IMPACT OF FEEDING 2500, 50,000 OR 100,000 IU OF VITAMIN D_3 DAILY ON FEEDLOT PERFORMANCE AND COOKED BEEF TENDERNESS

By

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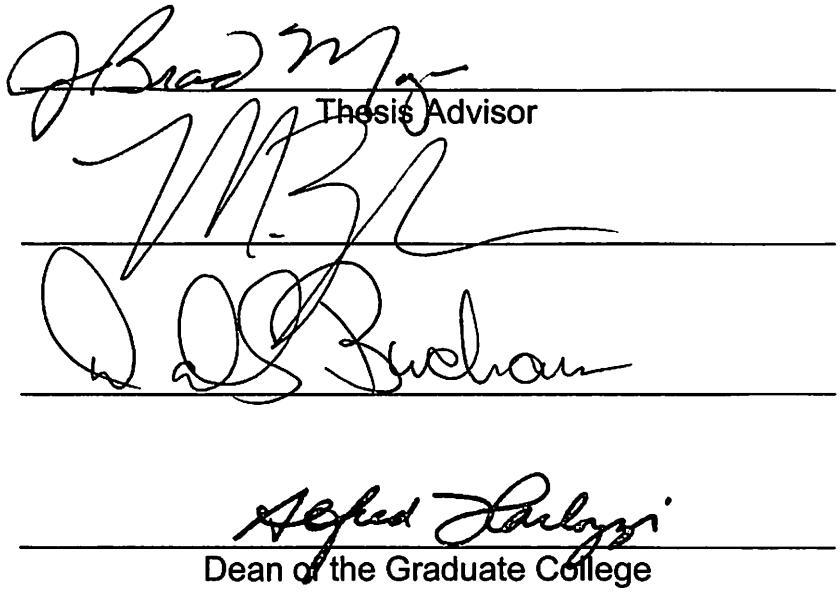

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FORMAT OF THESIS

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INTRODUCTION

As is well documented and understood, inadequate and inconsistent tenderness has plagued the beef industry for the last several decades (NBQA, 1995). Roughly 1 in 4 beef eating experiences is considered, by consumers; to be unacceptable in terms of tenderness by consumers (Smith et al., 1995). According to a study conducted by Miller and co-workers (2001), seventy-eight percent of consumers are willing to purchase beef steaks if the retailer guarantees them to be tender, and this could account for a $66.96/carcass premium when compared to beef carcasses classified as being tough (>4.9 kg Warner-Bratzler shear, WBS). Since the 1995 National Beef Quality Audit, the beef industry has implemented a multiple hurdle strategy to minimize the effects of poor palatability due to a lack of tenderness. With the onset of electrical stimulation (Savell et al., 1978), Tendercut® (Ludwig et al., 1997), mechanical tenderization (Hayward et al., 1980), enhancement solutions (Lansdell et al., 1995), and other tenderization methods, the beef industry has made great strides in improving the consistency of its beef products (Brooks et al., 2000). However, further methods of improving beef tenderness are crucial and need to be implemented into today's progressive beef processing industry.

Variation in tenderization is attributed to several factors such as the calpain proteases, μ-and m-calpain and their endogenous inhibitor, calpastatin.

It has long been known that aging meat for 14 days or longer has a positive influence on cooked tenderness (Nishimura et al. 1998), yet it is detrimental to shelf life and trim loss (Aberle, 2001). Pre-harvest supplementation of vitamin D_3 (VITD) to market-ready cattle has been shown to improve beef tenderness by increasing intracellular calcium concentrations, thereby hastening postmortem tenderization (Swanek et al., 1999). Swanek et al. (1999) and Karges et al. (1999) reported increased plasma calcium concentrations and a reduction in Warner Bratzler Shear Force (WBS) values when supplementing VITD for 4 to 6 days prior to harvest. However, both VITD studies displayed significantly declined intakes, decreased daily gain and lower gain to feed ratio when supplementing VITD at 4 to 6 million IU/hd/d for 4 to 6 days prior to harvest. Thus, the current research was conducted to determine the effects of feeding varying levels of VITD throughout the entire feeding period on feedlot performance, plasma and tissue calcium concentrations, longissimus tenderness, and meat sensory attributes.

Review of Literature

I. HISTORY OF VITAMIN D_3 RESEARCH

Rickets has been recognized as a disease since ancient times, but it was not until the time of the industrial revolution that rickets was thought to be of concern. The atmosphere of highly urbanized areas during the industrial revolution was filled with smoke from industrial plants. The limited exposure to sunlight combined with the common diets of most of the population lead to an epidemic of rickets within such areas around the globe. In a historical review by Feldman and co-workers (1997), it was reported that Sir Edward Mellanby decided to conduct research on the disease by studying the effects of oatmeal diets on dogs that were also restricted from ultraviolet light. The dogs developed severe rickets. Upon discovery of the vitamin A complex, first found in butterfat and cod liver oil, by McCollum and coworkers (1922), Mellanby (1919) decided to supplement the dogs with cod liver oil to test its potential impact on the prescence of rickets. The treatment cured the rickets disease, but Mellanby was unaware of any other possible substances and attributed the cure of rickets to vitamin A (Mellanby et al., 1919). McCollum did not concur with these reported findings and conducted further research involving fat soluble materials and their impact on rickets. His extended research demonstrated that cod liver oil was still an effective means to cure rickets even after destroying the activity of vitamin A by combined heat and aeration (McCollum et al., 1922). It was at this point that

McCollum determined that the rickets healing agent found within cod liver oil was due to a new and unknown substance that he termed "vitamin D". Based on the work done by Mellanby and McCollum VITD became known as the "essential nutrient".

Independent research conducted by Huldshinsky (1919) and Chick (1923) showed that children with rickets could be cured with exposure to ultraviolet rays. Later work done by Steenbock and Hart using lactating goats concluded that this phenomenon was due to ultraviolet lights ability to activate VITD activity, ultimately increasing calcium levels. This discovery brought on the idea that ultraviolet light could be used to irradiate and fortify foods. British researchers used irradiation on plant sterols as a means to isolate VITD, ergocalciferol (D_2). Further research done by Windaus and Bock (1923) concluded that there was another form of VITD that is present in the skin, cholecalciferol (D_3).

II. METABOLISM OF VITAMIN D_3

Almost twenty years after Mellanby's findings, Windaus and Bock (1937) synthesized the vitamin D precursor, 7-dehydrocholesterol, to form VITD (cholecalciferol) which gave researchers the structural characteristics of VITD. This conversion process naturally takes place in the plasma membrane of skin cells when the skin is exposed to ultraviolet irradiation. Activation of VITD does not occur until ring B of 7-dehydrocholesterol is broken (Feldman et al., 1997). This precursor has specific sites in the double bond system that enhance the absorption of ultraviolet light. This allows for the opening of the structure to form

cholecalciferol. In 1951, Hibbs and co-workers orally administered VITD to decrease the occurrence of milk fever in dairy cows. Early studies with low dosage levels (1-2 MIU/d) of VITD was not effective in increasing ionized serum calcium concentrations. This in turn led Hibbs and Pounden (1955) to increase the dose of VITD to (30 MIU/d) to find that it effectively increased serum calcium levels enough to reduce milk fever. Further research involved the effectiveness of VITD metabolites to also increase VITD in the blood. Bar et al. (1985) and Hodnett et al. (1992) conducted research that demonstrated the ability of intramuscularly administered 1a-hydroxy VITD and/or 25-Hydroxy-vitamin (25-$(OH)_2D_3$) to increase serum calcium and phosphorus levels in dairy cows. These results led researchers in the fields of animal nutrition and meat science to concur that VITD supplementation could elevate blood serum calcium levels high enough to cause increased activity of endogenous proteolytic enzymes. This brought on elevated calcium levels for postmortem activation of meat tenderization. Furthermore, hormonally inactive, naturally occurring dietary sources of the vitamin exist as VITD (cholecalciferol) and vitamin D_2 (ergocalciferol). These naturally occurring forms of VITD must be converted to the biologically active dihydroxycholecalciferol [1,25-$(OH)_2D_3$] form, which is produced by conversion of VITD to 25-$(OH)_2D_3$ via hydroxylation in the liver. Further metabolism in the kidney creates the active form 1,25-$(OH)_2$ D_3. This active form is the primary circulating form of VITD within blood plasma levels normally at 30 to 50 ng/ml (Feldman et al., 1997; Eisman et al., 1977; Best and Taylor, 1985). Hydroxylation during this phase may occur on either carbon 1, 25

or 26, with carbon 1 being the biologically active form of 1,25-$(OH)_2D_3$ (Feldman et al.,1997). This final hydroxylation occurs in the mitochondria within the proximal convoluted tubules of the kidney (Feldman et al., 1997; Chaney, 1997; DeLuca, 1979 and 1986; Norman, 1979). Best and Taylor (1985) reported the concentration of 1,25-$(OH)_2$ $D_3$is normally 20-50pg/ml in plasma with a half-life of fifteen hours in a human.

Researchers demonstrated that 1,25-$(OH)_2D_3$ was the active form of VITD through experimentation on anephric animals. The animals treated with 1,25-$(OH)_2D_3$ at biological levels responded with increased intestinal calcium absorption and bone calcium mobilization. Similar treatments of 25-$(OH)_2D_3$ doses did not receive a response. Carlsson et al. (1952) and Bauer et al. (1955) were the first to realize that a major function of VITD is to induce the mobilization of calcium to bone when required. Thus, those animals on low calcium diets have a rise in serum blood calcium levels due to VITD action on bone calcium mobilization.

III. VITAMIN D TOXICITY

There have been no reports of VITD toxicity due to prolonged exposure to the sun. It was reported by Holick et al. (1972) that nature has created a multiple hurdle system to prevent overproduction of VITD in the skin. Some authors suggest that this has created a correlation with latitude and skin pigmentation which limits the cutaneous production of VITD. In a review by Feldman and co-workers (1997), it was highlighted that Kreitmeir and Moll (1920) found large

amounts of ingested VITD in the diet resulted in VITD intoxication. As a result of excess VITD in a diet, 25-$(OH)_2D_3$ levels can be elevated from 30 to 50 ng/ml to 1000 ng/ml. Excess levels of 25-$(OH)_2D_3$ become competitive with 1,25-$(OH)_2$ D_3 for the vitamin D receptor. In effect high concentrations of 25-$(OH)_2D_3$ can induce reactions common of 1,25-$(OH)_2$ D_3. Thus this explains VITD intoxication in anephric humans or those without the ability to produce 1,25-$(OH)_2$ D_3.

It is understood in monogastric animals that VITD toxicity is associated with low levels of 1,25$(OH)_2D_3$. However, Horst and coworkers (1981) reported that in ruminants there is an opposite effect. VITD administered at 15 million IU intramuscularly in the ruminant significantly increased plasma levels of 1,25-$(OH)_2$ D_3. Horst and others concluded that this is the cause of VITD toxicity in ruminant animals. The level of toxicity can be dependent upon the type of VITD (vitamin D_3 or vitamin D_2), level of calcium and phosphorus in a diet, state of the kidney and composition of a diet. Elevated blood calcium levels due to VITD toxicity can cause calcification of the soft tissues and thinning of the bones. Also, weight loss, decreased appetite, and lowered blood phosphate levels are possible effects of VITD toxicity (Hoffman-LaRoche Inc., 1994). Toxicity can be reduced with the parallel supplementation of vitamin A or by thyroxine injections. Beckman et al. (1995) used rats to determine that dietary calcium restrictions in conjunction with VITD supplementation aided in the prevention of hypercalcemia. This combination lead to an increase in parathyroid hormone secretions which facilitated the depletion of 25-$(OH)_2D_3$ through metabolism to 1,25-$(OH)_2$ D_3 and then degradation to 24,25$(OH)_2D_3$. A contrasting diet with normal calcium levels

would decrease the effect of the parathyroid hormone. Accordingly, this down regulation enzyme activity resulted in high levels of 25-$(OH)_2D_3$ which could create a state of VITD intoxication. Wiegand and co-workers (2002) reported that blood plasma calcium levels consisting of 13 mg/l00 mL slightly decreased feed intake in pigs. Furthermore, Berry and co-workers (2000) reported steers supplemented with VITD at 6 mil IU/hd/d and elevated calcium for 5 d pre-harvest displayed significantly declined intakes, decreased daily gain and lower gain to feed. The later would concur with Karges et al., (2001) and Montgomery et al., (2002) who also reported decreased gains and reduced intake with supplemental VITD in feedlot beef animals.

IV. POSTMORTEM AGING

The beef industry's struggle with product inconsistency has been an ongoing battle for many years. In 1995, Smith and co-workers reported that a lack of consistently tender beef cost the industry $250 million annually. Thus, there is a constant incentive to improve beef tenderness. Some purchasers of beef products may specify a product that has been either dry aged or wet aged, in vacuum bags (Aberle, 2002). The aging process does bring about tenderization and flavor development, but must be balanced with the economical detriment of increased cooler shrink and trim loss due to increased storage time (Aberle, 2002). Postmortem aging of beef has been well documented as a means by which to increase tenderness through its association with endogenous proteolytic enzymes, primarily those being calcium activated (Eilers et al., 1996).

Enzymatic activation of μ-calpain causes myofibrilar fragmentation primarily by degradation of z-line structures. Dayton et al. (1981) and Goll et al. (1983) both conducted instrumental research that evidenced the prescence of calpain enzymes that were either dependent on micromolar or millimolar amounts of calcium for activation during postmortem aging. Koohmaraie et al. (1987) deemed that the more active of the calpain protease proteases was the micromolar fraction which are referred to as μ–calpains, and the less active form that requires millimolar amounts of calcium is referred to as m-calpains. In a study conducted by Xie et al. (1996), ribeye steaks from Wagyu-sired steers had significantly improved tenderness at day 10 compared to day 2 of wet aging as estimated by sensory panel and Warner-Bratzler Shear (WBS) measurements. In 1998, Weatherly reported that among aging periods of 4, 7, 10, 13, 16, 19, 22, 25, 28, 31 or 34 days, optimal tenderness was at 13 days using WBS. Also Smith et al.(1978) and Minks and Stringer (1972) had similar findings with subprimal rib cuts. In another study conducted by Eilers (1996), 256 strip loins and top sirloin steaks were aged for 6, 12, 18, or 24 days utilizing WBS and a trained sensory panel as measurements of tenderness and palatability. It was estimated that 12 days postmortem aging was required for "acceptable" tenderness of strip loin steaks (< 3.9 kg WBS), 24 days for "superior"(< 3.1 kg WBS). Top sirloin steaks required longer postmortem aging periods to achieve comparable WBS values.

V. CALCIUM ACTIVATED TENDERIZATION

As discussed earlier, calcium dependent proteases are most likely those involved with postmortem proteolysis of myofibrilar muscle proteins. Koohmaraie et al. (1990) identified the micromolar fraction as μ-calpain-dependent protease. He also identified the calpain inhibitor, calpastatin, which has been proven to inhibit proteolytic tenderization in cattle that are primarily of *Bos indicus* influence. Activity of μ–calpain is limited because it utilizes enzymatic activity to cause hydrolysis of calpastatin (Koohmaraie et al., 1992). One molecule of calpastatin is able to inhibit six molecules of calpain. In order for hydrolyses to occur greater quantities of calpain must exist over calpastatin (Shannon and Goll, 1985). Research by Pringle et al. (1998), concluded that Brahman cattle had greater calpastatin activity when compared to muscle from carcasses of Angus cattle.

Calcium-activated tenderization (CAT) is a postmortem procedure that has been successful in enhancing beef tenderness (Whipple and Koohmaraie, 1993). The CAT process utilizes a calcium chloride injection to activate the calpain proteinase system to enhance the degree of postmortem proteolysis (Koohmaraie et al., 1990). The tenderness of cuts retrieved from Bos indicus cattle could be increased with calcium injection, but were not equivalent to either Angus or F1 BrahmanxAngus. Koohmaraie et al. (1992) concluded that μ-calpain could be activated by calcium concentrations existing in postmortem muscle, and was the primary proteolytic enzyme active under normal postmortem conditions. Research conducted by Morgan et al. (1993), and Boehm et al.

(1998), backed up the original findings of Koohmaraie (1992). Wheeler and coworkers (1993) found that calcium chloride injected meat had increased tenderness via activation of the calpain proteolytic system. However, μ-calpain is autolytic, and causes self-degradation which decreases its ability to affect postmortem tenderization (Koohmaraie et al., 1990). Because of the later conclusion, Morgan et al. (1993) and Whipple and Koohmaraie (1992) utilized calcium chloride injections to cause activation of micro-calpains. In both studies, meat tenderness was greatly enhanced due to increased calcium levels.

VI. PREVIOUS RESEARCH WITH VITAMIN D_3 AND ITS RELATIONSHIP ON TENDERNESS

The importance of calcium ions was first demonstrated by Davey and Gilbert (1969) whereby they showed that the binding of calcium ions by the addition of ethylenediaminetetraacetic acid prohibited postmortem proteolysis. In an attempt to increase the amount of available calcium in post harvest muscles, researchers have been experimenting with the supplementation of VITD and its metabolites, 25-hydroxyvitamin D_3 and 1,25 dihydroxyvitamin D_3 (Montgomery et al., (2002) and Scanga et al., 2001). This elevated postmortem calcium is hypothesized to hasten the mechanisms of postmortem proteolysis, the breaking down of proteins by proteases. Swanek et al., (1997), reported a 12.6% increased plasma calcium concentrations for steers fed 5 mil IU/hd/day for 5 d ante-mortem against their unsupplemented counterparts. WBS values were 6.6% lower with 21.8% fewer tough steaks for steers supplemented VITD at 7 d

postmortem compared to steaks from CON animals. In experiment 2, by Swanek et. al (1997), steers supplemented with 7.5 M IU/hd/day for 10 d prior to harvest displayed lower (18% less) WBS values at 7 d postmortem aging and reduced tough steaks 23.3% and 22.5% at 14 and 21 postmortem aging times, respectively, compared to steaks from con carcasses. Also, Karges et. al. (1999) reported that supplementation of 6 M IU VITD for 4 to 6 d increased plasma calcium concentrations and decreased WBS values in *gluteus* and *longissimus* steaks with the longer feeding period having the strongest influence on increased tenderness. Likewise, Montgomery et al. (2000) supplemented feedlot-finished steers with either 5 or 7.5 M IU of VITD per day for 9 days and then withdrawn from supplementation the day prior to harvest, with increased ionized blood calcium levels 20-30% greater. Montgomery et al. (2002) supplemented steers with either 0, .5, 1, 2.5, 5, and 7.5 M IU/hd/d of VITD fed for 9 d consecutive pre-slaughter. In this study, plasma calcium increased linearly as level of VITD increased. Additionally, muscle calcium was increased by all VITD treatments when compared to controls (Montgomery et, al., 2002). As well, WBS values of strip loin steaks on 7 d postmortem aging period were reduced in animals fed either 0.5 or 7.5 M IU/hd/d when compared to CON steers. Top round steaks were more tender from cattle supplemented with 0.5, 1, and 5 x 10^6 IU/steer daily at 7 d, and all VITD treatments reduced WBS measurements for top round steaks at 10 d postmortem aging versus steaks from CON carcasses. The authors suggested that those animals that are inherently tough will benefit the most from the supplementation of VITD, and would have no impact on cattle that

innately produce tender beef. In contrast, heifers supplemented via oral bolus with either 1, 2, 3, 4 or 5 M IU/hd/d VITD for 2, 4, 6, or 8 d antemortem did increase serum Ca^{2+} concentrations compared with controls, but did not influence tenderness regardless of treatment or aging period (Scanga et.al., 2001). Foote and co-workers (2004) supplemented steers with either 1,25-$(OH)_2D_3$, 25-OH D_3 or VITD, and found that there was a trend for VITD and 25-OH D_3 to produce longissimus steaks with lower WBS values following 7 and 14 d postmortem aging, but 1,25-$(OH)_2D_3$ did not affect WBS values. In a similar study, 25-OH D3 was administered via bolus for either 4, 7, 21, or 35 d before slaughter (Wertz et. al., 2004). The bolus was sufficient to increase plasma 25-OH D_3 concentrations ($P < 0.0001$) through harvest, but the bolus did not improve plasma calcium concentrations, troponin-T degredation or WBS when compared to steaks from con animals.

VII. SLICE SHEAR FORCE METHOD FOR DETERMINING COOKED BEEF TENDERNESS

The use of WBS has proven to be an effective method of classifying and comparing the tenderness attributes of specific muscles and animals for the last decade. The standardization of this process was set forth by the National Cattlemen's Beef Association in 1994. Since, the need for more rapid methods of tenderness classification for packers and purveyors has been forthcoming. Shackelford and co-workers (1999) devised a method by which to classify the tenderness of longissimus thoracis and *longissimus lumborrum* muscles. The

slice shear force method (SSF), in contrast to WBS, can only by utilized on *longissimus* steaks of meat animals. This process automates the previous method of removing multiple 1.27 cm diameter cores from a *longissimus* steak. Now this can be done by obtaining 1 single slice from each steak. The dimensions of the single slice would be limited by ribeye size and muscle fiber orientation (Shackleford et, al., 1999). For high repeatability, a 5 cm sample of longissimus is obtained, and then is put into a standardized slice box specially designed for use with a double bladed knife that would pass through a longissimus muscle approximately parallel to the muscle fibers. Currently, a standardized 1 cm-thick, 5 cm-long slice from the lateral end of the longissimus muscle is used for this method (Shackleford et, al., 1999). Whereas WBS force uses a V-shaped blade, SSF uses a flat blade with the same thickness (1.016 mm) and degree of bevel (half-round) on the shearing edge. Shackelford et al., (1999) concluded that SSF was more strongly correlated with sensory panel tenderness rating than WBS (r.=.82 vs. .77) when measured at warm temperatures. In a subsequent study (Shackleford et al., 1999), 204 A-maturity carcasses were evaluated as duplicate samples with a repeatability coefficient of .89. In the same study, A-maturity carcasses (n=483) were classified into three groups (<23 kg, 23 kg to 40, and > 40 kg) based on SSF measurements at 3 day postmortem. These tenderness classifications differed in mean trained sensory panel tenderness ratings (7.3, 6.4, and 4.4, respectively) and the percentages (100%, 91%, and 28%) of those rated "Slightly Tender" or higher at 14 d postmortem.

VIII. SUMMARY

Increased intracellular calcium has been reported as a method by which to hasten postmortem aging. This has led researchers within the animal and meat science communities to look at the potential of VITD as a means to increase intracellular calcium. Previous VITD supplementation research has been reported as an effective means by which to increase tenderness, particularly those that are not inherently tender. However, research supplementing over 2 MIU/hd/d has evidenced negative effects on feedlot performance during feeding periods as short as 5 d pre-harvest. These negative effects have lead researchers to experiment with the supplemental feeding of VITD at lower levels (50,000 or 100,000 IU/hd/d) for the feeding duration as a potentially viable, cost effective method of improving cooked beef tenderness.

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IMPACT OF FEEDING 2500, 50,000 OR 100,000 IU OF VITAMIN D_3 DAILY ON FEEDLOT PERFORMANCE AND COOKED BEEF TENDERNESS

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ABSTRACT

One hundred eighty yearling steers (initial BW = 357± 28 kg) were used in a randomized complete block design to determine the effects of supplementing vitamin D_3 (VITD) throughout the finishing phase (146 and 181 d) on live animal performance and aged beef (7, 14, 21 d) *longissimus thoracic* (LT) tenderness. The current study compared the effects of 2500 (CON), 50,000 and 100,000 IU of VITD hd/d for the feeding duration. Compared to CON animals, no observed differences ($P > 0.05$) were noted for feed consumption (DMI), ADG, or carcass characteristics for animals supplemented with high levels of VITD. Plasma VITD (5.3, 17.3, 30.9 $\pm$ 0.8 ng/mL) and 25-hydroxyvitamin VITD (68.1, 97.0, and 117.0 $\pm$ 3.5 ng/mL) concentrations increased ($P < 0.0001$) as level of VITD supplementation increased (CON, 50,000 and 100,000 IU), respectively. Additionally, plasma 1,25-dihydroxyvitamin D_3 level numerically ($P = 0.13$) increased as dietary VITD supplementation increased. Liver and muscle levels

of VITD and metabolites did not differ ($P < 0.05$) among treatments. Compared to CON samples (d 7 and 14) steaks from carcasses of VITD supplemented steers displayed more desirable slice shear values ($P < 0.05$). However, advantage was negated with additional postmortem aging ($P > 0.05$). Also, there were corresponding tenderness advantages observed by trained sensory panelists as they rated CON samples tougher ($P < 0.05$) and less palatable than LT steaks from VITD treated carcasses. These findings suggest that VITD can be supranutritionally administered to beef cattle during the entire finishing phase while improving meat tenderness and maintaining live animal performance.

Key Words: Beef, Tenderness, Vitamin D, Performance

INTRODUCTION

Over the last decade researchers have looked at the potential of supplementing vitamin D_3 (VITD) during the feeding phase as a method by which to improve tenderness of cooked beef. It is hypothesized that increased dietary levels of VITD immediately prior to harvest will ultimately result in increased calcium concentrations in postmortem muscle (Koohmaraie et al., 1992). Takahashi and co-workers (1996, 1999) have proposed that the calcium theory of tenderization through postmortem aging is based on many myofibular changes resulting from elevated calcium levels (> .1 mm) resulting in the weakening of myofibular structures, such as Z-disks, linkages formed between actin and myosin at rigor mortis, titin and nebulin filaments, and the disassembly of desmin

intermediate filaments. This weakening of myofibular structures is thought to be the delicate relationship between available calcium and proteolytic enzyme activation, primarily due to μ-calpains activation (Koohmaraie, 1988; Goll et al., 1992).

Studies supplementing high doses (5 to 8 million IU per animal day) of VITD for 5 to 10 d antemortem improved Warner-Bratzler Shear force (WBS) of specific cooked beef cuts (Swanek et al., 1999; Montgomery et al., 2000, 2002). In a recent study conducted by Foote et al. (2004), oral dosing of VITD did not have a significant effect on cooked beef tenderness. Even with the potential for increased tenderness, there are numerous downfalls with using high levels of VITD even as brief as 5 to 10 d pre-harvest. These negative affects may include inferior live animal performance such as lower dry matter intake resulting in decreased growth performance and reduced hot carcass weights. Likewise, possible toxicological effects may occur during high VITD supplementation techniques applied immediately prior to harvest. These effects include high levels of VITD and its metabolites within the meat and an increased number of liver disposals (Montgomery et al., 2000, 2002; Foote et al., 2004). Thus, a method was hypothesized by which to lower the effective dose of VITD to eliminate any negative association with performance or toxicological effects while still achieving increased tenderness. This low dose, long term feeding method is thought to be more cost effective and have greater application within feeding systems.

MATERIALS AND METHODS

Animals

A total of 180 yearling steers (initial BW ~ 357 kg) were received at the Willard Sparks Beef Research Center in May, 2003. Upon arrival, steers were individually weighed and identified with an ear tag. Steers were then given ad libitum access to prairie hay and water until processing the next day. Based on initial weight, steers were stratified into 3 groups of 60 hd each. Within each respective weight block, steers were randomly allocated to 1 of 6 pens (n=10, each). Pens were 12.192 M X 30.48 M with 1.2 M of feed bunk per steer. Adjacent pens shared automatic waters. Each of the weight blocks were randomly assigned 1 of 3 dietary treatments: 2,500 IU (CON); 50,000 IU; or 100,000 IU VITD/steer per d. To minimize bias of cattle origin, cattle originating from a different source were equally distributed among pens and treatment groups.

Processing

On d 1 of the feeding period, each individual steer was weighed and given the following: vaccination with Titanium 5 L5^{TM1} and Vision 7 with SPUR^{TM2} (2 mL, sub-Q; Intervet Inc., Millsboro, DE); treatment with anthelmintics for internal and external parasites (7ml sub-Q; Ivomec-PlusTM3, Merial Limited, Iselin, NJ); and implantation with Revalor-S TM4 (20 mg TBA, 4 mg estradial; Intervet, Inc., Millsboro, DE). Steers were reimplanted with Revalor-S Tm4 and d 70. Weights were also recorded on d 35, 70, 105, 131, and 176. The heavy block was

chosen for blood sample recovery via venous puncture form the jugular into sterile 10 mL BD Vacutainer® (Beckton Dickinson & Co., Franklin Lakes, NJ) tubes containing sodium heparin. Plasma was then collected and frozen at 20°C for later analyses at The USDA-ARS National Animal Disease Center, Ames, IA.

Diet

All steers received a four stage diet (55%, 70%, 80%, and 87% DM of concentrate for 8, 6, 7, and 6 d, respectively) and a final finishing diet consisting of (DM basis) 80.7% rolled corn, 8.0% ground alfalfa hay, 3.0% fat, and 8.3% pelleted VITD supplement (Table 2). Cattle were fed twice daily at 0700 and 1300 hr. The supplemental VITD was administered as shown in Table 2. The OSU vitamin premix consisted of 19 parts fine ground corn to 1 part D3-500 [Roche Vitamins, Nutley Nj], to dilute the pure VITD to levels that would allow for the mixing of supplemental VITD directly into the pelleted supplement.

Dry matter intake was measured by collecting orts on appointed weigh days and at times of inclement weather. The listed treatment levels of 2,500 (con animals), 50,000 and 100,000 IU VITD were the established levels of intake. The samples were taken from each treatment and compiled by month. Assay of the supplements (Dr. Jonathon Wilson, Nutritional Pruducts, Inc., Parsippany, New Jersey) exhibited lower than anticipated levels of vitamin D3. Calculated average intake of VITD, based on assay results are displayed in Table 3.

Harvest

Visual appraisal of fat thickness combined with weight was the determining factor of optimum harvest time. Following 146 d of the feeding trial, the heavy and intermediate weight blocks were harvested at the commercial IBP/Tyson beef processing facility in Emporia, KS. The remaining group of steers were shipped and harvested after 181 days of feeding. All groups were shipped to Tyson Corporation in Emporia, Kansas. Oklahoma State University personnel accompanied cattle to the plant to maintain animal identification and obtain liver tissue samples upon evisceration for the heavy block. After a 30 hour chill period (1° C ± 1°) HCW, REA, fat thickness, KPH, marbling score, and USDA grades for quality and yield were recorded. The *longissimus thoracis* (LT) was retrieved from each carcass for analysis of sensory attributes and tenderness via the slice shear force method as described by Shackleford et al. 1999. Again the heavy block was utilized for collection of kidney and muscle tissue samples for procedure analyses of VITD and its metabolites as described by Montegomery et al. (2000).

LT sections (n=180) were brought to the Food and Agricultural Products Processing Center of Oklahoma State University, Stillwater OK. On d 4 postmortem, using a band saw, 2.54 cm thick steaks (n=3) were cut from each LT section and randomly assigned one of three postmortem aging periods (7, 14, or 21 d). Steaks were then vacuum packaged and stored at 4 °C for their respective aging period. After aging, steaks were frozen at -10°C for later analysis of tenderness of the longissimus dorsi via the slice shear force (SSF)

method. Also, another steak was removed, packaged, and aged 14 d postmortem and subsequently frozen for sensory analysis.

Objective Tenderness Determination

Prior to cooking (24 h), steaks were removed from the freezer and tempered at 4°C. Steaks were then cooked to an internal temperature of 70°C using an impingement convection oven (Lincoln Impinger, Model 1132-000-A, Lincoln Foodservice Products, Fort Wayne, IN). Cooked steaks were then stored overnight in a 5°C cooler. The SSF method developed by the Meat Animal Research Center, Clay Center, NE. was utilized. For maximum repeatability, a dual bladed knife was used to obtain a 1-cm-thick, 5-cm-long slice that is removed approximately parallel to the muscle fiber orientation on the lateral end of each longissimus steak as determined by USDA-ARS U.S. Meat Animal Research Center (MARC). Shear force values measured in kg were determined for each slice using a Universal Instron Testing Machine (Model 4502, Instron, Canton MS), with a SSF attachment.

Sensory Panel Evaluation

Steaks designated for sensory evaluation were aged for 14 d at 4°C and then frozen at -10°C. Before cooking, the steaks were thawed at 4°C (24 h) and then cooked as described for the SSF steaks. After reaching their appropriate internal temperature, steaks were placed in open zip lock bags and transferred to the sensory testing room. Five to six trained sensory panelists evaluated

samples from each steak for overall tenderness, juiciness, cooked beef flavor, off flavor and overall acceptance. Tenderness, juiciness and overall acceptance were evaluated using an 8 point scale (8 = extremely tender, extremely juicy, extremely acceptable; 1 = extremely tough, extremely dry, and extremely undesirable), while cooked beef flavor and off flavor was rated using a 3 point scale (3 = strong, 1 = not detectible).

Statistical Analysis

Data were analyzed as pen serving as the experimental unit. The PROC MIXED procedure of SAS was used to determine means and standard errors of means, with treatment level of VITD and weight block as fixed effects. For tissue and plasma samples, individual animal was considered the experimental unit. The PROC MIXED procedure of SAS was again used, but with load and treatment considered main effects, and pen and load*pen interactions within treatment considered random effects. A probability of less than .05 was considered significant.

RESULTS AND DISCUSSION

Feedlot Performance

Values for initial and interim BW, average daily gain (ADG), feed efficiency, and DMI are reported in Table 4. Initial body weight did not differ ($P > 0.05$) among VITD supplementation treatment groups. The final body weight for steers fed 100,000 IU of VITD/animal/d was 11 kg greater compared with CON

steers. Yet, there were no significant differences among treatments. The reduced level of VITD supplementation was successful in eliminating any negative affects toward growth performance as were reported by Karges et al. (2001), Scanga et al. (2001), and Montgomery et al. (2002). In all of these investigations, live animal feedlot performance was depressed as a result of VITD supplementation.

Average daily gain was based on a 4% shrink and applied both to interim and final body weight, and calculated by weigh period and by overall time on feed. Similar to final body weight, there were no differences in ADG ($P > 0.05$) among treatments. This would again be in contrast to findings from Scanga et al. (2001) in that that cattle receiving greater than 10×10^6 IU of VITD over an 8-d period immediately pre-harvest had significantly decreased ($P<0.05$) ADG than control cattle. In 2002, Montgomery et al. reported similar findings of decreased average daily gain ($P < 0.01$) when supplementing 5 and 7.5×10^6 IU VITD steer/d over the last 21 d of the feeding period.

In the current investigation, no observed differences among treatment groups for dry matter intake (DMI) were observed. Furthermore, no differences ($P > 0.05$) in feed efficiency (ADG:DMI) were observed when VITD levels of CON, 50,000; or 100,000 IU/hd/d were fed over the entire finishing phase. Montgomery et al. (2002) also reported no significant differences in daily feed intake with VITD supplementation. Even so, Montgomery et al. (2002) did observe a VITD supplementation x day interaction ($P < 0.02$) over a 9 d period, and reported that supplementing at levels of 2.5, 5, or 7.5 x 106 IU hd/d

decreased feed intake during d 7 and 8 compared with that of control steers ($P < 0.05$). Karges et al. (2001) reported numerically lower DMI for steers supplemented with VITD. In 2001, Scanga and co-workers found that 1×10^6 IU hd/d would decrease appetite after d 2 of supplementation. It appears that any degradative performance traits associated with supranutritional supplementation of VITD immediately prior to harvest can be overcome with long-term feeding of VITD.

Carcass Merit

Carcass merit characteristics as influenced by VITD supplementation are summarized in Table 5. Yield grade, quality grade, or maturity traits were not influenced by level of supplementation as was expected since there were no observed differences in live performance. This would comply with findings of Montgomery and co-workers (2002) who reported that hot carcass weight and dressing percentage were not affected by VITD supplementation for 9 d despite supplementation effects on average daily gain and daily feed intake.

Plasma and Tissue Concentrations

As shown in Table 6, VITD supplementation at 50,000 and 100,000 IU VITD hd/d significantly increased ($P < 0.05$) plasma VITD and 25-Hydroxy-vitamin D_3 (25-$(OH)_2D_3$) concentrations compared to CON steers. VITD and 25-$(OH)_2D_3$ plasma concentrations were 5.8 and 1.7 fold greater, respectively, in cattle receiving the 100,000 IU hd/d when compared to CON animals. Swanek et

al. (1999) also observed increased plasma calcium concentrations in VITD supplemented steers verses CON steers. Also, kidney tissue samples had significantly different ($P < 0.05$) concentrations of VITD and 25-$(OH)_2D_3$ with increasing level of supplementation. Concentrations of 1,25-Dihydroxyvitamin D_3 (1,25-$(OH)_2$ D_3) were numerically greatest in plasma liver tissue, and muscle tissue.

As the level of VITD supplementation increased, plasma calcium concentration also increased. This supports research done by Karges et al. (1999), Montgomery et al. (1999), and Swanek et al. (1999) who reported that increased levels of VITD supplementation subsequently increased plasma calcium concentrations. In 2001, Karges et al. reported blood plasma calcium concentrations were significantly greater ($P < 0.03$) for animals supplemented with 6×10^6 IU of VITD daily for 4 or 6 d prior to harvest, with cattle supplemented for 6 d having the greatest plasma calcium concentrations.

Slice Shear Force

Figure 1 represents the diet by postmortem aging interaction on slice shear force values of LT steaks. As traditionally is observed, LT steaks from CON Carcasses displayed a linear improvement in SSF values as postmortem aging time increased. However, as has been observed in previous studies investigating the impact of VITD supplementation on cooked beef tenderness (Swanek et al., 1999; Karges et al., 2001; Montgomery et al., 2000 and 2002), steaks from VITD supplemented cattle were more tender following short

postmortem aging times (i.e., ≤ 14 d) when compared to their CON steak counterparts. In fact, cattle supplemented with 100,000 IU VITD daily for the entire duration of the finishing period generated LT steaks which did not respond to postmortem aging. For example, postmortem aging was hastened for 100,000 IU steaks in that they displayed similar SSF values across all postmortem aging periods. It should be noted that following an extended aging period (21d), no differences in SSF tenderness values were observed across VITD treatment groups.

Sensory Evaluation

Figure 2 demonstrates the effect of VITD supplementation on sensory characteristics of tenderness, juiciness, cooked beef flavor, off flavor, and overall acceptance. Sensory panelists rated LT steaks from steers supplemented VITD as being more tender ($P < 0.05$) than their CON counterparts. However, there were not observed tenderness differences ($P > 0.05$) between LT steaks from steers supplemented with 50,000 or 100,000 IU hd/d VITD. This complies with Swanek et. al. (1999) who reported greater sensory panel ratings for tenderness from VITD treated steers when compared to CON steaks. Overall acceptance ratings of LT steaks from steers supplemented with 50,000 IU VITD hd/d were rated as being significantly higher ($P < 0.05$) when compared to CON. Steers supplemented with 100,000 hd/d VITD did not receive higher ($P > 0.05$) overall acceptance ratings compared to controls. Juiciness, cooked beef flavor, and off flavor were not significantly affected ($P > 0.05$) by dietary treatment of VITD.

IMPLICATIONS

The beef industry's efforts toward improving beef tenderness have helped to sustain market share with competing protein sources. In an effort to minimize unpleasant eating experiences for consumers much research involving the supplementation of VITD has been conducted as a way to further improve cooked beef tenderness for longissimus steaks that are not inherently tender. However, previous studies have observed detrimental effects toward feedlot performance and possible toxicological effects. With this in mind, the current study was developed to utilize the potential of VITD as a method of improving cooked beef tenderness while sustaining optimum feedlot performance.

Currently, the estimated cost of feeding 100,000 IU/hd/d is approximately $0.36 for a 150 d feeding period. The cost effectiveness of supplemental VITD at low levels for a typical beef finishing phase may have potential for producers, feeders, and packers to capture additional return through "guaranteed tender" branded beef programs. In conclusion, the supplemental feeding of VITD for the long term duration of the beef finishing phase appears to be an economically efficient, feedlot applicable way to improve beef tenderness while maintaining animal performance.

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Table 1. Diet composition and calculated nutrient value

Ingredients	% diet DM
Rolled corn	80.7
Ground alfalfa hay	8.0
Fat	3.0
Pelleted VITD supplement	8.3

Nutrient	DM basis
NE_m, Mcal/kg	2.03
NE_g, Mcal/kg	1.42
Crude protein, %	13.45
Calcium, %	0.70
Phosphorus, %	0.32
Potassium, %	0.60
Magnesium, %	0.16

Table 2. Vitamin D_3 supplement ingredients (% DM)[a] by treatment level

	VITD in Supplementation IU/hd/d		
Supplement	2,500 IU	50,000 IU	100,000 IU
Soybean meal	2.000	2.000	2.000
Cottonseed meal	2.000	2.000	2.000
Wheat midds	1.988	1.988	1.988
Limestone 38%	1.250	1.250	1.250
Salt	0.300	0.300	0.300
Vitamin A – 30,000 IU	0.011	0.011	0.011
Vitamin E – 50%	0.007	0.007	0.007
Rumensin – 80[b]	0.018	0.018	0.018
Zinc Sulfate	0.004	0.004	0.004
Manganous oxide	0.004	0.004	0.004
Copper sulfate	0.001	0.001	0.001
Selenium – 600	0.007	0.007	0.007
Urea	0.700	0.700	0.700
Tylan –40[b]	0.010	0.010	0.010
OSU vitamin premix	0.001	0.020	0.039

[a]Rumensin provided at the rate to supply 337.6 g/ton and Tylan provided at the rate to supply 96.5 g/t

[b]Elanco Animal Health, Greenfield, IN.

Table 3. Calculated average daily intake of assayed vitamin D_3 by target treatment consumed

Treatment	Assayed VITD level, IU/kg	Total VITD intake, IU/d
2,500 IU	4,748	3,607
50,000 IU	37,397	27,506
100,000 IU	88,998	70,075

Table 4. *Effect of vitamin D_3 supplementation on feedlot performance*

Item	VITD in Supplement IU/hd/d			SEM
	2,500 IU	50,000 IU	100,000 IU	
Pens	6	6	6	-
Initial weight, kg	356.0	357.0	356.0	0.47
Final BW, kg	593.0	589.0	602.0	4.28
Carcass adj. BW, kg[a]	610.0	618.0	616.0	3.49
DMI d 0 – finish, kg/d	9.41	9.50	9.63	0.18
ADG d 0 – finish, kg/d	1.49	1.53	1.56	0.03
Gain:Feed d 0 – finish, kg/kg	0.158	0.162	0.162	0.002
Carcass adj. ADG, kg/d	1.56	1.60	1.60	0.02
Carcass adj. gain:feed, kg/kg	0.174	0.181	0.180	0.003

[a]Carcass adj. BW calculated by dividing HCW by average dressing percent of each block.

Table 5. Effect of vitamin D_3 supplementation on carcass characteristics

	VITD in Supplement IU/hd/d			
Item	2,500	50,000	100,000	SEM
Hot carcass weight	384.00	389.00	388.00	2.19
12th rib fat thickness, cm	1.14	1.17	1.17	0.04
Longissimus muscle area, cm^2	87.92	86.18	87.40	1.33
Kidney, pelvic, and heart fat, %	2.20	2.20	2.30	0.10
USDA yield grade	2.30	2.40	2.20	0.08
Marbling[a]	380.00	376.00	380.00	7.04
Lean maturity[b]	172.00	180.00	174.00	3.08
Skeletal maturity[b]	159.00	158.00	156.00	3.69
USDA quality grade[c]	2.70	2.60	2.70	0.05

[a]Marbling score: 300 = slight[00], 400 = small[00].
[b]Maturity score: 100 = A, 200 = B.
[c]USDA quality grade: 3 = high select, 2 = low choice.

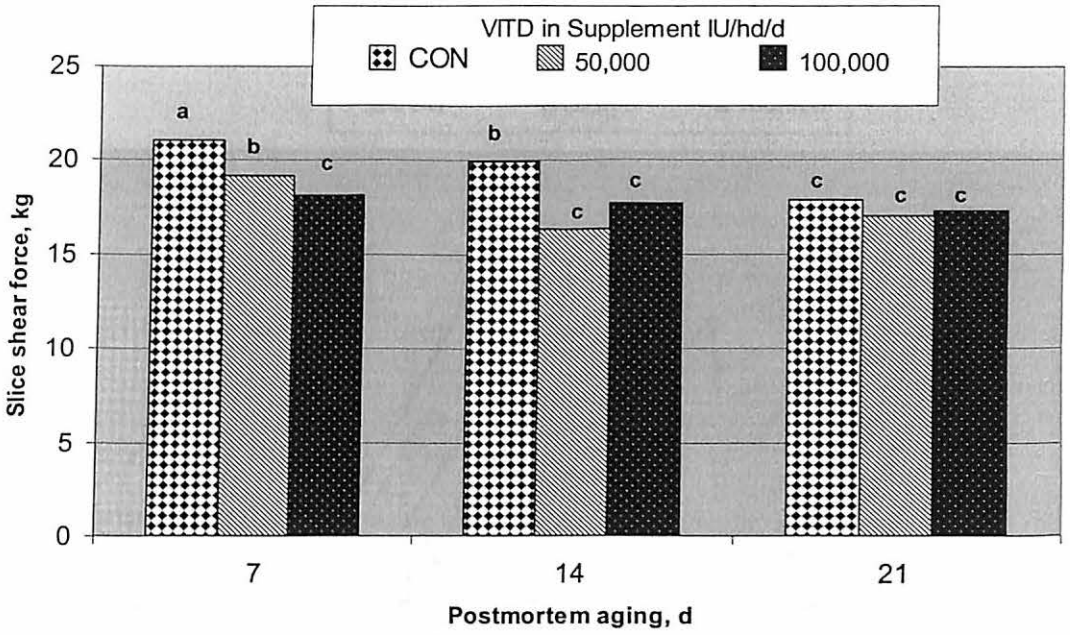
Table 6. Effect of treatment on vitamin D_3 and metabolite concentrations in plasma and tissue

	VITD in Supplement IU/hd/d				
	2,500	50,000	100,000	SEM	P>F
Plasma	60	60	60		
[a]D_3 (ng/gm)	5.32	17.25	30.19	0.82	<.0001
[b]$25D_3$ (ng/gm)	68.08	96.96	116.86	3.54	<.0001
[c]$1,25D_3$ (pg/gm)	53.81	46.63	60.15	4.68	0.13
Ca (mg%)	8.92	9.06	9.24	0.15	0.30
Mg (mg%)	1.69	1.68	1.73	0.07	0.89
Liver	44	44	41		
[a]D_3 (ng/gm)	38.11	45.93	47.11	3.79	0.12
[b]$25D_3$ (ng/gm)	6.95	8.52	13.58	2.87	0.35
[c]$1,25D_3$ (pg/gm)	138.20	97.28	134.05	18.29	0.22
Muscle	60	59	60		
[a]D_3 (ng/gm)	15.70	16.41	15.90	0.89	0.85
[b]$25D_3$ (ng/gm)	1.44	1.80	2.27	0.18	0.10
[c]$1,25D_3$ (pg/gm)	51.07	61.02	69.33	6.71	0.19
Kidney	57	57	41		
[a]D_3 (ng/gm)	5.51	25.05	39.71	3.75	<.0001
[b]$25D_3$ (ng/gm)	6.50	9.28	11.07	0.54	<.0001
[c]$1,25D_3$ (pg/gm)	126.95	156.17	131.82	37.28	0.83

[a]Vitamin D_3
[b]25-hydroxyvitamin D_3
[c]1,25-dihydroxyvitamin D_3

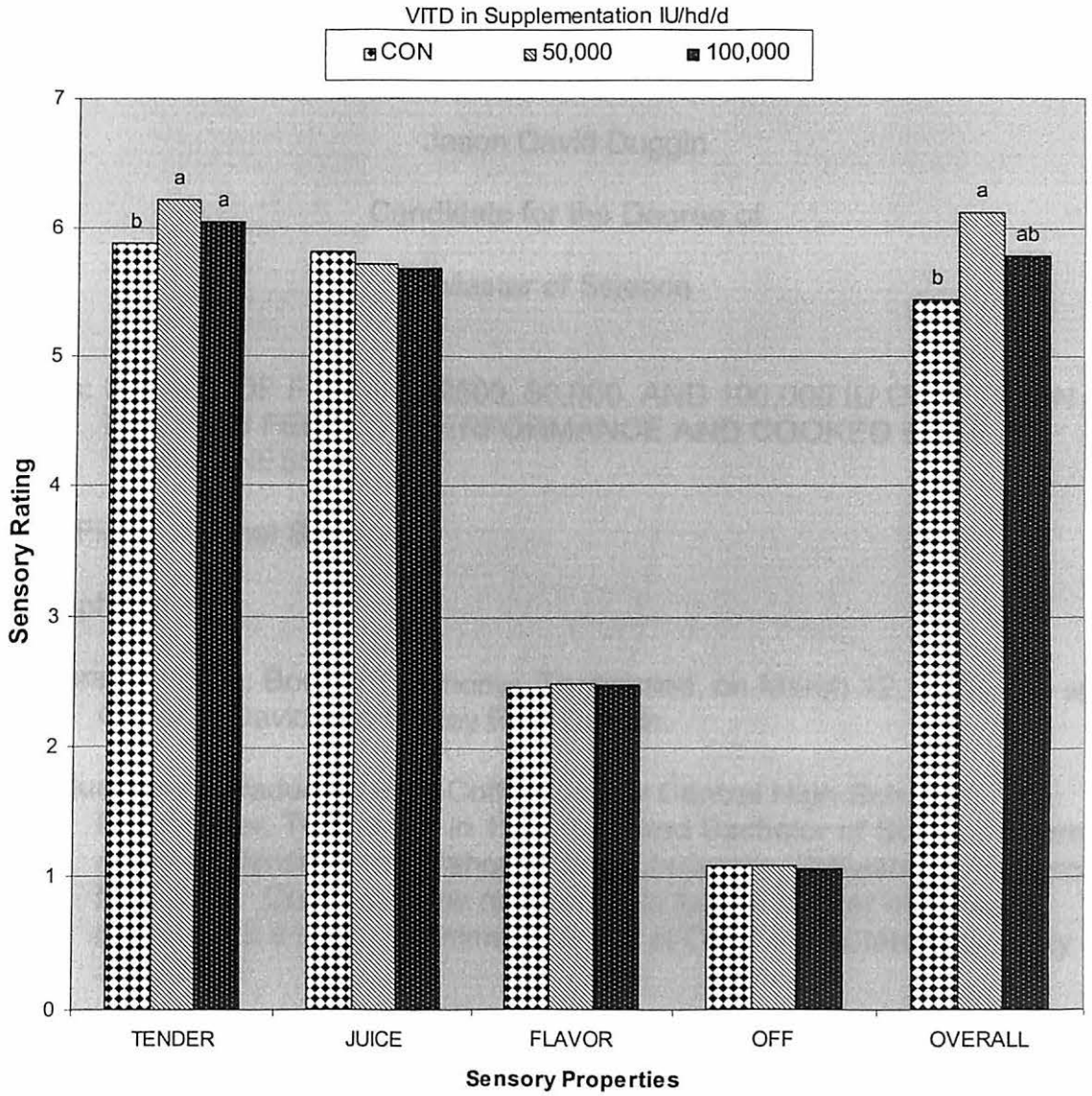
[abc] Means with different superscripts differ ($P < 0.05$)

Figure 1. Influence of postmortem aging and vitamin D_3 supplementation on *longissimus thoracis* slice shear force values.



Means with different superscripts differ ($P < 0.05$)

Figure 2. Influence of vitamin D_3 supplementation on sensory properties of *longissimus thoracis* steaks



[ab] Means with different superscripts differ ($P < 0.05$)

VITA

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Candidate for the Degree of

Master of Science

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