Effects of Postmortem Injection Time, Injection Level, and Concentration of Calcium Chloride on Beef Quality Traits[1]

T. L. Wheeler*, M. Koohmaraie*, J. L. Lansdell†, G. R. Siragusa*, and M. F. Miller†

*Roman L. Hruska U.S. Meat Animal Research Center, ARS, USDA, Clay Center, NE 68933-0166 and †Department of Animal Science, Texas Tech University, Lubbock 79409-2162

ABSTRACT: Two experiments were conducted to determine the optimum protocol for maximizing meat quality with $CaCl_2$ injection. Experiment 1 compared the effects of 30 min or 24 h postmortem injection of 175 m*M* $CaCl_2$ or water at 10% (wt/wt) to controls on various measures of meat quality. An injection of $CaCl_2$ reduced ($P < .05$) shear force values in all three beef muscles evaluated (longissimus, semimembranosus, and triceps brachii). Retail lean color was not affected ($P > .05$) by a $CaCl_2$ injection at 24 h postmortem, but was slightly darker ($P < .05$) for an injection at 30 min postmortem. Psychrophilic and total aerobic microbial counts were higher ($P < .05$) in 30-min than in 24-h treatments and were higher ($P < .05$) in $CaCl_2$- or water-injected semimembranosus muscle than in the control, but the treatment differences were minimal in 24-h injected meat. Experiment 2 compared the effects of 24-h postmortem injection of 200 m*M* or 250 m*M* $CaCl_2$ at either 5 or 10% (wt/wt) to controls on meat quality traits. Both 200 and 250 m*M* $CaCl_2$ reduced ($P < .05$) shear force values compared with the control. In addition, variation in shear force was decreased ($P < .05$) with $CaCl_2$ injection. Beef flavor intensity was slightly lower ($P < .05$) and off-flavor ratings were slightly higher ($P < .05$) in $CaCl_2$-injected meat, but the small differences were of no practical significance. Retail lean color was not affected ($P > .05$) by injection of the $CaCl_2$ at 24 h. Injecting 200 m*M* $CaCl_2$ at 5% (wt/wt) into 24 h postmortem meat can produce consistently tender meat without compromising other palatability or lean quality traits.

Key Words: Beef, Calcium Chloride, Color, Storage Life, Tenderness

J. Anim. Sci. 1993. 71:2965–2974

Introduction

Variability in meat tenderness is a major quality defect of current beef production practices (Morgan et al., 1991b; Smith et al., 1992). Thus, methodology is needed to assure consistently tender meat. It has been demonstrated that infusion or injection of a $CaCl_2$ solution (10% by weight of a 300 m*M* solution) into prerigor meat enhances and accelerates postmortem tenderization (Koohmaraie et al., 1988, 1989, 1990; Koohmaraie and Shackelford, 1991; Morgan et al., 1991a; Wheeler et al., 1991). Maximum tenderization can be accomplished by 1 d postmortem with this process. However, Morgan et al. (1991b) reported the average postmortem aging of retail beef was 17 d and a majority of retail beef cuts reached the retail case between 10 and 30 d postmortem. Wheeler et al. (1992) found that postrigor injection of beef with $CaCl_2$ (10% by weight of a 300 m*M* solution), if aged to 7 d postmortem, resulted in similar tenderization as prerigor injection. Morgan et al. (1991a) reported that 300 m*M* $CaCl_2$ resulted in bitter and metallic off-flavors. However, if the concentration and(or) the injection level of $CaCl_2$ could be reduced, flavor problems might be avoided. Thus, the objective of this research was to determine the minimum concentration and injection level of $CaCl_2$ that improves meat tenderization at 7 d postmortem.

[1]This research was supported partially by the Cattleman's Beef Promotion and Research Board through the Beef Industry Council of the National Live Stock and Meat Board. The authors express their gratitude to P. Ekeren, K. Theer, P. Tammen, C. Smith, K. Mihm, and B. Pacheco for their technical assistance in the execution of these experiments, and to C. Grummert for secretarial assistance. 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 others that may also be suitable.

Received April 8, 1993.

Accepted June 25, 1993.

Materials and Methods

Animals

The Roman L. Hruska U.S. Meat Animal Research Center Animal Care and Use Committee approved the use of the animals in this study. Seven Brahman × MARC III composite (1/4 Hereford, 1/4 Angus, 1/4 Pinzgauer, 1/4 Red Poll) and three 1/2 Charolais × 1/4 Sahiwal × 1/4 Angus steers (Exp. 1), and seven 1/2 Red Poll × 1/4 Nellore × 1/4 Hereford and three 1/2 Charolais × 1/4 Sahiwal × 1/4 Hereford heifers (Exp. 2), weighing approximately 340 kg, were given ad libitum access to a diet formulated to meet NRC requirements for optimum growth of beef cattle (NRC, 1984). The cattle were slaughtered at approximately 475 kg according to standard procedures.

Treatments

Experiment 1. At 30 min postmortem, the longissimus (**LM**), semimembranosus (**SM**), and Triceps brachii (**TB**) muscles were removed from one side of each carcass (alternating sides from carcass to carcass). The LM was removed between the 13th rib and 4th lumbar vertebrae. The LM and SM muscles were cut into three equal sections that were randomly assigned to control, water injected (10%, wt/wt), or $CaCl_2$ injected (10%, by weight of a 175 m*M* solution) within each muscle. The water injected and the water used to make $CaCl_2$ solutions was distilled and deionized, and was 23°C when injected. The TB muscles were cut into two equal sections, which were randomly assigned to control or $CaCl_2$ injected as described above. Muscle sections were injected with a hand stitch pump with four needles inserted into the cut at various locations to ensure even distribution of injected solution. Meat temperature at the time of the 30-min injection was 34°C. After injection, sections were allowed to drip for 5 min, then weighed to determine final percentage injection. After weighing, all injected and control sections were vacuum-packaged and chilled at 2°C until 24 h postmortem. At 24 h postmortem, the same three muscles were removed from the alternate sides, sectioned, and injected (meat temperature 2°C), weighed, and vacuum-packaged as described above. All control and treated sections were stored at 2°C until 7 d postmortem.

Experiment 2. At 24 h postmortem, the LM, SM, and TB muscles were removed from both sides of each carcass. The LM and SM muscles were cut into three sections that were randomly assigned to control, 200 m*M* $CaCl_2$, or 250 m*M* $CaCl_2$, within each muscle. The TB muscles were cut into two sections that were randomly assigned to control or injected with 250 m*M* $CaCl_2$ as described above. The muscles from left and right carcass sides were assigned alternately to injection at either 5 or 10% (wt/wt). After injection, sections were allowed to drip for 5 min, then weighed to determine final percentage injection. After weighing, all injected and control sections were vacuum-packaged and chilled at 2°C until 7 d postmortem.

At 7 d postmortem, the vacuum packages were opened and the drip loss (purge) was weighed. The sections from each muscle were cut into three, 2.54-cm thick steaks. Two of the steaks were frozen (−30°C) at 7 d postmortem. The third steak was placed on a plastic-foam tray, over-wrapped with polyvinyl chloride film, and displayed in a simulated retail case at 2°C under 2,153 lx of cool white fluorescent light continuously for 7 d (Exp. 1) or 5 d (Exp. 2). The distance from the light source to the meat surface was 34 cm. The steaks were evaluated for lean color (1 = bleached red, 4 = cherry red, 8 = very dark red) based on Standards for Beef Color, Research Report 336, New Mexico State University, and percentage of discoloration (1 = 0%, 4 = 20 to 59%, 7 = 100%) according to the procedures of Hunt et al. (1991), after a 3-d display by a five-member, trained panel. At the end of the retail display, the SM (Exp. 1) or LM (Exp. 2) muscles were removed from the packages and sampled for microbial counts.

Microbiological Determinations

The steaks were handled with sterile forceps during sampling for microbial assessment. A rectangular sample was cut from the center of each steak with a sterile scalpel on a sterile polypropylene cutting board. The sample was cut into 1-cm^3 cubes and 25 g was weighed into a stomacher bag. The samples were stomached in 225 mL of peptone buffer for 2 min. Samples were plated at 10^{-1} and 10^{-3} dilutions with a spiral plater on plate count agar (trypticase soy agar). Two plates were incubated at 20°C and two plates were incubated at 35°C for 48 h. Plates were counted and expressed as log colony-forming units (cfu) per gram of muscle.

Shear Force and Sensory Evaluations

Frozen steaks for Warner-Bratzler shear force determinations were stored at −30°C for 7 to 10 d before thawing and cooking. Warner-Bratzler shear force was determined as described previously (Wheeler et al., 1991). Steaks for sensory evaluation were prepared and evaluated as described by Crouse et al. (1989) for juiciness, tenderness, and beef flavor intensity. In Exp. 2, off-flavor was scored on a 4-point scale (1 = intense, 4 = none).

Statistical Analyses

Data were analyzed by ANOVA with the GLM procedure of SAS (1988). Experiment 1 was conducted as a split-plot design. The whole plot was time of injection (prerigor or postrigor) and the split plot was injection treatment (control, 175 m*M* $CaCl_2$, or water). The whole-plot error term was replication ×

Table 1. Least squares means for Warner-Bratzler shear force (kg) of three muscles at 7 days postmortem (Exp. 1)

Item	Longissimus	Semi-membranosus	Triceps brachii
Time[a]			
30 min	7.06	6.34[d]	6.08[d]
24 h	6.16	5.54[e]	4.87[e]
SEM	.17	.24	.20
P-value	.01	.05	.01
Treatment			
Control	7.48	6.47[d]	5.88[d]
$CaCl_2$[b]	5.58	5.56[e]	5.07[e]
Water[c]	6.76	5.79[de]	NM[f]
SEM	.21	.29	.20
P-value	.01	.03	.02
Interaction			
30 min			
Control	7.54[d]	6.58	6.49
$CaCl_2$[b]	5.89[e]	6.39	5.67
Water[c]	7.76[d]	6.06	NM[f]
24 h			
Control	7.44[d]	6.36	5.26
$CaCl_2$[b]	5.27[e]	4.73	4.47
Water[c]	5.77[e]	5.53	NM[f]
SEM	.29	.41	.28
P-value	.01	.21	.96

[a]Postmortem time of injection.
[b]175 m*M* at 10% by weight.
[c]10% by weight.
[d,e]Means in a column, within a main effect or interaction, lacking a common superscript letter differ ($P < .05$).
[f]Not measured.

Table 2. Least squares means for sensory traits of longissimus muscle at 7 days postmortem (Exp. 1)

Item	Tenderness[d]	Juiciness[d]	Beef flavor intensity[d]
Time[a]			
30 min	4.7	6.1	6.5
24 h	5.6	6.3	6.6
SEM	.15	.09	.09
P-value	.08	.25	.26
Treatment			
Control	5.0	6.3	6.6[ef]
$CaCl_2$[b]	5.8	6.3	6.7[e]
Water[c]	4.6	6.1	6.3[f]
SEM	.18	.10	.11
P-value	.01	.28	.03
Interaction			
30 min			
Control	4.9[f]	6.3[e]	6.6
$CaCl_2$[b]	5.4[ef]	6.4[e]	6.7
Water[c]	3.6[g]	5.7[f]	6.1
24 h			
Control	5.1[f]	6.3[e]	6.5
$CaCl_2$[b]	6.1[e]	6.3[e]	6.8
Water[c]	5.6[ef]	6.4[e]	6.5
SEM	.26	.15	.15
P-value	.02	.04	.09

[a]Postmortem time of injection.
[b]175 m*M* at 10% by weight.
[c]10% by weight.
[d]1 = Extremely tough, dry, or bland, 8 = extremely tender, juicy, or intense.
[e,f,g]Means in a column, within a main effect or interaction, lacking a common superscript letter differ ($P < .05$).

time of injection and the split-plot error term was the residual error. Experiment 2 was conducted as a split-plot design. The whole plot was percentage injection (5 or 10%) and the split plot was concentration of $CaCl_2$ (0, 200, or 250 m*M*). The whole-plot error term was replication × percentage of injection and the split-plot error term was the residual error. Means separation for a significant ($P < .05$) main effect was accomplished with the PDIFF option of the least squares procedure (a pairwise *t*-test). Because treatments were not balanced across all muscles, muscles were not statistically compared.

Results

Experiment 1

Shear force was reduced ($P < .05$) in $CaCl_2$-injected LM muscle compared with the control, regardless of the time of injection (Table 1). However, water injection reduced ($P < .05$) shear force of the LM when injected at 24 h postmortem, but not at 30 min. The SM and TB muscles treated at 24 h had lower ($P < .05$) shear force values than those injected at 30 min postmortem. In addition, control SM and TB had the highest ($P < .05$) shear force and calcium injected the lowest ($P < .05$) shear force, regardless of injection time. Water injection did not affect ($P < .05$) shear force of the SM, regardless of injection time.

Sensory tenderness ratings were higher ($P < .05$) for the LM injected with $CaCl_2$ at 24 h than for the control at either time or water injection at 30 min (Table 2). Injection of LM with water at 30 min resulted in the lowest ($P < .05$) tenderness ratings. Juiciness ratings were lowest ($P < .05$) in the LM injected with water at 30 min postmortem. Beef flavor intensity ratings were lower ($P < .05$) in LM injected with water than in $CaCl_2$-injected LM, but were not different ($P > .05$) than the control, regardless of treatment time.

Lean color scores after 3 d of retail display were darkest ($P < .05$) in LM injected with $CaCl_2$ at 30 min and lightest ($P < .05$) in LM injected with water at 30 min (Table 3). Lean color for the 30-min control LM was darker ($P < .05$) than for LM injected with $CaCl_2$ or water at 24 h. Control and $CaCl_2$ injected SM at 30 min had darker ($P < .05$) lean color than all other treatments. The TB muscle injected with $CaCl_2$ was darker ($P < .05$) than the control if treated at 30 min postmortem, but was not different ($P > .05$) when injected at 24 h. Treatment at 24 h did not affect ($P > .05$) lean color scores in any muscle. Percentage of discoloration was not affected ($P > .05$) by injection

Table 3. Least squares means for lean color scores and percentage discoloration after 3 days of retail display for three muscles (Exp. 1)

Item	Longissimus		Semimembranosus		Triceps brachii	
	Color[d]	Discolor.[e]	Color	Discolor.	Color	Discolor.
Time[a]						
30 min	5.4	1.0	6.0	1.2	6.5	1.4
24 h	5.0	1.2	4.8	1.3	5.8	2.2
SEM	.12	.09	.16	.12	.13	.37
P-value	.01	.11	.01	.43	.02	.10
Treatment						
Control	5.5	1.1	5.8	1.2	6.0	1.4
$CaCl_2$[b]	5.8	1.3	5.9	1.3	6.3	2.3
Water[c]	4.3	1.1	4.5	1.3	NM[j]	NM[j]
SEM	.14	.12	.19	.15	.13	.37
P-value	.01	.46	.01	.90	.12	.10
Interaction						
30 min						
Control	5.7[g]	1.0	6.3[f]	1.0	6.0[g]	1.3
$CaCl_2$[b]	6.6[f]	1.0	7.0[f]	1.0	7.0[f]	1.5
Water[c]	3.9[i]	1.1	4.7[g]	1.5	NM[j]	NM[j]
24 h						
Control	5.2[gh]	1.2	5.2[g]	1.4	6.0[g]	1.4
$CaCl_2$[b]	5.0[h]	1.5	4.8[g]	1.6	5.7[g]	3.1
Water[c]	4.7[h]	1.0	4.4[g]	1.0	NM[j]	NM[j]
SEM	.20	.16	.27	.21	.19	.52
P-value	.01	.21	.01	.05	.01	.19

[a]Postmortem time of injection.
[b]175 m*M* at 10% by weight.
[c]10% by weight.
[d]4 = cherry red, 5 = slightly dark red, 6 = moderately dark red, 7 = dark red.
[e]1 = none, 2 = slight (1–19%), 3 = small (20–39%).
[f,g,h,i]Means in a column, within a main effect or interaction, lacking a common superscript letter differ ($P < .05$).
[j]Not measured.

treatments, regardless of time of injection in any muscle.

In Exp. 1, psychrophilic and total aerobic microbial counts after 7 d of retail display were greater ($P < .05$) in meat from the 30-min treatments than in that from the 24-h postmortem treatments (Table 4). In addition, microbial counts were higher ($P < .05$) for meat injected with either $CaCl_2$ or water, but it seems this effect may have been due primarily to the 30-min injection treatment (although the interaction was not significant). Furthermore, the differences in microbial counts between the $CaCl_2$-treated meat at 24 h and control meat were not of practical significance.

Experiment 2

In Exp. 2, psychrophilic and total aerobic microbial counts were not affected ($P < .05$) by the presence, amount, or concentration of $CaCl_2$ (Table 4).

Injection amount (5 vs 10%) did not affect ($P > .05$) shear force in any muscle (Table 5). Shear force was lower ($P < .05$) in LM or SM injected with either concentration of $CaCl_2$ than in the control. In TB muscle with 5% injection, 250 m*M* $CaCl_2$ resulted in lower ($P < .05$) shear force, but for 10% injection there was no difference ($P > .05$) between control and 250 m*M* $CaCl_2$.

The injection amount did not affect ($P > .05$) sensory tenderness or juiciness ratings (Table 6). However, the 5% injection resulted in LM with higher ($P < .05$) beef flavor intensity and less ($P < .05$) off-flavor than the 10% injection. Sensory juiciness ratings of the LM were not affected ($P > .05$) by $CaCl_2$ concentration. However, both 200 and 250 m*M* $CaCl_2$ treatments resulted in higher ($P < .05$) tenderness ratings than the control. Beef flavor intensity was not different ($P > .05$) between control and 200 m*M* $CaCl_2$, but 250 m*M* $CaCl_2$ was lower ($P < .05$) in beef flavor intensity than either control or 200 m*M* $CaCl_2$. Off-flavors increased ($P < .05$) as $CaCl_2$ concentration increased in LM.

Sensory traits of the SM did not vary ($P > .05$) with injection amount. Calcium chloride concentration did not affect ($P > .05$) tenderness or juiciness ratings of SM. Beef flavor intensity decreased ($P < .05$) and off-flavors increased ($P < .05$) as $CaCl_2$ concentration increased in SM. Off-flavors were most often identified as "sour," "bitter," and(or) "salty."

The injection amount did not affect ($P > .05$) any sensory trait of TB muscle (Table 6). Tenderness and juiciness ratings of TB muscle were not affected ($P >$

Table 4. Least squares means for psychrophilic and total aerobic microbial counts after 7 days of retail display

Item	Log cfu/g[a] Psychrophilic count (20°C)	Total aerobic count (35°C)
	Exp. 1	
Time[b]		
30 min	7.76[e]	6.17[e]
24 h	5.99[f]	4.77[f]
SEM	.16	.22
P-value	.01	.01
Treatment		
Control	6.36[f]	4.48[f]
$CaCl_2$[c]	7.04[e]	5.88[e]
Water[d]	7.23[e]	6.04[e]
SEM	.19	.28
P-value	.01	.01
Interaction		
30 min		
Control	7.05	5.07
$CaCl_2$[c]	8.19	6.95
Water[d]	8.04	6.49
24 h		
Control	5.67	3.89
$CaCl_2$[c]	5.87	4.82
Water[d]	6.41	5.59
SEM	.26	.36
P-value	.21	.28
	Exp. 2	
Amount[g]		
5%	5.67	4.62
10%	5.65	4.91
SEM	.08	.09
P-value	.82	.48
Concentration		
Control	5.52	4.55
200 m*M* $CaCl_2$[h]	5.80	4.76
250 m*M* $CaCl_2$[h]	5.65	5.00
SEM	.09	.10
P-value	.11	.12
Interaction		
5%[g]		
Control	5.42	4.18
200 m*M* $CaCl_2$[h]	5.85	4.62
250 m*M* $CaCl_2$[h]	5.73	5.07
10%[g]		
Control	5.63	4.92
200 m*M* $CaCl_2$[h]	5.74	4.90
250 m*M* $CaCl_2$[h]	5.58	4.76
SEM	.13	.14
P-value	.36	.06

[a]Semimembranosus muscle (Exp. 1) and longissimus muscle (Exp. 2).
[b]Postmortem time of injection.
[c]175 m*M* at 10% by weight.
[d]10% by weight.
[e,f]Means in a column, within a main effect, lacking a common superscript letter differ ($P < .05$).
[g]Percentage injection (wt/wt).
[h]Injected at 24 h postmortem.

Table 5. Least squares means for Warner-Bratzler shear force (kg) of three muscles at 7 days postmortem (Exp. 2)

Item	Longissimus	Semimembranosus	Triceps brachii
Amount[a]			
5%	4.56	4.76	4.06
10%	4.45	4.28	4.03
SEM	.14	.18	NM[f]
P-value	.51	.06	.81
Concentration			
Control	5.42[c]	5.06[c]	4.25
200 m*M* $CaCl_2$[b]	4.20[d]	4.10[d]	NM[f]
250 m*M* $CaCl_2$[b]	3.89[d]	4.39[d]	3.83
SEM	.17	.22	NM[f]
P-value	.01	.02	.01
Interaction			
5%[a]			
Control	5.54	5.55	4.41[c]
200 m*M* $CaCl_2$[b]	4.34	4.13	NM
250 m*M* $CaCl_2$[b]	3.80	4.60	3.72[e]
10%[a]			
Control	5.31	4.57	4.10[cd]
200 m*M* $CaCl_2$[b]	4.05	4.07	NM[f]
250 m*M* $CaCl_2$[b]	3.98	4.18	3.96[de]
SEM	.24	.31	.11
P-value	.60	.34	.04

[a]Percentage of injection (wt/wt).
[b]Injected at 24 h postmortem.
[c,d,e]Means in a column, within a main effect or interaction, lacking a common superscript letter differ ($P < .05$).
[f]Not measured.

.05) by $CaCl_2$ concentration. However, 250 m*M* $CaCl_2$ injection resulted in lower ($P < .05$) beef flavor intensity and increased ($P < .05$) off-flavor ratings compared with those of the control TB muscle.

An interaction ($P < .05$) for retail lean color between injection amount and $CaCl_2$ concentration was detected because 200 m*M* $CaCl_2$ injected at 10% gave LM with lighter ($P < .05$) lean color than the control at 10%, but at 5% injection, 200 m*M* $CaCl_2$ was not different ($P > .05$) from the control (Table 7). Lean color of the SM was not affected ($P > .05$) by any treatment. Lean color of TB muscle injected at 10% was darker ($P < .05$) than with 5% injection, and 250 m*M* $CaCl_2$ resulted in lighter ($P < .05$) lean color than control TB muscle. Lean discoloration of LM was not affected ($P > .05$) by treatments. However, in SM, 10% injection resulted in less ($P < .05$) discoloration than 5% injection. In addition, control SM had less ($P < .05$) discoloration than $CaCl_2$-injected SM. An interaction ($P < .05$) between amount and concentration of $CaCl_2$ was detected in discoloration of TB muscle because at 5% there was no difference ($P > .05$) between control and 250 m*M* $CaCl_2$, but at 10%, 250 m*M* $CaCl_2$ injected TB muscle had more ($P < .05$) discoloration than control TB muscle.

The concentration of calcium in the LM tended ($P = .07$) to increase as injection amount increased (Table

Table 6. Least squares means for sensory traits of three muscles at 7 days postmortem (Exp. 2)[a]

Item	Longissimus				Semimembranosus				Triceps brachii			
	Tenderness	Juiciness	Flavor intensity	Off flavor[b]	Tenderness	Juiciness	Flavor intensity	Off flavor	Tenderness	Juiciness	Flavor intensity	Off flavor
Amount[c]												
5%	5.5	5.4	4.5[e]	3.0[e]	4.7	4.9	4.1	2.7	5.5	5.7	4.1	2.7
10%	5.6	5.4	4.2[f]	2.7[f]	4.8	4.9	4.1	2.6	5.6	5.7	4.0	2.6
SEM	.07	.07	.09	.06	.11	.12	.06	.05	.09	.12	.13	.08
P-value	.29	.68	.01	.01	.89	.52	.87	.12	.44	.81	.16	.07
Concentration												
Control	5.1[f]	5.2	4.6[e]	3.1[e]	4.6	4.9	4.4[e]	3.0[e]	5.4	5.6	4.3[e]	2.9[e]
200 m*M* $CaCl_2$[d]	5.6[e]	5.4	4.3[e]	2.8[f]	4.9	4.9	4.1[f]	2.6[f]	NM[h]	NM[h]	NM[h]	NM[h]
250 m*M* $CaCl_2$[d]	5.8[e]	5.5	4.0[f]	2.5[g]	4.7	4.9	3.8[g]	2.4[g]	5.6	5.8	3.7[f]	2.3[f]
SEM	.09	.09	.11	.08	.13	.14	.07	.06	.09	.12	.13	.08
P-value	.01	.15	.01	.01	.56	.88	.01	.01	.07	.12	.01	.01
Interaction												
5%[c]												
Control	5.1	5.3	4.6	3.2	4.5	4.9	4.3	2.9	5.4	5.6	4.2	2.8
200 m*M* $CaCl_2$[d]	5.6	5.4	4.4	2.9	4.8	4.9	4.1	2.7	NM[h]	NM[h]	NM[h]	NM[h]
250 m*M* $CaCl_2$[d]	5.8	5.5	4.3	2.8	4.9	5.1	3.8	2.5	5.6	5.8	3.9	2.5
10%[c]												
Control	5.1	5.2	4.5	3.1	4.7	5.0	4.5	3.0	5.5	5.6	4.2	2.9
200 m*M* $CaCl_2$[d]	5.7	5.4	4.3	2.7	5.0	4.8	4.0	2.5	NM[h]	NM[h]	NM[h]	NM[h]
250 m*M* $CaCl_2$[d]	5.9	5.5	3.7	2.2	4.5	4.7	3.8	2.3	5.6	5.8	3.5	2.2
SEM	.12	.13	.15	.11	.19	.20	.11	.09	.13	.17	.19	.11
P-value	.96	.82	.19	.14	.22	.57	.28	.16	.96	.86	.17	.12

[a]1 = extremely tough, dry, or bland; 8 = extremely tender, juicy, or intense.
[b]1 = intense, 2 = moderate, 3 = slight, 4 = none.
[c]Percentage of injection (wt/wt).
[d]Injected at 24 h postmortem.
[e,f,g]Means in a column, within a main effect or interaction, lacking a common superscript letter differ ($P < .05$).
[h]Not measured.

8). However, both $CaCl_2$ concentrations increased ($P < .05$) calcium content of the LM compared with the control, and 250 m*M* $CaCl_2$ resulted in a higher ($P < .05$) concentration of calcium than 200 m*M* $CaCl_2$. The $CaCl_2$ injection amount did not affect ($P > .05$) cooking loss in any muscle (Table 8). However, injection of both concentrations of $CaCl_2$ resulted in greater ($P < .05$) cooking loss in all muscles than in the control. Drip loss was not affected ($P > .05$) by injection amount in LM (Table 8). Drip loss was greatest ($P < .05$) in LM injected with 200 m*M* $CaCl_2$ and least ($P < .05$) in control LM. In SM, 10% injection had greater ($P < .05$) drip loss than 5% injection. As in LM, 200 m*M* $CaCl_2$ injected SM had the greatest ($P < .05$) drip loss and control SM the least ($P < .05$). The interaction of percentage of injected and concentration of $CaCl_2$ was significant ($P < .05$) for drip loss in TB muscle. The difference in drip loss between control and 250 m*M* $CaCl_2$ was twice as large ($P < .05$) in the 10% injection compared with the 5% injection.

Discussion

The effectiveness of injecting or infusing 300 m*M* $CaCl_2$ into prerigor meat for improving tenderness has been well established (Koohmaraie et al., 1988, 1989, 1990; Morgan et al., 1991a; Wheeler et al., 1991). Tenderness of postrigor meat also has been improved by soaking beef strips obtained at 1 d postmortem in 30 m*M* $CaCl_2$ for 24 h (Alarcon-Rojo and Dransfield, 1989), marinating steaks removed at 5 d postmortem in 150 m*M* $CaCl_2$ for 48 h (Whipple and Koohmaraie, 1993), or injecting LM muscle at 24 h postmortem with 300 m*M* $CaCl_2$ (Wheeler et al., 1992). By injecting at 24 h postmortem and aging until 7 d postmortem, it may be possible to decrease the level of $CaCl_2$ injected.

In Exp. 1, shear force of meat injected with 175 m*M* $CaCl_2$ at 30 min postmortem was not reduced as much compared with the control as has been reported for 300 m*M* $CaCl_2$, but was in agreement with the results of Koohmaraie et al. (1989). In fact, in contrast to the findings of Wheeler et al. (1992), $CaCl_2$ injection at 24 h postmortem resulted in greater reduction in shear force than when $CaCl_2$ was injected at 30 min postmortem. This discrepancy with previous work may be due to the lower concentration of $CaCl_2$ used (175 vs 300 m*M*). Although Exp. 1 confirms that injection of $CaCl_2$ into postrigor meat can be used to improve meat tenderness, the data indicate that 175 m*M* $CaCl_2$ at 10% (wt/wt) may not be a high enough concentration to ensure a consistent improvement in tenderness in all muscles. Even though the meat used in Exp. 2 was relatively tender, injection of 200 or 250 m*M* $CaCl_2$ at either 5 or 10% (wt/wt) reduced shear force of all three muscles. Sensory tenderness ratings were higher for $CaCl_2$-injected meat than for control meat from Exp. 2, but the difference was significant only for the LM muscle. Although the difference in mean shear force or tenderness rating between control and $CaCl_2$-injected meat was not great (because control meat was relatively tender), 60 to 100% (depending on the muscle) of $CaCl_2$-injected meat had shear force values < 5.0 kg in Exp. 1 (using 175 m*M* $CaCl_2$). In Exp. 2, 80 to 100% of the $CaCl_2$ injected meat (200 or 250 m*M* $CaCl_2$) had shear force values < 5.0 kg. In addition, both least squares analysis of the squared deviations from the mean for shear force and a Bartlett's test of homogeneity of variances for shear force indicated that $CaCl_2$ injection reduced the variability in shear force (data not shown). Thus, $CaCl_2$ injection greatly reduces the variation in tenderness and reduces or eliminates the occurrence of tough meat. Furthermore, the data also indicate that meat that is initially tender (< 5.0 kg) will not be over-tenderized by $CaCl_2$ injection.

Sodium chloride has long been used for its positive effects on preservation and flavor of meat. Most consumers consider the salty taste that sodium chloride imparts to meat as favorable. However, other chloride salts (e.g., $CaCl_2$) are not only salty, but also may taste bitter when used at the same levels as sodium chloride. The maximum amount of salt in meat products for desirable taste has been reported to be 2.3% for sodium chloride and .6% for $CaCl_2$ (Wierbicki et al., 1957). These data indicate that the amount of $CaCl_2$ injected in these studies (.05 to .3% in the meat) should not adversely affect meat flavor. Flavor evaluation of $CaCl_2$-injected meat has been conducted by trained descriptive attribute panels and trained flavor profile panels. Morgan et al. (1991a) reported that $CaCl_2$ injection (10% of a 300 m*M* solution) of cow meat resulted in greater flavor intensity ratings than in control cow meat. They also reported that panelists detected a greater percentage of metallic, bitter, and livery flavors in the $CaCl_2$-injected meat. However, Miller et al. (1986) reported that addition of $CaCl_2$ at .5% (wt/wt) as a substitute for sodium chloride in restructured steaks did not affect flavor desirability. St. Angelo et al. (1991) reported that meat from lamb carcasses infused at 10% (wt/wt) with 300 m*M* $CaCl_2$ had slightly increased salty and bitter flavor notes, but that desirable flavor attributes were not affected. However, when ascorbic acid was added to the infusate, the bitter flavor was not detected. Experiment 1 of the present study indicates that flavor was not a problem in meat injected with 175 m*M* $CaCl_2$. In Exp. 2, 200 and 250 m*M* $CaCl_2$ injected at 10% (wt/wt) increased off-flavor ratings. However, meat injected with 200 m*M* $CaCl_2$ at 5% (wt/wt) received similar beef flavor intensity and off-flavor ratings as the control meat. In agreement with our findings, generally little or no effect of $CaCl_2$ infusion or injection on juiciness

Table 7. Least squares means for lean color scores and percentage discoloration after 3 days of retail display for three muscles (Exp. 2)

Item	Longissimus		Semimembranosus		Triceps brachii	
	Color[a]	Discolor.[b]	Color	Discolor.	Color	Discolor.
Amount[c]						
5%	5.2	1.3	4.8	3.6[e]	5.3[f]	2.0
10%	5.2	1.5	5.0	3.0[f]	5.8[e]	2.8
SEM	.06	.10	.10	.18	.11	.17
P-value	.70	.24	.27	.03	.01	.02
Concentration						
Control	5.2	1.1	5.2	2.5[f]	5.7[e]	2.0
200 mM $CaCl_2$[d]	5.1	1.6	4.8	3.8[e]	NM[g]	NM[g]
250 mM $CaCl_2$[d]	5.2	1.5	4.7	3.7[e]	5.3[f]	2.8
SEM	.08	.12	.12	.22	.11	.17
P-value	.57	.12	.37	.01	.03	.01
Interaction						
5%[c]						
Control	5.1[ef]	1.1	4.9	2.6	5.5	1.9[f]
200 mM $CaCl_2$[d]	5.3[e]	1.3	4.9	4.1	NM[g]	NM[g]
250 mM $CaCl_2$[d]	5.3[e]	1.6	4.6	4.2	5.0	2.2[f]
10%[c]						
Control	5.4[e]	1.1	5.4	2.3	5.9	2.1[f]
200 mM $CaCl_2$[d]	4.9[f]	1.9	4.7	3.5	NM[g]	NM[g]
250 mM $CaCl_2$[d]	5.2[ef]	1.4	4.8	3.3	5.6	3.5[e]
SEM	.11	.17	.17	.31	.16	.24
P-value	.02	.10	.15	.66	.50	.05

[a]4 = cherry red, 5 = slightly dark red.
[b]1 = none, 2 = slight (1–19%), 3 = small (20–39%), 4 = modest (40–59%).
[c]Percentage of injection (wt/wt).
[d]Injected at 24 h postmortem.
[e,f]Means in a column, within a main effect or interaction, lacking a common superscript letter differ ($P < .05$).
[g]Not measured.

ratings has been reported in previous work (Morgan et al., 1991a). Thus, 5% (wt/wt) of a 200 mM $CaCl_2$ solution is the optimal amount to ensure tenderness improvement and minimize off-flavor potential. Certainly, the final test will be consumer evaluation of $CaCl_2$-injected meat that is seasoned normally.

Traditionally, lean color has been the main criterion for evaluating freshness of retail meat, and thus is a key component of consumer purchase decisions for retail beef. Maintenance of a bright red oxygenated lean color of fresh retail beef is dependent on several factors and is usually limited to 3 to 5 d of retail display. Salts are pro-oxidants and may cause discoloration when added to fresh meat in amounts as low as .5 to .75% NaCl (Huffman, 1980). Govindarajan (1973) reported that 3.0% NaCl accelerated pigment oxidation in fresh meat. Stewart et al. (1965) reported that 5.0% NaCl inhibited the metmyoglobin reducing activity. However, Miller et al. (1986) reported that $CaCl_2$ at .5 or 1.0% resulted in improved raw color scores and more desirable raw visual appearance of restructured beef steaks compared with the control steaks with no salt. In Exp. 1, retail lean color of $CaCl_2$-injected steaks was not different from controls if injected at 24 h postmortem. Water injection caused a "bleaching out" of pigments resulting in lighter lean color than in controls. Surface discoloration was not affected by 175 mM $CaCl_2$ injection regardless of time of injection. In Exp. 2, $CaCl_2$ injection at 5% (wt/wt) had no effect on lean color or discoloration scores.

It is possible that the insertion of injection needles into the meat during $CaCl_2$ injection could contaminate the meat and increase the microbial load, thus decreasing the shelf-life of the meat. However, it has been reported that blade tenderization of meat (representing an invasion of intact muscle similar to needle injection) did not reduce the microbiological quality or retail case-life of meat (Davis et al., 1977; Leak et al., 1987). Furthermore, Eilers et al. (1992) reported that $CaCl_2$ injection did not affect the microbial load of mature cow meat. The injection of calcium chloride into prerigor meat resulted in greater microbial counts than in that of postrigor meat, but postrigor injection had little effect on microbial counts compared with the control meat. This effect probably resulted from the higher temperature at the time of injection of the meat from the 30-min than from the 24-h injected meat.

The calcium concentration in the meat was increased with the injection of $CaCl_2$ and was greater for 250 mM than for 200 mM $CaCl_2$. In addition, 10% (wt/wt) injection tended to result in greater calcium concentration than 5%. The lack of a greater increase

Table 8. Least squares means for drip loss, cooking loss, and calcium concentration (Exp. 2)

Item	Longissimus			Semimembranosus		Triceps brachii	
	Calcium $\mu g/g$	Drip loss, %	Cooking loss, %	Drip loss, %	Cooking loss, %	Drip loss, %	Cooking loss, %
Amount[a]							
5%	198.1	5.8	31.9	4.7[d]	36.2	2.3	34.0
10%	249.3	5.9	32.9	6.4[c]	37.4	4.6	33.6
SEM	19.2	.28	.59	.35	.65	.24	.85
P-value	.07	.82	.34	.01	.20	.01	.71
Concentration							
Control	34.8[e]	2.0[e]	30.0[d]	3.5[e]	34.4[d]	1.4	32.3[d]
200 m*M* $CaCl_2$[b]	282.3[d]	9.5[c]	33.2[c]	7.7[c]	37.9[c]	NM[f]	NM[f]
250 m*M* $CaCl_2$[b]	353.9[c]	6.0[d]	34.1[c]	5.6[d]	38.2[c]	5.4	35.3[c]
SEM	23.5	.35	.72	.43	.80	.24	.85
P-value	.01	.01	.02	.01	.01	.01	.04
Interaction							
5%[a]							
Control	34.3	1.8	30.0	3.2	34.6	1.0[e]	32.2
200 m*M* $CaCl_2$[b]	267.7	9.9	31.4	6.6	36.9	NM[f]	NM[f]
250 m*M* $CaCl_2$[b]	292.4	5.8	34.4	4.3	37.0	3.6[d]	35.9
10%[a]							
Control	35.3	2.2	30.1	3.7	34.1	1.9[e]	32.5
200 m*M* $CaCl_2$[b]	297.0	9.2	34.9	8.8	38.9	NM[f]	NM[f]
250 m*M* $CaCl_2$[b]	415.5	6.2	33.7	6.8	39.3	7.3[c]	34.7
SEM	33.3	.49	1.02	.61	1.13	.35	1.21
P-value	.19	.42	.12	.29	.40	.01	.56

[a]Percentage of injection (wt/wt).
[b]Injected at 24 h postmortem.
[c,d,e]Means in a column, within a main effect or interaction, lacking a common superscript letter differ ($P < .05$).
[f]Not measured.

in calcium concentration between 5 and 10% injection may have been due to the inability of the meat to absorb the same proportion of the 10% injection as the 5% injection. The increase in calcium concentration due to $CaCl_2$ injection was proportional to previously reported data for injection of 300 m*M* $CaCl_2$ at 10% (Morgan et al., 1991a), and for infusion of 300 m*M* $CaCl_2$ at 10% (Koohmaraie et al., 1989). Injection of 200 m*M* $CaCl_2$ at 5% results in approximately 250 ppm of calcium in the meat, a sevenfold increase over controls. This increase in calcium concentration would provide a much-needed source of dietary calcium for the American consumer (Heaney and Barger-Lux, 1991).

Drip loss during vacuum-packaged storage represents the degree to which the meat was able to retain the added moisture from the injection. Obviously it was much higher for the injected treatments than for the control. The amount of drip loss was not proportional to injection level in all three muscles and the 200 m*M* treatment had greater drip loss than the 250 m*M* treatment. This effect was consistent with the increased water-holding capacity associated with increased salt content (Hamm, 1960; Sherman, 1961). The combined effect of greater drip and cooking loss in the injected treatments than in controls may explain the lack of difference between treatments in juiciness ratings of the cooked steaks. Previous research indicates there is no consistent effect on cooking loss. Wheeler et al. (1991) reported that cooking loss was increased with $CaCl_2$ injection. Koohmaraie et al. (1990) and Wheeler et al. (1992) found variable effects on cooking loss and Morgan et al. (1991a) reported no effect on cooking loss due to $CaCl_2$ injection.

Implications

These data indicate that injection of $CaCl_2$ can be performed on postrigor or prerigor meat to improve meat tenderness. The optimal combination of concentration and level of $CaCl_2$ for 24 h postmortem injection is 200 m*M* and 5% (wt/wt). Injection of 200 m*M* $CaCl_2$ at 5% (wt/wt) can be used to eliminate tough meat without compromising other meat quality traits.

Literature Cited

Alarcon-Rojo, A., and E. Dransfield. 1989. Effect of calcium ions on texture of beef during conditioning. Proc. Int. Congr. Meat Sci. Technol. (Copenhagen) 35:1141.

Crouse, J. D., L. V. Cundiff, R. M. Koch, M. Koohmaraie, and S. C. Seideman. 1989. Comparisons of *Bos indicus* and *Bos taurus* inheritance for carcass beef characteristics and meat palatability. J. Anim. Sci. 67:2661.

Davis, G. W., G. C. Smith, and Z. L. Carpenter. 1977. Effect of blade tenderization on storage life, retail caselife and palatability of beef. J. Food Sci. 42:330.

Eilers, J. D., A. M. Martin, J. B. Morgan, R. K. Miller, F. M. Mendez, D. S. Hale, G. R. Acuff, and J. W. Savell. 1992. Evaluation of calcium chloride and lactic acid injection on chemical, microbiological and descriptive attributes of mature cow beef. J. Anim. Sci. 70(Suppl. 1):115 (Abstr.).

Govindarajan, S. 1973. Fresh meat color. CRC Crit. Rev. Food Tech. 4:117.

Hamm, R. 1960. The biochemistry of meat hydration. Adv. Food Res. 10:356.

Heaney, R. P., and M. J. Barger-Lux. 1991. Food and Nutrition News. Vol. 63. p 2. Natl. Live Stock & Meat Board, Chicago, IL.

Huffman, D. L. 1980. Processing effects on fresh and frozen meat color. Proc. Recip. Meat Conf. 33:4.

Hunt, M. C., J. C. Acton, R. C. Benedict, C. R. Calkins, D. P. Cornforth, L. E. Jeremiah, D. G. Olson, C. P. Salm, J. W. Savell, and S. D. Shivas. 1991. Guidelines for meat color evaluation. Proc. Recip. Meat Conf. 44:3.

Koohmaraie, M., A. S. Babiker, A. L. Schroeder, R. A. Merkel, and T. R. Dutson. 1988. Acceleration of postmortem tenderization in ovine carcasses through activation of Ca^{2+}-dependent proteases. J. Food Sci. 53:1638.

Koohmaraie, M., J. D. Crouse, and H. J. Mersmann. 1989. Acceleration of postmortem tenderization in ovine carcasses through infusion of calcium chloride: Effect of concentration and ionic strength. J. Anim. Sci. 67:934.

Koohmaraie, M., and S. D. Shackelford. 1991. Effect of calcium chloride infusion on the tenderness of lambs fed a β-adrenergic agonist. J. Anim. Sci. 69:2463.

Koohmaraie, M., G. Whipple, and J. D. Crouse. 1990. Acceleration of postmortem tenderization in lamb and Brahman-cross beef carcasses through infusion of calcium chloride. J. Anim. Sci. 68: 1278.

Leak, F. W., J. D. Kemp, J. D. Fox, and B. E. Langlois. 1987. Effects of boning time, mechanical tenderization and partial replacement of sodium chloride on the quality and microflora of boneless dry-cured ham. J. Food Sci. 52:263.

Miller, M. F., G. W. Davis, S. C. Seideman, and C. B. Ramsey. 1986. Effects of chloride salts on appearance, palatability, and storage traits of flaked and formed beef bullock restructured steaks. J. Food Sci. 51:1424.

Morgan, J. B., R. K. Miller, F. M. Mendez, D. S. Hale, and J. W. Savell. 1991a. Using calcium chloride injection to improve tenderness of beef from mature cows. J. Anim. Sci. 69:4469.

Morgan, J. B., J. W. Savell, D. S. Hale, R. K. Miller, D. B. Griffin, H. R. Cross, and S. D. Shackelford. 1991b. National beef tenderness survey. J. Anim. Sci. 69:3274.

NRC. 1984. Nutrient Requirement of Beef Cattle (6th Ed.). National Academy Press, Washington, DC.

St. Angelo, A. J., M. Koohmaraie, K. L. Crippen, and J. Crouse. 1991. Acceleration of tenderization/inhibition of warmed-over flavor by calcium chloride-antioxidant infusion into lamb carcasses. J. Food Sci. 56:359.

SAS. 1988. SAS User's Guide: Statistics. SAS Inst. Inc., Cary, NC.

Sherman, P. 1961. The water binding capacity of pork. I. The influence of sodium chloride, pyrophosphate and polyphosphate on water absorption. Food Technol. 15:79.

Smith, G. C., J. W. Savell, R. P. Clayton, T. G. Field, D. B. Griffin, D. S. Hale, M. F. Miller, T. H. Montgomery, J. B. Morgan, J. D. Tatum, and J. W. Wise. 1992. The final report of the National Beef Quality Audit. Colorado St. Univ., Ft. Collins and Texas A&M Univ., College Station.

Stewart, M. R., B. K. Hutchins, M. W. Zipser, and B. M. Watts. 1965. Enzymatic reduction of metmyoglobin in ground beef. J. Food Sci. 30:487.

Wheeler, T. L., J. D. Crouse, and M. Koohmaraie. 1992. The effect of postmortem time of injection and freezing on the effectiveness of calcium chloride for improving beef tenderness. J. Anim. Sci. 70:3451.

Wheeler, T. L., M. Koohmaraie, and J. D. Crouse. 1991. Effects of calcium chloride injection and hot boning on the tenderness of round muscles. J. Anim. Sci. 69:4871.

Whipple, G., and M. Koohmaraie. 1993. Calcium chloride marination effects on beef steak tenderness and calpain proteolytic activity. Meat Sci. 33:265.

Wierbicki, E., V. R. Cahill, and F. E. Deatherage. 1957. Effects of added sodium chloride, potassium chloride, calcium chloride, magnesium chloride and citric acid on meat shrinkage at 70°C and of added sodium chloride on drip losses after freezing and thawing. Food Technol. 11:74.