EFFECT OF SUPPLEMENTAL VITAMIN D_3 AND ELECTRICAL STIMULATION ON QUALITY AND PALATABILITY CHARACTERISTICS OF FEEDLOT FINISHED BEEF STEERS

By

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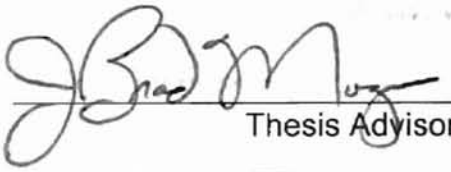

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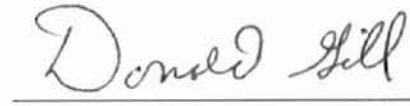

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FORMAT OF THESIS

This Thesis is presented in Journal of Animal Science style format, as outlined by the Oklahoma State University Graduate College Style Manual. The use of this format allows for independent chapters to be prepared suitable for submission to scientific journals.

CHAPTER 1

Introduction

Beef production no longer warrants commodity-based ideology. Continual loss of market share to other protein sources, since the late 1970's, has redirected the viewpoint of those involved with the beef industry. Producers are no longer able to deliver beef without regards for consumer acceptability. Consumer's are willing to purchase chicken, because "chicken is chicken" and offers a consistent product time after time. Beef however, has fallen short of providing consistently pleasant eating experiences. The 1995 National Beef Quality Audit listed low overall uniformity and consistency, inadequate tenderness, and low overall palatability as the top three aggregate concerns (Smith et al. 1995). Schroeder and Mark, (1999) stated that determining how to solve beef demand problems requires identifying the problem. Thus, there exists a need to address those issues that affect, or could plausibly improve beef's consistency, palatability, and tenderness.

Problems associated with tenderness have been identified as the primary culprit for palatability concerns. Dikeman, (1987), Savell and Shackelford, (1992), and Miller et al., (1995) all proved tenderness as the most important palatability attribute of beef. Brooks et al., (2000) reiterated findings from The Beef Customer Satisfaction Study (Neely et al., 1998, 1999; Lorenz et al., 1999; Savell et al., 1999), which demonstrated tenderness affects consumer's perception of taste. Moreover, Huffman et al., (1996) established the ability of

consumers to accurately evaluate tenderness. Culmination of these references, and many others demonstrate the extreme importance of beef tenderness characteristics.

Electrical stimulation has long been used as a means of improving meat tenderness. Research originating in the 1950's demonstrated the inherent ability of electrical stimulation to increase tenderness in meat products. Likewise, research conducted in the middle 1950's examined the effectiveness of supplememtal vitamin D_3 to increase serum calcium levels for the prevention of parturient paresis in dairy cows. Later, researchers who understood the necessity of calcium for the elucidation of natural proteolytic enzymes in muscle examined the ability of vitamin D_3 to increase tenderness in steaks from beef steers. Thus, this study was conducted in order to determine the effectiveness of supplemental vitamin D_3, in association with electrical stimulation on enhancing tenderness of steaks from feedlot-finished steers.

CHAPTER 2

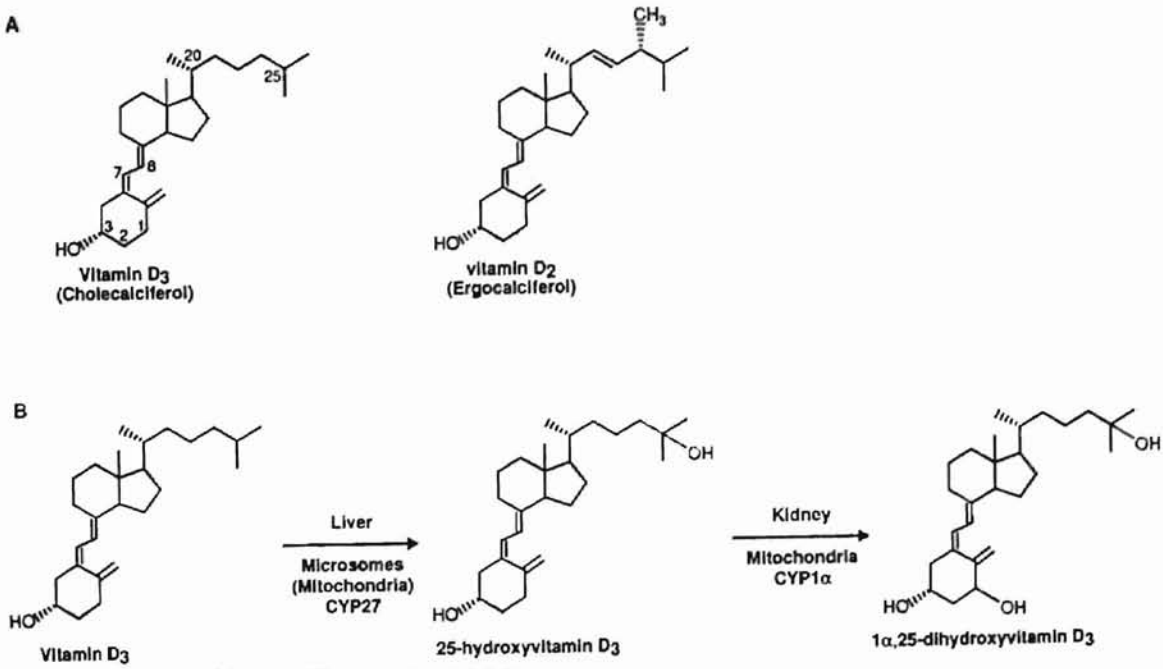
Literature Review

Vitamin D

Research involving dairy cows demonstrated the ability of orally administered Vitamin D_3 to decrease the occurrence of milk fever. Hibbs et al., (1951) concluded low dosage levels (1-2 MIU/d) of vitamin D_3 (VITD) would increase ionized serum calcium concentrations. However, supplementation at these low levels was ineffective at decreasing the development of parturient paresis in dairy cows. Thus, Hibbs and Pounden (1955) completed research which demonstrated VITD supplementation at relatively high dosage levels (5 – 30 MIU/d) effectively increased serum calcium levels enough to reduce milk fever. Likewise, later research proved the effectiveness of vitamin D metabolites to increase concentrations of hormonally active VITD in the blood. Bar et al., (1985) and Hodnett et al., (1992) conducted research that demonstrated the ability of intramuscularly administered 1α-hydroxyvitamin D_3 and/or 25-hydroxyvitamin D_3 to increase serum calcium and phosphorus levels in dairy cows. These results led to the postulation that vitamin D_3 supplementation could effectively enhance muscle tenderness in beef cattle. The presumption was VITD supplementation could elevate serum calcium levels high enough to cause increased activity of endogenous proteolytic enzymes. First, it is necessary to understand vitamin D metabolism.

Vitamin D metabolism

Vitamin D can be obtained from several sources. In vivo sources of vitamin D are obtained when skin is exposed to ultraviolet light. A cholesterol based 7-dehydrocholesterol molecule is localized in the skin. Exposure of the skin to sunlight (ultraviolet light 270-300 nm) proceeds to catalyze a reaction which converts this cholesterol-based molecule into a precursor form of active vitamin D. Also, hormonally inactive, naturally occurring dietary sources of the vitamin exist as vitamin D_3 (cholecalciferol), and a less defined vitamin D_2 (ergocalciferol). Activation of the precursor forms transpires via hydroxylation of the molecules first in the liver and then in the kidney to hormonally active 1,25 dihydroxyvitamin D_3 (1,25 $(OH)_2$ D_3). Figure A represents the inactive precursor forms of vitamin D. Figure B illustrates the steps necessary for formation of active vitamin D_3.



Adapted from Glennville et al., 1998.

Precursor forms of vitamin D exist very briefly in plasma circulation. The liver rapidly absorbs VITD and catalyzes hydroxylation of the 25th carbon via cytochrome P-450 containing enzymes. Originally, scientists viewed these enzymes as inner mitochondrial membrane bound systems. However, Madhok and DeLuca (1979) reported that rat liver microsomal fractions requiring NADPH, molecular oxygen, a flavoprotein, and a cytochrome P-450 was capable of 25-hydroxylation of VITD. Unfortunately this cytochrome P-450 has never been cloned. More recently, Axen et al. (1994) demonstrated microsomal fractions of pig hepatic cells contain the ability to cause 25-hydroxylation of VITD. Thus, current ideology suggests hydroxylation of VITD to its primary circulating form of 25-hydroxyvitamin D_3 (25 (OH) D_3) occurs in the liver by cytochrome P-450 membrane bound enzymes in either microsomal or mitochondrial cells. Hydroxylation of the 25th carbon is loosely regulated; furthermore, the fate of 25 (OH) D_3 depends upon the calcium needs of the animal. Animals with adequate amounts of circulating calcium convert 25 (OH) D_3 to 24,25 $(OH)_2$ D_3. However, if an immediate need for calcium exists in vivo, 1α-hydroxylation of 25 (OH) D_3 occurs in the kidney, to form active 1,25 dihydroxyvitamin D_3 (1,25 $(OH)_2$ D_3).

Hydroxylation of 25 (OH) D_3 to either active or biologically inactive steroid depends partially upon the current blood concentration of ionized calcium. If adequate amounts of plasma calcium exist, 25 (OH) D_3 is further hydroxylated to 24,25 $(OH)_2$ D_3. Likewise, if 1,25 $(OH)_2$ D_3 levels are increased in the blood, then inactivation of the steroid also begins with hydroxylation at carbon 24. Inactivation is accomplished via 25 (OH) D_3-24-hydroxylase enzymes. Tanaka et

al. (1977) stated that 24 hydroxylation renders 1,25 $(OH)_2$ D_3 ten times less biologically active. Increased 24-hydroxylase activity serves as a metabolic prevention for hypercalcemia. Moreover, 24 hydroxylation serves as the initial catabolic step of the 24-oxidation pathway carried out by VITD target tissues. 24-oxidation leads to the formation of calcitroic acid, which is the principal biliary excretory form of 1,25 $(OH)_2$ D_3. Makin et al. (1989) demonstrated the ability of vitamin D to induce calcitroic acid production in bone and kidney when high concentrations of active vitamin D exist in plasma circulation. Subsequently, researchers then proved the ability of vitamin D target organs, primarily kidney, intestine, and bone, to cause 24-oxidation under the influence of increased calcium levels in the blood. However, if an urgent need for calcium exists in vivo, 25 (OH) D_3 is further hydroxylated in the kidney to active vitamin D hormone.

Hydroxylation of 25 (OH) D_3 is highly regulated by ionized blood calcium levels via sensory receptors in the parathyroid gland. Rasmussen and DeLuca (1963) identified the parathyroid gland as the primary calcium-sensing organ in the body. Hypocalcemia, or a decrease in plasma calcium below metabolically stable levels, causes immediate stimulation of the parathyroid gland to secrete parathyroid hormone (PTH). PTH travels rapidly in the blood, and initiates a series of events that cause calcium to be absorbed into plasma circulation. The primary receptor for PTH is found in the kidney, where stimulation causes several reactions. First, PTH acts on the kidney to decrease phosphate absorption, which causes phosphate diuresis. The resulting decrease in plasma phosphorus concentration serves to aid calcium absorption by increasing the intestines ability

to absorb phosphate. Furthermore, PTH stimulates the kidney to initiate hydroxylation of 25 (OH) D_3 to active hormone (1,25 $(OH)_2$ D_3). Enzyme activity for formation of hormonally active vitamin D occurs in the proximal convoluted tubule cells of the kidney via mitochondrial cytochrome P-450 enzymes known as 25-hydroxyvitamin D-1α-hydroxylase. Moreover, PTH secretion decreases 24-hydroxylase activity, which further increase the amount of active hormone in the blood. All of the above mentioned reactions act harmoniously to increase serum calcium levels.

1,25 $(OH)_2$ D_3 acts directly on the intestines to increase calcium and phosphate absorption. Scientists are certain that both mechanisms are active calcium transport systems and both act independently of each other, but their mode of action has not been definitively explained. Wasserman and Feher (1977) finished research that led to the analysis of a calcium pump in the basolateral membrane, which was induced by 1,25 $(OH)_2$ D_3. However, Thomasset (1997) elucidated that 1,25 $(OH)_2$ D_3 stimulates the production of calbindin D9K, a gut protein that aids calcium absorption. None the less, 1,25 $(OH)_2$ D_3 effectively causes direct calcium absorption in the lower gut. Furthermore, 1,25 $(OH)_2$ D_3 works indirectly to increase serum calcium by stimulating target tissues, which contain vitamin D receptor sites.

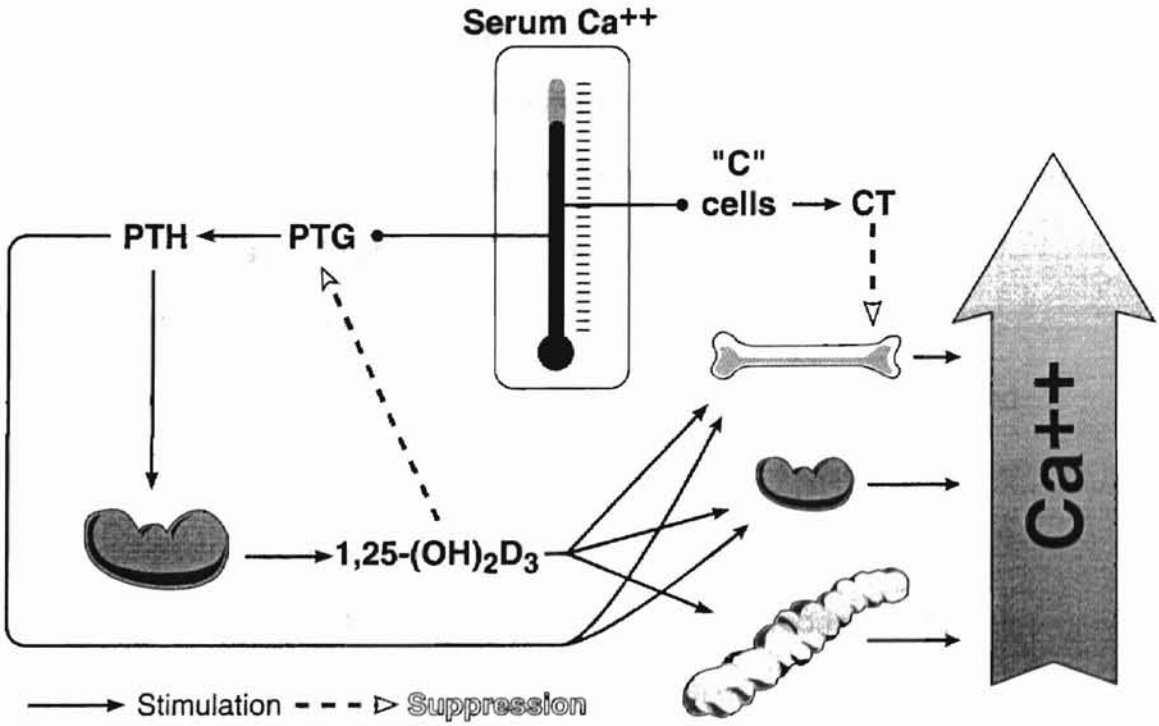
1,25 $(OH)_2$ D_3 and PTH synergetically cause reabsorption of calcium in the distal renal tubule. Moreover, both are required for osteoclastic activity to increase. Osteoclasts act on bone to cause resorption of minerals. However, osteoclasts lack specific PTH or 1,25 $(OH)_2$ D_3 receptor sites. Thus, resoprtion of

calcium from bone occurs via simultaneous stimulation of the osteoblasts. Osteoblasts stimulate osteoclast differentiation factors that convert promyelocytes to monocytes. Monocytes represent precursors for the formation of osteoclasts. Garabedian et al. (1974) proved the necessity of both hormones in order for calcium resorption to occur. In the previously mentioned research, vitamin D-deficient animals in the presence of PTH were unable to mobilize bone calcium. Similarly, parathyroidectomized animals could not mobilize bone calcium in the presence of 1,25 $(OH)_2$ D_3, unless PTH was introduced.

Serum calcium levels in conjunction with 1,25 $(OH)_2$ D_3 play a major role in controlling calcium homeostasis. Tightly regulated calcium levels range between 8-10 mg/dl in cattle. This minute range is closely maintained in order to prevent metabolic disorders, primarily hyper and hypocalcemia. Hypocalcemic conditions result in increased secretion of PTH as discussed earlier. However, excessive levels of serum calcium are known metabolically as hypercalcemia. If uncorrected, hypercalcemia can cause calcification of soft tissue primarily in the kidney, heart, aorta, and intestine, leading to organ failure and even death. Thus, downregulation of 1,25 $(OH)_2$ D_3 production and calcium absorption becomes important for vitality.

Increased plasma levels of 1,25 $(OH)_2$ D_3 and calcium act to suppress the production of PTH and calcium absorption into the blood stream. Downregulation of 1α-hydroxylation occurs 2-4 hours after exposure of 1,25 $(OH)_2$ D_3 to the system. Increased levels of 1,25 $(OH)_2$ D_3 close a negative feed back loop to the parathyroid glad, ceasing secretion of PTH. Moreover,

downregulation of 1α-hydroxlase activity in the kidney is reciprocally associated with increased hydroxylation of carbon 24. This step serves as the catabolic mechanism for inactivation of vitamin D hormone as discussed earlier. Likewise, an increase in serum calcium levels stimulates the production of calcitonin by the thyroid gland. Calcitonin directly affects osteoclastic activity on the skeleton by decreasing the ability of osteoclasts and osteocytes to mobilize calcium from bone. Figure c represents in vivo calcium homeostasis.



Adapted from Glennville et al., 1998.
PTG, parathyroid gland; PTH parathyroid hormone; C cells, parafollicular cells of the thyroid that secrete calcitonin (CT).

Increasing serum calcium levels enough to prevent parturient paresis was the primary objective of Hibbs' and Pounden's research involving supplemental VITD. Their monumental discoveries led researchers in the 90's to theorize supplemental VITD as an innate mechanism of improving muscle tenderness.

Swanek et al. (1999) first postulated VITD supplementation of feedlot-finished steers could increase longissimus muscle tenderness. In their study, feedlot steers supplemented with 5 million IU of VITD per day produced longissimus dorsi steaks with significantly lower shear force values after 7 days of postmortem age. Likewise, Montgomery et al. (2000) supplemented feedlot-finished steers with either 5 or 7.5 million IU of VITD per day for 9 days, 10 days prior to harvest. They demonstrated significant improvements in shear force values of top loin and strip loin steaks aged 14 days from VITD supplemented steers. Moreover, Karges et al. (1999) researched the effectiveness of supplemental VITD to increase tenderness in steaks from the round in addition to longissimus dorsi steaks. In their study, feedlot-finished steers were supplemented with either 4 or 6 million IU of VITD for 6 days prior to harvest. Their research demonstrated gluteus medius steaks from VITD supplemented steers exhibited significantly lower shear force values after 14 days of postmortem aging when compared with gluteus medius steaks from control steers. Also, VITD supplemented steers produced biceps femoris steaks with significantly lower shear force values at 7 days of postmortem age over biceps femoris steaks from control steers. In all of the previously mentioned studies, plasma calcium levels were increased 20-30% over ionized blood calcium levels of control cattle. The underlying premise for increasing serum calcium is its ability to activate endogenous proteolytic enzymes, primarily the calpains, which could further enhance muscle tenderness.

Vitamin D effects on muscle tenderness

Well established is the fact that postmortem aging increases muscle tenderness. This phenomenon is definitively associated with endogenous proteolytic enzymes. The primary enzymes to date are calcium dependent enzymes, and the cathepsins. Alkaline enzymes exist, but under postmortem conditions pH levels move towards acidic levels, thus a response from these enzymes is unlikely. Catheptic enzymes are very effective at degrading the most abundant myofibrilar protein, myosin, under in vitro conditions. Unfortunately, their innate ability to escape the lysosome in vivo has been proven to be difficult. Thus, research directed specifically at the calcium dependent enzymes has received the most attention.

Originally recognized as calcium activated factor (CAF), researchers concluded that calcium dependent proteases were most likely those involved with postmortem proteolysis of myofibrilar muscle proteins. Dayton et al. (1981) and Goll et al. (1983) both conducted instrumental research that evidenced the existence of calcium dependent proteolytic enzymes. Their research demonstrated the existence of proteases that were dependent on micromolar as well as millimolar concentrations of calcium for activation. Koohmaraie et al. (1987) conducted research that further identified the calcium dependent enzymes, as well as their inhibitor. He identified the millimolar calcium dependent enzyme as Ca^{++}-requiring calcium-dependent protease (CDP-II). In his research, CDP-II remained nearly constant throughout postmortem aging

periods. Therefore, he concluded it to be relatively inactive in meat tenderization under normal postmortem conditions. He then identified the micromolar fraction as Ca^{++}-requiring calcium-dependent protease (CDP-I). CDP-I lost enzymatic activity within 24 hours postmortem, due to autolysis. This loss of enzymatic activity coincided with hydrolysis of the enzymatic inhibiton, as well as the period of greatest myofibrilar fragmentation. Thus, he concluded that CDP-I could be activated by calcium concentrations existing in postmortem muscle, and was the primary proteolytic enzyme active under normal postmortem conditions. Subsequent research (Morgan et al. 1993; Thomson et al. 1996; Pringle et al. 1997; and Boehm et al. 1998) reiterated the original findings of Koohmaraie. The concurrent results from these experiments identified μ-calpains as those requiring micromolar concentrations of calcium for activation (10μ*M*); m-calpain was identified as requiring millimolar concentration of calcium (200-300μ*M*), and calpastatin was identified as the enzymatic fraction that inhibits calpain activity. The findings again concluded that sufficient calcium exists in postmortem muscle to cause activation of μ-calpain, but not m-calpain. Enzymatic activation of μ-calpain causes myofibrilar fragmentation primarily by degradation of z-line structures. However, μ-calpain activity is limited because it utilizes enzymatic activity to cause hydrolysis of calpastatin, its innate inhibitor. One molecule of calpastatin is able to inhibit six molecules of calpain. Thus, greater quantities of calpain must exist over calpastatin in order for hydrolysis to occur (Shannon and Goll, 1985). Moreover, μ-calpain is autolytic, and causes self-degradation which decreases its ability to affect postmortem tenderization. Hence, researchers

began looking for ways to activate m-calpains. Morgan et al. (1991) and Whipple and Koohmaraie (1992) utilized calcium chloride injections to elicit activation of m-calpains. In both studies muscle tenderness was significantly increased as a result of increased calpain activity in response to the elevated levels of calcium. Swanek et al. (1999) demonstrated that VITD supplementation significantly decreased both μ-calpain and calpastatin activity at 24 h postmortem, indicating an increase in proteolytic activity. Though not significant ($P < 0.07$), m-calpain levels were approaching significantly decreased activity levels at 24 h postmortem, indicating calcium concentrations reached levels high enough to activate m-calpain. These results conclude VITD supplemtation adequately increased calcium levels in postmortem muscle enough to elicit increased activity from the calpain/calpastatin system.

Electrical Stimulation

Harsham and Deatherage (1951) first patented electrical stimulation as a means of improving meat tenderness. Shortly after its introduction, electrical stimulation was relied upon for the prevention of cold shortening. Carse (1973) demonstrated the ability of electrical stimulation to effectively prevent cold shortening due to its ability to increase postmortem glycolysis. To date, extensive research involving electrical stimulation of beef carcasses has been performed. The benefits associated with electrical stimulation include increased tenderness of cooked beef (Bouton et al., 1980; Cross et al., 1979; McKeith et

al., 1980, 1981; Savell et al., 1978a,b, 1979), and improved lean color and lean maturity (McKeith et al., 1980, 1981; Savell et al., 1978b, 1979). These conclusions are associated with electrical stimulation's ability to increase the rate and extent of postmortem metabolism.

Electrical Stimulation Effects on Postmortem Carcass Characteristics

Electrical stimulation readily affects development of quality indicating characteristics of muscle. Lean color and texture both respond positively to postmortem electrical stimulation (Smith et. al., 1977; Savell et al.,1978a,b, 1979; Calkins et al., 1980; Riley et al., 1980; McKeith et al., 1981; Salm et al., 1981; Roeber et al., 2000). Improvements in lean color and texture are associated with electrical stimulation's ability to increase the rate and extent of postmortem metabolism. Moreover, Purchas, (1990), Jeremiah et al. (1991), and Watanabe et al. (1995) all stated that development of muscle color is related to ultimate muscle pH, which ranges between 5.4-5.7. Electrical stimulation affects ultimate pH primarily by increasing the rate at which postmortem metabolic products accumulate in muscle. Lactic acid is the primary product, and is necessary for pH decline in postmortem muscle. As pH declines, hydrogen ion concentration increases. This increase likely affects the spatial arrangement of myoglobin, the protein, responsible for color formation in meat. Myogolobin, under anaerobic conditions, exists as deoxymyoglobin. Deoxymyoglobin binds a water molecule, and causes muscle to appear purple in color. Exposure of muscle to oxygen, converts deoxymyoglobin to the oxymyoglobin state, which is associated with the

bright cherry red color of exposed lean surfaces. Moreover, the spatial arrangement of myoglobin is associated with the oxidation state of its internally bound heme iron molecule. In myoglobin, heme iron exists as either ferrous (Fe^{2+}) or ferric (Fe^{3+}) iron. The less reduced ferrous iron allows myoglobin to readily convert between the oxy and deoxy state, depending on oxygen status. However, the fully reduced ferric iron converts myoglobin to metmyoglobin. Conversion of myoglobin to metmyoglobin is irreversible, and causes brownish discoloration of meat.

Subjective and objective color measures are both improved by electrical stimulation. Objective color measurements are evaluated mechanically via CIE L*, a*, and b* values. Swatland et al. (1982) suggested that electrical stimulation increases light reflectance values, which is measured via L* values. Clydesdale and Francis (1971) suggested that b* values are inversely related to the amount of blue, which in turn, is positively associated with the amount of deoxymyoglobin. Roeber et al., (2000) demonstrated the ability of electrical stimulation treatments to increase L* a* and b* values over non-stimulated control sides. Also important from a color standpoint, is electrical stimulation's ability to decrease the incidence heat ring formation.

Normal postmortem muscle reaches an ultimate pH of 5.4-5.7 normally within 48 hours. Heat ring is associated with graded levels of pH across muscle areas. Ample research has been compiled to support electrical stimulation's ability to decrease the incidence of heat ring (Cross, 1979; Henrickson, 1979;

Orcutt et al., 1984; Savell et al., 1978a,b, 1979; Smith et al., 1977). The premise for electrical stimulation's ability to decrease this unfavorable color development is once again associated with its ability to increase the rate at which ultimate pH is achieved in postmortem muscle. Furthermore, electrical stimulation causes desirable increases in muscle tenderness.

Electrical Stimulation Effects on Tenderness

Theories associated with electrical stimulation's ability to increase muscle tenderness include prevention of cold shortening, physical disruption of muscle fibers, and increased proteolytic activity.

Prevention of cold shortening was influential in the development of electrical stimulation technologies. Early electrical stimulation research concluded tenderness is increased partially due to prevention of temperature induced shortening of pre-rigor muscle (Chrystall and Hagyard, 1976; Davey et al., 1976; Bouton et al., 1980). Cold shortening occurs when pre-rigor muscle with adequate ATP reserves is exposed to extremely low temperatures (0 °C). Exposure of pre-rigor muscle to cold temperatures decreases the ability of both the sarcoplasmic reticulum and the mitochondria to effectively bind calcium ions. Calcium then leaches into intercellular spaces, and in the presence of ATP, cold shortening occurs due to continual contraction of the muscle fibers. Electrical stimulation is effective in preventing cold shortening because it depletes the ATP reserves, and hastens rigor onset. Post-rigor muscle does not cold shorten because of an inadequate supply of ATP.

Physical disruption of muscle fibers has also been associated with electrical stimulation. Savell et al. (1978a) suggested that massive contraction which occurs during electrical stimulation, could possibly cause disruption of muscle fibers. However, McKeith et al. (1980) found no evidence of structural damage due to electrical stimulation. Likewise, George et al. (1980) performed histological tests that also showed no indication of gross structural changes due to electrical stimulation, and suggested electrical stimulation improves tenderness in meat via denaturation of sarcoplasmic proteins. While the previously mentioned mechanisms for increased tenderization due to electrical stimulation are in question, well accepted is the ideology that electrical stimulation enhances the effectiveness of proteolytic enzymes.

Increased proteolytic activity in electrically stimulated muscle has been collaborated (Smith et al., 1977; Savell et al., 1978b, 1979, Dransfield et al., 1992; Eilers et al., 1996; Ho et al., 1996; Hertog-Meischke et al., 1997). Electrical stimulation's ability to increase proteolytic activity stems from its ability to increase pH decline, and physically disrupt cellular membranes. First, electrical stimulation weakens the integrity of the sarcoplasmic reticulum, and subsequently increases extracellular calcium concentrations. This increase is sufficient to enhance activity of the calpain/calpastatin enzymes. Ho et al., (1996) utilizing SDS-PAGE demonstrated the ability of electrical stimulation to increase degradation of myofibrilar proteins. In that study, titin, nebulin, desmin, and troponin-T all showed increased protein precipitation in electrically stimulated muscle samples. The resulting increase in proteolytic degradation was

suggested to be the result of increased calpain activity. Furthermore, Watanabe and Devine (1996) proved the importance of ultimate pH on degradation of titin and nebulin. In their research, electrical stimulation affectively increased degradation of these proteins by increasing the rate at which ultimate pH was achieved. Thus proving the importance of increasing H^+ ion concentrations in order to cause lysis of cellular membranes. This coincides with suggested modes of action given with early electrical stimulation research. Savell et al., (1978b), and Dutson et al., (1980) provided evidence suggesting electrical stimulation increased free activity of lysosomal enzymes. They hypothesized, electrical stimulation disrupts lysosomal membranes both physically and chemically allowing catheptic enzymes to leach into the extracellular spaces, and cause increased proteolysis.

Sufficient evidence has been compiled to conclude both vitamin D supplementation, and electrical stimulation are effective means of improving meat quality. The National Cattlemen's Beef Association has challenged the beef industry to discover a way of providing quality beef. The previous discussion culminated ample research that demonstrated two very effective ways of achieving that goal. Thus, the following research was conducted in order to determine if vitamin D supplementation and electrical stimulation could synergetically improve quality in beef from feedlot-finished steers.

CHAPTER 3

EFFECT OF SUPPLEMENTAL VITAMIN D_3 AND ELECTRICAL STIMULATION ON QUALITY AND PALATABILITY CHARACTERISTICS OF FEEDLOT FINISHED BEEF STEERS

ABSTRACT

The objectives of this study were to determine the effects of supplemental vitamin D_3 and electrical stimulation on carcass characteristics of feedlot finished beef steers (n = 36). Steers were randomly assigned to either a control (CON) or vitamin D_3 (VITD) supplemented diet. Steers, assigned to the VITD group, were supplemented with 5.0 million IU of vitamin D_3 per day for 5 d, and given a two day withdrawal period prior to harvest. Within one hour postmortem, one side from each carcass was electrically stimulated. Electrically stimulated sides were alternated between each carcass. Temperature and pH measurements were taken at 0, 3, 6, 12, and 24 h post-electrical stimulation. Following a 36 h postmortem chilling, carcasses were ribbed at the 12/13th rib interface to obtain carcass data and CIE L*, a*, and b* values. Strip loins (IMPS # 180, NAMP) were removed from each side. Steaks (2.54 cm-thick) were cut from each strip and assigned to one of four age by internal cook temperature groups (7 d * 70°C, 7 d *74°C, 14 d * 70°C, and 14 d *74°C). Steaks were utilized for Warner-Bratzler shear force determination and sensory evaluation. Results indicated a diet by electrical stimulation by postmortem age interaction ($P < 0.04$) for Warner-Bratzler shear (WBS) force values of strip loin steaks. Electrically stimulated sides exhibited significantly lower pH values at 0, 3, 6, and 24 h than those from non-stimulated sides. Likewise, L*, a*, and b*, values were significantly higher

for electrically stimulated sides. Sensory panelists preferred steaks cooked to an internal temperature of 70 °C for juiciness and overall tenderness across all treatments ($P < 0.01$). However, steaks from electrically stimulated sides received lower ($P < 0.02$) juiciness and overall tenderness ratings. An interaction between internal cook temperature and electrical stimulation significantly ($P < 0.04$) affected sensory perception of flavor intensity. The later outcome is likely a result of internal degree of doneness which affects tenderness. Tenderness is highly correlated to perception of taste. These results conclude quality indicating characteristics benefit from electrical stimulation. Unfortunately, VITD supplementation had no significant affect on tenderness, and no synergetic effect of VITD and electrical stimulation was evidenced.

(Key Words: Vitamin D_3, Electrical Stimulation, Tenderness)

Introduction

In the 1995 National Beef Quality Audit, inadequate tenderness ranked second in the list of top 10 concerns (Smith et al., 1995). As a result of this study, the National Cattlemen's Beef Association requisitioned the development of a procedure that could improve beef tenderness. Tenderness has been identified as the single most important factor affecting consumer's perception of taste (Dikeman, 1987; and Savell and Shackelford, 1992). Savell et al. (1978b) demonstrated that electrical stimulation accelerates postmortem aging and has the potential to increase tenderness under shorter postmortem durations. Also pertinent to electrical stimulation is its ability to increase carcass temperature and subsequently hasten pH decline. Savell et al. (1979) showed that electrically

stimulated sides had significantly lower pH values at 1 h and 6 h postmortem when compared to non-stimulated sides.

Perhaps most appealing to beef tenderness are the results discovered by Swanek et al. (1999). Results from this study indicated that supplementation of finished beef cattle with 5 million IU of VITD 5 d prior to harvest decreased ($P < 0.05$) shear force values of longisimuss steaks aged for 7 d. It has been demonstrated that feeding supplemental VITD increases plasma calcium levels (Hibbs and Pounden, 1955). The underlying assumption of increasing calcium is the activation of relatively stable muscle proteases such as calpains. Koohmarie et al. (1987) demonstrated that μ-calpain is involved in postmortem aging, but under normal conditions there is insufficient calcium substrate to activate m-calpain. Therefore, the increase in serum calcium as a result of VITD supplementation could activate m-calpain proteases, which could further enhance beef tenderness. Thus, this experiment was conducted to evaluate the use of supplemental VITD in combination with electrical stimulation and their associated ability to improve beef quality characteristics.

Materials and Methods

Thirty-six feedlot finished steers (550 kg mean weight) of mixed origin were obtained from a commercial producer and transported to the Willard Sparks Beef Research Center at Oklahoma State University. Upon arrival, steers were randomly assigned to either a control (CON) or vitamin D_3 (VITD) supplemented diet, and given a 2 week adjustment period prior to supplementation. Steers assigned to the VITD treatment were supplemented with 5.0 million IU of vitamin

D_3 per day for 5 d. Supplementation began 7 d prior to harvest to provide a 2 d withdrawal period.

Steers were humanely harvested at the Food and Agricultural Products Center located on the Oklahoma State University campus. Within 1 h of exsanguination, steers were split into equal halves. One side from each carcass was electrically stimulated, and sides designated for stimulation were alternated between carcasses. Stimulated sides were subjected to 25 pulses of an electric current rated at 330 volts and 1.5 – 2.0 amps. Each pulse lasted 1 s, with 1 s of rest between each pulse. Carcass sides were chilled for 36 h before being ribbed at the 12/13th rib interface. Sides were given sufficient time to bloom before USDA yield and quality grade factors were collected by two Oklahoma State University personnel. During collection of carcass data, each side was also evaluated for lean color, texture, and firmness using an eight point scales (8 = extremely bright cherry red, firm, and fine textured; 1 = extremely dark red, soft, and course textured). After collection of carcass data, strip loins (IMPS # 180, NAMP) were removed from both sides of each carcass. The strips were labeled, vacuum packaged, and stored at 4 °C for 7 d before being frozen at -10 °C. Six steaks (2.54 cm thick) were then cut from the loin end of each frozen strip, using a ban saw. Steaks designated for 7 d age were immediately repackaged and transferred back to the freezer. Steaks assigned for 14 d age were vacuum packaged, allowed to thaw 24 h, then aged an additional 7 days at 4 °C.

Color evaluation

Following collection of carcass data, objective color measurements (CIE L*, a*, and b*) of the logissimus dorsi at the 12/13th rib interface were obtained using a Minolta colorimeter (Minolta Chroma Meter CR-300, Illuminant D65, Minolta Corp., Ramsey, NJ). The Minolta was calibrated for L* (lightness), a* (±, red to green), and b * (±, yellow to blue) measures using a white ceramic tile with specifications of Y = 94.8, x = 0.3153, and y = 0.3318. Each loin eye was measured in triplicate. The values listed are the average of the three measurements taken per side.

pH and Temperature Determination

pH and temperature measurements were obtained using a UniFET microprocessor pH/mV/°C system with an ISFET sensor (UniFET incorporated, San Diego, Ca.). Measurements were taken via probe penetration into the longissimus lomborum muscle posterior to the 13th rib. The initial pH and temperature measurements, for paired sides, were taken immediately after one side was electrically stimulated. Subsequent measurements were taken at 3, 6, 12, and 24 hrs post-stimulation.

Shear Force Determination

Steaks from each diet and electrical stimulation treatment were randomized into one of four postmortem age by internal cook temperature groups (7 d * 70°C, 7 d *74°C, 14 d * 70°C, and 14 d *74°C). Prior to cooking (24 h), steaks were removed from the freezer and tempered at 4 °C. Steaks were

cooked to their corresponding internal cook temperature using an impingement convection oven (Lincoln impinger, Model 1132-000-A, Lincoln Foodservice Products, Fort Wayne, IN). Internal temperature was monitored with a Koch T9100 digital thermometer. Cooked steaks were allowed to cool to 25 °C before four to six, 1.27-cm diameter cores were removed parallel to the muscle fiber orientation. Shear force values measured in kg were determined for each core using a Universal Instron Testing Machine (Model 4502, Instron, Canton MS), with a Warner-Bratzler attachment. Individual steak shear force values were quantified by averaging each steak's respective cores.

Sensory Panel Evaluation

Sensory panelists were trained using the methods described by Cross (1978). Steaks (n = 2) designated for sensory evaluation were aged for 14 d at 4 °C, and cooked to either 70 °C or 74 °C internal temperature. Steaks were cooked using the same methods described earlier. After reaching their appropriate internal temperature, steaks were placed in individual aluminum pouches and transferred to the sensory testing room. Six to eight trained sensory panelists evaluated samples from each steak for juiciness overall tenderness, connective tissue amount, and flavor intensity on an 8 point scale (8 = extremely juicy, tender, none, and intense; 1 = extremely dry, tough, abundant, and bland), and off flavor on a 4 point scale (4 = none, 1 = intense).

Statistical Analysis

Treatments (diet, electrical stimulation, postmortem age, and internal cook temp) were arranged in a split, split, split-plot in a completely randomized design. Diet was the main unit factor, electrical stimulation was the first split unit factor, postmortem age was the second split unit factor, and internal cook temperature was the third split unit factor. Analysis of variance was performed utilizing the Mixed procedures of SAS (version 7.0, 1999). Means were separated using least squares means and probability differences with $\alpha = 0.05$. Interactions were examined by using standard error values ordered by slice effects in the LS means statement.

Results and Discussion

Carcass Traits

Carcass characteristics are listed in Table 1. Cattle in the VITD treated group produced higher (P <0.05) kidney, pelvic, and heart fat (KPH) percentages when compared with carcasses from CON cattle. These results agree with Vargass et al. (1998) who reported higher (P <0.05) KPH percentage values for vitamin D_3 plus vitamin E supplemented cattle versus control cattle.

Subjective color measurements are listed in Table 2. Electrically stimulated sides exhibited higher subjective lean color scores (P <0.05) when compared with non-stimulated sides. These results are in agreement with previous studies. Savel et al. (1978) and McKeith et al. (1981) demonstrated that electrical stimulation significantly increased lean color scores up to 12 % over non-stimulated sides. Increase in lean color is indicative of an increase in rate and extent of postmortem glycolysis (Smith et al. 1977). Since dark lean is characterized as having a high pH, electrical stimulation's ability to increase the rate of postmortem glycolysis and effectively cause lactic acid build up is a preventive means of avoiding dark lean.

Objective color score values are listed in Table 3. VITD supplemented cattle had higher (P < 0.05) b* values across all treatment groups when compared to CON cattle. Likewise, across all treatments, electrically stimulated sides exhibited higher (P < 0.05) CIE L*, a*, and b* values in comparison to non-

stimulated sides. These outcomes are similar to the discoveries made by Orcutt et al. (1984) and Roeber et al. (2000).

Research has demonstrated that altering pH affects reflectance (Swatland et al. 1982). These results could be attributed to differences in reflectance of light wavelengths as a result of increased proportions of oxymyoglobin over deoxymyoglobin. Moreover, discoveries made by Clydesdale and Francis (1971) suggested that b* values are inversely related to the amount of blue, which in turn, is positively associated with the amount of deoxymyoglobin. The later research further supports the results from the current study.

pH and Temperature Measurements

Table 4 represents the effect of interaction ($P < 0.05$) between diet and stimulation on initial pH. In both the CON and VITD diets, electrically stimulated sides displayed lower ($P < 0.05$) initial pH values when compared with non-stimulated sides. Table 5 depicts the effects of diet or electrical stimulation on pH measurements taken subsequent to initial pH. Across all treatments, electrically stimulated sides exhibited lower ($P < 0.05$) pH values at 3, 6, and 24 h post stimulation versus non-stimulated sides. Table 6 illustrates the effects of diet or electrical stimulation on postmortem temperature measurements. CON cattle had a higher ($P < 0.05$) temperature reading at 3 h postmortem when compared to VITD supplemented cattle. Electrically stimulated sides had higher ($P < 0.05$) initial temperature measurements (0 h) when compared with non-stimulated sides. The later outcome is attributable to electrical stimulation's

ability to increase the rate of postmortem glycolysis and increase postmortem muscle temperature.

Rapid loss of postmortem muscle temperature decreases the ability of postmortem glycolysis to cause favorable color development in meat. Electrical stimulation increases postmortem glycolysis and muscle temperature, which effectively causes build up of the anaerobic glycolitic product, lactic acid. Increasing lactic acid decreases muscle pH. If ultimate pH is attained before muscle temperature falls to low development of desirable muscle color is more achievable. Savell et al. (1978a 1978b, 1979) has produced ample research to support this phenomenon.

Shear Force Evaluation

Table 7 represents the diet by electrical stimulation by postmortem age interaction on Warner-Bratzler shear force values of strip loin steaks. Steaks from CON and either electrically stimulated or non-electrically stimulated sides had 51.4 % and 50.0 % respectively shear below a 3.89 kg shear force threshold. Steaks from VITD and either electrically or non-electrically stimulated sides had 38.9 % and 47.2 % respectively shear below the same threshold. The predominate effect was between age periods, within electrical stimulation treatment and the same diet. Likewise, significant differences exist between shear force values across age and diet treatments together. These results are difficult to defend, since the table primarily shows an age effect. However, results indicated a significant ($P < 0.04$) Diet*ES*Age interaction. These results

are not concurrent with results of recent more promising research. Swanek et al. (1999) and Montgomery et al. (2000) both produced research demonstrating the ability of supplemental vitamin D_3 to increase tenderness in steaks aged 7, or 14 d. In the previously mentioned research electrical stimulation was not a treatment and could offer an explanation for the lack of a VITD response in our study. Although not certain, we believe the use of vitamin D_3 is beneficial. In our opinion, improvements in technology that allow for early identification of cattle with probable tenderness problems will produce a need for use of supplemental vitamin D_3.

Figure 1 illustrates the effect of internal cook temperature on Warner-Bratzler shear force. Across all treatments, steaks cooked to 70 °C had lower ($P < 0.05$) shear force values than steaks cooked to an internal temperature of 74 °C. This result is plausible since steaks cooked to a higher degree of doneness have considerably less juiciness for lubrication. Cross et al. (1976) demonstrated that an increase in internal cook temperature causes increased cooking loss, and this loss has a negative affect on tenderness. Wheeler et al. (1999a) produced results indicative of the same. He confirmed that an increase in degree of doneness from 60 °C to 80 °C caused a 1.31 kg increase in Warner-Bratzler shear force values for steaks categorized as being "tender". Likewise, he demonstrated that steaks allocated to a tough category had a 3.38 kg increase in shear force when cooked to a higher degree of doneness. Although our increase in internal temperature was not as drastic, it stands to reason that any increase in

degree of doneness should increase cooking loss and subsequently decrease tenderness.

Figures 2, 3, and 4 represent the minimum, mean, and maximum shear force values for all of the treatments in this experiment. The tables are included primarily to demonstrate the range of shear force values obtained.

Sensory Evaluation

Table 8 demonstrates the effects of internal temperature or electrical stimulation on sensory perception of juiciness, tenderness, connective tissue, and off-flavor. Sensory panelists gave higher ($P < 0.05$) juiciness, and overall tenderness ratings to steaks cooked to an internal temperature of 70 °C, as well as steaks from non-stimulated sides. Connective tissue amount and off-flavor were not significantly affected by internal cook temperature or electrical stimulation.

Table 9 represents the interaction ($P < 0.03$) effects of internal cook temperature and electrical stimulation on sensory perception of flavor intensity. Steaks, from electrically stimulated sides cooked to an internal temperature of 70 °C received higher ($P < 0.05$) flavor intensity ratings when compared with electrically stimulated 74 °C steaks. Also, non-stimulated steaks had increased ($P < 0.05$) flavor intensity ratings over stimulated steaks when cooked to an internal temperature of 74 °C. Our results for juiciness partially coincide with earlier findings. Savell et al. (1978b) and Stiffler et al. (1986) demonstrated that electrical stimulation caused decreased sensory ratings for juiciness. This effect

could be attributable to decreased water holding capacity of electrically stimulated muscle. However, in those same studies tenderness ratings were not significantly affected as they were with the electrically stimulated sides in our study.

Implications

Electrical stimulation improves color development in postmortem muscle, as evidenced by this research. However, as long as sufficient substrate exists for pH decline, and carcass temperature is reduced slowly muscle color should develop desirably. Vitamin D_3 supplementaion can increase tenderness in steaks from beef cattle. In this study vitamin D_3 supplementation had no significant bearing on increasing tenderness in strip loin steaks. The highest mean shear force in kg for strip steaks in this study is slightly above the desirable tenderness threshold. We believe the lack of a Vitamin D_3 response is partially due to the initial quality of the cattle. As technology improves, identification of problematic cattle will likely provide a need for the procedures examined in this study.

Table 1. Least squares means for the effect of diet on carcass characteristics of feedlot finished steers

	Diet	
Characteristic	CON	VITD
In wt, kg	545.3	556.0
Out wt, kg	556.0	560.4
Carcass wt, kg	340.3	345.5
Dressing %	61.2	61.7
REA, cm^{2a}	82.8	82.2
Fat, cm^{a}	1.5	1.7
KPH[a]	2.1[d]	2.4[c]
Yield Grade	3.2	3.6
Quality Grade[b]	419.7	425.8
% Choice	44.4	77.8
% Select	55.6	22.2

[a]Fat measure taken opposite the rib eye; REA = rib eye area; KPH = Kidney, pelvic, and heart fat percentage.

[b]300 = slight; 400 = small; 500 = modest.

[cd]Within a row, means without a common superscript letter differ ($P < 0.05$).

Table 2. Least squares means for the effect of electrical stimulation on subjective quality characteristics of feedlot finished steers

	Electrical Stimulation	
Characteristic	ES	NES
Lean Color[a]	6.53[b]	6.00[c]
Lean Firmness[a]	5.72	5.86
Lean Texture[a]	5.75	5.78

[a]Lean color: 8 = extremely bright cherry red, 1 = extremely dark red; Lean firmness: 8 = extremely firm, 1 = extremely soft; Lean texture: 8 = extremely fine textured, 1 = extremely coarse textured.

[bc]Withiiin a row, means without a common superscript letter differ ($P < 0.05$).

Table 3. Least squares means ± SEM for the effect of diet or electrical stimulation on CIE, L* a* b* values

Color measure	Diet		Electrical stimulation	
	CON	VITD	ES	NES
L*[a]	34.33 ± 0.52	35.32 ± 0.52	35.25 ± 0.40[c]	34.41 ± 0.40[d]
a*[b]	21.67 ± 0.28	22.34 ± 0.28	22.28 ± 0.23[c]	21.63 ± 0.23[d]
b*[b]	9.15 ± 0.15[d]	9.73 ± 0.15[c]	9.70 ± 0.14[e]	9.18 ± 0.14[f]

[a]"L" value is a measure of lightness (0 = black, 100 = white).

[b]"a" is a measure of green (-100) to red (+100). "b" is a measure of blue (-100) to yellow (+100). Both values represent their respective wavelength being reflected.

[cdef]Within a row, means without a common superscript letter differ ($P < 0.05$).

Table 4. Least squares means ± SEM for interaction between diet and electrical stimulation on initial pH

	Diet	
Electrical stimulation	CON	VITD
ES	6.31 ± 0.03[b]	6.23 ± 0.03[b]
NES	6.48 ± 0.03[a]	6.52 ± 0.03[a]

[ab]Within a column, means without a common superscript letter differ ($P < 0.05$). Within a row means are statistically similar ($P > 0.05$).

Table 5. Least squares means ± SEM for the effect of diet and electrical stimulation on pH measures subsequent to initial pH

h, post-stimulation	Diet		Electrical stimulation	
	CON	VITD	ES	NES
3	5.63 ± 0.03	5.66 ± 0.03	5.54 ± 0.03[b]	5.76 ± 0.03[a]
6	5.54 ± 0.03	5.55 ± 0.03	5.50 ± 0.03[b]	5.60 ± 0.03[a]
12	5.69 ± 0.05	5.67 ± 0.05	5.65 ± 0.04	5.70 ± 0.04
24	5.47 ± 0.01	5.48 ± 0.01	5.46 ± 0.01[b]	5.49 ± 0.01[a]

[ab]Within a row, means without a common superscript letter differ ($P < 0.05$).

Table 6. Least squares means ± SEM for the effect of diet and electrical stimulation on postmortem temperature measurements

h, post-stimulation	Diet		Electrical stimulation	
	CON	VITD	ES	NES
0	37.85 ± 0.27	38.52 ± .027	38.63 ± 0.20[a]	37.74 ± 0.20[b]
3	28.87 ± 0.36[a]	26.56 ± 0.36[b]	27.68 ± 0.35	27.75 ± 0.35
6	19.78 ± 0.30	19.37 ± 0.30	19.61 ± 0.27	19.54 ± 0.27
12	12.27 ± 0.34	12.91 ± 0.34	12.41 ± 0.31	12.76 ± 0.33
24	5.77 ± 0.17	6.01 ± 0.17	5.77 ± 0.15	6.01 ± 0.15

[ab]Within a row, means without a common superscript letter differ ($P < 0.05$).

Table 7. Effect of interaction between diet, electrical stimulation and age on Warner-Bratzler shear force values of strip loin steaks from feedlot finished steers

Electrical stimulation	d, postmortem age	Diet	
		CON	VITD
ES	7	4.21 ± 0.12[a]	4.33 ± 0.12[a]
	14	3.85 ± 0.12[b]	3.83 ± 0.12[b]
NES	7	4.17 ± 0.13[a]	4.08 ± 0.12[a]
	14	3.67 ± 0.12[b]	3.87 ± 0.12[b]

Values in kg, reported as least squares means ± SEM.

[ab]Within a column, means without a common superscript letter differ ($P < 0.05$).

Within a row, means are statistically similar ($P > 0.05$).

Table 8. Least squares means ± SEM for the effect of internal cook temperature or electrical stimulation on sensory perception of juiciness, overall tenderness, connective tissue amount, and off flavor*

Sensory characteristic	Temperature		Electrical stimulation	
	70 °C	74 °C	ES	NES
Juiciness*	5.66 ± 0.09[a]	4.60 ± 0.09[b]	5.02 ± 0.09[d]	5.25 ± 0.09[c]
Overall tenderness*	5.74 ± 0.09[a]	4.97 ± 0.09[b]	5.22 ± 0.09[d]	5.48 ± 0.09[c]
Connective tissue amount*	6.33 ± 0.08	5.94 ± 0.08	6.06 ± 0.08	6.21 ± 0.08
Off flavor*	3.93 ± 0.02	3.87 ± 0.02	3.88 ± 0.02	3.93 ± 0.02

*Juiciness: 8 = extremely juicy, 1 = extremely dry; overall tenderness: 8 = extremely tender, 1 = extremely tough; connective tissue amount: 8 = none, 1 = abundant; off flavor: 4 = none, 1 = intense.

[abcd]Within a row, means without a common superscript letter differ ($P < 0.05$).

Table 9. Least squares means ± SEM for the effect of interaction between internal cook temperature and electrical stimulation on sensory perception of flavor intensity*

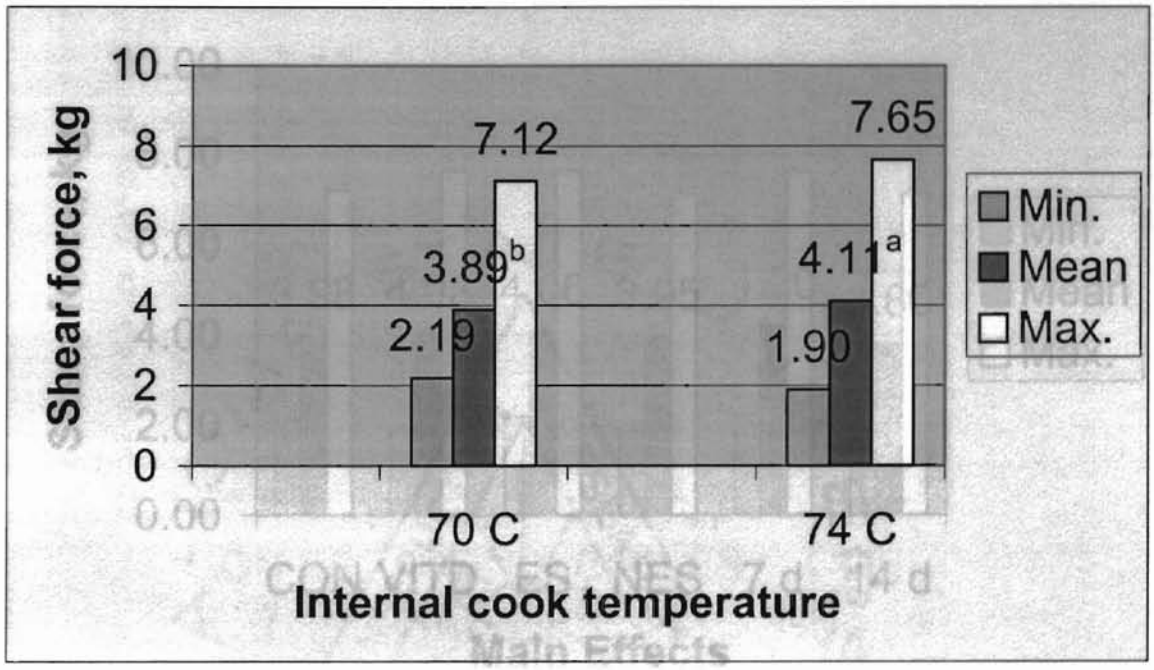
	Temperature	
Electrical stimulation	70 °C	74 °C
ES	5.53 ± 0.07^{ax}	5.14 ± 0.07^{by}
NES	5.44 ± 0.07^{ax}	5.32 ± 0.07^{ax}

*Flavor intensity: 8 = extremely intense, 1 = extremely bland.

^{ab}Within column, means without a common superscript letter differ ($P < 0.05$).

^{xy}Within a row, means without a common superscript letter differ ($P < 0.05$).

Figure 1. Range and mean for effect of internal cook temperature on Warner-Bratzler shear force values



[ab]Values without a common superscript letter differ ($P < 0.05$).

Figure 2. Range and mean for main effects of diet, electrical stimulation, and postmortem age on Warner-Bratzler shear force values

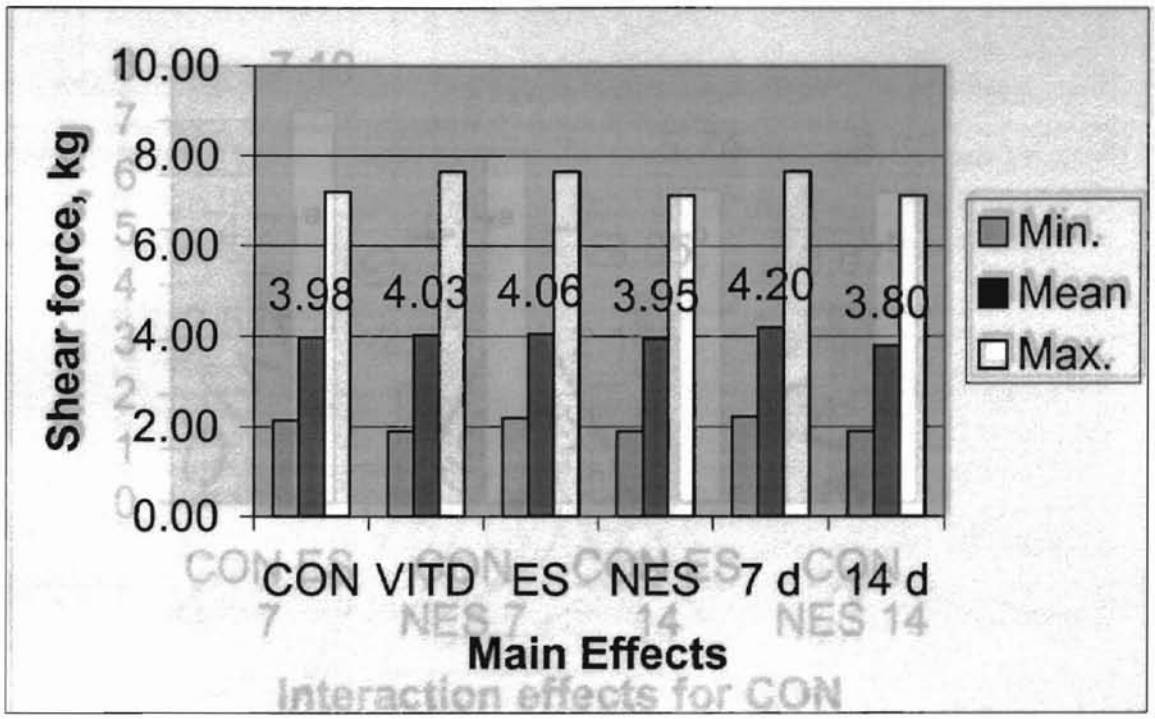
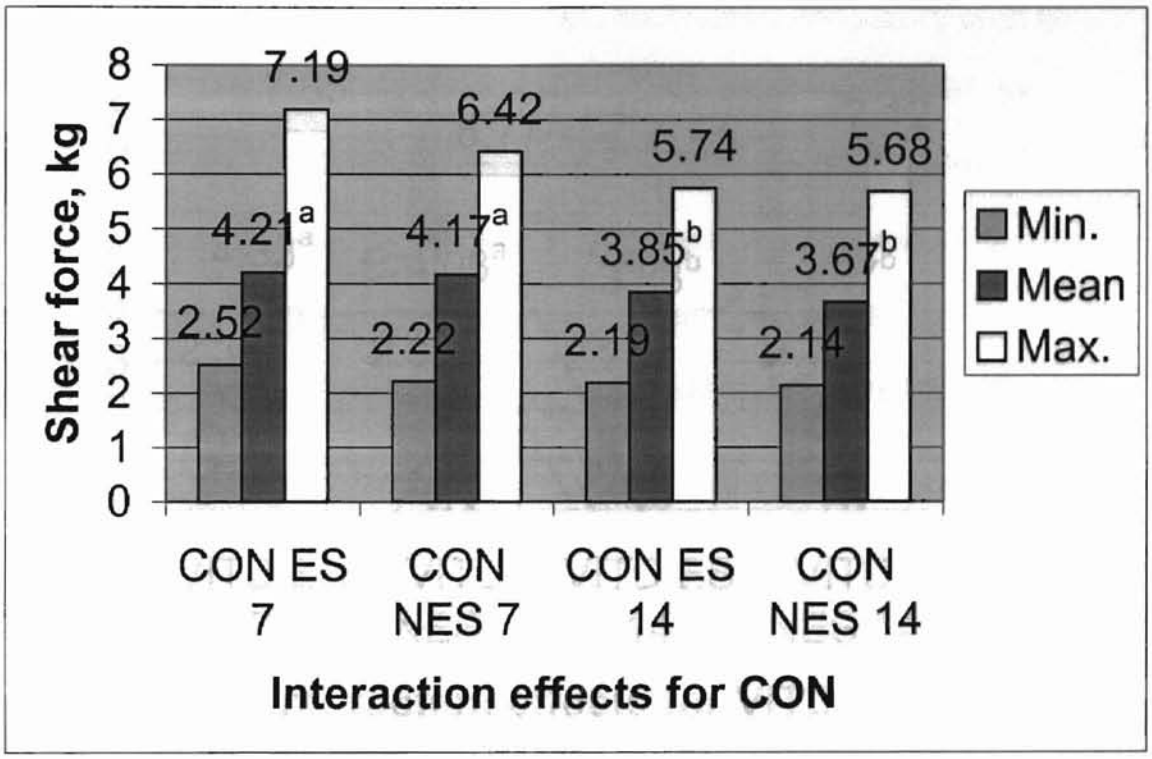
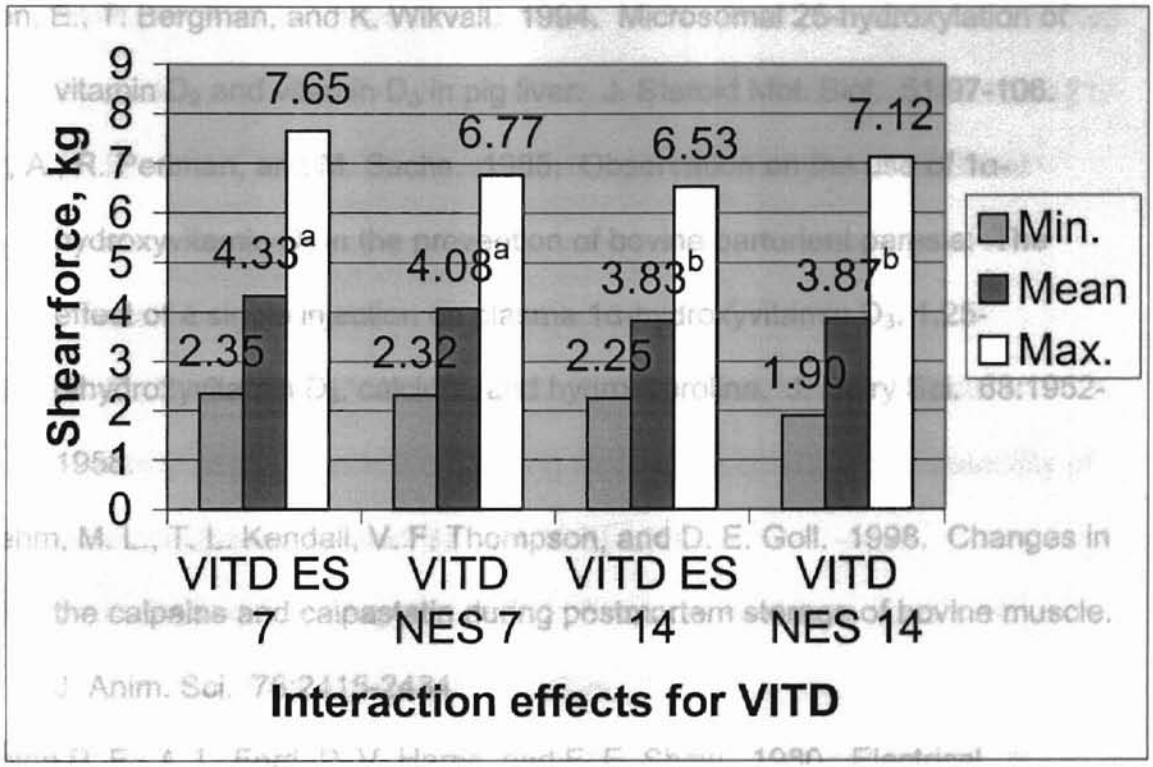


Figure 3. Range and mean for interaction effects of diet, electrical stimulation, and postmortem aging on Warner-Bratzler shear force values for CON steers



[ab]Values without a common superscript letter differ (P <0.05).

Figure 4. Range and mean for interaction effects of diet, electrical stimulation, and postmortem aging on Warner-Bratzler shear force values for VITD steers



[ab]Values without a common superscript letter differ (P <0.05).

Literature Cited

Axen, E., T. Bergman, and K. Wikvall. 1994. Microsomal 25-hydroxylation of vitamin D_2 and vitamin D_3 in pig liver. J. Steroid Mol. Biol. 51:97-106.

Bar, A., R. Perlman, and M. Sachs. 1985. Observation on the use of 1α-hydroxyvitamin D_3 in the prevention of bovine parturient paresis: The effect of a single injection on plasma 1α-hydroxyvitamin D_3, 1,25-dihydroxyvitamin D_3, calcium, and hydroxyproline. J. Dairy Sci. 68:1952-1958.

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.

Bouton P. E., A. L. Ford, P. V. Harris, and F. E. Shaw. 1980. Electrical stimulation of beef sides. Meat Science. 4:145-151.

Brooks, J. C., J. B. Belew, D. B. Griffin, B. L. Gwartney, D. S. Hale, W. R. Henning, D. D. Johnson, J. B. Morgan, F. C. Parrish, Jr., J. O. Reagan, and J. W. Savell. 2000. National Beef Tenderness Survey-1998. J. Anim. Sci. 78:1852-1860.

Calkins, C. R., J. W. Savell, G. C. Smith, and C. E. Murphey. 1980. Quality indicating characteristics of beef as affected by electrical stimulation and postmortem chilling time. J. Food Sci. 45:1330-1334.

Carse, W. A. 1973. Meat quality and the acceleration of postmortem glycolysis by electrical stimulation. J. Food Technol. 8:163-170.

Clydesdale, F. M., and F. J. Francis. 1971. The measurement of meat color. Food Prod. Dev: 5:87-91.

Cross, H. R., M. S. Stanfield, and E. J. Koch. 1976. Beef palatability as affected by cooking rate and final internal temperature. J. Anim. Sci. 43:114-121.

Cross, H. R. 1978. Meat cookery and sensory evaluation [American Meat Science Association, Results of Questionnaire]. Proceedings of the Annual Reciprocal Meats Conference AMSA. 30th:124.

Cross, H. R., G. C. Smith, A. W. Kotula, and D. A. Muse. 1979. Effects of electrical stimulation and shrouding method on qualith and palatability of beef carcasses. J. Food Sci. 44:1560-1564.

Chrystall, B. B., and C. J. Hagyard. 1976. Electrical stimulation and lamb tenderness. New Zealand J. Agri. Res. 19:7-11.

Dayton, W. R., J. E. Schollmeyer, R. A. Lepley, and L. R. Cortes. 1981. A calcium-activated protease possibly involved in myofibrillar protein turnover. Biochem. Biophys. Acta. 659:48-55.

Dayvey, C. L., K. V. Gilbert, and W. A. Carse. 1976. Carcass electrical stimulation to prevent cold shortening toughness in beef. New Zealand J. Agri. Res. 19:13-18.

Dransfield, E., D. J. Etherington, and M. A. J. Taylor. 1992. Modeling post-mortem tenerisation-II: Enzyme changes during storage of electrically stimulated and non-stimulated beef. Meat Sci. 31:75-84.

Eiler, 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.

Glenville, J., S. A. Strugnell, and H. F. DeLuca. 1998. Current understanding of the molecular actions of Vitamin D. Physiological Reviews. 78:1193-1231.

Goll, D. E., Y. Otsuka, P. A. Nagainis, J. D. Shannon, K. S. Sheridhar, and M. Maguruma. 1983. Role of muscle proteinases in maintenance of muscle integrity and mass. J. Food Biochem. 7:137-145.

Harsham, A., and F. E. Deatherage. 1951. Tenderization of meat. U. S. Patent 2,544,681.

Henrickson, R. L. 1979. Electricity and beef tenderness: a paper presented at the American Association of Meat Processors, Ceasars Palace, Las Vagas.

Hertog-Meischke, M. J. A., F. J. M. Smulders, J. G. van Logtestijn, and F. van Knapen. 1997. The effect of electrical stimulation on the water-holding capacity and protein denaturation of two bovine muscles. J. Anim. Sci. 75:118-124.

Hibbs, J. W., and W. D. Pounden. 1955. Studies on milk fever in dairy cows. IV. Prevention by sort-time, prepartum feeding of massive doses of vitamin D. J. Dairy Sci. 38:65-72.

Hibbs, J. W., W. D. Pounden, and W. E. Krauss. 1951. Studies on milk fever in dairy cows. III. Further studies on the effect of vitamin d on some of the

blood changes at parturition and the composition of colostrums in normal and milk-fever cows. J. Dairy Sci. 34:855-864.

Ho, C., M. H. Stromer, and R. M. Robson. 1996. Effect of electrical stimulation on postmortem titin, nebulin, desmin, and troponin-T degradation and ultrastructural changes in bovine longissimus muscle. J. Anim. Sci. 74:1563-1575.

Hodnett, D. W., N. A. Jorgensen, and H. F. DeLuca. 1992. 1α-hydroxyvitamin D_3 plus 25-hydroxyvitamin D_3 reduces parturient paresis in dairy cows fed high dietary calcium. J. Dairy Sci. 75:485-491.

Huffman, K. L., M. F. Miller, L. C. Hoover, C. K. Wu, H. C. Brittin, and C. B. Ramsey. 1996. Effect of beef tenderness on consumer satisfaction with steaks consumed in the home and restaurant. J. Anim. Sci. 74:91-97.

Jermiah, L. E., A. K. W. Tong, and L. L. Gibson. 1991. The usefulness of muscle color and pH for segregating beef carcasses into tenderness groups. Meat Science. 30:97-114.

Karges, K., J. B. Morgan, D. R. Gill, J. E. Breazile, and F. N. Owens. 1999. Effects of supplemental vitamin D_3 on feed intake and carcass characteristics of beef steers. In Review, J. Anim. Sci.

Koohmaraie, M, S. C. Seideman, J. E. Schollmeyer, T. R. Dutson, and J. D. Crouse. 1987. Effect of post-mortem storage on Ca++ dependent proteases, their inhibitor and myofibril fragmentation. Meat Science. 19:187-196.

Locker, R. H., and C. J. Hagyard. 1963. A cold shortening effect in beef muscle. J. Sci. Food Agric. 26:1721-1727.

Lorenzen, C. L., T. R. Neely, R. K. Miller, J. D. Tatum, J. W. Wise, J. F. Taylor, M. J. Buyck, J. O. Reagan, and J. W. Savell. 1999. Beef Customer Satisfaction: Cooking method and degree of doneness effects on the top loin steak. J. Anim. Sci. 77:637-644.

Madhok, T. C., and H. F. DeLuca. 1979. Characteristics of the rat liver microsomal enzyme system converting cholecalciferol to 25-hydroxycholecalciferol. Evidence for participation of cytochrome P-450. Biochem. J. 184:491-499.

Makin, G., D. Lohnes, V. Byford, R. Ray, and G. Jones. 1989. Target cell metabolism of 1,25 dihydroxyvitamin D_3 to calcitroic acid. Evidence for a pathway in kidney and bone involving 24-oxidation. Biochem. J. 262:173-180.

McKeith, F. K., J. W. Savell, and G. C. Smith. 1981. Tenderness improvement of the major muscles of the beef carcass by electrical stimulation. J. Food Sci. 46:1774-1776.

Mckeith, F. K., G. C. Smith, T. R. Dutson, R. L. Hostetler, and Z. L. Carpenter. 1980. Electrical stimulation of intact or split steer or cow carcasses. J. Food. Prot. 43:795-798.

Montgomery, J. L., F. C. Parrish, Jr., D. C. Beitz, R. L. Horst, E. J. Huff-Lonergan, and A. H. Trenkle. 2000. The use of vitamin D3 to improve beef tenderness. J. Anim. Sci. 78:2615-2621.

Morgan, J. B., J. W. Savell, D. S. Hale, R. K. Miller, D. B. Griffin, H. R. Cross, and S. D. Shackelford. 1991. National beef tenderness survey. J. Anim. Sci. 69:3274-3283.

Morgan, J. B., T. L. Wheeler, M. Koohmaraie, J. W. Savell, and J. D. Crouse. 1993. Meat tenderness and the calpain proteolytic system in longissimus muscle of young bulls and steers. J. Anim. Sci. 71:1471-1476.

Neely, T. R., C. L. Lorenzen, R. K. Miller, J. D. Tatum, J. W. Wise, J. F. Taylor, M. J. Buyck, J. O. Reagan, and J. W. Savell. 1998. Beef Customer Satisfaction: Role of cut, USDA quality grade, and city on in-home consumer ratings. J. Anim. Sci. 76:1027-1033.

Neely, T. R., C. L. Lorenzen, R. K. Miller, J. D. Tatum, J. W. Wise, J. F. Taylor, M. J. Buyck, J. O. Reagan, and J. W. Savell. 1998. Beef Customer Satisfaction: Cooking method and degree of doneness effects on the top round steak. J. Anim. Sci. 76:653-660.

Orcutt, M. W., T. R. Dutson, D. P. Cornforth, and G. C. Smith. 1984. Factors affecting the formation of a dark coarse band ("Heat-Ring") in bovine longissimus muscle. J. Anim. Sci. 58:1366-1375.

Pringle, T. D., S. E. Williams, B. S. Lamb, D. D. Johnson, and R. L. West. 1997. Carcass characteristics, the calpain proteinase system, and aged tenderness of Angus and Brahman crossbred steers. J. Anim. Sci. 75:2995-2961.

Purchase, R. W. 1990. An assessment of the role of pH differences in determining the relative tenderness of meat from bulls and steers. Meat Science. 27:129-140.

Rasmussen, H. and H. F. DeLuca. 1963. Calcium homeostasis. Ergebnisse Physiol. 53:107-173.

Riley, R. R., J. W. Savell, G. C. Smith, and M. Shelton. 1980. Quality, appearance and tenderness of electrically stimulated lamb. J. Food Sci. 45:199-203.

Roeber, D. L., R. C. Cannel, K. E. Belk, J. D. Tatum, and G. C. Smith. 2000. Effects of a unique application of electrical stimulation on tenderness, color, and quality attributes of the beef longissimuss muscle. J. Anim. Sci. 78:1504-1509.

Salm, C. P., E. W. Mills, E. S. Reeves, M. D. Judge, and E. D. Aberle. 1981. Effect of electrical stimulation on muscle characteristics of beef cattle fed a high energy diet for varying lengths of time. J. Food Sci. 46:1284-1289.

Savell, J. W., T. R. Dutson, G. C. Smith, and Z. L. Carpenter. 1978a. Structural changes in electrically stimulated beef muscle. J. Food Sci. 43:1606-1609.

Savell, J. W., G. C. Smith, and Z. L. Carpenter. 1978b. Beef quality and palatability as affected by electrical stimulation and cooler aging. J. Food Sci 43:1666-1668.

Savell, J. W., G. C. Smith, Z. L. Carpenter, and F. C. Parrish Jr. 1979. Influence of electrical stimulation on certain characteristics of heavy-weight beef carcasses. J. Food Sci. 44:911-913.

Savell, J. W., C. L. Lorenzen, T. R. Neely, R. K. Miller, J. D. Tatum, J. W. Wise, J. F. Taylor, M. J. Buyck, and J. O. Reagan. 1999. Beef Customer Satisfaction: Cooking method and degree of doneness effects on the top loin steak. J. Anim. Sci. 77:645-652.

Schroeder, T. C., and D. R. Mark. 1999. How can the beef industry recapture lost consumer demand? Proceedings of the American Society of Animal Science 1999. 1-13.

Shannon, J. D., and D. E. Goll. 1985. Properties of a protein that is purified from bovine skeletal muscel that inhibits the Ca^{2+}-dependent protease. Intracellular Prot. Catab. 257.

Smith, G. C., T. R. Dutson, Z. L. Carpenter, and D. A. Suter. 1977. Using electrical stimulation to tenderize meat. Proc. Meat Ind. Res. Conf. 29:147-155.

Smith, G. C., J. W. Savell, H. G. Dolezal, T. G. Field, D. R. Gill, D. B. Griffin, D. S. Hale, J. B. Morgan, S. L. Northcutt, and J. D. Tatum. 1995. The final report of the National Beef Quality Audit. Colorado Stat University, Fort Collins, Texas A&M University, College Station, and Oklahoma State University, Stillwater, NCA, Englewood, CO.

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

Swanek, S. S., J. B. Morgan, F. N. Owens, D. R. Gill, C. A. Strasia, H. G. Dolezal, and F. K. Ray. 1999. Vitamin D3 supplementation of beef steers increases Longissimus tenderness. J. Anim. Sci. 77:874-881.

Swatland, H. J. 1982. Meat color of pork chops in relation to pH and adductor capacitance of intact carcasses. J. Anim. Sci. 54:264-267.

Tanaka, Y., L. Castillo, H. F. Deluca, and N. Ikekawa. 1977. The 24-hydroxylation of 1.25 dihydrrxyvitam D_3. J. Biol. Chem. 252:1421-1424.

Thomasset, M. Calbindin-D9k. 1997. In: Vitamin D, edited by D. Feldman, F. H. Glorieux, and J. W. Pike. San Diego, CA: Academic, chapt. 14, p. 223-232.

Thomson, B. C., P. M. Dobbie, K. Singh, and P. A. Speck. 1996. Post-mortem kinetics of meat tenderness and the components of the calpain system in bull skeletal muscle. Meat Science. 44:151-157.

Vargas, D. N., H. Han, J. B. Morgan, and H. G. Dolezal. 1998. Effect of supplementing feedlot steers with vitamin D3 and E on carcass traits, shelf-life attributes and longissimus muscle tenderness. Masters Thesis, Oklahoma State University, Stillwater, Oklahoma.

Wasserman, R. H., and J. J. Feher. 1977. Vitamin D-dependent calcium binding proteins. In: Calcium Binding Proteins and Calcium Function, edited by R. H. Wasserman, R. A. Corradino, E. Carafoli, R. H. Kretsinger, D. H. MacLennan, and S. L. Siegel. New York: Elsevier/North-Holland, p. 292-302.

Watanabe, A., C. C. Daly, and C. E. Devine. 1995. The effects of the ultimante pH of meat on tenderness changes during ageing. Meat Science. 42:67-78.

Watanabe, A., and C. E. Devine. 1996. Effect of meat ultimate pH on rate of titin and nebulin degradation. Meat. Sci. 42:407-413.

Wheeler, T. L., S. D. Shackelford, and M. Koohmaraie. 1999a. Tenderness classification of beef: III. Effect of the interaction between end point temperature and tenderness on Warner-Bratzler shear force of beef longinssimus. J. Anim. Sci. 77:400-407.

Wheeler, T. L., S. D. Shackelford, and M. Koohmaraie. 1999b. Tenderness classification of beef: IV. Effect of USDA quality grade on the palatability of "tender" beef longissimus when cooked well done. J. Anim. Sci. 77:882-888.

VITA

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Master of Science

Thesis: EFFECT OF SUPPLEMENTAL VITAMIN D_3 AND ELECTRICAL STIMULATION ON QUALITY AND PALATABILITY CHARACTERISTICS OF FEEDLOT FINISHED BEEF STEERS

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