and time postmortem for the activities of CDP-I, -II and inhibitor ($P > .05$).

Cathepsin B and cathepsin B + L activities both pre- and post-column were not affected ($P > .05$) by breed cross, postmortem aging or the interaction of breed cross with postmortem aging (Table 4). Whipple et al. (1990) found no difference in cathepsin B and B + L activity between AH and Sahiwal \times AH cattle at 1 and 14 d postmortem. Moreover, Wheeler et al. (1990) found that Brahman and H cattle did not differ in cathepsin B and B + L activity either at 0 or at 14 d postmortem.

Cooking loss and cooking rate were not affected by breed cross ($P > .05$), postmortem aging or the interaction of breed cross with postmortem aging (Table 5). Shear force was higher and MFI values were lower for the 5/8 Brahman crossbreds ($P < .05$). Shear force has been reported to be higher in cattle of *Bos indicus* inheritance at 1, 14 (Whipple et al., 1990) and 7 d (Crouse et al., 1989) postmortem. Whipple et al. (1990) reported that MFI values were lower in 5/8 Sahiwal than in AH

crossbreds at 1, 3, 7 and 14 d postmortem. Wheeler et al. (1990) reported that shear force was higher for meat from Brahman than for meat from Hereford cattle. In the present study, shear force was highest at d 1 and lowest at d 14, whereas MFI values were lower at d 1 and 3 than on d 7 and 14 ($P < .05$). Breed cross and postmortem aging time did not interact to affect shear force or MFI values ($P > .05$).

Differences in fiber type may result in differences in tenderness between breed by affecting collagen density. However, Whipple et al. (1990) reported that total and percentage soluble collagen did not differ between AH and Sahiwal \times AH cattle. In their study, no differences were detected between breed types for fiber type distribution or fiber area.

Implications

The activity of calcium-dependent protease inhibitor is related to aging. The increased activity of calcium-dependent protease inhibitor in carcasses from Brahman crossbred cattle may account for differences in tenderness

TABLE 5. COOKING LOSS, COOKING RATE, SHEAR FORCE AND MYOFIBRIL FRAGMENTATION INDICES OF THE LONGISSIMUS MUSCLE OF ANGUS-HEREFORD AND 5/8 BRAHMAN CROSSBRED HEIFERS AT 1, 3, 7 AND 14 DAYS POSTMORTEM

Item	Cooking loss, %	Cooking rate, g/min	Shear force, kg	Myofibril fragmentation index
Breed cross				
Angus-Hereford	23.2	9.3	6.3 ^b	58.7 ^a
5/8 Brahman	21.8	9.3	7.4 ^a	53.1 ^b
SEM	.7	.3	.3	2.0
Day postmortem				
1	22.0	9.1	8.6 ^a	40.5 ^b
3	21.9	9.4	7.4 ^b	47.9 ^b
7	22.0	9.8	6.3 ^b	64.9 ^a
14	24.1	8.9	4.9 ^c	70.4 ^a
SEM	1.0	.4	.4	2.9
Interaction				
Probability level	.96	.64	.87	.35
Angus-Hereford, d 1	22.7	8.8	8.0	45.6
Angus-Hereford, d 3	23.0	9.1	6.6	53.6
Angus-Hereford, d 7	22.4	10.2	6.0	64.5
Angus-Hereford, d 14	24.8	9.1	4.5	71.3
5/8 Brahman, d 1	21.3	9.4	9.2	35.4
5/8 Brahman, d 3	20.8	9.6	8.2	42.2
5/8 Brahman, d 7	21.7	9.5	6.7	65.3
5/8 Brahman, d 14	23.4	8.8	5.4	69.6
SEM	1.4	.6	.6	4.1

^{a,b,c}Means in the same column, within a main effect or interaction, with superscripts that do not have a common superscript letter differ ($P < .05$).

between *Bos indicus* breed crosses and *Bos taurus* breed crosses. Therefore, development of methods to improve the tenderness of beef from Brahman crossbred cattle should involve decreasing the activity of calcium-dependent protease inhibitor.

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