

# Effect of Prerigor Freezing and Postrigor Calcium Chloride Injection on the Tenderness of Callipyge Longissimus<sup>1</sup>

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**ABSTRACT:** The effect of rapid prerigor freezing and postrigor freezing and postrigor calcium chloride injection on the tenderness of callipyge longissimus was studied. Ewe and wether lambs ( $n = 49$ ; 1/2 Dorset  $\times$  1/2 Romanov) were grain-fed and slaughtered at approximately 250 d of age. Based on leg conformation scores, 23 of the carcasses had the callipyge phenotype. Within each phenotype, approximately one-half of the carcasses were chilled conventionally (24 h at  $-2^{\circ}\text{C}$ ). At approximately 17 min postmortem, the remaining carcasses were submersed in liquid nitrogen for 15 min and then held at  $-2^{\circ}\text{C}$  for 4 d. At 1 d postmortem for carcasses chilled conventionally and at 4 d postmortem for carcasses frozen in liquid nitrogen, the longissimus muscles from both sides were removed. The longissimus from one side of each carcass was vacuum-packaged and aged ( $1^{\circ}\text{C}$ ) conventionally for 7 or 14 d. The remaining muscles were injected with a 2.22% solution of food-grade calcium chloride at 5% by weight, vacuum-packaged, and aged as above. Liquid nitrogen freezing was effective in limiting sarcomere shortening (1.99 vs 1.63  $\mu\text{m}$ ;  $P < .05$ ). Warner-Bratzler shear force values of callipyge longissimus were 222 and 232% of that of normal longissimus after 7 and 14 d postmortem,

respectively ( $P < .001$ ). Also, trained panel tenderness rating was decreased by 49.4% in untreated callipyge longissimus after 14 d postmortem. Liquid nitrogen, calcium chloride injection and their combination did not affect d-14 longissimus shear force and sensory tenderness for normal lambs because untreated muscles were already tender. Liquid nitrogen freezing improved the shear force and sensory tenderness rating of callipyge longissimus by 30 and 86.2% after 14 d postmortem, respectively. Calcium chloride injection improved the shear force and sensory tenderness of callipyge longissimus by 36.7 and 86.2% after 14 d postmortem, respectively ( $P < .001$ ). The most effective treatment for mitigating the callipyge effect on tenderness was the combination (freezing and calcium chloride injection) treatment, which improved the shear force and sensory tenderness by 51.2 and 124.2% after 14 d postmortem, respectively ( $P < .001$ ). We conclude that either treatment can effectively mitigate the negative effect of callipyge phenotype on longissimus tenderness. Callipyge lamb carcasses subjected to the combination of prerigor liquid nitrogen freezing, postrigor calcium chloride injection, and 14 d postmortem storage had tenderness similar ( $P > .05$ ) to that of normal, untreated carcasses after 14 d of postmortem storage.

Key Words: Callipyge, Tenderness, Calcium Chloride, Freezing Techniques

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## Introduction

The callipyge phenotype greatly improves feed efficiency (Jackson et al., 1997a), dressing percentage, and carcass composition (Koohmaraie et al., 1995, 1996b; Jackson et al., 1997b). These advantages, combined with absence of dystocia, presents the lamb industry with an attractive method of efficiently producing lean meat. The major drawback to the callipyge condition is its negative effect on meat tenderness (Koohmaraie et al., 1995, 1996b; Field et al., 1996; Shackelford et al., 1997). The negative effect seems to be particularly great in the longissimus, whereas the toughness of callipyge leg muscles is eliminated by oven roasting (Shackelford et al.,

<sup>1</sup>Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of other products that may also be suitable. We gratefully acknowledge the technical assistance of P. Ekeren, K. Mihm, P. Tammen, and M. Thoesen for execution of these experiments and the secretarial assistance of M. Bierman.

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1997). Before the lamb industry can use callipyge lambs, processes must be implemented to mitigate and possibly eliminate its negative effect on longissimus tenderness.

On the basis of their findings on the relationship between sarcomere length (**SL**) and longissimus tenderness during the first 24 h after slaughter (Wheeler and Koohmaraie, 1994; Koohmaraie et al., 1996a), Koohmaraie (1996) suggested that freezing the carcasses immediately after slaughter and then storing at just below freezing to prevent thaw rigor might prevent toughness development. The objective of the present experiment was to determine the effectiveness of rapid prerigor freezing, postrigor injection with calcium chloride, and their combination in mitigating the negative effects of the callipyge phenotype on longissimus tenderness.

### Materials and Methods

Crossbred (1/2 Dorset  $\times$  1/2 Romanov) wether and ewe lambs ( $n = 49$ ) were grain-fed and slaughtered at approximately 250 d of age. Carcasses were dressed conventionally, and hot carcass weight was measured (mean = 28.4 kg). Based on leg conformation scores, 23 of the carcasses were determined to have the callipyge phenotype. Within each phenotype, about one-half of the carcasses were chilled conventionally (24 h at  $-2^{\circ}\text{C}$ ). At approximately 17 min postmortem, the remaining carcasses were submersed in liquid nitrogen for 15 min. The liquid nitrogen treatment was accomplished by immersing the lamb carcass, suspended by the achilles tendon, into an insulated stainless-steel cylinder (195-cm height and 41-cm diameter) that contained sufficient liquid nitrogen to totally immerse the carcass. Frozen carcasses were then held at  $-2^{\circ}\text{C}$  for 4 d.

To monitor temperature in carcasses frozen in liquid nitrogen, a thermocouple wire was inserted into the center of the longissimus at the 10th rib, and temperature was determined at 1-min intervals during liquid nitrogen freezing and at 1, 2, 3, 4, 5, 10, 15, 30, 45, 105, and 165 min after a carcass was removed from the liquid nitrogen tank using a handheld thermometer (Cole-Parmer, Vermont Hills, IL). Because of technical difficulties associated with properly positioning the end of the thermocouple wire in the center of the longissimus and keeping the thermocouple wire properly positioned throughout the freezing process, valid temperature data were available for 10 of the liquid nitrogen carcasses. For conventionally chilled carcasses, longissimus temperature was determined at 30, 45, 60, 120, 180, and 1,440 min postmortem using a handheld thermometer and needle thermocouple.

At 1 d postmortem, the longissimus (4th rib to last lumbar vertebra) was removed from both sides of the

conventionally chilled carcasses. Muscles from one side of each carcass were vacuum-packaged and aged ( $1^{\circ}\text{C}$ ) conventionally for 7 or 14 d. The remaining muscles were injected with a 2.22% (200 mM) solution of food-grade calcium chloride using a commercial pickle injector. Injection level was targeted to be 5%. Injected cuts were vacuum-packaged and aged as above.

At 4 d postmortem, bone-in loins were removed from both sides of the frozen carcasses. Loins were thawed for 24 h at  $1^{\circ}\text{C}$ . At 5 d postmortem, the longissimus was removed. Muscles from one side of each carcass were vacuum-packaged as above. The remaining muscles were injected with calcium chloride, vacuum packaged, and aged as above.

At 7 d postmortem, 11 chops (2.54 cm thick) were removed from each muscle beginning at the posterior end. The first four chops and the second four chops were assigned alternately for evaluation of Warner-Bratzler shear force (three chops) and sarcomere length (one chop) at 7 or 14 d postmortem. The last three chops were used for trained sensory panel analysis. Day-14 chops were vacuum-packaged and aged ( $1^{\circ}\text{C}$ ). At 14 d postmortem, sensory panel chops were frozen ( $-30^{\circ}\text{C}$ ) and stored up to 1 mo before analysis.

For determination of SL, a cube (.4  $\times$  .4  $\times$  2 cm) was removed from the medial end, center, and lateral end of each chop parallel with the longitudinal axis of the muscle fibers and fixed according to Koolmees et al. (1986). Eight fibers were teased from each cube and SL was measured using the neon laser (Spectra Physics, Eugene, OR) diffraction method described by Cross et al. (1980). Sarcomere length was not determined on calcium chloride-injected samples.

For determination of Warner-Bratzler shear force, chops were broiled on Farberware Open Hearth electrical broilers (Farberware, Bronx, NY) to an internal temperature of  $70^{\circ}\text{C}$ . Chops were chilled for 24 h at  $4^{\circ}\text{C}$  to facilitate removal of cores. Two cores (diameter = 1.27 cm) were removed from each chop parallel with the longitudinal orientation of the muscle fibers. Cores were sheared with a Warner-Bratzler attachment using an Instron Universal Testing Machine (Instron, Canton, MA) with a 50-kg load cell and crosshead speed of 200 mm/min.

For trained sensory panel analysis, chops were thawed for 24 h at  $5^{\circ}\text{C}$  and broiled to an internal temperature of  $70^{\circ}\text{C}$ . Each panelist received three cubes (1.3  $\times$  1.3  $\times$  1.9 cm) from each sample. Sensory panelists rated cubes for tenderness, amount of connective tissue, juiciness, and lamb flavor intensity on 8-point scales (1 = extremely tough, abundant, dry, and bland and 8 = extremely tender, little, juicy and intense). Off-flavor was scored on a 4-point scale (1 = strong and 4 = none). One sample from each treatment combination was served per day.

Warner-Bratzler shear force data were analyzed as a split-split-plot design (SAS, 1988). Carcasses served

as the whole plot, with whole-plot treatments of phenotype (normal vs callipyge) and chilling method (conventional vs liquid-nitrogen frozen). The split-plot treatment was injection (control vs  $\text{CaCl}_2$ ), and the split-split-plot treatment was aging period (7 vs 14 d). Sarcomere length data were analyzed as a split-plot design. Carcasses served as the whole plot, with whole-plot treatments of phenotype and chilling method. The split-plot treatment was aging period. Sensory panel data were analyzed as a split-plot design. Carcasses served as the whole plot, with whole-plot treatments of phenotype and chilling method. The split-plot treatment was injection. Additionally, specific contrasts were conducted to compare various treatment combinations using one-way ANOVA.

## Results and Discussion

There is a remarkable agreement among results of different experiments from various institutions on the tenderness of lamb longissimus exhibiting the callipyge phenotype. These studies have collectively indicated that meat from callipyge longissimus has a high shear force value and low sensory score and improves minimally during extended postmortem storage (Koochmaraie et al., 1995, 1996b; Field et al., 1996; Shackelford et al., 1997). Taylor and Koochmaraie (unpublished data) examined the ultrastructural changes in the longissimus of normal lamb and callipyge lambs. Compared to longissimus from normal lamb, callipyge longissimus showed very little degradation of intermediate filaments and had few myofibril breaks. The lack of response to postmortem storage is due to minimal changes in the proteins whose degradation is the cause of ultrastructural changes that result in postmortem tenderization. Koochmaraie et al. (1995) stated that the extent of degradation of troponin-T, desmin, nebulin, and titin in callipyge longissimus after 21 d postmortem was comparable to that of longissimus of normal lamb after 1 d postmortem. The reduced rate and extent of postmortem proteolysis is due to reduced activity of the calpain proteolytic system by an 82, 86, and 108% increase in calpastatin activity immediately after slaughter and after 7 and 21 d postmortem, respectively (Koochmaraie et al., 1995). The differences between the tenderness of longissimus of normal and callipyge lamb is not due to sarcomere length (Koochmaraie et al., 1995), collagen content (Field et al., 1996), or collagen (hydroxylsypyrindinoline) cross-linking (Field et al., 1996).

Wheeler and Koochmaraie (1994) demonstrated that lamb longissimus has an intermediate shear force value immediately after slaughter (5.07 kg), toughens during the first 24 h (maximum toughness was achieved at 9 to 24 h; 8.66 kg), and then becomes tender during postmortem storage at 4°C (3.10 kg).

Because SL decreased (from at-death lengths of 2.24  $\mu\text{m}$  to 24-h postmortem lengths of 1.69  $\mu\text{m}$ ) as shear force increased, we concluded that sarcomere shortening during rigor development is the cause of lamb longissimus toughening from 0 to 24 h postmortem. We provided further support for the accuracy of our hypothesis by demonstrating that preventing sarcomere shortening also prevented meat toughening (Koochmaraie et al., 1996a). Based on these and other observations, Koochmaraie (1996) proposed a method of producing consistently tender lamb by preventing sarcomere shortening. The proposed method included rapid freezing of carcasses immediately after slaughter (the goal is to produce conditions that result in SL of not less than 2.2  $\mu\text{m}$ ) and storage at  $-5^\circ\text{C}$  (to deplete ATP and, thus, prevent thaw shortening) for a period of time necessary to drop longissimus pH to  $< 5.8$ . The objective of the present experiment was to test the feasibility of the above proposal in mitigating the toughness of callipyge longissimus. An additional objective was to determine the effectiveness of post-rigor injection with calcium chloride, singularly, or combined with the freezing treatment in mitigating the toughness of callipyge longissimus.

We attempted to freeze carcasses at  $-30^\circ\text{C}$  with various levels of air movement combined with hanging carcasses by aitch bone or achilles tendon. The most effective method was submersion in liquid nitrogen. Effectiveness was defined as freezing that results in post-rigor sarcomere length of not less than 2  $\mu\text{m}$ .

The pattern of temperature decline in longissimus of conventionally chilled and liquid-nitrogen-frozen lamb carcasses is shown in Figure 1. Approximately 17 min after slaughter, carcasses were submersed in liquid nitrogen for 15 min. At the immersion time, the temperature of the longissimus was  $40^\circ\text{C}$ , did not change for about 4 min, and then declined very slowly. At about 35 min after slaughter, the temperature of the longissimus was  $-2.3^\circ\text{C}$  and continued to decline until it reached  $-14.5^\circ\text{C}$  at 62 min after slaughter.

After 4 d at  $-2^\circ\text{C}$ , the longissimus had reached its final pH (mean pH value of 5.63 and SD of .10). This protocol was effective in limiting the reduction in

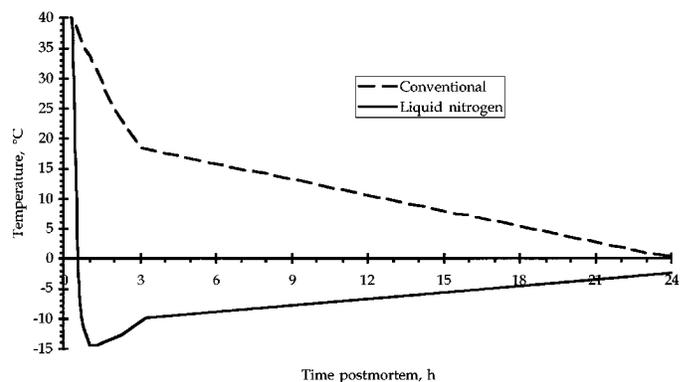


Figure 1. Temperature decline of conventionally chilled carcasses and carcasses frozen in liquid nitrogen.

Table 1. Mean squares for sarcomere length (SL)

Source <sup>a</sup>	df	SL
Phenotype	1	.03
Treatment	1	3.13***
Phenotype × treatment	1	.01
Carcass (phenotype × treatment)	45	.02
Aging	1	.04
Phenotype × aging	1	.01
Treatment × aging	1	.03
Phenotype × treatment × aging	1	.00
Residual	45	.03

<sup>a</sup>Phenotype (normal vs callipyge); treatment (conventional chilling vs liquid nitrogen freezing); aging (7 vs 14 d postmortem).

sarcomere length. The sarcomere length of longissimus from carcasses frozen in liquid nitrogen was 1.99  $\mu\text{m}$  as compared to 1.63  $\mu\text{m}$  in conventionally chilled carcasses ( $P < .05$ ). No other factor affected SL (Table 1). Phenotype, chilling method, and injection interacted to affect shear force and sensory tenderness scores (Tables 2 and 3). Consistent with previous observations (Koochmaraie et al., 1996a), limiting sarcomere shortening produced meat with lower shear force value and higher tenderness sensory scores (Figures 2, 3, and 4). Shear force value and sensory tenderness of callipyge longissimus was improved by 30 and 86.2% after 14 d postmortem, respectively, by liquid nitrogen freezing alone compared to conventionally chilled callipyge longissimus (Figures 2, 3, and 4). Because longissimus of normal lamb is already tender, liquid nitrogen freezing had minimal effect on its shear force or sensory tenderness.

Table 2. Mean squares for Warner-Bratzler (W-B) shear force

Source <sup>a</sup>	df	W-B shear force
Phenotype	1	209.03***
Treatment	1	39.88***
Phenotype × treatment	1	23.62***
Carcass (phenotype × treatment)	45	1.12***
Injection	1	48.04***
Phenotype × injection	1	22.33***
Treatment × injection	1	2.16*
Phenotype × treatment × injection	1	2.56*
Aging	1	9.83***
Phenotype × aging	1	2.36*
Treatment × aging	1	.62
Phenotype × treatment × aging	1	.48
Injection × aging	1	.02
Phenotype × injection × aging	1	.84
Treatment × injection × aging	1	.02
Phenotype × treatment × injection × aging	1	.11
Residual	135	.41

<sup>a</sup>Phenotype (normal vs callipyge); treatment (conventional chilling vs liquid nitrogen freezing); injection (not injected vs calcium chloride); aging (7 vs 14 d postmortem).

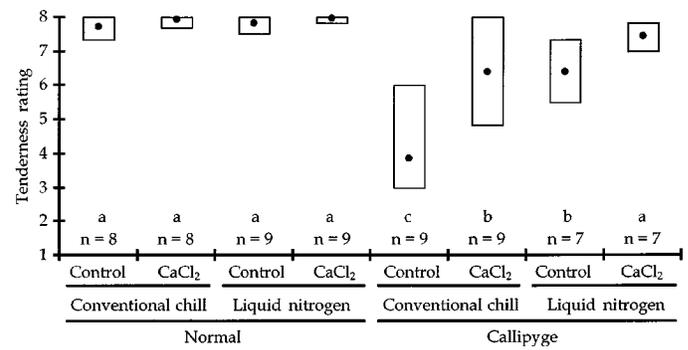


Figure 2. Effects of prerigor freezing of lamb carcasses with liquid nitrogen and postrigor calcium chloride injection on expert sensory panel tenderness ratings of lamb at 14 d postmortem. Dots indicate the mean for each subclass. Vertical bars indicate the range for each subclass. Letters above the x-axis indicate statistical differences; means not bearing a common letter differ (SEM = .22;  $P < .05$ ).

Since the first report (Koochmaraie et al., 1988), injection of cuts of meat or infusion of carcasses with a solution of food-grade calcium chloride has been shown to consistently enhance tenderness of meat that is not already tender (Koochmaraie et al., 1989, 1990; Koochmaraie and Shackelford, 1991; Wheeler et al., 1991, 1992, 1993, 1996; Kerth et al., 1995; Lansdell et al., 1995; Miller et al., 1995; Wulf et al., 1996). Consistent with these reports, postrigor injection of longissimus with calcium chloride improved ( $P < .05$ ) shear force (36.7% improvement) and sensory tenderness (86.2% improvement) of callipyge lamb, but calcium chloride injection had minimal effect on similar traits for longissimus of normal lamb (Figures 2, 3, and 4).

Because liquid nitrogen freezing prevents or limits the development of toughness caused by rigor-induced sarcomere shortening and calcium chloride improves tenderness predominantly by enhancing postmortem proteolysis through activation of calpains (Koochmaraie et al., 1988), the combination of these two treatments should have an additive effect on ultimate meat tenderness if neither treatment alone maximized tenderness. Clearly, this was the case for callipyge longissimus. The data reported in Figures 2, 3, and 4 best demonstrate the synergistic actions of these two treatments. The combination treatment improved ( $P < .001$ ) the shear force and sensory tenderness of callipyge longissimus by 48.8 and 92.3% after 14 d postmortem, respectively (Figures 2, 3, and 4). Consistent with the individual treatment effects, the combination treatment had minimal effect on indices of meat tenderness in longissimus of normal lamb.

Based on these findings, we conclude that either liquid nitrogen freezing or calcium chloride injection treatment can effectively mitigate the negative effect

Table 3. Mean squares for sensory traits<sup>a</sup>

Source <sup>b</sup>	df	TEN	ACT	JUI	LFI	OF
Phenotype	1	.39	.01	1.45	.08	.62
Treatment	1	.23	.01	.01	1.24	.34
Phenotype × treatment	1	.02	.01	.43	.01	.15
Carcass (phenotype × treatment)	4	.10	.01	.72	.84	.34
Injection	1	16.34***	.01	1.78*	.12	3.73***
Phenotype × injection	1	10.75***	.01	.11	.95	.12
Treatment × injection	1	2.51*	.01	.01	.07	.16
Phenotype × treatment × injection	1	2.00*	.01	.39	.09	.02
Residual	54	.40	.01	.42	.43	.28

<sup>a</sup>TEN = tenderness; ACT = amount of connective tissue; JUI = juiciness; LFI = lamb flavor intensity; OF = off-flavor.

<sup>b</sup>Phenotype (normal vs callipyge); treatment (conventional chilling vs liquid nitrogen freezing); injection (not injected vs calcium chloride).

of callipyge phenotype on longissimus tenderness. Callipyge lamb subjected to the combination of liquid nitrogen freezing, calcium chloride injection, and 14 d postmortem storage had tenderness similar ( $P < .05$ ) to that of normal, untreated lamb after 14 d of postmortem aging.

None of the factors tested affected sensory panel scores for amount of connective tissue or lamb flavor intensity. The only factor that affected juiciness or off-flavor scores was calcium chloride injection, which increased juiciness (5.7 vs 5.3;  $P < .05$ ) and decreased (2.6 vs 3.1;  $P < .001$ ) off-flavor scores.

The methodologies reported here should enable the lamb industry to benefit from many of the advantages of callipyge lamb. However, a number of issues will have to be resolved before the lamb-processing industry can use one or more of these technologies. One such issue is the type of modification needed in the processing plant to allow freezing of lamb carcasses at chain speeds. We envision a system in which hanging

lamb carcasses are carried through a liquid nitrogen vat in much the same manner as hanging pork carcasses are carried through hot water for dehairing. Other issues include the effect of these treatments on other meat quality traits such as color, drip loss, and protein solubility.

### Implications

These results indicate that untreated callipyge longissimus has low indices of meat tenderness and likely would not meet consumer expectation. Freezing callipyge carcasses in liquid nitrogen immediately after slaughter or postrigor injection with calcium chloride can effectively mitigate the negative effect of callipyge phenotype on longissimus tenderness. Callipyge lamb subjected to the combination of liquid nitrogen freezing, calcium chloride injection, and 14-d postmortem storage had tenderness similar to

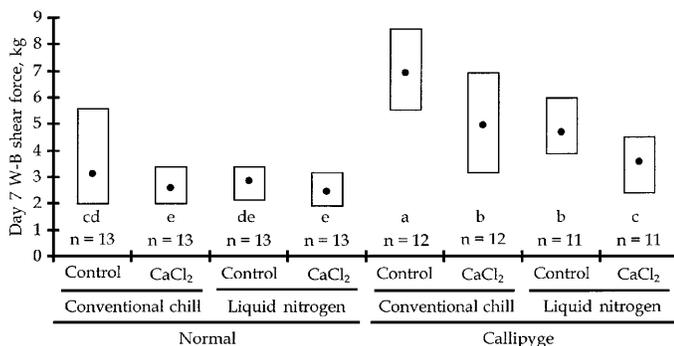


Figure 3. Effects of prerigor freezing of lamb carcasses with liquid nitrogen and postrigor calcium chloride injection on Warner-Bratzler (W-B) shear force values of lamb at 7 d postmortem. Dots indicate the mean for each subclass. Vertical bars indicate the range for each subclass. Letters above the x-axis indicate statistical differences; means not bearing a common letter differ (SEM = .18;  $P < .05$ ).

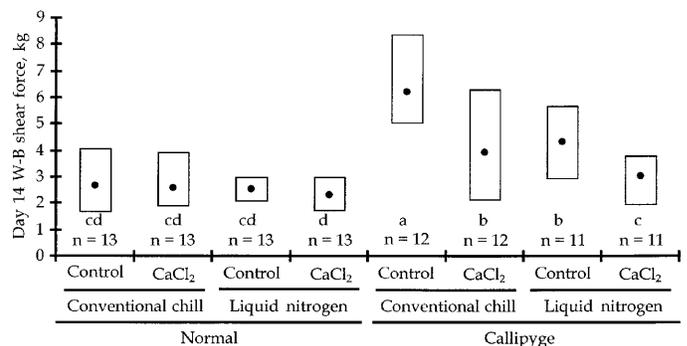


Figure 4. Effects of prerigor freezing of lamb carcasses with liquid nitrogen and postrigor calcium chloride injection on Warner-Bratzler (W-B) shear force values of lamb at 14 d postmortem. Dots indicate the mean for each subclass. Vertical bars indicate the range for each subclass. Letters above the x-axis indicate statistical differences; means not bearing a common letter differ (SEM = .18;  $P < .05$ ).

that of normal, untreated lamb after 14 d of postmortem aging.

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