Reprinted from J. Food Sci.
56(5): 1130–1135 & 1140

Predictors of Beef Tenderness: Development and Verification

S.D. SHACKELFORD, M. KOOHMARAIE, G. WHIPPLE, T.L. WHEELER, M.F. MILLER, J.D. CROUSE, and J.O. REAGAN

ABSTRACT

Equations were developed to predict beef longissimus dorsi (LD) tenderness after postmortem refrigerated aging. Warner-Bratzler shear force (WBSF) and myofibril fragmentation indices (MFI) were determined at 1, 3, 7 and 14 days postmortem on LD of Angus-Hereford (AH, n = 8) and 5/8 Brahman crossbred (n = 8) heifer carcasses. Correlation coefficients between WBSF and MFI were −0.91, −0.74, −0.63, and −0.40 at 1, 3, 7 and 14 days postmortem, respectively. None of the traits measured correlated significantly with 14-day WBSF ($P > 0.05$). A three-variable prediction equation that included 24-hr calcium-dependent protease (CDP) inhibitor activity, 0–hr CDP-I activity and 24-hr cystatin activity accounted for 63% of the variation in 14-day WBSF.

INTRODUCTION

CONSUMER DEMAND for beef is influenced by many factors including palatability and leanness (Cross et al., 1986). Variation in beef tenderness has been reported to be a primary concern of the U.S. beef industry (Morgan et al., 1991). Present technologies do not allow for objective determination of postmortem aged-beef tenderness in live animals. Before selection for tenderness can be employed, those characteristics responsible for tenderness must be identified. Many factors have been associated with tenderness in beef such as marbling (Tatum et al., 1982; Riley et al., 1983b; Smith et al., 1987), subcutaneous fat thickness (Dolezal et al., 1982; Riley et al., 1983a, b), catheptic enzymes (Calkins et al., 1987), calcium-dependent proteases and their inhibitor (Koohmaraie, 1988).

The objectives of our study were to determine what factors are related most highly to postmortem tenderness of beef and to develop and verify equations to predict tenderness of aged beef so antemortem selection techniques could be invoked

MATERIALS & METHODS

ANGUS-HEREFORD (AH, n = 8) and 5/8 Brahman × AH (n = 8) crossbred heifers were weaned at 6 to 8 mo of age, fed an alfalfa haylage-corn silage diet for 4 mo and fed a corn-corn silage diet until slaughter at 15 to 17 mo of age. The alfalfa haylage (48.6% as fed) -corn silage (49.6% as fed) diet contained 32.75% dry matter (DM), 2.18 Mcal/kg metabolizable energy (ME), 60.30% total digestible nutrients (TDN) and 11.80% crude protein (CP). The corn (53.5% as fed) -corn silage (43.1% as fed) diet contained 65.8% DM, 3.08 Mcal/kg ME, 85.2% TDN and 10.30% CP. Heifers were assigned randomly to one of two groups that were slaughtered two weeks apart to facilitate collection of data. Temperature and pH of the longissimus dorsi (LD) were determined at 0, 3, 6, 9, 12, and 24 hr postmortem. Temperature and pH were determined at stratified points along the length (sixth thoracic vertebrae to the sixth lumbar vertebrae) of the LD to eliminate location effects. Within each breed, each postmortem measurement occurred the same number of times at any location. Temperature values were obtained using an electronic digital probe (Digital Multimeter, Model 8020A, John Fluke Mfg. Co., Inc., Mountlake Terrace, WA 98043) at three different depths (1/3, 1/2 and 2/3 the distance across the LD) of the LD and averaged to further eliminate location effects. To determine pH, 2.5 g of LD was homogenized in 10 volumes of 5 mM iodoacetate containing 150 mM KCl as described by Bendall (1973) with an electronic pH meter (PHM62 Standard pH Meter, Radiometer America, Inc., Cleveland, OH 44145).

Fifty-gram LD samples were taken from the 12th rib region of the left side of each carcass for determining calcium-dependent protease (CDP)-I, -II and inhibitor activities at 0 and 24 h postmortem. Activities were determined on fresh samples according to Koohmaraie (1990) except dialysis was used to adjust the ionic strength of the supernatant prior to ion exchange chromatography. Activities were expressed as the amount of CDP caseinolytic activity in 50g 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 hr at 25°C. One unit of inhibitor activity was defined as the amount that inhibited one unit of CDP-II activity.

At 24 hr 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, ribeye area, percentage kidney, pelvic and heart fat, USDA yield grade, skeletal maturity, lean maturity, overall maturity and marbling were recorded. Heat ring, lean color, lean firmness and lean texture were scored on a 1 to 8 point scale (8 = nondetectable, bleached, firm or fine and 1 = severe, dark, soft or coarse).

At 24 hr postmortem, the portion of the LD (right side of carcass) from the 7th thoracic vertebra to the 5th lumbar vertebra was cut into 2.54-cm steaks (n = 12) and vacuum packaged in Cryovac® B550 bags (Cryovac Division of W.R. Grace & Co., Duncan, SC 29334). Steaks were assigned to 1, 3, 7, and 14 days postmortem vacuum aging by stratifying storage time along the length of the longissimus muscle.

Steaks were broiled to internal temperature 40°C, turned, and broiled to internal temperature 70°C on electric broilers (Farberware Open-hearth Broilers, Model 450N, Kidde, Inc., Bronx, NY) for determination of Warner-Bratzler shear force (WBSF) at 1, 3, 7, and 14 days postmortem. Internal temperature was monitored by iron constantan thermocouple probes attached to a potentiometer (Honeywell Potentiometer Multipoint Recorder, Model 112). Weights were recorded before and after cooking for determination of cooking loss (%) and cook rate (g/min). Steaks were tempered 24 hr at 4°C (Crouse and Koohmaraie, 1990a) and 6-1.3 cm cores were removed from each steak, parallel to the longitudinal orientation of muscle fibers. Cores were sheared with a Warner-Bratzler shear device attached to a universal testing machine (Model 1122, Instron Universal Testing Machine, Instron Corp., Canton, MA 02021) equipped with a Microcon computer. Crosshead speed was 5 cm/min and fail criterion was 75%.

At 1, 3, 7 and 14 days postmorten myofibril fragmentation indices (MFI) were determined on fresh muscle samples according to Culler et al. (1978). Samples for determining catheptic enzyme activities were frozen in liquid Nitrogen at 0, 1, 3, 7, and 14 days postmortem and stored at −70°C prior to extraction. Muscle extracts were prepared from 5g of LD according to Etherington et al. (1987). The homogenate was allowed to stand for 1 hr prior to centrifugation at 25,000 x g for 30 min to remove debris. The supernate was filtered

Author Shackelford, formerly with the Univ. of Georgia, Athens, is now with the Meats & Muscle Biology Section, Dept. of Animal Science, Texas A&M Univ., 348 Kleborg Center, College Station, TX 77843. Authors Koohmaraie, Whipple (formerly with Kansas State Univ.), Wheeler (formerly with Texas A&M Univ.), and Crouse are with the U.S. Dept. of Agriculture, ARS, Roman L. Hruska U.S. Meat Animal Research Center, P.O. Box 166, Clay Center, NE 68933. Author Miller's present address: Texas Tech Univ., Dept. of Animal Science, Lubbock, TX 79409. Author Reagan's present address: National Live Stock & Meat Board, 444 N. Michigan Ave., Chicago, IL 60611. Address reprint requests to Dr. M. Koohmaraie.

Table 1—Means, standard deviations and ranges for tenderness measures[a]

	Angus-Hereford	5/8 Brahman
Warner-Bratzler shear force (kg)		
day1	7.95	9.21
	(2.25)	(1.77)
	5.18-11.45	7.13-12.38
day 3	6.64	8.18
	(1.34)	(1.81)
	4.82-8.33	4.75-10.47
day 7	6.03	6.66
	(1.37)	(2.03)
	4.15-8.53	3.06-9.34
day 14	4.48	5.36
	(0.96)	(1.57)
	3.56-6.73	3.21-8.22
Myofibril fragmentation index		
day 1	45.6	35.4
	(11.2)	(7.6)
	33-61	24-48
day 3	53.6	42.2
	(12.9)	(9.6)
	29-75	31-57
day 7	64.5	65.3
	(12.2)	(13.8)
	41-74	49-83
day 14	71.3	69.6
	(12.9)	(10.1)
	51-87	50-84

[a] Means did not differ between breeds (P>0.05).

through glass wool and 2 mL of supernate was allowed to react (end-over-end mixing) for 2 hr with 2 mL of S-carboxymethylated-papain-Sepharose (Koohmaraie and Kretchmar, 1990) in a mini-column (Bio-Rad Econo-column, Bio-Rad Laboratories, 1414 Harbour Way South, Richmond, CA 94804) to remove cystatin. The resin was prepared from CNBr-activated Sepharose (Pharmacia LKB, 800 Centennial, Ave., Piscataway, NJ 08855) which was coupled to papain (Sigma Chemical Co., P.O. Box 14508, St. Louis, MO) according to Anastasi et al. (1983). Protein concentration of the pre-column supernatant and post-column eluate were determined spectrophotometrically with Bicinchoninic Acid (BCA) protein assay reagant (Pierce, Rockford, IL) according to Smith et al. (1985). Activities of cathepsins B and B + L were determined according to Kirschke et al. (1983) 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 15 min incubation at 37°C. Activities were expressed as nmole . min^{-1} . g $muscle^{-1}$. Cystatin activity was expressed as the ratio of post- to pre-column B+L activity.

The MAXR technique of stepwise regression (SAS, 1985) was used to develop regression equations for determination of WBSF and MFI at 1, 3, 7 and 14 postmortem. The MAXR technique selected the model with the given number of independent variables that had the maximum coefficient of determination (R^2) for the given dependent variable. Models were then evaluated for goodness of fit based on significance ($P < 0.05$) of each partial coefficient of regression. We chose to evaluate only those models with 5 or less variables as the inclusion of greater numbers of variables would have resulted in models with insignificant partial slopes.

Regression equations were verified on the beef populations of Wheeler et al. (1990) and Whipple et al. (1990b). Due to some differences in variables measured in our study and those determined in the referenced studies, many of our models could not be tested. This is mainly because we used some techniques in our study that were not available when the research of Wheeler et al. (1990) and Whipple et al. (1990b) was conducted. The animals (n = 20) used by Wheeler et al. (1990) were H and Brahman steers while those (n = 27) of Whipple et al. (1990b) were AH, 3/8 Sahiwal X H, A or AH and 5/8 Sahiwal X H, A or AH steers and heifers.

RESULTS & DISCUSSION

CHARACTERISTICS of the carcasses we used were reported by Shackelford et al. (1991). Means, standard deviations and ranges of tenderness values are reported by breed and time postmortem in Table 1. Simple correlation coefficients of the dependent variables with WBSF and MFI are presented in Table 2. Correlation coefficients between WBSF and MFI were −0.91, −0.74, −0.63, and −0.40 at 1, 3, 7 and 14 days postmortem, respectively (data not shown). None of the traits correlated significantly with 14-day WBSF. This contradicted the findings of Whipple et al. (1990a) who reported a strong positive correlation for CDP inhibitor activity at 24 hr postmortem and a strong negative correlation for MFI at 14 days

Table 2—Simple correlation coefficients[a] *of various parameters with Warner-Bratzler shear force and myofibril fragmentation index*

	Warner-Bratzler shear force				Myofibril fragmentation index			
Parameter	1[b]	3	7	14	1	3	7	14
Live wt	0.40	0.14	−0.03	0.04	−0.34	−0.26	−0.04	0.26
Hot carcass wt	0.34	0.17	−0.06	0.09	−0.28	−0.30	0.05	0.22
12th rib adjusted fat thickness	−0.18	−0.43	−0.08	−0.15	0.29	0.34	−0.17	−0.15
Rib-eye area	0.01	−0.01	−0.39	0.03	0.01	−0.34	0.07	−0.41
Kidney, pelvic and heart fat (%)	0.04	0.21	0.26	0.02	0.04	0.16	−0.31	−0.09
Marbling score	−0.50	−0.51	−0.40	−0.28	0.52	0.39	0.07	0.34
Temp 0 hr[c]	−0.20	−0.24	0.10	−0.28	0.22	0.35	0.36	0.54
Temp 3 hr	−0.46	−0.15	−0.69	−0.20	0.46	−0.17	0.38	−0.62
Temp 6 hr	−0.30	−0.18	−0.37	−0.10	0.51	0.08	−0.02	−0.38
Temp 9 hr	0.09	−0.05	−0.01	0.29	0.04	−0.33	−0.12	−0.55
Temp 12 hr	0.06	−0.08	0.13	0.20	0.14	0.08	−0.34	−0.21
Temp 24 hr	0.07	−0.14	0.42	−0.12	−0.17	0.34	−0.08	0.49
pH 0 hr	0.35	0.02	0.17	0.12	−0.47	−0.33	−0.18	−0.15
pH 3 hr	0.47	0.04	0.35	0.17	−0.58	−0.25	−0.41	−0.01
pH 6 hr	0.29	0.54	0.33	0.36	−0.47	−0.61	−0.02	−0.39
pH 9 hr	0.36	0.23	0.55	0.15	−0.39	−0.10	−0.52	−0.17
pH 12 hr	−0.39	−0.10	−0.58	−0.01	0.51	−0.15	0.18	−0.74
pH 24 hr	0.02	−0.01	−0.06	−0.17	−0.05	0.21	−0.01	0.17
CDP-I 0 hr	−0.07	−0.28	−0.22	−0.32	−0.03	−0.06	0.10	0.03
CDP-II 0 hr	0.19	0.19	0.20	0.07	−0.26	−0.22	−0.26	−0.01
CDP inhibitor 0 hr	0.09	−0.13	−0.23	−0.08	−0.05	0.04	−0.07	0.16
CDP-I 24 hr	0.54	0.28	0.43	0.04	−0.68	−0.22	−0.17	0.31
CDP-II 24 hr	−0.13	0.18	−0.35	0.15	0.21	−0.29	−0.01	−0.45
CDP inhibitor 24 hr	0.35	0.48	−0.01	0.39	−0.38	−0.69	−0.12	−0.57
Cystatin 0 hr	0.44	0.50	0.61	0.21	−0.40	−0.17	−0.39	0.22
Cystatin 24 hr	0.56	0.35	0.62	0.30	−0.46	−0.12	−0.53	0.37
Cystatin 72 hr	0.29	0.25	0.35	−0.02	−0.27	−0.05	−0.35	0.28
Cystatin 168 hr	0.42	0.24	0.47	0.10	−0.36	0.04	−0.48	0.40
Cystatin 336 hr	0.22	0.23	0.35	−0.03	−0.14	0.06	−0.28	0.34

[a] Correlations >0.47 or < − 0.47 are significant (P<0.05).
[b] Day postmortem.
[c] Hr postmortem.

Table 3—Prediction equations for 1-day Warner-Bratzler shear force

Number of variables significance	Independent variables[a]	Intercept	β Value	R-Square	Individual significance	Overall
1	Cystatin 24	5.5448	1.2576	0.31	0.03	0.05
2	Cystatin 24	1.1155	1.5032	0.55	0.01	0.01
	CDP inh 24		0.0356		0.02	
3	Cystatin 24	4.1228	1.4950	0.68	0.01	0.01
	CDP inh 24		0.0509		0.01	
	CDP-II 24		−0.0819		0.05	
4	Cystatin 24	−5.6032	1.5867	0.81	0.01	0.001
	CDP inh 24		0.0638		0.01	
	CDP-II 24		−0.1152		0.01	
	Temp 12		0.6192		0.02	
5	Cystatin 24	18.6973	1.7665	0.85	0.01	0.001
	CDP inh 24		0.0782		0.01	
	CDP-II 24		−0.1538		0.01	
	Temp 12		0.8277		0.01	
	pH 9		−4.9847		0.11	

[a] Cystatin 24 is the ratio of post- to pre-column B+L activity at 24 hr postmortem. CDP inh 24 is the activity of CDP inhibitor per 50g LD at 24 hr postmortem. CDP-II 24 is the activity of CDP-II per 50g LD at 24 hr postmortem. Temp 12 is LD temp (°C) at 12 hr postmortem. pH 9 is LD pH at 9 hr postmortem.

Table 4—Prediction equations for 3-day Warner-Bratzler shear force

Number of variables significance	Independent variables[a]	Intercept	β Value	R-Square	Individual significance	Overall
1	pH 6	−20.8419	5.0275	0.29	0.04	0.05
2	Cystatin 0	0.8767	1.3407	0.55	0.02	0.01
	CDP inh 24		0.0333		0.02	
3	CDP inh 24	6.9014	0.0468	0.81	0.01	0.001
	Cystatin 0		1.3616		0.01	
	CDP-I 0		−0.1117		0.01	
4	CDP-I 0	11.3155	−0.1219	0.88	0.01	0.0001
	Cystatin 0		1.2957		0.01	
	CDP inh 24		0.0387		0.01	
	Adj Fat		−5.0719		0.03	
5	CDP-I 0	35.2680	−0.1317	0.91	0.01	0.0001
	Cystatin 0		1.2808		0.01	
	CDP inh 24		0.0371		0.01	
	Adj Fat		−6.2358		0.01	
	pH 24		−4.1243		0.10	

[a] pH 6 is LD pH at 6 hr postmortem. Cystatin 0 is the ratio of post- to pre-column B + L activity at 0 hr postmortem. CDP inh 24 is the activity of CDP inhibitor per 50g LD at 24 hr postmortem. CDP-I 0 is the activity of CDP-I per 50g LD at 0 hr postmortem. Adj Fat is the 12th rib adjusted fat thickness (mm). pH 24 is LD pH at 24 hr postmortem.

postmortem with 14-day WBSF. This was most likely due to less variation within the population of cattle in our study than within the population of cattle of Whipple et al. (1990a). A strong negative correlation between degree of myofibril fragmentation and WBSF has been well documented (Olson and Parrish, 1977; Culler et al., 1978; Calkins et al., 1980; Davis et al., 1980; Cole and Davis, 1981; Hawkins et al., 1987). Because MFI is a result, rather than a cause, of postmortem aging, MFI and WBSF were used as dependent variables for development of regression equations.

Marbling correlated ($P < 0.05$) −0.50, −0.51, and 0.52 with WBSF at days 1 and 3 MFI at day 1, respectively. Correlation coefficients indicated lower LD temperature corresponded with higher WBSF and lower MFI values, but few correlations were significant. Many correlations ($P < 0.05$) were determined between pH and WBSF and MFI; however, the direction of the correlations varied from one pH measurement to another. Through 9 hr postmortem, higher pH resulted in higher WBSF and lower MFI values. At 12 hr postmortem, higher pH resulted in lower 7-day WBSF, higher 1-day MFI values and lower 14-day MFI values. The contradiction between correlations of pH at 12 hr postmortem and MFI values at days 1 and 14 was due partially to the limited variation in ultimate pH in these animals and was indicated by the low correlation of 1- to 14-day MFI values ($r = -0.11$).

Calcium-dependent protease-I, -II and inhibitor activities at 0 h postmortem did not correlate ($P > 0.05$) with MFI or WBSF. However, CDP-I activity at 24 hr postmortem correlated ($P < 0.05$) 0.54 and −0.68 to 1-day WBSF and MFI, respectively. Therefore, those animals that had lost the most CDP-I activity during the first 24 hr had gone through the most proteolysis as revealed by MFI. That is, 24 hr CDP-I activity related inversely to improved tenderness. Warner-Bratzler shear force at 3 days postmortem and 3- and 14-day MFI were 0.48, −0.69, and −0.57 correlated with 24-hr CDP inhibitor activity, respectively. Whipple et al. (1990a) reported 24-hr CDP inhibitor activity correlated 0.66 and −0.66 to WBSF and sensory panel tenderness scores, respectively.

Cystatin (an inhibitor of lysosomal cysteine proteinases) activity up to day 7 correlated ($P < 0.05$) with MFI and WBSF. However, at day 14, cystatin activity slightly correlated ($P > 0.05$) with WBSF and MFI. This suggested that if lysosomal enzymes were involved in postmortem aging, their contribution to the aging process had ended by day 14 postmortem. However, postmortem storage (0, 1, 3, 7 and 14 days) did not affect ($P > 0.05$) pre-(before cystatin removal) and post-column (after cystatin removal) cathepsin B and B+L activity (Shackelford et al., 1991). Calkins et al. (1987) reported a 5-variable equation that included cathepsins B and H and β-glucuronidase total and specific activities within 1 hr postmortem. That equation accounted for 46% of the variation in WBSF at day 7 postmortem. However, cystatin was not removed from the aliquots which were assayed in that study. Thus, relationships may have been due to differences in the level of cystatin inhibition rather than activity of the catheptic enzymes.

The best 1- to 5-variable equations for predicting WBSF at 1, 3, 7 and 14 days postmortem are reported in Tables 3, 4, 5, and 6. Cystatin activity at 24 hr postmortem accounted for 31% of the variation in 1-day WBSF. Major increases in R^2

Table 5—Prediction equations for 7-day Warner-Bratzler shear force

Number of variables significance	Independent variables[a]	Intercept	β Value	R-Square	Individual significance	Overall
1	Temp 3	21.2034	−0.4609	0.48	0.01	0.01
2	Temp 3	−12.2156	−0.3909	0.62	0.01	0.01
	pH 9		5.6566		0.05	
3	Temp 3	6.4985	−0.4179	0.76	0.01	0.001
	Cystatin 0		1.2862		0.01	
	Temp 9		0.6001		0.02	
4	Cystatin 24	−40.2444	1.3668	0.87	0.01	0.0001
	pH 6		6.2813		0.01	
	Temp 12		0.6905		0.01	
	CDP-II 24		−0.0555		0.02	
5	Cystatin 24	−32.5931	1.1422	0.90	0.01	0.0001
	pH 6		5.8303		0.01	
	Temp 12		0.6503		0.01	
	CDP-II 24		−0.0392		0.08	
	Temp 3		−0.1504		0.15	

[a] Temp 3 is LD temperature (°C) at 3 hr postmortem. pH 9 is LD pH at 9 hr postmortem. Cystatin 0 is the ratio of post- to pre-column B+L activity at 0 hr postmortem. Temp 9 is LD temperature (°C) at 9 hr postmortem. Cystatin 24 is the ratio of post- to pre-column B+L activity at 24 hr postmortem. pH 6 is LD pH at 6 hr postmortem. Temp 12 is LD temperature (°C) at 12 hr postmortem. CDP-II 24 is the activity of CDP-II per 50g LD at 24 hr postmortem.

Table 6—Prediction equations for 14-day Warner-Bratzler shear force

Number of variables significance	Independent variables[a]	Intercept	β Value	R-Square	Individual significance	Overall
1	CDP inh 24	2.9665	0.0181	0.15	0.14	0.14
2	CDP inh 24	7.6824	0.0285	0.42	0.02	0.05
	CDP-I 0		−0.0867		0.03	
3	CDP inh 24	5.9891	0.0345	0.63	0.01	0.01
	CDP-I 0		−0.0959		0.01	
	Cystatin 24		0.6922		0.03	
4	CDP inh 24	−0.7947	0.0327	0.73	0.01	0.01
	CDP-I 0		−0.0880		0.01	
	Cystatin 24		0.8367		0.01	
	Temp 9		0.3483		0.08	
5	CDP inh 24	20.8325	0.0330	0.78	0.01	0.01
	CDP-I 0		−0.0952		0.01	
	Cystatin 24		0.8182		0.01	
	Temp 9		0.3272		0.09	
	pH 24		−3.8101		0.18	

[a] CDP inh 24 is the activity of CDP inhibitor per 50g LD at 24 hr postmortem. CDP-I 0 is the activity of CDP-I per 50g LD at 0 hr postmortem. Cystatin 24 is the ratio of post- to pre-column B+L activity at 24 hr postmortem. Temp 9 is LD temperature (°C) at 9 hr postmortem. pH 24 is LD pH at 24 hr postmortem.

Table 7—Prediction equations for 1-day myofibril fragmentation index

Number of variables significance	Independent variables[a]	Intercept	β Value	R-Square	Individual significance	Overall
1	CDP-I 24	56.27	−0.54	0.46	0.01	0.01
2	CDP-I 24	−3.98	−0.46	0.58	0.01	0.01
	Temp 6		2.59		0.08	
3	CDP-I 24	5.87	−0.49	0.67	0.01	0.01
	Temp 6		4.50		0.02	
	Temp 12		−3.23		0.09	
4	Live weight	292.85	−0.23	0.87	0.01	0.0001
	Temp 6		6.52		0.01	
	Temp 12		−6.20		0.01	
	pH 6		−34.20		0.01	
5	Live weight	365.31	−0.23	0.92	0.01	0.0001
	Temp 6		6.29		0.01	
	Temp 12		−5.37		0.01	
	pH 6		−28.60		0.01	
	pH 9		−20.38		0.05	

[a] CDP-I 24 is the activity of CDP-I per 50g LD at 24 hr postmortem. Temp 6 is LD temperature (°C) at 6 hr postmortem. Temp 12 is LD temperature (°C) at 12 hr postmortem. Live weight is the weight of the live animal (kg) prior to slaughter. pH 6 is LD pH at 6 hr postmortem. pH 9 is LD pH at 9 hr postmortem.

occurred with addition of successive variables to the model until the fifth variable was added. The R^2 of the 5 variable equation was only slightly higher than the 4-variable equation and the partial coefficient of regression of the fifth variable was not significant ($P > 0.05$).

For 3-day WBSF a 3-variable equation that included 24-hr CDP inhibitor activity, 0-hr cystatin activity and 0-hr CDP-I activity had an R^2 of 0.81 (Table 4). As models were developed for prediction of 7-day WBSF, all variables included in the best 3-variable model (temp at 3 hr, cystatin at 0 hr, and temp at 9 hr postmortem) were not included in the best 4-variable model (cystatin at 24 hr, pH at 6 hr, temp at 12 hr and CDP-II activity at 24 hr; Table 5). Each variable was replaced by a closely related variable, usually a repeated measure of the same variable. Models that included cystatin activity could not be tested against the populations of Whipple et al. (1990b) and Wheeler et al. (1990) because it was not measured in those studies.

The best 1-variable model for predicting WBSF at day 14 (Table 6) was not significant ($P > 0.05$). The best 2-variable model, which included 24-hr CDP inhibitor activity and 0-hr CDP-I activity, accounted for 42% of the variation in 14-day

Table 8—Prediction equations for 3-day myofibril fragmentation index

Number of variables significance	Independent variables[a]	Intercept	β Value	R-Square	Individual significance	Overall
1	CDP inh 24	79.94	−0.30	0.47	0.01	0.01
2	CDP inh 24	92.27	−0.32	0.55	0.01	
	Cystatin 24		−3.88		0.16	
3	CDP inh 24	170.10	−0.30	0.68	0.01	0.01
	Cystatin 0		−7.42		0.04	
	Temp 9		−4.22		0.04	
4	CDP inh 24	155.78	−0.32	0.79	0.01	0.001
	cystatin 0		−8.28		0.01	
	Temp 9		−4.68		0.02	
	KPH fat (%)		9.29		0.04	
5	CDP inh 24	124.37	−0.36	0.84	0.01	0.001
	Cystatin 0		−8.11		0.01	
	Temp 9		−4.24		0.02	
	KPH fat (%)		10.59		0.02	
	CDP-I 0		0.37		0.10	

[a] CDP inh 24 is the activity of CDP inhibitor per 50g LD at 24 hr postmortem. Cystatin 24 is the ratio of post- to pre-column B+L activity at 24 hr postmortem. Cystatin 0 is the ratio of post- to pre-column B+L activity at 0 hr postmortem. Temp 9 is LD temperature (°C) at 9 hr postmortem. KPH fat (%) is the percentage kidney, pelvic and heart fat. CDP-I 0 is the activity of CDP-I per 50g LD at 0 hr postmortem.

Table 9—Prediction equations for 7-day myofibril fragmentation index

Number of variables significance	Independent variables[a]	Intercept	β Value	R-Square	Individual significance	Overall
1	Cystatin 24	82.44	−7.26	0.28	0.04	0.05
2	Cystatin 24	337.38	−6.16	0.47	0.05	0.05
	pH 9		−46.77		0.05	
3	Cystatin 24	305.54	−7.15	0.63	0.02	0.01
	pH 3		−26.00		0.03	
	Temp 12		−4.47		0.03	
4	Cystatin 24	333.84	−7.92	0.71	0.01	0.01
	Temp 12		−5.91		0.01	
	pH 3		−28.69		0.02	
	Temp 24		2.53		0.12	
5	Cystatin 168	898.07	−8.24	0.84	0.01	0.001
	pH 3		−45.85		0.01	
	CDP-I 0		0.87		0.01	
	Temp 12		−3.75		0.02	
	pH 24		100.66		0.02	

[a] Cystatin 24 is the ratio of post- to pre-column B+L activity at 24 hr postmortem. pH 9 is LD pH at 9 hr postmortem. pH 3 is LD pH at 3 hr postmortem. Temp 12 is LD temperature (°C) at 12 hr postmortem. Temp 24 is LD temperature (°C) at 24 hr postmortem. Cystatin 168 is the ratio of post- to pre-column B+L activity at 168 hr postmortem. CDP-I 0 is the activity of CDP-I per 50g LD at 0 hr postmortem. pH 24 is LD pH at 24 hr postmortem.

Table 10—Prediction equations for 14-day myofibril fragmentation index

Number of variables significance	Independent variables[a]	Intercept	β Value	R-Square	Individual significance	Overall
1	pH 12	781.36	−131.16	0.54	0.01	0.01
2	pH 12	1198.71	−161.20	0.75	0.01	0.0001
	pH 9		−46.21		0.01	
3	pH 12	1216.11	−155.57	0.82	0.01	0.0001
	pH 9		−37.54		0.02	
	pH 6		−17.03		0.05	
4	pH 9	799.95	−59.00	0.91	0.01	0.0001
	CDP-I 24		0.55		0.01	
	CDP inh 24		−0.20		0.01	
	pH 12		−73.94		0.02	
5	pH 9	813.57	−56.50	0.95	0.01	0.0001
	CDP-I 24		0.64		0.01	
	CDP inh 24		−0.27		0.01	
	pH 12		−80.74		0.01	
	CDP-II 24		0.28		0.05	

[a] pH 12 is LD pH at 12 hr postmortem. pH 9 is LD pH at 9 hr postmortem. pH 6 is LD pH at 6 hr postmortem. CDP-I 24 is the activity of CDP-I per 50g LD at 24 hr postmortem. CDP inh 24 is the activity of CDP inhibitor per 50g LD at 24 hr postmortem. CDP-II 24 is the activity of CDP-II per 50g LD at 24 hr postmortem.

WBSF (Table 6). This model was tested against the populations of Wheeler et al. (1990) and Whipple et al. (1990b). Correlations of predicted 14-day WBSF with actual 14-day WBSF in the respective populations were 0.77 and 0.54. The best model for prediction of 14-day WBSF was a 3-variable equation that included 24-hr CDP inhibitor activity, 0-hr CDP-I activity and 24-hr cystatin activity. Unfortunately, again this model could not be tested on the other beef populations.

The best 1- to 5-variable equations for predicting MFI at 1, 3, 7 and 14 d postmortem are reported in Tables 7, 8, 9 and 10, respectively. All partial coefficients of regression in the best 5-variable model for predicting 1-day MFI were significant. This model contained live weight, LD temperature at 6 and 12 hr postmortem and pH at 6 and 9 hr postmortem (Table 7). These correlations were possibly due to relationships between pH, temperature and activities of endogenous proteinases.

Activity of CDP inhibitor at 24 hr accounted for 47% of the variation in 3-day MFI values. The best two-variable model for 3-d MFI contained a variable (24-hr cystatin activity) which

Table 11—Simple correlation coefficients of predicted[a] and actual Warner-Bratzler shear force and myofibril fragmentation indices of Whipple et al. (1990a)

Number of variables	Warner-Bratzler shear force		Myofibril fragmentation index			
	1[b]	14	1	3	7	14
1	ND[d]	0.66	−0.34	0.66	ND	0.52
2	ND	0.54	−0.24	ND	ND	0.46
3	ND	ND	−0.24	ND	ND	0.45
4	ND	ND	−0.19	ND	ND	0.50
5	ND	ND	−0.14	ND	ND	0.53
Special[c]	0.45	0.54	0.80	0.69	0.51	0.75

[a] Predicted values were calculated with the prediction equations generated in this study. The equations used are presented in Tables 2 and 5 through 9.

[b] Days postmortem.

[c] Special refers to a 2-variable equation, consisting of CDP inhibitor activity at 24 hr postmortem and CDP-I activity at 0 hr postmortem, which was developed in the present study to predict each of the dependent variables. These equations are:

1-day WBSF = 9.0584 + 0.0319(24 hr CDP Inh) − 0.0583(0 hr CDP-I)
14-day WBSF = 7.6824 + 0.0285(24 hr CDP Inh) − 0.0867(0 hr CDP-I)
1-day MFI = 46.129 − 0.1632(24 hr CDP Inh) + 0.1779(0 hr CDP-I)
3-day MFI = 60.530 − 0.3401(24 hr CDP Inh) + 0.3568(0 hr CDP-I)
7-day MFI = 56.657 − 0.0845(24 hr CDP Inh) + 0.2580(0 hr CDP-I)
14-day MFI = 73.201 − 0.2722(24 hr CDP Inh) + 0.3952(0 hr CDP-I).

[d] ND = Could not be determined because these equations contained independent variables which were not measured by Whipple et al. (1990a).

had a nonsignificant partial coefficient ($P > 0.05$). However, all partial coefficients of regression were significant for the best 3- and 4-variable models for 3-day MFI ($R^2 = 0.68$ and 0.79, respectively). The best model for 7-day MFI, which had an R^2 of 0.84, was a 5-variable model that included 7-day cystatin activity, 3-hr pH, 0-hr CDP-I activity, 12-hr temperature and 24-hr pH.

The best 1- to 3-variable models for 14-day MFI were composed solely of pH measurements (Table 10). The 5-variable model contained the activities of CDP-I, -II and inhibitor at 24 hr and pH at 9 and 12 hr postmortem. When these models were tested on the population of Whipple et al. (1990b), moderate correlations (about 0.5) were found between actual and predicted MFI (Table 11).

The model containing 24-hr CDP inhibitor activity and 0-hr CDP-I activity was found accurate at predicting 14-d WBSF in somewhat diverse populations. Therefore we decided to test the relationship of these two parameters to WBSF at 1 and 7 days postmortem and MFI at 1, 3, 7, and 14 days postmortem. Equations were developed to predict the tenderness measurements using 24-hr CDP inhibitor activity and 0-hr CDP-I activity. Correlation coefficients for values predicted with these "special" equations to actual values collected by Whipple et al. (1990b) are reported in Table 11. Also reported in Table 11 are correlation coefficients for the best 1- to 5-variable models for each dependent variable determined by Whipple et al. (1990b). The 1-variable model for predicting 14-day WBSF (Table 6), which contained 24-hr CDP inhibitor activity, predicted values that correlated 0.66 to actual values from Whipple et al. (1990b) (Table 11). However, when this 1-variable model was applied to the population of Wheeler et al. (1990), a nonsignificant ($P > 0.05$) correlation of 0.13 was determined. This could have been because Wheeler et al. (1990) determined activities of the components of the CDP system on frozen samples. Freezing has been shown to reduce the activity of CDP inhibitor (Koohmaraie, 1990).

Estimated 7-day WBSF values, which were determined with an equation including 24-hr CDP inhibitor activity and 0-hr CDP-I activity [7-day WBSF = 9.1481 + 0.0057 (24-hr CDP Inhibitor) − 0.0508(0-hr CDP-I)], were 0.58 correlated to actual WBSF in the population of Wheeler et al. (1990).

In predicting MFI at day 1 and 14, the "special equation" using 24-hr CDP inhibitor and 0-hr CDP-I activities was superior to the best 1- to 5-variable equations (Table 11). However, the 1-variable equation for 3-day MFI, which consisted of 24-hr CDP inhibitor activity, was similar to the "special" equation in its ability to predict 3-day MFI in the Whipple et al. (1990b) population. When prediction equations including temperature were tested on the cattle population of Whipple et al. (1990b), correlations were negative between actual and predicted 1-day MFI values (Table 11). The best 1- to 5-variable equations for 14-day MFI, which included pH measurements, resulted in predicted values that correlated moderately with actual values.

There has been much interest in evaluating genetic potential for tenderness in live animals. Wheeler and Koohmaraie (1991) reported the CDP system may be accurately quantified in limited (5g) muscle samples. Crouse and Koohmaraie (1990b) demonstrated that MFI measurements could be performed accurately on muscle biopsy explants. Measurement of the components of the CDP system or MFI on muscle biopsies may have potential use as a breeding animal selection technique.

CONCLUSIONS

THE ACTIVITIES of calcium-dependent protease inhibitor at 24 hr and calcium-dependent protease-I at 0 hr postmortem were valuable predictors of postmortem tenderness across different beef populations. Also, cystatin activity seemed to be of value in predicting tenderness in our study and should be further investigated in other cattle populations. The strong relationship of the calcium-dependent proteolytic system to postmortem tenderness indicates that reseach to improve tenderness should be conducted by manipulating this system.

REFERENCES

Anastasi, A., Brown, M.A., Kembhavi, A.A., Nicklin, M.J.H., Sayers, C.A., Sunter, D.C., and Barrett, A.J. 1983. Cystatin, a protein inhibitor of cysteine proteinases. Biochem. J. 211: 129.

Bendall, J.R. 1973. Postmortem changes in muscle. In *Structure and Function of Muscle*, (Ed.) G.H. Bourne, Vol. 2, p. 243. Academic Press, New York.

Calkins, C.R., Davis, G.W., and Sanders, W.L. 1980. Fragmentation index of raw muscle as a tenderness predictor of steaks from USDA commercial and utility carcasses. J. Food Sci. 45: 111.

Calkins, C.R., Seideman, S.C., and Crouse, J.D. 1987. Relationships between rate of growth, catheptic enzymes and meat palatability in young bulls. J. Anim. Sci. 64: 1448.

Cole, A.B. Jr., and Davis, G.W. 1981. Influence of postmortem aging period on the fragmentation index of bovine longissimus muscle. J. Food Sci. 46: 644.

Cross, H.R., Savell, J.W. and Francis, J.J. 1986. National Consumer Retail Beef Study. Proc. 39th Ann. Recip. Meat Conf. 39: 112.

Crouse, J.D., and Koohmaraie, M. 1990a. Effect of post-cooking storage conditions on shear-force values of beef steaks. J. Food Sci. 55: 858.

Crouse, J.D. and Koohmaraie, M. 1990b. Effect of time of sampling postmortem on myofibril fragmentation index of meat. J. Food Sci. 55: 254.

Culler, R.D., Parrish, F.C. Jr., Smith, G.C., and Cross, H.R. 1978. Relationship of myofibril fragmentation index to certain chemical, physical and sensory characteristics of bovine longissimus muscle. J. Food Sci. 43: 1177.

Davis, G.W., Dutson, T.R., Smith, G.C., and Carpenter, Z.L. 1980. Fragmentation procedure for bovine longissimus muscle as an index of cooked steak tenderness. J. Food Sci. 45: 880.

Dolezal, H.G., Smith, G.C., Savell, J.W., and Carpenter, Z.L. 1982. Comparison of subcutaneous fat thickness, marbling and quality grade for predicting palatability of beef. J. Food Sci. 47: 397.

Etherington, D.J., Taylor, M.A.J., and Dransfield, E. 1987. Conditioning of meat from different species. Relationship between tenderizing and levels of cathepsin B, cathepsin L, calpain I, calpain II and β-glucuronidase. Meat Sci. 20: 1.

Hawkins, R.R., Davis, G.W., Cable, J.K., Jr., and Ramsey, C.B. 1987. Fragmentation index as an early postmortem predictor of beef tenderness. J. Anim. Sci. 64: 171.

Kirschke, H., Wood, L., Roisen, F.J., and Bird, J.W.C. 1983. Activity of lysosomal cysteine proteinase during differentiation of rat skeletal muscle. Biochem. J. 214: 871.

Koohmaraie, M. 1988. The role of endogenous proteases in meat tenderness. Proc. 41st Ann. Recip. Meat Conf. 41: 89.

Koohmaraie, M. 1990. Quantification of Ca^{2+}-dependent protease activities by hydrophobic and ion-exchange chromatography. J. Anim. Sci. 68: 659.

Koohmaraie, M., and Kretchmar, D.H. 1990. Comparisons of four methods for quantification of lysosomal cysteine proteinase activities. J. Anim. Sci. 68: 2362.

Morgan, J.B., Savell, J.W., Hale, D.S., Miller, R.K., Griffin, D.B., Cross, H.R., and Shackelford, S.D. 1991. National beef tenderness survey. J. Anim. Sci. 69: 3274.

Olson, D.G., and Parrish, F.C. Jr. 1977. Relationship of myofibril fragmentation index to measures of beefsteak tenderness. J. Food Sci. 42: 506.

Riley, R.R., Savell, J.W., Murphey, C.E., Smith, G.C., Stiffler, D.M., and Cross, H.R. 1983a. Effects of electrical stimulation, subcutaneous fat

—Continued on page 1140

PREDICTION OF AGED BEEF TENDERNESS. . .From page 1135

thickness and masculinity traits on palatability of beef from young bull carcasses. J. Anim. Sci. 56: 584.
Riley, R.R., Savell, J.W., Murphey, C.E., Smith, G.C., Stiffler, D.M., and Cross, H.R. 1983b. Palatability of beef from steer and young bull carcasses as influenced by electrical stimulation, subcutaneous fat thickness and marbling. J. Anim. Sci. 56: 592.
SAS, 1985. *SAS User's Guide: Statistics*. Statistical Analysis System Institute, Inc., Cary, NC.
Shackelford, S.D., Koohmaraie, M., Miller, M.F., Crouse, J.D., and Reagan, J.O. 1991. An evaluation of tenderness of the longissimus muscle of Angus by Hereford vs Brahman crossbred heifers. J. Anim. Sci. 69: 171.
Smith, G.C., Savell, J.W., Cross, H.R., Carpenter, Z.L., Murphey, C.E., Davis, G.W., Abraham, H.C., Parrish, F.C., Jr., and Berry, B.W. 1987. Relationship of USDA quality grades to palatability of cooked beef. J. Food Qual. 10: 269.
Smith, P.K., Krohn, R.I., Hermanson, G.T., Malia, A.K., Gartner, F.H., Goeke, N.M., Olson, B.J., and Klenk, D.C. 1985. Measurement of protein using Bicinchoninic Acid. Anal. Biochem. 150: 76.
Tatum, J.D., Smith, G.C., and Carpenter, Z.L. 1982. Interrelationships between marbling, subcutaneous fat thickness and cooked beef palatability. J. Anim. Sci. 54: 777.
USDA. 1989. Official United States Standards for Grades of Carcass Beef. Agr. Marketing Service, United States Department of Agriculture, Washington, DC.
Wheeler, T.L., and Koohmaraie, M. 1991. A modified procedure for simultaneous extraction and subsequent assay of calcium-dependent and lysosomal protease systems from a skeletal muscle biopsy. J. Anim. Sci. 69: 1559.
Wheeler, T.L., Savell, J.W., Cross, H.R., Lunt, D.K., and Smith, S.B. 1990. Mechanisms associated with the variation in tenderness of meat from Brahman and Hereford cattle. J. Anim. Sci. 68: 4206.
Whipple, G., Koohmaraie, M., Dikeman, M.E., and Crouse, J.D. 1990a. Predicting beef-longissimus tenderness from various biochemical and histological muscle traits. J. Anim. Sci. 68: 4193.
Whipple, G., Koohmaraie, M., Dikeman, M.E., Crouse, J.D., Hunt, M.C., and Klemm, R.D. 1990b. Evaluation of attributes that affect longissimus muscle tenderness in *Bos taurus* and *Bos indicus* cattle. J. Anim. Sci. 68: 2716.
Ms received 11/14/90; revised 2/6/91; accepted 4/11/91.