

Effects of biological type and dietary fat treatment on factors associated with tenderness: II. Measurements on beef semitendinosus muscle

P. S. Kuber*, J. R. Busboom*¹, S. K. Duckett†, P. S. Mir‡, Z. Mir‡, R. J. McCormick§, C. T. Gaskins*, J. D. Cronrath*, D. J. Marks*, and J. J. Reeves*

*Washington State University, Pullman 99163-6310; †University of Idaho, Moscow 83844-2330;

‡Agriculture and Agri-food Canada, Lethbridge, AB T1J 4B1; §University of Wyoming, Laramie 82071

ABSTRACT: The objective of this study was to evaluate attributes in semitendinosus muscle (ST) associated with tenderness in divergent breeds—Wagyu (W; n = 12), Limousin (L; n = 12), and Wagyu × Limousin cross cattle (W×L; n = 12)—fed two dietary treatments (0 or 6% sunflower oil, DM basis). A randomized complete block repeated measures design with a 3 × 2 factorial arrangement of treatments was used to measure effects of breed, diet, block, and associated interactions. Cattle were fed barley-based diets for an average of 259 d. Temperature and pH were measured at 0, 1, 3, 6, 12, and 24 h postmortem (PM). Steaks from the ST were removed 24 h postmortem, vacuum-packaged, aged (1, 3, 7, 14, 28, and 56 d postmortem) at 2°C, and frozen (−40°C) until analyzed. Dietary treatment did not ($P > 0.10$) affect Warner-Bratzler shear force (WBSF), collagen amount (OH-PRO) or cross-linking (HP), temperature, or pH. Steaks from W×L aged 14 d postmortem

had lower ($P < 0.05$) WBSF values than L (W were intermediate). Cooking time was longer ($P < 0.01$) in W and W×L than in L; however, breed did not affect ($P > 0.10$) cooking loss. Cooking time was not influenced by diet, but steaks from cattle fed 6% sunflower oil had lower ($P < 0.05$) cooking losses. Temperature decreased more ($P < 0.05$) rapidly, and pH more slowly ($P < 0.05$), in W and W×L than L in the first 24 h postmortem. Limousin steaks were lighter (higher L*) and more yellow (higher b*) in color than steaks from W and W×L ($P < 0.05$). The control diet (no oil added) resulted in steaks that were lighter ($P < 0.05$) than the treatment diet (6% added sunflower oil). Neither breed nor diet affected ($P > 0.10$) OH-PRO or HP concentration. The results of this study indicate that biological type differences may not be as great in the ST as in longissimus muscle; thus, to increase tenderness in ST, emphasis may need to be placed on processing and cooking techniques rather than genetic selection.

Key Words: Beef, Connective Tissue, Limousin, Semitendinosus, Tenderness, Wagyu

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Introduction

Tenderness has been a targeted area of research because of its importance to consumer perception of beef palatability (Morgan et al., 1991; Boleman et al., 1997). Typically, studies investigate the effect of attributes associated with tenderness in longissimus muscle steaks, but few have focused on cuts of the round and chuck. The semitendinosus from the round is traditionally regarded as a cut high in connective tissue and, subsequently, tough when cooked using dry-heat (Powell et al., 2000). Collagen is a major component of connective tissue and, as an animal matures, toughness increases due to an increase in collagen cross-linking

between the collagen molecules, providing structural support (McCormick, 1994; Bosselmann et al., 1995).

Temperature and pH decline during the first 24 h postmortem are inversely related (Bendall, 1978). Eilers et al. (1996) reported that ultimate pH at 24 h postmortem was related to tenderness, and that higher longissimus muscle pH values at 24 h postmortem produced less-tender longissimus, gluteus medius, and semimembranosus muscle steaks. Page et al. (2001) reported that L*, a*, and b* values were negatively correlated with muscle pH, and values for a* and b* were more highly correlated with muscle pH than were values for L* (Wulf and Wise, 1999; Page et al., 2001). Moreover, Wulf et al. (1997) found that b* values (yellowness) had a stronger positive relationship to tenderness than L* values (lightness). Thus, of the color values, b* values may be the best indicator of beef tenderness. Therefore, the objective of this study was to evaluate connective tissue amount and cross-linking, temperature, pH, and color attributes and their effects

¹Correspondence: 126 Clark Hall (phone: 509-335-2880; fax: 509-335-1082; e-mail: busboom@wsu.edu).

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on beef tenderness in semitendinosus steaks from steers of divergent breeds (Wagyu, Limousin, and Wagyu × Limousin) fed diets formulated with 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 conformed to the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (Consortium, 1988).

Thirty-six steers, representing Wagyu (n = 12), Limousin (n = 12), and Wagyu × Limousin (**W×L**; n = 12) were blocked by initial BW into three blocks (replicates), 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 basis). Diets were described in detail by Mir et al. (2002). All diets were barley-based and the sunflower oil treatments were applied during the last 95 d of the backgrounding phase (159 d) and all the finishing phase (100 d). Cattle were weighed and humanely harvested at the Washington State University Meats Laboratory, and carcasses were chilled for 24 h at 0 to 2°C.

Internal semitendinosus (**ST**) pH and temperature were measured at 0, 3, 6, 12, and 24 h postmortem using a hand-held pH monitor (Model pH 10, Cole-Parmer, Niles, IL). At 24 h postmortem, the ST was removed from the right side of each carcass and cut into eight 2.54-cm-thick steaks. Steaks were aged for 1, 3, 7, 14, 28, and 56 d postmortem for Warner-Bratzler shear force (**WBSF**), 14 d postmortem for trained sensory panel, and 1 d postmortem for fresh meat color evaluation. An additional 1.25-cm-thick steak, aged 1 d postmortem, was cut for connective tissue analysis. Steaks for WBS, sensory panel, and connective tissue analyses were vacuum-packaged, aged for the appropriate time, and frozen (−40°C) for subsequent 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). Percentage cooking loss and cooking time were recorded. 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 using 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 were served to a nine-member trained (AMSA, 1995) sensory panel. Panelists evaluated each steak for initial tenderness, sustained tenderness, initial juiciness, sustained juiciness, beef flavor intensity, and off-flavor using a 10-cm (0 = extremely tough, dry, bland, and no off-flavor to 10 = extremely tender, juicy, intense beef flavor, and pronounced off-flavor) unstructured line scale for each independent attribute measured.

Fresh Meat Color Attributes

A MiniScan XE LAV (Hunter Lab, Reston, VA) spectrophotometer was used to obtain L* (lightness; higher the L* value, the lighter the color), a* (red-green spectrum; higher the a* value, the redder the color), and b* (yellow-blue spectrum; higher the b* value, the more yellow the color) values on d-1 steaks using D65 illuminant and the 10° standard observer settings. Steaks were cut and allowed to bloom (10 to 15 min), and then 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.

Connective Tissue

Muscle samples (5 g wet weight) were removed from steaks with the exclusion of epimysial connective tissue, and dried in an oven at 100°C for 24 h. Dried ST 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 determination of hydroxyproline. Collagen content (**OH-PRO**) was calculated by multiplying the measured weight of hydroxyproline by 7.25. Hydroxylysylpyridinoline (**HP**) cross-link concentration (HP concentration is directly related to collagen cross-linking) for ST tissue samples was measured after the filtration step in hydroxyproline determination. Concentrations of HP and vitamin B₆ standard were determined using a modified HPLC procedure developed by Eyre et al. (1984).

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

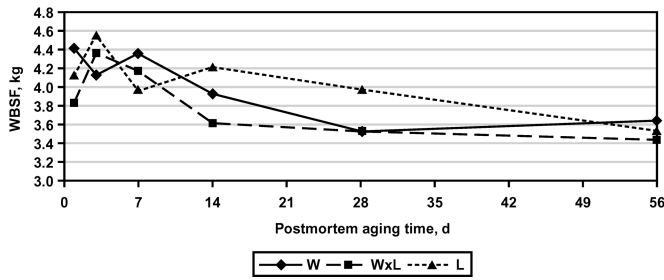


Figure 1. Effect of cattle breed type (W = Wagyu; L = Limousin; and W×L = Wagyu × Limousin) on Warner-Bratzler shear force (WBSF) values of semitendinosus steaks during postmortem aging. There was a main effect of postmortem aging time ($P < 0.0001$; SEM = 0.09), and an interactive effect of breed × postmortem aging time ($P < 0.05$; SEM = 0.16) on WBSF.

were analyzed using GLM procedures in SAS (SAS Inst. Inc., Cary, NC), with breed, diet, block, and breed × diet included in the model. For pH, temperature, and WBSF, the model was extended for the repeated measure, which was time postmortem. 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 replaced time postmortem in the model. 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 compare the relationship between WBSF and sensory panel tenderness values using PROC CORR.

Results and Discussion

The primary intent of this study was to evaluate attributes associated with ST tenderness in divergent biological types of cattle. Thus, the cattle selected represented a moderate-framed breed (Wagyu) that is heavily marbled (Yamazaki, 1981; Lunt et al., 1993) and a large-framed, faster growing, heavy-muscled breed (Limousin) that is very lean (Wulf et al., 1996). As reported in Kuber et al. (2004), Limousin steers were faster growing, more heavily muscled, and leaner in composition than Wagyu and W×L steers, and Wagyu steers had higher marbling scores and subsequently higher quality grades than W×L, which were subsequently higher than for Limousin. Moreover, Kuber et al. (2004) reported that dietary treatment had no adverse effects on carcass quality traits.

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

Shear force decreased ($P < 0.0001$) over postmortem aging time in all breeds (Figure 1). The interaction of

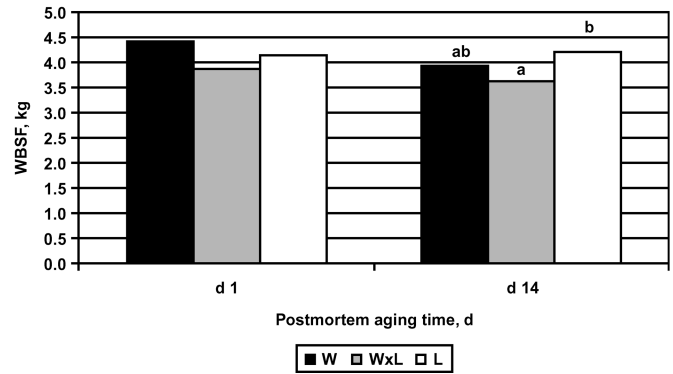


Figure 2. Comparison of breed type (W = Wagyu; L = Limousin; and W×L = Wagyu × Limousin) on Warner-Bratzler shear force (WBSF) values of semitendinosus steaks after 1 and 14 d of postmortem aging. Bars that do not have a common superscript letter differ ($P < 0.05$; SEM = 0.17).

breed × day was significant ($P < 0.05$), prompting an evaluation of d-1 and d-14 samples. Shear force values for W×L samples (Figure 2) aged 1 d postmortem were numerically ($P > 0.10$) lower than both Wagyu and Limousin, and, after 14 d of postmortem aging, W×L were more tender ($P < 0.05$) than Limousin, whereas Wagyu did not differ from other breeds. Dietary treatment did not affect ($P > 0.10$) WBSF (data not shown).

Trained sensory panel scores were not affected ($P > 0.10$) by breed (Table 1), diet, or breed × diet (data not shown). Busboom et al. (1993) showed that longissimus muscle steaks from Wagyu steers were more palatable than steaks from Angus and Longhorn steers, and, in our companion paper (Kuber et al., 2004), Wagyu longissimus muscle steaks received higher sustained tenderness scores than Limousin steaks. In the present study, ST samples were different with respect to WBSF, yet the trained panel did not detect differences, possibly due to textural property differences associated with cuts higher in connective tissue. Likewise, longissimus muscle steaks are more highly correlated with WBSF than steaks from other muscle groups, which were low to moderately correlated with WBSF (Deatherage and Garnatz, 1952; Cover et al., 1962; Sharrah et al., 1965). Furthermore, the difference between W×L and Limousin for WBSF was small and not consistent across aging times. Although this difference was detectable by a mechanical instrument, it may not have been large enough for the sensory panel to detect, especially when considering the random variation that may have existed between the steaks selected for WBSF and sensory panel analysis.

Breed did not influence ($P > 0.10$) cooking loss; however, steaks from Wagyu and W×L required more ($P < 0.01$) time on the open-hearth grill than Limousin (Table 2). The increased cooking time may have negatively influenced palatability attributes in Wagyu and W×L. Differences in steak cross-sectional diameter and tem-

Table 1. Sensory panel scores of semitendinosus muscle steaks from Wagyu (W), Wagyu × Limousin (W×L), and Limousin (L) steers fed diets with or without supplemental sunflower oil

Panelist scores ^a	W	W×L	L	SEM
Number of steaks	12	12	12	—
Initial tenderness	6.38	6.37	6.13	0.32
Sustained tenderness	6.29	6.24	5.79	0.34
Initial juiciness	5.57	5.95	5.91	0.44
Sustained juiciness	5.04	5.25	5.13	0.44
Beef flavor intensity	4.73	4.84	4.74	0.21
Off-flavor	0.77	0.66	0.89	0.09

^a0 = extremely tough, dry, bland, and no off-flavor to 10 = extremely tender, juicy, intense beef flavor, and pronounced off-flavor.

perature conductivity may have influenced cooking time, but these would need to be further investigated. The addition of sunflower oil at 6% to the diet decreased ($P < 0.05$) cooking loss when compared to the diet without added sunflower oil, yet cooking time was not ($P > 0.10$) influenced by diet.

Connective Tissue

Collagen content and cross-linking is sometimes associated with decreased tenderness (McCormick, 1999). Total OH-PRO did not differ ($P > 0.10$) among breeds (Table 2). More importantly, differences in the amount of collagen cross-linking (Table 2), measured directly by the amount of HP, did not differ ($P > 0.10$) between 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 ST steaks from cattle of the same sex and relative age indicates that breed differences in tenderness could not be attributed to variation in collagen amount or the amount of cross-linking. Diet also did

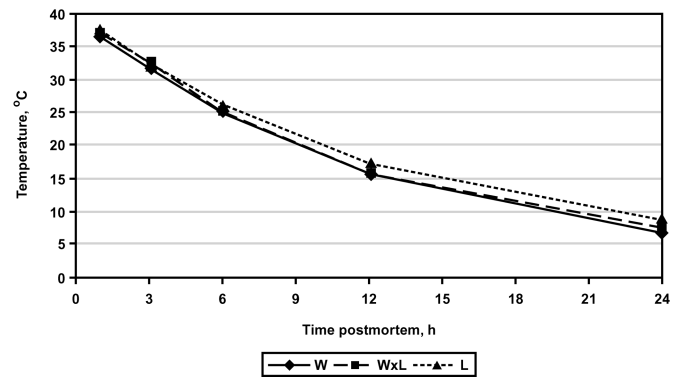


Figure 3. Effect of cattle breed type (W = Wagyu; L = Limousin; and W×L = Wagyu × Limousin) on temperature decline and ultimate (24 h) temperature in semitendinosus muscle. There were main effects of breed type ($P < 0.01$; SEM = 0.26) and time postmortem ($P < 0.0001$; SEM = 0.30) for temperature decline, and the main effect of breed type ($P < 0.001$; SEM = 0.35) on ultimate (24 h) muscle temperature.

not affect OH-PRO or HP ($P > 0.10$; data not shown), which was consistent with the results for longissimus muscle steaks (Kuber et al., 2004).

Temperature and pH

Regardless of breed, both temperature and pH declined ($P < 0.0001$) over the first 24 h postmortem. This was expected, and the decline curves (Figures 3 and 4) indicate that a favorable environment for anaerobic glycolysis, rigor development, and completion at 24 h postmortem was achieved. No ($P > 0.10$) interactions existed with regard to temperature and pH. During the first 24 h postmortem, temperature (Figure 3) decreased ($P < 0.05$) more rapidly, and pH (Figure 4) more slowly, in Wagyu and W×L compared to Limousin. A slower temperature decline would be expected to result

Table 2. Hunter-Lab miniscan color analysis, cooking attributes, % collagen (OH-Pro) and hydroxylslylpyridinoline (HP) cross-link concentration of semitendinosus 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, %	35.8	34.5	35.9	0.48	36.0 ^x	34.7 ^y	0.39
Cook time, min/100 g	17.8 ^x	16.5 ^x	12.8 ^y	1.00	15.7	15.6	0.40
Muscle color							
L*	42.9 ^y	43.0 ^y	45.4 ^x	0.37	44.2 ^x	43.3 ^y	0.30
a*	14.3	14.9	14.8	0.38	14.3	15.0	0.31
b*	13.2 ^y	13.8 ^y	15.1 ^x	0.32	14.0	14.1	0.26
Collagen and collagen cross-linking							
OH-Pro, μ/mg tissue	3.4	3.5	3.9	0.19	3.8	3.4	0.20
HP:OH-Pro, mol	0.41	0.38	0.38	0.03	0.41	0.38	0.03

^{x,y}Within a row, means without a common superscript letter differ ($P < 0.05$).

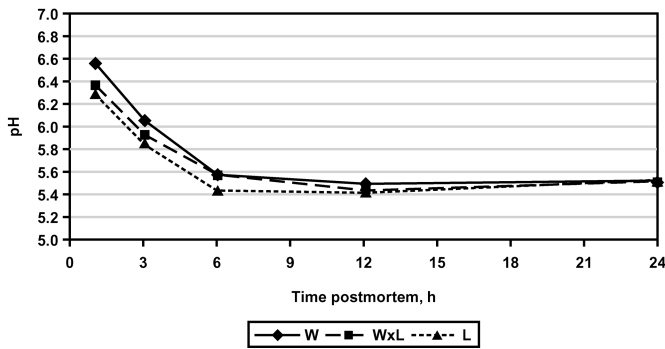


Figure 4. Effect of cattle breed type (W = Wagyu; L = Limousin; and W×L = Wagyu × Limousin) on pH decline in semitendinosus muscle. There were main effects of breed type ($P < 0.05$; SEM = 0.03) and time postmortem ($P < 0.0001$; SEM = 0.03) on pH decline.

in an increased rate of postmortem glycolysis and, consequently, a more rapid pH decline (Bendall, 1978). Temperature at 24 h postmortem was also lower ($P < 0.001$) in Wagyu and W×L compared to Limousin (Figure 3). Fat thickness did not differ among breeds, indicating that differences in muscle size between Wagyu- and Limousin-influenced steers (Kuber et al., 2004) probably contributed to the breed differences in rate of temperature and subsequent pH decline, as well as ultimate temperature at 24 h postmortem. The difference in 24-h postmortem temperature did not result in a difference in ultimate pH, and a pH range of 5.4 to 5.6 was achieved in all breeds by 24 h postmortem.

Eilers et al. (1996) reported that muscle temperature was not closely related to steak tenderness, but higher pH values at 24 h postmortem produced less-tender beef. In the present study, however, 24-h pH (ultimate) did not differ among breeds, but d-14 WBSF values differed between W×L and Limousin (Figure 2). The effect of the rate of temperature and pH decline on the calpain proteolytic system could in part explain this result. The calpain proteolytic system, responsible for postmortem degradation of muscle proteins (Koochmarai, 1988, 1992a), is affected by the rate of temperature and pH decline. Autolysis of μ -calpain is increased as pH decreases from 7.0 to 5.8 and slowed as temperature decreases from 25 to 5°C (Koochmarai, 1992b). In the present study, pH declined more slowly and muscle temperature more rapidly in the ST of Wagyu and W×L than Limousin, which, according to Koochmarai (1992b), would contribute to less autolysis of calpains. This in turn could contribute to greater postmortem myofibrillar proteolysis and more-tender meat. In the present study, W×L had lower ($P < 0.05$) and Wagyu tended to have lower ($P = 0.21$) WBSF values after 14 d of aging than Limousin.

Fresh Meat Color

Steaks from Limousin carcasses were lighter (higher L^*) and more yellow (higher b^*) in color than steaks

from Wagyu or W×L carcasses ($P < 0.05$; Table 2), but breed did not ($P > 0.05$) affect ST redness (a^*). Wulf and Wise (1999) reported that as marbling score increased, L^* values increased (increased lightness), suggesting that because the L^* values are based on a white/black (light/dark) spectrum and that intramuscular fat is white, higher levels of marbling will result in lighter lean color readings. Page et al. (2001) also reported that as quality grade (marbling score) increased, L^* values increased. Furthermore, Wulf and Wise (1999) reported an even stronger relationship between pH and L^* values, indicating that lighter colored beef would have a lower pH. In either case, Wagyu and W×L ST steaks were higher in pH (Figure 4) and higher in marbling than Limousin. Although not measured specifically in the ST, the degree of marbling in Wagyu-influenced ST steaks was visually higher than that of Limousin-influenced steaks, following the same pattern as the marbling in the longissimus muscle (Kuber et al., 2004). Thus, marbling clearly does not explain the difference in L^* values among breeds. Moreover, lean maturity, which has been reported to be negatively correlated (Orcutt et al., 1984; Wulf and Wise, 1999) with L^* values, was probably not responsible because cattle in the current study were of approximately the same chronological age.

When pH decreases rapidly before the muscle has been chilled, sarcoplasmic and myofibrillar proteins are partially denatured, resulting in pale color (Ledward et al., 1992). Semitendinosus muscle from Limousin carcasses had the slowest temperature decline, most rapid pH decline, and the highest L^* values. Thus, rate of pH decline could at least in part explain the difference in L^* values. Muscle fiber types were not determined in this study, but breed effects on the prevalence of fiber types (Goto et al., 1994; Ozawa et al., 2000) could also have contributed to the observed differences in lean color values.

Several studies (Purchas, 1990; Jeremiah et al., 1991; Watanabe et al., 1996) have reported that slightly dark beef with a moderately high pH tends to be tougher than brighter colored meat with a normal ultimate pH. Moreover, Wulf et al. (1997) found that b^* values (yellow) had a stronger positive relationship to tenderness than L^* values (lightness). By contrast, beef from Limousin had the highest L^* and b^* values and yet tended to be the toughest in the present study. All beef in the present study, however, was acceptable in color, and ultimate pH did not differ among breeds.

Diet did not affect ($P > 0.10$) a^* or b^* values of ST steaks. Steaks from steers fed the control diet were lighter (higher L^* ; $P < 0.05$) than those from steers fed the diet containing 6% supplemental sunflower oil, but the difference was small (44.2 vs. 43.3) and probably of limited practical importance.

Implications

In semitendinosus muscle, breed differences in Warner-Bratzler shear force were not supported by sensory

panel data, nor were they explained by hydroxyproline, hydroxylysylpyridinoline, ultimate pH, or color attributes. Rates of postmortem temperature and pH decline may have had some influence on the rate and extent of shear force decline. This study indicates that biological type differences may not be as great in the semitendinosus as in longissimus muscle. Thus, to increase tenderness in semitendinosus, emphasis may need to be placed on processing and cooking techniques rather than genetic selection. Moreover, feeding oils high in linoleic acid does not seem to affect palatability or lean color attributes of the semitendinosus.

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