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Differences in Cathepsin B + L and Calcium-Dependent Protease Activities Among Breed Type and Their Relationship to Beef Tenderness

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DIFFERENCES IN CATHEPSIN B + L AND CALCIUM-DEPENDENT PROTEASE ACTIVITIES AMONG BREED TYPE AND THEIR RELATIONSHIP TO BEEF TENDERNESS[1]

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ABSTRACT

Activities of acidic proteases (cathepsin B + L) and neutral, calcium-dependent proteases (CDP) were quantified to determine whether differences in proteolytic activity could explain differences in meat tenderness among breed types. Steers (n = 32) of known percentage Angus (A) and Brahman (B) breeding were used to establish differences in meat tenderness (A; 3/4A-1/4B; 1/2A-1/2B; 1/4A-3/4B). Samples were removed from the longissimus muscle within 1 h postmortem and within 2 h were frozen for subsequent determination of cathepsin B + L, CDP-I, CDP-II and CDP-inhibitor activities. Warner-Bratzler shear (WBS) was assessed after 1, 5 and 10 d of postmortem aging. Taste panel evaluations, conducted on steaks that were subjected to 5 d of aging, detected no differences. At d 1, WBS did not differ among breed types; however, by d 10 of aging, steaks from Angus steers were more tender ($P < .05$) than steaks from 1/2B and 3/4B steers. The Angus and 1/4B steaks had significantly more ($P < .05$) cathepsin B + L activity than the 3/4B. The CDP had no relationship with WBS; however, CDP-inhibitor was positively related to d-1 WBS ($r = .41$, $P < .05$). Cathepsin B + L activity was negatively related to WBS at d 10 ($r = -.44$, $P < .05$). These data suggest that differences in meat tenderness among breed types may be explained partially by differences in proteolytic enzyme activity.

(Key Words: Beef, Tenderness, Cathepsins, Proteolysis, Brahman, Angus.)

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Introduction

Research has shown that steers containing Angus breeding are perceived to be more tender than steers containing Brahman breeding (Damon et al., 1960; Ramsey et al., 1963; Luckett et al., 1975; Peacock et al., 1980, 1982; Williams et al., 1987, 1988). These differences in tenderness suggest that factors affecting tenderness are influenced by breed type.

Proteolytic degradation of muscle proteins occurs during the postmortem aging process (Asghar and Bhatti, 1987; Ouali et al., 1987). However, some questions still remain as to which enzymes are responsible for proteolytic breakdown, Ca^{++}-dependent proteases (Dayton et al., 1975; Koohmaraie et al., 1986, 1987; Calkins and Seideman, 1988) and(or) lysosomal proteases (Dutson and Lawrie, 1974; Moeller et al., 1976, Calkins et al., 1987; Calkins and Seideman, 1988). Cathepsin B is a lysosomal protease that degrades myosin (Schwartz and Bird, 1977), troponin T (Noda et al., 1981) and actin (Hirao et al., 1984). The Ca^{++}-dependent proteases (CDP) are present in two distinct forms, one requiring 50 to 70 μM Ca^{++} (CDP-I) and the second requiring 1 to 5 mM Ca^{++} (CDP-II) for activation (Mellgren,

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1980; Dayton et al., 1981; Szpacenko et al., 1981; Inomata et al., 1984; Karlsson et al., 1985). The CDP have been shown to degrade myosin (Ishiura et al., 1979), Z-disk (Dayton et al., 1976a,b), troponin-T (Olson et al., 1977; Koohmaraie et al., 1986) and desmin (Zeece et al., 1983; Koohmaraie et al., 1986).

This study was conducted to examine the relationship of CDP and lysosomal enzymes with breed type and beef tenderness.

Experimental Procedure

Thirty-two steers of known percentage Angus (A) and Brahman (B) breeding (A, 3/4A-1/4B, 1/2A-1/2B, 1/4A-3/4B) were slaughtered at the University of Florida Meat Laboratory. Samples of the longissimus muscle between 95 g and 250 g were obtained within 1 h of slaughter. Five to ten grams of the sample were designated for determination of cathepsin B + L activity; the remainder was used to determine CDP enzyme and inhibitor activities. The muscles were trimmed of fat and connective tissue, cut into 1-cm × 1-cm pieces, immediately frozen in liquid nitrogen and stored at −70°C. After sample collection, the samples were shipped with dry ice to the University of Nebraska for enzyme assays.

Carcasses were chilled at 0 and 2°C for 24 h, ribbed and graded for USDA quality and yield factors. These data were discussed in detail by Huffman et al. (1990). The strip loin (IMPS 180) was removed immediately after grading and fabricated into 2.5-cm steaks. Beginning at the anterior end, steaks one and three were assigned to sensory panel evaluation, steak two to 5-d Warner-Bratzler shear (WBS) force determination, steaks four and five to 1- and 10-d WBS (randomly) and steaks six and seven to sarcomere length and fragmentation index analysis (randomly). Steaks were vacuum-packaged in oxygen barrier bags.[4] The three steaks designated for WBS determination were aged at 1°C. Steaks for sensory panel evaluation and analysis of sarcomere length and fragmentation were aged 5 d postmortem. All steaks were subsequently frozen at −18°C until laboratory determinations could be performed. Loin steaks for WBS and sensory analysis were thawed 18 h at 2 to 4°C and then broiled[5] to an internal temperature of 70°C (AMSA, 1978). Internal temperatures were monitored using copper-constantan thermocouples attached to a potentiometer.[6] Steaks used for WBS determination were cooled to 21°C and eight cores (1.27 cm diameter) were removed parallel to fiber orientation and sheared on a Warner-Bratzler shearing device.

Cooked loin steaks for sensory evaluation were cut into 1.27-cm^2 samples and served warm to an 8- to 10-member trained sensory panel (AMSA, 1978). Steak samples were evaluated for flavor (8 = extremely intense to 1 = extremely bland), juiciness (8 = extremely juicy to 1 = extremely dry), tenderness (8 = extremely tender to 1 = extremely tough), amount of detectable connective tissue (8 = none to 1 = abundant) and off-flavor (6 = none detected to 1 = extreme off-flavor). Panelists rated each trait with an integer value from the appropriate scale.

The steak for sarcomere length and fragmentation index analyses was trimmed of all subcutaneous fat and epimysial connective tissue and cut while frozen into 7-mm cubes. Sarcomere length was determined by homogenizing 5 g of frozen sample in 25 ml of cold .25 *M* sucrose solution. A drop of homogenate was placed on a microscope slide and covered with a cover slip. The slide was placed on a stage and a helium-neon laser[7] light (.95 mW) was directed through individual myofibrils. Fifteen diffraction patterns were measured and the equation of Cross et al. (1981) was used to convert the measurements to sarcomere length in micrometers.

Fragmentation index was performed with modifications according to the procedure outlined by Davis et al. (1980). This involved homogenizing 10 g of meat in 50 ml of cold homogenizing solution (.25 *M* sucrose, .02 *M* KCl) at full speed for 45 s using a homogenizer[8]. The homogenate then was filtered through a 250-μm mesh screen. After a 40-min drying period, the screens were weighed and the fragmentation index was calculated according to Davis et al. (1980).

The CDP enzyme and inhibitor activities were determined using the methods of Kooh-

[4]Cryovac B620; W. R. Grace and Co., Duncan, SC.
[5]Farberware Open-Hearth; Farberware, Inc., Bronx, NY.
[6]Speedomax-165, Leeds and Northrup; North Wales, PA.
[7]Model 155, Spectra Physics, Inc., Mt. View, CA.
[8]Virtis 23, The Virtis Co., Gardiner, NY.

maraie et al. (1987) with slight modifications. The frozen samples were removed from storage (–70°C) and impact-fractured into small pieces. The samples were weighed (95 to 200 g) and immediately homogenized in 2.5 volume of extraction buffer (100 m*M* Tris-base, 10 m*M* 2-Mercaptoethanol, 5 m*M* EDTA, pH adjusted to 8.3 with 1 *M* HCl) in a chilled glass blender jar with a blender.[9] Homogenization was performed three times for 30 s at low speed setting and once at high speed setting for 30 s with a 30-s cooling period between each homogenization. The homogenate was centrifuged at 25,000 × g_{max} for 90 min. The supernatant was filtered through four layers of cheesecloth and then through glass wool. The pH of the supernatant was adjusted to 7.5 with 1 *M* HCl; then it was centrifuged for 90 min at 50,000 × g_{max}. The supernatant was filtered through glass wool and chromatographed on a DEAE-Sephacel column that had been equilibrated with elution buffer (20 m*M* Tris-Base, 5 m*M* EDTA, 10 m*M* Mercaptoethanol, pH adjusted to 7.5 with HCl). The bound proteins were eluted with a 400-ml continuous gradient of NaCl. The gradient was started with the elution buffer and the ending buffer consisted of elution buffer and 500 m*M* NaCl. The flow rate of the elution was 30 ml/h and 110 fractions (6.5 ml) were collected from the column.

The CDP enzymes and inhibitor activities were assayed using the methods of Koohmaraie et al. (1987) with slight modifications. The CDP-I and CDP-II enzyme activities were assayed using .5 ml from fractions 40 to 110 and 1.5 ml of Ca^{++}-assay media (100 m*M* Tris-Base, 1 m*M* NaN_3, 5 m*M* $CaCl_2$, 5 mg/ml casein, pH adjusted to 7.5 with 1 *M* acetic acid). A sample blank containing .5 ml of sample and 1.5 ml of EDTA assay media (100 m*M* Tris-Base, 1 m*M* NaN_3, 10 m*M* EDTA, 5 mg/ml casein, adjusted to pH 7.5 with 1 *M* acetic acid) accompanied each reaction tube. The reaction mixtures were incubated for 45 min at 25°C; incubation was then stopped with 2.0 ml of 5% trichloroacetic acid. The mixture was centrifuged at 35,000 × g_{max} for 4 min, then absorption of the supernatant was read at A_{278}. The CDP enzyme activity was determined according to the following formula: CDP activity = [A_{278} in Ca^{++} assay] – [A_{278} in EDTA assay].

The inhibitor was assayed by taking a 1-ml aliquot each of fractions 25 to 70. The aliquots were heated at 90°C for 3.5 min then centrifuged at 2,000 × g_{max} for 30 min; the final volume of the supernatant was recorded. A dilution factor was determined to calculate inhibitor activity in 1 ml of the supernatant. The ensuing measurements were made from each fraction to determine inhibitor activity: a) +CDP: .5 ml of CDP enzyme alone in Ca^{++} assay media; b) Fraction alone: fraction being assayed for inhibitor in Ca^{++} assay media and c) Fraction + (+CDP): fraction and +CDP incubated in Ca^{++} assay media. The reaction media was incubated and read to determine activity as described above. Inhibitor activity was determined according to the following formula: Inhibitor activity = +CDP alone – [(+CDP) + fraction] – [fraction alone × dilution factor]. The CDP enzymes and inhibitor activities were reported as amount of caseinolytic activity in 200 g of muscle.

The cathepsin B + L samples were prepared for assay using the procedures of Moeller et al. (1976). The samples (5 to 10 g) were removed from storage (–70°C) and impact-fractured into smaller (1 × 1 cm^2) pieces. Samples were mixed with 40 ml of .25 *M* sucrose containing .02 *M* KCl and homogenized for 50 s at full speed with a homogenizer[8]. The homogenate was filtered through cheesecloth and pH was adjusted to 7.3 with 1 *N* KOH. The solution was centrifuged at 5,000 × g_{max} for 5 min at 2°C. After the supernatant was collected, the pellet was resuspended in 35 ml of KCl-sucrose solution and centrifugation was repeated. The supernatants were combined and protein concentration was determined using the Biuret method. Samples were diluted to .3 mg/ml with .1% Brij 35-1m*M* NaN_3 reagent.

Cathepsin B + L total activities were determined using the procedures of Barrett (1980) with some slight modification. Assay tubes containing .25 ml of assay buffer (.352 *M* KH_2PO_4, .048 *M* Na_2HPO_4, 4 μ*M* disodium EDTA, pH adjusted to 6.0 with 1 *M* HCl, and 8 μ*M* cysteine base) and .5 ml of the diluted enzyme sample were incubated for 6 min at 40°C. The assay was started by adding .25 ml of .02 m*M* substrate solution (.01 *M* Z-Phe-Arg-4-methyl-7-coumarylamide and .1% Brij 35-1 m*M* NaN_3 reagent) and allowed to

[9]Waring Commercial Blender, Dynamics Corp. America, New Hartford, CT.

TABLE 1. LEAST SQUARES MEANS OF BREED TYPE FOR TASTE PANEL TENDERNESS, WARNER-BRATZLER SHEAR AND SARCOMERE LENGTH

Trait	Angus	3/4 Angus 1/4 Brahman	1/2 Angus 1/2 Brahman	1/4 Angus 3/4 Brahman	SE
Taste panel[a] tenderness	5.30[b]	5.06[b]	4.71[b]	4.88[b]	.31
WBS[d] on d 1, kg	5.35[b]	5.92[b]	5.13[b]	6.55[b]	.54
WBS on d 5, kg	4.92[b]	4.87[b]	5.08[bc]	6.55[c]	.49
WBS on d 10, kg	3.44[b]	4.26[bc]	4.99[c]	5.49[c]	.40
Sarcomere length	1.72[b]	1.71[b]	1.68[b]	1.72[b]	.05
FI[e]	324.0[b]	358.1[b]	480.0[b]	402.3[b]	25.90

[a]Tenderness scores were evaluated using an 8-point scale with 8 being extremely tender and 1 being extremely tough.

[b,c]Means in the same row with the same superscript letter do not differ ($P > .05$).

[d]Warner-Bratzler shear.

[e]Fragmentation index.

incubate for 30 min. The reaction was stopped by adding 1 ml of standard-blank stop solution (.1 *M* sodium chloroacetate in a buffer containing .03 *M* $NaC_2H_3O_2$, .001 *M* NaN_3, and .07 *M* CH_3COOH, adjusted to pH 4.3 with 1 *M* HCl).

Activity for cathepsin B + L was expressed as the total amount of enzyme in 10 g of muscle capable of hydrolyzing the specific substrate. Total activity for cathepsin B + L was determined using 7-amino-4-methylcoumarin as a fluorescent tag on the substrate. The fluorimeter was zeroed against the standard-blank stop solution and set to read 100 arbitrary units with .5 μ*M* 7-amino-4-methylcoumarin in the standard-blank stop solution. Cathepsin B and cathepsin L activities were reported as a combined total because both enzymes have been reported to hydrolyze the substrate (Barrett and Kirschke, 1981). The data were analyzed using a complete randomized design for analysis of variance. Least significant differences were used to determine differences between treatment means. Correlations were examined to determine the relationships between treatments and measurements (Steel and Torrie, 1980).

Results

Effects of breed type on taste panel tenderness and Warner-Bratzler shear values (WBS) are presented in Table 1. Steaks from the four breed types received similar taste panel tenderness ratings, although steaks from Angus cattle tended to be most tender. Earlier research (Ramsey et al., 1963; Peacock et al., 1980) showed that steaks from Angus steers received higher taste panel tenderness ratings than steaks from Brahman steers.

Breed types did not differ in WBS at d 1; however, WBS differed between breed types at d 5 and d 10 (F-tests were $P < .10$ and $P < .05$, respectively). Steaks from Angus and 1/4 Brahman had lower ($P < .05$) d-5 shear values than steaks from 3/4 Brahman steers. The present research is in agreement with larger studies by Williams et al. (1987, 1988) and Johnson et al. (1990) in which WBS values were lower from Angus and 1/4 Brahman steers than from 3/4 Brahman. These results parallel the taste panel tenderness ratings (Table 1) that used 5 d for aging except that the magnitude of differences between treatments are greater.

Shear values from 1/2 Brahman steaks did not differ from Angus and 1/4 Brahman at d 5. Williams et al. (1988) reported that WBS following 5 d of aging were comparable in Angus, 1/4 Brahman and 1/2 Brahman steaks. In our study, Angus steaks aged for 10 d had lower ($P < .05$) WBS values than comparable steaks from 1/2 and 3/4 Brahman carcasses. The d-10 shear values indicate a trend, although not consistent enough to be significant, of increasing shear values with decreasing percentages of Angus breeding in 1/4, 1/2 and 3/4 Brahman steers. These results indicate that carcasses containing higher percentages of Brahman breeding are less tender than purebred Angus following postmortem aging.

Breed type did not have a significant effect on sarcomere lengths or fragmentation index (Table 1). Therefore, differences in shear values between breeds cannot be attributed to differences in sarcomere length or fragmentation.

Change in WBS in response to aging among the four breed types from d 1 to d 10

TABLE 2. LEAST SQUARES MEANS OF CHANGES IN WARNER-BRATZLER SHEAR VALUES IN RESPONSE TO AGING BY BREED TYPE

Trait	Angus	3/4 Angus 1/4 Brahman	1/2 Angus 1/2 Brahman	1/4 Angus 3/4 Brahman	SE
WBS[a] on d 1–5, kg	.43[b]	1.05[b]	.05[b]	.00[b]	.55
WBS on d 5–10, kg	1.49[b]	.61[bc]	.09[c]	1.09[bc]	.40
WBS on d 1–10, kg	1.92[b]	1.66[b]	.13[c]	1.06[bc]	.46

[a]Warner-Bratzler shear.

[b,c]Means in the same row with the same superscript letter do not differ ($P > .05$).

differed ($P < .10$, Table 2). Steaks from the Angus and 1/4 Brahman had greater ($P < .05$) decreases in shear values from d 1 to d 10 than 1/2 Brahman. Changes in shear values from d 1 to d 10 were not significant between Angus, 1/4 Brahman and 3/4 Brahman; however, the total reduction of shear tended to increase with percentage of Angus. No significant differences were observed in the overall change in WBS in response to aging between d 1 and d 5 or d 5 and d 10 among the four breed types, except for a tendency for greater ($P < .05$) WBS reduction in Angus carcasses than in 1/2 Brahman carcasses between d 5 and d 10 of aging. Differences in aging response were not substantial enough to fully explain differences in shear values among breeds at d 10. Therefore, the differences in d-10 shear between Angus and 3/4 Brahman were due to the cumulative effects of breed type and aging period on shear force.

Data in Table 3 show total activities of the proteolytic enzymes. There were no differences among the breed types for CDP-I and CDP-II activity. The CDP inhibitor activity was greater ($P < .05$) in 3/4 Brahman than in the Angus and 1/2 Brahman carcasses. Okitani et al. (1976) suggested that CDP inhibitor could control proteolytic activity of the Ca^{2+}-activated enzyme in skeletal muscle cells in vivo. Consequently, the in vivo activity of CDP could be influenced by the amount of inhibitor.

There were differences ($P < .01$) for cathepsin B + L total activity among carcasses from the four breed types. Cathepsin B + L total activities were higher ($P < .05$) in Angus carcasses than in carcasses containing Brahman breeding. The 1/4 Brahman carcasses had higher ($P < .05$) cathepsin B + L total activity than did 3/4 Brahman carcasses, and there was a definite trend for decreasing cathepsin B + L total activity with decreasing percentages of Angus breeding. This is opposite the trend for increasing shear values with decreasing percentages of Angus breeding at d 10.

Table 4 presents the simple correlation coefficients between total enzyme activities and selected tenderness measurements. The data for CDP-II activity are not discussed

TABLE 3. LEAST SQUARES MEANS AND STANDARD ERRORS FOR TOTAL ACTIVITIES OF Ca^{++}-DEPENDENT PROTEASES (CDP), CDP-INHIBITOR AND CATHEPSIN B + L ENZYMES AMONG BREED TYPE

Enzyme	Angus	3/4 Angus 1/4 Brahman	1/2 Angus 1/2 Brahman	1/4 Angus 3/4 Brahman
CDP I	75.97[a]	68.06[a]	54.18[a]	80.05[a]
	(12.72)[d]	(13.60)	(14.69)	(12.72)
CDP II	50.84[a]	56.75[a]	61.88[a]	66.85[a]
	(7.26)	(7.76)	(8.38)	(7.26)
CDP-inhibitor	129.11[a]	167.09[ab]	134.11[a]	208.71[b]
	(22.67)	(24.24)	(26.18)	(22.67)
Cathepsin B + L total activity	5.15[a]	4.35[b]	3.64[bc]	3.37[c]
	(.26)	(.26)	(.20)	(.26)

[a,b,c]Means in the same row with the same superscript letter do not differ ($P > .05$).

[d]Standard errors given in parentheses.

TABLE 4. CORRELATION COEFFICIENTS AMONG TOTAL ACTIVITY OF CDP ENZYMES, CDP-INHIBITOR, CATHEPSIN B + L AND SELECTED TENDERNESS MEASURES

Trait	CDP-I[b] activity	CDP-II[b] activity	CDP-Inhibitor[c]	Total cathepsin B + L activity[d]
Tenderness (d 5)	.230	–.269	–.005	–.055
WBS[a] on d 1	.102	.222	.407**	–.061
WBS on d 5	–.008	.281	.105	–.179
WBS on d 10	–.305*	.152	.098	–.441**
WBS on d 1–5	.133	–.116	.347*	.150
WBS on d 5–10	.386**	.168	.008	.333**
WBS on d 1–10	.450**	.050	.298	.418**

[a]Warner-Bratzler shear.

[b]Caseinolytic activity from 200 g of muscle.

[c]Inhibition of CDP activity from 200 g of muscle.

[d]Activity from 10 g of muscle.

*$P < .10$.

**$P < .05$.

because research has shown that the physiological concentration of Ca^{++} in the muscle is below the concentration required to activate this enzyme (Ridgeway and Ashley, 1967).

Sensory panel tenderness ratings were not associated with CDP-I activity. Similar results were found between CDP-I and WBS at d 1 and 5. These data suggest that CDP-I activity did not influence postmortem tenderness changes in the muscle between d 1 and d 5. This contradicts results by Calkins and Seideman (1988), who reported that CDP-I activity was related to d-1 shear force values. The CDP inhibitor was, however, significantly correlated (r = .41) with d-1 WBS. This positive relationship indicates that increased levels of inhibitor activity were associated with increased WBS values. Therefore, during the first 24 h postmortem, the amount of inhibitor activity could play a major role in muscle proteolysis by regulating CDP action and limiting activity. The CDP-I activity had a strong ($P < .10$) negative correlation with d-10 WBS (r = –.31) and a significant association with changes in WBS from d 5 to d 10 and d 1 to d 10 (r = .39 and r = .45, respectively). There also appeared to be an association ($P < .10$) between CDP inhibitor activity and the changes in WBS in response to aging from d 1 to d 5 (r = .35).

Cathepsin B + L activity had a negative association (r = –44; $P < .01$) with WBS at d 10. Relationships at d 1 and d 5 were lower ($P > .10$); however, there was a trend for increasing correlations between total activity and WBS as postmortem aging proceeded to d 10. These data suggest that cathepsin B + L activity could have a substantial influence on changes in shear value during postmortem aging. Cathepsin B + L total activity was ($P < .05$) correlated with changes in WBS due to aging from d 5 to d 10 and d 1 to d 10 (r = .33, r = .42, respectively). This implies that increasing levels of cathepsin B + L total activity were associated with increases in tenderness in response to aging. Calkins and Seideman (1988) reported that cathepsins B + L were related to the overall (d 1 to d 14) changes in shear force and strongly correlated with the changes that occurred during aging from d 3 to d 6. These data suggest that cathepsin B + L activity could have a predominant influence on changes in tenderness during postmortem aging.

Discussion

Warner-Bratzler shear values were affected by breed type. Angus consistently had lower WBS values than 3/4 Brahman during postmortem aging. Although changes in WBS due to aging were not significantly different between Angus and 3/4 Brahman, the original tenderness differences plus the effects of aging were enough to cause differences in tenderness at d 10.

The CDP enzymes are widely distributed in the cytoplasm of vertebrate tissues (Asghar and Bhatti, 1987). The optimal pH and temperature for activity of CDP enzymes, which have been shown to degrade some myofibrillar proteins, are at a neutral pH and

near 37°C (Dutson, 1983); therefore, the physiological condition of carcasses immediately following slaughter should be optimum for maximum CDP enzyme activity. Data in the current study suggest that differences in WBS at d 1 cannot be due to CDP enzyme concentration. However, CDP enzyme activity may be regulated in muscle tissue by the presence of an endogenous inhibitor (Okitani et al., 1976; Waxman and Krebs, 1978). The large amount of CDP inhibitor activity in the 3/4 Brahman may have contributed to the slightly increased WBS on d 1. The ratio of CDP activity to CDP inhibitor could influence postmortem tenderness at d 1.

It is difficult to explain the high correlations between CDP-I and inhibitor activities with changes in WBS after 24 h. Koohmaraie et al. (1987) reported that approximately 50% of the maximal CDP-I activity was lost during the first 24 h postmortem. Some of this decrease may occur as a result of the inability of partially autolyzed CDP to bind to the ion exchange column (Koohmaraie, 1990). The drop in muscle temperature and pH associated with postmortem aging has been shown to reduce CDP-I activity by 93.6% from optimal temperature and pH conditions (Calkins and Rhynalds, 1989). Koohmaraie et al. (1986) concluded that at 5°C, only 24 and 28% of M CDP maximal activity was retained at pH 5.5 and 5.8, respectively. Perhaps muscle offers some protective benefit to CDP at lower pH. Alternatively, a prolonged aging time (10 d) may allow sufficient low-level CDP activity, even at these sub-optimum conditions, to enhance tenderness. Changes in postmortem muscle occurring after 1 d cannot, therefore, be totally credited to CDP enzyme activity.

Activity of cathepsin B + L enzymes is maximal at acidic pH of 4.0 to 6.0 (Dutson, 1983). The ultimate pH of beef carcasses usually is 5.6 to 5.8. Therefore, maximal activation of cathepsin B should occur following rigor and during aging. Difference in WBS among breed types at d 5 and 10 in this study could be attributed partially to cathepsin B + L activity. Angus carcasses had the greatest cathepsin B + L activity and the largest decrease in WBS in response to the 10-d aging period. Similar conclusions could be drawn by comparing 1/4 Brahman with carcasses containing 1/2 and 3/4 Brahman breeding. Therefore, the increasing levels of cathepsin B + L activity found with increasing percentages of Angus breeding could have influenced the WBS response to postmortem aging.

Conclusion

Breed type had a bearing on postmortem tenderization. The CDP enzymes may influence tenderness postmortem, although the level of CDP inhibitor could have affected the in vivo CDP activity. Therefore, regulation of CDP activity by its endogenous inhibitor could influence meat tenderness during early postmortem aging. Breed type did not alter the level of CDP activity; however, WBS and cathepsin B + L total activity differed among breed types. Breed type and cathepsin B + L enzyme activity may influence the postmortem tenderization process. The amount of cathepsin B + L activity increased as the percentage of Angus breeding increased in the carcasses. Thus, the level of cathepsin B + L activity could contribute to the differences in tenderness perceived between Angus and Angus × Brahman crossbred steers.

The factors that controlled the level of cathepsin B + L or CDP inhibitor activity have not been determined in vivo. Angus steers appear to have factors that result in greater levels of endogenous enzyme activity than Angus × Brahman crossbred steers.

Implications

Because differences in breed type may partially be explained by enzyme activities, in vivo control of enzyme activities and genetic selection of animals with a propensity for proteolysis may improve meat tenderness. This approach might be used to improve specific breed types and(or) to enhance the tenderness of beef from animals used for production of lean meat.

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