ELSEVIER

Meat Science 63 (2003) 325–331

MEAT SCIENCE

www.elsevier.com/locate/meatsci

Measuring the effects of phenotype and mechanical restraint on proteolytic degradation and rigor shortening in callipyge lamb longissimus dorsi muscle during extended aging

P.S. Kuber[a], S.K. Duckett[b], J.R. Busboom[a,*], G.D. Snowder[c], M.V. Dodson[a], J.L. Vierck[a], J.F. Bailey[b]

[a]*Department of Animal Sciences, Washington State University, Pullman, WA 99164-6310, USA*
[b]*Department of Animal and Veterinary Science, University of Idaho, Moscow, ID 83844-2330, USA*
[c]*US Sheep Experiment Station, Dubois, ID 83423, USA*

Received 27 December 2001; received in revised form 29 March 2002; accepted 29 March 2002

Abstract

The purpose of this study was to determine if tenderness of callipyge (CLPG) longissimus dorsi muscle (LM) could be improved by: (1) extending the aging period to 48 days postmortem or (2) preventing rigor shortening by clamping. In CLPG and normal (NML) chops respectively, initial Warner-Bratzler shear values (WBS) were lower ($P<0.05$) in clamped (CL) (5.5 and 3.6 kg) compared to unclamped (UCL) (7.4 and 4.9 kg) LM. In CLPG, an acceptable WBS (3.6 kg) was reached at 48 days PM, whereas, NML lambs reached an acceptable level (3.8 kg) by 3 days PM. Sarcomere lengths (SL) of CL (1.68 μm) were longer ($P<0.05$) than for UCL (1.44 μm) and were negatively correlated with WBS ($r=-0.55$; $P<0.1$). The appearance of Troponin-T (TNT) degradation product coincided with tender WBS values; 3 days postmortem in NML UCL and 48 days postmortem in CLPG. In conclusion, clamping reduced WBS possibly by reducing rigor shortening. Extended aging resulted in CLPG LM with acceptable WBS values, concurrent with the appearance of TNT degradation products. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Tenderness; Callipyge lamb; Troponin-T

1. Introduction

The US sheep industry could benefit from the positive production traits of superior feed efficiency and yield of retail cuts offered by callipyge (CLPG) lambs (Shackelford, Wheeler, & Koohmaraie, 1998). Busboom, Wahl, and Snowder (1999) reported that CLPG genetics have the potential to decrease the cost of lamb to consumers and increase profitability in the lamb industry. The specific muscle hypertrophy associated with CLPG genetics results in an increase in the area of the longissimus dorsi muscle (LM) and in the size of the leg muscles (Clare, Jackson, Miller, Elliot, & Ramsey, 1997; Duckett, Klein, Leckie, Thorngate, Busboom, & Snowder, 1998; Jackson, Miller, & Green, 1997a; Koohmaraie, Shackelford, Wheeler, Lonergan, & Doumit, 1995). In addition to the increase in muscle mass, an increase in hot carcass weight and dressing percentage (12 and 8%, respectively), with no difference in live weight at time of harvest between CLPG and normal (NML) lamb (Duckett, Klein, Leckie, et al., 1998a), indicates the potential for higher economic return to US sheep producers. However, Clare et al. (1997) reported that consumers found 40% of all CLPG LM chops unacceptable for tenderness. Numerous studies have shown that CLPG LM is tougher initially (1 day postmortem) than NML LM. The phenotypic (CLPG vs NML) differences resulting in a variation in tenderness are due to higher calpastatin activity in the muscle tissue of CLPG compared to NML lamb (Clare et al., 1997; Duckett, Klein, Leckie, et al., 1998; Duckett, Snowder, & Cockett, 2000; Koohmaraie et al., 1995). Calpastatin is the specific inhibitor of the calpain system, which is responsible for the postmortem (PM) proteolytic breakdown of muscle proteins. Calpastatin has been shown to alter both the rate (Koohmaraie et al., 1995)

* Corresponding author. Tel.: +1-509-335-2880; fax: +1-509-335-1082.

E-mail address: busboom@wsu.edu (J.R. Busboom).

0309-1740/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0309-1740(02)00089-X

and extent of PM proteolysis (Geesink & Koohmaraie, 1999) in CLPG lambs. Wheeler and Koohmaraie (1994) reported that NML lamb LM at time of harvest is moderate in tenderness, that rigor shortening toughens the meat, and that proteolysis tenderizes the meat during postmortem storage resulting in more tender meat after 14 days of aging than at harvest. Further, Wheeler and Koohmaraie (1994) reported that increases in shear force values between harvest and 1 day PM are related to decreases in sarcomere length, a result of rigor shortening. In a study which measured extended aging (56 day PM) in lamb (CLPG vs. NML) biceps femoris muscle, Geesink and Koohmaraie (1999) reported that extended aging may be a viable option to tenderize meat that is tough after 14 days of PM aging. Therefore, the objectives of this study were to evaluate changes in shear force, myofibrillar proteolysis, and sarcomere length in LM of CLPG lamb as compared to NML lamb after applying postharvest treatments of: (1) extended PM aging time or (2) prevention of rigor shortening.

2. Materials and methods

2.1. Experimental design and animal management

The University of Idaho Animal Care and Use Committee approved the use of animals in this study. Three CLPG and three NML lambs (78 kg) were obtained from the US Sheep Experiment Station at Dubois, ID. The lambs were 7/8 Columbia and 1/8 Dorset and were sired by a heterozygous CLPG ram. The lambs were rested for a 12 day period in which they were given ad libitum access to a dehydrated alfalfa pellet, corn and soybean meal mixture (35:60:5% as fed; 12% CP). After the rest period, the lambs were deprived of feed for 24 h and harvested at the University of Idaho Meat Laboratory.

2.2. Post-slaughter carcass measurements and sampling

At 15 min PM, subcutaneous fat which covered the right LM between the first and last lumbar vertebrae was removed. The right LM was then separated from the vertebral body and the lateral spinous processes but remained attached to the carcass at each end. Two stainless steel plates (15 cm×9 cm×3 mm thick; 25 °C) were placed on the inside and outside of the muscle to sandwich the LM according to Wheeler and Koohmaraie (1994). These plates were clamped in the center and on each end with enough pressure to prevent the LM from shortening. Next, the clamped muscle section (CL) was removed by cutting transversely across the LM at the end of the plates and chilled at 1 °C for 24 h.

Carcasses with the left LM intact were chilled at 1 °C for 24 h (UCL). At 0, 3, 6, 12, and 24 h PM, the temperature of the LM was recorded, and 3 and 5 g LM samples were removed from over the 5th to 12th ribs of the left side of each carcass. The 3 g samples were homogenized in 30 ml deionized water for pH determination (Cole-Parmer, Niles, IL). The 5 g samples were quick frozen in liquid nitrogen and stored at −70 °C for subsequent ATP, proximate (% moisture and ash) and mineral (total Ca, Fe, Mg, and Zn mg/100 g of tissue) analyses. Both proximate and mineral analyses were conducted using AOAC (1990) procedures. ATP (μmol/g) was determined using a Sigma Test Kit (Kit #366, Sigma, St. Louis, MO), which measured the amount of ATP via oxidation of NADH to NAD.

At 24 h PM, loin eye area (LEA) of the left side was measured. The left LM was removed from the carcass between the first and last lumbar vertebrae and cut into chops (one 1.25 and one 2.54 cm thick chop for each aging time). The chops were vacuum packaged, aged at 4 °C for 1, 3, 6, 12, 24, and 48 days PM, and then frozen at −20 °C. Clamped LM samples from the right side (24 h PM) were cut into chops (1.25 and 2.54 cm thick), vacuum packaged, and frozen at −20 °C. In this study the intent was to age both CL and UCL samples for 1, 3, 6, 12 24, and 48 days PM. However, clamping altered the shape of the LM, reducing the number of cores that could be obtained for WBS. The decision was made that it would be more important to accurately determine (with a confident number of samples) the effect of clamping on the initial shear force of day 1 CL and UCL samples.

2.3. Calpastatin activity (CA)

A 5 g sample was removed from the left LM at 15 min PM and placed in pre-rigor buffer for the determination of CA according to Duckett, Klein, Leckie, et al. (1998). One unit of CA is defined as the amount of calpastatin that inhibited one unit of m-calpain activity. Calpain activity is reported as the caseinolytic activity that resulted in an increase in absorbance of 1 unit at 278 nm after a 60 min incubation at 25 °C.

2.4. Sarcomere length (SL)

Muscle samples (left LM) were placed in cold (2 °C) 0.2 M cacodylate-buffered, 1.5% glutaraldehyde fixative and minced to obtain uniform 1 mm cubes. The tissue was allowed to remain in the fixative overnight. Tissue samples were washed in two changes of buffer for 30 min each and placed in a 2% osmium tetraoxide solution for 2 h. Tissue samples were washed a second time in two changes of buffer before being dehydrated in a graded series of 15 min ethanol changes (40%–50%–70%–85%–95%) followed by two changes in 100% ethanol for 30 min each. Tissue samples were placed in a 50/50 ethanol/propylene oxide solution for 30 min, straight propylene oxide for 30 min and then a 50/50

propylene oxide/epoxy resin (uncovered) overnight. The next morning, tissue samples were transferred to pure epoxy resin and placed in a vacuum oven. A vacuum was drawn on the tissue samples for approximately 30 min before the oven was activated, at which point the samples were allowed to polymerize at 70 °C for 8 h. The samples were then sliced into blocks and thin sectioned using an ultramicrotome (Ultramicrotome—LKB Ultratome III). The resulting sections were placed on a 3 mm transmission electron microscope grid and stained with lead citrate and urinal acetate before viewing with the transmission electron microscope (Transmission Electron Microscope—JEOL 1200EX-II). Electron micrograph pictures were standardized to a linear scale equal to 1 μm, which allowed comparisons of SL between micrographs.

2.5. Troponin-T (TNT) degradation

Degradation of TNT at all PM aging times was determined using Western blotting procedures according to Huff-Lonergan, Mitsuhashi, Parrish, and Robson (1996). Briefly, whole muscle samples (right LM) were prepared from the 1.25 cm thick chops. 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). Sixty micrograms of whole muscle preparation were loaded onto 15% polyacrylamide slab separating gels with a 5% polyacrylamide stacking gel. The gels were run at room temperature for 45 min at a constant voltage of 200 and 60 mA/gel. The gels were transferred to Immuno-Blot PVDF membranes (#162–0177, Bio-Rad, Hercules, CA) for 1 h at 100 V and 340 mA, using a Mini Trans-Blot Cell (Bio-Rad, Hercules, CA) equipped with a bio-ice cooling apparatus. The complete transfer of proteins in the 30 kDa molecular weight range was verified by a Kaleidoscope pre-stained molecular weight marker (Bio-Rad, Hercules, CA) and by subsequent staining of the 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 perioxidase (Sigma, St. Louis, MO; 1:5,000) as the secondary antibody and a chemiluminescent detection system (Pierce Super Signal® Substrate; Pierce, Rockford, IL). Degradation of TNT was measured by a Bio-Rad Fluor-S MultiImager (Bio-Rad, Hercules, CA), based on image analysis using Western blots. A subjective evaluation of the appearance of the 30 kDa TNT primary degradation product was used to compare treatment (NML vs CLPG and UCL vs CL).

2.6. Warner-Bratzler shear force (WBS)

Shear force analysis was conducted according to the guidelines of the American Meat Science Association (AMSA, 1995). Chops (2.54 cm) from the UCL and CL LM were thawed at 4 °C for 24 h and broiled to an internal temperature of 71 °C using Farberware Open Hearth Grills (Model R4550; Farberware, Bronx, NY). The internal temperature was monitored with a Digi-Sense temperature logger (Cole Parmer, Niles, IL). The chops were cooled at room temperature (22 °C) for a 4 h period and four cores (1.27 cm) were removed parallel to the muscle fiber. The cores were sheared using a Texture Analyser (TA-XT2; Texture Technologies Corp., Scarsdale, NY) equipped with a WBS blade. Crosshead speed was set at 20 cm/min.

2.7. Statistical analyses

A completely randomized split-plot design was used to evaluate the effect of phenotype and clamping. Animal within phenotype was used to test the effect of phenotype and residual error was used to test the effect of clamping as well as the phenotype by clamping interaction. A completely randomized repeated measures design was used to analyze pH, temperature, proximate composition (% moisture, % ash), mineral composition (Fe, Mg, Zn, and Ca^{2+}), ATP, and WBS. For the repeated measures analysis, animal within phenotype was used to test the effect of phenotype and residual error was used to test the effect of time as well as phenotype by time. Overall correlation coefficients were used to evaluate the relationship of sarcomere length to WBS. All data were analyzed using the GLM and CORR procedures of SAS (SAS Institute, Cary, NC).

3. Results and discussion

3.1. CLPG versus NML lamb

Proximate (% moisture and % ash) and mineral (Fe, Mg, and Zn mg/100 g of tissue) composition (Table 1) did not differ ($P>0.1$) for phenotype (CLPG or NML) or time (0, 3, 6, 12, and 24 h). Levels of total Ca^{2+} tended ($P=0.20$) to increase from 0 to 24 h PM (+1.0 mg/100 g of tissue) over time in both NML and CLPG, and at a slightly faster ($P=0.18$) rate in NML lamb than CLPG (6.59 and 5.91 mg/100g, respectively). ATP levels decreased ($P<0.0001$) over time (0 h = 2.00 μmol/g vs 24 h = 0.05 μmol/g on average). CLPG lambs (1.385 μmol/g) tended to have higher ($P=0.15$) levels of ATP than NML lambs (1.120 μmol/g). Supporting this tendency, CLPG lambs had a more rapid ($P<0.05$) pH decline than NML lambs, indicating they had a more rapid anaerobic glycolytic rate (Fig. 1). Koohmaraie et al. (1995) reported that phenotype had no affect on the rate of LM pH decline or ultimate pH. However, in the present study the tendency for elevated ATP levels in

Table 1
Proximate and mineral composition between phenotype and time postmortem (PM)

Item	Phenotype		*P*-value	Time PM (h)				*P*-value
	Normal	Callipyge		0	3	6	24	
% Moisture	69.77±0.83	70.36±1.1	0.67	70.8±1.7	69.4±1.3	70.9±1.2	69.2±1.2	0.67
% Ash	1.12±0.02	1.13±0.02	0.83	1.10±0.037	1.13±0.029	1.13±0.026	1.16±0.026	0.63
Ca (mg/100 g)	6.60±0.30	5.91±0.38	0.18	5.39±0.60	7.20±0.47	6.02±0.421	6.40±0.421	0.14
Fe (mg/100 g)	0.351±0.028	0.398±0.036	0.33	0.414±0.057	0.38±0.045	0.38±0.040	0.33±0.040	0.60
Mg (mg/100 g)	0.529±0.124	0.383±0.158	0.48	0.49±0.25	0.57±0.20	0.21±0.18	0.55±0.18	0.47
Zn (mg/100 g)	0.273±0.025	0.283±0.031	0.82	0.31±0.05	0.31±0.04	0.24±0.03	0.26±0.03	0.52
ATP (μmol/g)	1.21±0.07	1.39±0.07	0.1021	2.0±0.1	1.9±0.1	1.3±0.1	0.1±0.1	0.0001

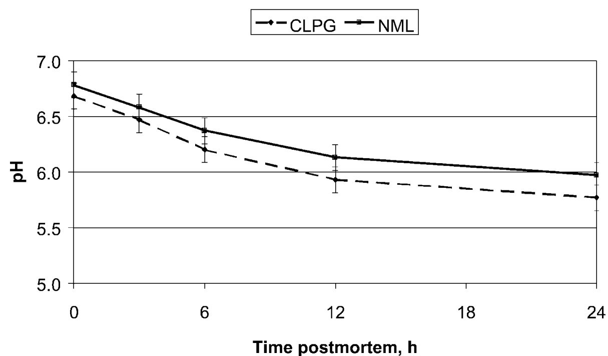

Fig. 1. Effect of callipyge (CLPG) and normal (NML) lamb phenotype on pH decline over time postmortem (0–24 h) in longissimus dorsi muscle (LM). Statistical data: phenotype ($P<0.05$, SEM = 0.05) and time ($P<0.0001$, SEM = 0.08).

CLPG lamb may have resulted in a greater rate and extent of anaerobic glycolysis, thus elevating lactic acid levels and resulting in a more rapid pH decline and lower ultimate pH. Temperature decline ($P<0.0001$) over time was normal, and no phenotypic differences were detected (Fig. 2). Koohmaraie et al. (1995) also reported that temperature did not differ between phenotypes. Although in theory an increase in muscle mass through specific muscle hypertrophy (Koohmaraie et al., 1995; Jackson, Miller, & Green, 1997b) may be enough to create differences in heat dissipation, the phenotypic expression of the CLPG gene does not appear to affect the rate of temperature decline in the LM. This may potentially be due to a reduction in subcutaneous fat and its insulating effects in CLPG versus NML lamb (Jackson et al., 1997a). As expected, phenotypic expression of the CLPG gene in this study increased ($P<0.05$) muscling, as measured by an increase in LEA in CLPG (9.4 cm^2) versus NML (7.0 cm^2) lambs.

Chops from CLPG lambs had higher ($P<0.0001$) WBS values than NML chops (Fig. 3). Miller, Carr, Ramsey, Crockett, and Hoover, (2001) reported that in beef, WBS values of 4.0 kg or less resulted in 94% consumer satisfaction for tenderness. In the present study, WBS values of less than 4.0 kg were achieved at 48 days PM in CLPG (3.6 kg), but in only 3 days PM (3.8 kg) in NML lambs. Numerous studies (Duckett, Klein, Dodson & Snowder, 1998b; Duckett, Klein, Leckie et al., 1998; Duckett et al., 2000; Koohmaraie et al., 1995; Shackelford et al., 1998) have shown that CLPG chops required more force to shear initially (day 1), and that WBS values for CLPG lamb decline more slowly than for NML lamb. A normal PM aging curve was achieved in this study ($P<0.0001$), evidenced by a decrease in WBS during extended aging (Fig. 3). The aging by phenotype interaction for WBS was significant ($P<0.1$); WBS values for NML lambs declined more rapidly than for CLPG during the first 12 days PM, but from 12 to 48 days PM the decline in WBS was greater in CLPG than for NML lambs (Fig. 3). Although CLPG lamb eventually reached an acceptable level (<4.54 kg) by 48 days PM, these values were still higher than the WBS values of NML lamb achieved as early as 6 days PM. Other studies (Duckett, Klein, Dodson, & Snowder, 1998; Dickett, Kein, Leckie et al., 1998; Duckett et al., 2000; Koohmaraie et al., 1995; Shackelford et al., 1998) reported that CLPG LM chops improve only minimally in tenderness during PM aging (up to 14 days PM), however, these studies did not age CLPG LM chops to the extent of 48 days PM, as in this study.

Differences in tenderness as measured by mechanical shear force have been attributed to levels of CA (Koohmaraie et al., 1995). Calpastatin activity (Fig. 4) for CLPG (3.95) was 58% higher ($P<0.05$) than for NML (2.49) lambs. These data agree with previous studies (Clare et al., 1997; Duckett, Klein, Dodson, & Snowder, 1998; Duckett et al., 2000; Koohmaraie et al., 1995) that reported higher CA in CLPG compared to NML lambs. Koohmaraie et al. (1995) suggested that the induced muscle hypertrophy of the CLPG phenotype is associated with higher CA and a slower rate and extent of PM proteolysis resulting in a less tender LM chop compared to NML lamb. Comparatively LM CA and WBS were positively correlated ($r=0.70$; $P<0.12$), indicating that higher levels of CA will inhibit the rate of PM proteolysis and offer less tender lamb LM. Duckett et al. (2000) reported that WBS values at day 6

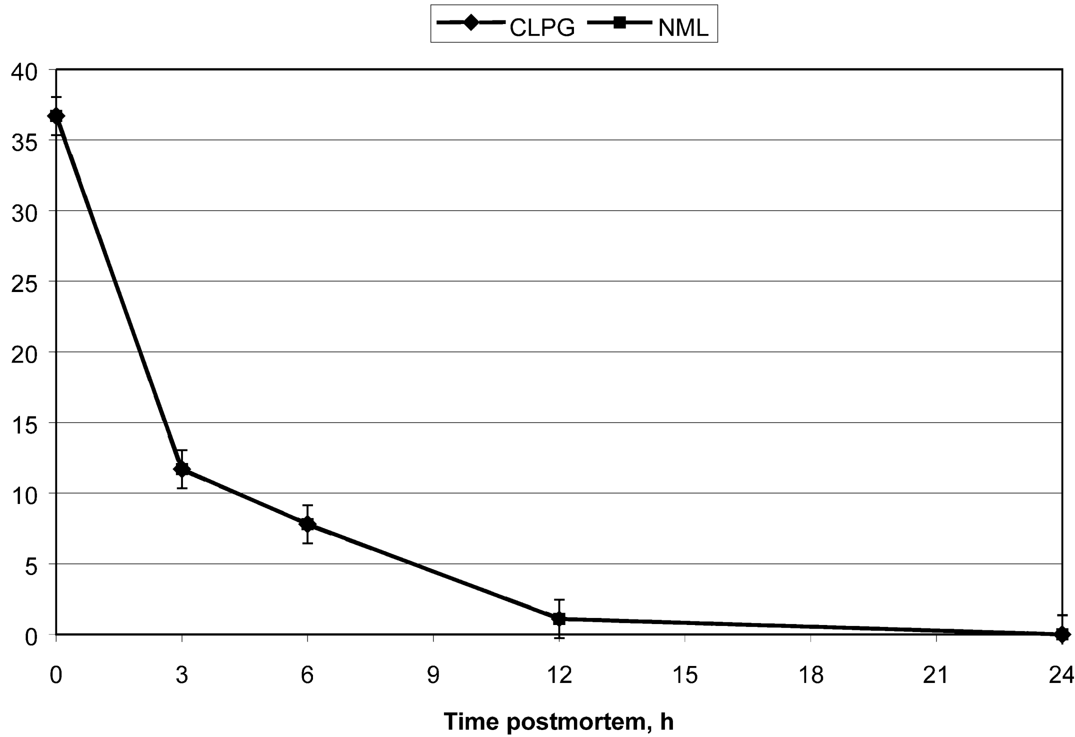

Fig. 2. Effect of callipyge (CLPG) and normal (NML) lamb phenotype on temperature (°C) decline over time postmortem (0–24 h) in longissimus dorsi muscle (LM). Statistical data: phenotype ($P>0.1$, SEM = 0.60) and time ($P<0.0001$, SEM = 0.95).

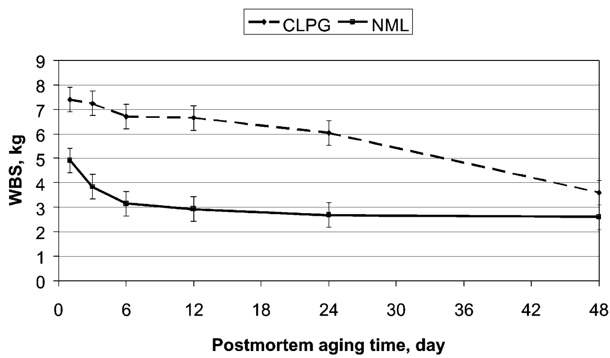

Fig. 3. Effect of callipyge (CLPG) and normal (NML) lamb phenotype on Warner-Bratzler shear force (WBS) during postmortem aging (1, 3, 6, 12, 24, and 48 days) in longissimus dorsi muscle (LM). Statistical data: phenotype ($P<0.0001$, SEM = 0.21), aging ($P<0.0001$, SEM = 0.36) and phenotype×aging ($P<0.1$, SEM = 0.51).

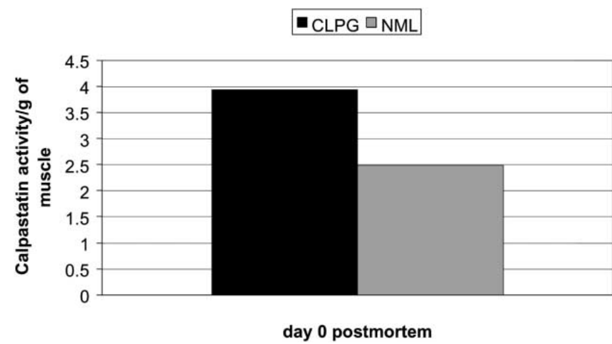

Fig. 4. Effect of callipyge (CLPG) and normal (NML) lamb phenotype on calpastatin activity (CA: units of activity/g of muscle) in longissimus dorsi muscle (LM) (day 0 postmortem). CLPG were 58% higher ($P<0.05$, SEM = 0.24) in CA than NML lamb LM.

PM and CA at 24 h PM in lamb LM were highly correlated ($r=0.72$) and that in beef, (Whipple, Koohmaraie, Dikeman, & Crouse, 1990) CA measured at 24 h PM was highly correlated ($r=0.66$) with 14 day WBS. The strong positive correlations in these studies, as well in the present study support the theory that high CA is associated with high WBS values.

Western Blot analysis, which measured the rate and extent of TNT degradation, paralleled the results reported for WBS and CA, as the 30 kDa degradation fragment of TNT appeared early in samples with low shear force values and low CA values (Fig. 5). An intense band for the 30 kDa primary degradation fragment of TNT appeared at 3 days PM in NML compared to 48 days PM in CLPG, indicating that the rate of TNT degradation was greater in NML than in CLPG lamb. Correspondingly, chops from NML lambs reached an acceptable WBS after only 3 days of PM aging, whereas CLPG chops did not reach an acceptable WBS level until 48 days PM (Fig. 3). Additionally, CLPG lamb had a higher level of CA than NML lamb (Fig. 4). Taylor and Koohmaraie (1998) suggested that CLPG LM is tough due to a lack of PM proteolysis. The difference between the rate and extent of proteolytic degradation of TNT in the current study may be due to

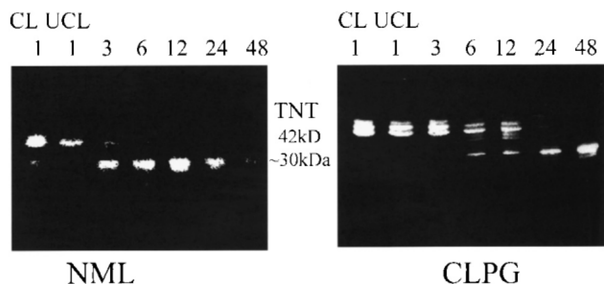

Fig. 5. Western blot analysis for the determination of rate and extent of proteolytic degradation differences of troponin-T (TNT) as measured by the appearance of the 30 kDa TNT fragment (the primary degradation product of TNT) of callipyge (CLPG) and normal (NML) lamb longissimus dorsi muscle (LM) over time postmortem (PM). Poly-acrylamide gel lane set up: lane 1 = clamped (CL) day 1 PM, lane 2 = unclamped (UCL) day 1 PM, lane 3–7 = UCL 3, 6, 12, 24, and 48 day PM, respectively.

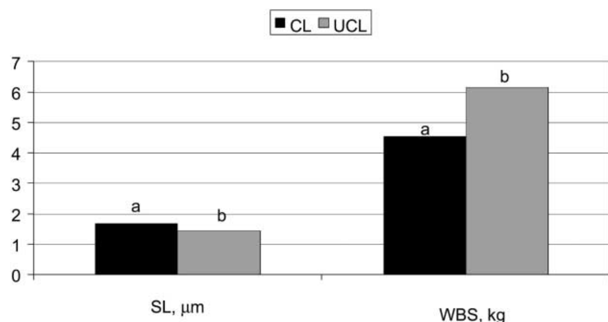

Fig. 6. Sarcomere length (SL) and Warner-Bratzler shear force (WBS) of clamped (CL) and unclamped (UCL) lamb longissimus muscle. Statistical data: SL ($P<0.01$, SEM = 0.03), WBS ($P<0.05$, SEM = 0.47) and the correlation between SL and WBS ($r=-0.55$).

higher levels of CA (58%) in CLPG than in NML lambs. Moreover, by 48 days PM the primary degradation product in NML lamb (visible at 3 days PM) was further degraded into secondary degradation products, which were not present on the gel matrix and subsequently did not transfer to the PVDF membrane. Sarcomere length did not differ ($P>0.1$) between NML and CLPG LM (data not shown). Koohmaraie et al. (1995) also reported that SL did not differ between NML and CLPG lamb LM concluding that differences in tenderness were not influenced by a reduction in SL.

3.2. Clamped versus unclamped muscle

In CLPG and NML chops, respectively, initial WBS was lower ($P<0.05$) in CL (5.5 and 3.6 kg) compared to UCL (7.4 and 4.9 kg). Clamping reduced ($P<0.05$) WBS values across phenotype by 1.6 kg (UCL = 6.15 kg; CL = 4.55 kg, Fig. 6). Sarcomere lengths (SL) were longer ($P<0.05$) for CL (1.68 µm) than for UCL (1.44 µm) (Fig. 6). Moreover, TEM pictures of UCL muscle exhibited a constant gradient of striation between repeating z disks, whereas CL LM had breaks in the striation pattern at each repeating z disk (Fig. 7). It is hypothesized that the breaks in striation (Fig. 7) may be due to a reduction in the overlapping of the thick and thin filaments of the sarcomere at the completion of rigor in CL LM when compared to UCL LM. Alternatively, the reduction in WBS in CL LM may have been due to tearing of the sarcomere at the z-disk. In either case further studies will be necessary to evaluate the effects of clamping on the microstructure of the sarcomere.

Koohmaraie, Shackelford, and Wheeler (1998) reported that (from 0 to 24 h PM) SL decreased, as shear force increased and concluded that sarcomere shortening during rigor development is the cause of toughening of lamb LM during the first 24 h PM. In the present study, SL was negatively correlated with WBS ($r=-0.55$; $P<0.1$), indicating that as SL increased, the force to shear the sample decreased, confirming the results of Koomaraie et al. (1998). As demonstrated in Fig. 5, CL versus UCL LM resulted in no apparent difference in the extent of proteolysis of TNT day 1 PM. This, along with the TEM pictures (Fig. 7) implies that the difference in WBS between CL and UCL day 1 samples is most likely due to a physical disruption of the myofibrillar structure during rigor. Preventing the sarcomere from shortening during rigor has previously been shown to prevent meat toughening (Koohmaraie, Shackelford, & Wheeler, 1996).

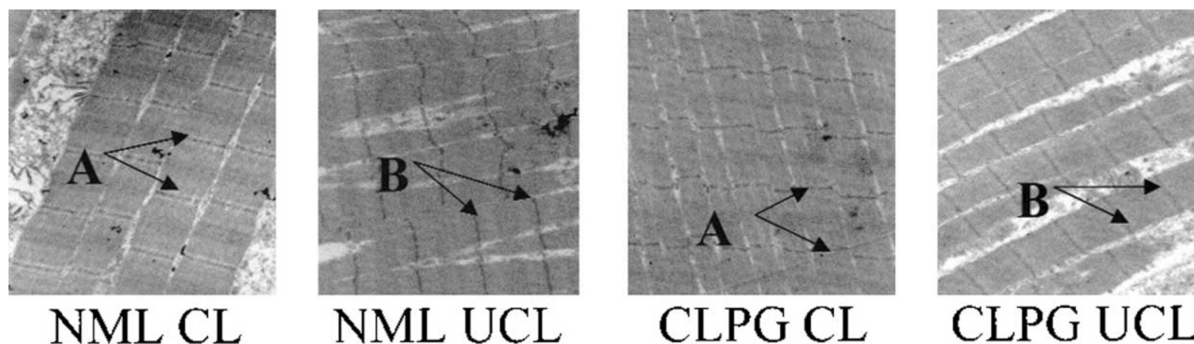

Fig. 7. Transmission electron micrograph for estimation of sarcomere length (SL). A = clamped (CL) sarcomere; B = unclamped (UCL) sarcomere. Statistical data: SL ($P=0.1071$, SEM = 0.09). NML, normal; CLPG, callipyge.

In conclusion, extended aging (48 days) resulted in CLPG LM with acceptable WBS values, concurrent with the appearance of TNT degradation products, but CLPG LM chops still had higher WBS values than NML chops even at 48 days PM. Clamping the LM prior to the onset of and during rigor reduced initial shear force values by 1.6 kg and although not significant, the effect of rigor on sarcomere shortening was reduced. The inverse relationship of WBS and SL support the theory that if a reduction in rigor shortening is accomplished the end result may be a reduction in WBS.

4. Implications

Some muscles from callipyge lambs, have inherent physiological differences rendering them less tender. This study indicates that extended aging can improve tenderness of CLPG LM. Further investigation into the optimum length of PM aging between 24 and 48 days PM is warranted. Altering day 1 sarcomere length during rigor by muscle restraint did not affect TNT degradation, but it did lower initial WBS values, therefore further research is required to determine the effect of PM aging on lamb with varying sarcomere lengths.

Acknowledgements

I would like to acknowledge and thank the supporting work of Sue Pritchard, Lynn Forzcek, Dessa Dal Porto, and Dr. Elizabeth Huff-Lonergan, for their technical and procedural assistance.

References

AMSA. (1995). *Research guidelines for cookery, sensory evaluation and instrumental tenderness measurements of fresh meat*. Chicago, IL: American Meat Science Assoc.

AOAC. (1990). *Method number 968.08*. *Official methods of analysis* (15th ed.). Arlington, VA: Association of Official Analytical Chemists.

Busboom, J. R., Wahl, T. I., & Snowder, G. D. (1999). Economics of callipyge lamb production. *Journal of Animal Science*, *77*(Suppl. 2), 243–248.

Clare, T. L., Jackson, S. P., Miller, M. F., Elliot, C. T., & Ramsey, C. B. (1997). Improving tenderness of normal and callipyge lambs with calcium chloride. *Journal of Animal Science*, *74*, 377–385.

Duckett, S. K., Klein, T. A., Dodson, M. V., & Snowder, G. D. (1998). Tenderness of normal and callipyge lamb aged fresh or after freezing. *Meat Science*, *49*, 19–26.

Duckett, S. K., Klein, T. A., Leckie, R. K., Thorngate, J. H., Busboom, J. R., & Snowder, G. D. (1998). Effect of freezing on calpastatin activity and tenderness of callipyge lamb. *Journal of Animal Science*, *76*, 1869–1874.

Duckett, S. K., Snowder, G. D., & Cockett, N. E. (2000). Effect of the callipyge gene on muscle growth, calpastatin activity, and tenderness of three muscles across the growth curve. *Journal of Animal Science*, *78*, 2836–2841.

Geesink, G. H., & Koohmaraie, M. (1999). Postmortem proteolysis and calpain/calpastatin activity in callipyge and normal lamb biceps femoris during extended postmortem storage. *Journal of Animal Science*, *77*, 1490–1501.

Huff-Lonergan, E., Mitsuhashi, T., Parrish, F. C. Jr., & Robson, R. M. (1996). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting comparisons of purified myofibrils and whole muscle preparations for evaluating titin and nebulin in postmortem beef muscle. *Journal of Animal Science*, *74*, 779–785.

Jackson, S. P., Miller, M. F., & Green, R. D. (1997a). Phenotypic characterization of Rambouillet sheep expressing the *callipyge* gene: II. Carcass characteristics and retail yield. *Journal of Animal Science*, *75*, 125–132.

Jackson, S. P., Miller, M. F., & Green, R. D. (1997b). Phenotypic characterization of Rambouillet sheep expressing the *callipyge* gene: III. Muscle weights and muscle weight distribution. *Journal of Animal Science*, *75*, 133–138.

Koohmaraie, M., Shackelford, S. D., & Wheeler, T. L. (1996). Effects of a β-adrenergic (L-644,969) and male sex condition on muscle growth and meat quality of callipyge lambs. *Journal of Animal Science*, *74*, 70–79.

Koohmaraie, M., Shackelford, S. D., & Wheeler, T. L. (1998). Effect of pre-rigor freezing and post-rigor calcium chloride injection on the tenderness of callipyge longissimus. *Journal of Animal Science*, *76*, 1427–1432.

Koohmaraie, M., Shackelford, S. D., Wheeler, T. L., Lonergan, S. M., & Doumit, M. E. (1995). A muscle hypertrophy condition in lamb (callipyge): characterization of effects on muscle growth and meat quality traits. *Journal of Animal Science*, *73*, 3596–3607.

Miller, M. F., Carr, M. A., Ramsey, C. B., Crockett, K. L., & Hoover, L. C. (2001). Consumer thresholds for establishing the value of beef tenderness. *Journal of Animal Science*, *79*, 3062–3068.

Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (1998). Can the genetic antagonisms of callipyge lamb be overcome? *Proceedings Reciprocal Meat Conference*, *51*, 125–132.

Taylor, R. G., & Koohmaraie, M. (1998). Effects of postmortem storage on the ultrastructure of the endomysium and myofibrils in normal and callipyge longissimus. *Journal of Animal Science*, *76*, 2811–2817.

Whipple, G. M., Koohmaraie, M., Dikeman, M. E., & Crouse, J. D. (1990). Predicting beef longissimus tenderness from various biochemical and histological muscle traits. *Journal of Animal Science*, *68*, 4193–4199.

Wheeler, T. L., & Koohmaraie, M. (1994). Prerigor and postrigor changes in tenderness of ovine longissimus muscle. *Journal of Animal Science*, *72*, 1232–1238.