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Meat Science 70 (2005) 347–356
MEAT SCIENCE
www.elsevier.com/locate/meatsci

Factors influencing tenderness in steaks from Brahman cattle

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Received 4 November 2004; received in revised form 25 January 2005; accepted 25 January 2005

Abstract

The objective of this study was to identify a set of explanatory variables for Warner–Bratzler shear force and myofibril fragmentation indices after 7, 14, and 21 d of aging; and sensory tenderness after 14 d of aging of steaks from Brahman cattle. Insoluble collagen was negatively associated ($P < 0.001$) with all tenderness traits across aging periods, and regression coefficients ranged from 5.69 ± 0.49 to 9.12 ± 0.29 N for Warner–Bratzler shear force. The effect of lean color score ($P < 0.05$) in analyses of unadjusted traits was diminished when data were adjusted for contemporary group (calves of the same sex, fed in one pen, and slaughtered the same day). Insoluble collagen may be of special importance and offer a unique opportunity to improve palatability of steaks from purebred Brahman cattle.
Published by Elsevier Ltd.

Keywords: Beef; Brahman; Collagen; Tenderness

1. Introduction

There are many advantages of using Brahman crossbred cattle in different parts of the world, but there are some widely known undesirable palatability attributes which reduce the value of cattle with Brahman background. Among the most important of these undesirable attributes is the reputation for inadequate tenderness in the middle meats. Since the report of Crouse, Cundiff, Koch, Koohmaraie, and Seideman (1989), the US beef industry has persistently identified toughness of steaks from Brahman carcasses as a final product problem for consumers. Consequently, lower prices for feeder and fed cattle have been realized for cattle with identifiable Brahman background. It is therefore appropriate to investigate all aspects of tenderness in Brahman cattle for potential improvement. Characterization of tenderness measures in purebred populations is an important initial step in finding a solution to this problem. Genetic control of tenderness measures is low in purebred Brahman (Riley et al., 2003a), but appears to be somewhat higher in crossbred populations (Gregory, Cundiff, & Koch, 1995; O'Connor, Tatum, Wulf, Green, & Smith, 1997). A major palatability difference between *Bos indicus* and *Bos taurus* cattle appears to be the result of calpastatin activity in postmortem muscle (Pringle, Williams, Lamb, Johnson, & West, 1997; Whipple et al., 1990b). Proper management of the carcass may be more important for controlling palatability, especially tenderness (Robinson, Ferguson, Oddy, Perry, & Thompson, 2001). Postmortem intervention strategies such as electrical stimulation or alternate methods of carcass suspension (Koohmaraie, 1996; Thompson, 2002) especially with regard to different muscles (Koohmaraie, Kent, Shackelford, Veiseth, & Wheeler, 2002) may be effective

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0309-1740/$ - see front matter. Published by Elsevier Ltd.
doi:10.1016/j.meatsci.2005.01.022

for improving tenderness in Brahman carcasses. The objective of the present study was to identify a set of factors that influence different measures of tenderness of longissimus steaks from Brahman carcasses.

2. Materials and methods

2.1. Animals

The original experiment design and animal population was described by Riley et al. (2002). In brief, Brahman sires were progeny tested by mating to Brahman cows in the herd at the Subtropical Agricultural Research Station (STARS) near Brooksville, FL. Initially, four or five sires were used each year from 1994 through 1998 in single-sire breeding herds of approximately 30 cows. In all years but the first, a sire used the previous year was again used. In 1999, the American Brahman Breeders Association requested that the experiment be continued for two additional years in order for the breed to participate in the various aspects of the National Cattlemen's Beef Association Carcass Merit Project. The data for the current study came from calves that were born in 1998 through 2001. Brahman bulls ($n = 12$) were loaned to the station for this project or semen (for $n = 3$ bulls) was provided for artificial insemination of cows.

2.2. Feeding and slaughter procedures

All experimental procedures were evaluated for compliance with all appropriate regulations and approved by the local Institutional Animal Care and Use Committee. Calves were born in spring of each year and immediately after weaning at approximately 7 months of age, both steers (all males were castrated at birth) and heifers went through a brief conditioning period and then were placed in the STARS feed yard and adjusted to a feeding regimen previously described (Riley et al., 2002). When the median back fat (as measured by real time ultrasound) of a pen of cattle was 10 mm, the entire pen was slaughtered under normal commercial procedures in Central Florida. Sires averaged 31.2 progeny with records; these ranged from $n = 12$ (sire was killed by lightning during the breeding season) to $n = 53$.

2.3. Traits evaluated

Hot carcass weight was recorded at slaughter. Approximately 18–24 h after slaughter, carcasses were evaluated by trained University of Florida personnel. Factors affecting USDA yield and quality grades were: 12th rib fat thickness adjusted according to USDA (1990) guidelines and ribeye area. Color, texture, and firmness of the longissimus muscle were subjectively evaluated at the interface of the 12th and 13th ribs on the carcass. Lean color was scored on a scale from 1 to 8 (1 = dark pink; 5 = cherry red; 8 = very dark red). Lean texture and lean firmness were scored on 1–7 scales (1 = very fine, very firm; 7 = extremely coarse, extremely soft). Maturity scores (lean, skeletal, and overall) were evaluated numerically where A = 100 to 199, and B = 200 to 299. Marbling score was evaluated numerically: Devoid = 100 to 199; Traces = 200 to 299; Slight = 300 to 399; Small = 400 to 499; Modest = 500 to 599; Moderate = 600 to 699. Carcass hump height was measured from the most dorsal point of the hump to the dorsal edge of the ligamentum nuchae.

After grading (18–24 h after slaughter), the strip loin from the left side of each carcass was removed and sent to the University of Florida Meats Laboratory, where each was fabricated into steaks. Steaks were assigned to the various analyses in this manner, from posterior to anterior: (1) 24 h calpastatin, (2) and (3) 14 d sensory panel analyses, (4) 14 d Warner–Bratzler shear force, (5) 14 d myofibril fragmentation index, (6) 7 d Warner–Bratzler shear force, (7) 7 d myofibril fragmentation index and sarcomere length, (8) 21 d Warner–Bratzler shear force, (9) 21 d myofibril fragmentation index, (10) 24 h collagen, (11) percentage of raw lipids. Steaks for assay of myofibril fragmentation indices after all aging periods, sarcomere length, and percentage of raw lipids were cut 1.26 cm thick; all others were 2.54 cm thick.

Calpastatin activities (24 h) were determined according to the procedures of Koohmaraie (1990) with slight modifications described by Riley et al. (2003a). Total lipids were obtained on oven-dried steak samples by diethyl ether extraction (Method 985.15 AOAC, 1995), and recorded weights were used to determine percentages. Muscle collagen was estimated by determining hydroxyproline content of 5 g samples of longissimus muscle. Samples for collagen assays were frozen 24 h post slaughter. Samples were homogenized in a food processor and subsequently heated in a water bath (77 °C) for 63 min in 0.25 strength Ringer's solution (Hill, 1966). After centrifugation (12,000 rpm for 20 min at 4 °C with SS 34 rotor), the supernatant and residue fractions were individually hydrolyzed in 6 M HCl for 18 h at 15 psi (121 °C). Hydroxyproline content of each hydroxilate was determined according to Bergman and Loxley (1963). Total and soluble collagen was calculated according to Goll, Hoekstra, and Bray (1964). Sarcomere length of longissimus samples was determined by homogenizing a 5 g sample in 25 ml of a 0.25 M sucrose solution. A drop of the homogenate was placed on a slide and covered with a slip. Ten diffraction patterns were measured for each sample with a helium-neon laser (Model 155, Spectra Physics, I. C., Mt. View, CA) light (0.95 W) transmitted through individual myofibrils. The equation of Cross, West, and Dutson (1980) was used to convert measurements to micrometers.

Steaks were aged (7, 14, or 21 d), vacuum-packaged in barrier bags (Cryovac B620, Sealed Air Corp., Duncan, SC) and placed in a −40 °C freezer for 30–60 days until analyses were conducted. Steaks were thawed for 18 h at 4 °C. A 30 gauge TT thermocouple was placed in the geometric center of thawed steaks, which were placed on Farberware Open-Hearth grills (Model 455N, Yonkers, NY). Steaks were heated to an internal temperature of 35 °C, turned and cooked to a final internal temperature of 71 °C (AMSA, 1995). After the steaks were cooled to 21 °C, 6–8 cores (1.27 cm in diameter) were removed parallel to fiber orientation from each steak. Peak shear force was measured on each core using a Warner–Bratzler shearing device (crosshead speed = 200 mm/m) attached to an Instron Universal Testing Machine (Instron Corporation, Canton, MA). Warner–Bratzler shear forces for the different aging periods were evaluated as the average of 6–8 shears from the same steak.

Myofibril fragmentation indices of uncooked samples aged 7, 14, and 21 d were determined according to the procedures of Calkins and Davis (1978), accomplished by measuring the percentage of homogenized muscle that passed through a screen. Samples that had undergone protein degradation passed through the screen to a greater extent and had lower values. These were used to estimate the extent of protein degradation that had occurred during the aging process.

Steaks for sensory evaluation were cooked in the same manner as steaks used in shear force evaluations. After cooking, samples were evaluated by a trained sensory panel of 8–10 members (AMSA, 1995). Six samples (two 1.27 cm cubes per sample) were evaluated by each panelist per session. Two steaks per animal were cooked for sensory evaluation and six animals were evaluated per session. Cooked samples were wrapped in heavy foil and placed into warmed casserole dishes until all steaks were cooked. Then samples were cut into 1.27 cm cubes and two cubes were placed into warmed glassware in insulated yogurt makers, which were then presented to the panelist. Samples were evaluated for overall tenderness on a scale of 1 through 8 (1 = extremely tough; 8 = extremely tender). The averages of the responses of the panel were used as the dependent variables for statistical analyses.

2.4. Statistical analyses

Individual animals were experimental units. The experimental design was a progeny test of Brahman sires. Relationships of measures of tenderness with other variables were initially investigated by examining correlation coefficient estimates generated by the CORR procedures of SAS (SAS Inst. Inc., Cary, NC). Data were subsequently analyzed using stepwise regression techniques of the REG procedures of SAS. The criterion for independent variable entry into the model was a probability value of 0.15 or less associated with the F statistic; all variables were reevaluated for inclusion with the same criterion with the entry of each additional variable into the model.

Separate sets of analyses were conducted. The first was conducted using the unadjusted values of the different measures of tenderness. The REG procedures of SAS do not permit the fitting of fixed or random effects, therefore the second set was conducted using tenderness residuals as dependent variables. These residuals were produced by mixed models in which contemporary group was fitted as a fixed effect and sire of calf was fitted as a random effect. Contemporary group ($n = 39$) was defined as cattle of the same sex, fed in the same pen, and slaughtered on the same day. Contemporary group size ranged from 10 to 14 animals.

Within each set of analyses (dependent variables unadjusted or adjusted), two regressions were conducted. The first utilized all available information as independent variables. A subsequent run was conducted using independent variables that would be available at the time the carcasses were graded (approximately 24 h after slaughter) to assess the predictive value of those (relatively) easily obtained carcass measures at that time.

Additional analyses were conducted separately at a common animal age, 12th rib back fat, marbling score, and carcass weight (i.e., these regressions were forced into the model without considering their significance). Only minor differences in results were produced with this procedure, and final analyses were conducted without forcing any variable into the model.

The final models were chosen based on consideration of both the coefficients of determination (model R^2) and root mean square errors. Regression coefficients and SE from analyses of myofibril fragmentation indices, Warner–Bratzler shear force, and sensory panel overall tenderness values after 14 d aging were determined.

3. Results

3.1. Descriptive statistics

Sample sizes, unadjusted means, SEM, and CV for the dependent and independent variables in this study are presented in Table 1. Relationships between measures of tenderness and other traits were initially explored by examining simple correlations that are shown in Table 2. Although many of these coefficients were significant, the strongest relationships appeared to be those of collagen and hump height with shear force and overall tenderness. Table 3 presents simple correlation estimates between the seven measures of tenderness in this study. There were large positive coefficients within traits (myofibril fragmentation index and Warner–Bratzler shear force) across aging periods. Sensory panel overall

Table 1
Descriptive statistics for traits of Brahman carcasses

Trait	No.	Mean	SEM	CV
Warner–Bratzler shear force, N				
Day 7	467	55.12	0.98	38.0
Day 14	468	49.04	0.69	31.1
Day 21	467	44.72	0.39	35.0
Myofibril Fragmentation Index[a]				
Day 7	456	413.57	5.51	28.4
Day 14	468	301.35	6.32	45.4
Day 21	468	220.02	6.62	65.1
Overall tenderness[b]	467	4.86	0.03	14.7
Hot carcass weight, kg	468	274.17	1.61	13.8
12th rib fat thickness, mm	468	12.56	0.16	27.3
Ribeye area, cm^2	468	71.87	36.87	11.1
Lean maturity[c]	468	159.89	0.99	13.4
Skeletal maturity[c]	468	152.56	0.48	6.8
Lean color[d]	468	4.69	0.06	27.0
Lean texture[e]	468	3.35	0.03	21.6
Lean firmness[e]	468	3.25	0.03	20.2
Marbling score[f]	468	316.92	2.55	17.4
Hump height, cm[g]	457	13.73	0.84	22.6
Raw lipids, %	466	2.89	0.07	48.6
Total collagen, mg per g muscle	466	3.49	0.08	48.1
Insoluble collagen, mg per g muscle	466	2.88	0.06	45.7
Calpastatin, units per g muscle (24 h)	451	2.68	0.06	45.9
Sarcomere length (24 h), μm	459	1.76	0.003	3.9

[a] Myofibril fragmentation index was determined by the procedures of Calkins and Davis (1978).
[b] Overall tenderness on samples aged 14 d measured on scale from 1 to 8: 1 = extremely tough, 4 = slightly tough, 5 = slightly tender, 8 = extremely tender.
[c] Maturity scored approximately 24 h after slaughter: A = 100 to 199; B = 200 to 299.
[d] Lean color scored approximately 24 h after slaughter from 1 to 8: 1 = dark pink; 4 = slightly light cherry red; 5 = cherry red; 8 = very dark red.
[e] Lean texture and lean firmness scored from 1 to 7: 1 = very fine, very firm; 3 = moderately fine, moderately firm; 4 = slightly coarse, slightly soft; 7 = extremely coarse, extremely soft.
[f] 200 to 299 = Traces; 300 to 399 = Slight; 400 to 499 = Small.
[g] Hump height was measured on the carcass from the most dorsal point of the hump to the dorsal edge of the ligamentum nuchae.

Table 2
Simple correlations of tenderness traits with other carcass and palatability traits of Brahman cattle

Days of aging	MFI			WBSF			OT
	7	14	21	7	14	21	14
Carcass weight, kg	0.01	**0.14**	**0.14**	**−0.21**	**−0.21**	**−0.15**	0.04
12th rib fat thickness, mm	−0.02	**0.11**	0.07	**−0.29**	**−0.25**	**−0.27**	**0.11**
Ribeye area, cm^2	**0.16**	**0.26**	**0.33**	**−0.18**	**−0.22**	**−0.10**	0.01
Lean maturity	−0.03	**−0.10**	−0.06	0.07	**0.10**	**0.15**	**−0.12**
Skeletal maturity	**−0.23**	**−0.19**	**−0.17**	**−0.28**	**−0.26**	**−0.24**	0.03
Lean color	**−0.30**	**−0.32**	**−0.37**	**−0.30**	**−0.18**	**−0.25**	**0.16**
Lean texture	**−0.13**	**−0.25**	**−0.28**	**0.19**	**0.18**	**0.20**	**−0.12**
Lean firmness	**−0.17**	**−0.20**	**−0.17**	**−0.12**	**−0.12**	**−0.13**	**0.09**
Marbling score	**0.08**	**0.15**	**0.11**	**−0.13**	**−0.18**	**−0.13**	0.07
Hump height, cm	**0.26**	**0.24**	**0.23**	**0.52**	**0.44**	**0.43**	**−0.21**
Raw lipids, %	−0.06	−0.03	**−0.12**	**−0.20**	**−0.12**	**−0.16**	0.07
Collagen, mg per g muscle							
Total	**0.19**	0.02	0.05	**0.66**	**0.56**	**0.82**	**−0.36**
Insoluble	**0.19**	0	0.03	**0.66**	**0.57**	**0.83**	**−0.37**
Calpastatin, units/g muscle	**−0.16**	**−0.09**	**−0.25**	**−0.12**	−0.05	−0.06	−0.01
Sarcomere length, μm	**0.12**	**0.12**	**0.18**	−0.02	−0.07	**−0.08**	**0.16**

MFI = myofibril fragmentation index; WBSF = Warner–Bratzler shear force; OT = sensory panel overall tenderness.
$|r| > 0.07$ differ from 0 ($P < 0.05$) and are bold type.

Table 3
Simple correlations among measures of tenderness of Brahman cattle

Days of aging	MFI		WBSF			OT
	14	21	7	14	21	14
MFI						
Day 7	**0.69**	**0.62**	**0.22**	**0.23**	**0.26**	**−0.23**
Day 14		**0.86**	−0.03	0.06	0.04	**−0.21**
Day 21			0.02	−0.02	0.06	**−0.12**
WBSF						
Day 7				**0.71**	**0.78**	**−0.43**
Day 14					**0.76**	**−0.47**
Day 21						**−0.46**

MFI = myofibril fragmentation index; WBSF = Warner–Bratzler shear force; OT = sensory panel overall tenderness.
$|r| > 0.07$ differ from 0 ($P < 0.05$) and are bold type.

tenderness was negatively correlated with Warner–Bratzler shear force (all aging periods). Not all were strongly correlated; note particularly the estimates of correlation of d 14 and d 21 myofibril fragmentation indices with shear force after the different aging periods. Both Tables 2 and 3 seem to suggest that the actual physical breakdown of muscle (myofibril fragmentation index) measures a different aspect of tenderness than either shear force or overall tenderness.

3.2. Unadjusted dependent variables

Results from stepwise regression analyses of all unadjusted data are shown in Tables 4–6. The amount of insoluble collagen had pronounced effects on Warner–Bratzler shear force and overall tenderness values, as it was the first independent variable selected for inclusion after all aging periods. A 1 mg increase in insoluble collagen was associated with over 5.0 N increase in d 14 shear force (Table 5); this association

Table 4
Regression coefficient estimates, model R^2 values, and root mean square errors from models of traits related to tenderness of Brahman longissimus after 7 days aging

Variable	MFI	WBSF (N)
Intercept	612.95 ± 99.45	121.12 ± 12.95
Lean color	−16.12 ± 4.64	− 3.33 ± 0.59
Lean texture	−13.50 ± 7.58	3.53 ± 0.98
Insoluble collagen	51.57 ± 22.38	8.34 ± 0.59
Ribeye area	2.65 ± 0.65	− 0.29 ± 0.10
Lean firmness	−22.87 ± 8.27	
Skeletal maturity	− 1.40 ± 0.56	− 0.29 ± 0.10
Fat thickness		− 0.49 ± 0.20
Hump height		− 0.49 ± 0.29
Total collagen	−29.26 ± 17.59[a]	
Calpastatin	−10.72 ± 4.32	
Lean maturity		− 0.10 ± 0.03[b]
Raw lipids, %		− 1.18 ± 0.49
Model R^2	0.19	0.56
Root mean square error	107.1	14.02

MFI = myofibril fragmentation index; WBSF = Warner–Bratzler shear force. Empty cells indicate regressions that were among those that were excluded from final models ($P > 0.15$).
Coefficient estimates are significant ($P \leqslant 0.05$) unless superscripted.
[a] Regression approached significance ($0.05 < P \leqslant 0.10$).
[b] Regression approached significance ($0.10 < P \leqslant 0.15$).

Table 5
Regression coefficient estimates, model R^2 values, and root mean square errors from models of traits related to tenderness of Brahman longissimus after 14 days aging

Variable	MFI	WBSF (N)	OT
Intercept	556.69 ± 117.25	90.42 ± 10.10	3.57 ± 1.01
Lean color	−28.41 ± 4.98		0.09 ± 0.03
Lean texture	−25.03 ± 8.22	1.57 ± 0.79[a]	
Insoluble collagen		5.69 ± 0.49	−0.17 ± 0.02
Ribeye area	4.13 ± 0.78	−0.29 ± 0.10	
Lean firmness	−28.07 ± 8.78		0.07 ± 0.05[b]
Skeletal maturity	− 1.08 ± 0.61[a]	−0.29 ± 0.10	
Fat thickness	4.30 ± 1.73	−0.49 ± 0.20	
Hump height	4.88 ± 2.08		
Sarcomere length			1.42 ± 0.46
Lean maturity			−0.003 ± 0.001[a]
Slaughter age	− 0.46 ± 0.21		−0.002 ± 0.001[a]
Model R^2	0.27	0.391	0.183
Root mean square error	118.1	11.97	0.65

MFI = myofibril fragmentation index; WBSF = Warner–Bratzler shear force; OT = sensory panel overall tenderness.
Empty cells indicate regressions that were among those that were excluded from final models ($P > 0.15$).
Coefficient estimates are significant ($P \leqslant 0.05$) unless superscripted.
[a] Regression approached significance ($0.05 < P \leqslant 0.10$).
[b] Regression approached significance ($0.10 < P \leqslant 0.15$).

Table 6
Regression coefficient estimates, model R^2 values, and root mean square errors from models of traits related to tenderness of Brahman longissimus after 21 days aging

Variable	MFI	WBSF (N)
Intercept	123.10 ± 183.51	45.11 ± 6.38
Lean color	−29.12 ± 4.86	−1.08 ± 0.39
Lean texture	−19.13 ± 8.16	1.57 ± 0.39
Insoluble collagen		9.12 ± 0.29
Ribeye area	4.70 ± 0.75	
Lean firmness	−22.90 ± 8.99	−1.18 ± 0.59[a]
Skeletal maturity		−0.10 ± 0.04
Fat thickness		−0.29 ± 0.10
Hump height	9.87 ± 1.99	
Total collagen	6.37 ± 3.35[a]	
Calpastatin	−21.26 ± 4.59	
Sarcomere length	177.11 ± 83.24	
Slaughter age	− 0.97 ± 0.21	
Marbling score	0.16 ± 0.11[b]	
Model R^2	0.403	0.714
Root mean square error	111.1	8.43

MFI = myofibril fragmentation index; WBSF = Warner–Bratzler shear force.
Empty cells indicate regressions that were among those that were excluded from final models ($P > 0.15$).
Coefficient estimates are significant ($P \leqslant 0.05$) unless superscripted.
[a] Regression approached significance ($0.05 < P \leqslant 0.10$).
[b] Regression approached significance ($0.10 < P \leqslant 0.15$).

was larger for d 7 and d 21 shear force (8.34 ± 0.59 and 9.12 ± 0.29 N, respectively; Tables 4 and 6). Lean color regression coefficient estimates for myofibril fragmentation index (−16.12 ± 4.64 and −29.12 ± 4.86 for 7 and 21 d aging) and for shear force values (−3.33 ± 0.59 and −1.08 ± 0.39 N for 7 and 21 d aging, respectively) were consistent with the results for 14 d aging. After 14 d aging, higher lean color scores (indicating darker red lean) were associated with increased tenderness (decreased myofibril fragmentation index values and increased panel tenderness scores). Regression coefficient estimates for lean firmness score were similar in sign and magnitude as those for lean color score; higher lean firmness values (softer lean) were associated with improved tenderness for all traits except d 14 shear force. Most independent variables did not explain variation in tenderness across all traits and aging periods. Increases in ribeye area were associated with less myofibril degradation, but lower shear force values after the different aging periods (7 and 14 d only for shear force values).

3.3. Dependent variables pre-adjusted for contemporary group effects

Analyses of residuals for the different tenderness measures (adjusted for contemporary group) resulted in generally fewer independent variables in the final stepwise regression models and lower model R^2 values (Tables 7–9). Insoluble collagen was the first variable retained for each of the dependent tenderness variables; its association in each case was with reduced tenderness (to increase myofibril fragmentation indices and shear force, and to lower overall tenderness values). Although lean color score was an important regression in the analyses of unadjusted data, it approached significance only for d 21 myofibril fragmentation index (Table 9). This suggests that the explanatory value of lean color score may have been associated with differences among contemporary groups.

Table 7
Regression coefficient estimates, model R^2 values, and root mean square errors from models of pre-adjusted traits related to tenderness of Brahman longissimus after 7 days aging

Variable	MFI	WBSF (N)
Insoluble collagen	66.62 ± 16.73	4.90 ± 0.49
Sarcomere length	−132.78 ± 61.91	−18.24 ± 7.85
Raw lipids, %		− 0.79 ± 0.39
12th rib fat thickness	2.50 ± 1.25	
Total collagen	− 34.82 ± 13.13	
Carcass weight		− 0.05 ± 0.02
Skeletal maturity	1.27 ± 0.63	
Model R^2	0.125	0.241
Root mean square error	76.11	9.71

MFI = myofibril fragmentation index; WBSF = Warner–Bratzler shear force.
Empty cells indicate regressions that were among those that were excluded from final models ($P > 0.15$).
All coefficient estimates are significant ($P \leqslant 0.05$).

Table 8
Regression coefficient estimates, model R^2 values, and root mean square errors from models of pre-adjusted traits related to tenderness of Brahman longissimus after 14 days aging

Variable	MFI	WBSF (N)	OT
Insoluble collagen	19.25 ± 3.75	3.04 ± 0.49	−0.39 ± 0.13
Sarcomere length		−21.87 ± 7.45	
Raw lipids, %		− 0.88 ± 0.39	
12th rib fat thickness	3.69 ± 1.45		
Total collagen			0.20 ± 0.10[a]
Lean maturity		0.06 ± 0.03	−0.002 ± 0.002[b]
Lean firmness			0.09 ± 0.05[a]
Carcass weight	0.34 ± 0.22		
Ribeye area	1.02 ± 0.70[b]		
Skeletal maturity	1.33 ± 0.62		
Calpastatin activity			−0.09 ± 0.05
Model R^2	0.092	0.148	0.105
Root mean square error	79.5	9.22	0.58

MFI = myofibril fragmentation index; WBSF = Warner–Bratzler shear force; OT = sensory panel overall tenderness.
Empty cells indicate regressions that were among those that were excluded from final models ($P > 0.15$).
Coefficient estimates are significant ($P \leqslant 0.05$) unless superscripted.
[a] Regression approached significance ($0.05 < P \leqslant 0.10$).
[b] Regression approached significance ($0.10 < P \leqslant 0.15$).

Table 9
Regression coefficient estimates, model R^2 values, and root mean square errors from models of pre-adjusted traits related to tenderness of Brahman longissimus after 21 days aging

Variable	MFI	WBSF, N
Insoluble collagen	24.30 ± 3.03	7.06 ± 0.29
Sarcomere length		−14.42 ± 5.49
Raw lipids, %	4.43 ± 2.61[b]	− 0.49 ± 0.29[b]
12th rib fat thickness	2.18 ± 1.10	
Lean maturity	− 0.29 ± 0.17[b]	
Lean firmness		− 0.98 ± 0.59[a]
Ribeye area	0.91 ± 0.53	
Lean texture		0.98 ± 0.59[a]
Lean color	− 5.78 ± 3.86[b]	
Model R^2	0.148	0.544
Root mean square error	64.2	6.77

MFI = myofibril fragmentation index; WBSF = Warner–Bratzler shear force.
Empty cells indicate regressions that were among those that were excluded from final models ($P > 0.15$).
Coefficient estimates are significant ($P \leqslant 0.05$) unless superscripted.
[a] Regression approached significance ($0.05 < P \leqslant 0.10$).
[b] Regression approached significance ($0.10 < P \leqslant 0.15$).

3.4. Grading information only – unadjusted dependent variables

When analyses were conducted with those independent variables that were available up to the time of grading, characteristics of lean at the interface of the 12th

and 13th ribs appeared to be the most important explanatory variables (Tables 10–12), particularly lean color score and ribeye area. There was an association of higher lean color scores (darker red) with improved tenderness measures in all cases. Higher lean texture and firmness scores (increasingly coarse and soft muscle) were also associated with improved tenderness scores. Regression coefficient estimates indicated that larger ribeye areas were associated with less myofibril degradation, but lower shear force values after 14 d aging. Model R^2 values ranged from 0.06 to 0.38 for these analyses and root mean square errors for each trait were largest for these analyses.

Table 10
Regression coefficient estimates, model R^2 values, and root mean square errors from models (analyses considered only variables available on day carcass was graded) of traits related to tenderness of Brahman longissimus after 7 days aging

Variable	MFI	WBSF, N
Intercept	691.27 ± 109.53	172.60 ± 15.20
Lean color	−21.64 ± 4.52	− 4.71 ± 0.69
Lean firmness	−16.92 ± 8.35	− 1.96 ± 1.28[b]
Lean texture		5.98 ± 1.18
Ribeye area	2.64 ± 0.65	− 0.39 ± 0.10
Skeletal maturity	− 1.21 ± 0.55	− 0.39 ± 0.10
12th rib fat thickness		− 0.88 ± 0.29
Hump height		− 0.79 ± 0.29
Marbling score	0.16 ± 0.10[b]	
Slaughter age	− 0.40 ± 0.19[a]	
Model R^2	0.16	0.307
Root mean square error	108.4	17.56

MFI = myofibril fragmentation index; WBSF = Warner–Bratzler shear force.
The independent variables considered in these analyses were only those that were available at the time the carcasses were graded (approximately 24 h post-slaughter).
Empty cells indicate regressions that were among those that were excluded from final models ($P > 0.15$).
Coefficient estimates are significant ($P \leqslant 0.05$) unless superscripted.
[a] Regression approached significance ($0.05 < P \leqslant 0.10$).
[b] Regression approached significance ($0.10 < P \leqslant 0.15$).

Table 11
Regression coefficient estimates, model R^2 values, and root mean square errors from models (analyses considered only variables available on day carcass was graded) of traits related to tenderness of Brahman longissimus after 14 days aging

Variable	MFI	WBSF, N	OT
Intercept	509.76 ± 122.43	148.78 ± 12.36	5.07 ± 0.31
Lean color	−29.04 ± 5.01	− 1.77 ± 0.60	0.10 ± 0.03
Lean firmness	−25.42 ± 9.18	− 2.06 ± 0.98[b]	0.10 ± 0.05[a]
Lean texture	−24.14 ± 8.28	3.73 ± 0.88	−0.14 ± 0.05
Ribeye area	4.09 ± 0.78	− 0.39 ± 0.10	
Skeletal maturity	− 1.05 ± 0.61	− 0.39 ± 0.10	
12th rib fat thickness	3.55 ± 1.79	− 0.49 ± 0.20	
Hump height	5.10 ± 2.08	− 0.39 ± 0.20[b]	
Marbling score	0.17 ± 0.11[b]	− 0.02 ± 0.01[b]	
Slaughter age	− 0.48 ± 0.22		
Lean maturity			−0.003 ± 0.002[b]
Model R^2	0.278	0.254	0.064
Root mean square error	118.1	13.24	0.70

MFI = myofibril fragmentation index; WBSF = Warner–Bratzler shear force; OT = sensory panel overall tenderness.
The independent variables considered in these analyses were only those that were available at the time the carcasses were graded (approximately 24 h post-slaughter).
Empty cells indicate regressions that were among those that were excluded from final models ($P > 0.15$).
Coefficient estimates are significant ($P \leqslant 0.05$) unless superscripted.
[a] Regression approached significance ($0.05 < P \leqslant 0.10$).
[b] Regression approached significance ($0.10 < P \leqslant 0.15$).

Table 12
Regression coefficient estimates, model R^2 values, and root mean square errors from models (analyses considered only variables available on day carcass was graded) of traits related to tenderness of Brahman longissimus after 21 days aging

Variable	MFI	WBSF, N
Intercept	490.28 ± 96.64	91.60 ± 12.06
Lean color	−36.49 ± 4.67	−2.26 ± 0.60
Lean firmness	−19.19 ± 8.79	−1.96 ± 1.08
Lean texture	−28.08 ± 7.86	3.92 ± 0.98
Ribeye area	5.33 ± 0.76	−0.20 ± 0.10[a]
Skeletal maturity		−0.29 ± 0.07
12th rib fat thickness		−0.69 ± 0.20
Hump height	7.58 ± 1.99	
Marbling score	0.20 ± 0.11[a]	
Slaughter age	− 1.11 ± 0.21	
Lean maturity		0.08 ± 0.03
Model R^2	0.377	0.193
Root mean square error	115.0	14.12

MFI = myofibril fragmentation index; WBSF = Warner–Bratzler shear force.
The independent variables considered in these analyses were only those that were available at the time the carcasses were graded (approximately 24 h post-slaughter).
Empty cells indicate regressions that were among those that were excluded from final models ($P > 0.15$).
Coefficient estimates are significant ($P \leqslant 0.05$) unless superscripted.
[a] Regression approached significance ($0.05 < P \leqslant 0.10$).

3.5. Grading information only – dependent variables pre-adjusted for contemporary group effects

Pre-adjustment of data for contemporary group (year, sex, pen, slaughter date) effects and considering independent variables available on day of grading deteriorated the explanatory value of all independent variables. The number of variables retained in regression models was greatly reduced (data not shown); three analyses retained two or fewer independent variables. The highest model R^2 value was 0.121 for d 14 shear force; the remainder ranged from 0.008 for d 7 myofibril fragmentation index to 0.037 for d 14 myofibril fragmentation index. Regression coefficient estimates (not presented) were of smaller magnitude than those from other analyses. The reduced influence of lean color score on tenderness measures appeared to be associated with removal of contemporary group effects.

4. Discussion

4.1. Collagen

Several decades ago, connective tissue was identified as an important aspect of beef tenderness (Cross, Carpenter, & Smith, 1973). Total collagen amount (but not percentage soluble collagen) was reported to be a significant explanatory variable of shear force and overall tenderness of longissimus from heifers from 10 months to cows 27 years of age (Reagan, Carpenter, & Smith, 1976). Total collagen content did not effectively explain among-breed (including both *Bos indicus* and *Bos taurus*) tenderness differences (Johnson, Huffman, Williams, & Hargrove, 1990; Norman, 1982; Whipple et al., 1990b). However, breed differences in the solubility of collagen have been detected (Norman, 1982). Collagen content to date has not been shown to be an effective explanatory variable for tenderness (Oddy, Harper, Greenwood, & McDonagh, 2001). However, of the three primary sources of variation in tenderness (sarcomere length, connective tissue content, and protein proteolysis) of aged longissimus (Koohmaraie et al., 2002), the connective tissue component appeared to have the most influence on the different measures of tenderness in these data. This may be a unique characteristic of tenderness in Brahman, or it may be a characteristic of single-breed populations; most of the experimental populations utilized in similar work have been multi-breed or crossbred. Its importance is supported by its presence in analyses of all tenderness measures across all aging periods; this was consistent with the lack of influence of aging on amount or solubility of collagen reported by Koohmaraie, Seideman, Schollmeyer, Dutson, and Crouse (1987). The influence of insoluble collagen content appeared to increase when contemporary group effects were removed from the phenotypes (Tables 7–9). Increases in postmortem aging may be associated with diminishing myofibrillar influence on tenderness, and therefore increasing influence of stromal proteins because the latter are less affected by aging than the myofibrillar proteins. Inadequate sample size did not permit estimation of additive genetic control of this trait; however, these results suggest that differences in insoluble collagen content may be associated with different Brahman genotypes. Insoluble collagen content may represent an exploitative opportunity for improvement of palatability of Brahman steaks, possibly through identification of parents with desirable collagen genotypes or identification of carcasses at high risk for poor tenderness.

4.2. Lean color

The association of lean color score with six of the seven measures of tenderness was marginal; as lean color score increased (darker red), tenderness improved. The cause of this unexpected result was uncertain. Wulf and Page (2000) reported increased Warner–Bratzler shear force and decreased sensory panel tenderness means associated with low b^* (yellow relative to blue) values, but reported no differences in shear force for low and high L^* (more black relative to white) values. There were no dark cutting carcasses in data from the present study. Colorimeter readings have been examined for a possible role in palatability assessment of beef carcasses (Johnston, Reverter, Ferguson, Thompson, & Burrow, 2003; Wulf & Page, 2000; Wulf & Wise, 1999). Both L^* and a^* values differed between *Bos taurus* and *Bos indicus* carcasses (Wulf, O'Connor, Tatum, & Smith, 1997) and were used to separate carcasses into tenderness groups, but no color differences were reported between these types of carcasses in a separate study (Page, Wulf, & Schwotzer, 2001). Results from the present study suggest that lean color score effects may be confounded with differences in management of cattle or carcasses because of its diminished importance in analyses of tenderness residuals (contemporary group effects removed). This appears to be consistent with plant differences in colorimeter readings (and possible differential rates of pH decline) reported by Page et al. (2001).

4.3. Fat deposition

We did not detect strong relationships of measures of intramuscular fat (as indicated by marbling score, percentage of raw lipids, and 12th rib fat thickness) with the measures of tenderness in this study. This is consistent with the established weak relationship of marbling and tenderness (Wheeler, Cundiff, & Koch, 1994). Jones and Tatum (1994) accounted for 9% and 5.1% of the variation in Warner–Bratzler shear force and muscle fiber tenderness (after 10 d of aging), respectively, with marbling score. Fat deposition traits appeared to be influential on Warner–Bratzler shear force essentially when they represented the only data available.

4.4. Inhibition of proteolysis

Although the elevated levels of postmortem calpastatin activity in *Bos indicus* muscle (Pringle et al., 1997) appeared to be a point of influence on tenderness, results from the present study indicate that calpastatin activity may only be helpful to explain tenderness differences in across-breed (when Brahman is included) comparisons (O'Connor et al., 1997; Whipple, Koohmaraie, Dikeman, & Crouse, 1990a; Wheeler, Savell, Cross, Lunt, & Smith, 1990), rather than within-breed (Riley et al., 2003a, 2003b).

4.5. Effectiveness of information available on day of grading

When stepwise regression analyses were conducted while excluding all independent variables that would not normally be available at the time carcasses were graded,

model fit diminished substantially for shear force after all aging periods and overall tenderness, but only slightly for d 7 and 21 myofibril fragmentation index (compare Model R^2 and root mean square error values). Lean color score and ribeye area showed some explanatory value for these tenderness measures, and maybe to a lesser degree lean texture score. However, when contemporary group effects were removed, none of the independent variables effectively explained tenderness.

5. Conclusions

Insoluble collagen must be an important component of tenderness in Brahman longissimus. There may be differential collagen content and solubility associated with genetic differences in growth rate and maturation that should be explored. Genetic and environmental mechanisms of influencing this component must be considered. Any explanatory value for subjective lean color score must be tempered by the apparent association with some aspect of the environment (contemporary groups). Little of the information that is only available at the time of USDA carcass grading explains variation in, or predicts tenderness. Finally, although higher calpastatin activity of Brahman longissimus may be responsible for relative (to other breeds) toughness of Brahman steaks, it appears to explain very little of the within-breed variation in tenderness measures across aging periods. Results from this study highlight the complexity of the beef tenderness issue. The traits evaluated in this study may represent distinct aspects of tenderness with largely different associations with other beef traits; it seems unlikely that a single locus could exert substantial influence over all seven.

Acknowledgements

Appreciation is extended to the American Brahman Breeders Association and the Florida Brahman Association for loaned bulls; and to D.B. Sartain, M.L. Rooks, E.J. Bowers, V.E. Rooks, E.L. Adams, and the University of Florida Meats Laboratory and STARS staffs for technical support. This research was supported by the Florida Agricultural Experiment Station, and approved for publication as Journal Series No. R-10685. Mention of a trade name, proprietary product, or specified equipment does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of other products that may be suitable.

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