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ACCELERATION OF POSTMORTEM TENDERIZATION IN LAMB AND BRAHMAN-CROSS BEEF CARCASSES THROUGH INFUSION OF CALCIUM CHLORIDE[1]

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ABSTRACT

A study involving 24 lamb carcasses was conducted to determine the effects of electrical stimulation and $CaCl_2$ infusion on meat shear forces. Infusion of lamb carcasses with $CaCl_2$ (.3 *M* at 10% of live weight) accelerated postmortem tenderization such that postmortem storage beyond 24 h to ensure meat tenderness was not necessary. Low-frequency electrical stimulation of lamb carcasses had no effect on shear force and was not a necessary step prior to $CaCl_2$ infusion. Twelve carcasses obtained from Brahman × Hereford or Angus crossbred cattle were used to determine whether $CaCl_2$ also would accelerate postmortem aging of carcass beef. Within 45 min of slaughter, a section of longissimus muscle from one side was injected with .3 *M* $CaCl_2$ solution. The corresponding longissimus muscle from the other side served as a control. Results indicated that 1) $CaCl_2$ injection of beef longissimus muscle accelerated postmortem tenderization as determined by shear force on d 1, 2) shear force value of noninjected longissimus muscle decreased by 2.8 kg (from 9.03 kg at d 1 to 6.23 at d 14) during 14 d of postmortem storage, whereas shear value of $CaCl_2$-injected samples decreased by only about 1 kg (from 6.09 at d 1 to 5.06 at d 14); 3) $CaCl_2$-injected samples had higher cooking loss at d 1 but not at d 14 postmortem; 4) $CaCl_2$ injection had no effect on cooking rate; and 5) $CaCl_2$ injection had a clear and definable effect on the activities of components of the Ca^{2+}-dependent proteolytic system, but it had no effect on the activities of Cathepsins B and B + L.

(Key Words: Tenderness, Proteases, Aging, Proteolysis.)

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Introduction

Crossbreeding has become a widely accepted means of improving efficiency of beef production. The economic value of *Bos indicus* breeds of cattle in crossbreeding programs in semitropical and tropical climates has been well established Carroll et al., 1955; Cole et al., 1963; Crockett et al., 1979).

Tenderness is the predominant quality determinant and probably the most important organoleptic characteristic of meat (Weir, 1960; Lawrie, 1966; Moeller et al., 1977). Meat from *Bos indicus* crosses may have objectionable tenderness ratings (Ramsey et al. 1963; Koch et al., 1982; Crouse et al., 1987, 1989).

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Infusion of ovine carcasses with $CaCl_2$ immediately after death accelerates the postmortem aging process such that postmortem storage of carcasses beyond 24 h to ensure acceptable tenderness values is no longer necessary (Koohmaraie et al., 1988b, 1989). The objectives of the research reported here were to determine 1) whether electrical stimulation is a necessary step prior to $CaCl_2$ infusion of carcasses and 2) whether $CaCl_2$ infusion could improve tenderness in *Bos indicus* carcasses.

Materials and Methods

Experiment 1. Effect of Electrical Stimulation and/(or) $CaCl_2$ Infusion of Carcasses

Animals. Twenty-four lambs (1/2 Merino × 1/2 Finnsheep, n = 12; 1/2 Finnsheep × 1/4 Dorset × 1/4 Rambouillet, n = 12; male, 8 to 12 mo old, 34 to 50 kg live weight) were slaughtered in groups of four, one lamb for each of the four treatments. Treatments included 1) control, slaughtered according to the normal procedures; 2) infused i.v. immediately after death with .3 *M* $CaCl_2$ at a volume equal to 10% of live weight; 3) electrically stimulated immediately after death (2 Hz; 100 volts, total of 360 pulses, 10 s on, 10 s off; total time = 180 s and 4) electrically stimulated prior to infusion with .3 *M* $CaCl_2$ at a volume equal to 10% of live weight.

Infusion and Sampling. Infusion and sampling were done according to the procedures described by Koohmaraie et al. (1989).

Experiment 2. $CaCl_2$ Injection of Beef Loins

Animals. Twelve 5/8 Brahman × Hereford or Angus (four steers and eight heifers, about 18 mo of age) were slaughtered according to normal procedures. Within 45 min after slaughter, a section of the longissimus muscle 38 cm in length (from first to the sixth lumbar vertebra) from one side was needle-injected with 400 to 500 ml of .3 *M* $CaCl_2$ (five injection sites) using a pumping device (Koohmaraie et al., 1989). The corresponding longissimus muscle section of the other side served as a control. After completion of the injection process, the sides were transferred to a cooler (–1°C). After 24 h, the loins from injected and noninjected sides were removed and cut into steaks 2.54 cm thick; shear force was determined at 1 and 14 d postmortem. After 24 h, samples also were obtained to measure Ca^{2+}-dependent proteases and their inhibitor activities, lysosomal enzyme activities, and calcium content.

Shear Force Determination. Two steaks 2.54 cm thick (fresh, never frozen) were cooked according to AMSA (1978) Guidelines. Steaks were broiled on a Farberware "Open Hearth" broiler[4]. Internal temperature was monitored with iron constantan wire thermocouples attached to a Honeywell 112 Potentiometer. Steaks were turned at 40°C and removed from the broiler at 70°C. Cooked steaks were stored in ventilated polyethylene bags for 24 h at 2 to 4°C before coring. Six 1.27-cm cores were cut such that the fiber direction of the muscle was parallel to the length of the core. Cores were sheared with an Instron 1132/Microcon II Universal Testing Instrument[5] equipped with a Warner-Bratzler type shear blade.

Ca^{2+}-Dependent Proteases and Their Inhibitor

Ca^{2+}-dependent proteases and their inhibitor were extracted and chromatographed; their activities were determined on 100 g of fresh longissimus muscle according to the procedure described by Koohmaraie et al. (1988b). For low calcium-requiring (**CDP-I**) and high calcium-requiring (**CDP-II**) proteases, one unit of activity was defined as the amount of enzyme that catalyzed an increase of 1.0 absorbance unit at 278 nm in 60 min at 25°C. For CDP inhibitor, one unit was defined as the amount of inhibitor that inhibited one unit of CDP-II activity. All activities are reported per 100 g of wet muscle tissue.

Lysosomal Enzymes. Lysosomal proteases were prepared according to the procedure described by Bechet et al. (1986). Briefly, muscle samples were homogenized (Polytron[6], 3 × 15 s, 1/2 speed) in 7 volumes of extraction solution (250 m*M* sucrose, 150 m*M* KCl and 1

[4]Model 450N, Farberware, Bronx, NY.
[5]Instron Corp., Caton, MA.

m*M* EDTA, pH 7.2). The extract was centrifuged at 1,000 × g_{max} for 10 min and than at 4,000 × g_{max} for another 20 min without being removed from the centrifuge. The supernatant was passed through glass wool and centrifuged at 25,000 × g_{max} for 30 min to collect a lysosome-enriched fraction. This fraction then was lysed with 50 m*M* sodium acetate buffer, pH 5.0, containing 1 m*M* EDTA and .2% Triton X-100 and frozen at –20°C for at least 24 h. After thawing, the lysosomal lysate was clarified by centrifugation at 32,000 × g_{max} for 40 min. After measurement of protein content, the activity was determined fluorimetrically according to the procedure of Kirschke et al. (1983). The lysosomal lysate was assayed with N-CBZ-L-Arginyl-L-Arginine 7-amido-4 methylcoumarin[7] (**Z-Arg-Arg-NHMec**) and N-CBZ-L-Phenylalanyl-L-arginine 7-amido-4 methylcoumarin[7] (**Z-Phe-Arg-NHMec**). The activity against Z-Arg-Arg-NHMec was designated as Cathepsin B activity and activity against Z-Phe-Arg-NHMec was designated as B + L activity (Kirschke et al., 1983). Fluorescence was measured in a filter fluorometer[8]. Zero was set with 100 m*M*-chloroacetate buffer, and the reading for .1 μ*M* aminomethylcoumarin[9] in the same buffer was obtained. Activities are expressed as pmoles of product released·min^{-1}·g^{-1} muscle.

Mineral Extraction. Water-extractable calcium was determined according to the procedure described by Nakamura (1973a,b).

Statistical Analysis. Data were analyzed by least squares procedures (SAS, 1985) with $CaCl_2$ injection serving as the whole-plot effect and days postmortem representing a split-plot effect.

Results

Effect of Electrical Stimulation and/(or) $CaCl_2$ Infusion of Carcasses

In our previous experiments, prior to infusion with $CaCl_2$, the carcasses were stimulated electrically (Koohmaraie et al., 1988b, 1989). The main purpose of the electrical stimulation was to deplete ATP so that Ca^{2+}-induced contraction with subsequent toughening would not occur after $CaCl_2$ infusion. Because high-frequency electrical stimulation (i.e., 60 Hz) alone will result in meat tenderization, low-frequency electrical stimulation was used (Takahashi et al., 1984, 1987) to avoid this effect. In this experiment, $CaCl_2$ infusion accelerated postmortem tenderization with and without prior electrical stimulation (shear force at d 1), so we concluded that electrical stimulation is not a necessary step prior to $CaCl_2$ infusion of carcasses (Table 1). Results also indicated that low-frequency electrical stimulation did not cause a reduction in shear force (Table 1). Shear force values 1 d postmortem were 8.9 and 9.7 for control samples and samples given low-frequency electrical stimulation, respectively. Therefore, in the second experiment (i.e., $CaCl_2$ injection of beef carcasses) electrical stimulation was not used. In addition, results indicated that $CaCl_2$ infusion did not affect cooking loss or cooking rate (Table 1).

$CaCl_2$ Injection of Beef Loins. Carcass characteristics of animals used for $CaCl_2$ injection of bovine longissimus muscle are reported in Table 2 and results of $CaCl_2$ injection are reported in Table 3. Consistent with previous observations of $CaCl_2$ infusion of lamb carcasses (Koohmaraie et al., 1988b, 1989), $CaCl_2$ injection of bovine longissimus muscle resulted in 1) significant elevation in longissimus muscle calcium concentration 2) acceleration of postmortem tenderization as determined by shear force on d 1, 3) higher cooking loss at d 1 but not at d 14, 4) significant reductions in the activities of CDP-I, CDP-II and CDP inhibitor, and 5) no effect on the activities of Cathepsins B and B + L. Calcium chloride-injected muscles at d 1 postmortem had shear force values similar to those of noninjected muscles at d 14. The difference between d 1 and d 14 shear force values was 2.8 kg for noninjected muscles vs 1.0 kg for $CaCl_2$-injected samples. $CaCl_2$ injection had no effect on the activities of Cathepsins B and B + L but a clear and definable effect on the activities of CDP-I and CDP-II (loss of activity probably because of autoproteolysis) as noted before (Koohmaraie et al., 1988a,b, 1989).

Discussion

Injected calcium can exert its effect (i.e., acceleration of postmortem tenderization)

[6]Brinkmann Instruments, Westbury, NY.
[7]Bachem, Inc., Torrance, CA.
[8]American Instrument Co., Silver Spring, MD.
[9]Sigma Chemical Co., St. Louis, MO.

TABLE 1. EFFECT OF LOW-FREQUENCY ELECTRICAL STIMULATION ON $CaCl_2$ INFUSION IN OVINE CARCASSES (LEAST SQUARE MEANS)

Treatment[a] (n = 6)	Aging, d	Traits: Shear force, kg	Cooking loss, %	Cooking rate, g/min	Water extractable calcium µg/g
CON	1	8.9	24.6	6.9	16
CON	7	5.4	29	5.8	
CON + $CaCl_2$	1	4.1	26.1	5.9	519
CON + $CaCl_2$	7	3.5	31.4	5.4	
LFES	1	9.7	23.6	5.9	16
LFES	7	6.1	28.1	6.2	
LFES + $CaCl_2$	1	3.4	29.6	6.1	477
LFES + $CaCl_2$	7	3.8	30.7	6.4	
$P > F$ of treatment[b]		.01	.15	.57	.01
$P > F$ of aging		.01	.01	.53	
$P > F$ of interaction of treatment and aging		.01	.73	.49	
SE		.5	1.9	.5	26

[a]Treatment: CON = controls (slaughtered according to the normal procedures); CON + $CaCl_2$ = animals were infused with .3 *M* $CaCl_2$ at 10% of live weight; LFES = low-frequency electrical stimulation (2Hz, 100 volts, total of 360 pulses, 10 s on, 10 s off); LFES + $CaCl_2$ = electrically stimulated, then infused with .3 *M* $CaCl_2$ at 10% of live weight.

[b]Effect of both $CaCl_2$ and LFES.

through various mechanisms. The original experiments (Koohmaraie et al., 1988b) were designed to provide an exogenous source of calcium to activate Ca^{2+}-dependent proteases, and we still believe that calcium probably functions in that way (Koohmaraie et al., 1989). It is important to recognize that CDP-II is remarkably stable under normal postmortem conditions (Vidalenc et al., 1983; Ducastaing et al., 1985; Koohmaraie et al., 1987). Other than $CaCl_2$ infusion (Koohmaraie et al., 1988b, 1989) and $CaCl_2$ injection (Table 3), there are no documented conditions that can affect CDP-II so dramatically. Based on these observations (total loss of CDP-I activity and significant reduction in CDP-II activities), we believe that $CaCl_2$ infusion or injection results in activation of CDP-I and CDP-II, which eventually results in their loss of activities because of autolysis (for review, see Koohmaraie et al., 1989). Both CDP-I and CDP-II undergo autolysis in the presence of calcium,

TABLE 2. BOVINE CARCASS CHARACTERISTICS

Trait	Mean	SD	Min.	Max.
Hot carcass wt, kg	334	46	251	407
Lean color[a]	4.17	.58	3	5
Lean firmness[b]	6.42	.9	5	8
Lean texture[c]	5.92	.9	4	7
Lean maturity[d]	149.2	6.7	140	160
Skeletal maturity[d]	157.5	3.3	140	170
Overall maturity[d]	153.3	2.2	140	165
Marbling[e]	387	60	310	530
Adjusted fat thickness, cm	1.45	.41	.76	2.29
Ribeye area, cm^2	70.9	6.4	62.6	81.3
Estimated kidney, heart and pelvic fat, %	3.29	.78	2	4.5
Yield grade	3.77	.78	2.2	4.9

[a,b,c]Scored: 1 = very dark, soft or coarse through 8 = very light cherry red, very firm or very fine.

[d]Scored: 100 through 199 = A.

[e]Scored: 300 through 399 = slight and 400 through 499 = small.

TABLE 3. EFFECT OF $CaCl_2$ INJECTION OF BOVINE LOINS ON CALCIUM CONTENT, COOKING PARAMETERS AND PROTEASE ACTIVITIES (LEAST SQUARE MEANS)

	Control		Calcium-injected			Probability levels		
Trait	d 1	d 14	d 1	d 14	SE	Treatment	Aging	Interaction
Water-extractable Cal, μg/g	11		1,346		95.8	.01		
Shear force, kg	9.03	6.23	6.09	5.06	.15	.01	.01	.01
Cooking loss, %	19.36	20.47	25.09	22.50	.67	.01	.28	.01
Cooking rate, g/min	9.26	9.33	9.15	8.95	.25	.56	.80	.58
CDP-I[a]	62.1		1.9		3.5	.01		
CDP-II[b]	131.4		34.9		6.8	.01		
CDP inhibitor[c]	166.1		17.3		11.6	.01		
Cathepsin B[d]	24.6		25.2		.8	.56		
Cathepsin B + L[d]	35.8		30.4		1.1	.25		

[a]Low-calcium-requiring Ca^{2+}-dependent protease. Total activity/100 g muscle (caseinolytic assay).

[b]High-calcium-requiring Ca^{2+}-dependent protease. Total activity/100 g muscle (caseinolytic assay).

[c]Total activity/100 g muscle (inhibition of casein hydrolysis by CDP-II).

[d]pmole of product released $\cdot min^{-1} \cdot mg\ protein^{-1}$.

and continued incubation in the presence of calcium results in loss of enzymatic activity (Guroff, 1964; Suzuki et al., 1981a,b; Hathaway et al., 1982; Mellgren et al., 1982; Parkes et al., 1985; DeMartino et al., 1986; Imajoh et al., 1986; Inomata et al., 1986; Crawford et al., 1987). However, the loss of enzymatic activity by autolysis is highly temperature-dependent (Koohmaraie et al., 1989) and reduced significantly in the presence of substrate (DeMartino et al., 1986).

Calcium chloride also can exert its effect by other mechanisms, such as destabilization of muscle proteins. The $CaCl_2$-induced destabilization of proteins appears to be due mainly to the effect of Ca^{2+} ions. Von Hippel and Schleich (1969) have reported that Ca^{2+} is particularly effective in decreasing the stability of the native conformation of fibrillar proteins in water (promoting unfolding) and is also an effective destabilizer of the native conformation of globular proteins. Therefore, it is possible that the $CaCl_2$-induced tenderization could be due to the destabilizing effect of Ca^{2+} ions on proteins. However, based on the information currently available (Koohmaraie et al., 1988a,b, 1989), we suggest that $CaCl_2$-induced tenderization of meat probably is due to activation of Ca^{2+}-dependent proteases, but we cannot rule out other possible mechanisms. Further experimentation will ascertain the accuracy of this hypothesis. The activities of Cathepsins B and B + L probably are not related to tenderization induced by $CaCl_2$ injection or infusion of carcasses.

Implications

Elevation of calcium concentration in the skeletal muscle by infusion of whole carcasses or direct injection into muscle eliminates the requirement for postmortem storage beyond 24 h to ensure meat tenderness.

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