EFFECT OF SUPPLEMENTING FEEDLOT STEERS WITH VITAMINS D_3 AND E ON CARCASS TRAITS, SHELF-LIFE ATTRIBUTES AND LONGISSIMUS MUSCLE TENDERNESS

By

DAVID NORMAN VARGAS

Bachelor of Science

California Polytechnic State University

San Luis Obispo, California

1996

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
degree of
MASTER OF SCIENCE
May, 1999

OKLAHOMA STATE UNIVERSITY

EFFECT OF SUPPLEMENTING FEEDLOT STEERS WITH VITAMINS D_3 AND E ON CARCASS TRAITS, SHELF-LIFE ATTRIBUTES AND LONGISSIMUS MUSCLE TENDERNESS

Thesis Approved:

J. Brad Morgan
Thesis Adviser

Helen Dolezal

Fredrick K. Ray

Wayne B. Powell
Dean of the Graduate College

PREFACE

This study was conducted to investigate the combined effects of supranutritional supplementation of beef steers with vitamins E and D_3. Over the last 20 years the beef industry has experienced dramatic market share losses to competing protein sources, attributable to diminishing product quality and consistency. Previous research (Arnold et al., 1992) has demonstrated that supplementing finishing diets of feedlot steers with vitamin E is an effective tool for extending the retail shelf-life of fresh beef. Additionally, recent research by Swanek et al. (1999), has shown that supplementing the diets of feedlot steers with vitamin D_3 was a possible means of improving beef tenderness. The purpose of this study was to determine if simultaneous supplementation of vitamin E and D_3 to feedlot steers is a plausible means of improving the shelf-life stability and tenderness of beef.

I sincerely thank the members of my master's committee - - Drs. J. Brad Morgan, H. Glen Dolezal and Fred Ray for their guidance and support over the last two years. To Linda Guenther and Kris Novotany – Thank you for all your valuable advice, both professionally and personally – Now I'll try to put it to good use. I also thank Dr. John Wagner of Continental Beef Research for his cooperation, financial support and professional advice during this research.

TABLE OF CONTENTS

List of Tables

List of Figures

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 I

Introduction

The continual loss of market share to poultry and pork over the last 20 years has created a paradigm shift in the minds of beef producers, packers and retailers. Today, instead of producing commodity beef with little concern for quality, those involved in the beef production chain must focus on satisfying consumer expectations ... each and every time. Today's domestic and international beef consumers demand more in the area of beef quality than ever in the past. The beef consumers' quality equation now contains additive factors such as safety, nutrition and convenience as well as dependability. Increased competitiveness within the beef industry due to economic pressure has also caused the formation of many marketing alliances that strive to produce high-quality beef products. The objective of most alliances is to improve profits through product differentiation. By producing a "branded" product that is consistently superior in terms of tenderness, juiciness and flavor, alliances have the opportunity to take advantage of customer loyalty. Despite these efforts to improve quality, the 1995 National Beef Quality Audit still found that low overall uniformity and consistency, inadequate tenderness and low overall palatability were the top three producer-influenced quality concerns of beef processors, purveyors, restaurateurs and retailers. Additionally, at the 1994 NCBA National Beef Tenderness Symposium, Dr. Jeff Savell of Texas A&M University reported that one out of every four steaks provides a less than desirable eating

experience. It has also been reported that approximately $520 million are lost annually due to product that is either discounted or discarded at the retail case because of discoloration. These findings, coupled with relentless consumer demands have prompted the beef industry to search for new and innovative ways to produce high-quality beef products that consistently clear the quality hurdle.

Many contemporary meat science research efforts have focused on the possibility of using antioxidants as a means of extending the shelf-life of beef. It has been well established that supplementing feedlot cattle with vitamin E provides an effective means of delaying the onset of oxidative discoloration in fresh beef products. Through its activity as a biological antioxidant, vitamin E is able to counteract the effects of free radical agents that would otherwise initiate the onset of meat discoloration. Research conducted at the University of Wisconsin, at Madison by Arnold et al. (1992) has shown that supplementing feedlot cattle with supranutritional levels of vitamin E extends the color display life of gluteus medius steaks from 1.6 to 3.8 d when compared to cattle receiving a conventional diet. The cost of feeding vitamin E for 100 days is approximately $4.00/hd, but its potential benefit could save the industry millions of dollars annually. By extending the shelf-life of beef, retailers are able to avoid costly discounts and discards, allowing them to realize the full value of their products.

Recent research at Oklahoma State University has suggested that including supplemental vitamin D_3 in finishing rations for the final 5 to 10 days immediately prior to harvest aids in improving the tenderness and consistency of

beef. Vitamin D_3's affect is manifested through its ability to cause an increase in calcium absorption at the small intestine. Use of this mechanism has been extensively studied by the dairy industry as a way to reduce the incidence of milk fever. One proposed theory is that once in the muscle, calcium provides the necessary fuel to increase the activity of naturally-occurring, calpain proteases (i.e., calpains) which are responsible for postmortem tenderization. Although the number of studies investigating the effect of vitamin D_3 on meat tenderness is limited, the preliminary results appear to be promising.

It has long been known that aging meat for 14 days or longer has a positive influence on tenderness; however it is also detrimental to shelf-life -- impairing the ability of beef to maintain its attractive bright cherry-red color over time. Through the use of these two vitamins, we hope to optimize the color-tenderness relationship of beef. Feeding vitamin E will allow us to take advantage of extended retail display periods while vitamin D_3 should help in maximizing tenderness, improving the overall quality of beef. The objective of this study was to examine the effects of simultaneous supplementation of vitamins E and D_3 to feedlot cattle on beef color and meat tenderness.

CHAPTER II

Literature Review

Vitamin D

Vitamin D, also known as the "sunshine vitamin", is synthesized in the skin catalyzed by the action of ultraviolet light. A cholesterol based molecule (7-Dehydrocholesterol), is present in large amounts in the skin and acts as the precursor for Vitamin D_3. When exposed to sunlight, the B-ring of 7-dehydrocholesterol is opened between the 9 and 10 positions by the activity of UV rays and a double bond forms between carbons 10 and 19, hence vitamin D_3 is formed (McDowell, 1989).

Vitamin D_3 (cholecalciferol) plays a major role in maintaining calcium homeostasis (Sachs et al., 1987; Bar et al., 1988; Goff et al., 1991; Hodnett et al. 1992). The active metabolite of vitamin D (1,25-Dihydroxyvitamin D_3 [1,25 $(OH)_2$ D_3]), is formed via the action of the liver and kidney. Once in the blood, vitamin D_3 rapidly accumulates in the liver and is hydroxylated to 25-hydroxyvitamin D [25-OHD], the major circulating metabolite of vitamin D. Next, under the influence of parathyroid hormone (PTH), it is further hydroxylated by the kidney to 1,25-dihydroxy D_3 [1,25 $(OH)_2$ D_3] (DeLuca, 1981; Horst, 1986; Heldenberg, 1992). Much of the current information available regarding the role of vitamin D in calcium homeostasis was elucidated during studies conducted to develop methods of reducing the incidence of parturient paresis (i. e., milk fever).

Parturient paresis is a metabolic disorder caused by hypocalcemia that occurs in dairy cows at the onset of lactation due to overwhelming calcium

demands. Studies have shown that periods of calcium stress result in increases of PTH secretion and 1,25 dihydroxyvitamin D_3 production (Dunham et al. 1971; Bar et al., 1988; Gaynor et al., 1989; Goff et al., 1991; Weaver et al., 1993). These two hormones work synergistically to maintain normal calcium levels in the blood. Parathyroid release of PTH, in response to low plasma Ca^{++} levels, is responsible for initiating bone calcium resorption. $1,25(OH)_2D_3$ then augments this response by increasing Ca^{++} absorption in the gut (Goings et al., 1974; Horst et al., 1978; , Horst, 1986; Gaynor et al., 1989; Hodnett et al., 1992). In the previously mentioned study (Goings et al., 1974), it was demonstrated that feeding a low calcium prepaturient diet was an effective prophylactic method for decreasing parturient paresis. A Ca^{++} deficient diet fed to pregnant cows prior to parturition apparently stimulated the release of increased PTH and further renal hydroxylation of 1α-$(OH)D_3$ to its active metabolite, $1,25(OH)_2D_3$ via the activity of 1α-hydroxylase.

There are at least two different mechanisms under which Ca^{++} absorption takes place. During normal physiological conditions calcium is absorbed from the lumen of the gut via passive diffusion (Bronner, 1987). However during periods of calcium stress, absorption across the duodenal brush occurs via facilitated transport, apparently the result of increased $1,25(OH)_2D_3$ levels. The increased rate of Ca^{++} absorption has been attributed to the effects of the vitamin D-dependent calcium-binding protein, calbindin D-28K (CaBP). It has been suggested that $1,25(OH)_2D_3$ is associated with increased activity of calbindin D-

28K (Wasserman et al., 1984; Bronner, 1990). Calbindin actually acts as a ferry, transporting Ca^{++} molecules from the luminal side, across the cytosol, to the basolateral side of the intestinal cell where it is extruded into the extracellular fluid at the serosal side. The activity of calbindin is capable of increasing intercellular Ca^{++} absorption by as much as 60-fold. As eluded to above, this saturable, translocation process, which typically occurs in the duodenum and upper jejunum, is subject to nutritional and physiological regulation by vitamin D (Bronner, 1987).

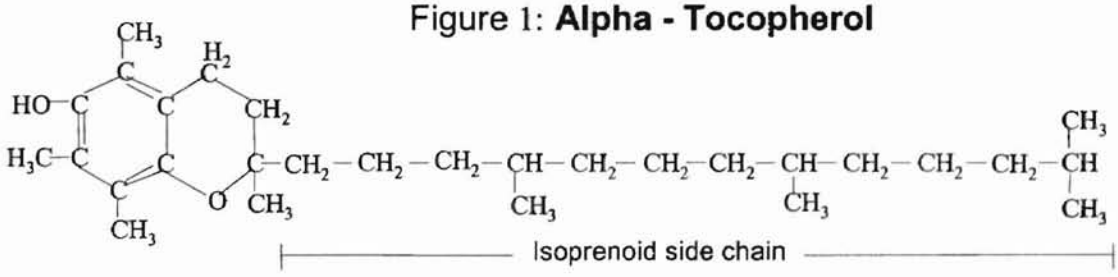
In earlier studies (Spencer et al., 1978; Thomasset, 1979), augmented Ca^{++} absorption was found prior to the appearance of increased calbindin. Because of these findings, some researchers (Rasmussen et al., 1982) have argued for a "liponomic" action of vitamin D, suggesting that the 1,25-$(OH)_2D_3$ interaction with the intestinal cell alters the membrane fluidity, allowing more Ca^{++} to enter the cell. It has also been proposed by Bronner (1987) that because CaBP activity is increased as early as one hour (Buckley et al., 1980) after administration of 1,25-$(OH)_2D_3$, that this response may be at least partially attributed to the action of previously synthesized calbindin. Despite this controversy surrounding the possible mechanisms for increased calcium absorption, Bronner (1987) concludes that calcium translocation across the intestinal cell can indeed be expedited by the action of 1,25-$(OH)_2D_3$.

Vitamin E

Properties and Metabolism

All animal species, including humans require vitamin E. However, there has been much debate among researchers and producers as to the conditions that necessitate vitamin E supplementation as well as the amounts needed to be included in the diet. Vitamin E activity in foods originates from several compounds of plant origin, referred to as tocopherols and tocotrienols. There are eight naturally occurring forms, four tocopherols (α, β, λ, δ) and four tocotrienols (α, β, λ, δ). Differences between the four forms are due to differences in the placement of methyl groups on the phenyl ring. Of these four, α-tocopherol has the highest biological activity, and therefore is the predominant form used in feedstuffs and direct supplementation to livestock (figure 1) .

Figure 1: **Alpha - Tocopherol**



The tocopherol molecule contains three asymmetric methyl groups which are located at the 2 position of the phenyl ring and the fourth and eighth carbons

of the isoprenoid side chain. The *d*-form of α-tocopherol, found in plants, has all three of these methyl groups aligned on the same side of isoprenoid chain and is referred to as the *RRR*-form. The chemically synthesized form (*dl*-form) of α-tocopherol contains an equal number of *R* and *S* configurations across the three methyl group locations. This form is referred to as the *all-rac* (all racemic) form and is the form most commonly used to supplement livestock feeds.

Similar to other tocopherols, α-tocopherol is a yellow oil which is resistant to heat but readily oxidized. Tocopherols are vulnerable to destruction via oxidation and this process can be exacerbated by heat, moisture, rancid fat, Cu and iron. Alpha-tocopherol provides feedstuffs and cellular membranes with an excellent source of protection against oxidative agents; however, after serving as an antioxidant, its biological activity is terminated.

Naturally occurring tocopherol forms are subject to degradation in the gastrointestinal tract, while the acetate ester form travels through the system intact. Because of this, it is a common manufacturing practice to acetylate the *all-rac*-α-tocopherol form in order to prevent premature destruction in the digestive tract. During absorption through the intestinal wall the majority of acetate is cleaved from the tocopherol molecule, thus allowing vitamin E to exert its biological effect as an antioxidant (McDowell, 1989).

Vitamin E is fat-soluble and its absorption is inextricably related to lipid digestion. Whether vitamin E is present in the digestive tract as a free alcohol or in the acetylated form, it is absorbed as the alcohol. Following hydrolyzation of

the ester-form in the gut wall, the free alcohol enters the lacteals and is transported via the lymph to the blood. Vitamin E absorption is seemingly dependent upon the vitamin E status of animals. It has been reported that ruminants deficient in vitamin E absorb 50 - 75% of their tocopherol intake while animals with sufficient vitamin E levels absorb only 20 - 30%. Research by Combs (1981) indicated that a large variety of lipid digestion disorders may cause impaired vitamin E absorption.

Tocopherol absorption rates vary in accordance to their biological activity ($\alpha>\lambda>\beta>\delta$). α-tocopherol is the most readily absorbed, with λ-absorption equal to approximately 85% of the α-form, although it is more rapidly excreted. It is typically true that most of the vitamin E activity in the blood and tissue is α-tocopherol (Ullrey, 1981). Once in the general circulation, vitamin E is commonly attached to lipoproteins in the globulin fraction within cells and is found in most appreciable quantities in the mitochondria and microsomes.

The uptake and tissue retention of tocopherol remains unclear; however, it is known that very little storage occurs in the body. In contrast to vitamin A, no appreciable amounts of vitamin E are stored in the liver. The minute amounts of tocopherol that does persist in the body is rapidly exhausted by polyunsaturated fatty acids (PUFA) in the tissue, with the rate of disappearance paralleling PUFA intakes. Once flowing through the circulatory system, tocopherol is distributed to a variety of body tissues with the largest proportion residing in adipose tissues. After entering the intracellular matrix vitamin E is further distributed to the

subcellular fractions with the highest concentrations found in membranous organelles that have highly active oxidation-reduction systems (i.e., mitochondria and microsomes). Arnold et al. (1993a) conducted a study to evaluate the subcellular distribution of α-tocopherol in supplemented and non-supplemented cattle and found that although tocopherol concentrations were higher in muscle from supplemented cattle, the proportional distribution within specific organelles did not differ.

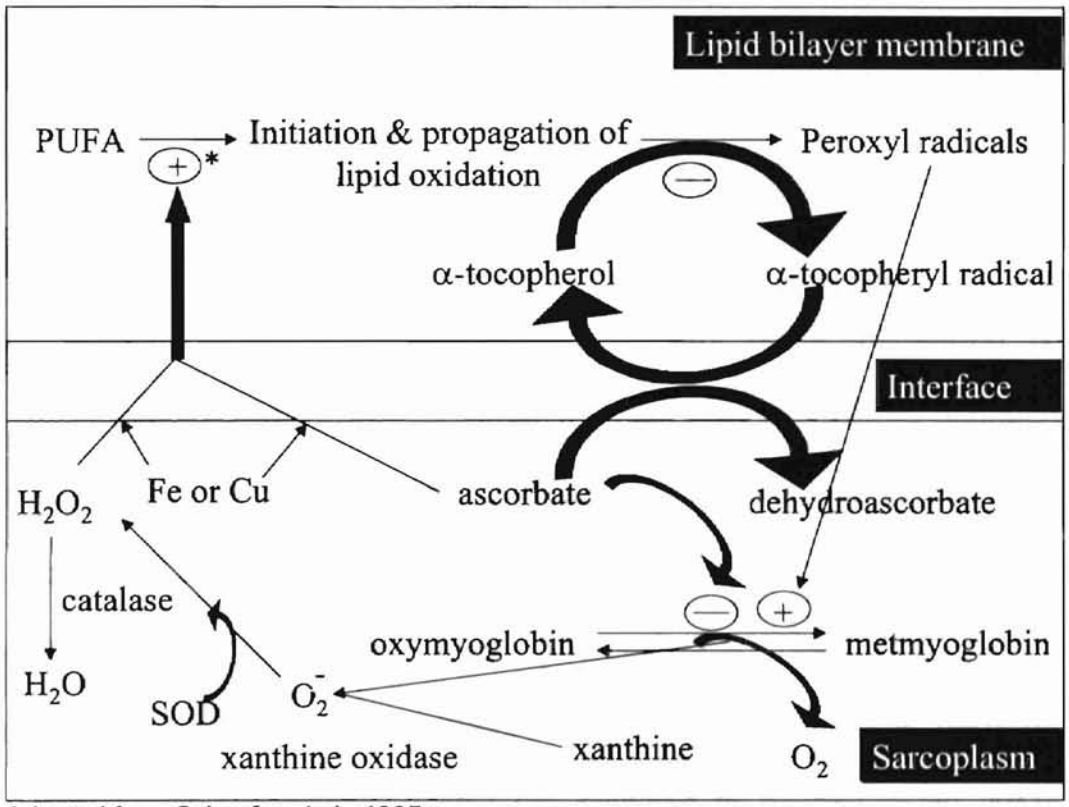
Vitamin E as an Antioxidant

Vitamin E has several functions, however, most importantly is its ability to serve as an intercellular and intracellular antioxidant. By acting as a defense mechanism, α-tocopherol protects cellular membranes from the destructive effects of reactive oxygen and free radicals that initiate oxidation of intracellular PUFA. Vitamin E prevents the oxidation of membranal phospholipids by neutralizing free radicals such as peroxides which would otherwise cause the onset of oxidation (Burton and Traber, 1990). Schaefer and co-workers (1995) have described the influence of vitamin E on myoglobin and lipid stability and proposed a model for their mode of action (Fig. 2).

Cellular membranes are extremely susceptible to oxidative destruction because of their high intramembranal concentrations of polyunsaturated fatty acids and the close proximity of oxygen, transition metals and peroxidases (Vladimirov et al., 1980). A large portion of α-tocopherol's efficacy as an antioxidant can be attributed to its position within the lipid bilayer. The position

of its chromal ring, at the polar portion of the phospholipid, allows its isoprenoid side chain to interact with the unsaturated fatty acyl chains of the phospholipids in the interior of the membrane (Fukuzawa and Fujii, 1992).

Figure 2. **Proposed model of oxidation-reduction relationships in beef.**



Adapted from Schaefer et al., 1995.
* + = prooxidant, - = antioxidant

Donation of a hydrogen atom from the hydroxyl group on carbon 6 of an α-tocopherol molecule quenches free radical species and stabilizes the tocopheryl radical in several quinone configurations (Farrell, 1988). This antioxidant function of vitamin E not only protects its resident cell's membrane from oxidative

destruction but also reduces the incidence of migratory free radical damaging the membranes of adjacent cells.

Cells that have insufficient quantities of intramembranal antioxidants are much more susceptible to oxidation by free radicals. Severe membranal oxidation results in cellular leakage of creatine and different transaminases into the plasma. Cells found in voluntary skeletal muscle that recruit high amounts of lipids for energy and locomotion are at greater risk of oxidative damage than cells from less active muscles when vitamin E is sparse.

Meat Color

Meat color is the single-most important factor influencing consumer purchase decisions (Kropf, 1980). Bright-cherry red and slightly bright-cherry red are the two colors most commonly associated with beef that consumers consider acceptable, and any deviations from these colors causes consumers to discriminate against those products (Sherbeck et al., 1995).

Discoloration of meat occurs when the muscle pigments, deoxymyoglobin (DMb) and oxymyoglobin (OMb) are oxidized to metmyoglobin (MMb). The pigment associated with the deep purple color of beef before it is exposed to oxygen is DMb. In this reduced state, a H_2O molecule is attached to myoglobin's free-binding site. After meat is cut and exposed to air for 10 to 15 minutes, oxygen displaces the H_2O molecule at the sixth binding site and oxymyoglobin is formed. In this state the meat has a desirable, bright-cherry red color. Following prolonged exposure to oxygen for 24 hours or more, oxymyoglobin is oxidized to metmyoglobin, which causes the meat to discolor and become brown. This

reaction occurs when the oxygen is replaced by a hydroxy (OH) molecule and the iron molecule found in myoglobin's porphoryn ring is converted from the ferrous (Fe^{+2}) to the ferric (Fe^{+3}) state (Lui et al., 1995).

Oxidation rates are very dynamic and considerable variation exists between different muscles from the same animal. O'Keefe and Hood (1982) ranked four beef muscles based on their propensity to oxidize and noted the following order: psoas major > gluteus medius > longissimus dorsi > semimembranosus. The brightness of beef and its display case-life are dependent upon both the depth of oxygen penetration as well as the number of myoglobin molecules with an attached oxygen. Oxygen penetration is primarily determined by the oxygen consumption rate of the tissue. It is also influenced by the degree of oxygen diffusion into the product and the oxygen pressure at the meat surface (Brooks, 1929a). Oxygen in postmortem muscle is used by substrates, coenzymes, and enzymes involved with mitochondrial respiration; therefore, as meat ages and these biological entities are depleted or degraded, the tissue oxygen consumption rate will subsequently decrease (De Vore and Solberg, 1974). As with all biological systems, a decrease in either pH or temperature will limit the activity of the above factors, and in turn also cause a decline in oxygen consumption rates (Bendell, 1972).

Bendell and Taylor (1972) reported variation in oxygen consumption rate between meat from different species and attributed these differences to varying intracellular nicotinamide adenine dinucleotide (NAD) concentrations. In the same study, they also identified differences in oxygen consumption rates

between muscles from the same species (beef), and indicated that this variation was most likely a result of variation in mitochondrial density, enzyme activity and NAD content.

The oxidation of deoxymyoglobin to oxymyoglobin and then to metmyoglobin is a reversible process. Dean and Ball (1960) reported the existence of an inherent metmyoglobin reduction system after they observed a decline in surface metmyoglobin content in meat stored under aerobic conditions. They indicated that metmyoglobin is converted to oxymyoglobin by "natural processes"; however the exact mechanism of this change is not known. Following this pioneer metmyoglobin reduction study, many researchers (Stewart et al., 1965; Giddings, 1974; Ledward, 1985; Echevarne et al., 1990), have continued to investigate the role of endogenous pigment reducing systems in the maintenance of meat color. Many relationships have been proposed between metmyoglobin reducing activity, oxygenation rate, oxygen consumption rate, myoglobin content, NAD concentration or succinic dehydrogenase activity and the color stability of beef. Stewart et al. (1965) indicated it is the balance between the autoxidation of the ferrous pigments to metmyoglobin and the enzymatic reduction of ferric metmyoglobin that imposes the most significant influence on muscle color. Other researchers (Giddings, 1974; Ledward, 1984) have concluded that because the autoxidation of various muscles within an animal occurs at similar rates (Ledward, 1977), that it is the inherent reducing ability of each particular cut that determines the relative rate of metmyoglobin formation. This appears even more logical when one considers the variation that

exists in discoloration rates of intact meat relative to minced product. Mincing meat destroys the reducing system, decreasing the variation in rates of discoloration, but also increasing the rates of metmyoglobin formation (Ledward, 1977).

In contrast to the above, some researchers (O'Keefe and Hood, 1982; Renerre and Labas (1987a, b) champion the idea that oxygen consumption rates play the biggest role in determining discoloration rates in meat. High oxygen consumption rates promote the reduction of oxymyoglobin to its' deoxygenated state. Because myoglobin is more susceptible to oxidation than oxymyoglobin, when oxygen consumption rates are high, there is a subsequent increase in the rate of metmyoglobin formation. It has also been suggested that oxygen consumption rates may affect meat color by altering the depth of metmyoglobin formation (Madhavi and Carpenter, 1993). When oxygen consumption rates are high, oxygen molecules do not penetrate deeply into the meat allowing metmyoglobin to be formed near the surface, causing rapid deterioration of the product. Ledward (1985) agreed that oxygen consumption rate was an important factor in determining the rate of discoloration between muscles, but indicated it was the combination of oxygen consumption rate and pigment reducing activity that affected the overall color stability of meat.

Another factor, reported to influence the rate of metmyoglobin formation, is the intracellular NAD concentration. Research by Watts et al. (1966) has shown that the reduction of both myoglobin and oxygen is regulated by NAD. In the same study, the addition of NAD to meat caused an increase in

metmyoglobin reduction. This finding led Watts to postulate that NAD may be a limiting factor in the metmyoglobin reducing system. Supporting these results, Saleh (1967) and Saleh and Watts (1968) reported that the addition of NAD or NADH alone, improved the rate of metmyoglobin reduction. However, the authors also pointed out that because pyridine nucleotides are the key intermediates in the transportation of reducing equivalents, that the rate in which they are reduced controls the overall rate of metmyoglobin formation.

More recently, Lanari and Cassens (1991) conducted a study to evaluate the differences in mitochondrial activity between the longissimus dorsi (LD) and gluteus medius (GM) muscles of Holstein and crossbred cattle. Steaks from Holstein and crossbred cattle were used because of their documented (Faustman and Cassens, 1991) differences in shelf-life stability, hoping that comparisons made between the two would lead to a better understanding of some of the biological factors responsible for the variation in muscle color stability. Mitochondrial content of the LD and GM were higher for muscles from crossbred cattle when compared to their Holstein counterparts, indicating that elevated mitochondrial activity may have a favorable affect on color stability. These results dispute earlier findings by Renerre (1984), who suggested that muscles with high mitochondrial content will be highly oxidative and consequently less color stable.

Lanari and Cassens (1991) also found that, independent of breed type, oxygen consumption rates (OCR) of the GM were higher than that of the LD muscle. This was not unexpected considering that mitochondria are among the

most highly oxidative organelles within a cell. These particular findings indicate that OCR is the primary determinant of metmyoglobin formation. However, while Holstein muscle had fewer numbers of mitochondria, it also had higher rates of oxygen consumption, suggesting that OCR may not be solely dependent on mitochondrial content.

Lanari and Cassens (1991) also investigated the effect of metmyoglobin reducing activity (MRA) on the color stability of beef muscles. They found that for both breeds, MRA in the mitochondria was higher for GM than for LD muscles. Additionally, regardless of muscle type, Holstein mitochondria displayed more reducing activity than muscles from crossbred steers. Therefore, because the color-labile muscle (GM) and breed (Holstein) had the highest MRA, the authors concluded that the affect of this reducing activity on the shelf-life of beef was of little importance.

Lipid Oxidation

Lipid oxidation is also a contributor to the deterioration of meat quality and has been correlated to the oxidation of the myoglobin pigments, although the precise relationship is not known (Greene, 1969; Faustman et al., 1989b). Buckley et al. (1989) reported that lipid oxidation occurs primarily in phospholipid-rich membranes. Gray (1978) described lipid oxidation as occurring when unsaturated fatty acids in the cell membrane react with oxygen via a free radical chain mechanism and produce fatty acyl hydroperoxides (peroxides), which are the primary products of oxidation. This occurrence then

initiates other secondary reactions that cause the degradation of membrane lipids, leading to oxidative rancidity. The breakdown of the primary lipid oxidation product (hydroperoxides) to hydroxy and alkoxy radicals, coupled with the cleavage of fatty acid chains, produces volatile compounds such as alchohols and hydrocarbons, which generate the odor associated with oxidative rancidity (Gray 1978).

Enser (1987) noted that the resistance of meat products to the effects of oxidative destruction is dependent upon the ratio of unsaturated to saturated fatty acids as well as the level of antioxidants present in the tissue. This knowledge has led many researchers (Mitsumoto et al., 1991; Faustman et al., 1989b; Arnold et al., 1993b) to investigate the effects of supplemental antioxidants on lipid oxidation and meat color.

Vitamin E Supplementation

Vitamin E supplementation has been shown to be an effective method for improving the color stability of poultry (Webb et al, 1972; Bartov et al., 1983), pork (Tsai et al., 1978; Buckley et al., 1980; Buckley et al., 1989), and beef (Faustman et al., 1989a, b; Arnold et al., 1993; Lanari et al., 1993).

Webb (1972) showed that feeding broilers 220 IU of vitamin E/kg feed for 12 days prior to slaughter reduced thiobarbituric acid (TBA) levels relative to meat from non-supplemented birds. Bartov (1983) fed 12-week old turkey males supplemental α-tocopherol (5 and 45 mg/kg diet) and found a significant

improvement in the lipid stability of their breast meat. It was also noted that although the low levels of vitamin E were unable to suppress TBA levels, they did prevent undesirable changes that would otherwise be detected by sensory panelist.

Buckley et al. (1989) fed thirty pigs (avg. wt = 40 kg) one of four different experimental rations: 1) short-term α-tocopherol supplementation, 2) long-term α-tocopherol supplementation, 3) long-term natural mixed tocopherol supplementation, and 4) oxidized corn oil supplementation. They found that the lipids of the microsomes and mitochondria from muscle of long-term α-tocopherol-supplemented pigs were the most resistant to the effects of oxidation, while short-term supplementation had no effect on lipid stability. Based on these findings, Buckley concluded that α-tocopherol in sarcoplasmic membranes was responsible for the decreased oxidation and enhanced stability of intramembranal polyunsaturated fatty acids in pork. In an earlier study, Buckley and Connolly (1980) supplemented 10 market-ready hogs with 800 mg/hd/d of vitamin E for the last seven feeding days prior to harvest and compared the subsequent TBA values and shelf-life stability to 10 non-E-supplemented hogs. Their experimental data showed that ground pork from hogs supplemented with vitamin E had significantly lower TBA values relative to controls. These results were independent of packaging type; either oxygen permeable film or vacuum packaged.

Tsai et al. (1978) fed 32 hogs fed one of eight different treatment diets containing a variety of combinations and concentrations of α-tocopherol and ascorbic acid. Results showed that, in contrast to ascorbate, vitamin E supplementation increased the tissue α-tocopherol content and improved the stability of fresh adipose tissue. It was also noted that as supplementation levels of vitamin E increased there was an ensuing decrease in the efficacy of α-tocopherol to resist lipid oxidation.

Several researchers have investigated the effects of supranutritional supplementation of vitamin E on shelf-life and lipid oxidation characteristics of beef (Faustman et al., 1989a; Lanari et al., 1993; Arnold et al., 1993; Sherbeck et al., 1995). Faustman et al. (1989a) conducted a study using 34 Holstein steers to determine if vitamin E supplementation impacted sirloin steak color. Half of the experimental steers received a diet containing 370 IU α-tocopheryl acetate/hd/d from the time they weighed 110 kg until harvest at 545 kg. Color measurements (for L, a and b) were determined for sirloin steaks using a Hunterlab tristimulus colorimeter. At day 0 there were no detectable differences in objective color measurements. As time progressed 'a' and chroma values decreased for steaks from both groups; however, steaks from the vitamin E supplemented group resisted color changes more tenaciously than control steaks. Faustman et al. (1989a) attributed the difference in lean color between the two groups to more rapid pigment oxidation in the control steaks. It was then

concluded that supplementing Holstein steers with vitamin E was an effective means for improving the shelf-life stability of steaks.

Lanari et al. (1993) fed six Holstein steers one of two different high-moisture corn based diets. Three of the steers were supplemented with 2,100 IU α-tocopheryl acetate while the remaining three served as the control group. After a 24 h chill period, strip loins and top round primals were removed from the right side of each carcass, and used to investigate the effects of repeated freeze-thaw cylces on lean color. Results showed that a* and saturation index values remained constant for steaks from the E-supplemented group after two freeze-thaw cycles, while steaks from the control group reached their lowest values after the first cycle. Based on these data, Lanari concluded that vitamin E supplementation to Holstein steers effectively delayed surface discoloration of steaks subjected to repeated freeze-thaw cycles.

In a trial conducted by Sherbeck et al. (1995) 80 crossbred steers were fed a conventional feedlot diet supplemented with 500 IU/hd/d of dl-α-tocopheryl acetate throughout the entire feeding period. From these, 20 USDA Choice carcasses were randomly selected and compared against 20 commodity Choice carcasses selected from the plant inventory. Strip loins, round knuckles and bulk combo-packed chuck rolls (used for ground chuck) were followed through the processing chain to two separate retail outlets in Fort Collins, CO. Upon arrival at the outlet stores the primals were cut or ground and prepared for retail display. Once in the display case, disappearance rates for each of the cuts was

monitored. Retail packages that were either discounted or discarded were also quantified. Data revealed that T-bone steaks from E-supplemented cattle maintained a desirable lean color throughout the display period while those from control steers developed an undesirable dark-red or brown color by the end of display. Ground chuck from control cattle discolored more rapidly over the 2 - 7 d display period than did their E-treated counterparts. Data showed that vitamin E supplementation reduced the percentage of retail cuts discounted and discarded relative to controls over the 7 d display period. Based on these data, Sherbeck concluded that supplementation of feedlot cattle with vitamin E provides sufficient shelf-life advantages to improve the profitability and competitiveness of beef as a protein source.

Arnold et al. (1993) conducted a study to ascertain the effect of vitamin E supplementation on beef quality. Holstein (n=36) and crossbred (n=30) steers were blocked by frame size and randomly assigned to breed pens in groups of six or five, respectively. Two pens of both breed groups were then randomly assigned to each of three vitamin E treatments (0 IU/hd/d, 500 IU/hd/d and 2000 IU/hd/d). Steers were then serially slaughtered after 211, 232 and 252 d on feed. Data revealed that vitamin E supplementation improved the color stability and extended the shelf-life of longissimus lumborum (LL) and gluteus medius (GM) steaks. The formation of metmyoglobin was also reduced and delayed in steaks aged for 7 or 21 d from E-supplemented cattle. Visual assessment of LL and GM steaks revealed that shelf-life was extended 4.1 and 2.8 d, respectively. Arnold's et al. (1993) data also showed that lipid oxidation was inhibited in steaks

from those cattle supplemented with vitamin E. They also noted that although there were no substantial color differences following an 18 day display period between steaks from cattle fed 500 and 2000 IU/hd/d, there were noticeable differences between the two treatments when aged for 21 days. Therefore, they concluded that steaks stored or displayed for extended periods could benefit from higher doses of vitamin E.

CHAPTER III

Effect of Supplementing Feedlot Steers with Vitamins D_3 and E on Carcass Traits, Shelf-Life Attributes and Longissimus Muscle Tenderness

D. N. Vargas, H. Han, J. B. Morgan and H. G. Dolezal

Oklahoma State University, Stillwater, OK 74074

ABSTRACT

Cattle (n=119) of mixed origin were divided into four dietary treatment groups: control (CON), vitamin D_3 (VITD), vitamin E (VITE) and vitamin E and D_3 (COMBO). Treatments receiving vitamin E (i.e., VITE and COMBO) were supplemented with 1,000 IU/hd/d for the final 54 d of the feeding period. Additionally, steers in the VITD and COMBO groups received 6 million IU/hd/d of VITD for the final 6 d immediately prior to slaughter. Longissimus lumbarum (LL) samples from carcasses were divided into four sections and stored in refrigerated conditions for 7, 14, 21 or 28 d postmortem. At the end of each aging period LL sections were cut into three 2.54 cm steaks for Warner-Bratzler shear force determination, shelf-life analysis and sensory evaluation. Tissue samples from each section were excised for α-tocopherol and calcium analysis. Retail color evaluation was conducted twice daily using a three-member trained panel and a Minolta colorimeter. Tissue oxidation was estimated at the beginning and end of each seven d retail display period using the thiobarbituric acid (TBA) assay. Mean Warner-Bratzler shear force values across all aging periods were higher ($P < .05$) for CON steaks compared to other treatments (CON 3.88 kg, VITD 3.65 kg, VITE 3.63 kg, COMBO, 3.57 kg). At 7 d

postmortem, VITD steaks had lower shear force values ($P < .05$) than VITE steaks. Regression analysis showed steaks from carcasses of the VITD and COMBO groups required fewer aging days to become "very tender" (shear force $\leq$ 3.86 kg) relative to CON and VITE treatments (7.4 and 9.8 vs 15.8 and 11.8, respectively). Lean color scores were higher ($P < .05$) at display d 4, 5 and 6 for steaks from cattle fed vitamin E. Minolta colorimeter mean a* values were higher ($P < .05$) for VITE and COMBO steaks when compared to CON and VITD treatments; while b* values were higher ($P < .05$) for steaks from cattle in the VITE group relative to all other treatments. Mean TBA values were lower ($P < .05$) for VITE and COMBO groups compared to CON and VITD (.112 and .059 vs .237 and .372 mg MDA/kg of fresh tissue, respectively). Regression analysis showed that vitamin E supplementation provided an additional .2 to .7 d of retail case-life acceptability relative to longissimus steaks in the CON and VITD groups. Therefore, in order to optimize the color-tenderness relationship of beef, it is recommended that cattle be supplemented with both vitamin D_3 and E.

Introduction

Changes in consumer preferences in recent years have compelled the beef industry to re-evaluate its product quality as well as all influencing factors throughout the production chain. In 1991, the first National Beef Quality Audit (NBQA) was conducted, from which it was found that the top concern of beef purveyors, restaurateurs and retailers was excessive external fat. Between the time this information was disseminated to the industry and 1995 (Boleman et al.,

1995), excessive external fat levels had dropped out as the top quality concern – the second NBQA concluded the industry was making progress. However, now the top three quality concerns included 1) low overall uniformity and consistency of beef products, 2) inadequate tenderness, and 3) low overall palatability. Production practices between the two audits had effectively reduced the amount of external fat on carcasses, but has also decreased the degree of intramuscular marbling – a major determinant of beef tenderness and palatability.

Because of these production changes, researchers have been forced to find new, alternative ways to influence beef tenderness and palatability. Perhaps the most promising work in this area is research by Swanek et al. (1999) which has suggested the possibility of using large doses of dietary vitamin D_3 to enhance beef tenderness. Results from this study concluded that supplementing finishing cattle with 5 million IU/hd/d of vitamin D_3 for the final 5 to 10 d prior to harvest decreased ($P < .05$) shear force values of longissimus steaks aged for only 7 d. Vitamin D_3 promotes increased calcium levels in muscle which is associated with accelerated postmortem tenderization. Application of findings such as these should facilitate the delivery of consistently tender beef to retail outlets, even under "short-aged" conditions.

Maintaining beef's desirable, bright cherry-red color during its retail display presents an entirely different set of quality challenges. In fact, in 1993 the National Cattlemen's Association estimated that approximately $520 million are lost annually due to the discoloration of retail beef products. In effort to recapture some of this "lost value" current research has focused on the use of

antioxidants to extend the retail case-life of beef. Wescott (1997) illustrated that by decreasing the rate of lipid oxidation and lean discoloration, a $4.00 investment of supplemental vitamin E ensures that less beef cuts are discounted or discarded due to premature browning, resulting in $30.00 to $35.00 of savings per carcass marketed through commercial retail outlets.

It is well established that storing carcasses in refrigerated conditions for 14 d or more improves the tenderness of beef. However the cost of improved tenderness is decreased retail case-life. When beef is aged for long periods it loses inherent factors that would otherwise contribute to lean color and lipid stability. Therefore the objective of the current study is to determine if simultaneous supplementation of vitamins D_3 and E is an effective means of stabilizing beef color while maximizing meat tenderness.

Materials and Methods

Cattle (n=119) of mixed origin were fed (124 d) a high concentrate finishing diet at a commercial feedyard in Lamar, CO. At the beginning of the trial, steers were divided into four dietary treatment groups: control (CON), vitamin D_3 (VITD), vitamin E (VITE) and vitamin D_3 and E (COMBO). Cattle receiving vitamin E (i.e., VITE and COMBO) were supplemented with 1,000 IU/hd/d for the final 54 d of the feeding period. Steers receiving vitamin D_3 were supplemented with 6 million IU/hd/d for 6.5 d immediately prior to harvest. Cattle were harvested at a commercial facility in Dodge City, KS where carcass data (quality and yield grade factors) were collected after a 36 h chill period. Strip

loins from the right side of each carcass were individually identified then transported to the Food and Agricultural Products Center for further analysis. After seven d postmortem, strip loins were fabricated into four 8.9 cm sections. The cranial section was further fabricated into three 2.54 cm steaks for Warner-Bratzler shear force, shelf-life and sensory analysis. Tissue samples (10 g) were excised to determine tissue α-tocopherol and calcium concentration. Base-line Thiobarbituric acid (TBA) values were established (Witte et al., 1970) for 50% of the samples at the beginning of each display period and compared to values obtained after seven d of evaluation in the retail case. Shear force and sensory steaks were individually vacuum packaged and frozen until the time of analysis. The remaining three strip sections were then aged at 4.4° C for either 14, 21 or 28 d. At the end of each respective aging period samples were treated using the above protocol.

Shelf-Life Analysis

Steaks were individually placed on Styrofoam trays, overwrapped with polyethylene film and displayed in two retail cases located beneath fluorescence lamps (Sylvania Cool-White, 75 watts) emitting 175 foot candles for seven d. Case temperatures ranged from 2 to 3° C. Steaks were randomly arranged in both retail display cases at the beginning of each display day in attempt to avoid the variability of light exposure within each case. A three-member trained visual panel evaluated steaks for lean color, fat color, percent discoloration and overall

appearance (Tables 1 and 2). Steaks were evaluated twice daily, once at 8 a.m. and again at 5 p.m. Objective color measurements were determined using the L* a* and b* color space setting of a Minolta colorimeter (Minolta Chroma Meter CR-300, Minolta Corp., Ramsey, NJ) immediately following each visual appraisal meeting. Measurements were taken at three different locations on each steak and averaged to obtain one L, a* and b* value per steak. Attempts were made to avoid large flakes of intramuscular fat.

Shear Force Evaluation

Steaks were randomized, thawed at 4° C for 12 to15 h, then cooked to an internal temperature of 70° C using an impingement convection oven (Lincoln Impinger, Model 1132-000-A). Cooked steaks were then allowed to cool to 25° C before removing six cores per steak parallel to the orientation of the muscle fibers. Shear force values 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 calculated by averaging the shear force values generated by each steak's respective cores.

Sensory Panel Evaluation

Panelist were trained for one week immediately prior to the beginning of the sensory panel session. During training, panelist were calibrated for all sensory characteristics using the method described by Cross (1978). Steaks

used for panel evaluation were cooked using the same protocol as for shear force determination. After reaching 70° C, steaks were individually placed in aluminum pouches and transported to the sensory evaluation laboratory. Six to eight trained sensory panelist evaluated steaks for overall tenderness, juiciness, connective tissue amount, flavor intensity, cooked beef fat and off-flavor.

Tocopherol Tissue Concentrations

Concentrations of α-tocopherol in meat tissues were determined after soponification and extraction, by reverse-phase high pressure liquid chromatography (Liu et al., 1996) and fluorescence detection.

Statistical Analysis

Analysis of data was performed using the GLM procedure of SAS Institute, Inc. (1985). Means were separated using LS means and probability differences. Regression analyses were performed for Warner-Bratzler shear force and shelf-life data in order to determine days of aging necessary for steaks to become "very tender" and days of retail case acceptability.

Results and Discussion

Live Performance

Simple statistics for live performance are presented in table 3. Initial weight, dry matter intake and feed efficiency were all statistically similar ($P > .05$). Cattle supplemented with VITD for the last 6 d of the feeding period (i.e., VITD and COMBO) had lighter ($P<.05$) final weights compared to steers in the CON and VITE groups. Furthermore, cattle receiving supplemental VITD had lower average daily gains relative to their non-VITD-supplemented counterparts. Although dry matter intakes between treatment groups were statistically similar, the reduced average daily gain of cattle supplemented with VITD is undoubtedly due to the sharp decrease in feed intake noted during the final 2 d of feeding. It is hypothesized that these marked decreases in dry matter intake are a result of a brief period of hypercalcemia caused by VITD supplementation.

Carcass Traits

Cattle in the VITD group produced carcasses with higher ($P<.05$) preliminary yield grades relative to the COMBO treatment; while carcasses from the CON and VITE groups were intermediate (Table 4). Inexplicably kidney, pelvic and heart fat percentage was higher ($P<.05$) in carcasses from COMBO cattle when compared to the CON group. All other carcass traits were not influenced by vitamin supplementation.

Tissue Tocopherol Concentrations

Supplementing steers with VITE resulted in a 31.7% increase in α-tocopherol tissue concentrations compared to tissue from non-VITE-supplemented cattle (Table 5). Tissue samples from cattle supplemented with vitamin E (i.e., VITE and COMBO) had higher ($P<.05$) α-tocopherol levels than their non-E-supplemented counterparts. Interestingly, within both "non-E-supplemented" (i.e., CON and VITD) and "E-supplemented" (i.e., VITE and COMBO) groups, tissue tocopherol concentrations were higher ($P < .05$) for treatments fed supplemental VITD. These data suggest a possible linkage between metabolic tocopherol absorption and VITD supplementation.

Previous research (Faustman et al., 1989a; Arnold et al., 1993 and Chan et al., 1995) has indicated that tissue α-tocopherol levels between 3.0-4.0 μg/g are necessary to improve the lipid stability of beef. Many researchers have reached these suggested threshold levels by supplementing cattle for at least 100 d prior to harvest (Arnold et al., 1992; Mitsumoto et al., 1993, Sanders et al., 1997) suggesting that extended supplementation periods are necessary to generate a favorable shelf-life response. However, in the present study, tissue α-tocopherol concentrations providing desirable shelf-life results (1.91 μg/g), were reached after only 54 d of VITE supplementation.

Visual Panel

Panelist lean color scores were higher ($P < .05$) for steaks from cattle fed vitamin E (i.e., VITE and COMBO) on display d 4, 5 and 6, compared to their non-VITE-supplemented counterparts (Figure 3). These data suggests that "short-term" supplementation of feedlot cattle with VITE is a plausible way to reduce the rate of metmyoglobin formation (i.e., browning) in steaks aged for various time periods prior to retail display. In the present study, fat color data is not presented because it closely paralleled the rate of lean color deterioration.

The percentage of lean discoloration was not affected ($P > .05$) by dietary treatment. However, panelist perceived less ($P < .05$) discoloration in steaks aged 7 and 14 d relative to those aged for more extended periods (Figure 4). Vitamin E supplementation did improve the overall appearance of steaks regardless of aging period. Steaks from cattle supplemented with vitamin E (i.e., VITE and COMBO) maintained higher ($P < .05$) overall appearance scores when compared to the VITD treatment (Figure 5). Additionally, VITE steaks tended ($P = .07$) to have higher overall acceptability ratings relative to the non-supplemented CON group.

The degree of tissue oxidation (as evidenced by TBA results) was lower ($P < .05$) for steaks from cattle supplemented with VITE than either CON or VITD treatments across all aging periods (Table 6). Following a 7 d postmortem storage period, VITE and COMBO steaks had nondetectable levels of malenaldehyde while CON and VITD samples produced .007 and .184 mg/kg, respectively. Steaks from cattle in the VITD group consistently had the highest

TBA values at the end of each aging period. After a 14 d aging period TBA values for CON, VITE and COMBO treatments were statistically similar, while VITD steaks underwent a higher ($P < .05$) degree of lipid oxidation. More ($P < .05$) malonaldehyde equivalents were detected in CON and VITD treatments after being aged for 21 d relative to steaks from cattle supplemented with VITE aged for an equivalent time period. After being aged for 28 d, the VITE and COMBO treatments experienced less ($P < .05$) oxidation than the VITD steaks stored for the same time period. Additionally, the oxidative stability of steaks in the COMBO group was higher ($P < .05$) than that of CON. These data indicate that VITE supplementation was effective in decreasing the degree of tissue oxidation in steaks aged for various time periods and displayed under retail conditions for 7 d.

Objective Color Measurements

Results from Minolta colorimeter readings show that steaks from the CON, VITD and VITE groups all had L* values higher ($P < .05$) than the COMBO treatment (Figure 6). This indicates that across all aging periods COMBO steaks were consistently less bright than all other treatments. Steaks from carcasses in the VITE and COMBO groups generated higher ($P < .05$) a* values regardless of aging period (Figure 7). Because a* provides a measurement of the degree of redness, these data suggest that "short-term" supplementation of VITE was effective in prolonging the time that beef appeared cherry-red in the retail case. Minolta b* values were higher ($P < .05$) for steaks in the VITE group relative to all

other treatments following 7 and 28 of storage, and tended to be higher after 14 and 21 d aging periods (Figure 8). Similarly, b* values for COMBO steaks were higher ($P < .05$) relative to CON and VITD treatments after 14 d of aging and tended to be higher following a 7 d storage period prior to display. However, after more extended aging periods, COMBO b* values declined below those of VITD steaks and were statistically similar CON steaks. Surprisingly, b* values for all treatments tended to increase with extended aging. Data of this nature have not previously been reported, and currently no explanation exists for this phenomenon.

Vitamin E supplementation extended the retail display-life of steaks from .2 to .7 d relative to CON and VITD groups (Figure 9). This equates to 5 to 17 h of additional display time, providing beef retailers the opportunity to realize the full value of their beef products. These results are similar to those from Arnold et al. (1992) who found that supplementing feedlot cattle with 1,140 IU/d for 67 d or 1,200 IU/d for 38 d effectively extended the retail shelf-life of longissimus lumbarum steaks by 2.5 and 4.8 d, respectively.

Tissue Calcium Concentrations

Tissue calcium concentrations were elevated as a result of VITD supplementation (Table 7). Samples from cattle in the VITD group had higher ($P <. 05$) calcium concentrations compared to the CON and VITE treatments. Likewise, tissue from the COMBO treatment had elevated ($P < .05$) calcium concentrations relative to the CON group and tended to be higher than the VITE

group. Additionally, data from correlation analysis showed a moderate inverse relationship (r = -.58) between tissue calcium concentration and Warner-Bratzler shear force values, indicating that as calcium levels are elevated, shear force values subsequently decrease. These data support the proposed hypothesis of Swanek et al. (1999), that VITD supplementation improves beef tenderness through the provision of additional calcium which possibly accelerates postmortem proteolytic activity.

Shear force analysis

Steaks from CON carcasses were tougher (P < .05) than all other treatments across all aging periods (Table 8). After 7 d of postmortem storage VITD steaks were (P < .05) more tender than VITE steaks and tended (P = .06) to have lower shear force values than those from the CON group (Figure 10). These results are in agreement with Swanek et al. (1999) who found VITD supplementation reduced both shear force values and tenderness variation after a 7 d aging period. In the present study, cattle in the VITD and COMBO treatment groups experienced a 50% reduction in feed intake during the last 2 d of the feeding period. This decline in feed intake is presumably due to the metabolic affect of VITD supplementation. With elevated serum calcium levels it is possible that cattle experienced a brief period of hypercalcemia, causing them to consume less feed. Therefore, although VITD supplementation did improve the tenderness of steaks aged for 7 d, supplementing cattle with levels of VITD that maintain normal intakes may further augment this response.

Shear force did not differ for steaks aged for more extended time periods (Figures 11, 12 and 13). However, regression analysis determined that steaks from cattle supplemented with vitamin D (i.e., VITD and COMBO) required fewer aging days to become "very tender" (WBS $\leq$ 3.86 kg) relative to CON and VITE treatments (Figure 14). This indicates that VITD supplementation can be used to accelerate the aging process and improve the tenderness of beef products provided to consumers.

Sensory Evaluation

Results from sensory evaluation are presented in table 9. In this study no significant differences were detected by panelist for juiciness, beef fat flavor, overall tenderness, connective tissue amount, flavor intensity or off-flavor. This suggests that differences in tenderness found in shear force evaluation were too small to be noticed by panelist.

Conclusions

Supranutritional supplementation of vitamins to finishing cattle has recently been of great interest to many researchers. Many studies have successfully improved the retail color stability of beef by providing high levels of vitamin E in feedlot diets. Furthermore, it has recently been shown that feeding VITD in doses above nutritional requirements provides a favorable tenderness

response. The current study illustrates that feeding VITD in conjunction with VITE is an effective means of accelerating the postmortem tenderization process while simultaneously stabilizing the color of beef. Optimization of the color-tenderness relationship of beef should allow the industry to provide consumers with a high-quality beef product.

Implications

One of the most formidable challenges currently facing the United States beef industry is recapturing market share that has been lost to competing protein sources. Much of this decline can be attributed to decreased eating quality of beef which has resulted from adjustments in production practices aimed at decreasing excessive carcass fat levels. Because identification of genetic lines that are capable of producing high-quality (i.e., high marbling) carcasses with minimal external fat has proven difficult, research efforts have focused on finding alternative methods to improve beef quality. The present study demonstrates that dietary supplementation of vitamins E and D_3 to feedlot steers may be one such tool for improving the appearance and tenderness of beef. However, in order to avoid decreased feed intake immediately prior to harvest, further research is needed to determine the appropriate level and duration of vitamin D_3 supplementation.

Table 1. Visual evaluation scales for lean and fat color

Lean Color (oxygenated)	Fat Color
8 - Bright cherry-red	8 - Creamy white
7 - Moderately bright cherry-red	7 - Mostly creamy white
6 - Cherry-red	6 - Slightly tan
5 - Slightly dark-red	5 - Tan
4 - Moderately dark-red or brown	4 - Slightly brown
3 - Dark red or brown	3 - Moderately brown
2 - Very dark-brown	2 - Brown or slightly green
1 - Extremely dark-brown or green	1 - Dark brown or green

Table 2. Visual evaluation scales for percent discoloration and overall appearance

Percent Surface Discoloration	Overall Appearance
7 - None	7 - Extremely desirable
6 - 1-10	6 - Desirable
5 - 11-25	5 - Slightly desirable
4 - 26-50	4 - Acceptable
3 - 51-75	3 - Slightly undesirable
2 - 76-99	2 - Undesirable
1 - Complete	1 - Extremely undesirable

Table 3. Treatment means for live performance data

	Treatment			
Characteristic	CON	VITD	VITE	COMBO
Initial wt., kg	471±21	472±23	470±21	469±22
Final wt., kg	577±22	569±28	575±24	565±23
DMI, kg	9.3±.05	9.0±.73	8.8±.36	8.5±.54
ADG, kg	1.66±.10	1.52±.09	1.64±.06	1.5±.07
Feed/gain, kg	5.65±.37	5.87±.13	5.40±.22	5.73±.35

	Prob. > F		
Characteristic	VITD[a]	VITE[b]	COMBO[c]
Initial wt	.9285	.1180	.5093
Final wt.	.0324	.3199	.7435
DMI	.3116	.1917	.9498
ADG	.0259	.6120	.9162
Feed/gain	.2142	.3580	.7815

[a]VITD and COMBO vs. CON and VITE.
[b]CON and VITD vs. VITE and COMBO
[c]VITE vs. COMBO.

Table 4. Treatment least squares means for carcass characteristics

	Treatment				
Characteristic	CON	VITD	VITE	COMBO	SEM
PYG[a]	3.40[de]	3.69[d]	3.43[de]	3.33[e]	.11
Yield Grade	3.07[d]	3.56[d]	3.19[d]	3.12[d]	.19
Marbling[b]	474[d]	408[d]	405[d]	434[d]	53.08
KPH, %	1.96[ef]	2.14[def]	2.21[de]	2.27[d]	.09
Overall Maturity[c]	159[d]	160[d]	157[d]	156[d]	1.83
Ribeye, cm^2	87.1[d]	85.6[d]	83.0[d]	85.4[d]	.28
Hot carcass wt, kg	372[d]	369[d]	375[d]	367[d]	12.61

[a]PYG = Preliminary yield grade.
[b]300 = slight; 400 = small; 500 = modest.
[c]Overall maturity = average of skeletal and lean maturity; 1 = A; 2 = B.
[def]Means within row with different superscripts differ ($P < .05$).

Table 5. Treatment tissue α-tocopherol concentrations

Treatment	Tocopherol[a]	SEM
CONTROL	1.25[e]	.08
VITD	1.63[d]	.08
VITE	1.91[c]	.09
COMBO	2.31[b]	.08

[a]Concentration measured as μg of tocopherol/g of tissue.

[bcde]Means within column with different superscripts differ ($P < .05$).

Table 6. Treatment least squares means for TBA values[a] stratified by aging period

Treatment	CONTROL	VITD	VITE	COMBO	SEM
7 d age	.007[bc]	.184[b]	-.083[c]	-.128[c]	.06
14 d age	.197[c]	.439[b]	.116[c]	.075[c]	.06
21 d age	.293[bc]	.340[b]	.139[cd]	.095[d]	.06
28 d age	.450[bc]	.525[b]	.275[cd]	.196[d]	.05

[a]mg malonaldehyde equivalents/kg fresh muscle.
[bcd]Means within row with different superscripts differ ($P < .05$).

Table 7. Treatment tissue calcium concentrations

Treatment	Calcium[a]	SEM
CONTROL	8.70[d]	.22
VITD	10.10[b]	.22
VITE	9.09[cd]	.21
COMBO	9.55[bc]	.23

[a]Concentration measured as μg/g of fresh tissue.
[bcd]Means within column with different superscripts differ ($P < .05$).

Table 8. Treatment least squares means for Warner-Bratzler shear force values

Treatment	Shear Force, kg	SEM
CON	3.88[a]	.04
VITD	3.65[b]	.05
VITE	3.63[b]	.04
COMBO	3.57[b]	.04

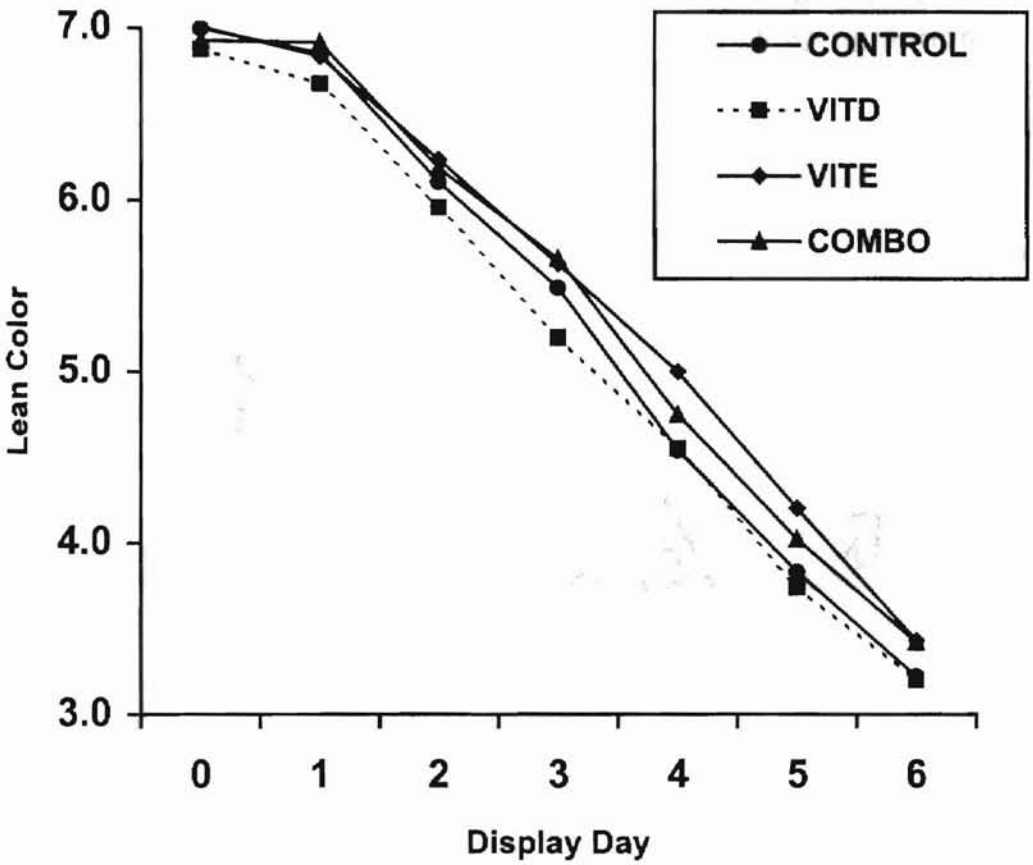
[ab]Means within column with different superscripts differ (P < .05).

Table 9. Treatment least squares means[a] for sensory characteristics

	Treatment				
Characteristic[b]	CON	VITD	VITE	COMBO	SEM
Juiciness	4.87	4.85	4.92	4.90	.07
Beef fat flavor	.67	.70	.67	.68	.03
Overall tenderness	5.43	5.29	5.51	5.34	.08
Connective tissue amount	5.32	5.19	5.34	5.28	.07
Flavor intensity	5.13	5.12	5.21	5.11	.05
Off-flavor	3.89	3.86	3.85	3.90	.02

[a]Means within row with different superscripts differ ($P < .05$).
[b]Juiciness: 1=extremely dry, 8=extremely juicy; Beef fat flavor: 0=none, 2=very strong; Overall tenderness: 1=extremely tough, 8=extremely tender; Connective tissue amount: 1=abundant, 8=none; Flavor intensity: 1=extremely bland, 8=extremely intense; Off-flavor: 1=intense, 4=none.

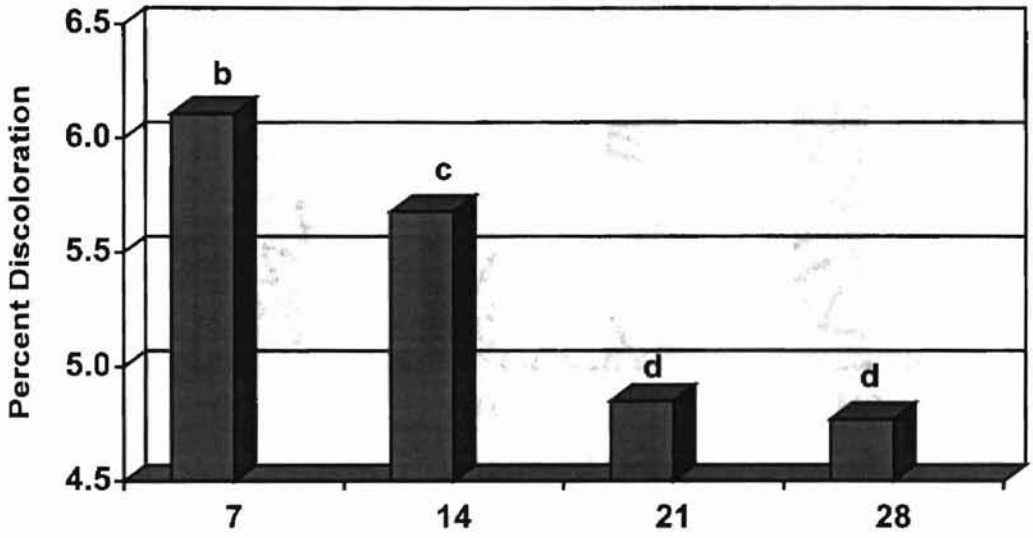
Figure 3. **Effect of treatment and display on lean color means.**



Treatment DAY	0	1	2	3	4	5	6
CON	a	a	cd	f	j	m	o
VITD	a	b	d	g	j	m	o
VITE	a	ab	c	ef	h	k	n
COMBO	a	a	c	e	i	l	n

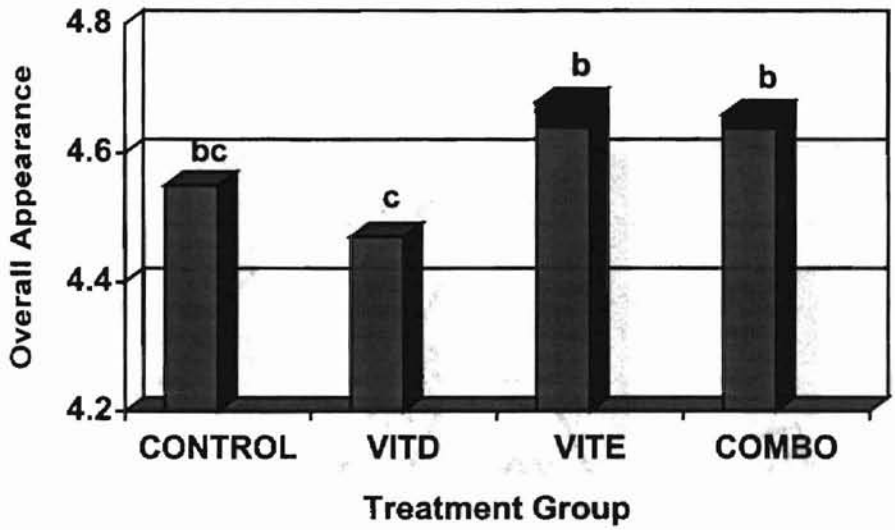
[a-o]Means with different letters differ ($P < .05$).

Figure 4. **Percent discoloration means[a] across all treatments and display days stratified by aging period**



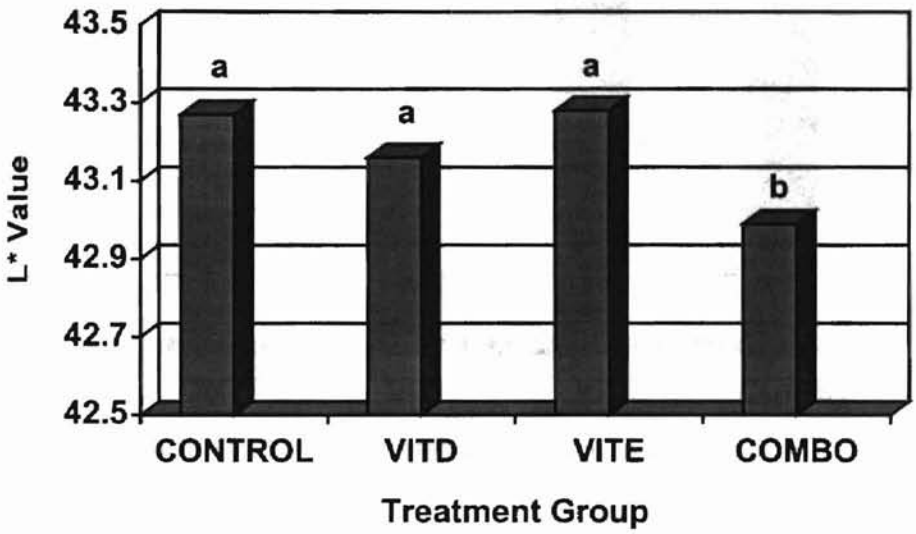
[a]Percent discoloration: 7 = none, 1 = complete.
[bcd]Means without common superscripts differ (P < .05).

Figure 5. **Overall appearance[a] means across all aging periods and display days stratified by treatment**



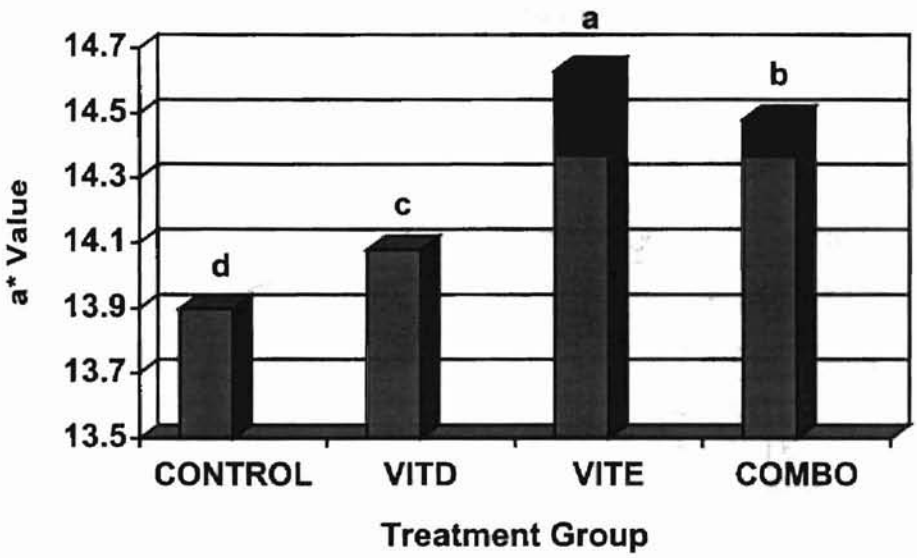
[a]Overall Appearance: 7 = extremely desirable, 1 = extremely undesirable.
[bc]Means with different superscrips differ (P < .05).

Figure 6. **Mean L* values across all aging periods and display days stratified by treatment**



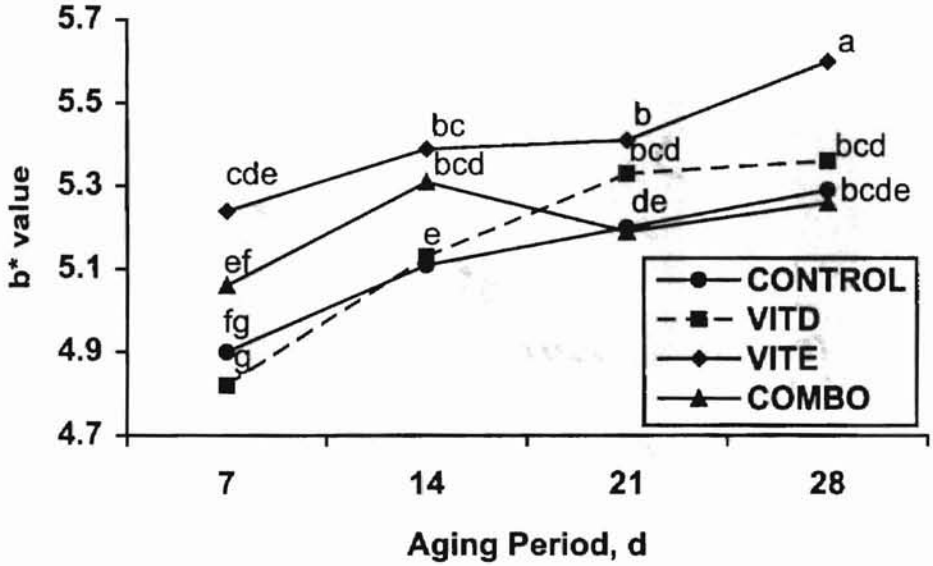
[ab]Means with different superscripts differ (P < .05).

Figure 7. **Mean a* values across all aging periods and display days stratified by treatment**



[abcd]Means with different superscripts differ (P < .05).

Figure 8. **Effect of treatment and aging period on mean b* values**



[abcdefg]Means with different superscripts differ (P < .05).

Figure 9. **Days of lean color acceptability (greater than or equal to 4.5) in retail display case stratified by treatment**

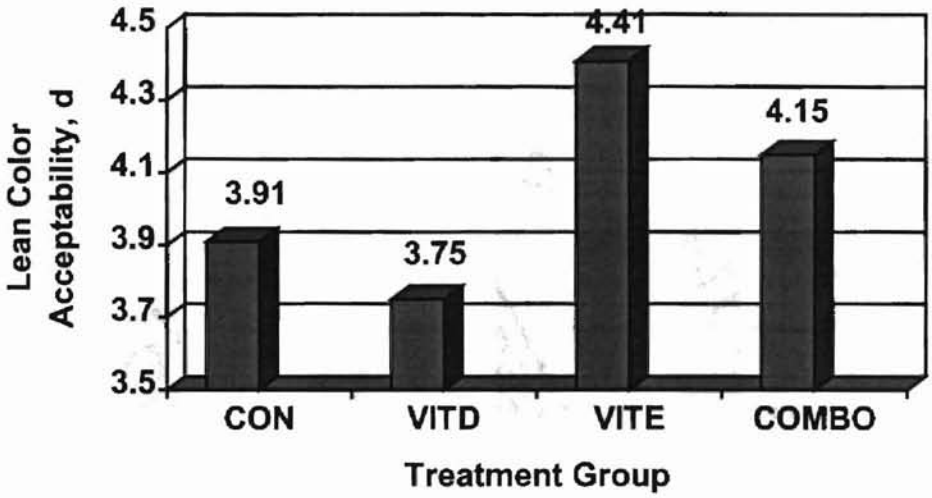
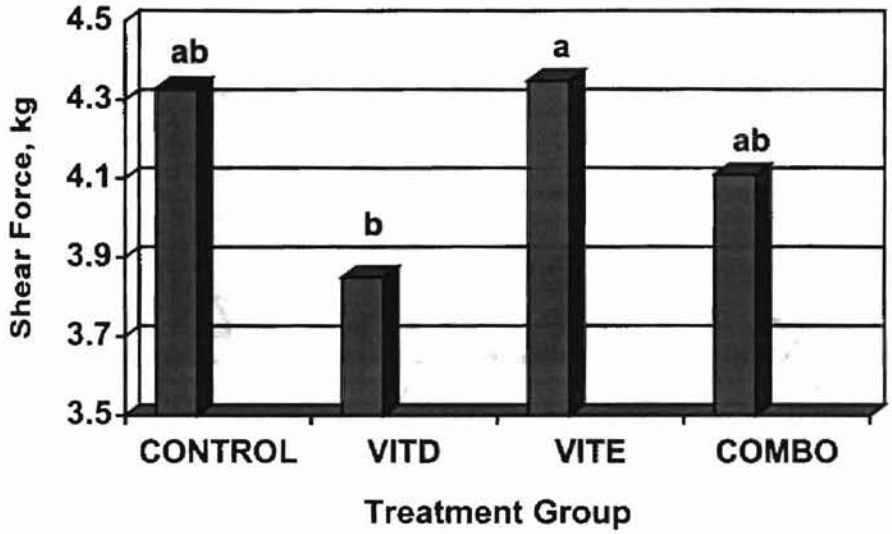
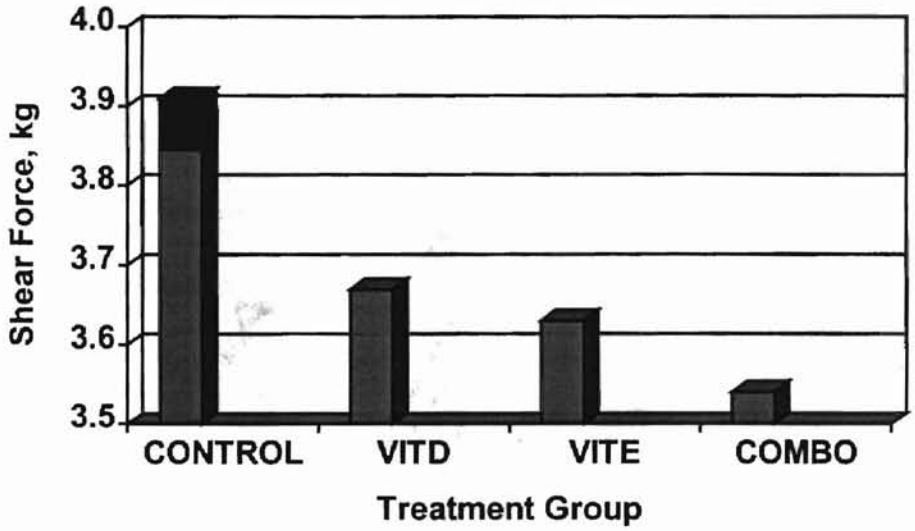


Figure 10. **Treatment Warner-Bratzler shear force means for steaks aged 7 days**



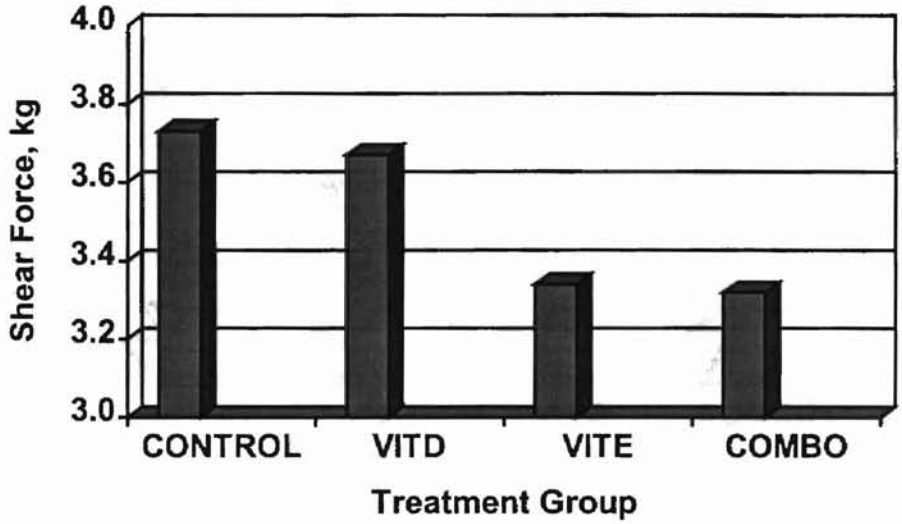
[ab]Means with different superscripts differ (P < .05).

Figure 11. **Treatment Warner-Bratzler shear force means [a] for steaks aged 14 days**



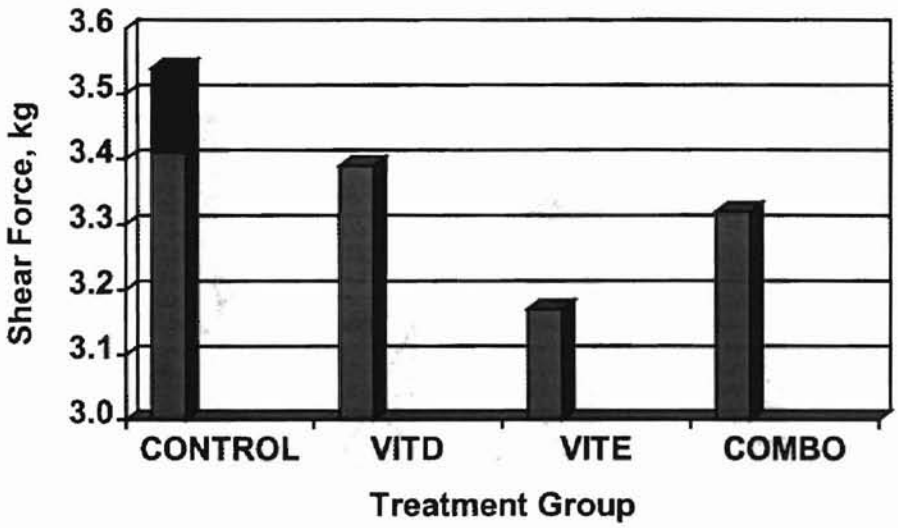
[a]Means with different superscripts differ (P < .05).

Figure 12. **Treatment Warner-Bratzler shear force means[a] for steaks aged 21 days**



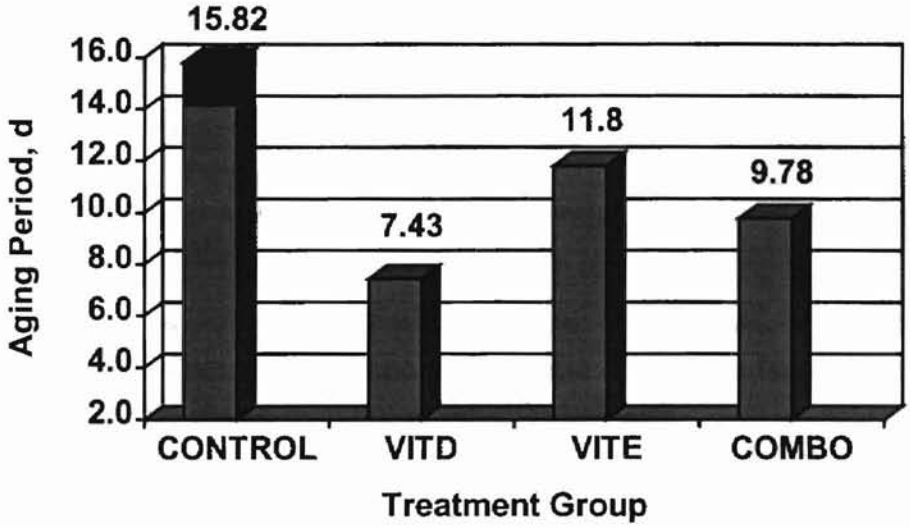
[a]Means with different superscripts differ (P<.05)

Figure 13. **Treatment Warner-Bratzler shear force means[a] for steaks aged 28 days**



[a]Means with different superscripts differ ($P < .05$).

Figure 14. **Aging days required for steaks to become "very tender" (shear force equal to or less than 3.86 kg)**



CHAPTER IV

Literature Cited

Arnold, R. N., K. K. Scheller, S. C. Arp, S. N. Williams, D. R. Buege and D. M. Schaefer. 1992. Effect of Long- or Short-Term Feeding of α-Tocopheryl Acetate to Holstein and Crossbred Beef Steers on Performance, Carcass Characteristics and Beef Color Stability. J. Anim. Sci. 70:3055.

Arnold, R. N., Arp, S. C., Scheller, K. K., Williams, S. N. and Schaefer, D. M. 1993a. Tissue Equilibration and Subcellular Distribution of Vitamin E Relative to Myoglobin and Lipid Oxidation in Displayed Beef. J. Anim. Sci. 71:105.

Arnold R. N., K. K. Scheller, S. C. Arp, S. N. Williams, and D. M. Schaefer. 1993b. Dietary α-Tocopheryl Acetate Enhances Beef Quality in Holstein and Beef Breed Steers. J. Food Sci. 58:28.

Bar, A., S. Striem, R. Perlman, and M. Sachs. 1988. Use of 1α-Hydroxyvitamin D_3 in Prevention of Bovine Parturient Paresis. 8, Maternal and Neonatal Plasma Calcium, Parathyroid Hormone, and Vitamin D Metabolites Concentrations. J. Dairy Sci. 71:2723.

Bartov, I., D. Basker, and S. Angel. 1983. Effect of Dietary Vitamin E on the Stability and Sensory Quality of Turkey Meat. Poultry Sci. 62:1224.

Bendell, J. R. 1972. Consumption of Oxygen by the Muscles of Beef Animals and Related Species, and its Effect on the Colour of Meat. I. Oxygen Consumption in Pre-Rigor Muscle. J. Sci. Food Agric. 23:61.

Bendell, J. R. and A. A. Taylor. 1972. Consumption of Oxygen by the Muscles of Beef Animals and Related Species. II. Consumption of Oxygen by Post-Rigor Muscle. J. Sci. Food Agric. 23:707.

Boleman, S. L., S. J., Boleman, W. W. Morgan, D. S. Hale, D. B. Griffin, J. W. Savell, R. P. Ames, M. T. Smith, J. D. Tatum and T. G. Field. 1998. National Beef Quality Audit – 1995: Survey of Producer-related Defects and Carcass Quality and Quantity Attributes. J. Anim. Sci. 76:96.

Brooks, J. 1929. Postmortem Formation of Methaemoglobin in Red Muscle. Biochem. J. 23:1391.

Bronner, F. 1987. Intestinal Calcium Absorption: Mechanisms and Applications. J. Nutr. 117:1347.

Bronner, F et al. 1990. Intracellular Calcium Regulation. New York: Wiley-Liss

Buckley, D. J., J. I. Gray, A. Asghar, J. F. Price, R.L. Crackel, A.M. Booren, A. M. Pearson, and E. R. Miller. 1989. Effects of Dietary Antioxidants and Oxidized Oil on Membranal Lipid Stability and Pork Quality. J. Food Sci. 54:1193.

Buckley, J. and J. F. Connolly. 1980. Influence of Alpha-Tocopherol (Vitamin E) on Storage Stability of Raw Pork and Bacon. J. Food Protection. 43:265.

Burton, G. W. and M. G. Traber. 1990. Vitamin E: Antioxidant Activity, Biokinetics and Bioavailability. Annual Review of Nutrition. 10:357.

Chan, W. K. M., K. Hakkarainen, C. Faustman, D. M. Schaeffer, K. K. Scheller and Q. Lui. 1995. Color Stability and Microbial Growth Relationships in Beef as Affected by Endogenous α-Tocopherol. J. Food Sci. 60:966.

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.

Dean, R. W. and C. O. Ball. 1960. Analysis of Myoglobin Fractions on the Surface of Beef Cuts. Food Technol. 14:271.

DeLuca, H. 1981. Recent Advances in the Metabolism of Vitamin D. Annual Review of Physiology. 43:199.

DeVore, D. P. and Soleburg. 1974. Oxygen Uptake in Postrigor Bovine Muscle. J. Food Sci. 39:22.

Dunham, J. R., and G. Ward. 1971. Influences of Calcium Intake and Vitamin D Supplementation on the Composition of Lactating Cows Blood. J. Dairy Sci. v. 54, no. 6.

Echevarne, C., M. Renerre and R. Labas. 1990. Metmyoglobin Reductase Activity in Bovine Muscles. Meat Sci. 27:161.

Enser, M. 1987. What is Lipid Oxidation. Fd. Sci. Technol. Today. 1:151.

Farrell, P. M. 1988. Vitamin E. In Modern Nutrition in Health and Disease. Shils and V. Young (Ed.). Lea & Febiger, Philadelphia, PA. p. 340.

Faustman C., R. G. Cassens, D. M. Schaefer, D. R. Buege, and K. K. Scheller. 1989a. Vitamin E Supplementation of Holstein Steer Diets Improves Sirloin Steak Color. J. Food Sci. 54:485.

Faustman C., R. G. Cassens, D. M. Schaefer, D. R. Buege, S. N. Williams, and K. K. Scheller. 1989b. Improvement of Pigment and Lipid Stability in Holstein Steer Beef by Dietary Supplementation with Vitamin E. J. Food Sci. 54:858.

Faustman, C. and R. G. Cassens. 1991. The Effect of Cattle Breed and Muscle Type on Discoloration and Various Biochemical Parameters in Fresh Beef. J. Anim. Sci. 69:184.

Fukuzawa, K. and T. Fujii. 1992. Peroxide Dependent and Independent Lipid Peroxidation: Site-Specific Mechanisims of Initiation by Chelated Iron and Inhibition by α-Tochopherol. Lipids. 27:227.

Gaynor, P. J., F. J. Mueller, J. K. Miller, N. Ramsey, J. P. Goff, and R. L. Horst. 1989. Parturient Hypocalcemia in Jersey Cows Fed Alfalfa Haylage-Based Diets with Different Cation to Anion Ratios. J. Dairy Sci. 72:2525.

Giddings, G. G. 1974. Reduction of Ferrimyoglobin in Meat. Critical Reviews in Food Tech. 5:143

Goff, J. P., T. A. Reinhardt, and R. L. Horst. 1991. Enzymes and Factors Controlling Vitamin D Metabolism and Action in Normal and Milk Fever Cows. J. Dairy Sci. 74:4022.

Goings, R. L., N. L. Jacobson, D. C. Beitz, E. T. Littledike, and K. D. Wiggers.1974. Prevention of Parturient Paresis by a Prepartum, Calcium-Deficient Diet. J. Dairy Sci. v. 57, no. 10.

Gray, J. I. and A. M. Pearson. 1978. Rancidity and Warmed-Over Flavor. Adv. Meat Res. 3:221.

Greene, B. E. 1969. Lipid Oxidation and Pigment Changes in Raw Beef. J. Food Sci. 34:110.

Heldenburg, D., Tenenbaum, G. and Weisman, Y. 1992. Effect of Iron on serum 25-Hydroxyvitamin D and 24,25-dihydroxyvitamin D concentrations. Am. J. Clin. Nutr. 56:533.

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.

Horst, R. L., Jorgensen, N. A. and DeLuca, H. F. 1978. Plasma 1,25-Dihydroxyvitamin D and Parathyroid Hormone Levels in Paretic Dairy Cows. Am. J. Physiol. 235 (6): E634.

Horst, R. L. 1986. Regulation of Calcium and Phosphorus Homeostasis in the Dairy Cow. J. Dairy Sci. 69:604.

Kropf, D. H. 1980. Effects of Retail Display Conditions on Meat Color. Reciprocal Meat Conference Proceedings. 33:15.

Ladikos, D. and Lougovois, V. 1990. Lipid Oxidation in Muslce Foods: A Review. Food Chemistry. 35:295.

Lanari M. C., and R. G. Cassens. 1991. Mitochondrial Activity and Beef Muscle Color Stability. J. Food Sci. 56:1476

Lanari M. C., R. G. Cassens, D. M. Schaefer, and K. K. Scheller. 1993. Dietary Vitamin E Enhances Color and Display Life of Frozen Beef from Holstein Steers. J. Food Sci. 58:701.

Ledward, D. A., C. G. Smith, H. M. Clarke and M. Nicholson. 1977. Relative Role of Catalysts and Reductants in the Formation of Metmyoglobin. Meat Sci. 1:149.

Ledward. 1984. Haemoproteins in Meat and Meat Products. In Developments in Food Proteins – III(Hudson, B. J. F.(Ed.)). Applied Science Publishers, London, 33.

Ledward, D. A. 1985. Post-Slaughter Influences on the Formation of Metmyoglobin in Beef Muscles. Meat Sci. 15:149.

Lui, Q., K. K., Scheller and D. M. Schaefer. 1996. Technical Note: A simplified Procedure for Vitamin E Determination in Beef muscle. J. Anim. Sci. 74:2406.

Lui, Q., M. C. Lanari and Schaefer, D. M. 1995. A Review of Dietary Vitamin E Supplementation for Improvement of Beef Quality. J. Anim. Sci. 73:3131.

Madhavi, D. L. and C. E. Carpenter. 1993. Aging and Processing Affect Color, Metmyoglobin Reductase and Oxygen Consumption of Beef Muscles. J. Food Sci. 58:939.

McDowell, L. R. 1989. Vitamins in Animal Nutrition. Academic Press Inc. p. 55 – 131.

Mitsumoto, M., R. G. Cassens, D. M. Schaefer, R. N. Arnold and K. K. Scheller. 1991a. Improvement of Color and Lipid Stability in Beef Longissimus with Dietary Vitamin E and Vitamin C Dip Treatment. J. Food Sci. 56:1489.

Mitsumoto, M. R., R. N. Arnold, D. M. Schaefer and R. G. Cassens. 1993. Dietary versus Postmortem Supplementation of Vitamin E on Pigment and Lipid Stability in Ground Beef. J. Anim. Sci. 71:1812.

NBQA. 1991. Executive Summary. The National Beef Quality Audit. Colorado State University, and Texas A&M University.

NBQA. 1995. Executive Summary: The National Beef Quality Audit. Colorado State University, Oklahoma State University and Texas A&M University.

NCA. 1993. Executive Summary: Strategic Alliances Field Study. National Cattlemen's Assoc., Englewood, CO.

O'Keefe, M. and D. E. Hood. 1982. Biochemical Factors Influencing Metmyoglobin Formation on Beef from Muscles of Differing Colour Stability. Meat Sci. 7:209.

Rasmussen, H., T. Matsumoto, O. Fontaine, and D. B. P. Goodman. 1982. The Role of Changes in Membrane Lipid Structure in the Action of 1,25-$(OH)_2D_3$. Fed. Proc. 41:72.

Renerre, M. and R. Labas. 1987a. Biochemical Factors Influencing Metmyoglobin Formation in Beef Muscle. Meat Sci. 19:151.

Renerre, M. and R. Labas. 1987b. Oxydo-reduction de la myoglobine et couleur de la viande. Sciences des Aliments. 7 hors-serie. VIII:27

Sachs, M., R. Perlman, and A. Bar. 1987. Use of 1α-Hydroxyvitamin D_3 in the Prevention of Bovine Parturient Paresis. IX. Early and Late Effects of a Single Injection. J. Dairy Sci. 70:1671.

Saleh, B. A. H. 1967. The Role of Pyridine Nucleotides, Substrates and Intermediates in the Enzymatic Reduction of Metmyoglobin in Ground Beef. Ph.D. Thesis. Florida State University.

Saleh, B and B. M. Watts. 1968. Substrates and Intermediates in the Enzymatic Reduction of Metmyoglobin in Ground Beef. J. Food Sci. 33:353.

Sanders, S. K., J. B. Morgan, D. M. Wulf, J. D. Tatum, S. N. Williams and G. C. Smith. 1997. Vitamin E Supplementation of Cattle and Shelf-Life of Beef for the Japanese Market. J. Anim. Sci. 75:2634.

Sherbeck J. A., D. M. Wulf, J. B. Morgan, J. D. Tatum, G. C. Smith, and S. N. Williams. 1995. Dietary Supplementation of Vitamin E to Feedlot Cattle Affects Beef Retail Display Properties. J. Food Sci. 60:250.

Spencer, R., M. Chapman, P. W. Wilson, and D. E. M. Lawson. 1978. The Relationship between Vitamin D - Stimulated Calcium Transport and Intestinal Calcium-Binding Protein in the Chicken. Biochem. J. 170:93.

Stewart, M. R., B. K. Hutchins, M. W. Zipser and B. M. Watts. 1965. Enzymatic Reduction of Metmyglobin in Ground Beef. J. Food. Sci. 30:487.

Swanek, S. S., J. B. Morgan, F. N. Owens, D. R. Gill, C. A. Strasia, H. G. Dolezal and F. K. Ray. 1999. Vitamin D_3 Supplementation of Beef Steers Increases Longissimus Muscle Tenderness. J. Anim. Sci. 77:874

Thomasset, M., P. Cuisinier-Gleizes, and H. Mathieu. 1979. 1,25-dihydroxycholecalciforal: Dynamics of the Stimulation of Duodenal Calcium-Binding Protein, Calcium Transport and Bone Calcium Mobilization in vitamin D - in Calcium Deficient Rats. Febs Lett. 107:91.

Tsai, T. C., G. H. Wellington, and W. G. Pond. 1978. Improvement in the Oxidative Stability of Pork by Dietary Supplementation of Swine Rations. J. Food Sci. 43:193.

Ullrey, D. E. 1972. Vitamin E for Swine. J. Anim. Sci. 53:1039

Vladimirov. Y. A., V. I. Olenev, T. B. Suslova and Z. P. Cheremisina. 1980. Lipid Peroxidation in Mitochondrial Membranes. Adv. Lipid Res. 17.:173.

Wasserman, R. H., C. S. Fullmer and F. Shimura. 1984. Calcium Absorption and the Molecular Effects of Vitamin D_3. In: Vitamin D: Basic and Clinical Aspects. Ed. by Rajiv Kumar.

Watts, B. M., J. Kendrick, M. W. Zipser, B. Hutchins and B. Saleh. 1966. Enzymatic Reducing Pathways in Meat. J. Food Sci. 31:855.

Weaver, V. M., and J. Welsh. 1993. 1, 25-Dihydroxycholecalciferol Supplementation Prevents Hypocalcemia in Magnesium-Deficient Chicks. J. Nutr. 123:764.

Webb. J. E., C. C. Brunson and J. D. Yates. 1972. Effects of Feeding Antioxidants on Rancidity Development in Pre-Cooked, Frozen Broiler Parts. Poultry Sci. 51:1601.

Wescott, E. A. 1997. Vitamin E: An Assessment of the "Quality & Economic" Gains Achieved through Supplementation of α-Tocopheryl Acetate in Feedlot Steers. M.S. Thesis. Oklahoma State University.

VITA

David Vargas

Candidate for the Degree of

Master of Science

Thesis: EFFECT OF SUPPLEMENTING FEEDLOT STEERS WITH VITAMINS D_3 AND E ON CARCASS TRAITS, SHELF-LIFE ATTRIBUTES AND LONGISSIMUS MUSCLE TENDERNESS

Major Field: Food Science

Biographical:

Personal Data: Born in Madera, California, On December 23, 1972, the son of Ron and Joan Vargas.

Education: Graduated from Madera High School, Madera, California in June, 1991; received Bachelor of Science degree in Animal Science from California Polytechnic State University, San Luis Obispo, California in June 1996. Completed the requirements for the Master of Science degree with a major in Food Science at Oklahoma State University in May, 1999.

Experience: Raised on a ranch in Madera, California; employed as warehouse laborer during summers; employed by California Polytechnic State University feedmill as an undergraduate, 1992 - 1996; employed by Oklahoma State University as a graduate research assistant; Oklahoma State University, Animal Science Department, 1996 to present.

Professional Memberships: American Society of Animal Science, American Meat Science Association.