INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA

UMI®
800-521-0600

DISSERTATION

POSTMORTEM AND PRE-HARVEST INTERVENTIONS TO IMPROVE THE TENDERNESS AND CONSISTENCY OF BEEF

Submitted by

John A. Scanga

Department of Animal Sciences

In partial fulfillment of the requirements

for the Doctor of Philosophy Degree

Colorado State University

Fort Collins, Colorado

Fall 1999

COLORADO STATE UNIVERSITY

October 29, 1999

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY JOHN A. SCANGA ENTITLED "POSTMORTEM AND PRE-HARVEST INTERVENTIONS TO IMPROVE THE TENDERNESS AND CONSISTENCY OF BEEF" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Gary C. Smith

Advisor

Department Head

ABSTRACT OF DISSERTATION

Postmortem and Pre-Harvest Interventions To Improve the Tenderness And Consistency Of Beef

Because the per capita consumption of beef continues to decline and consumers are consistently dissatisfied with the tenderness and palatability of beef, mechanisms to improve these attributes are imperative to the sustainability of the beef industry. The objectives of this research were to examine the effects of postmortem beef marination and pre-harvest oral supplementation with 7-dehydrocholestrol (vitamin D_3) on cooked beef tenderness. In the first study, 28 USDA Select top sirloin steaks were randomly assigned to a control group (CT) or one of five marination treatments: 1) 150 mM calcium chloride (CA); 2) 10% solution of beef-flavoring/seasoning mixture (FL); 3) CA and FL (CF); 4) 2.5% sodium phosphate and FL (PF); and, 5) tap water (TW). Steaks were marinated in vacuum pouches, aged for 7 days, cooked to 70° C and evaluated by members of a trained sensory panel. Marination with CA, compared to CT or TW, did not affect tenderness ratings, but increased ($P < .05$) bitter and metallic flavors compared to CT or TW treatments. Use of FL, alone or in conjunction with CA or sodium phosphate, increased ($P < .05$) tenderness and juiciness ratings and reduced ($P < .05$) bitterness and metallic flavors compared to CT, CA and TW marinades. The second study included 191 heifers that were assigned (n = 6) to negative controls or supplemented via oral bolus with one of 7 levels of vitamin D_3 (1, 2, 3, 4, 5 million IU D_3/d, 2 million IU D_3/d plus 75 g $CaCO_3$ or 4 million IU D_3/d plus 75 g $CaCO_3$) for 2, 4, 6 or 8 days antemortem. Individual feedlot performance, carcass data and total serum Ca^{2+} concentrations were collected and Warner-Bratzler Shear (WBS) force was

measured at 2, 7, 14 and 21 days postmortem for *longissimus* steaks (8 steaks/carcass) cooked to 70° or 85° C. Supplementation with vitamin D_3 generally decreased daily feed intake (as-fed), and reduced ($P < .05$) average daily gains, compared with controls during the 8 d supplementation period. Supplementation with 1, 2, 3, 4, or 5 million IU D_3/d for at least 2 d increased ($P < .05$) total blood serum Ca^{2+} concentrations compared to those for controls, however, supplementation with any level of vitamin D_3, for any length of time up to 8 d did not improve ($P > .05$) WBS force of steaks cooked to 70 or 85° C at 2, 7, 14, or 21 d of postmortem aging compared with controls. Marination of beef, in vacuum pouches, was an effective method that improved the tenderness of beef. Oral supplementation of cattle with vitamin D_3 (at high or low doses) for 2 to 8 d before harvest increased total blood serum Ca^{2+} concentration, but did not improve cooked beef tenderness.

John A. Scanga
Department of Animal Sciences
Colorado State University
Fort Collins, CO 80523
Fall 1999

ACKNOWLEDGEMENT

It is a well-known fact that the process, trials and tribulations of obtaining any advanced degree are mentally, physically and emotionally taxing. Undoubtedly an experience that would be unbearable without the help, support and training provided by extensive numbers of dedicated and tolerant individuals.

I would like to specifically thank the members of my graduate committee. Dr. Keith Belk who tolerated my training and always pushed me to attain a higher level of knowledge and professionalism. It was a great experience to work with Dr. Belk as a student and I am anxiously anticipating a similar experience as his colleague. Dr. Gary Smith, who with his hard work and dedication, developed the Meat Science Department at CSU to what it is today. I am eternally indebted to him for the opportunities that made my graduate training truly unique and make the program at CSU the finest in the country. Finally, I would like to thank Dr. Daryl Tatum for being the most human person in the field of Meat Science that I have known. Regardless of the situation or issue, he always was direct, honest (sometimes brutally) and trustworthy. Both my wife and I thank him for the wonderful job opportunity.

As much as this degree was an individual effort, it also required the support of the team. The team being all the graduate students (especially the meat heads) and support staff in the Department of Animal Sciences that helped me somewhere,

somehow during my graduate training. I would like to wish all of them good luck and good fortune in their endeavors to come.

Finally, I would like to acknowledge the people that built and sustained CSU and developed this institution and made my university experience a true pleasure. I will unquestionably always be indebted and faithful to CSU. GO RAMS!

DEDICATION

I would like to dedicate my dissertation to several people, all of whom are the most important individuals in my life.

First to my wife Chauna, thank you for your unselfish understanding and sharing of my time with work and your unconditional love. You are my one true love and my passions in life begin with you.

Secondly to my family, without your never-ending love and support this achievement would have been much more difficult and much less satisfying.

To my parents, the financial life-blood of any student, thank you for your support, love and guidance. Above this, thank you for raising me in such a way that I appreciate what each day brings, work hard for what I want and take nothing for granted. Additionally, the opportunities and experiences that I was subjected to as a child and young adult; everyone should be so fortunate as to have what I did. "Thank you" and "I love you" are the only words I can say, but somehow these do not describe the appreciation and gratefulness that I feel, or justifiably express my gratitude for you.

Rebecca and Lisa, I have learned so much and shared so much with the two of you that I cannot begin to list the details of why I am so grateful. The greatest lesson I have taken from both you is that happiness is wealth. Thank you for always being there and I wish you both more happiness and wealth than can be conceived.

TABLE OF CONTENTS

CHAPTER PAGE

SIGNATURE PAGE ii
ABSTRACT OF THESIS iii
ACKNOWLEDGEMENTS v
DEDICATION vii
TABLE OF CONTENTS viii
LIST OF TABLES x
LIST OF FIGURES xi

I. INTRODUCTION 1

II. REVIEW OF THE LITERATURE 3
CALCIUM DEPENDENT PROTEASES 3
"ENHANCEMENT" OF BEEF PRODUCTS 6
VITAMIN D_3 SUPPLEMENTATION 8
JUSTIFICATION OF RESEARCH 11

III. PALATABILITY OF BEEF STEAKS MARINATED WITH SOLUTIONS OF CALCIUM CHLORIDE, PHOSPHATE, AND (OR) BEEF FLAVORING 13
ABSTRACT 13
INTRODUCTION 14
MATERIALS AND METHODS 16
RESULTS AND DISCUSSION 18
CONCLUSIONS 22

CHAPTER PAGE

IV. SUPRANUTRITIONAL ORAL SUPPLEMENTATION WITH VITAMIN D_3 AND CALCIUM TO IMPROVE BEEF TENDERNESS 23

ABSTRACT 23

INTRODUCTION 24

EXPERIMENTAL PROCEDURES 25

RESULTS AND DISCUSSION 29

IMPLICATIONS 38

LITERATURE CITED 40

LIST OF TABLES

TABLE		PAGE
Table 3.1.	Means ± standard deviation for sensory panel ratings for overall juiciness, overall tenderness, muscle fiber tenderness and connective tissue amount..	19
Table 3.2	Means ± standard deviation for sensory panel ratings for bitterness, metallic taste, saltiness, beefy flavor and soapiness..	21
Table 3.3	Means ± standard deviation for percent marinade penetration (uptake), percent weight loss during cooking, final product yield and the pH of the marination solution	22
Table 4.1.	Means for final live weight, average daily gain, dressing percentage, and total serum calcium for cattle supplemented with 0, 1, 2, 3, 4, 5 million IU of vitamin D_3, 2 million IU of vitamin D_3 plus 75g $CaCO_3$, or 4 million IU of vitamin D_3 plus 75g $CaCO_3$, regardless to duration of supplementation prior to slaughter	30
Table 4.2.	Means for initial live cattle weight, final live weight, average daily gain, hot carcass weight, dressing percentage, and USDA marbling score of cattle that received no vitamin D_3, ≤ 10 million IU or > 10 million IU of vitamin D_3, within 8 d of slaughter..	33
Table 4.3.	Least squares means ± standard error for average daily gain (ADG), hot carcass weight (HCW), dressing percentage (DRESS) and Warner-Bratzler shear force (WBS) for steaks aged 2 d and cooked to 70° C from cattle that received 2 or 4 million IU of vitamin D_3/d with or without 75 g of additional calcium carbonate for up to 8 d pre-harvest	34

LIST OF FIGURES

FIGURE PAGE

Figure 2.1. Model of activation of calpains and tenderization.. 4

Figure 4.1 Trend lines of daily feed intake, as fed, for cattle that were supplemented, via oral bolus, with (a) no vitamin D_3, b) 1 million IU/d, (c) 2 million IU of vitamin D_3/d, (d) 3 million IU of vitamin D_3/d, (e) 4 million IU of vitamin D_3/d, (f) 5 million IU of vitamin D_3/d, (g) 2 million IU of vitamin D_3/d plus 75 g $CaCO_3$, and (h) 4 million IU of vitamin D_3/d plus 75 g $CaCO_3$. 31

Figure 4.2. Dose response, over time, for total serum Ca^{2+} for cattle that were supplemented with no vitamin D_3, 1 million IU of vitamin D_3/d, 2 million IU of vitamin D_3/d, 3 million IU of vitamin D_3/d, 4 million IU of vitamin D_3/d, 5 million IU of vitamin D_3/d, 2 million IU of vitamin D_3/d plus 75 g $CaCO_3$ or 4 million IU of vitamin D_3/d plus 75 g $CaCO_3$. 36

Figure 4.3. Formation and hydroxylation of vitamin D_3 from 7-dehydrocholesterol through the metabolic actions of ultra-violet light, liver enzymes and kidney enzymes, and its role in regulating serum calcium levels, calcitonin and parathyroid hormone..... 39

CHAPTER I

Introduction

It is predicted that the 1999 per capita consumption of beef will slip to 29.1 kg, down 26% from 1977 (USDA, 1999). It has been documented that this steady decline in beef's market share is due, in part, to consumers' dissatisfaction with the tenderness, consistency and overall palatability of beef (Moeller and Courington, 1998). Since 1991, the beef industry has intently focused on improving these organoleptic attributes, as they are considered the most important factors affecting consumers' perception of taste (Savell et al., 1987, 1989). In light of these facts, the beef industry, especially the National Cattlemen's Beef Association (NCBA), has directed research funds towards studies focused on improving, predicting and enhancing the tenderness and palatability of beef products in an attempt to regain market share.

In spite of NCBA's research and rhetorical efforts to improve beef's tenderness and palatability, an eight-city audit of retail, beef loin steaks (George et al., 1999) found little evidence of improvement in U.S. beef tenderness since the National Beef Tenderness Survey (Morgan et al., 1991) was conducted. This fact, plus the continued loss of beef's market share, provides incentives for developing new technologies for predicting and enhancing the tenderness and palatability of beef. Among the new technologies are HunterLab's BeefCam System (Wyle et al., 1999) and dietary supplementation of feedlot cattle with supranutritional levels of vitamin D_3 (Swanek et

al., 1999), which have been shown to predict and improve, on average, the overall palatability and tenderness of beef, respectively. Warner-Bratzler shear (WBS) force analysis at 1 or 2 d postmortem (Shackelford et al., 1997) has also been shown to be an effective method for predicting ultimate beef tenderness. The application of calcium-chloride (Kerth et al., 1995), sodium tripolyphosphate, sodium lactate or sodium chloride (Vote et al., 1999; Papadopoulos et al., 1991) via needle injection has also been shown to improve the tenderness and consistency of cooked beef steaks.

All of these technologies influence the tenderness or palatability of beef and could have application in today's consumer driven and brand-focused beef industry. Specifically, a system similar to that described by Tatum et al. (1999), which relied on use of carcass sorting technologies plus several tenderness enhancing practices, could become an integral part of the process of delivering branded beef that is certified and(or) guaranteed to be tender to end-users.

The following research studies describe efforts to examine, as possible palatability intervention technologies, the effectiveness of vacuum-pouch marination with $CaCl_2$, phosphate and/or beef flavoring solutions to enhance the overall palatability of beef-steaks and dietary supplementation of vitamin D_3 to feedlot cattle.

CHAPTER II

Review of the Literature

CALCIUM DEPENDENT PROTEASES

Aging of meat at refrigerated temperatures has been shown to improve both subjective and objective ratings for beef tenderness (Smith et al., 1978; Eilers et al., 1996). The mechanism of this improvement in postmortem tenderness has not been fully defined, but research indicates that the calcium dependent proteases (Dayton et al., 1976; Koohmaraie, 1988; Boehm et al., 1998) and(or) the cathepsins (Moeller et al., 1977; Dutson, 1983; Ouali et al., 1987) are the two proteolytic systems that are most probably involved.

The premise that cathepsins play a substantial role in tenderizing normally handled meat, especially in early postmortem tenderization, has been disproved; if catheptic enzyme tenderization of meat occurs, it is in carcasses stored for 7-10 d postmortem and(or) at temperatures above 15° C (Koohmaraie, et al., 1988a, b; Boehm et al., 1998). The strongest evidence supporting the latter conclusion arises from the findings of SDS-PAGE studies that report no degradation of actin or myosin, both proteins that are susceptible to catheptic proteolysis, in muscle stored at 0° C to 4° C (Olson and Parrish, 1977; Olson et al., 1977; Koohmaraie et al., 1984a,b,c). Because of these findings, attention in this literature review will be directed towards the effects and

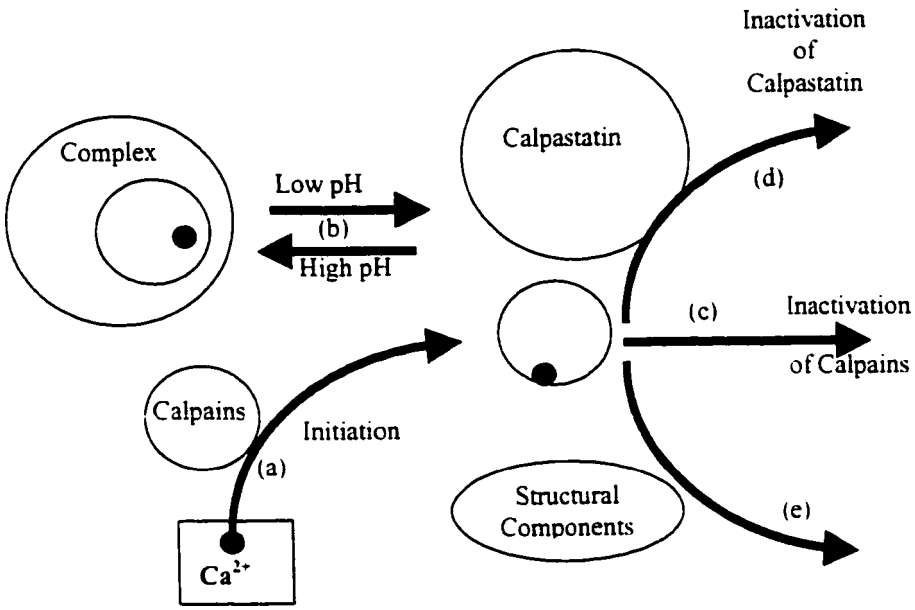


Figure 2.1. Model of activation of calpains and tenderization. Taken from Dransfield, (1993)

Step (a): Initiation. The inert calpains are activated by the rise in calcium ion concentration and enter into the tenderization system.

Step (b) Binding. The equilibrium of the binding of calpains to calpastatin determines the level of free activated-calpains which increases as the pH declines.

Step (c) Inactivation of free activated-calpains. Decay of free activated-calpains by autolysis.

Step (d) Inactivation of calpastatin (it should be noted that the model makes no distinction between proteolysis by calpains of the complexed vs. free calpastatin, but, for clarity, inactivation is shown only for free calpastatin.

Step (e) Tenderization. Proteolysis of structural components by calpains causes tenderization.

mechanisms of calpains (calcium dependent proteases) in tenderizing conventionally stored and handled meat.

Calpains were first described by Davey and Gilbert (1969), who demonstrated that by chelating the calcium ions in muscle with ethylenediamine-tetraacetic acid (EDTA), the weakening of muscle fibers and the disappearance of the Z-

disk could be prevented. Since the publication of these original research findings, studies have indicated that calpains play a major role in postmortem proteolysis, and that 90% of the postmortem proteolysis that occurs during the first 7 to 10 d under refrigerated conditions (0 - 4° C) is due to CDP (Koohmaraie, 1988; Goll et al., 1992 a,b; Taylor et al., 1995a).

Calcium dependent proteases are further classified (as CDP-I and CDP-II) based upon their functionality. Both forms of the enzyme contain a catalytic subunit and a 30,000 dalton subunit that is the same for both enzymes, whereas the catalytic subunits are the products of different genes (Koohmaraie, 1988). There are also no differences in the temperature and pH, for optimum activity, of μ- calpain and m-calpain, but μ-calpain requires a very low level of calcium for 50% activation when compared to m-calpain (Koohmaraie, 1988). There is also a third, very important component of this calcium-dependent system, calpastatin. The mode of action of calpastatin (CDP-inhibitor) is not fully understood, but it is apparent that the binding of calpains and calpastatin requires Ca^{2+} and that the calcium concentration for inhibition differs for μ-calpain vs. M-calpain. The concentration of calcium ions required for inhibition appears to be the same as is needed for catalytic activity of the two respective proteases (Koohmaraie, 1988). The interaction of μ-calpain, m-calpain and calpastatin is diagrammed in Figure 2.1 (Dransfield, 1993). It is important to note that measurement of calpastatin at 24 h postmortem has been shown to be related to ultimate meat tenderness (Koohmaraie et al., 1996a, b).

There has been extensive debate surrounding which of the calpains is responsible for the tenderization of meat during aging. Koohmaraie (1988) concluded that, because

the maximum activity of μ-calpain and m-calpain occurs at 25° C and a pH of 7.5, both conditions that do not exist in postmortem muscle, and because of the low concentration of free calcium in muscle, the majority of postmortem proteolysis was due to the actions of μ-calpain. The free calcium concentration in living muscle would not be expected to rise above 10 μM (Penny, 1980), but the total calcium content in meat is 1500 μM (Dransfield, 1993). The free calcium concentration in meat has been estimated to range from 100 μM (Hattori and Takahashi, 1992) to 500 μM (Penny, 1980), due to the leaking of calcium out of the sarcoplasmic reticulum which occurs in response to depletion of ATP. In addition to the increase of free muscle Ca^{2+} during rigor, Boehm et al. (1998) reported that the activity of m-calpain declines to 83% of at-death activity at 1 d postmortem and to 63% of at-death activity at 7 d postmortem. In comparison, μ-calpain activity declines rapidly to 20% of its at-death activity at 1 d postmortem, and to less than 4% of its at-death activity at 7 d postmortem. Because of these findings, it now seems apparent that a majority of the tenderization that occurs during postmortem aging is a result of the rise in free muscle Ca^{2+} during rigor and the concomitant activation of m-calpain.

This brief review is not intended to be all-encompassing because excellent, comprehensive literature reviews on this subject have been published by Penny (1980), Koohmaraie (1988), Dransfield (1992), Boehm et al. (1998) and Goll et al. (1998).

"Enhancement" of Beef Products

The term "enhancement", (meaning, injecting salt solutions into fresh meat) evolved from commercialization of the process and was so named by pork packers. The

process is now widely used and pork products treated by use of the process are widely accepted by consumers. "Enhancement" improves the palatability and consistency of cooked pork in addition to the profitability as it increases (by 5 to 25%) the weight of the "enhanced" product. It is of current interest to determine potential efficacy of use of this technology for beef.

Injection of beef with solutions containing $CaCl_2$ (Tatum et al., 1999; Morris et al., 1997; Wulf et al., 1996; Miller et al., 1995; Kerth et al., 1995; Lansdell et al., 1995; Eilers et al., 1994;Wheeler et al., 1993; Wheeler et al., 1992); lactic acid (Morris et al., 1997; Eilers et al., 1994); sodium phosphate, sodium chloride and sodium lactate (Vote et al., 1999; Papadopoulos et al., 1991) has been shown to increase both the sensory and mechanical tenderness of cooked products from injected beef cuts. But, while meat tenderness has been increased by injection of such solutions, meat flavor has not always been enhanced; sensory panels have detected off-flavors with the injection with 10% (wt/wt) solutions of 300 mM $CaCl_2$ (Morris et al., 1997; Eilers et al., 1994; Wheeler et al., 1993). Other research studies have shown that injection with lesser amounts of calcium [5% (wt/wt) solutions of 200 mM $CaCl_2$] improved the juiciness, flavor and overall palatability of beef (Lansdell et al., 1995; Miller et al., 1995); so, perhaps, its usefulness depends on the concentration at which it is added to products. The effectiveness of injection, as a tenderness enhancing agent, was studied as an intervention strategy in the Palatability Assurance Critical Control Points (PACCP) model (Tatum et al., 1999). In that study, cuts of beef from carcasses identified to be inferior in tenderness could be subjected to an enhancement procedure and subsequently marketed as a more tender, consistent and palatable end-product.

Although injection has been shown to be an effective system for delivery of a tenderness enhancing agent, USDA-FSIS (1999) recently proposed that the category of meat products in which presence of *E.* coli O157:H7 constitutes adulteration be broadened to include all products that have been injected, mechanically tenderized, chopped, ground or minced. As a result of this USDA-FSIS proposed ruling, alternative, non-invasive mechanisms of improving the tenderness of whole muscle beef products are needed. One possible tenderness-improvement technology that has been proven effective is marination (Whipple and Koohmaraie, 1992a,b). Whipple and Koohmaraie (1992a,b) marinated beef steaks in 150 mM $CaCl_2$ for 48 h, observed lower peak Warner-Bratzler shear (WBS) force measurements; they indicated that this tenderness improvement was due to increased proteolysis resulting from greater calpain activity.

From the reviewed literature, it is apparent that $CaCl_2$, sodium lactate and sodium phosphate are effective enhancers of beef tenderness, whether injected or used as a marinade. Because of the recent proposed federal ruling categorizing injected meat products as adulterated if contaminated with *E. coli* O157:H7, marination of whole muscle beef items would be more beneficial than injection of products from a food safety perspective, as a tenderness-enhancing intervention strategy.

Vitamin D_3 Supplementation

Vitamin D_3 and its role in calcium metabolism, especially in relation to therapy for parturient paresis (milk fever) in dairy cattle, can be traced to the 1940's (Hibbs et al., 1946, from Hibbs and Pounden, 1954). Hibbs and Pounden (1954), in an attempt to

prevent milk fever, concluded that supplementation to prepartum Jersey cows, of 5 to 30 million IU of vitamin D_3 for 3-8 days prepartum, increased blood serum Ca^{2+} concentration compared to that in cows that received no vitamin D_3 supplementation. Since publication of results of this early work, researchers have verified the finding that oral supplementation of vitamin D_3 to cattle increases serum Ca^{2+} concentrations (Swanek et al., 1999; Montgomery et al., 1998; Montgomery et al., 1997).

Vitamin D_3 is inappropriately named. It is not actually (by modern definition) a vitamin because it can be produced endogenously (as 7-dehydrocholesterol) and converted, initially by UV light, into its active metabolite forms, $25(OH)D_3$ and $1,25(OH)_2D_3$ hydroxylase (Dusso and Brown, 1998; Mayes 1996; Haussler and McCain, 1977). In its active form, vitamin D is essential for normal calcium and phosphorus homeostasis, and for the normal development and maintenance of bone (Dusso and Brown, 1998; Mayes 1996). The most biologically active form of vitamin D_3 [$1,25(OH)_2D_3$] is produced from 7-dehydrocholesterol, which is photolytically catalyzed by UV radiation to form $25(OH)D_3$ (Previtamin D_3), which is further hydroxylated in the liver by the mitochondrial enzyme 25-hydroxylase (Dusso and Brown, 1998; Mayes 1996; Haussler and McCain, 1977). The next and most important step is the conversion of $25(OH)D_3$ to the most biologically active form, $1,25(OH)_2D_3$, which occurs in the kidney and is catalyzed by the enzyme 1α-hydroxylase (Dusso and Brown, 1998; Mayes 1996; Haussler and McCain, 1977). Because of the potent effect of this form of vitamin D_3, it is normally found in circulating concentrations between 25 and 70 pg/ml, 1,000 times lower than the levels of its precursor, $25(OH)D_3$ (Dusso and Brown, 1998). The active metabolite is then distributed to tissues through the circulatory system, bound to

either albumin or D binding protein (DBP), to its primary target, the intestine, where it enhances the absorption of calcium. As blood calcium levels increase and return to normal, $1,25(OH)_2D_3$ acts via a negative feedback mechanism to down-regulate parathyroid hormone (PTH) production and to reduce the activity of 1α-hydroxylase in the kidney and to increase the activity of 24-hydroxylase, the primary enzyme involved in $1,25(OH)_2D_3$ degradation (Dusso and Brown, 1998; Haussler and McCain, 1977). The levels of circulating blood calcium and this regulatory system are stringently controlled to aid in the preservation of skeletal integrity and facilitate calcium homeostasis (Dusso and Brown, 1998; Haussler and McCain, 1977).

Using results of previous research studies describing relationships of muscle calcium concentration and the activation of the CDP, Swanek et al. (1999), Ducket et al. (1998), and Montgomery et al. (1997) hypothesized that by increasing serum Ca^{2+} levels in live animals, subsequent Ca^{2+} levels in postmortem muscle also would be increased, and that higher intramuscular concentrations of Ca^{2+} would increase the activity of calpains and improve the tenderness of postmortem meat. This hypothesis was tested by Montgomery et al. (1997), who concluded that oral bolusing of cattle with 5 or 7.5 million IU of vitamin D_3 for 8 d before slaughter reduced peak WBS force values for 14 d postmortem muscle compared to that for controls. The same hypothesis also was investigated by Swanek et al. (1999) who found that feeding vitamin D_3 for 7 or 10 d at levels of 5 or 7.5 million IU/d, respectively, also reduced peak WBS force values for 7 d postmortem muscle compared to that for controls. Unfortunately though, in addition to improving tenderness, dietary supplementation with vitamin D_3 also has been reported to reduce daily feed intake of cattle fed 2.5 to 5 million IU/d for 10 d before slaughter

(Montgomery et al., 1998). Additional research conducted by Karges et al. (1998) indicated that feeding cattle 5, 7.5, 15 or 75 million IU of vitamin D_3 reduced dry matter intake after 6, 5, 4 or 2 d of supplementation, respectively. Although early reports are promising, there are issues that must be addressed with the use of vitamin D_3 before recommendations can be made for widespread industry implementation of this tenderization technique. One such remaining obstacle is lack of approval for supranutritional use of vitamin D_3 in food animals by the U.S. Food and Drug Administration. A second obstacle is the effect of vitamin D_3 supplementation on the performance of feedlot cattle. Even if FDA approves use of vitamin D_3 supplementation for beef tenderization purposes, it is not likely to be used commercially if it has deleterious effects on feedlot cattle performance.

Justification of Research

Scientists have concluded, through research, that by increasing the available calcium concentration in meat, it is possible to increase the activity of the calpain system and subsequently increase the tenderness of postmortem beef. As per capita consumption of beef continues to decline and consumers continue to be dissatisfied with the average quality and consistency beef products, the need for technology to enhance meat tenderness and total palatability management increases. The success of "enhancing" fresh meat is evident in the pork retail case and it does not seem far-fetched to envision similar success if used with beef items. The addition of management practices that could improve tenderness; i.e., feeding vitamin D_3, could be integrated into a total quality/palatability management system, or used to establish a brand identity for a tenderized beef product. The use of these technologies and their potential application by

the beef industry remain to be determined. To expedite the implementation of these technologies, each must first be proven to be effective through sound and repeated science.

CHAPTER III

Palatability of Beef Steaks Marinated with Solutions of Calcium Chloride, Phosphate, and (or) Beef Flavoring

ABSTRACT

The objectives of this study were to evaluate the efficacy of marination for increasing consumer acceptability of beef. Steaks from 28 USDA Select top sirloin butts were randomly assigned to one of six marination treatments: 1) negative control (CT), 2) 150 mM calcium chloride (CA), 3) 10% solution of beef-flavoring/seasoning mixture (FL), 4) CA and FL (CF), 5) 2.5% sodium phosphate and FL (PF), or 6) tap water (TW). Steaks were marinated in vacuum pouches, aged for 7 days, cooked to 70° C and evaluated by a trained sensory panel. Marination with CA did not affect tenderness ratings, but increased ($P < .05$) bitter and metallic flavors compared to CT or TW treatments. Use of FL, alone or in conjunction with CA or sodium phosphate, increased ($P < .05$) tenderness and juiciness ratings and reduced ($P < .05$) bitterness and metallic flavors compared to CT, CA and TW marinades. Marination of beef, in vacuum pouches, is an effective method the palatability and yields of cooked beef steaks.

Key Words: Beef, Tenderness, Calcium Chloride, Phosphate, Marination,

INTRODUCTION

To overcome the problems associated with tough beef, researchers have investigated technologies that could be used to improve tenderness, reduce tenderness variability and increase consumer's satisfaction with beef. The benefits of using chemical meat-enhancing agents (phosphate, $CaCl_2$ and papain) have been well documented (Wang et al., 1957; Kerth et al., 1995; Morris et al., 1997) and the success of "enhanced" (injected with salt solutions) retail products, especially pork, is apparent in the U.S. meat industry. In addition to enhancing the flavor, tenderness and consumer acceptance of retail meat products, the ability to produce and sell value-added and water-added retail beef provides the beef industry access to a large and growing marketing opportunity.

Sodium phosphate is commonly used in meat processing and has been documented to increase protein solubility and the water-binding ability of meat (Hellendoorn, 1962; Trout and Schmidt, 1986). Smith et al. (1984) concluded that injection of brine containing sodium tripolyphosphate into pork longissimus increased juiciness and reduced Warner-Bratzler shear force values, and that injection of such a solution, when used with beef semimembranosus increased juiciness. Addition of sodium phosphate (as either pyrophosphate or hexametaphosphate) also has been reported to prevent rigor mortis and increase tenderness of freshly slaughtered beef (Streitel et al., 1977).

Calcium chloride injection into beef has been identified as a means for increasing beef tenderness (Kerth et al., 1995; Wheeler et al., 1991; Whipple and Koohmaraie, 1992a,b). However, a 10% injection of 0.3 M calcium chloride into beef has been shown

to have an adverse effect on palatability, imparting a bitter, metallic and sour taste to the cooked product (Eilers et al., 1994; Morris et al., 1997). To compensate for the undesirable flavor characteristics associated with the use of calcium chloride, flavoring agents--used in conjunction with calcium chloride injection--have been shown to mask the off-flavors (Morris et al., 1997).

Benefits of marinating (Whipple and Koohmaraie, 1992a,b) or injecting (Kerth et al., 1995; Koohmaraie et al., 1990; Morgan et al., 1991a) beef cuts from 30 min to 5 d postmortem with calcium chloride to increase tenderness by enhancing calcium-activated proteolysis are well documented. However, implementation of this technology has not progressed rapidly, probably due to the compromised flavor associated with its use (Eilers et al., 1994; Morris et al., 1997) and because of the recent USDA-FSIS proposed rule that would classify injected, mechanically tenderized, chopped, ground or minced product that is contaminated with *E. coli* O157:H7 as "adulterated" (USDA-FSIS, 1999). In the present study, a solution of beef-flavoring/seasoning mixture was added to solutions containing either phosphate or calcium chloride to determine: 1) if marination of beef retail cuts can be used as an effective mechanism to apply chemical enhancing agents, 2) if phosphate solutions can be used to increase the tenderness, juiciness and overall palatability of cooked beef steaks, and 3) if off-flavors associated with the use of a calcium chloride solution (as a marinade) can be masked using a flavoring/seasoning agent.

MATERIALS AND METHODS

Product Selection

Top sirloin butts were obtained 24 h postmortem from the right side of USDA Select beef carcasses (n = 28) in a commercial beef packing facility. Vacuum-packaged top sirloin butts were transported to the Colorado State University Meat Laboratory and fabricated into six steaks (2.54 cm thick) from the *gluteus medius* (excluding end cuts). Steaks then were trimmed to yield portions weighing 170 ± 3 g.

Treatments

One steak from each top sirloin butt was assigned randomly to either a control (no marinade) or one of five marination-treatment groups: 1) calcium chloride, 2) flavoring/seasoning mixture, 3) calcium chloride and beef-flavoring/seasoning mixture, 4) sodium phosphate and beef-flavoring/seasoning mixture, and, 5) tap water. All solution volumes were added to the steaks to equal 25% of raw cut weight (25% wt/wt). Calcium chloride marination **(CA)** was applied via a pH 7.26 solution of 150 mM food grade calcium chloride (Spectrum, Gardena, CA). The flavoring/seasoning marinade **(FL)** treatment was applied via a 10% solution of a pH 5.29 flavoring/seasoning agent (Williams Seasoning, Inc., Product No. B01144, Lenexa, KS) that contained 48% salt, 24% hydrolyzed soy protein, 15% maltodextrin, 7% dried beef stock and 6% spices and flavoring. The combined calcium chloride and beef flavor/seasoning marinade **(CF)** contained equal volumes of 150 mM calcium chloride and 10% beef-flavoring/seasoning mixture solutions, pH 5.07; the sodium phosphate and beef flavor/seasoning marinade **(PF)** contained equal parts of 2.5% sodium phosphate (BRIFISOL® 85 Instant, BK-Ladenburg Corp., Lodi, CA) and 10% beef-flavoring/seasoning mixture solutions, pH

6.79. All solutions were applied to the steaks in vacuum pouches to determine if the effect of chemical additives could be observed without using an invasive, surface penetrating process (i.e., needle injection).

Packaging and Cooking

Steaks were individually weighed and vacuum packaged with nothing (control) or with the appropriate marination treatment solution in 20.3 x 25.4 cm vacuum bags (.75 mil nylon and 2.25 mil polyethylene bag) with an oxygen transmission rate of 3.5 $cm^3 \cdot (.065\ m^2)^{-1} \cdot (24\ h)^{-1}$ at 21°C (Koch Supplies Inc., Kansas City, MO), aged for 7 d at 2°C, frozen and stored at -28.6°C. Steaks were then thawed for 24 h at 2°C, weighed and cooked on an electric char-broiler (Model 0B51, Hobart Corporation, Troy, OH). Each steak was turned during cooking every 4 min and the temperature was monitored during cooking using a digital probe thermometer (Atkins Technical Inc., Gainesville, FL) until a final internal temperature of 70°C was reached. Cooked steaks were then reweighed to determine weight loss during cooking.

Sensory Evaluation

Warm samples were evaluated by members of a trained (Cross et al., 1978) eight-member sensory panel, for juiciness, muscle fiber tenderness, connective tissue amount and overall tenderness using 8-point rating scales (8 = extremely juicy, extremely tender, none or extremely tender; 1 = extremely dry, extremely tough, abundant or extremely tough, respectively). Additionally, flavors (metallic, salty, bitter, beefy and soapy) were evaluated on a 3-point scale (0 = none detectable, 1 = slightly detectable and 2 = very strong) by the same trained sensory panel following AMSA (1995) guidelines. Panelists were trained to detect flavors using the procedures of Meilgaard et al. (1991).

Statistical Analysis

Individual panelist ratings for each organoleptic trait were averaged to determine the sensory ratings of each organoleptic trait for each steak. Random effects analysis of variance was conducted using the general linear models procedures of SAS (1988) for a split-plot design which included the random effect of top sirloin butt. Data were analyzed using a model that included the mean sensory rating as the dependent variate, the independent whole plot of top sirloin butt and the fixed, independent effects for marination treatment (CT, CA, FL, CF, PF and TW) within subprimal as the split plot. When appropriate, means were separated using Tukey's studentized range test.

RESULTS AND DISCUSSION

Sensory Characteristics

Benefits of marinating (Whipple and Koohmaraie, 1992a,b) or injecting (Koohmaraie *et al.*, 1990; Morgan *et al.*, 1991a; Kerth et al., 1995) beef cuts with calcium chloride to increase tenderness through enhanced calcium-activated proteolysis have been well documented; yet, due to compromised flavor (St. Angelo *et al.*, 1991; Eilers *et al.*, 1994), practical implementation of this technology has not progressed rapidly.

Steaks marinated with beef-flavoring, either solely or in combination with $CaCl_2$ or phosphate, were rated (Table 3.1) by panelists as being juicier and more tender (for both overall tenderness and muscle fiber tenderness) and as having less detectable connective tissue ($P < .05$) than steaks from the two control groups (no marinade or tap water) and than steaks from the treatment that included only $CaCl_2$. These results are

contradictory to those reported by Whipple and Koohmaraie (1992a,b) who found that tenderness was increased when steaks were marinated in $CaCl_2$ for 2 or 5 days. Among steaks treated with marinades that included a beef-flavoring component, there were no differences ($P < .05$) in juiciness, overall tenderness, muscle fiber tenderness or connective tissue content (Table 3.1).

Table 3.1. Means ± standard deviation for sensory panel ratings of individual steaks (N = 168) for overall juiciness[a], overall tenderness[b], muscle fiber tenderness[c] and connective tissue amount[d]

Treatment	Juiciness	Overall Tenderness	Muscle Fiber Tenderness	Connective Tissue Amount
No marinade (control)	4.64 ± .51[z]	4.34 ± .84[z]	4.74 ± .73[z]	4.32 ± .85[z]
25%[e] 150 mM $CaCl_2$	4.95 ± .73[z]	4.68 ± .86[z]	4.98 ± .78[z]	4.65 ± .88[z]
25% 10% beef-flavoring	5.56 ± .64[y]	5.24 ± .75[y]	5.53 ± .69[y]	5.12 ± .79[y]
25% $CaCl_2$ and beef-flavoring	5.52 ± .50[y]	5.23 ± .68[y]	5.53 ± .55[y]	5.17 ± .72[y]
25 % phosphate and beef-flavoring	5.44 ± .61[y]	5.41 ± .83[y]	5.74 ± .68[y]	5.37 ± .76[y]
25% tap water control	4.85 ± .53[z]	4.37 ± .87[z]	4.68 ± .77[z]	4.41 ± .89[z]

[a] Overall juiciness was scored on an 8-point scale, 1 = extremely dry, 8 = extremely juicy.
[b] Overall tenderness was scored on an 8-point scale, 1 = extremely tough, 8 = extremely tender.
[c] Muscle fiber tenderness was scored on an 8-point scale, 1 = extremely tough, 8 = extremely tender.
[d] Connective tissue amount was scored on an 8-point scale, 1 = abundant, 8 = none.
[e] All solutions were added to pouches containing the steaks to equal 25% of the weight of the raw product (25% wt/wt).
[y,z] Means in the same column, lacking common superscript letters, differ ($P < .05$).

Trained panelist flavor scores indicated that steaks marinated with $CaCl_2$ had the most ($P < .05$) bitter and metallic off-flavors compared to steaks in the two control groups and in all other marinade treatments, whereas steaks marinated in solutions that contained beef-flavoring, either solely or in combination with other additives, were less bitter ($P < .05$) and less metallic ($P < .05$) than non-marinated steaks and steaks marinated in tap water (Table 3.2). The addition of a beef-flavoring agent masked the bitter and metallic

flavors that were detected in steaks marinated with 150 mM $CaCl_2$. Additionally, steaks marinated with solutions that contained a beef-flavoring agent also were described as having a beefier ($P < .05$) flavor than steaks from all other marinade treatments (Table 3.2). Although the addition of beef-flavoring to the marinade reduced the bitter and metallic off-flavors, not surprisingly, it also dramatically increased ($P < .05$) the saltiness of the cooked, marinated steaks compared to steaks from the two control (no marinade or tap water) groups and steaks marinated with $CaCl_2$. Mean panelist ratings for the presence of a soapy off-flavor in the cooked marinated steaks were below 1.0 for all marinade treatments, and the steaks marinated in solutions that contained a beef-flavoring agent were rated as being less soapy ($P < .05$) than steaks in the non-marinated control group (Table 3.2).

Steak Yields

Steaks were weighed before marination, before cooking and following cooking to determine the uptake of marinade into the raw steak during marination and the weight lost during cooking. Steaks marinated in solutions of higher pH, with strong buffering capacities, should have increased water binding ability compared to those steaks that were marinated in solutions with a pH close to, or below, the isoelectric point of meat. In this study (Table 3.3), steaks marinated in a solution of high pH that contained phosphate and beef flavoring absorbed more ($P < .05$) marinade solution than control steaks or steaks marinated with $CaCl_2$ alone or beef-flavoring alone (but not than steaks marinated with both $CaCl_2$ and beef-flavoring). Control steaks (no marinade or tap water) had the greatest ($P < .05$) weight loss from marination/packaging to cooking. Steaks in the no-marinade control group and those that were marinated in a solution that contained

phosphate and beef flavoring had lower ($P < .05$) weight loss during cooking than steaks in all other treatments and steaks in the tap water control group. Because of the loss of water during marination, the control (no marinade or tap water) steak weight loss during cooking was similar ($P > .05$) to that sustained in all other marination treatments, but control (no marinade or tap water) steak final yields were lower ($P < .05$) than final yields for steaks marinated in solutions of beef-flavoring, either solely or in combination with $CaCl_2$ or phosphate (Table 3.3).

Table 3.2. Means ± standard deviation for sensory panel ratings of individual steaks (N = 168) for bitterness[a], metallic taste[b], saltiness[c], beefy flavor[d] and soapiness[e]

Treatment	Bitter	Metallic	Salty	Beefy	Soapy
No marinade (Control)	.57 ± .21[x]	.27 ± .14[x]	.05 ± .09[z]	.41 ± .16[z]	.08 ± .13[w]
25%[f] 150 mM $CaCl_2$	.74 ± .28[x]	.49 ± .21[x]	.05 ± .08[z]	.40 ± .22[z]	.06 ± .09[wx]
25% 10% beef-flavoring	.23 ± .15[z]	.12 ± .12[z]	1.15 ± .22[y]	.84 ± .24[y]	.02 ± .05[xy]
25% $CaCl_2$ and beef-flavoring	.33 ± .20[z]	.10 ± .11[z]	1.27 ± .26[w]	.77 ± .23[y]	.00 ± .02[yz]
25 % Phosphate and beef-flavoring	.31 ± .14[z]	.06 ± .08[z]	1.35 ± .30[x]	.82 ± .18[y]	.00 ± .00[z]
25% tap water	.52 ± .22[x]	.28 ± .18[x]	.00 ± .02[z]	.41 ± .18[z]	.06 ± .08[wxy]

[a] Bitterness was scored on an 3-point scale, 0 = not bitter, 2 = extremely bitter.
[b] Metallic taste was scored on an 3-point scale, 0 = not metallic, 2 = extremely metallic.
[c] Saltiness was scored on an 3-point scale, 0 = not salty, 2 = extremely salty.
[d] Beefy flavor was scored on an 3-point scale, 0 = extremely weak, 2 = extremely beefy.
[e] Soapiness was scored on an 3-point scale, 0 = not soapy, 2 = extremely soapy.
[f] All solutions were added to pouches containing the steaks to equal 25% of the weight of the raw product (25% wt wt).
[w,x,y,z] Means in the same column, lacking common superscript letters, differ ($P < .05$).

CONCLUSIONS

This study indicated that the use of marination effectively enhanced the palatability characteristics of beef steaks, especially when marinade solutions contained a beef-flavoring additive. In addition to improving perceived tenderness, marinades that incorporated a beef-flavoring agent also reduced off-flavors and increased cooked product yields. Marination of beef steaks, especially with solutions containing a beef-flavoring agent, is an effective and practical means to improve cooked beef palatability.

Table 3.3. Means ± standard deviation of individual steaks (N = 168) for percent marinade penetration (uptake)[a], percent weight loss[b] during cooking, final product yield[c] and the pH of the marination solution

Treatment	Marinade Uptake (%)	Weight loss during cooking (%)	Final Yield (%)	Marination Solution, pH
No marinade (Control)	-4.7 ± 1.7^{z}	24.9 ± 4.0^{yz}	71.6 ± 4.0^{z}	-
25%[d] 150 mM $CaCl_2$	-1.7 ± 2.2^{y}	27.0 ± 3.9^{y}	71.8 ± 4.4^{z}	7.26
25% 10% beef-flavoring	2.8 ± 2.1^{x}	25.3 ± 4.4^{y}	76.7 ± 4.5^{y}	5.29
25% $CaCl_2$ and beef-flavoring	3.8 ± 1.6^{wx}	25.8 ± 4.2^{y}	77.1 ± 4.8^{y}	5.07
25 % phosphate and beef-flavoring	4.6 ± 3.1^{w}	22.3 ± 4.5^{z}	81.2 ± 4.1^{x}	6.79
25% tap water	-3.5 ± 3.5^{z}	27.4 ± 4.7^{y}	70.0 ± 6.2^{z}	-

[a] Percent marinade uptake was calculated using weights taken prior to marination and before cooking.
[b] Percent weight loss was calculated using weights taken immediately prior to and following cooking.
[c] Percent final yield was calculated using weights taken prior to marination and immediately following cooking.
[d] All solutions were added to pouches containing the steaks to equal 25% the weight of the raw product (25% wt/wt).
[w,x,y,z] Means in the same column, lacking a common superscript letter, differ ($P < .05$).

CHAPTER IV

Supranutritional Oral Supplementation with Irradiated 7-Dehydrocholestrol and Calcium to Improve Beef Tenderness

ABSTRACT

Intrinsic, calcium dependent proteases deteriorate the ultrastructure of muscle early postmortem, and increase tenderness. Due to the calcium dependent nature of calpains, it has been hypothesized that oral supplementation with vitamin D_3 will increase both muscle Ca^{2+} content and the activity of muscle proteases, and thus, the tenderness of cooked beef. Individual heifers (n = 191) were assigned to a negative control group or groups supplemented via oral bolus with one of 7 levels of vitamin D_3 (1, 2, 3, 4, 5 million IU D_3/d, 2 million IU D_3/d plus 75 g $CaCO_3$ or 4 million IU D_3/d plus 75 g $CaCO_3$) for 2, 4, 6 or 8 days antemortem. Individual feedlot performance, serum Ca^{2+} levels and carcass data were collected and 8 steaks/carcass were used to obtain Warner-Bratzler shear (WBS) force was values measured at 2, 7, 14 and 21 days postmortem for *longissimus* steaks cooked to 70° or 85° C. Supplementation with vitamin D_3 generally decreased daily feed intake (as-fed), and reduced ($P < .05$) average daily gains compared to controls during the 8 d supplementation period. Additionally, supplemented cattle had numerically higher dressing percentages, indicating less fill at the time of slaughter, as

carcass weights and USDA yield grades did not differ ($P > .05$) across treatment groups. Supplementation with 1, 2, 3, 4, or 5 million IU D_3/d, for at least 2 d, increased ($P < .05$) total blood serum Ca^{2+} concentrations compared with controls, while cattle that received additional dietary Ca^{2+}, in the form of $CaCO_3$ had the lowest blood serum Ca^{2+} concentration. Although blood serum Ca^{2+} was increased, supplementation with any level of vitamin D_3 for any length of time up to 8 d, did not improve ($P > .05$) WBS force at 2, 7, 14, or 21 d of postmortem aging compared to controls when steaks were cooked to final internal temperatures of either 70° or 85° C. Results indicated that oral supplementation with vitamin D_3 (at high or low doses) for 2 to 8 d before slaughter increased total blood serum Ca^{2+} concentration, but oes not improve cooked longissimus tenderness.

Key Words: Vitamin D_3, Beef, Tenderness, Calcium, Calpains

INTRODUCTION

The Beef Industry Long Range Plan Task Force (NCBA, 1999) concluded that consumer demand for beef products could be increased by enhancing the quality and consistency, especially tenderness of retail beef products. Also, the National Beef Tenderness Survey concluded that toughness was a problem among a variety of retail beef cuts (Morgan et al., 1991b). George et al. (1999) reported findings similar to those of the 1991 National Beef Tenderness survey and concluded that the beef industry has made little progress in improving retail beef tenderness, especially for beef products from carcasses of USDA Select and low Choice grades.

In order to slow the decline in beef's market share and per capita consumption of beef, researchers have aggressively sought methods to predict and improve the tenderness of beef. Supplementation of cattle with supranutritional levels of vitamin D_3 immediately pre-harvest is one method that has been explored. Montgomery et al. (1997) reported that steers, orally bolused with 5 and 7.5 million IU D_3/d for 8 days before slaughter, had greater concentrations of plasma calcium and produced steaks with lower Warner-Bratzler shear values than did untreated control cattle. More recently, Swanek et al. (1999) reported that cooked beef tenderness was improved through dietary supplementation of cattle with vitamin D_3 at a rate of 5 million IU/d for 7 days or of 7.5 million IU/d for 10 d.

The objectives of this study were to 1) evaluate the effects of dietary vitamin D_3 supplementation on feedlot performance and blood calcium levels, 2) to determine the effect of vitamin D_3 on postmortem tenderness, and 3) to develop a recommendation for the optimum dose level and duration of administration of vitamin D_3 for purposes of improving tenderness of cooked beef.

EXPERIMENTAL PROCEEDURES

In order to minimize breed to breed variability, 192 Charolais x Hereford heifers from a single source and of similar size were selected from a commercial feedlot in eastern Colorado. Following selection, cattle were shipped to Colorado State University's Beef Nutrition Unit and allowed to acclimate to feedlot conditions for 5 days. Cattle were acclimated to the finishing ration and returned to full-feed following the five-day period, at which time cattle were weighed, tagged and randomly assigned to

fill the cells of an 8x4 factorial design. Cattle were assigned to one of eight vitamin D_3 and $CaCO_3$ dose combination levels [control, 1 million IU D_3/d, 2 million IU D_3/d, 3 million IU D_3/d, 4 million IU D_3/d, 5 million IU D_3/d, 2 million IU D_3/d plus 75 g CaCO $(2+)_3$ and 4 million IU D_3/d plus 75 g $CaCO_3$ (4+)]. Within each dose level, cattle (n = 6) were supplemented for either 2, 4, 6 or 8 days before slaughter.

Boluses were manually prepared using gelatin capsules (Torpac Inc., Fairfield, NJ) and Rovimix® D_3 500 (Roche Vitamins and Fine Chemicals, Nutley, NJ) or ground limestone (Colorado Lien Company, Fort Collins, CO; minimum of 33% calcium). Vitamin D_3 was administered in the form of irradiated 7-dehydrocholesterol. Boluses were filled and individually weighed and verified to contain 1, 2, 3, 4, or 5 million IU of vitamin D_3 or 75g of $CaCO_3$.

Cattle were moved through a hydraulic squeeze chute daily and dietary supplements were administered via oral bolus. Control cattle were processed daily, but did not receive a placebo bolus. On day 7 of the trial, a single heifer was removed -- due to illness -- from the treatment group that was to receive 2 million IU D_3/d plus 75 g $CaCO_3$ for 8 d.

Negative controls and cattle that were to receive vitamin D_3 for 8 d, from each dosage level (n = 6 per cell) were segregated and fed apart from the remaining cattle in 8 small pens (one pen for controls and each vitamin D_3 dose level). On a daily basis, all feed remaining in the bunk was removed, weighed, and average daily feed intake was calculated for each pen and is reported as average daily intake per head (Kg fed – Kg remaining/6 hd/pen). These pens were used to evaluate the level of feed intake for controls and treatment groups receiving vitamin D_3 for 6 hd/pen over 8 d. The remaining

cattle (n = 144) were co-mingled and equally divided into two pens and fed as single groups.

Twenty-four hours following administration of the final bolus, final live weights were obtained, blood was collected via jugular venipuncture using Vacutainer™ evacuated blood collection vials (Becker Dickinson and Company, Franklin Lakes, NJ), and cattle were shipped (~48 km) to, and processed at, a commercial beef packing facility in Greeley, CO.

Blood samples were allowed to clot overnight and centrifuged at 1300 x g for 15 m using a Beckman, Model TJ6 centrifuge (Beckman Instruments, Inc., Palo Alto, CA), and the resulting serum was removed, frozen (-17° C) and stored for subsequent total serum Ca^{2+} determination.

Individual cattle were followed through slaughter, chilled for 36 h and all USDA Quality and Yield Grade factors were determined by USDA personnel. Hunter Miniscan® (Hunter Laboratories, Reston, VA) CIE L*, a* and b* values were obtained from the exposed surface of the longissimus between the twelfth and thirteenth ribs of the right side of each carcass.

Following carcass data collection, striploins were collected from the right side of each carcass and transported to the CSU Meats laboratory. Striploins were further fabricated to yield 8 steaks (2.54 cm thick), from which four sets of two adjacent steaks were randomly assigned to one of four postmortem aging periods (2, 7, 14 or 21 d). Paired steaks were then randomly assigned to one of two endpoint cooking temperatures (70°C and 85°C). Steaks were aged at 15° C, and when the predetermined aging period had elapsed, frozen and held at -11°C until subsequent analysis. Steaks were removed

from the freezer and allowed to thaw for 24 h at 15°C, broiled on a Hobart Char Broiler, (model CB 51, Hobart, Troy, OH) to the specified end-point temperature and allowed to cool to room temperature (35°C).

When steaks reached room temperature, a minimum of seven 1.27-cm diameter cores were removed from each steak parallel to the muscle fiber orientation and peak shear force was determined for each core using a Warner-Bratzler shear (WBS) machine. The peak shear force values for the cores obtained from each steak were then averaged to determine mean steak shear force (AMSA, 1995).

Frozen blood samples were packed on ice and shipped to Oklahoma State University for total serum Ca^{2+} concentration determination. Samples were digested by combining 1 ml of serum with 5 ml of HNO_3 and heated using a microwave digestion system (CEM Corporation, Matthews, NC). Following acid digestion, total serum Ca^{2+} concentrations were determined using spectroscopy and are reported as mg of Ca^{2+}/dl of serum.

Data were analyzed using the General Linear Models procedure of SAS, using a model appropriate for the 8x4 factorial design (SAS, 1988). The dose by time interaction was not significant ($P > .05$) and was therefore removed from the analysis model. Subsequent analyses were performed including only the main effects of supplementation treatment level, regardless of days of administration. Additionally, treatment groups that did not receive additional dietary Ca^{2+} were pooled into three subclasses, (1) cattle that received no vitamin D_3, (2) cattle that received 10 million IU or less (total dose over 8 days) of vitamin D_3, or (3) cattle that received more than 10 million IU (total dose over 8

days) of vitamin D_3. When significant main effects of treatment level or total dose group occurred ($P < .05$), means were separated using a protected Tukey's HSD method.

RESULTS AND DISCUSSION

Feedlot Performance

At the onset of the trial, there were no differences ($P > .05$) in the weight of the cattle between the 32 control or treatment groups. Following the eight-day supplementation period, finished weights were obtained and average daily gains were calculated. Cattle that were supplemented with 4 million IU D_3/d plus 75 g $CaCO_3$ (4+) had lower ($P < .05$) final weights than cattle that had received 3 million or fewer IU D_3/d, and cattle that received 4 million IU D_3/d plus 75 g $CaCO_3$ over the eight-day supplementation period had a net loss in live weight and lower average daily gains than control cattle or cattle that were supplemented with 1 million IU D_3/d (Table 4.1).

Because the length of time that vitamin D_3 was administered pre-harvest or the interaction of time pre-harvest and vitamin D_3 dose did not influence feedlot performance ($P > .05$), the treatment subclasses were reclassified to reflect three pooled treatment groups of cattle that received (1) no vitamin D_3, (2) 10 million IU D_3 (total cumulative dose over 8 d) or less, and (3) more that 10 million IU D_3 (total cumulative dose over 8 d). Among the three pooled control and treatment groups, there were no differences ($P > .05$) in initial live weight, final live weight, hot carcass weight, dressing percentage, USDA Yield Grade or USDA marbling score. However, cattle that received >10 million IU of vitamin D_3 over the 8 d period did have lower ($P < .05$) ADG than negative control cattle, whereas cattle that received 10 million IU of vitamin D_3 or less over the 8 d period

had intermediate daily gains and their gain did not differ ($P > .05$) from that of control cattle or cattle receiving the higher vitamin D_3 dose.

Table 4.1. Arithmetic means for final live weight (FWT), average daily gain (ADG), dressing percentage (DRESS), and total blood serum calcium concentration (Ca^{2+} mg/dl) for cattle (N = 24) supplemented with 0, 1, 2, 3, 4, 5 million IU of vitamin D_3, 2 million IU vitamin D_3 plus 75g $CaCO_3$, or 4 million IU vitamin D_3 plus 75g $CaCO_3$, regardless of the length of time that cattle were subjected to pre-harvest supplementation with vitamin D_3

Trt[a]	FWT (kg)	ADG (kg/d)	DRESS (%)	Ca^{2+} (mg/dl)
0	516^{y}	2.2^{y}	59.8^{z}	11.3^{z}
1	508^{y}	1.7^{y}	60.0^{z}	13.3^{y}
2	507^{y}	1.1^{yz}	60.1^{z}	13.2^{vw}
3	502^{y}	1.0^{yz}	60.1^{z}	12.0^{wxyz}
4	496^{yz}	0.2^{yz}	60.9^{yz}	12.9^{xwz}
5	500^{yz}	$(0.1)^{yz}$	60.8^{yz}	12.7^{wxy}
2+[bc]	487^{yz}	1.0^{yz}	60.5^{z}	11.7^{yz}
4+[d]	470^{z}	$(0.7)^{z}$	61.9^{yz}	12.0^{xyz}
RSD	36.05	2.66	1.71	1.27

[a] Treatment level indicates the daily supplemental dose of vitamin D_3.
[b] N = 23
[c] Cattle received 2 million IU D_3 d plus 75 g $CaCO_3$
[d] Cattle received 4 million IU D_3 d plus 75 g $CaCO_3$
[y,z] Means, within a column, but lacking common superscript letters, differ ($P < .05$).

Feed Intake

In order to determine feed intake patterns for cattle supplemented with vitamin D_3, daily feed consumption (as-fed) was collected for cattle that received a dose of vitamin D_3 on each of the 8 days pre-harvest. Trend lines, from single observations, are shown in Figure 4.1 for each of the eight treatment groups. Cattle that were

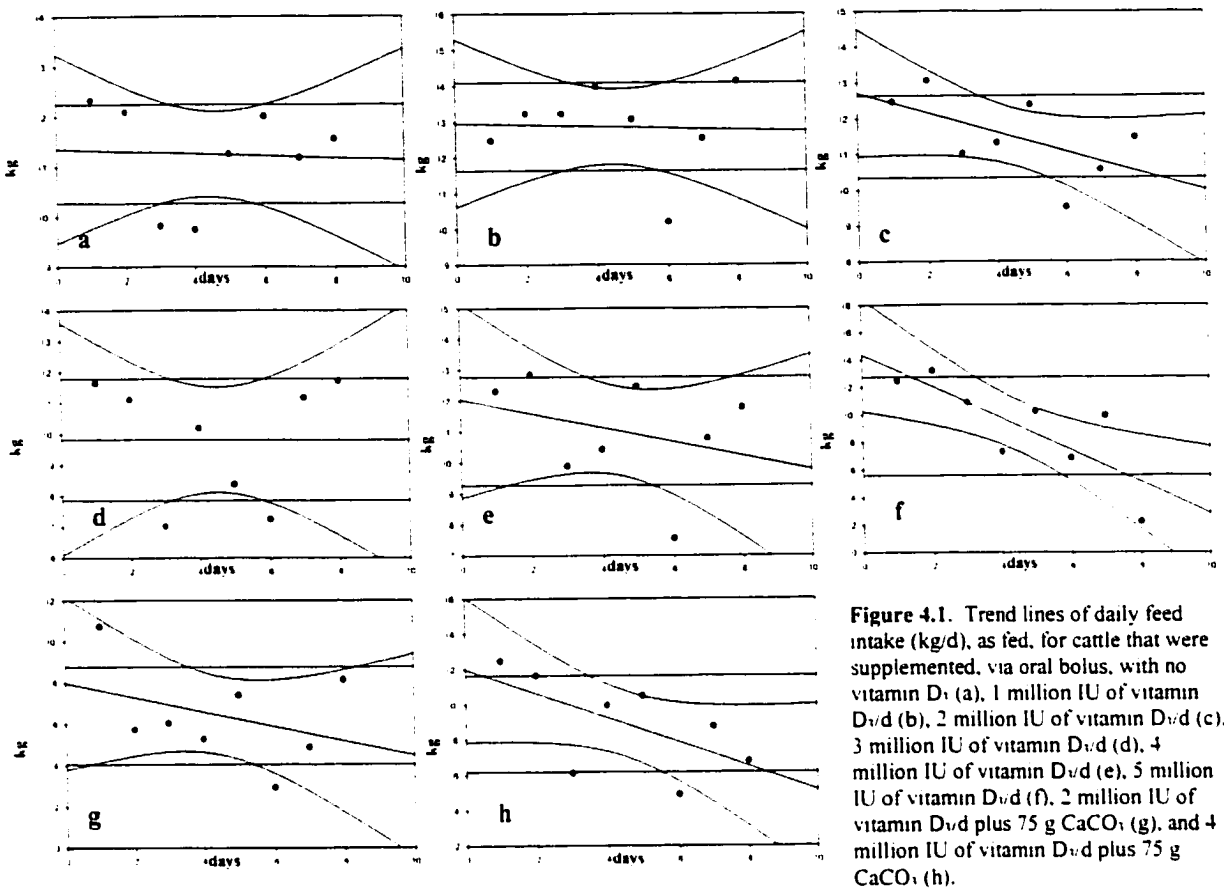


Figure 4.1. Trend lines of daily feed intake (kg/d), as fed, for cattle that were supplemented, via oral bolus, with no vitamin D_3 (a), 1 million IU of vitamin D_3/d (b), 2 million IU of vitamin D_3/d (c), 3 million IU of vitamin D_3/d (d), 4 million IU of vitamin D_3/d (e), 5 million IU of vitamin D_3/d (f), 2 million IU of vitamin D_3/d plus 75 g $CaCO_3$ (g), and 4 million IU of vitamin D_3/d plus 75 g $CaCO_3$ (h).

supplemented with 2, 4, 5, 2+ and 4+ million IU D_3/d exhibited decreased daily feed consumption during the supplementation period. These data suggested that following day 2 of supplementation, the appetite of cattle receiving more than 1 million IU D_3/d declined, and the reduction in feed consumption was amplified by administration of added dietary calcium (in the form of $CaCO_3$). This pattern of reduced daily feed intake could be indicative of cattle that manifested acute toxicity from the supranutritional levels of vitamin D_3 provided. When additional calcium was added to the diet, the impact on feed intake was amplified, suggesting that the combination of increased vitamin D_3 and additional dietary calcium magnified the toxic effect.

Total Serum Calcium

Twenty-four hours following administration of the last bolus, blood samples were collected via jugular venipuncture and analyzed for total serum calcium content. Cattle that were supplemented with 1, 2 or 5 million IU D_3/d had higher ($P < .05$) total serum calcium levels than negative control cattle; cattle that were supplemented with 1 million IU D_3/d, regardless of days of administration, had the highest ($P < .05$) serum Ca^{2+} concentration (Table 4.1). Cattle that were given additional dietary calcium (in the form of $CaCO_3$) plus 2 or 4 million IU D_3/d, had the lowest ($P < .05$) blood serum Ca^{2+} concentrations following 8 d of supplementation. These results were consistent with reports from Hibbs and Pounden (1954) and Swanek et al. (1999) that stated that serum Ca^{2+} levels increased when cattle were fed supranutritional levels of vitamin D_3. Vitamin D_3 was successfully administered via oral bolus in the present study, and feedlot heifers responded metabolically to supranutritional supplementation with vitamin D_3.

Total blood serum Ca^{2+} concentration, among the three pooled, cumulative dose, treatment groups, are reported in Table 4.2. Cattle that received vitamin D_3, whether it was a large total dose (> 10 million IU) or a smaller dose (10 million IU or less) over 8 d, had higher ($P < .05$) total blood serum Ca^{2+} concentrations than did control cattle. This latter finding demonstrated the effectiveness of vitamin D_3 in manipulating the circulating concentration of Ca^{2+}, but also indicated that there was a level of administration that achieved the greatest increase in total blood Ca^{2+} while at the same time, minimizing the cost of supplementation.

In order to determine the optimum dose level and time of administration, total blood serum Ca^{2+} concentrations were analyzed by dose over the course of the 8 possible

days of supplementation (Figure 4.2). It appeared that supplementation with either 1 or 2 million IU D_3/d for 8 d produced the maximum concentration of blood serum Ca^{2+}, in this study. However, it also was apparent that supplementation with either 1, 2, or 4 million IU D_3/d for only 2 d also effectively increased ($P < .05$) total blood serum Ca^{2+} concentrations. Again, it was observed that when additional dietary calcium was provided, total blood serum Ca^{2+} concentrations were the lowest, numerically, and lower ($P < .05$) than that for cattle that received only 1 or 2 million IU D_3/d for the same length of time. From an economic standpoint, supplementation with a lower dose of vitamin D_3 for fewer total days, while attaining an equal increase in blood Ca^{2+} concentrations, would require fewer total IU of vitamin D_3 and would thus, be less expensive to incorporate into a production system.

Table 4.2. Means for final live weight (FWT), average daily gain (ADG), dressing percentage (DRESS), and total serum calcium concentration (Ca^{2+} mg.dl) for cattle that received no vitamin D_3, < 10 million IU of vitamin D_3 or > 10 million IU of vitamin D_3, within 8 d of slaughter

Trt	N	FWT (kg)	ADG (kg/d)	DRESS	Ca^{2+} (mg/dl)
0	24	516	2.2^{y}	59.8	11.3^{z}
< 10 MIL	54	510	1.4^{yz}	60.1	12.9^{y}
> 10 MIL	66	496	0.3^{z}	60.7	12.8^{y}
$\sqrt{MSE}$	-	37.05	2.66	1.73	1.38

[a] Treatment level indicates the total amount of vitamin D_3 administered over 8 days.
[y,z] Means, within a column, lacking common superscript letters, differ ($P < .05$).

Calcium carbonate ($CaCO_3$) was included in two of the treatment groups to explore the possibility that cattle had adequate, endogenous levels of vitamin D_3 to

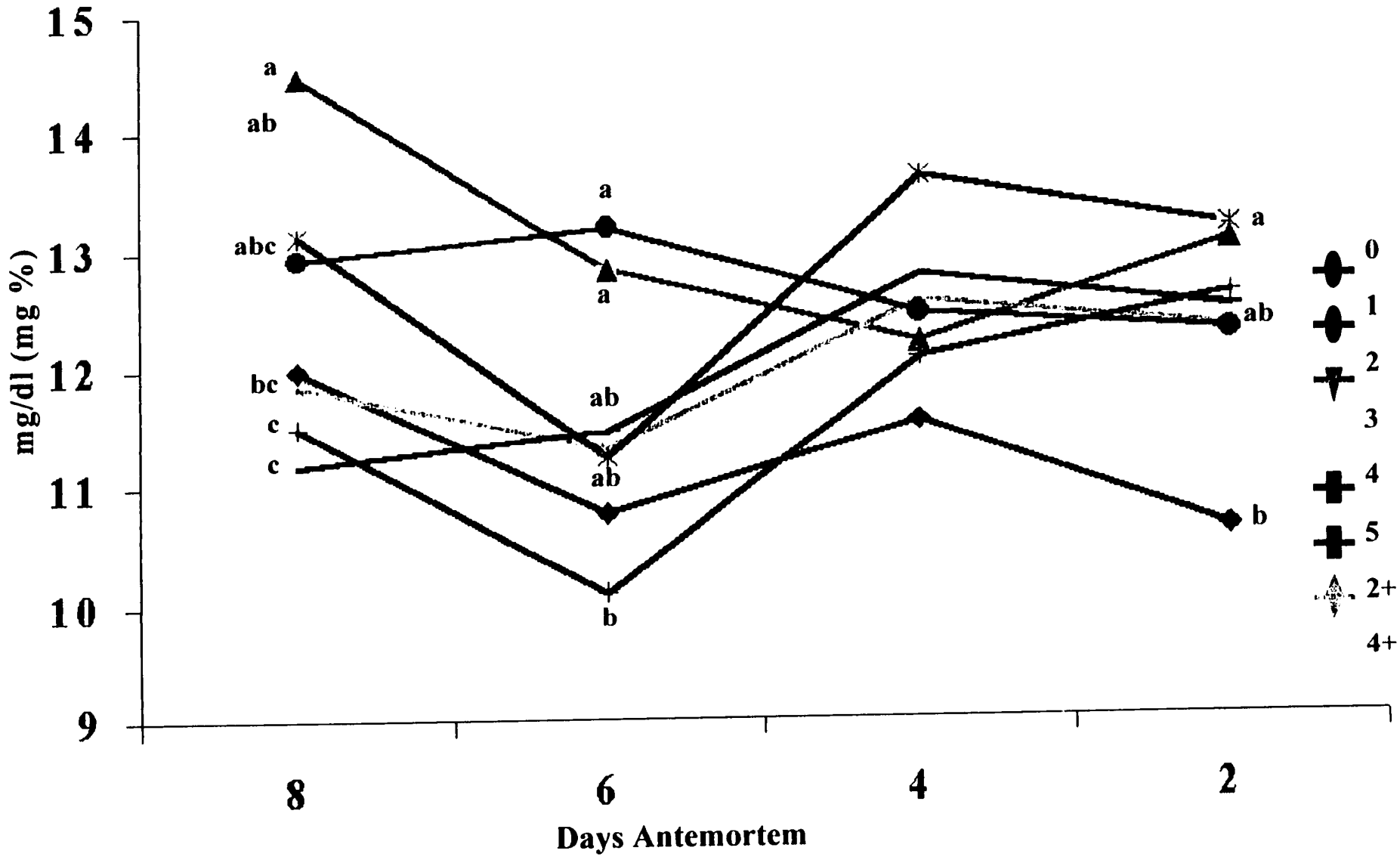
absorb greater amounts of calcium than were normally available in the diet. If the latter hypothesis were true, it would be feasible to suggest that Ca^{2+} supplementation alone could increase blood and muscle Ca^{2+} concentrations sufficiently to increase calpain activity and postmortem tenderness. The latter hypothesis was based on the work of Ducket et al. (1998), who found that drenching of cattle 3-6 h pre-harvest with a solution of calcium propionate increased cooked meat tenderness at 4 and 7 d postmortem. In the present study, supplementing cattle with both supranutritional levels of vitamin D_3 and $CaCO_3$ lowered ($P < .05$) total serum Ca^{2+} content following d 6 of supplementation (Figure 4.2). Cattle that received 2 or 4 million IU D_3/d and additional dietary calcium were compared with cattle that received 2 or 4 million IU D_3/d and no additional calcium (Table 4.3). The administration of 75 g of $CaCO_3$ daily for up to 8 d reduced ($P < .05$) HCW and dressing %, increased ($P = .0589$) WBS force for their steaks after aging them for 2 d postmortem and cooking them to 70° C. Contrary to our original hypothesis, supplementation with $CaCO_3$, plus bolusing them with 2 or 4 million IU D_3/d, reduced ($P < .05$) total blood serum Ca^{2+} concentrations.

Table 4.3. Least squares means ± standard error for average daily gains (ADG), hot carcass weight (HCW), dressing percentage (DRESS), Warner-Bratzler shear force (WBS) and total serum calcium concentration (Ca^{2+} mg dl) for steaks aged 2 d and cooked to 70° C from cattle that received 2 or 4 million IU of vitamin D_3/d with or without 75 g of additional $CaCO_3$ for up to 8 d pre-harvest

Treatment	N	ADG (kg/d)	HCW (kg)	DRESS (%)	WBS (kg)	Ca^{2+} (mg/dl)
No Added Calcium	48	.67 ± .38	303 ± 2.90^{z}	60.5 ± .24^{y}	6.18 ± .23^{t}	13.0 ± .19^{z}
Added Calcium	47	.10 ± .38	292 ± 2.92^{y}	61.3 ± .25^{z}	6.81 ± .23^{u}	11.8 ± .19^{y}

[a] N = 43 for each treatment level.
[y,z] Means, within the same column, lacking a common superscript letter, differ ($P < .05$).
[t,u] Means, within the same column, lacking a common superscript letter, differ ($P = .0589$).

Figure 4.2. Dose response, over time, for total serum Ca^{2+} for cattle that were supplemented with no vitamin D_3, 1 million IU of vitamin D_3/d, 2 million IU of vitamin D_3/d, 3 million IU of vitamin D_3/d, 4 million IU of vitamin D_3/d, 5 million IU of vitamin D_3/d, 2 million IU of vitamin D_3/d plus 75 g $CaCO_3$ or 4 million IU of vitamin D_3/d plus 75 g $CaCO_3$. Means, within day, lacking common superscript letters, differ ($P < .05$).



Based on the aforementioned information, and the biochemical mechanism through which vitamin D_3 is synthesized and regulates blood calcium (Figure 4.3), it was concluded that the abundance of dietary calcium provided to the animal actually inhibited the synthesis and actions of vitamin D_3. This presumably occurred at two levels, (1) Ca^{2+} inhibits the activity of 25 Hydroxylase and (2) Ca^{2+} inhibits the activity of 1 α-Hydroxylase. Both of these enzymes perform a regulatory role in the production of metabolically active vitamin D_3 [1,25$(OH)_2$-D_3]. Because these key regulatory enzymes were probably inhibited, the supplemented vitamin D_3 (7-Dehydorcholesterol) and intrinsic vitamin D_3 were most likely not converted to metabolically active vitamin D_3, resulting in a net reduction in total blood serum Ca^{2+} concentration.

Cooked Steak Tenderness

Boneless top loin steaks were aged for 2, 7, 14 and 21 d following slaughter and Warner-Bratzler shear force analyses were conducted at two end-point cooking temperatures (70° C and 85° C). Again, duration of administration of vitamin D_3 had no effect ($P > .05$) on WBS force, thus means were evaluated across the 8 control and dosage levels, regardless of days of administration. There were no differences ($P > .05$) in mean shear force measures for steaks from control vs. Treated cattle (irrespective of vitamin D_3 dose) cooked to 70° C at 2, 7, 14 or 21 d postmortem. Additionally, there were no differences ($P > .05$) in mean shear force measures for steaks from control vs. Treated cattle (irrespective of vitamin D_3 dose) cooked to 85° C at 2, 7 or 14 d postmortem. Steaks aged 21 d and cooked to 85° C from cattle that were supplemented with 5 million IU D_3/d had lower ($P < .05$) mean shear values than comparably aged and

cooked steaks from cattle supplemented with 1 million IU D_3/d or 4 million IU D_3/d plus 75 g $CaCO_3$.

Warner-Bratzler shear force values also were stratified by pooled, cumulative vitamin D_3 dose treatment groups and no differences ($P > .05$) in mean shear force were found across aging periods or end-point degrees of doneness. Contrary to earlier reports (Swanek et al., 1999, Montgomery et al., 1997), vitamin D supplementation did not improve cooked longissimus tenderness in our study even though it did generally increase total serum Ca^{2+} concentrations.

It is important to note that, in this study, aging period and end-point cooking temperatures had significant impacts on WBS force (data not shown). These findings are consistent with the well accepted conclusions that beef longissimus steaks become more tender with increased aging time (Eilers et al., 1996) and less tender as degree of doneness increases (Wulf et al., 1996).

IMPLICATIONS

These results indicate that supplementing cattle with vitamin D_3 (7-dehydrocholestrol) does not influence (improve or impair) postmortem cooked beef tenderness. It is apparent that more work is necessary to determine if vitamin D_3 has an impact on beef tenderness, and if so, does the positive impact of tenderness outweigh the negative effect on feedlot performance and result in tenderness improvements that are sufficient to offset the cost of vitamin D_3 supplementation.

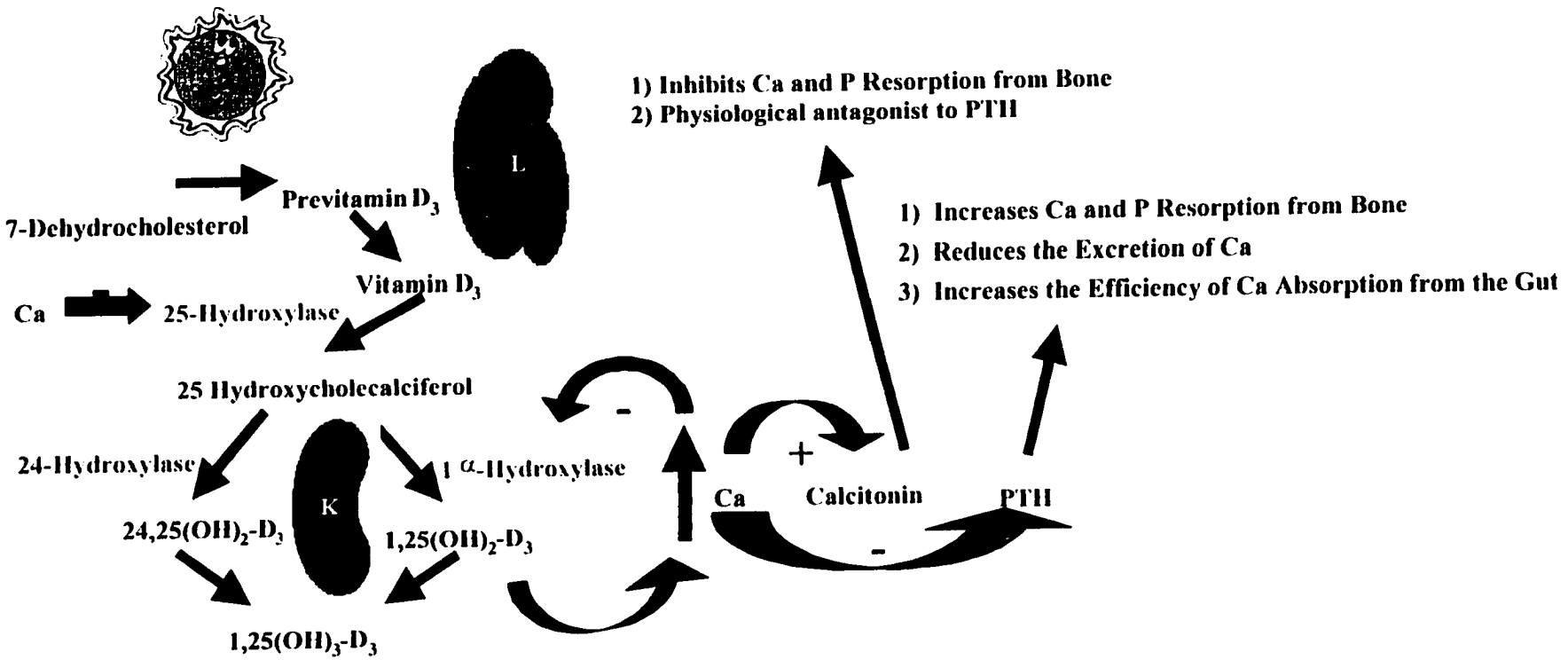


Figure 4.3. Formation and hydroxylation of vitamin D_3 from 7-Dehydrocholesterol through the metabolic actions of ultra-violet light as well as, liver (L) and kidney (K) enzymes, and its role in regulating serum calcium levels, calcitonin and parathyroid hormone (PTH).

LITERATURE CITED

AMSA. 1995. Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of meat. American Meat Science Association and National Live Stock and Meat Board, Chicago, IL.

Boehm, M. L., T. L. Kendall, V. F. Thompson, and D. E. Goll. 1998. Changes in the calpains and calpastatin during postmortem storage of bovine muscle. J. Anim. Sci. 76:2415-2434.

Cross, H. R., R. Moen, and M. S. Stanfield. 1978. Training and testing of judges for sensory analysis of meat quality. Food Technol. 32:48-52.

Dayton, W. R.., D. E. Goll, M. G. Zeece, R. M. Robson, and W. J. Reville. 1976. A Ca^{2+}activated protease possibly involved in myofibrillar protein turnover. purification from porcine muscle. Biochem. 15:2150-2158.

Davey C. L., and K. V. Gilbert. 1969. Studies in Meat Tenderness. 7. Changes in the fine structure of meat during aging. J. Food Sci. 34:69-74.

Deatherage, F. E. 1963. The effects of water and inorganic salts on tenderness. In: Proc. Campbell's Soup Company, Meat Tenderness Symposium, Camden, NJ. pp. 45-68.

Diles, J. J. B., M. F. Miller, and B. L. Owen. 1994. Calcium chloride concentration, injection time, and aging period effects on tenderness, sensory, and retail color attributes of loin steaks from mature cows. J. Anim. Sci. 72:2017-2021.

Dransfield, E. 1992. Modeling Post-mortem tenderization—IV: Role of calpains and calpastatin in conditioning. Meat Sci. 34:217-234.

Ducket, S. K.., T. A. Klein, J. G. Andrae, and W. K. Sanchez. 1998. Pre-harvest tenderization through oral calcium gel administration. J. Anim. Sci. 76, (Suppl.):156.

Dusso, A. S., and A. J. Brown. Mechanisms of vitamin D action and its regulation. Amer. J. Kidney Disease. 32(Suppl. 2):S13-S24.

Dutson, T. R. 1983. Relationship of pH and temperature to disruption of specific muscle proteins and activity of lysosomal protease. J. Food Biochem. 7:223-245

Eilers, J. D., J. B. Morgan, A. M. Martin, R. K. Miller, D. S. Hale, G. R. Acuff, and J. W. Savell. 1994. Evaluation of calcium chloride and lactic acid injection on chemical, microbiological and descriptive attributes of mature cow beef. Meat Sci. 38:443-451.

Eilers, J. D., J. D. Tatum, J. B. Morgan, and G. C. Smith. 1996. Modification of early-postmortem muscle pH and use of postmortem aging to improve beef tenderness. J. Anim. Sci. 74:790-798.

George, M. H., J. D. Tatum, K. E. Belk, and G. C. Smith. 1999. An audit of retail beef loin steak tenderness conducted in eight U.S. cities. J. Anim. Sci. 77:1735-1741.

Goll, D. E., F. G. Taylor, J. A. Christiansen, and V. F. Thompson. 1992a. Role of proteases and protein turnover in muscle growth and meat quality. Proc. 44th Annu. Recip. Meat Conf. pp 25-36. National Live Stock and Meat Board. Chicago, IL.

Goll, D. E., V. F. Thompson, R. G. Taylor, and J. A. Christiansen. 1992b. Role of the calpain system in muscle growth. Biochimie. 74:225-237.

Goll, D. E., V. F. Thompson, R. G. Taylor, and A. Ouali. 1998. The calpain system and skeletal muscle growth. Can. J. Anim. Sci. 78:503-512.

Harrell, R. A., T. D. Bidner, and E. A. Icaza. 1978. Effect of altered muscle pH on beef tenderness. J. Anim. Sci. 46:1592-1596.

Hattori, A., and K. Takahashi. 1982. Calcium-induced weakening of skeletal muscle Z-disks. J. Biochem. 92:381-390.

Haussler, M. R., and T. A. McCain. 1977. Basic and clinical concepts related to vitamin D metabolism and action. New England J. Medicine. 297:974-983.

Hellendoorn, E. W. 1962. Water-binding capacity of meat as affected by phosphates. Food Technol. 16:119-124.

Hibbs, J. W., and W. D. Pounden. 1954. Studies on milk fever in dairy cows. IV. Prevention by short-time, prepartum feeding of massive doses of vitamin D. J. Dairy Sci. 38:65-72.

Karges, K., F. N. Owens, D. Gill, and J. B. Morgan. 1998. Effects of vitamin D_3 levels on feed intake by yearling steers. J. Anim. Sci. 76 (Suppl.):361.

Kerth, C. R., M. F. Miller, and C. B. Ramsey. 1995. Improvement of beef tenderness and quality traits with calcium chloride injection of beef loins 48 hours postmortem. J. Anim. Sci. 73:750-756.

Koohmaraie, M., W. H. Kennick, E. A. Elgasim, and A. F. Anglemeier. 1984a. Effect of pre-rigor pressurization on the activity of calcium-activated factor. J. Food Sci. 49:680-684.

Koohmaraie, M., W. H. Kennich, E. A. Elgasim, and A. F. Anglemeier. 1984b. Effect of postmortem storage on muscle protein degradation: Analysis by SDS-polyacrylamide gel electrophoresis. J. Food Sci. 49:292-293.

Koohmaraie, M., W. H. Kennick, A. F. Anglemeier, E. A. Elgasim and T. K. Jones. 1984c. Effect of postmortem storage on cold-shortened bovine muscle: Analysis by SDS-polyacrylamide gel electrophoresis. J. Food Sci. 49:290-291.

Koohmaraie, M. 1988. The role of endogenous proteases in meat tenderness. Proc. 41st Annu. Recip. Meat Conf. pp. 89-100. National Live Stock and Meat Board. Chicago, IL.

Koohmaraie, M., A. S. Babiker, R. A. Merkel, and T. R. Dutson. 1988a. Role of Ca^{2+}-dependent proteases and lysosomal enzymes in postmortem changes in bovine skeletal muscle. J. food Sci. 53:1253-1257.

Koohmaraie, M., S. C. Siedeman, H. E. Schollmeyer, T. R. Dutson, And A. S. Babiker. 1988b. Factors associated with the tenderness of three bovine muscles. J. Food Sci. 53:407-410.

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. 68:1278-1283.

Koohmaraie, M., M. E. Doumit, and T. L. Wheeler. 1996a. Meat toughening does not occur when rigor shortening is prevented. J. Anim. Sci. 74:2935-2942.

Koohmaraie, M., S. D. Shackelford, and T. L. Wheeler. 1996b. Effects of a β-adrenergic agonist (L-644,969) and male sex condition on muscle growth and meat quality of callipyge lambs. J. Anim. Sci. 74:70-79.

Lansdell, J. L., M. F. Miller, T. L. Wheeler, M. Koohmaraie, and C. B. Ramsey. 1995. Postmortem injection of calcium chloride effects on beef quality traits. J. Anim. Sci. 73:1735-1740.

Mayes, P. A. 1996. Structure and Function of Lipid Soluble Vitamins. In: Harper's Biochemistry (24th edition). pp. 617-618. Appleton and Lange, Connecticut.

Meilgaard, M., G. V. Civille, and B. T. Carr. 1991. Sensory Evaluation Techniques (2nd ed.). pp. 135-186. CRC Press, Inc. Boca Raton, FL.

Miller, M. F., K. L: Huffman, S. Y. Gilber, L. L. Hamman, and C. B. Ramsey. 1995. Retail consumer acceptance of beef tenderized with calcium chloride. J. Anim. Sci. 73:2308-2314.

Moeller, P. W., P. A. Fields, T. R. Dutson, W. A. Landmann, and Z. L. Carpenter. 1977. High temperature effects on lysosomal enzyme distribution and fragmentation of bovine muscle. J. Food Sci. 42:510-512.

Moeller, R. J. and S. Courington. 1998. Branded Beef Study. National Cattlemen's Beef Association, Englewood, CO.

Montgomery, J. L., F. C. Parrish, Jr., D. C. Beitz, R. L. Horst, E. J. Huff-Lonergan, and A. H. Trenkle. 1997. The use of vitamin D_3 to improve beef tenderness. In: Proc. 50th Reciprocal Meat Conference, Ames, IA. p 168.

Montgomery, J. L., M. A. Carr, C. R. Kerth, G. G. Hilton, L. L. Behrends, E. R. Behrends, and M. F. Miller. 1998. Final Report: Dietary Modifications Using Vitamin D_3 to Improve Beef Tenderness. National Cattlemen's Beef Association, Englewood, CO.

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. 69:4469-4476.

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. 69:3274-3283.

Morris, C. A., R. L. Theis, R. K. Miller, G. R. Acuff, and J. W. Savell. 1997. Improving the flavor of calcium chloride and lactic acid injected mature beef top round steaks. Meat Sci. 45:531-537.

NCBA. 1999. Beef Industry Long Range Plan Task Force Report. Beef Industry Long Range Plan Task Force, National Cattlemen's Beef Association, Englewood, CO.

Olson, D. G., and F. G. Parrish, Jr. 1977. Relationship of myofibril fragmentation index to measure of beef steak tenderness. J. Food Sci. 42:506-509.

Olson, D. G., F. C. Parrish, Jr., W. R. Dayton, and D. E. Goll. 1977. Effect of postmortem storage and calcium activated factor on myofibrillar proteins of bovine skeletal muscle. J. Food Sci. 42:117-124.

Ouali, A. 1984. Sensitivity to ionic strength of Mg-Ca-enhanced ATPase activity as an index of myofibrillar aging in beef. Meat Sci. 11:79-88.

Ouali, A., N. Garrel, C. Obled, C. Deval, C. Valin, and I. F. Penny. 1987. Comparative action of cathespin D, B, H, L and of a new lysosomal cysteine proteinase on rabbit myofibrils. Meat Sci. 19:83-100.

Papadopoulos L. S., R. K. Miller, L. J. Ringer and H. R. Cross. 1991. Sodium lactate effect on sensory characteristics, cooked meat color and chemical composition. J. Food Sci. 56:621-631.

Penny, I. F. 1980. The enzymology of Conditioning. In: Developments in Meat Science. R. F. Lawrie (ed.). Applied Science Publishers Ltd., London.

SAS. 1988. SAS/STAT® User's Guide (Release 6.03). SAS Inst. Inc., Cary, NC.

Savell, J. W., R. E. Branson, H. R. Cross, D. M. Stiffler, J. W. Wise, D. B. Griffin, and G. C. Smith. 1987. National consumer retail beef study: Palatability evaluations of beef loin steaks that differed in marbling. J. of Food Sci. 52:517-519.

Savell, J. W., H. R. Cross, J. J. Francis, J. W. Wise, D. S. Hale, D. L. Wilkes, and G. C. Smith. 1989. National consumer retail beef study: Interaction of trim level, price and grade on consumer acceptance of beef steaks and roasts. J. of Food Qual. 12:251-273.

Shackelford, S. D., T. L. Wheeler, and M. Koohmaraie. 1997. Tenderness classification of beef: I. Evaluation of beef longissimus shear force at 1 or 2 days postmortem as a predictor of aged beef tenderness. J. Anim. Sci. 75:2417-2422.

Smith, G. C., G. R. Culp, and Z. L. Carpenter. 1978. Postmortem aging or beef carcasses. J. Food Sci. 43:823-826.

Smith, L. A., S. L. Simmons, F. K. McKeith, P. J. Betchel, and P. L. Brady. 1984. Effects of sodium tripolyphosphate on physical and sensory properties of beef and pork roasts. J. Food Sci. 49:1636-1637.

Streitel, R. H., H. W. Ockerman, and V. R. Cahill. 1977. Maintenance of beef tenderness by inhibition of rigor mortis. J. Food Sci. 42:583-585.

Swanek, S. S., J. B. Morgan, F. N . Owens, D. R. Gill, C. A. Strasia, H. G. Dolezal and F. K. Ray. 1999. Vitamin D_3 supplementation of beef steers increases longissimus muscle tenderness. J. Anim. Sci. 77:874-881.

Tatum, J. D., K. E. Belk, M. H. George, and G. C. Smith. 1999. Identification of quality management practices to reduce the incidence of retail beef tenderness problems: Development and evaluation of a prototype quality system to produce tender beef. J. Anim. Sci. 77:2112-2118.

Taylor, R. G., G. H. Geesink, V. F. Thompson, M. Koohmaraie, and D. E. Goll. 1995. Is Z-disk degradation responsible for postmortem tenderization? J. Anim. Sci. 73:1351-1367.

Terrell, R. N., C. G. Ming, J. A. Jacobs, G. C. Smith, and Z. L. Carpenter. 1981. Effect of chloride salts, acid phosphate and electrical stimulation on pH and moisture loss from beef clod muscles. J. Anim. Sci. 53:658-662.

Trout, G. R. 1984. Effect of ionic strength, phosphate type, pH and cooking temperature on meat protein functionality. Ph.D. Dissertation. Colorado State Univ., Fort Collins.

Trout, G. R. and G. R. Schmidt. 1986. Effect of phosphate on the functional properties of restructured beef rolls: The role of pH, ionic strength, and phosphate type. J. Food. Sci. 51:1416-1422.

USDA. 1996. Institutional Meat Purchase Specifications. USDA Agricultural Marketing Service, Livestock and Seed Division, Livestock and Meat Standardization Branch, United State Department of Agriculture, Washington, DC.

USDA. 1999. Food Consumption, Prices and Expenditures, 1970-1997; 1999. United States Department of Agriculture, Washington DC.

USDA-FSIS. 1999. FSIS issues policy statement on beef and E. coli O157:H7 (January 19, 1999). USDA-Food Safety and Inspection Service. Washington DC.

Vote, D. J., W. J. Platter, J. D. Tatum, G. R. Schmidt, K. E. Belk, G. C. Smith, and N. C. Speer. 1999. Injection of beef strip loins with solutions containing sodium tripolyphosphate, sodium lactate, and sodium chloride to enhance palatability. J. Anim. Sci. In-Press.

Wang, H., C. E. Wier, M. L. Birkner, and B. Ginger. 1957. The influence of enzyme tenderizers on the structure and tenderness of beef. Proc. 9th Res. Conf., American Meat Institute Foundation, p. 72.

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. 69:4871-4875.

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. 70:3451-3457.

Wheeler, T. L., M. Koohmaraie, J. L. Lansdell, G. R. Siragusa, and M. F. Miller. 1993. Effects of postmortem injection time, injection level and concentration of calcium chloride on beef quality traits. J. Anim. Sci. 71:2965-2974.

Whipple G. and M. Koohmaraie. 1992a. Freezing and calcium chloride marination effects on beef tenderness and calpastatin activity. J. Anim. Sci. 70:3081-3085.

Whipple G. and M. Koohmaraie. 1992B. Calcium chloride marination effects on beef steak tenderness and calpain proteolytic activity. Meat Sci. 33:265-275

Wulf, D. M., J. B. Morgan, J. D. Tatum, and G. C. Smith. 1996. Effects of animal age, marbling score, calpastatin activity, subprimal cut, calcium injection, and degree of doneness on the palatability of steaks from limousin steers. J. Anim. Sci. 74:569-576.

Wyle, A. M., D. L. Roeber, R. C. Cannell, K. E. Belk, M. Goldberg, J. D. Tatum, and G. C. Smith. 1999. Final Report: Effectiveness of Using the HunterLab BeefCam System to Sort Beef Carcasses into Differing Product Lines Across Four Beef Packing Facilities and From Both Source Verified and Non-source Verified Fed Cattle, Based on Projected Tenderness of Subsequently Aged and Cooked Product. National Cattlemen's Beef Association, Englewood, CO.