

# Effects of biological type and dietary fat treatment on factors associated with tenderness: I. Measurements on beef longissimus muscle

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**ABSTRACT:** The objective was to evaluate chemical, mechanical, and sensory attributes associated with tenderness in divergent cattle breeds—Wagyu (W; n = 12), Limousin (L; n = 12) and F<sub>1</sub>-cross (W×L; n = 12)—fed two dietary treatments (0 or 6% sunflower oil (DM basis)). A randomized complete block repeated measures design in a 3 × 2 factorial arrangement of treatments was used, and effects of breed, diet, block, and associated interactions were tested. Cattle were fed barley-based diets for an average of 259 d. Twenty-four hours postmortem (PM), steaks from the longissimus muscle (LM) were sliced, vacuum-packaged, aged (1, 3, 7, 14, 28, and 56 d PM) at 2°C, and frozen (−40°C) until analyzed. Wagyu steaks had lower ( $P < 0.05$ ) Warner-Bratzler shear force (WBSF) values than L steaks across all aging times. At 1 d PM, W steaks required slightly more ( $P > 0.05$ ) force to shear than W×L or L (0.30 and 0.11 kg, respectively); however, by d 14 PM, W steaks required 0.77 kg less ( $P < 0.05$ ) force to shear than L. Wagyu steaks received higher ( $P < 0.05$ ) sensory

panel sustained tenderness scores at d 14 PM than L. The pH decline was slower ( $P < 0.05$ ), and temperature decline more ( $P < 0.05$ ) rapid, in W carcasses than L or W×L carcasses. Breed and diet did not affect ( $P > 0.10$ ) free calcium levels (FCL) over time (0, 1, 3, 7, and 14 d PM), 0-h calpastatin activity (CA), d-1 percent collagen (OH-PRO), or d-1 collagen cross-linking (HP). Western blot analysis for the presence of the troponin-T (TNT) 30-kDa fragment, conducted only on samples from steers fed the 0% sunflower oil diet, demonstrated more proteolysis by d 3 PM in L than W or W×L. Overall, breed differences in mechanical and sensory measures of tenderness were not explained by FCL, CA, OH-Pro, and HP. Even though the initial appearance of the TNT 30-kDa fragment was greater in L, linear slopes for appearance of TNT degradation product across aging time were greater for W and W×L ( $P < 0.01$  and  $P = 0.056$ , respectively) than for L, suggesting that tenderness differences due to breed may have been facilitated by more-rapid proteolytic degradation over time.

Key Words: Beef, Collagen, Limousin, Tenderness, Troponin-T, Wagyu

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## Introduction

Consumer satisfaction depends on consistent beef palatability, especially tenderness. Thus, consumers were willing to pay more for guaranteed tender beef (Boleman et al., 1997). Differences in beef tenderness have been mainly attributed to myofibrillar and connective tissue proteins and intramuscular fat. Yet, no single factor explains greater than 50% of the variation in tenderness (Koohmaraie, 1996). Factors such as sex,

age, breed, and muscle location affect muscle proteins and play an integral role in beef tenderness (McCormick, 1994; Huff-Lonergan et al., 1996; Koohmaraie, 1996). Some tenderness differences are due to collagen content (McCormick, 1999), and, as an animal matures, toughness increases due to an increase in collagen cross-linking (McCormick, 1994; Bosselmann et al., 1995). Postmortem myofibrillar protein degradation, attributed to the calpain system, is also thought to play a crucial role in meat tenderness (Koohmaraie, 1996). Tough beef samples have a slower rate of troponin-T degradation (Huff-Lonergan et al., 1996), and breakdown of troponin-T may directly contribute to postmortem tenderization by weakening the thin filament (Taylor et al., 1995). Calpastatin, the specific muscle protease inhibitor of the calpain system, is important to the

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rate and extent of postmortem degradation (Koochmar-  
aie et al., 1995). The calpain system is activated by  
increasing levels of calcium; half-maximal activation of  
 $\mu$ - and m-calpain requires 3 to 50  $\mu$ M and 300 to 800  
 $\mu$ M concentration of free calcium, respectively (Goll et  
al., 1995), and beef contains calcium concentrations of  
630 to 970  $\mu$ M at 10 to 14 d postmortem (Parrish et  
al., 1981). Therefore, the objective of this study was to  
evaluate the relationship of chemical, mechanical, and  
sensory attributes related to beef tenderness in longis-  
simus muscle steaks from steers of divergent breeds  
(Wagyu, Limousin, and Wagyu  $\times$  Limousin) fed diets  
containing 0 or 6% sunflower oil.

## Materials and Methods

### *Design, Feeding, and Sample Collection*

The experimental protocol was approved by the  
Washington State University Animal Care and Use  
Committee (Protocol No. 2832). All procedures con-  
formed to the *Guide for the Care and Use of Agricultural  
Animals in Agricultural Research and Teaching* (Con-  
sortium, 1988).

Thirty-six steers born in the spring of 1998, repre-  
senting Wagyu (n = 12), Limousin (n = 12), and Wagyu  
 $\times$  Limousin (W $\times$ L; n = 12), were obtained at weaning  
(November 30, 1998). Steers were grouped by initial  
BW into three blocks, and two steers per breed group  
within block were randomly assigned to one of six pens.  
Pens within block were randomly assigned to one of  
two dietary treatments (0 or 6% sunflower oil (DM ba-  
sis)). Diets were described in detail by Mir et al. (2002).  
All diets were barley-based and the sunflower oil treat-  
ments were applied during the last 95 d of the back-  
grounding phase (159 d) and all of the finishing phase  
(100 d). Cattle were weighed and humanely slaughtered  
at the Washington State University Meats Laboratory,  
and carcasses were chilled for 24 h at 0 to 2°C.

Longissimus muscle (LM) samples were collected  
from the 12th and 13th ribs at 15 min postexsanguina-  
tion for measurement of calpastatin activity (CA) and  
at 0, 1, 3, 7, and 14 d postmortem for determination of  
free calcium concentration (FCL). Internal LM pH and  
temperature were measured at 0, 3, 6, 12, and 24 h  
postmortem using a hand-held pH monitor (Cole-  
Parmer, Niles, IL). At 24 h postmortem, cold carcass  
weight; LM area; adjusted 12th-rib fat thickness; and  
percentage of kidney, pelvic, and heart fat were mea-  
sured and used to determine quality and yield grades.  
Then, the entire boneless strip loin was removed from  
the right side and cut into eight 2.54-cm-thick steaks.  
The steaks were aged for 1, 3, 7, 14, 28, and 56 d post-  
mortem for determination of Warner-Bratzler shear  
force (WBSF), 14 d postmortem for trained sensory  
panel, and 1 d postmortem for fresh meat color evalua-  
tion. In addition, five 1.25-cm-thick steaks were cut and  
aged 1 d postmortem for connective tissue, as well as  
aged 1, 3, 7, and 14 d postmortem for troponin-T (TNT)

degradation product analysis. After separation into  
treatment groups, steaks were vacuum-packaged, aged  
for the appropriate time, and frozen (-40°C) for subse-  
quent analysis.

### *Free Calcium Level*

Intramuscular FCL was measured according to Par-  
rish et al. (1981) with the following procedural modifi-  
cations. Briefly, 0.5-g samples were minced by hand  
and placed in screw-top ultracentrifuge tubes. One mil-  
liliter of monoiodoacetic acid was added, tubes were  
capped, and hand-shaken vigorously. Suspended sam-  
ples were centrifuged for 2 h at 105,718  $\times$  g. Then, 1  
mL of the supernatant was removed and mixed with  
0.1 mL of 27.5% trichloroacetic acid, vortexed, and cen-  
trifuged at approximately 20°C for 20 min at 1,750  $\times$   
g. After centrifugation, 1 mL of distilled/deionized H<sub>2</sub>O  
was filtered with Millex-Gp 0.22-micron syringe filters  
(Millipore, Bedford, MA) into 10-mL test tubes, followed  
by 1 mL of the prepared FCL sample, and brought  
to a 5-mL total volume with distilled/deionized H<sub>2</sub>O.  
Prepared FCL samples were evaluated using standards  
of the appropriate calcium concentrations (0.15, 0.35,  
0.7, and 1.4 mM standard concentrations), which were  
prepared concurrently. All samples and standards were  
evaluated using inductively coupled plasma spectro-  
scopy (Thermo-Jarell-Ash, Franklin, MA).

### *Calpastatin Activity*

A 5-g sample was removed from the LM at 15 min  
postexsanguination and placed in prerigor buffer for  
the determination of CA according to procedures of  
Duckett et al. (1998). One unit of CA is defined as the  
amount of calpastatin required to inhibit one unit of  
m-calpain activity. Calpain activity is reported as the  
caseinolytic activity that resulted in an increase in ab-  
sorbance of one unit at 278 nm after a 60-min incubation  
at 25°C.

### *Fresh Meat Color Attributes*

A MiniScan XE LAV (Hunter Lab, Reston, VA) spec-  
trophotometer was used to obtain L\* (lightness; higher  
the L\* value, the lighter the color), a\* (red-green spec-  
trum; higher the a\* value, the redder the color), and  
b\* (yellow-blue spectrum; higher the b\* value, the more  
yellow the color) values on steaks aged 1 d using D65  
illuminant and the 10° standard observer settings.  
Steaks were cut, allowed to bloom for 15 to 20 min,  
and color assessment was conducted. Readings were  
obtained at six locations on the exposed lean surface of  
each steak, avoiding large pieces of connective tissue  
or fat particles. The six readings for each steak were  
averaged for statistical analysis.

### *Warner-Bratzler Shear Force and Cooking Evaluation*

Warner-Bratzler shear force analysis was conducted  
according to AMSA (1995) guidelines. Steaks were

thawed for 24 h at 4°C, and broiled to an internal temperature of 71°C on Farberware Open Hearth grills (model R4550; Farberware, Bronx, NY). Steaks were weighed before and after broiling in order to calculate cooking loss percentages. Steaks were cooled to room temperature (approximately 22°C), six 1.27-cm-diameter cores were removed parallel to the muscle fiber, and cores were shorn perpendicular to the longitudinal axis of the fibers. Peak shear force was measured using a Texture Analyzer (TA-XT2; Texture Technologies Corp., Scarsdale, NY), equipped with a WBSF attachment at a crosshead speed of 200 mm/min.

#### *Trained Sensory Panel*

Steaks were thawed for 24 h at 4°C, cooked to an internal temperature of 71°C on Open Hearth grills (Model R4550; Farberware), trimmed of all external fat and major connective tissue, and cut into 1- × 1- × 2.54-cm samples. Samples identified with randomized three-digit numbers were served to a nine-member trained (AMSA, 1995) sensory panel seated in individual booths under fluorescent lighting. Six samples were served per session in randomized order at approximately 3-min intervals. Panelists evaluated each steak for initial tenderness, sustained tenderness, initial juiciness, sustained juiciness, beef flavor intensity, and off-flavor using a 10-cm unstructured line scale for each independent attribute (0 = extremely tough, dry, bland, and no off-flavor to 10 = extremely tender, juicy, intense beef flavor, and pronounced off-flavor).

#### *Connective Tissue*

Muscle samples (5 g wet weight) were removed from steaks with the exclusion of epimysial tissue, and dried in an oven at 100°C for 24 h. Dried LM was weighed and ground using a mortar and pestle. Ground samples were then hydrolyzed for 15 h with 20 vol of 6 N HCl according to Woessner (1961) for the determination of hydroxyproline. Collagen content (**OH-PRO**) was calculated by multiplying the measured weight of hydroxyproline by 7.25. Hydroxylsypyrindinoline (**HP**) cross-link concentration (HP concentration is directly related to collagen cross-linking) was measured after the filtration step in hydroxyproline determination. Concentrations of HP and vitamin B<sub>6</sub> standard were determined using a modified HPLC procedure developed by Eyre et al. (1984).

#### *Troponin-T Degradation*

Degradation of TNT across postmortem aging times was determined using Western blotting procedures according to Huff-Loneragan et al. (1996). Preliminary studies looking at diet indicated that adding sunflower oil to the diet did not alter proteolytic degradation of the muscle. With that in mind, Western blot analysis was conducted in duplicate and only on the non-oil diet

treatment groups. Briefly, whole muscle samples were prepared from 1.25-cm-thick steaks. The protein content of the extracts was determined using the Bio-Rad DC protein assay (Bio-Rad, Hercules, CA), and all samples were adjusted to a constant protein level (6.4 mg/mL) using distilled deionized water. Sixty micrograms of the whole muscle preparations were loaded onto 15% polyacrylamide slab separating gels with a 5% polyacrylamide stacking gel. The gels were run at room temperature (approximately 20°C) for 45 min at a constant voltage of 200 and 60 mA per gel. Gels were transferred to Immuno-Blot PVDF membranes (No. 162-0177; Bio-Rad), using a Mini Trans-Blot Cell (Bio-Rad) equipped with a bio-ice cooling apparatus for 1 h at 100 V and 340 mA. Complete transfer of proteins in the 30-kDa molecular weight range was verified using a Kaleidoscope prestained molecular weight marker (Bio-Rad) and subsequent staining of gels after transfer. The Western blotting procedure used anti-troponin-T JLT-12 (Sigma, St. Louis, MO; 1:10,000) as the primary antibody, anti-mouse IgG labeled with peroxidase (Sigma; 1:5,000) as the secondary antibody, and a chemiluminescent detection system (Pierce Super Signal Substrate; Pierce, Rockford, IL). Quantification of TNT degradation was measured based on image analysis of Western blots using a Bio-Rad Fluor-S MultiImager (Bio-Rad), which measured the blots for band density/mm<sup>2</sup>. Ratios for analysis were calculated by dividing the density/mm<sup>2</sup> of a standard (a 30-kDa primary TNT degradation product from lamb LM aged for 6 d postmortem) into the sample density/mm<sup>2</sup>. Verification of equal protein loading and complete transfer of low-molecular weight proteins was accomplished through 1) Coomassie staining of the transferred gel and 2) Bio-Rad colloidal gold staining of the PVDF membrane after Western blot analysis. The rate of TNT proteolytic degradation was based on the regression coefficients of TNT degradation product appearance over aging time postmortem.

#### *Statistical Analysis*

A randomized complete block design with a 3 × 2 factorial arrangement of treatments with the main effects of breed and diet was used to evaluate factors associated with tenderness. The steers were initially blocked by BW into light-, medium-, and heavyweight groups within each breed (four for each breed by weight distinction), and then two steers were assigned to one of two dietary treatments, completing the design. Data were analyzed using GLM procedures in SAS (SAS Inst. Inc., Cary, NC) with breed, diet, block, and breed × diet included in the model. Individual steer served as the experimental unit. For pH, temperature, FCL, WBSF, and proteolytic degradation rate of TNT, the model was extended for the repeated measure of aging time. Sensory data were analyzed as a split-plot design with the same structure as the repeated measures design described above. The lone difference was that panelist



**Table 1.** Live weight and carcass traits of Wagyu (W), Wagyu × Limousin (W×L), and Limousin (L) steers fed diets with or without supplemental sunflower oil

Item	W	W×L	L	SEM	No oil diet	Oil diet	SEM
Number of animals	12	12	12	—	18	18	—
Live weight, kg	477 <sup>z</sup>	499 <sup>y</sup>	596 <sup>x</sup>	5.5	518	529	4.5
Cold carcass weight, kg	299 <sup>z</sup>	323 <sup>y</sup>	388 <sup>x</sup>	3.7	334	340	3.1
Longissimus muscle area, cm <sup>2</sup>	82 <sup>y</sup>	86 <sup>y</sup>	106 <sup>x</sup>	2.0	92	92	1.6
Kidney, pelvic, and heart fat, %	3.4 <sup>x</sup>	3.0 <sup>x</sup>	2.4 <sup>y</sup>	0.17	2.6 <sup>y</sup>	3.2 <sup>x</sup>	0.14
Adjusted fat thickness, cm	0.90	1.04	0.83	0.065	0.84	1.00	0.054
Yield grade	2.2 <sup>x</sup>	2.3 <sup>x</sup>	1.5 <sup>y</sup>	0.13	2.0	2.0	0.10
Marbling score <sup>a</sup>	867 <sup>x</sup>	583 <sup>y</sup>	471 <sup>z</sup>	18.3	643	637	15.0
Quality grade <sup>b</sup>	20.3 <sup>x</sup>	17.3 <sup>y</sup>	16.1 <sup>z</sup>	0.25	17.9	17.8	0.21

<sup>a</sup>400 = Slight<sup>0</sup>; 500 = Small<sup>0</sup>; 600 = Modest<sup>0</sup>; 700 = Moderate<sup>0</sup>; and 800 = Slightly abundant<sup>0</sup>.

<sup>b</sup>16 = high Select; 17 = low Choice; 18 = average Choice; 19 = high Choice; and 20 = low Prime.

<sup>x,y,z</sup>Within a row, means without a common superscript letter differ ( $P < 0.05$ ).

replaced time postmortem in the model. Linear, quadratic, and cubic effects for TNT degradation rate were determined using orthogonal polynomials. Differences due to breed, diet, and block were tested using the between-animal-error term, and differences due to the effect of time postmortem were tested using the within-animal-error term. Breed means were compared using LSD. Overall correlation coefficients were calculated to evaluate relationships among chemical, mechanical, and sensory attributes associated with tenderness using the correlation procedure of SAS.

## Results and Discussion

### Carcass Data

The primary intent of this study was to evaluate attributes associated with tenderness in divergent biological types of cattle. Thus, a moderate-framed breed that is heavily marbled (Wagyu) and a large-framed, faster growing, heavy-muscled breed that is very lean (Limousin) were selected. Breed influenced many carcass and some tenderness traits. Live weight and cold carcass weights increased ( $P < 0.0001$ ) as the percentage of Limousin influence increased (Table 1). Further, LM area was larger ( $P < 0.0001$ ) in Limousin than Wagyu or W×L, and kidney, pelvic, and heart fat was lower ( $P < 0.01$ ) for Limousin than Wagyu or W×L. Adjusted fat thickness tended to be lower ( $P < 0.10$ ) in Limousin than W×L, (Wagyu were intermediate), and, concomitantly, yield grade values were numerically lower in Limousin (1.5) compared to Wagyu (2.2) or W×L (2.3). However, yield grades for all breeds were within an acceptable range, indicating a relatively high retail cut yield. These results indicated that Limousin steers were faster growing, more heavily muscled, and leaner in composition than Wagyu and W×L steers, and Wagyu steers had higher ( $P < 0.0001$ ) marbling scores and higher quality grades than W×L, which were higher than for Limousin. Wulf et al. (1996) reported that Limousin steers produced high-cutability carcasses (94% were Yield Grade 1 or 2), whereas Wagyu (Yamazaki,

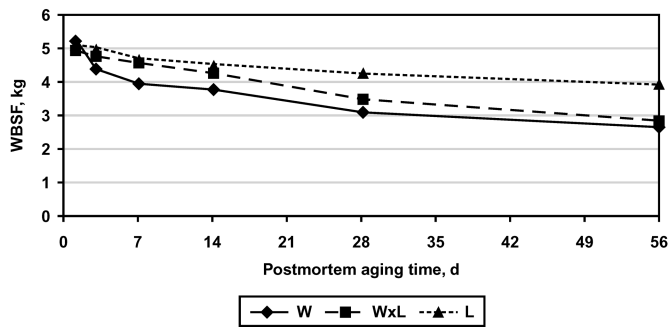
1981; Lunt et al., 1993) cattle are characterized by their ability to deposit extensive amounts of intramuscular fat (marbling) and as a result are very palatable.

The dietary fat treatments were applied primarily to evaluate the effect of supplemental fat on fatty acid composition, which is reported elsewhere (Mir et al., 2001). The only effects of dietary treatment (Table 1) were that added sunflower oil increased ( $P < 0.001$ ) kidney, pelvic, and heart fat percentage and tended to increase ( $P < 0.10$ ) adjusted fat thickness. Marks et al. (2001) reported that the addition of yellow grease (3:1 ratio of unsaturated to saturated fatty acids) increased ADG, marbling score, and percentage of internal fat. Yellow grease would be comparable to the addition of sunflower oil to the diet in the current study, and it appears that added dietary oil had no major adverse effects on carcass quality traits.

### Warner-Bratzler Shear Values, Trained Sensory Panel, and Cooking Attributes

Shear force values decreased ( $P < 0.0001$ ) over time, exhibiting a normal aging curve in all breeds (Figure 1). Wagyu had lower ( $P < 0.05$ ) WBSF across all postmortem aging times than Limousin (W×L were intermediate). The interaction of breed × time postmortem for WBSF approached significance ( $P = 0.11$ ), prompting an evaluation of d-1 vs. d-14 WBSF values. Breed groups aged 1 d postmortem were not different ( $P = 0.77$ ); however, by 14 d postmortem, Wagyu were more ( $P < 0.05$ ) tender than Limousin, whereas W×L did not differ from either breed (Figure 2).

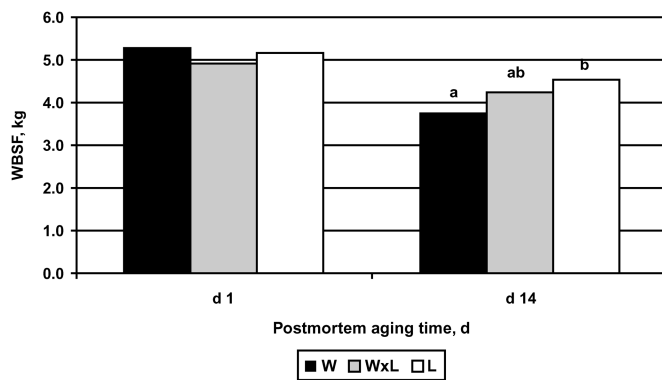
Both Wagyu and W×L tended to receive higher ( $P = 0.13$ ) initial tenderness scores (more tender) than Limousin (Table 2). After mastication, Wagyu steaks received higher ( $P < 0.05$ ) sustained tenderness scores than Limousin (W×L were intermediate). Both initial tenderness and sustained tenderness were negatively correlated with d-14 WBSF values ( $r = -0.45$  and  $r = -0.51$ , respectively). Thus, as WBSF values increased, both initial tenderness and sustained tenderness values decreased. Busboom et al. (1993) showed that



**Figure 1.** Effect of cattle breed type (W = Wagyu; L = Limousin; and W×L = Wagyu × Limousin) on Warner-Bratzler shear force (WBSF) values of longissimus steaks during postmortem aging. There was a main effect of postmortem aging time ( $P < 0.0001$ ; SEM = 0.13) and breed ( $P < 0.05$ ; SEM = 0.18), and an interactive effect of breed × postmortem aging time ( $P = 0.11$ ; SEM = 0.23) on WBSF force.

steaks from Wagyu steers were more palatable than steaks from Angus and Longhorn steers. In this study, differences in tenderness were detected by WBSF and sensory panel evaluation, yet breed type did not ( $P > 0.10$ ) affect any other sensory attributes measured.

Breed did not ( $P > 0.10$ ) influence cooking loss percentages; however, Wagyu and W×L required more ( $P < 0.01$ ) time on the open hearth grill than Limousin to reach 71°C internal temperature (Table 3). Moreover, neither diet nor breed × diet affected ( $P > 0.10$ ) WBSF or trained sensory panel scores (data not shown). Addition of 6% sunflower oil in the finishing diet slightly decreased ( $P < 0.10$ ) cooking loss compared to the diet without added sunflower oil, but diet did not ( $P > 0.10$ ) affect cooking time (Table 3) or palatability (data not shown).



**Figure 2.** Comparison of breed type (W = Wagyu; L = Limousin; and W×L = Wagyu × Limousin) on Warner-Bratzler shear force (WBSF) values of longissimus steaks after 1 and 14 d of postmortem aging. Bars that do not have a common superscript letter differ ( $P < 0.05$ ; SEM = 0.22).

**Table 2.** Sensory panel scores of longissimus muscle steaks from Wagyu (W), Wagyu × Limousin (W×L), and Limousin (L) steers fed diets with or without supplemental sunflower oil

Panelist scores <sup>a</sup>	W	W×L	L	SEM
Number of steaks	12	12	12	—
Initial tenderness	6.9	6.8	5.9	0.35
Sustained tenderness	6.3 <sup>x</sup>	5.9 <sup>xy</sup>	4.8 <sup>y</sup>	0.39
Initial juiciness	6.4	6.5	6.0	0.34
Sustained juiciness	5.9	5.4	5.1	0.32
Beef flavor intensity	5.7	6.5	5.2	0.26
Off-flavor	0.4	0.3	0.4	0.07

<sup>a</sup>0 = extremely tough, dry, bland, and no off-flavor to 10 = extremely tender, juicy, intense beef flavor, and pronounced off-flavor.

<sup>x,y</sup>Within a row, means without a common superscript letter differ ( $P < 0.05$ ).

### Temperature and pH

During the first 24 h postmortem, temperature (Figure 3) declined ( $P < 0.05$ ) more rapidly, and pH (Figure 4) more slowly, in Wagyu compared to Limousin or W×L. Differences in muscle mass between Wagyu and Limousin potentially influenced the rate of temperature decline. Bendall (1978) reported that a slower temperature decline would be expected to result in an increased rate of postmortem glycolysis, resulting in a more rapid pH decline. Regardless of breed, time postmortem was significant ( $P < 0.001$ ) for both temperature and pH decline, which would be expected in normal temperature and pH decline curves. This would indicate a favorable environment for anaerobic glycolysis, rigor development and completion at 24 h postmortem. A breed × diet interaction ( $P < 0.05$ ) indicated that carcasses from steers with Limousin breeding (W×L and Limousin) fed a diet with supplemental sunflower oil retained more heat during the first 24 h postmortem (data not shown), whereas those fed no supplemental oil chilled faster. The combination of an increase ( $P < 0.0001$ ) in muscle mass of Limousin steers, as well as an increase ( $P < 0.10$ ) in adjusted fat thickness in steers fed a diet with supplemental sunflower oil vs. those fed a diet free of sunflower oil (Table 1), may partially explain the increase in heat retention. Although the rate of decline was different, ultimate temperature and pH did not ( $P > 0.10$ ) differ, and an acceptable temperature (4 to 6°C) and pH (5.4 to 5.6) range was achieved at 24 h postmortem in all breeds. Overall, there was little relationship between postmortem temperature and pH decline and tenderness.

### Calpastatin Activity and Free Calcium Levels

There was no effect ( $P > 0.10$ ) of breed (Figure 5) or diet (data not shown) on 0-h CA. Several studies have indicated that 24-h CA has a strong inverse relationship with tenderness as measured by WBSF and trained sensory panel evaluation at 14 d postmortem (Whipple et al., 1990; Shackelford et al., 1994). A conflicting study

**Table 3.** Objective color analysis and cooking attributes of longissimus muscle steaks from Wagyu (W), Wagyu × Limousin (W×L), and Limousin (L) steers fed diets with or without supplemental sunflower oil

Item	W	W×L	L	SEM	No oil diet	Oil diet	SEM
Number of steaks	12	12	12	—	18	18	—
Cooking loss, %	28.6	28.9	29.3	0.57	29.5	28.2	0.47
Cook time, min/100 g	11.0 <sup>x</sup>	11.0 <sup>x</sup>	9.6 <sup>y</sup>	0.30	10.7	10.5	0.20
Muscle color							
L*	38.7	37.9	39.0	0.61	38.6	38.5	0.50
a*	19.9	20.8	20.8	0.42	20.4	20.6	0.34
b*	16.4	17.2	17.6	0.40	16.8	17.3	0.33

<sup>x,y</sup>Within a row, means without a common superscript letter differ ( $P < 0.05$ ).

by Wulf et al. (1996) reported that 24-h CA was not a good indicator of d-14 tenderness in Limousin steers. Limousin genetics were represented in two of the three treatment groups in the present study; so, based on results of Wulf et al. (1996), we chose to measure 0-h CA rather than 24-h. However, 0-h CA did not explain breed differences in WBSF (Figure 5) or trained sensory panel tenderness scores (Table 2).

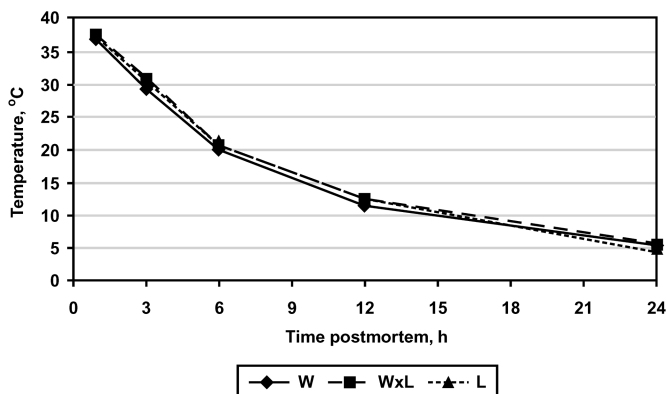
Breed (Figure 6) and diet (data not shown) did not affect ( $P > 0.10$ ) FCL over time. However, time was significant ( $P < 0.0001$ ) across all breeds resulting in FCL concentrations greater than 300  $\mu\text{M}$  by 3 d postmortem. Jeacocke (1983) reported that free calcium concentrations rose from 0.1  $\mu\text{M}$  to greater than 100  $\mu\text{M}$  as muscle entered rigor (1 to 24 h postmortem). Parrish et al. (1981) reported that beef LM reaches 630 to 970  $\mu\text{M}$  FCL by 10 to 14 d postmortem. The current study indicated that the time frame from 3 to 7 d postmortem would allow for sufficient levels of free calcium (300 to 800  $\mu\text{M}$ ) to half-maximally activate m-calpain under optimum conditions according to Goll et al. (1995). Day-

ton (1982) reported that the m-calpain activation requires levels in the 200 to 300  $\mu\text{M}$  range. Boehm et al. (1998) reported that at-death and 7-d-postmortem m-calpain had about the same FCL requirement (330 and 350  $\mu\text{M}$ , respectively) for half-maximal activity.

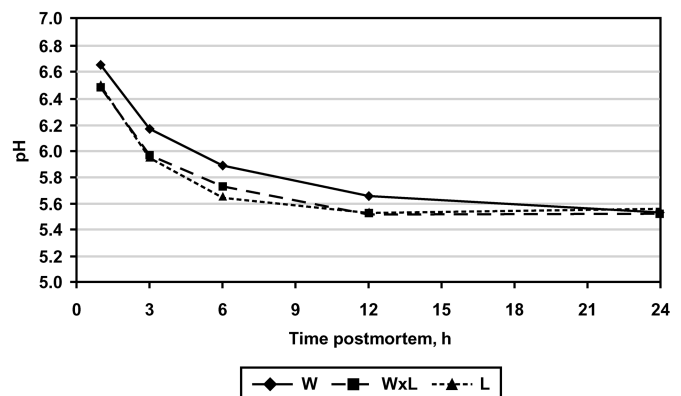
#### Fresh Meat Color

Breed did not ( $P > 0.10$ ) affect L\*, a\*, and b\* values (Table 3). However, Limousin steaks tended ( $P = 0.12$ ) to be more yellow (higher b\* values) than steaks from Wagyu steers. Color (L\*, a\*, and b\*) attributes have been linked to beef carcass palatability (Wulf et al., 1997). Wulf and Page (2000) reported that selection for carcasses based on quality grade in combination with muscle color and pH increased the accuracy of selecting palatable carcasses. In the current study, WBSF and color values (L\*, a\*, and b\*) were not significantly correlated ( $r = 0.11, -0.16, \text{ and } -0.03$ , respectively).

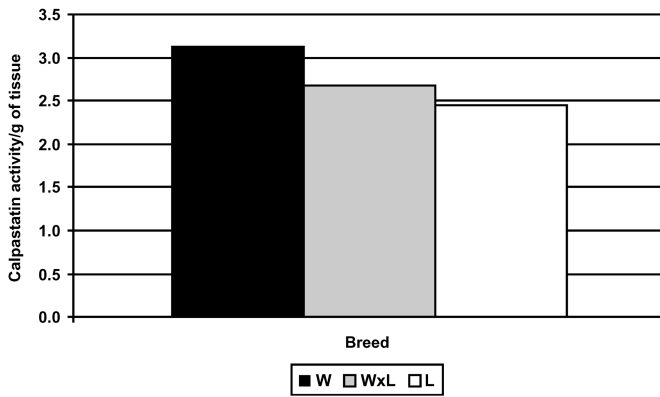
Diet did not ( $P > 0.10$ ) affect L\*, a\*, and b\* values (Table 3). Similarly, steers fed yellow grease diets compared to control diets did not differ in visual color score or in L\*, a\*, or b\* values (Marks et al., 2001). The



**Figure 3.** Effect of cattle breed type (W = Wagyu; L = Limousin; and W×L = Wagyu × Limousin) on temperature decline in the longissimus muscle. There were main effects of breed type ( $P < 0.05$ ; SEM = 0.21) and time postmortem ( $P < 0.0001$ ; SEM = 0.21), and an interactive effect of breed × diet ( $P < 0.05$ ; SEM = 0.29) on temperature decline.



**Figure 4.** Effect of cattle breed type (W = Wagyu; L = Limousin; and W×L = Wagyu × Limousin) on pH decline in the longissimus muscle. There were main effects of breed type ( $P < 0.05$ ; SEM = 0.04) and time postmortem ( $P < 0.0001$ ; SEM = 0.04) on pH decline.

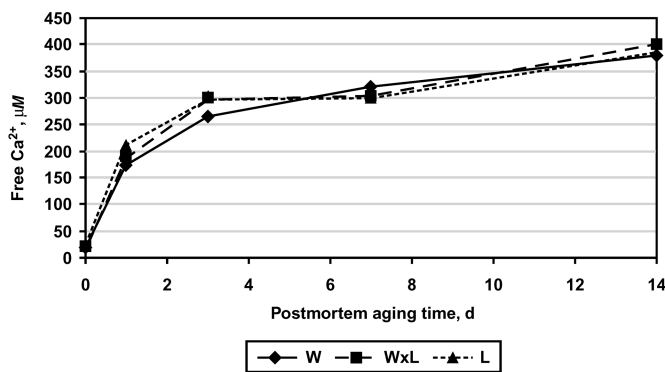


**Figure 5.** Effect of cattle breed type (W = Wagyu; L = Limousin; and W×L = Wagyu × Limousin) on calpastatin activity at 0 h postmortem. There was no main effect of breed type ( $P > 0.10$ ; SEM = 0.23) on calpastatin activity.

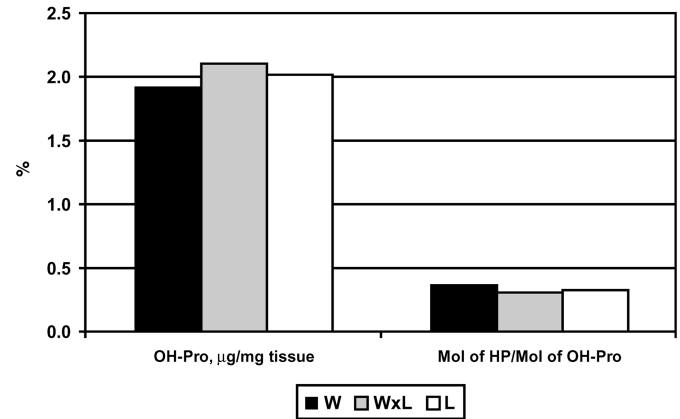
current study, as well as Marks et al. (2001), indicates that color attributes are comparable in cattle fed supplemental oil diets vs. diets without supplemental oil.

#### Connective Tissue

Collagen content and cross-linking are sometimes associated with decreased tenderness (McCormick, 1999). In the present study, OH-PRO (Figure 7) did not ( $P > 0.10$ ) differ among breeds. More importantly, amount of collagen cross-linking was not ( $P > 0.10$ ) different among the breed types. Wide differences in cross-link type and concentration occur between different tissues and muscle types (McCormick, 1994). Maiorano et al. (1993) reported that sex and age differences affect the amount of collagen and cross-linking in sheep. The current study with LM steaks from cattle of the same sex and relative age indicates that breed differences in tenderness could not be attributed to variation in collagen



**Figure 6.** Effect of cattle breed type (W = Wagyu; L = Limousin; and W×L = Wagyu × Limousin) on free calcium ( $Ca_2$ ) level in longissimus muscle during postmortem aging. There was a main effect of postmortem aging time ( $P < 0.0001$ ; SEM = 13.8), but not breed ( $P > 0.10$ ; SEM = 5.46) on free calcium level.

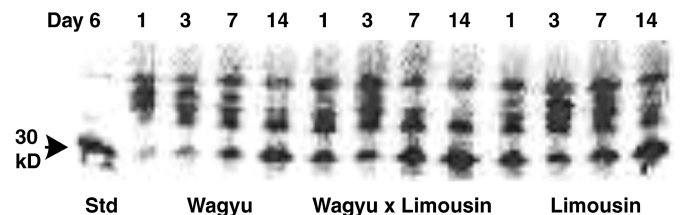


**Figure 7.** Effect of cattle breed type (W = Wagyu; L = Limousin; and W×L = Wagyu × Limousin) on percentage of total collagen (OH-PRO) and connective tissue cross-linking (HP). There were no main effects of breed type on OH-PRO ( $P > 0.10$ ; SEM = 0.11) or HP ( $P > 0.10$ ; SEM = 0.03).

amount or the amount of cross-linking. Furthermore, diet did not ( $P > 0.10$ ) affect collagen content or cross-linking (data not shown).

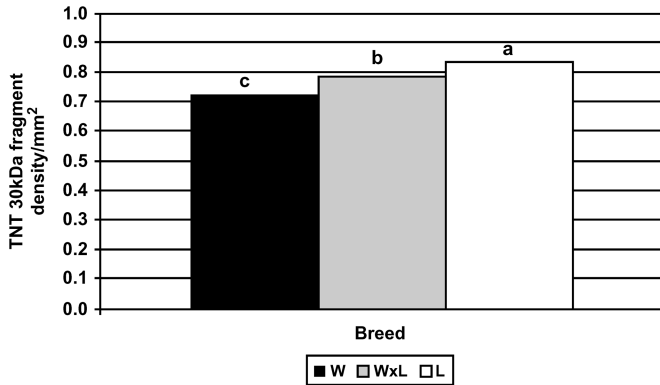
#### Proteolytic Degradation

Proteolytic degradation postmortem plays an integral role in the tenderness of beef. In Figure 8, Western blot analysis visually shows that the appearance of the TNT 30-kDa fragment increased during postmortem storage (1, 3, 7, and 14 d). Further, it appeared that the band density was greater in Limousin and W×L than in Wagyu earlier during storage (1 and 3 d postmortem). Hence, the visual band density values conflicted with WBSF and trained sensory panel values. Quantification of the TNT 30-kDa fragment was necessary to evaluate the relationship of tenderness mea-



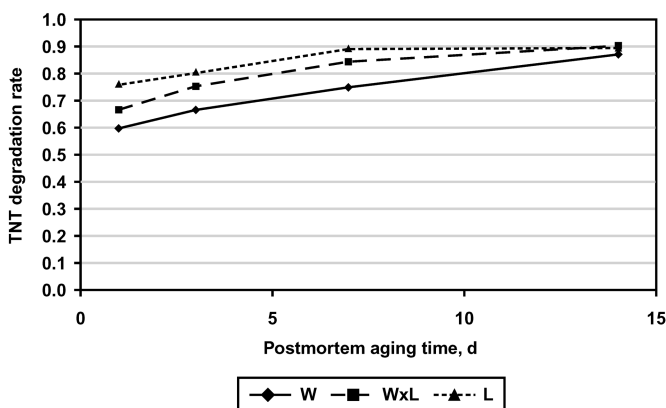
**Figure 8.** Western blot analysis of troponin-T (TNT) proteolytic degradation. The standard (Std) is a 6-d-postmortem lamb longissimus muscle sample with a significant amount of the 30-kDa TNT fragment. Samples represent longissimus muscle samples from Wagyu, Wagyu × Limousin, and Limousin steers after 1, 3, 7, and 14 d of postmortem aging. The arrow from the TNT 30-kDa next to the Std label points to the location of the primary proteolytic degradation product of TNT on the Western blot.





**Figure 9.** Effect of cattle breed type (W = Wagyu; L = Limousin; and W×L = Wagyu × Limousin) on appearance of troponin-T (TNT) 30-kDa fragment across postmortem aging time. Bars that do not have a common superscript letter differ ( $P < 0.10$ , SEM = 0.03).

sures (WBSF and trained sensory panel values), band density, and the rate of proteolytic degradation. Using Western blot analysis and quantification of band density for each sample, the appearance of the primary degradation product TNT 30-kDa fragment increased ( $P < 0.0001$ ) over subsequent days postmortem (1, 3, 7, and 14 d). This is in agreement with previous studies (Huff-Lonergan et al., 1995; Ho et al., 1997), which reported that the TNT 30-kDa fragment intensified with increasing time postmortem, regardless of animal, sex, and age. Band density values were measured to determine the amount of degradation (Figure 9), and the rate of appearance in the 30-kDa TNT fragment during aging (Figure 10). The density of the 30-kDa fragment differed ( $P < 0.10$ ) among breeds. The LM from Limousin steers produced bands that were more intense at each aging time than W×L, which were subse-



**Figure 10.** Effect of cattle breed type (W = Wagyu; L = Limousin; and W×L = Wagyu × Limousin) on troponin-T (TNT) degradation rate during postmortem aging. The linear slope for W was greater than for L ( $P < 0.01$ ; SEM = 0.03), and the linear slope for W×L tended to be greater than for L ( $P = 0.055$ ; SEM = 0.03).

quently more intense than Wagyu. The greater band density in the Limousin and W×L in comparison to Wagyu may be due to a potential variation in Limousin TNT isoforms, causing them to bind with more affinity to the JLT-12 primary antibody used in the Western blot procedure and elicited a more intense chemiluminescent reaction. This suggestion is supported by verification of equal protein loading and uniform transfer through transferred gel and PVDF membrane staining (described in Materials and Methods). Analysis of isoform differences will need to be investigated further in order to verify this hypothesis.

The rate of appearance of the TNT 30-kDa fragment across all aging times, measured by the linear slope, was greater for Wagyu ( $P < 0.010$ ) and W×L ( $P = 0.0546$ ) than Limousin. Furthermore, Wagyu had lower WBSF and had more desirable sensory panel sustained tenderness values at 14 d postmortem than Limousin. This corroborates previous findings that the rate of increase in band intensity of the 30-kDa fragment differed among animals, and more-tender samples exhibited a more-rapid postmortem appearance of the 30-kDa fragment (Olson and Parrish, 1977; Huff-Lonergan et al., 1995). Although W×L were not different ( $P > 0.05$ ) from Limousin in WBSF and sustained tenderness, they were numerically more desirable in both attributes, and proteolytic degradation rate also tended to be higher for W×L than for Limousin.

## Implications

Free calcium levels, calpastatin activity, total collagen, and hydroxylslypyridinoline cross-link concentration did not explain the enhanced tenderness in longissimus steaks from Wagyu compared with Limousin steers. Even though the initial appearance of the troponin-T 30-kDa fragment was greater in Limousin samples, the linear slopes for appearance of troponin-T degradation during postmortem aging was more rapid for Wagyu and Wagyu × Limousin than for Limousin. Thus, tenderness differences attributed to breed type may be a function of the rate of proteolytic degradation during postmortem aging.

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