

# EVALUATION OF ATTRIBUTES THAT AFFECT LONGISSIMUS MUSCLE TENDERNESS IN *BOS TAURUS* AND *BOS INDICUS* CATTLE<sup>1,2</sup>

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## ABSTRACT

Biological tenderness differences between longissimus muscles (LM) from *Bos indicus* and *Bos taurus* breeds were evaluated. Steers and heifers of Hereford × Angus (H × A, n = 10), 3/8 Sahiwal × H, A or H × A (3/8 SAH, n = 6) and 5/8 Sahiwal × H, A or H × A (5/8 SAH, n = 11) crosses were utilized. Muscle temperature and pH were monitored every 3 h for the first 12 h and at 24 h. Samples were obtained within 1 h and at 24 h postmortem from the LM for determination of calcium-dependent protease (CDP) -I and -II and CDP inhibitor (INH) activities. At 1 and 14 d postmortem, LM samples were removed for determining cathepsin B and B + L activity, soluble and total collagen, sarcomere length, muscle-fiber histochemistry, shear force and sensory-panel traits. Data were analyzed using least squares procedures with fixed effects of breed cross, sex and their interaction. No significant breed cross effects were observed for carcass traits or rates of pH and temperature decline. Steaks from H × A had lower ( $P < .05$ ) shear-force values and higher ( $P < .05$ ) sensory scores for tenderness at 1 and 14 d postmortem than steaks from 3/8 and 5/8 SAH. Correspondingly, 5/8 SAH had lower ( $P < .05$ ) myofibril fragmentation indices than H × A at 1, 3, 7 and 14 d postmortem. Breed cross effects were not significant for sarcomere length, fiber types, soluble and total collagen, cathepsin B and B + L specific activity, CDP-I and -II activity and INH activity within 1 h postmortem. However, INH total activity/100 g of muscle was greater ( $P < .01$ ) at 24 h postmortem for 5/8 SAH ( $208.8 \pm 14.8$ ) and 3/8 SAH ( $195.6 \pm 19.3$ ) than for H × A ( $136.3 \pm 14.9$ ). For H × A, SDS-PAGE revealed that by d 1 desmin had been subjected to proteolysis, and by d 14 desmin could not be detected, but a 30,000-dalton component was clearly evident. However, in 5/8 SAH, desmin remained visible at d 14 without a 30,000-dalton component appearing. This reduced protein hydrolysis may account for less tender meat in SAH; INH apparently influences this process.

(Key Words: Cattle, Tenderness, Proteases, Histochemistry, Protein Degradation, Aging.)

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## Introduction

Cattle producers utilize crossbreeding programs to take advantage of hybrid vigor. *Bos indicus* breeds are used frequently to maximize heterosis, especially in semitropical and tropical climates where these breeds provide additive advantages for heat and disease resistance (Cole et al., 1963; Crockett et al., 1979). However, meat from *Bos indicus* breeds of cattle often is less tender than meat from *Bos taurus* cattle (Koch et al., 1982; McKeith et

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al., 1985b; Crouse et al., 1987, 1989). Because tenderness is a major palatability trait that determines consumer acceptability, it is important to understand what causes meat from these animals to be less tender.

There have been many attempts to explain the variation in beef tenderness. The effects of animal age (Davis et al., 1979), breed (Koch et al., 1982), nutritional regimen (Dikeman et al., 1985), days fed high-concentrate diets (Tatum et al., 1980) and the use of growth promotants (Unruh et al., 1986) on meat tenderness have been explored. In such cases, factors that influence tenderness may include USDA quality grade, USDA yield grade, rates of pH and temperature decline, sarcomere length, total and soluble collagen, muscle histochemical traits, and activity of proteases thought to be involved in postmortem tenderization (calcium-dependent protease and cathepsins). These variables all have some relationship to tenderness; however, most studies evaluating tenderness variation have been narrowly focused. Our objectives were to examine all of these biological traits in an attempt to explain why meat from *Bos indicus* crossbred cattle is less tender than meat from *Bos taurus* cattle.

#### Materials and Methods

**Experimental Animals.** Seven heifers and four steers of 5/8 Sahiwal  $\times$  Hereford (H), Angus (A) or H  $\times$  A (5/8 SAH), three heifers and three steers of 3/8 Sahiwal  $\times$  H, A or H  $\times$  A (3/8 SAH) and five heifers and five steers of H  $\times$  A crosses were utilized. Calves were weaned at 6 to 8 mo of age and fed an alfalfa haylage and corn silage diet for 4 mo. Cattle then were fed a corn and corn silage finishing diet until 15 to 17 mo of age. Cattle were selected randomly and slaughtered (three to four animals each week for a 7-wk period). This was done so that enough time and instruments would be available to assay for calcium-dependent protease activity on fresh muscle samples. Within 1 h postmortem, longissimus muscle (LM) samples were taken from alternate sides at the fifth to sixth rib region. Alternate sides were utilized for sampling to account for any variation that may occur in rate of temperature decline because of different amounts and positions of kidney and pelvic fat. Carcasses were not electrically stimulated but were chilled at  $-1^{\circ}\text{C}$  for 24 h. Carcass data (USDA, 1976) were collected; the

semitendinosus (ST; center portion) and LM (sixth rib to the fifth lumbar region) muscles were removed from the same sides that were previously sampled.

**Temperature and pH Determinations.** Temperature of the LM and ST and pH of the LM were determined at < 1, 3, 6, 9, 12 and 24 h postmortem. The procedure of Bendall (1973) was used to determine pH on 2.5 g of tissue. Temperature of the LM was monitored using an electronic digital probe at the 11th rib region. Three temperature measurements were recorded at approximately 5.7, 2.5 and .3 cm depths from the dorsal surface of the LM, and an average temperature was calculated. Temperatures of the ST were determined by a thermometer located in the center of the muscle for 24 h.

**Calcium-Dependent Protease and Inhibitor Activities.** Activities of calcium-dependent protease (CDP)-I and -II and CDP inhibitor (INH) were determined on 100 g of fresh LM at < 1 h and 24 h postmortem according to the procedures of Koochmarai et al. (1989). Proteins were eluted at the rate of 30 ml/h with an 840-ml linear gradient (0 to 500 mM NaCl in elution buffer; Figure 1). The INH was eluted at approximately 75 mM NaCl (fractions 45 to 62), whereas CDP-I was eluted at 125 mM NaCl (fractions 67 to 78); CDP-II was eluted in fractions 105 to 118. Activities were expressed as the amount of CDP caseinolytic activity in 100 g of muscle. One unit of DEAE-sephacel-purified-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  $25^{\circ}\text{C}$ . One unit of INH activity was defined as the amount that inhibited 1.0 unit of DEAE-sephacel-purified CDP-II activity.

**Cathepsin B and B + L Activities.** Lysosomal-enriched fractions were prepared from 5 g of LM taken at 1 and 14 d postmortem according to the method of Bechet et al. (1986). Protein concentration of the supernatant was determined according to Markwell et al. (1978). Activities of cathepsins B and B + L were assayed according to procedures of Kirschke et al. (1983), using amino-methylcoumarin 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 30-min incubation at  $37^{\circ}\text{C}$ . Specific activities were expressed as pmole-mg of pro-

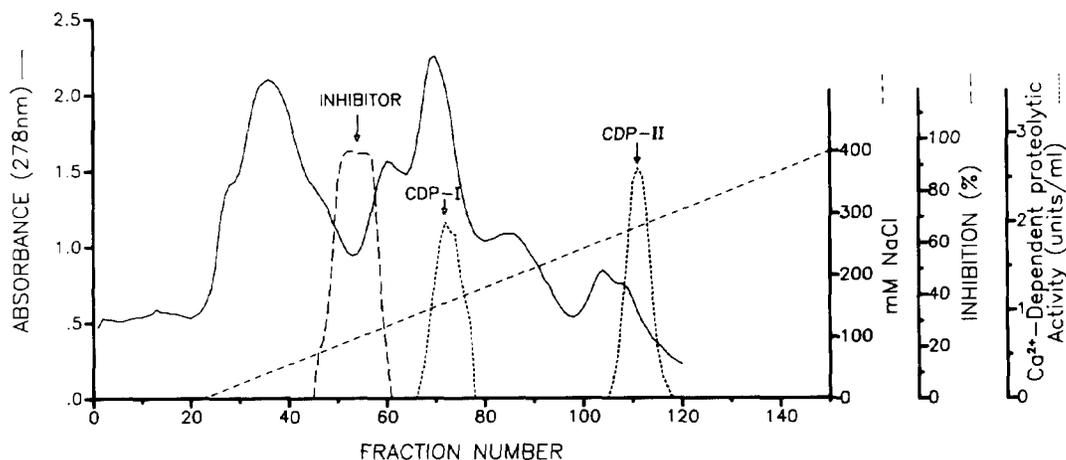


Figure 1. Elution profile of calcium-dependent protease (CDP)-I, CDP-II and inhibitor from DEAE-Sephacel column. The bound proteins were eluted at the rate of 30 ml/h with an 840 ml linear gradient of 0 to 400 mM NaCl in elution buffer.

tein<sup>-1</sup>·min<sup>-1</sup>.

**Myofibril Fragmentation Index Measurements.** Samples were excised from the LM at 24 h postmortem and were vacuum-aged for 3, 7 and 14 d. Myofibril fragmentation indices (MFI) were determined on fresh muscle at 1, 3, 7 and 14 d postmortem by the method of Culler et al. (1978).

**Determination of Water-Soluble Calcium and Zinc.** Water-extractable calcium and zinc were determined on 5 g of muscle according to the method of Nakamura (1973a), using specific atomic absorption.

**Muscle-Fiber Histochemistry and Fiber Diameter Determinations.** The simultaneous histochemical procedure of Solomon and Dunn (1988) was slightly modified. Longissimus samples were removed from the center of the muscle at the eighth rib region at 24 h postmortem. Several 1-cm<sup>3</sup> samples were frozen on cork in isopentane at -147°C and stored at -70°C. Cryostat sections, 10 μm thick, were cut and allowed to air dry. A 45-min, β-NADH (pH 7.4) incubation (37°C) was applied prior to a 5-min acid incubation (pH 4.1). An ATPase incubation (pH 9.4) was conducted for 30 min at 37°C. Fibers were classified as βR, αR or αW according to Ashmore and Doerr (1971). Fiber areas were measured by Microcomp PM<sup>7</sup> interactive image analysis for planar morphometry. Fiber

diameters were calculated from the area measured for each fiber. The area occupied by each of the three fiber types was reported as a percentage of total area measured.

**Sarcomere Length Measurements.** Sarcomere lengths were determined on fixed LM obtained at 24 h postmortem using a neon laser according to Cross et al. (1981).

**Total and Soluble Collagen Content.** Samples of LM were analyzed on d 1 and 14 postmortem for soluble and insoluble collagen (Hill, 1966). Hydroxyproline content was determined by the method of Bergman and Loxley (1963). Hydroxyproline contents of the soluble and insoluble portions were multiplied by factors of 7.52 and 7.25, respectively, according to Cross et al. (1973). Values are reported per gram of wet tissue.

**Muscle Composition.** Fat and dry matter percentages were determined on 3 to 5 g of LM taken at 24 h postmortem according to AOAC (1975) procedures.

**Myofibril Isolation and SDS-PAGE.** Myofibrils were isolated according to the method of Olson et al. (1976) at < 1 h, 1 and 14 d postmortem; protein concentration was determined by biuret procedures. Electrophoretic techniques of Laemmli (1970) were followed. Myofibrillar proteins were separated using a discontinuous gradient slab gel with 7.5 to 15% acrylamide. The acrylamide to bisacrylamide ratio was 37.5:1. The stacking gel consisted of 4% acrylamide. Eighty micro-

<sup>7</sup>Southern Micro Instruments, Atlanta, GA.

grams of protein were applied to each well and 8 milliamperes (mA) were used per gel until protein samples had focused; then current was increased to 24 mA/gel.

**Warner-Bratzler Shear and Sensory-Panel Evaluations.** At 24 h postmortem, six steaks, 2.54 cm thick, were removed from the loin beginning at approximately the first lumbar vertebra, vacuum-packaged, aged for the appropriate time period (1 or 14 d) and frozen at -30°C. Steaks were thawed and cooked on Farberware open-hearth broilers to an endpoint temperature of 70°C. For Instron Warner-Bratzler shear (WBS) determinations, steaks were tempered for approximately 24 h at refrigerator temperature. Six 1.3-cm cores were removed parallel to the muscle fibers with a hand coring device. For sensory-panel-tenderness scores, cooked steaks were wrapped in foil and held at 70°C (<30 min) in a preheated oven. In four sessions (two slaughter groups/session), eight panelists evaluated three (1.3 × 1.3 × 2.5 cm) cubes/steak with an 8-point scoring system according to procedures outlined by AMSA (1978).

**Statistical Analysis.** Analysis of variance was performed using the General Linear Models procedure of SAS (1985) that included the fixed effects of breed, sex and their interaction. Means were separated by least squares procedures.

**Results and Discussion**

The cattle utilized had similar ( $P > .10$ ) USDA (1976) quality and yield grades, with averages of high Select and 3.2, respectively. Also, no differences ( $P > .05$ ) were found for lean color; lean firmness; lean texture; maturity scores; dressing percentage and percentage of kidney, pelvic and heart fat (data not presented). Least squares means and standard deviations for growth, carcass and LM chemical traits are given in Table 1. Breed cross effects were observed for average daily gain, with 5/8 SAH gaining the slowest ( $P < .01$ ). The H × A crosses were heavier ( $P < .05$ ) at slaughter than 5/8 SAH (502 vs 448 kg, respectively). There were no breed cross marbling differences, which contradicts some reports. Cole et al. (1963) reported differences in marbling and carcass grades between *Bos taurus* and *Bos indicus* cattle, with marbling scores of high Modest and avg Slight, respectively. Crouse et al. (1989) found that as

TABLE 1. LEAST SQUARES MEANS (±SE) FOR GROWTH, CARCASS AND CHEMICAL TRAITS BY BREED CROSS, AND BREED × SEX INTERACTION

Breed	N	Traits <sup>a</sup>							
		Avg daily gain, kg	Slaughter wt, kg	Hot carcass wt, kg	Adjusted fat thickness, cm	Ribeye area, cm <sup>2</sup>	Marbling <sup>b</sup>	Ether extract, %	Heat ring <sup>c</sup>
H × A	11	.96 ± .03 <sup>h</sup>	476 ± 20 <sup>de</sup>	288 ± 13 <sup>eg</sup>	1.43 ± .17 <sup>def</sup>	74.2 ± 3.6 <sup>d</sup>	351 ± 37	5.4 ± .6	6.6 ± .4 <sup>def</sup>
3/8 SAH	6	.88 ± .04 <sup>h</sup>	529 ± 20 <sup>d</sup>	329 ± 13 <sup>d</sup>	1.57 ± .17 <sup>de</sup>	75.8 ± 3.6 <sup>d</sup>	276 ± 48	4.0 ± .8	7.6 ± .4 <sup>d</sup>
5/8 SAH	6	.81 ± .03 <sup>i</sup>	500 ± 26 <sup>de</sup>	320 ± 16 <sup>de</sup>	1.93 ± .22 <sup>d</sup>	77.6 ± 4.7 <sup>d</sup>	357 ± 37	4.7 ± .6	6.0 ± .5 <sup>ef</sup>
Breed × sex			438 ± 26 <sup>ef</sup>	267 ± 16 <sup>g</sup>	.81 ± .22 <sup>f</sup>	64.5 ± 4.7 <sup>de</sup>			5.7 ± .5 <sup>f</sup>
H × A-heifer	5		417 ± 17 <sup>f</sup>	264 ± 11 <sup>g</sup>	1.27 ± .14 <sup>ef</sup>	64.3 ± 3.1 <sup>e</sup>			7.1 ± .4 <sup>de</sup>
H × A-steer	5		479 ± 22 <sup>de</sup>	302 ± 14 <sup>def</sup>	1.10 ± .19 <sup>f</sup>	76.8 ± 4.1 <sup>d</sup>			6.8 ± .5 <sup>def</sup>
3/8 SAH-heifer	3								
3/8 SAH-steer	3								
5/8 SAH-heifer	7								
5/8 SAH-steer	4								

<sup>a</sup>Traits without a superscript letter did not differ ( $P > .05$ ) for main effect in column.

<sup>b</sup>USDA marbling score: 200-299 = slight, 300-399 = small.

<sup>c</sup>Presence of heat ring: 1 = severe, . . . 8 = none.

<sup>d,e,f,g</sup>Means within a column without a common superscript letter differ ( $P < .05$ ).

<sup>h,i</sup>Means within a column without a common superscript letter differ ( $P < .01$ ).

TABLE 2. BREED LEAST SQUARES MEANS ( $\pm$ SE) FOR WARNER-BRATZLER SHEAR VALUES, COOKING LOSSES, AND SENSORY-PANEL SCORES OF LONGISSIMUS STEAKS

Traits	Breed cross		
	H $\times$ A	3/8 SAH	5/8 SAH
Day 1			
Shear force, kg	7.0 $\pm$ .5 <sup>b</sup>	9.3 $\pm$ .7 <sup>c</sup>	9.6 $\pm$ .5 <sup>c</sup>
Cooking loss, %	27.7 $\pm$ 1.0 <sup>b</sup>	30.0 $\pm$ 1.3 <sup>bc</sup>	31.6 $\pm$ 1.0 <sup>c</sup>
Juiciness <sup>a</sup>	5.3 $\pm$ .1	5.2 $\pm$ .2	5.1 $\pm$ .1
Flavor intensity <sup>a</sup>	4.6 $\pm$ .1	4.6 $\pm$ .1	4.6 $\pm$ .1
Ease of fragmentation <sup>a</sup>	4.7 $\pm$ .2 <sup>e</sup>	3.7 $\pm$ .2 <sup>f</sup>	3.6 $\pm$ .2 <sup>f</sup>
Connective tissue amount <sup>a</sup>	4.4 $\pm$ .2 <sup>e</sup>	3.4 $\pm$ .2 <sup>f</sup>	3.4 $\pm$ .2 <sup>f</sup>
Overall tenderness <sup>a</sup>	4.6 $\pm$ .2 <sup>e</sup>	3.6 $\pm$ .3 <sup>f</sup>	3.6 $\pm$ .2 <sup>f</sup>
Day 14			
Shear force, kg	4.7 $\pm$ .3 <sup>b</sup>	6.4 $\pm$ .4 <sup>c</sup>	7.7 $\pm$ .3 <sup>d</sup>
Cooking loss, %	26.1 $\pm$ 1.0	28.0 $\pm$ 1.3	29.6 $\pm$ 1.0
Juiciness <sup>a</sup>	5.4 $\pm$ .1	5.3 $\pm$ .1	4.8 $\pm$ .1
Flavor intensity <sup>a</sup>	4.9 $\pm$ .1	4.7 $\pm$ .1	4.7 $\pm$ .1
Ease of fragmentation <sup>a</sup>	6.0 $\pm$ .2 <sup>b</sup>	5.0 $\pm$ .2 <sup>c</sup>	4.4 $\pm$ .2 <sup>d</sup>
Connective tissue amount <sup>a</sup>	5.9 $\pm$ .2 <sup>e</sup>	4.7 $\pm$ .2 <sup>f</sup>	4.2 $\pm$ .2 <sup>f</sup>
Overall tenderness <sup>a</sup>	5.9 $\pm$ .2 <sup>b</sup>	5.0 $\pm$ .2 <sup>c</sup>	4.4 $\pm$ .2 <sup>d</sup>

<sup>a</sup>A score of 6 = moderately juicy, moderately intense, moderately easy, traces and moderately tender; . . . 4 = slightly dry, slightly bland, slightly difficult, moderate and slightly tough; . . . 1 = extremely dry, extremely bland, extremely difficult, abundant and extremely tough.

<sup>b,c,d</sup>Means within a row without a common superscript letter differ ( $P < .05$ ).

<sup>e,f</sup>Means within a row without a common superscript letter differ ( $P < .01$ ).

percentage of Brahman or Sahiwal inheritance increased, carcass weights and marbling decreased. In addition, *Bos indicus* crosses have been reported to have less tender meat than *Bos taurus* crosses, even when marbling scores are held constant (Koch et al., 1988).

A sex effect was observed for adjusted fat thickness; heifers had more ( $P < .05$ ) fat cover (1.55 vs 1.16 cm) than steers. Significant sex  $\times$  breed group interactions occurred for slaughter weight, hot-carcass weight, adjusted-fat thickness, ribeye area and incidence of heat ring (Table 1). The H  $\times$  A steers were heavier at slaughter ( $P < .05$ ; 529 kg) than 3/8 SAH steers (438 kg) and 5/8 SAH heifers (417 kg); other sex  $\times$  breed cross groups were intermediate in weight. For hot-carcass weights, 3/8 SAH steer (267 kg) and 5/8 SAH heifer (264 kg) carcasses were lighter ( $P < .05$ ) than carcasses of H  $\times$  A steers and 3/8 SAH heifers. The 3/8 SAH heifers had more ( $P < .05$ ) fat cover than the other *Bos indicus* crosses, with H  $\times$  A possessing an intermediate amount of finish. The 5/8 SAH heifers and 3/8 SAH steers had similar ribeye areas, but 5/8 SAH heifer ribeyes were smaller ( $P < .05$ ) than those from the other breed crosses. The incidence of heat ring occurred more frequently in 3/8 SAH steers (lower numeric

score;  $P < .05$ ) than in H  $\times$  A steers and 5/8 SAH heifers, likely because of less fat cover on 3/8 SAH steer carcasses.

No breed cross differences ( $P > .10$ ) occurred for temperature and pH or temperature with less than 2/10 difference in pH among the breed crosses at any of the time periods (data not presented). Overall rates of temperature decline in LM and ST (Figure 2) show that temperature in ST was initially ( $< 1$  h) lower; however, ST temperature was higher after 6 h postmortem. Twenty-four-hour temperatures of the ST and LM were 4.8 and 1.3°C, respectively. In the LM, 3-h pH was 6.4 and ultimate pH was 5.5 (Figure 3); these are in the normal range (Bouton, 1973).

Sensory panel evaluation scores and WBS values for LM steaks are given in Table 2. The H  $\times$  A crosses had lower ( $P < .05$ ) WBS values and higher ( $P < .01$ ) sensory panel scores for ease of fragmentation, connective-tissue amount and overall tenderness at 1 and 14 d postmortem. These breed cross differences agree with those reported by Koch et al. (1982). By d 14, 3/8 SAH were more tender than 5/8 SAH, which indicates a greater response to aging. Steaks from H  $\times$  A and 3/8 SAH had approximately a 30% decrease in WBS values between 1 and 14 d of aging.

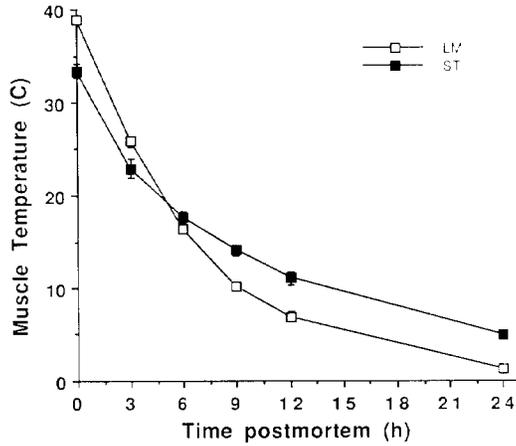


Figure 2. Overall temperature decline of longissimus and semitendinosus muscles.

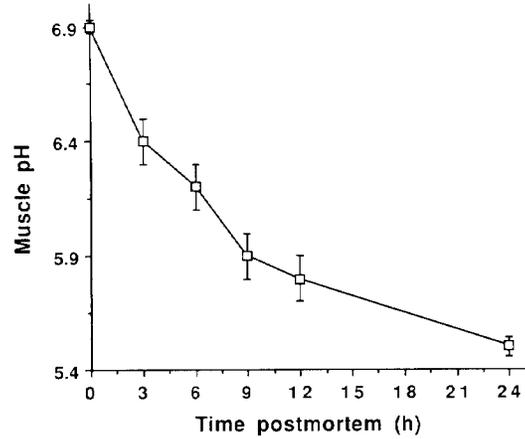


Figure 3. Longissimus muscle pH decline.

However, WBS values for 5/8 SAH steaks decreased only 20% and were still unacceptable (> 5.0 kg) after 14 d of aging. In addition, 5/8 SAH had a higher ( $P < .05$ ) cooking loss than  $H \times A$  at d 1 but no differences were seen at d 14. Our WBS results agree closely with those of Crouse et al. (1989) in which WBS values at 7 d postmortem were 4.4, 5.6, 6.6, and 8.4 kg for  $H \times A$ , 1/4 SAH, 1/2 SAH and 3/4 SAH crosses, respectively.

Table 3 reveals that breed crosses were similar ( $P > .05$ ) for semitendinosus steak WBS values and cooking loss at either 1 or 14 d postmortem. Therefore, it appears that tenderness differences between *Bos indicus* and *Bos taurus* are greater for certain muscles than for others. However, there was a trend ( $P = .08$ ) for 5/8 SAH steaks to be less tender at d 14. Dransfield et al. (1981) found that the ST initially was slightly less tender than the LM, but after aging the LM was only half as tender

as the ST. In that same study, a 5°C rise in temperature produced a significant increase in the rate of tenderization. These results indicate that more research needs to be conducted on enzyme activity and its relationship to tenderness of the ST and other muscles.

Longissimus tenderness is highly correlated with MFI (Olson et al., 1976; Koohmaraie et al., 1987). Figure 4 contains MFI for LM of breed cross at 1, 3, 7 and 14 d postmortem. The  $H \times A$  had higher ( $P < .05$ ) MFI values than 5/8 SAH at all times. Furthermore, 3/8 SAH had higher ( $P < .05$ ) MFI values than 5/8 SAH at d 7 and d 14. At d 14, 5/8 SAH had only a slightly larger MFI value than  $H \times A$  had at d 1 postmortem. This is reflected also in LM WBS values, which were similar at d 14 for 5/8 SAH and d 1 for  $H \times A$ . Because many changes occur with postmortem aging within the first 24 h (Marsh et al., 1981; Koohmaraie et al., 1987), we could speculate that the LM of  $H \times A$  already may have undergone

TABLE 3. BREED LEAST SQUARES MEANS ( $\pm$ SE) FOR WARNER-BRATZLER SHEAR VALUES AND COOKING LOSSES OF SEMITENDINOSUS STEAKS<sup>a</sup>

Traits	Breed cross		
	$H \times A$	3/8 SAH	5/8 SAH
Day 1, shear force, kg	5.8 $\pm$ .3	6.5 $\pm$ .4	6.1 $\pm$ .3
Day 14, shear force, kg	4.4 $\pm$ .3	4.9 $\pm$ .3	5.3 $\pm$ .3
Day 1, cooking loss, %	37.8 $\pm$ 1.0	38.1 $\pm$ 1.2	39.9 $\pm$ .9
Day 14, cooking loss, %	34.8 $\pm$ 1.4	35.2 $\pm$ 1.8	37.5 $\pm$ 1.4

<sup>a</sup>No differences ( $P > .05$ ).

TABLE 4. BREED LEAST SQUARES MEANS ( $\pm$ SE) FOR LONGISSIMUS SARCOMERE LENGTH, CALCIUM AND ZINC CONTENT, COLLAGEN AND CATHEPSIN B AND B + L SPECIFIC ACTIVITY<sup>a</sup>

Traits	Breed cross		
	H x A	3/8 SAH	5/8 SAH
Day 1			
Sarcomere length, $\mu$ m	1.83 $\pm$ .06	1.76 $\pm$ .08	1.75 $\pm$ .06
Calcium content, $\mu$ g/g	10.8 $\pm$ .7	8.6 $\pm$ .7	9.5 $\pm$ .5
Zinc content, $\mu$ g/g	9.5 $\pm$ .5	8.5 $\pm$ .6	8.9 $\pm$ .5
Soluble collagen, %	13.6 $\pm$ .6	14.1 $\pm$ .8	14.2 $\pm$ .6
Total collagen, mg/g	2.9 $\pm$ .1	2.6 $\pm$ .1	2.8 $\pm$ .1
Cathepsin B, pmol-mg <sup>-1</sup> .min <sup>-1</sup>	32.4 $\pm$ 2.9	27.9 $\pm$ 3.8	34.3 $\pm$ 2.9
Cathepsin B + L, pmol-mg <sup>-1</sup> .min <sup>-1</sup>	41.7 $\pm$ 2.7	38.3 $\pm$ 3.5	44.4 $\pm$ 2.7
Day 14			
Soluble collagen, %	16.7 $\pm$ 1.2	14.1 $\pm$ 1.4	16.5 $\pm$ 1.1
Total collagen, mg/g	3.1 $\pm$ .1	2.7 $\pm$ .2	3.0 $\pm$ .1
Cathepsin B, pmol-mg <sup>-1</sup> .min <sup>-1</sup>	30.5 $\pm$ 1.8	29.0 $\pm$ 2.5	34.7 $\pm$ 1.7
Cathepsin B + L, pmol-mg <sup>-1</sup> .min <sup>-1</sup>	41.5 $\pm$ 3.3	41.0 $\pm$ 4.6	46.6 $\pm$ 3.1

<sup>a</sup>No differences ( $P > .05$ ).

tenderization prior to the 24-h tenderness measurements.

Sarcomere length is related to tenderness, especially in cases of severe shortening (Marsh and Leet, 1966; Harris, 1976). Because no significant sarcomere length differences were observed in our study (Table 4), this factor did not contribute to breed cross differences in tenderness. Sarcomere lengths ranged from 1.75 to 1.83  $\mu$ m, which are similar to LM sarcomere measurements made by Hunt and Hedrick (1977) and McKeith et al. (1985a).

Likewise, no differences ( $P > .05$ ) were found among breed crosses for total and percentage of soluble collagen at 1 and 14 d postmortem (Table 4). Therefore, we concluded that neither solubility nor quantity of collagen were contributing factors in the tenderness differences observed among breed crosses in our study. Other researchers have found that collagen characteristics were not significantly related to tenderness, especially within the same muscle from animals similar in age (Dransfield, 1977; Seideman et al., 1987). Thus, myofibrillar components may be the major contributor to tenderness variation of the animals in this study. Researchers that have reported a relationship between collagen solubility and tenderness (Hall and Hunt, 1982) have looked at treatment effects beyond those in our study.

Differences in the percentages of fiber types and their sizes have been suggested as affecting meat tenderness (Calkins et al., 1981; Ockerman et al., 1984; Seideman and Crouse,

1986). However, we found no differences ( $P > .05$ ) among breed crosses for fiber type percentage or percentage fiber area (Figure 5). Moody et al. (1970) also found that fiber diameter was not related to tenderness. Melton et al. (1975) reported that red-fiber area was related to animal weight and fat deposition, but not to palatability traits. Therefore, from their results and ours, histological traits did not help explain tenderness differences in LM steaks.

In addition to fiber size and number, the mineral content of different fiber types might be related to tenderness. Of particular interest

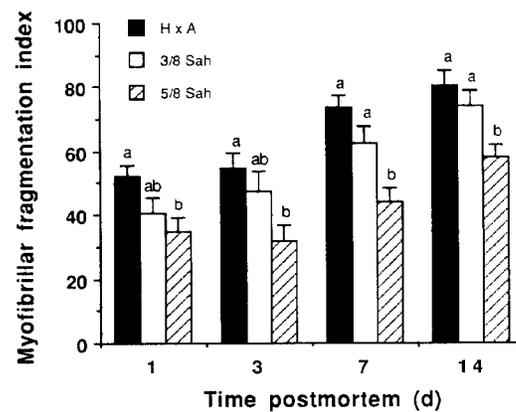


Figure 4. Longissimus myofibril fragmentation indices at 1, 3, 7 and 14 d postmortem by breed cross. Bars without a common superscript letter differ ( $P < .05$ ) within days postmortem.

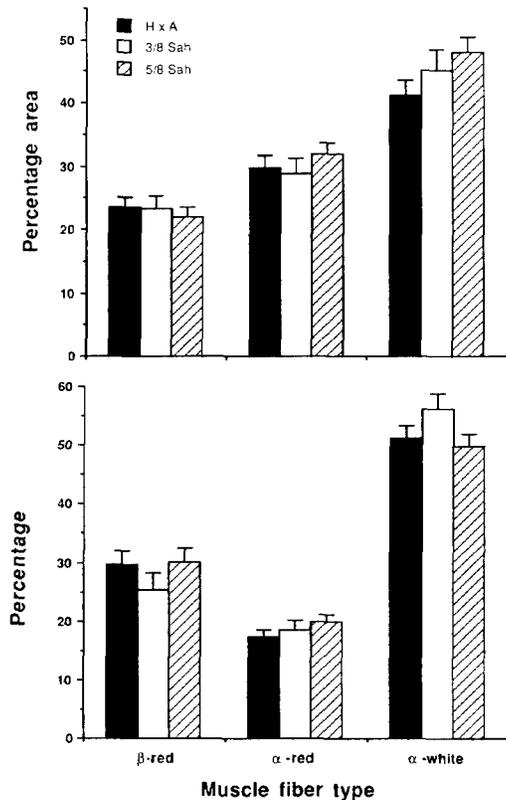


Figure 5. Percentage of fiber area and fiber type percentage of longissimus muscle by breed cross.

is  $\text{Ca}^{2+}$  concentration because of its requirement for CDP activation. The sarcoplasmic reticulum is less developed in red fibers than in white fibers (Gauthier, 1970); therefore, red muscles could contain less  $\text{Ca}^{2+}$ . However, mitochondria, which are more abundant in red muscle, could be supplying free  $\text{Ca}^{2+}$  postmortem (Buege and Marsh, 1975; Mickelson, 1983). We determined water-soluble free  $\text{Ca}^{2+}$  at d 1 postmortem and found no differences ( $P > .05$ ) among breed crosses (Table 4). Therefore,  $\text{Ca}^{2+}$  content does not appear to be a limiting factor.

Guroff (1964) reported that  $\text{Zn}^{2+}$  inhibits CDP activity. Koochmariaie (1990b) found that infusion of ovine carcasses with  $\text{ZnCl}_2$  inhibited the aging response such that CDP-I activity did not decline by d 1 postmortem, as it normally does. We measured  $\text{Zn}^{2+}$  concentrations; no differences ( $P > .05$ ) were found (Table 4). However, because no significant breed cross differences were seen in fiber type

percentage, differences in overall mineral content would not be expected.

Another aspect of tenderization is the activity of the proteases involved in postmortem aging. One proteolytic system that may be involved is the lysosomal enzyme system. Cathepsins are located within the lysosomal membrane and may not be released during the normal storage period. LaCourt et al. (1986) found no indication of lysosomal rupture with  $> 3$  wk of aging. When muscle samples were subjected to exogenous cathepsins B, D and L, degradation of myofibrillar proteins, including myosin and actin, occurred (Schwartz and Bird, 1977; Matsukura et al., 1981; Mikami et al., 1987; Ouali et al., 1988). However, Bandman and Zdanis (1988) failed to detect any myosin degradation from muscle aged for 21 d. It still is controversial whether or not cathepsins are involved in postmortem tenderization under normal conditions. Therefore, we determined whether catheptic-enzyme activity differed between SAH and  $\text{H} \times \text{A}$ . The lysosomes were isolated and then lysed to prevent their inhibition by known cysteine protease inhibitors (Kirschke et al., 1983; Barrett, 1987), because as much as 70 to 75% of catheptic-enzyme activity can be inhibited during extraction (Etherington et al., 1987). We found no significant breed-cross effects for cathepsin B or B + L specific activity (Table 4) at either d 1 or d 14. Therefore, it appears that cathepsins were not involved in our breed-cross differences in meat tenderization with 14 d of aging.

Another proteolytic system implicated in postmortem tenderization is the CDP system. The myofibrillar proteins hydrolyzed in vitro by CDP closely mimic changes observed under normal postmortem conditions (Dayton et al., 1976; Olson et al., 1977; Elgasim et al., 1985; Koochmariaie et al., 1988). Davey and Gilbert (1969) considered that meat aging was due to disruption and possible dissolution of Z-disk material, leading to a weakening of intermyofibrillar linkages. With the addition of EDTA to chelate  $\text{Ca}^{2+}$ , these changes did not occur. For activation, CDP-I requires 50 to 70  $\mu\text{M}$   $\text{Ca}^{2+}$  concentrations, but once autolyzed, it requires less  $\text{Ca}^{2+}$  for activity; CDP-II requires 1 to 5  $\text{mM}$   $\text{Ca}^{2+}$  for activity (Mellgren, 1980; Dayton et al., 1981). In living muscle, the very low level of free  $\text{Ca}^{2+}$  ( $< .01$   $\text{mM}$ ) may regulate or limit indiscriminate destruction of the Z-disk by CDP in vivo. However, Nakamura

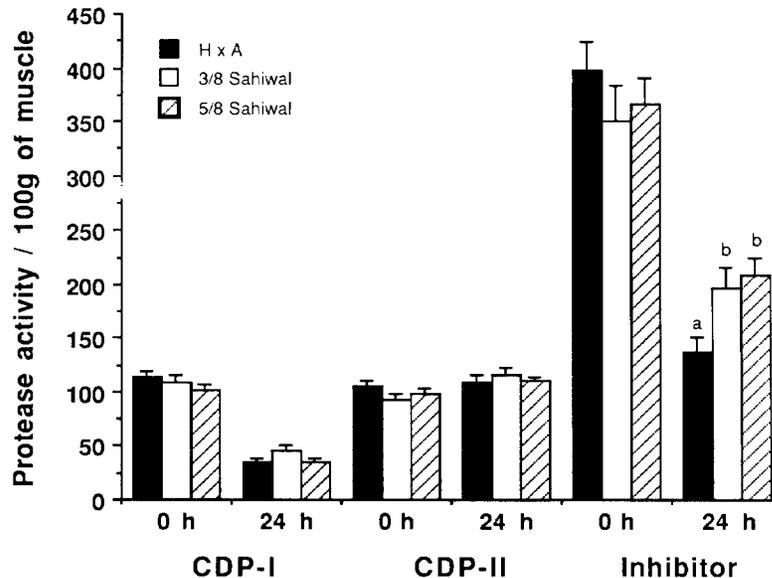


Figure 6. Longissimus calcium-dependent protease-I, -II and CDP-inhibitor activities at < 1 and 24 h postmortem by breed cross. Bars without a common superscript letter differ ( $P < .05$ ).

(1973a,b) and Mickelson (1983) have shown that sarcoplasmic reticulum membranes lose their ability to sequester  $Ca^{2+}$  postmortem because of a drop in temperature and a depletion of ATP. Therefore,  $Ca^{2+}$  concentrations would increase postmortem. As indicated in Table 4, no breed-cross differences in  $Ca^{2+}$  content existed, and the range of 8.6 to 10.8  $\mu\text{g/g}$  (.21 to .27 mM) was sufficient  $Ca^{2+}$  to activate CDP-I, but not CDP-II.

In addition to mimicking proteolysis seen in postmortem aging, CDP has been localized at the Z-disk and sarcolemma and vaguely at the M-line in chicken skeletal muscle (Ishiura et al., 1980). In beef cardiac muscle, CDP was observed at the Z-disk and sarcolemma (Lane et al., 1985). Kleese et al. (1987) found CDP to be localized in crotaline (rattlesnake) striated muscle at the I-band. Also, because of the cross-reaction between bovine and crotaline species, they concluded that both CDP and INH in remotely related organisms have very similar structures.

We determined activities of CDP-I, -II and INH within 1 h and at 24 h postmortem. No significant differences in activity/100 g of muscle were seen for CDP-I and -II at < 1 and 24 h, and in INH at < 1 h (Figure 6). However, INH activity at 24 h was lower ( $P < .05$ ) for H

$\times$  A ( $136.3 \pm 14.9$ ) than for 3/8 SAH ( $195.6 \pm 19.3$ ) and 5/8 SAH ( $208.8 \pm 14.8$ ). Koohmaraie et al. (1987, 1989) observed decreases in CDP-I and INH activity with postmortem aging; decreased activity was associated with increased tenderness. Previous researchers have measured CDP and INH activities only on frozen tissue; however, because INH is susceptible to freezing (Otsuka and Goll, 1987; Koohmaraie, 1990a), its true activity likely has been underestimated. In our study, CDP and INH activities were determined on fresh muscle, which provided new insight into the relationships among CDP, INH and tenderness. The initial CDP and INH activities were similar among breed crosses. Although breed crosses showed the same amount of CDP-I activity decline by 24 h, a significant difference in tenderness was still observed. Differences in tenderness and INH activity at 24 h between SAH and H  $\times$  A indicated that a relationship might exist between these two factors. Apparently, the CDP INH mechanism differs for SAH and H  $\times$  A, resulting in a decreased ability of CDP-I to hydrolyze myofibrillar proteins in SAH.

For H  $\times$  A, SDS-PAGE revealed that by d 1 desmin had been subjected to proteolysis, and by d 14 desmin could not be detected (Figure



Figure 7. SDS-PAGE of myofibrillar proteins isolated from longissimus muscle at <1 h, 1 and 14 d postmortem by breed cross.

7). Instead, a 30,000-dalton component was clearly evident. For 3/8 SAH, desmin was more intense at d 1 than for H x A, but by d 14, the 3/8 SAH gel resembled that of the H x A. However, in 5/8 SAH, desmin remained at d 14 and no 30,000-dalton component appeared. A 95,000-dalton component appeared by d 14 in all breed crosses, indicating that its appearance may not be associated with tenderness. With beef stored at 2°C, the gradual disappearance of desmin and the appearance of 110,000-, 95,000- and 30,000-dalton polypeptides has been reported (Koohmaraie et al., 1984; Xiong and Anglemier, 1989). MacBride and Parrish (1977) found that the major difference between tough and tender meat was associated with the presence of a 30,000-dalton component. In a study comparing tenderness traits between Friesian and Friesian x Brahman bulls, the Z-disk region was more stable after aging in the Brahman crosses (Purchas, 1972). These reports indicate that myofibrillar-protein hydrolysis is important in tenderization; this also is reflected in our MFI values (Figure 3). As the amount of protein hydrolysis increases, the myofibrils are more likely to fragment, thus increasing MFI. The myofibrillar proteins that probably are responsible for the amount of fragmentation

are desmin and those associated with the Z-disk region.

Reduced protein hydrolysis appears to account for less tender meat in SAH; the CDP INH mechanism may be influencing this process. We do not think that this is solely due to increased inhibitory action on CDP-I by INH in vivo. The binding of INH to CDP occurs in the presence of  $Ca^{2+}$ , and it can be reversed by adding EDTA during extraction (DeMartino and Croall, 1985). We think that if there had been more inhibition CDP-I activity would have been greater at 24 h in SAH because less CDP-I would have been autolyzed.

It is thought that INH is hydrolyzed by CDP (Imajoh and Suzuki, 1985; Mellgren et al., 1985, 1986); however, if CDP and INH are not sequestered in close proximity to one another, hydrolyzation of INH likely could not occur. We found that INH activity in SAH did not decline as much by 24 h as it did in H x A. This may have been due to CDP and INH being in different locations. However, in studies involving skeletal and cardiac muscles of various species, no localization differences were found (Ishiura et al., 1980; Lane et al., 1985).

In the presence of sufficient  $Ca^{2+}$ , CDP may undergo autolysis (Hathaway et al., 1982;

Coolican and Hathaway, 1984; Demartino et al., 1986; Crawford et al., 1987); this occurs more rapidly in the absence of a substrate or INH. Therefore, autolysis of CDP-I among breed crosses may have been different. Autolysis of CDP-I could have been greater in SAH, thus decreasing its ability to hydrolyze INH or myofibrillar proteins. However, because the rate of this process is dependent on the availability of a substrate or the presence of INH, a localization difference also is possible.

Another explanation could be that unknown protease(s) are involved. This has been proposed by other researchers to explain the loss in activity in CDP-I and INH (Vidalenc et al., 1983; Koochmarai, 1990a). When CDP-I was inhibited by  $ZnCl_2$  infusion, thus preventing autolysis, Koochmarai (1990b) proposed that a protease possibly hydrolyzed CDP-I between d 1 and d 14 postmortem. Also, no change was detected in INH activity with  $ZnCl_2$  infusion; this adds credence to the idea that CDP hydrolyzes INH. Therefore, unknown protease(s) may be preventing CDP-I from hydrolyzing INH and myofibrillar proteins in SAH. Also, protease(s) could be present that hydrolyze INH in  $H \times A$  but not in SAH. This would explain the decline in INH activity and, thus, lower the availability of INH to inhibit CDP-I protein hydrolysis in  $H \times A$  but not in SAH.

From this discussion, it is apparent that much is unknown about the interaction between CDP and INH. Our study suggests that there is a relationship between this mechanism and postmortem tenderization.

#### Implications

Less tender longissimus muscles of *Bos indicus* cattle compared with *Bos taurus* cattle apparently was due to reduced postmortem proteolysis of myofibrillar proteins in *Bos indicus* cattle. This reduced proteolysis was associated with higher activity of calcium-dependent protease inhibitor in *Bos indicus* cattle. Limited published research to date has examined the activity of calcium-dependent protease or its inhibitor as a possible explanation for less tender meat in *Bos indicus* cattle than in *Bos taurus* cattle. Because aging *Bos indicus* longissimus steaks for 14 d did not result in acceptable tenderness, other tenderization techniques may be necessary. The reasons that *Bos indicus* cattle have higher inhibitor

activity and the mechanism(s) involved in its regulation are unknown. Therefore, more research is needed to understand and possibly control the mechanism(s).

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