EFFECT OF VITAMIN D_3 SUPPLEMENTATION ON FEED INTAKE AND CARCASS CHARACTERISTICS OF BEEF STEERS

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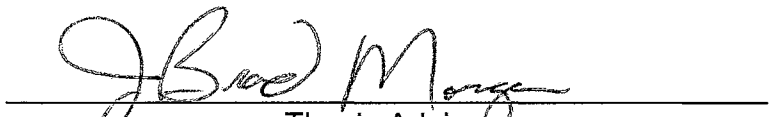

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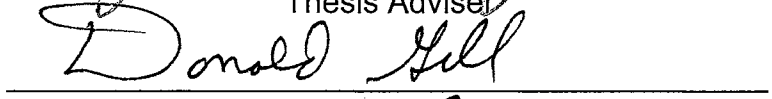

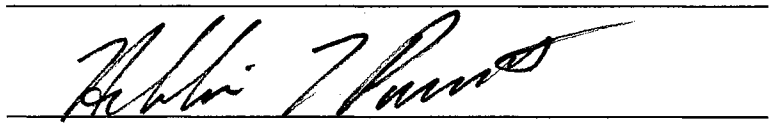

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FORMAT OF THESIS

This Dissertation is presented in the Journal of Animal Science style and format , as outlined by the Oklahoma State University graduate college style manual. The use of this format allows the independent chapters to be suitable for submission to scientific journals. Two papers have been prepared from the data collected for research to partially fulfill the requirements for the degree of Doctor of Philosophy. Each paper is complete in itself with an abstract, introduction, material and methods, results and discussion, implications and literature cited.

Introduction

Inadequate and inconsistent tenderness has been a problem in the beef industry as stated in the 1995 National Beef Quality Audit. This problem represents substantial economic losses to the industry on annual basis. Consequently, tenderness has become a major area of research in terms of developing new techniques/procedures that would enhance the tenderness of meat products consumers purchase. Only in recent years have researchers begun to truly understand the science underlying meat tenderness from an enzymatic standpoint.

For the most part, research has demonstrated that postmortem tenderization is attributed to the calcium dependent proteolytic enzymes, μ-and m-calpain and their protease inhibitor calpastatin. The activity of the enzymes is dependent upon intracellular calcium concentrations, the higher the calcium concentrations the greater activity level exhibited by the proteases. Injecting carcasses with a solution of calcium chloride has resulted in increased tenderness through the activation of the calpain protease system. Although, this type of system is useful in activation of the enzymes, consumer acceptability is low due to the off flavor of meat that has been injected. Thus, the problem that arises is how to increase intracellular calcium concentrations of muscle prior to animal harvest to emulate postmortem aging.

Pre-harvest supplement of Vitamin D_3 (VITD) to market-ready cattle pre-harvest has been shown to improve beef tenderness. The current research was conducted to determine the effects of supplemental VITD of beef steers on dry

matter intakes, blood mineral levels, muscle pH, water holding capacity and tenderness of Longissimus dorsi (LD), Gluteus medius (GM) and Biceps femoris (BF) steaks aged for varying lengths of times.

Review of Literature

I. HISTORICAL PERSPECTIVE

Vitamin D is believed to have been first produced from ocean dwelling plankton and has been estimated by researchers to have been synthesized by life forms for over 1 billion years. Currently it is believed that the primary function of vitamin D in vertebrates is to maintain extracellular fluid concentrations of calcium and phosphorus within a normal range (Feldman et al., 1997). Vitamin D has been demonstrated to perform this function by increasing the efficiency of absorption of calcium and phosphorous from the small intestine as well as from bone and kidney reabsorption (Holick, 1997).

At the turn of the century, the disease rickets was prevalent in a large epidemic proportion of the populations of Europe and the United States. It was not until later that it was realized that this disease had great nutritional implications. At this time in history, many scientists in the field of nutrition were beginning to realize that a diet could not be based solely on carbohydrates, protein, fat and salts. This realization prompted the first experiments by McCollum with rats in which he demonstrated that a substance present in butterfat and cod liver oil was necessary for the growth of animals that were maintained on a chemically defined diet. This fat-soluble substance was given the name of vitamin A (DeLuca, 1979).

Sir Edward Mellanby, had successfully produced rickets in dogs maintained on an oatmeal diet and in the absence of ultraviolet light. Mellenby (1919) utilized cod liver oil to treat dogs and concluded that the healing of rickets

was due to the action of the fat soluble vitamin A. McCollum however, suspected than the antirachitic activity discovered by Mellenby was different then the antixerophthalmia activity he had observed in cod liver oil. By bubbling oxygen through cod liver oil while at the same time heating the oil, McCollum et al. (1922) was able to determine that this resulted in the destruction of vitamin A activity, yet the properties of cod liver oil in the prevention and cure of rickets remained. His conclusion was that this represented a new fat-soluble vitamin which he called vitamin D (DeLuca, 1979). It was not until after the discovery that the relationship of ultraviolet light irradiation (Steenbock and Black, 1924) in conjunction with vitamin D played a role in prevention of rickets, that foods began to be fortified with vitamin D, resulting in the elimination of rickets as a major medical problem.

Following the discovery of vitamin D, emphasis was then placed in learning the overall functions of vitamin D. Currently there are two known basic functions of vitamin D. The first is that vitamin D is required in the prevention of two major bone diseases; rickets in children and osteomalacia in adults. The second involves not only vitamin D but also the parathyroid hormone, in the prevention of the neuromuscular dysfunction termed hypocalcemic tetany. The bone disorder diseases affect the osteoblasts and chondrocytes of the epiphyseal plate preventing proper mineralization of the bone matrix necessary to provide the structural rigidity needed for skeletal formation. With neuromuscular dysfunction, ionized calcium is needed in the extracellular fluid for normal excitation and relaxation of muscle by nerves. When vitamin D is deficient

there is insufficient calcium in the extracellular fluid resulting in continuous excitation of muscle causing hypocalcemic tetany (DeLuca, 1986).

The failure of bone calcification primarily results from a deficiency in vitamin D causing an inadequate concentration of two mineral elements, calcium and phosphorus, in the plasma (Underwood and DeLuca, 1984; Weinstein et al., 1984). Researchers agree that vitamin D functions for the most part by increasing plasma calcium and phosphorus levels to a saturation point in relation to the mineral component of bone. In order for such an elevation of calcium and phosphorus in plasma to occur, vitamin D must act to stimulate intestinal absorption of calcium independent of phosphorus (DeLuca, 1986). Furthermore, vitamin D plays a crucial role in the mobilization of calcium from bone to support plasma calcium concentration. This is primarily a function of the parathyroid hormone but the action of the peptide hormone requires that vitamin D hormone be present (Rasmussen et al., 1963; Garabedian et al., 1974; Feldman et al., 1997).

II. CHEMISTRY AND METABOLISM

Within this section, discussions will focus in five primary areas: 1) vitamin D metabolism, 2) vitamin D 25-hydroxylase, 3) 1,25-hydroxyvitamin D, 4) 24,25-hydroxyvitamin and 5) vitamin D binding protein. According to Norman (1979) there are currently eight different known metabolites of vitamin D: 7-dehydrocholesterol, cholecalciferol, ergocalciferol, 25-dihydroxycholcalciferol, 25, 26-dihydroxycholcalciferol, 1, 24, 25-trihydroxycholcalciferol, 24, 25-

dihydroxycholcalciferol, and 1, 25-dihydroxycholcalciferol. The two most common forms of vitamin D are ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3). Ergocalciferol is produced from a plant steroid whereas cholecalciferol is derived from a precursor referred to as 7-dehydrocholesterol originating from cholesterol. Cholecalciferol is synthesized in the body and found in large concentrations in the skin, intestinal wall and other tissues. The chemical compound of vitamin D is very closely related to the four ring structures of other steroids. This ring system that is common to all steroids is called cyclopentanoperhydrophenanthrene. The official nomenclature of for vitamin D defined by the International Union of Pure and Applied Chemistry relating it to the steroid nucleus.

Vitamin D occurs as colorless crystals, which are insoluble in water but soluble in alcohol and other organic solvents. Vitamin D needs to be stabilized to prevent oxidation from occurring which can be increased during heat, moisture and possible interactions with trace minerals. Shelf life of vitamin D for the cholecalciferol form can keep up to one year permitting proper conditions are utilized (Hoffman-LaRoche Inc., 1994). Some of the best dietary sources of vitamin D are saltwater fish, liver and egg yolk (Chaney, 1997) with the potency of vitamin D measured in terms of milligrams of cholecalciferol (1 μg cholecalciferol or ergocalciferol = 40 IU).

Vitamin D in the skin is referred to as a provitamin which implies that 7-dehydrocholesterol in the epidermis undergoes a conversion to the compound referred to as cholecalciferol through the process of ultraviolet irradiation. This photochemical process occurs in the plasma membrane of skin cells with

approximately 50% of previtamin being converted to vitamin D within 2 hours (Holick, 1997). Vitamin D activity does not become biologically active until ring B of the provitamin (7-dehydrocholesterol) is broken. The provitamin has specific locations in the double bond system that in turn enhances the absorption of light in the wavelength of ultraviolet range. This then allows the ring structure to be opened by a photochemical reaction to form the structure of cholecalciferol. Thus, as long as the skin is exposed to adequate sunlight there is little dietary requirement for vitamin D (Holick, 1997; Chaney, 1997; Norman, 1979; DeLuca, 1979).

It has been hypothesized that as the level of skin pigmentation in human populations tends to increase with their proximity to the equator, serving as a mechanism to prevent vitamin D intoxication by filtering out excessive solar radiation. Whereas lighter pigmentation skin will have the opposite effect on the activity of vitamin D (Voet and Voet, 1995). However, Loomis (1967) speculated that the melanin pigmentation in humans developed for the purpose of regulating prevention of over production of vitamin D in the skin due to over exposure to sunlight. The thought behind this is that once previtamin D is formed in the skin it can iosmerize to vitamin D or can absorb ultraviolet B radiation and isomerize into two different inactive forms. Thus, sunlight itself can regulate the total production of vitamin D in the skin by causing this photodegradation of previtamin D and vitamin D. Individuals that receive adequate sunlight during the spring, summer, and fall are generally not at risk for developing vitamin D deficiency. Vitamin D can be stored for long periods in the fat depots within the body and

released on as needed basis. However, there is evidence that elderly people are at high risk for developing vitamin D deficiency due to lack of exposure to sunlight as well as aging significantly decreases the ability of the skin to produce vitamin D due to the decline in 7-dehydrocholesterol in the epidermis (Holick, 1997).

Technically, vitamin D should probably be considered a hormone rather than a vitamin since it can be synthesized in the skin from the compound 7-dehydrocholesterol and then goes through a two stage transformation to form active vitamin D 1,25(OH_2)D. From activation in the kidney, it is transferred to target cells found in bone, kidney and the intestinal tract where it performs different biological functions. As a hormone its secretion must be regulated in a feedback fashion by the substances that it affects. Vitamin D production is in fact very closely regulated by serum levels of both calcium and phosphorus (Norman, 1979; DeLuca and Schnoes, 1976), parathyroid hormone, growth hormones, sex hormones and vitamin D itself (Norman, 1979; DeLuca, 1982).

Both ergocalciferol and cholecalciferol are metabolized identically in that they are both carried to the liver where the 25-hydroxy derivative is formed. This reaction takes place in the endoplasmic reticulum and requires NADPH, Mg, oxygen, and cytoplasmic factor (Bhattacharyya, 1974) as well as 25 hydroxylase resulting in hydroxylation on carbon 25 to yield 25-dihydroxycholecalciferol (Ponchon, 1969.) The 25-dihydroxycholecalciferol is the primary circulating form of vitamin D with normal levels in the blood plasma of this metabolite 30 to 50 ng/ml (Feldman et al., 1997; Eisman et al., 1977; Best and Taylor, 1985) and a

half life of 15 d. The 25-dihydroxycholecalciferol undergoes further hydroxylation on either carbon 1, 25, or 26 with hydroxylation of carbon 1 being the biological active form of 1-α,25-dihydroxycholecalciferol which occurs in the mitochondria within the proximal convoluted tubules of the kidney utilizing 1,25 hydroxylase (Feldman et al., 1997; Chaney, 1997; DeLuca, 1979 and 1986; Norman, 1979). The concentration of 1,25(OH_2)D is typically 20-50 pg/ml in plasma with a half-life of 15 hours in man (Best and Taylor, 1985). Once formed in the kidney it is then transported elsewhere in the body where it is involved in the metabolism of calcium and phosphorus. The kidney will also function as the exclusive site of vitamin D production in animals with the exception that in pregnant animals the placenta has the ability to synthesize vitamin D (DeLuca, 1988). Activation of vitamin D occurring in the kidney supports the concept that the kidney is the endocrine organ for the production of this hormonally active form of vitamin D and is the principle control point for the metabolism of the active form of vitamin D (Feldman et al., 1997; Norman, 1979; DeLuca, 1979) as well as the regulation of normal calcium and phosphorus homeostasis. In addition, elements of the calcium transport systems including calbindin-D28, calbindin-D9, and the plasma membrane calcium pumps are present in the distal tubule, where the vitamin D receptors are also found (Kumar, 1997).

Research has determined that there are two known sites of production of the enzyme 25 hydroxylase; the smooth endoplasmic reticulum and mitochondria. The smooth endoplasmic reticulum enzyme has been determined to be a cytochrome P450 mixed-function oxidase requiring NADPH cytochrome

c/P450 reductase (Yoon and DeLuca, 1980). Whereas, according to Barre (1997) the hepatic mitochondria enzyme has been determined to be a sterol 27-hydroxylase that is also a mixed-function oxidase requiring ferrodoxin and ferrodoxin reductase. The half life of sterol 27-hydroxylase mRNA has been reported to be between 18 and 24 h suggesting a slow response to regulators such as bile acids, growth hormone and insulin (Stravitz et al., 1996). The sterol 27-hydroxylase will exhibit a very high capacity to bind vitamin D with an affinity that is many times higher than the circulating steady state concentration of the substrate. Thus, the liver regulation of the enzyme appears to take on an environment that is favorable for the efficient transformation of vitamin D into 25-dihydroxycholecalciferol. In contrast, the smooth endoplasmic reticulum enzyme was at one time thought to be a high affinity, low capacity enzyme but has since been indicated to be an enzyme with low substrate specificity (Barre, 1997).

According to Norman (1979) regarding the activity and regulation of 1,25(OH) hydroxylase enzyme (cytochrome $P450_1$), Ghazarian determined that the source of oxygen introduced into the 1 α position of 25(OH)D was molecular oxygen rather than water demonstrating that 1,25(OH) hydroxylase is a mixed-function oxidase just as 25-hydroxylase. Mixed function oxidases catalyze the consumption of one molecule of oxygen per molecule of substrate: one atom of oxygen goes to the product while the other is reduced to water by a hydrogen donor. For mitochondria, mixed-function oxidases which are the electrons for the reduction of molecular oxygen, are derived from NADPH that are generated from the TCA cycle. An important aspect involved in the regulation of $1,25(OH_2)D$ is

that it appears to be self-regulating in accordance with the calcium needs of the animal. However, it has been suggested that the enzymatic activity of 1,25(OH_2)D is affected by an array of things such as changes in serum calcium concentration, serum phosphorus, parathyroid hormone, calcitonin and other associated factors like pH (Norman, 1979). Current understanding regarding the regulation process of 1-hydroxylase is divided into four principle areas: 1) the chemical signals to the kidney 2) response of kidney cells to these signals 3) effects on the activity of the mitochondria renal 1-hydroxylase system and 4) increased or decreased rate of production of 1,25(OH_2)D.

In the course of regulation, both calcium and phosphorus ions are needed for some activity, however, elevated concentrations can serve to inhibit 1-hydroxylase. In mammalian and avian species, vitamin D binding protein has been observed to decrease 25-dihydroxycholecalciferol entry into the kidney cell for further metabolism (Henry, 1979). It has been recognized that localization of the production of 1,25(OH_2)D in the kidney in vitamin D deficiencies indicates higher activity of the 1,25(OH) hydroxylase than occurs in vitamin D replete animals (Warner and Tenenhouse, 1985). It is speculated that the effect of 1,25(OH_2)D on the 1,25(OH) hydroxylase enzyme activity may possibly be mediated by a transcriptional down regulation of the message much in the same manner that up regulation of 24-hydroxylase activity occurs (Henry, 1997). Parathyroid hormone also plays an important role in the regulation of 1,25(OH_2)D by the stimulation of the 1,25(OH) hydroxylase activity which has been found in both avian (Henry, 1981) and mammalian kidney cells (Tanka and DeLuca,

1984). This effect of up regulation of 1,25(OH_2)D by PTH could possibly operate through gene transcription mechanisms although this has not been demonstrated; although gene transcription mechanisms do mediate this partly by cAMP signaling pathway.

Although 1,25(OH_2)D is the active hormone, Madhok et al. (1977) determined that the major product coming from 25(OH_2)D is 24,25(OH_2)D, is a dihydroxyvitamin D metabolite whose production occurs through the action of 24-hydroxlase (cytochrome $P450_{24}$ or CYP24). Both the 24-hydroxlase and 1,25(OH) hydroxylase are enzymes that are closely regulated, with their expression occurring in the kidney. For instance, during conditions of high PTH/low calcium the activity of 1,25(OH) hydroxylase is up-regulated and 24-hydroxlase is down-regulated. With high vitamin D/high calcium 24-hydroxlase is up-regulated and 1,25(OH) hydroxylase is down regulated (Omdahl and May, 1997; DeLuca, 1979; Norman, 1979).

The combined actions of 1,25(OH_2)D and vitamin D receptor (VDR) can act to increase transcription of the CYP24 gene. Thus, cellular expression of CYP24 is directly related to the VDR. The majority of cells that contain VDR will express a base level of CYP24 or react to 1,25(OH_2)D levels by initiating synthesis of the enzyme in the kidney and small intestine (Matkovits and Christakos, 1995). The feedback action appears to be a paracrine/autocrine mechanism involving cellular 1,25(OH_2)D in conjunction with calcium levels. Protein kinase C also appears to have a role in the up-regulation of the gene CYP24 (Omdahl and May, 1997). Furthermore, the removal of ovaries in rats

resulted in the increased renal expression of CYP24 mRNA (Matkovits and Christakos, 1995). From numerous studies (Feldman et al., 1997) it has been shown that PTH functions to increase 1,25(OH_2)D while decreasing CYP24 activity in the kidney. With elevated PTH levels 1,25(OH_2)D production is stimulated while PTH also functions to decrease expression of VDR and CPY24 mRNA. By limiting these two functions it in turn promotes decreased transcription and continued 1,25(OH_2)D production during high PTH levels.

Vitamin D binding protein (DBP) binds and transports vitamin D and its metabolites through serum and reflects the free hormones (unbound). These hormones are regarded as the only ones that are biological active. The bound hormones serve as a reservoir of hormones that can be available to the free pool on an as needed basis. DBP is a component of serum globulin and has an affinity for binding 25(OH)D, 24,25(OH)D, 25,26(OH), 1,25(OH_2)D, and cholecalciferol, in that order. However, a recent study suggests that the binding of these metabolites could be diminished by mono- and polyunsaturated fatty acids, which also bind DBP (Bouillan et al., 1992). According to Cooke and Haddad (1997), Green and Wahli indicated that this binding to fatty acids suggest that DBP may also be a carrier of fatty acids to peroxisome proliferator-activated receptors. These receptors are newly discovered members of the steroid receptor family. DBP has a circulating level of 4-8 μM in normal human plasma with a half life of 2.5 to 3 days in plasma and a production rate of approximately 10 mg/kg of body weight. Recent studies summarized by Cooke and Haddad (1997) indicated that DBP has a high affinity for G-actin and that the action of

DBP in conjunction with gelsolin (which severs actin filaments into oligomers thus increasing the number of growing filament sites) on actin filament results in degradation.

III. MECHANISMS OF ACTION

Perhaps one of the more significant observations was the discovery of DBP in target tissues that appeared to be responsible for nuclear localization of 1,25(OH_2)D. This eventually led to the cloning of vitamin D receptor (VDR) in 1987 which in turn established a clear relationship between steroid and thyroid hormones and vitamin D. It appears that the distribution of VDR, which are believed to reside in the nucleus prior to activation by 1,25(OH_2)D, are wide spread with findings in parathyroid gland, bone, kidneys, pancreas, placenta, ovary, testis, mammary gland and heart. In addition, more and more possibilities are being uncovered for potential therapeutic roles for 1,25(OH_2)D and its analogs as anticancer agents (Feldman et al., 1997).

The VDR exists in relatively low abundance in target tissues and cultured cells at a concentration that parallels the fact that it is a potent transcription regulatory molecule (Pike et al., 1979). Estimations of receptors abundance range from 500 to over 25,000 copies of VDR/cell. Two important observations regarding DNA binding properties of the VDR were made that reflected the role of 1,25(OH_2)D in the receptor activation process (Hunziker et al., 1983). First, VDR was capable of binding DNA in the absence of ligand. Secondly, the affinity

of the receptor for DNA was quantitatively increased following formation with 1,25(OH_2)D.

The cloning of the VDR gene, which lies on human chromosome 12, gave way to the general description of receptor segments to include A, B, C, D, E, and F domains (Pike, 1997). Segment A/B includes residues of the amino terminal to the DNA binding domain. The C region contains the actual DNA binding protein domain. The hinge region lies between the C region and the ligand-binding domain and is referred to as the D region. Finally, the carboxyl-terminal region that contains the ligand-binding domain in the activated receptors is referred to as E/F region. These different domains illustrate the role of the VDR as a major center for other transcription factors that contribute to the DNA binding function of the VDR as well as its transcription regulating functions. One interesting note regarding the activation site is that in sex steroid receptors a key event in their activation is the dissociation of an inhibitory complex composed of heat shock proteins (Pratt et al., 1988). In contrast, the VDR is not found associated with heat-shock proteins (Pike, 1997).

It is hypothesized that at least one role of 1,25(OH_2)D might be to promote an increase in affinity for the VDR receptor. Typically 1,25(OH_2)D binds to VDR forming a receptor-hormone complex that in turn binds to vitamin D response element (VDRE), which are short DNA sequences. To that the VDR binds and exerts its effects on transcription. In conjunction, the retinoid X receptor (RXR) forms a VDR-RXR complex that is needed for the expression VDRE that in turn exerts its effects on transcription. Sone et al. (1991) demonstrated that in the

absence of DNA the affinity of the VDR for the nuclear accessory factor increased 9-fold in response to 1,25(OH_2)D supporting the hypothesis stated previously.

There are a variety of mechanisms and factors that regulate VDR abundance and how it affects target cells that 1,25(OH_2)D acts upon. Three important concepts that emphasize this are the following: 1) VDR abundance determines the level of hormone response and the abundance of VDR is regulated by many physiological signals; 2) there are differences of VDR regulation among species and between various target tissues of the same species; 3) several different cellular mechanisms are involved in VDR regulation (Feldman et al., 1997). A variety of factors effect VDR levels in target cells including 1,25(OH_2)D itself, other vitamin D metabolites, hormones, estrogens, growth factors and intracellular second messenger systems. However, other investigations have examined the role of serum calcium in the up-regulation of VDR. Normal levels of serum calcium are necessary for 1,25(OH_2)D-induced up-regulation of kidney VDR. According to Feldman et al. (1997) Vuorio, (1990) observed that an increase in serum calcium alone, absent exogenous vitamin D, resulted in a 2-fold increase in kidney VDR. In avian parathyroid glands an increase in dietary calcium alone produced a 60% increase in VDR mRNA. With vitamin D treatment and high level of dietary calcium, a substantial, 6-8 fold increase in VDR mRNA was detected by Russell et al. (1993). It appears that both calcium and phosphorus intake play a role in the regulation of VDR levels in target tissues in conjunction with PTH and 1,25(OH_2)D levels. Levels of

1,25(OH_2)D seem to up-regulate VDR, whereas the role of PTH is not yet understood in this overall process. VDR is not distributed equally in the adult rat intestine; highest expressions are observed in the duodenum within the crypts and gradually decreases throughout the small intestine (Feldman et al., 1979).

The two major target tissues of 1,25(OH_2)D are the intestine and the kidney, with one of the largest effects in the induction of the calcium binding protein, calbindin, the first major identifiable target of 1,25(OH_2)D action. There are two major classes of calbindin: a protein of 28,000 molecular weight (calbindin-D28) which contains four high affinity calcium binding sites, and protein of 9,000 molecular weight (calbindin-D9) which contains two calcium binding sites. Calbindin-D28 is found in the highest concentration in avian intestine whereas calbindin-D9 is present in highest concentrations in mammalian intestines (Christakos et al., 1997).

One of the significant effects of 1,25(OH_2)D is to increase the synthesis of intestinal calbindin that occurs primarily in the cytoplasm of absorptive cells in avian intestine. A very strong correlation has been detected between the level of calbindin and an increase in intestinal calcium transport in the chick (Taylor and Wasserman, 1969; Feher and Wasserman, 1979). In the intestine, calcium binds to calmodulin at the brush border membrane, followed by calcium binding to calbindin due its much higher affinity for calcium and greater concentration. Calbindin will then transport calcium to the basolateral membrane where calcium will be actively transported out of the cell into the lamina via the plasma membrane calcium pump. Furthermore, calbindin-D28 acts the same way in the

kidney as it does in the intestine, serving as a vehicle for transport of calbindin-D28 across the cell to be extruded out at the basolaterol pole by the plasma membrane calcium pumps (Christakos et al., 1997). There have also been reports that cabindin-D28 activity has been observed in other areas such as: bone, pancreas, testes, nervous tissue, eggshell gland, and uterus (Feldman et al., 1997).

According to Thomasset (1997) cabindin-D9 is not closely related to calbindin-D28 although, calbindin-D9 has the same general effect in that it has two binding sites for calcium. Calbindin-D9 is found in the small intestine with largest concentrations existing in the duodenal and decreases to the point that calbindin-D9 is not found in the large intestine at all. This distribution pattern correlates with the calcium pattern of absorption throughout the small intestine in conjunction with VDR. There are indications that dietary calcium can effect calbindin-D9 levels in the intestine independent of renal stimulation by 1,25(OH_2)D (Bronner et al., 1986). Increasing the calcium concentrations has resulted in increased production of calbindin-D9 mRNA in the presence and absence of 1,25(OH_2)D in rat intestinal organ cultures. Aside from calbindin-D9 calcium binding properties there has not been any other physiological function established for this protein at this time.

IV. ABSORPTION, TRANSPORT AND EXCRETION OF VITAMIN D

According to Norman (1997), genomic responses due to 1,25(OH_2)D are generated much the same way that other steroids are. Briefly, the hormone is

produced by a stimulus, then circulates in the blood bound to a protein carrier (DBP) that then delivers it to target tissues where the hormone enters into the cell via a receptor on the surface membrane. The hormone is then transported to the nucleus, undergoing activation via phosphorylation, binds to a transcription activation site where the hormone binds to VDRE on the DNA to modulate the expression of hormone sensitive genes. This results in either production or repression of mRNA, which will then result in the formation of new proteins needed for the biological response. Contrarily, nongenomic actions have been demonstrated in intestine, liver, and muscle. It is believed that 1,25(OH_2)D either interacts with protein kinase C (PKC) or mediates changes in the intracellular region of PKC in a variety of cells.

The process where 1,25(OH_2)D mediates the "rapid hormonal stimulation of calcium transport" which has been termed transcaltachia will briefly be described. This process has only been documented in the vitamin D replete chicken and has not been observed in a vitamin D deficient chicken. In this model described by Norman (1997) calcium is absorbed across the brush border membrane of the epithelial cell, packaged in endocytic vesicles that are transported along microtubules to eventually undergo fusion with a lysosome. The lysosome continues to move along the microtubules until it fuses with the basal lateral membrane where upon stimulation of the calcium channels by 1,25(OH_2)D the fused lysosome vesicles undergo exocytosis, resulting in the extrusion of calcium out of the cell near the vascular system. The time it takes for calcium to be absorbed from the lumen of the duodenum to extrusion from the

cell into the vascular system is within 2 minutes after stimulation with 1,25(OH_2)D. In addition, binding of 1,25(OH_2)D to the membrane surface receptor may result in an increase of one or more second messengers systems (cAMP, DAG, IP_3, PKC, or intracellular Ca) which then respond in the opening of calcium channels thus, possibly resulting in transcaltachia if this is the biological response.

Vitamin D from the diet is absorbed from the intestinal tract. For ruminants vitamin D metabolism begins prior to absorption in that the rumen microbes are capable of degrading vitamin D into inactive metabolites (Hoffman-LaRoche Inc., 1994) thus explaining the higher vitamin D requirements of ruminants. Exactly where in the intestinal tract vitamin D is absorbed is not clear, but absorption of vitamin D from an alcoholic solution appeared to be in the duodenal of the small intestine (Schachter et al., 1964). However, when absorption deals with a fatty solution, absorption occurs in the ileum area of the small intestine were there is longer retention time of food in the distal portion of the intestine with bile salts being required for optimum absorption (Norman, 1979). Once absorbed, vitamin D is incorporated into a chylomicron which will then be transported to the tissues or bone via the lymphatic system. Vitamin D may also be transported on a vitamin D transport protein called transcalciferin or DBP (Hoffman-LaRoche Inc., 1994) which appears in the α-globulin fraction of electrophoresis (DeLuca, 1982).

The primary source of excretion appears to be in the feces which accounts for virtually all the vitamin D given in a physiological dose (Neville and DeLuca, 1966) with less then 4% being excreted in the urine (Gray et al., 1974). The bile

fluid accounts for as much as 30% of a physiological dose that can appear within 48 hours after dosing occurs.

V. INTESTINAL CALCIUM ABSORPTION

The entry of calcium from the intestinal lumen across the brush border membrane into the enterocyte is a downhill diffusion process involving the basilateral calcium pumps(Fullmer, 1992). Vitamin D is necessary in order for the intestine to adjust its efficiency of absorption of calcium in order to meet the skeletal needs of the animal. If this need is not satisfied then skeletal calcium will be sacrificed for the maintenance of plasma calcium concentration. Thus, bone mass is decreased which gives rise to thin bones contributing to osteoporosis. Therefore, vitamin D is heavily involved in calcium economy of the body.

In the past it has been questioned whether the intestinal response to 1,25-$(OH)_2D$ involves a nuclear-mediated reaction or a function by direct action on the membranes (DeLuca, 1986). The basis for this controversy is the failure of actinomycin D to prevent the intestinal response to 1,25-$(OH)_2D_3$ (Bikle et al., 1978). It does however, prevent the response of the bone calcium mobilization system (Tanaka and DeLuca, 1971). The lack of actinomycin block has been used to argue that the 1,25-$(OH)_2D_3$ will function in the intestine by a non-nuclear mediated mechanism (Rasmussen et al., 1982). However, further research has indicated that in fact 1,25-$(OH)_2D_3$ functions in the intestine at least largely by a nuclear mediated process. This process involves transcription and

translation of specific genes that code for calcium and phosphorus transport protein (DeLuca, 1986).

The stimulation of calcium absorption through the actions of vitamin D is an active process that requires energy to support the transfer of calcium from the lumen of the intestine to the extracellular fluid against an electro-chemical potential gradient (Wasserman et al., 1963). There are two types of independent processes that regulate calcium absorption from the small intestine. The first is a saturable process that is regulated by vitamin D and calcium binding protein. The second is concentration dependent moving calcium along the length of the intestinal tract (Behar and Kerstein, 1976). In addition, if the intestinal brush border is rendered permeable to calcium with the antibiotic fiflipin, then the vitamin D deficient intestine is able to transport calcium much like that of intestines from animals that are not vitamin D deficient.

Vitamin D binds to a cytoplasmic receptor protein which is transferred into the nucleus, where it undergoes a molecular weight change, induces the transcription of messenger RNA of a specific gene(s) and then codes proteins responsible for intestinal calcium transport. These proteins in turn function at the brush border membrane, in the cytosol, and at the basal lateral membrane to bring about the transport of calcium across the intestinal wall (DeLuca, 1986). Therefore, scientists agree that the major site of action of vitamin D is at the intestinal brush border in regard to calcium absorption (Martin and DeLuca, 1969; Wasserman and Kallfelz, 1962; Feldman et al., 1997).

Two proteins have been identified that are stimulated by 1,25-$(OH)_2D_3$, calbindin-D28 and calbindin-D9 (Feldman et al., 1997; DeLuca, 1986). Wasserman and Feher (1977) concluded that intestinal calcium binding proteins are largely if not entirely vitamin D dependent. However, at one time it was not certain that the appearance of calcium binding protein had a high correlation with calcium transport. This stems from the argument that the proteins do not appear at the right time nor diminish at the right time to account for intestinal calcium transport. Consequently, it was perceived by scientists that calcium binding proteins were not the sole mediators of the vitamin D induced intestinal calcium transport system. However, Corradino (1973) results indicated that 1,25-$(OH)_2D_3$ appeared to function in the initiation of the formation of calcium binding proteins. Additionally, Bishop et al., (1984) research indicates that intestinal calcium binding proteins do make their appearance prior to calcium transport in a well-controlled chick intestinal organ culture system.

Recently, Wasserman et al. (1997) concluded that very little transcellular transport and absorption of calcium occurs in the absence of 1,25-$(OH)_2$ D_3. It has been demonstrated that after dosing with 1,25-$(OH)_2$ D_3 to a deficient animal there is a rapid increase (30 minutes) in calcium entry into the cell. However, the entire completion of the vitamin D stimulated transcellular transfer and absorption occurs some time (3-4 h) later. This lag time is the transcription phase for calbindin proteins. These proteins assist in the transfer of calcium through the cytosol while serving as buffers for the maintenance of intercellular calcium concentrations at safe levels. The extrusion of calcium from the cell is controlled

by its availability to the plasma membrane calcium pumps via 1) free calcium diffusion and 2) release from calbindin. It has been repeatedly shown (Kumar, 1997) that pump numbers are increased by the stimulation of pump synthesis through the action of 1,25-$(OH)_2$ D_3 in the intestine. However, the exact mechanism for this response is uncertain. One possibility for this increase could be related to an increase in the amount of mRNA for the pump in the segments of the small intestine. Typically this increase occurs within three to four hours after the administration of 1,25-$(OH)_2$ D_3 and the effect is dose dependent.

VI. INTESTINAL PHOSPHATE TRANSPORT

Phosphate transport is an active process in which phosphate is carried across the intestinal brush-border by a sodium-phosphate cotransport system. This process is stimulated by 1,25-$(OH)_2$ D_3 in nephrectomized animals but not by 25-OH-D, demonstrating that 1,25-$(OH)_2$ D_3 is the active form. In addition, research has indicated that sodium is required for entry of phosphate across the intestinal epithelium (DeLuca, 1979). According to Wasserman, (1997), Berner et al. (1976) results further supported that the entry of phosphate into the mucosa is dependent upon sodium which led to a sodium/phosphate cotransporter being identified by Peerce (1989). A sodium gradient across the membrane contributes the energy for the active transport of phosphorus that is established and maintained by the basolateral ATP dependent Na^+/K^+ pump. One action of 1,25-$(OH)_2$ D_3 on phosphate absorption appears to be the stimulation of the synthesis of additional cotransporter units (Wasserman, 1997).

Low blood phosphorus has been shown to stimulate production and accumulation of 1,25-$(OH)_2$ D_3 plus ionized calcium levels will be increased which in turn suppresses the parathyroid hormone while high serum phosphate decreases 1,25-$(OH)_2$ D_3 (DeLuca, 1979; Silver and Naveh-Many, 1997). Lack of the parathyroid hormone causes increased phosphate retention by the kidney whereas the presence of parathyroid hormone results in the blocking of phosphate reabsorption. Both of these factors will then help to increase plasma phosphorus since 1,25-$(OH)_2$ D_3 will stimulate the mobilization of phosphate from bone (Chen et al., 1972) and absorption of phosphorus from the small intestine (Castillo et al., 1975). In addition, the kidney has a unique mechanism independent of vitamin D or parathyroid hormone where the efficiency of renal tubular absorption of phosphorus is enhanced under conditions of hypophosphatemia (Steele and DeLuca, 1976).

DeLuca (1979) summarized how vitamin D can function in both arenas as a calcium regulating and a phosphorus-regulating hormone. The signal to raise plasma calcium comes from the stimulation of secretion of the parathyroid hormone which causes not only higher levels of parathyroid hormone to be present but also higher levels of vitamin D can be found in the blood. The parathyroid hormone causes an increased excretion of phosphorus in the urine which negates the vitamin D stimulation of intestinal phosphorus absorption. Contrarily, calcium absorption in the intestine and kidney will be increased by vitamin D and parathyroid hormone presence. Thus, under hypocalcemic conditions, plasma calcium will rise and phosphate will stay constant. In the case

of hypophosphatemic conditions, the parathyroid hormone is suppressed. Therefore vitamin D will have little effect in mobilizing calcium and phosphate from bone, but will stimulate intestinal calcium and phosphate transport. With low blood phosphorus, the kidney will readjust to have maximum phosphorus reabsorption. Thus, in the case of hypophosphatemic there will be an increase in plasma phosphorus due to the intestinal effect of vitamin D and the changes in reabsorption in the kidney.

VII. REGULATION BY PARATHYROID HORMONE

Because serum calcium levels are closely involved in the regulation of synthesis of 1,25-dihydroxycholecalciferol, it is reasonable to conclude that the parathyroid hormone is involved in the calcemic regulation. The secretion of parathyroid hormone is regulated by small changes in the extracellular fluid calcium concentrations. The synthesis of 1,25-$(OH)_2$ D_3 is also closely regulated by calcium (Silver and Naveh-Many, 1997). The compound 1,25-$(OH)_2$ D_3 acts in conjunction with parathyroid hormone, which is produced when there is low serum calcium levels. Parathyroid hormone plays a major role in the regulation of vitamin D activity. High parathyroid hormone levels will stimulate the production of 1,25-$(OH)_2$ D_3 while low levels of parathyroid hormone result in the formation of an inactive form of 24,25-$(OH)_2$ D_3. Once formed 1,25-$(OH)_2$ D_3 has the capability to act on as many as 35 different target tissues as well as over 50 genes are known to be regulated by 1,25-$(OH)_2$ D_3 (Norman, 1997). In the bone, vitamin D

and parathyroid hormone act together promoting bone resorption by stimulating osteoblast formation and activity (Chaney, 1997).

When low serum calcium levels are present parathyroid hormone and vitamin D levels are increased acting to enhance calcium absorption largely by increasing the efficiency of intestinal calcium absorption in addition to bone resorption. 1,25-$(OH)_2$ D_3 will also potently decrease the transcription of the parathyroid hormone. This action was first demonstrated in vivo in bovine parathyroid cells where 1,25-$(OH)_2$ D_3 led to a decrease in parathyroid hormone mRNA levels thus decreasing parathyroid hormone secretion (Silver and Neveh-Many, 1997). It has also been determined that 1,25-$(OH)_2$ D_3 in physiologically relevant doses leads to an increase in VDR mRNA levels in the parathyroid glands in contrast to a decrease in the parathyroid hormone mRNA. Furthermore, it was also determined by these scientists that VDR mRNA was found to be in the same concentration in the parathyroid gland as in the duodenum (Naveh-Many et al., 1990).

A unique characteristic of the parathyroid gland is its sensitivity to small changes in serum calcium that lead to large changes in parathyroid secretion. Silver and Naveh-Many (1997) indicated that small decreases in serum calcium in turn leads to large increases (3-fold over that of controls) in parathyroid hormone mRNA levels. On the other hand, high serum calcium levels appear to have no effect on parathyroid hormone mRNA levels suggesting that the parathyroid gland is geared to responding to hypocalcemia and not hypercalcemia. With normal to high serum calcium levels production of

parathyroid hormone is blocked (Chaney, 1997) allowing 1,25-$(OH)_2$ D_3 to be converted to 24,25-dihydroxycholecalciferol, which is thought to be the specific mineralization form in bones and in embryonic development in chicks (Henry et al., 1977). In the absence of parathyroid hormone and 1,25-$(OH)_2$ D_3 bone resorption is inhibited and calcium excretion is enhanced.

During periods of calcium stress parathyroid hormone activates renal mitochondria 1 α-hydroxylase enzymes converting 25-OH-D_3 to 1,25-$(OH)_2$ D_3 which causes the inactivation of both renal and extrarenal 24- and 23-hydroxylases which convert the 25-OH-D_3 to inactive metabolites (Goff et al., 1991). During periods of no calcium stress the parathyroid hormone has little activity and the 1 α-hydroxylase enzymes are rendered non-active allowing the stimulation of 24- and 23-hydroxylases.

VIII. BONE CALCIUM

Vitamin D has long been associated with cartilage and bone metabolism. In fact, the use of vitamin D in the treatment of rickets and osteomalacia has been used for over 60 years. The principle disturbance caused by vitamin D deficiency is the failure of maturation of cartilage and reduced mineralization of cartilage. According to Boyan et al. (1997) deficiencies such as rickets causes the hypertrophic cell zone, which deals with growth, to greatly increase in size. Following normal restoration of 1,25-$(OH)_2$ D_3 resulted in normal mineralization of the extracellular matrix and the healing of rickets.

It is speculated that both 1,25-$(OH)_2$ D_3 and 24,25-$(OH)_2$ D_3 are involved in the effects on growth plate cartilage possibly by acting on specific target cell. The growth plate gives the impression of being a target tissue for these metabolites because it is one of the few tissues in the body in which cells are found together at essentially the same maturation state (Boyan et al., 1997). In addition, VDR receptors have been identified in cartilage as well as receptors for 24,25-$(OH)_2$ D_3 thus it is possible that many of the effects in cartilage are via VDR pathways, involving changes in gene transcription and mRNA.

It has been demonstrated *in vivo* that 1,25-$(OH)_2$ D_3 is about 10 times more effective than vitamin D itself in regard to inducing bone calcium mobilization (Tanaka et al., 1973). It has been further demonstrated that nephrectomizing animals, which prevents 1-hydroxylation of 25-OH-D_3, will prevent bone calcium mobilization in response to 25-OH-D_3 and vitamin D. However, this does not prevent the bone mineral mobilization response to the 1,25-$(OH)_2$ D_3 providing evidence that 1,25-$(OH)_2$ D_3 is the metabolically active form in bone as well as in the intestine (Holick et al., 1972).

The exact mechanism of how 1,25-$(OH)_2$ D_3 brings about mobilization of calcium from bone is not fully understood. It is not clear whether this involves osteoclastic resorption, osteocytic osteolysis or osteoblastic mediated transfer of calcium from bone fluid to extracellular fluid (DeLuca, 1982). What is clear is that actinomycin D given prior to dosage of 1,25-$(OH)_2$ D_3 will block mobilization of calcium from bone but, when given after 1,25-$(OH)_2$ D_3 it can no longer block that

system, indicating that transcription of DNA is involved in this response (Tanaka and DeLuca, 1971).

Urinary hydroxyproline excretion has been used as an index from bone reabsorption in cattle. The bone resorption of calcium is thought to slightly contribute to the increase of calcium concentration observed after vitamin D supplementation. Reasoning for this is that plasma hydroxyproline concentrations are either reduced (Hove et al., 1983) or remain unchanged during periods of hypercalcemic (Goff et al., 1986).

IX. BLOOD CALCIUM

Blood calcium concentrations are normally between 8-12 mg/dl in cattle and are closely regulated. There are three primary factors contributing to this regulation of calcium levels: two peptide hormones - parathyroid hormone and calcitonin, and vitamin D itself (Feldman et al., 1997). Calcitonin along with parathyroid hormone function in a delicate relationship with vitamin D to control blood calcium and phosphorus levels (Hoffman-LaRoche Inc., 1994). Calcitonin functions to regulate hypercalcemia by decreasing calcium absorption from the intestine as well as decreasing bone resorption and renal calcium reabsorption (McDowell, 1992). As previously mentioned parathyroid hormone stimulates the production of 1,25-$(OH)_2$ D_3 in response to low blood calcium levels but the intestinal absorption of calcium responds only to 1,25-$(OH)_2$ D_3 and does not respond to parathyroid hormone (Garabedian et al., 1974). The parathyroid hormone will cause the uptake of intestinal calcium indirectly by causing the

production of 1,25-$(OH)_2$ D_3. The activity of the 1 α-hydroxylase enzymes, which are responsible for the conversion of 25-OH-D_3 to 1,25-$(OH)_2$ D_3, can also be activated by low blood calcium or phosphorus concentration. On the other hand, when high plasma 1,25-$(OH)_2$ D_3 concentrations are present it can have an inhibitory effect on renal 1 α-hydroxylase and a stimulatory effect on tissue 24- and 23-hydroxlases (Engstrom et al. 1987). This is an example of the check and balance system that exists for the production and catabolism of vitamin D with the majority of the control falling on the activity of the renal 1 α-hydroxylase. Thus, the production of vitamin D can be adjusted according to the calcium needs of the animal (Hoffman-LaRoche Inc. 1994).

Effects of vitamin D on milk fever in dairy cows have long been established. Hibbs and Pounden (1955) demonstrated that oral doses of vitamin D at 5, 10, 20, and 30 million IU of vitamin D for 3 – 8 days prepartum resulted in increased blood calcium by 1.9, 1.0, 1.9 and 2.3 mg/dl, respectively. Contrarily, Horst and Littledike (1979) illustrated that four weekly injections of vitamin D at a dose of 15 million IU resulted in increasing blood calcium by 3.5 mg/dl. Blood calcium rose 8 to 10 days after the initial injection and increased until peaking 3 days after the final injection.

Sachs et al., (1987) gave a single injection to dairy cows for the prevention of parturient paresis. A single injection of 1,25-hyroxyvitamin D_3 resulted in increasing blood calcium by 33% starting 4 days after the injection. The concentration of 1,25-hyroxyvitamin D_3 peaked 24 to 48 h post-injection then dropped to the initial concentrations at 5 d post-injection. This decline in vitamin

D concentration post injection is supportive of Bar et al. (1985) who determined that the biological half life of vitamin D is approximately 2.1 days.

Intermuscular injections with 1,25-hyroxyvitamin D_3 resulted in greater blood levels of 1,25-hyroxyvitamin D_3 than oral administration of the vitamin. This suggests that oral administration may have an effect on the intestinal tract to increase the absorption of calcium (Hove et al., 1983). However, one could speculate that utilizing an oral dose of 1,25-hyroxyvitamin D_3 to raise blood calcium levels could possibly result in actually lowering the 1 α-hydroxylase enzyme concentration thus having less feedback inhibition of the enzyme due to 1,25-hyroxyvitamin D_3 circulating in the blood.

X. VITAMIN D TOXICITY

With the elaborate controls on the metabolism of vitamin D, it is difficult and unusual for vitamin D toxicity to even occur. However, excessive intake of vitamin D will produce a variety of effects as result of abnormal elevation of blood calcium. The main pathological effect of a massive dose of vitamin D is calcification of the soft tissues and demineralization that results in thinning of the bones. Some common observations of vitamin D toxicity are loss of appetite, weight loss, elevated blood calcium and lowered blood phosphate (Hoffman-LaRoche Inc., 1994). It has been shown that cows receiving 30 million IU of vitamin D_2 orally for 11 d developed anorexia, reduced rumination, and depression (NRC, 1987).

Parenteral administration of 15 million IU of vitamin D_3 in a single dose caused toxicity and death in pregnant dairy cows (Littledike and Horst, 1982). Whereas, an oral dose of 20 to 30 million IU of vitamin D_2 daily for 7 days caused little or no toxicity in pregnant dairy cows (Hibbs and Pounden, 1955) probably due to the fact that the form D_3 is much more potent then the D_2 form. The rumen microbes are able to metabolize vitamin D to the inactive form of 10-keto-19-nor vitamin D, thus explaining some of the differences observed between oral and parenteral dosing (Hoffman-LaRoche Inc., 1994). The toxic dose of vitamin D is variable and greatly depends on the duration of intake and level as it appears to be a cumulative toxicity.

In certain geographical areas of the world a problem occurs with plant-induced calcinosis, which is a disease characterized by calcium salts being deposited in the soft tissues. Destruction of connective tissues occurs followed by mineralization, which involves magnesium, calcium and phosphorus. This is a problem that represents large economic losses in milk and meat production in certain areas of the world such as Argentina where 10 to 30% of the cattle show signs of this problem (Hoffman-LaRoche Inc., 1994).

Through out history, fatal intoxication of vitamin D is possible but the probability is unlikely due to differences in tolerability from one individual to another individual. Doses ranging from 1000 IU/kg to 30,000 IU/kg of body weight have shown to have side affects with the most common being the following: anorexia, loss of weight, gastrointestinal discomfort, vomiting, diarrhea and sometimes deep depression has occurred (Reed et al., 1940; Hoffman-

LaRoche Inc., 1994). It appears kidneys are the most affected organs in the body followed by the lungs, thyroid, left ventricle, and the liver. Another interesting side note is that subjects that received a high level of supplementation with vitamin D were noted to have increased libido (Reed et al., 1940). It is hypothesized that the sex hormones might actually interfere with the conversion of 25(OH)D to 1,25 $(OH)_2$ D thus being intimately involved in the regulation of vitamin D metabolism and action (Norman, 1979).

The incidence and severity of vitamin D toxicity can be based on one or more of the following circumstances: 1) the dose per unit of body weight, 2) the duration of administration, 3) composition of the diet, 4) species susceptibility, 5) individual susceptibility, 6) age of individual, 7) the vehicle in which the vitamin was administered, 8) route of administration and 9) source of the vitamin. The incidence of intoxication in humans is relatively low with the threshold stated to be 20,000 units per kg of body weight per day (Reed et al., 1940). On the other hand, the tolerable upper intake level defined as the maximal level of nutrient intake that is unlikely to pose risks of adverse health effects to almost all individuals in the target group was recently stated as 50 μg/d which translates into 2,000 IU of vitamin D per d (Nutrition Today, 1997).

Vitamin D toxicity may occur due to any one of three forms of vitamin D specifically, the vitamin D parent compound, 25(OH)D, or 1,25-$(OH)_2$ D. Several factors can influence toxicity such as the concentration of the vitamin D metabolite itself, degradation pathway, and the capacity of the DBP. The toxicity effect is harder to manage with vitamin D_2 or vitamin D_3 than it is with its

metabolites 25(OH)D or 1,25-$(OH)_2$ D_3. For the most part this is due to the large storage capacity and half life of the parent compound which can range from 20 days to months in contrast to days or hours for the metabolites (Thys-Jacobs et al., 1997). Thus, the hypercalcemia of parent vitamin D overdose can last for as long as 18 months, long after its dosing has been discontinued which is due to its slow release from fat depots.

The toxicity that arises in the target tissues from either the parent vitamin D or 25(OH)D is due to25(OH)D. Shepard and DeLuca (1980) found that large doses of vitamin D_3 resulted in excessive concentrations of vitamin D_3 and 25(OH)D but not in 1,25-$(OH)_2$ D_3. Unlike 1,25-$(OH)_2$ D_3 whose production is tightly regulated in the kidney, the production of 25(OH)D is not closely monitored by the liver. Therefore, the high capacity of the liver for hydroxylation of vitamin D and the poor regulation lends itself to allow for massive amounts of 25(OH)D to be generated from large amounts of vitamin D (Thys-Jacobs et al., 1997). High concentrations of 25(OH)D results in competition for binding sites on the VDR as well as serving as an up-regulator of intestinal VDR. Thus, 25(OH)D is able to produce biological effects similar to those of 1,25-$(OH)_2$ D on intestine and bone. Beckman et al. (1995) determined with rats that dietary calcium restrictions in conjunction with vitamin D treatments actually helped in the prevention of hypercalcemia by increased parathyroid hormone secretions which facilitated the elimination of 25(OH)D via metabolism to 1,25-$(OH)_2$ D and then degradation to 24,25(OH)D. In contrast, a normal calcium diet plus vitamin D resulted in toxicity

by decreased levels of parathyroid hormone causing down regulation of enzyme activity and thus, higher concentrations of 25(OH)D occurred.

XI. MUSCLE TENDERIZATION

Calpains are calcium dependent proteolytic systems that are widely distributed in mammalian tissues and apparently participate in a variety of cellular functions mediated by calcium, including a variety of muscle cell differentiation. Two types of calpain showing distinct calcium sensitivity exist. They are designated as μ-calpain and m-calpain according to their requirements of μM and mM levels of calcium for activity. μ-Calpain is highly sensitive to calcium requiring concentrations only in the micromolar range for activity and is the only calpain found in human platelets and erythrocytes. m-Calpain is found in many tissues, especially liver and muscle, and is relatively insensitive to calcium levels requiring concentrations in the millimolar range for full activity. In addition, a highly specific endogenous inhibitor calpastatin also exists in a wide array of tissues and cells controls activities of the calpains. Calpastatin possesses four inhibitory domains, which account for the maximal inhibitory stoichiometry of 1 calpastatin molecule per 4 calpain molecules (Kwak et al., 1993; McClelland and Hathaway, 1991; Goll et al., 1992). Calcium dependent binding of calpastatin occurs at two locations on the calpains thus inhibiting activity. However, calpastatin is very competitive inhibitor of calpains thus it will also must bind to a third site, the active site, on the calpains. During high calcium concentrations this active site is exposed, calpastatin will bind to this site immediately and inactivate

the enzyme. When concentrations of calcium are high enough this system of inhibition is overwhelmed and eventually leads to activation of the calpains (Goll et al., 1992).

Calpains are cytosol proteinases found primarily in the mitochondria and on the myofibril. They have been found to have an absolute dependence for calcium for proteolytic activity. Both μ-calpain and m-calpain are heterodimers having a native molecular weight of approximatley 110,000 consisting of an 80 kDa catalytic subunit and 30 kDa regulatory subunit. Calpains are well known for being very susceptible to calcium induced autolysis. Prolonged exposure to sufficient calcium results in loss of proteolytic activity and destruction of the enzymes (Koohmaraie, 1992a; Croall and DeMartino, 1991; Goll et al., 1992).

Much effort has gone into trying to understand how calpains function in muscle tissue. Koohmaraie (1992a) and Goll et al. (1992) stated that there is a debate on how calpains could possibly function in muscle tissue due to: 1) the calcium requirement for proteolytic activity of calpains (typically 10 μM for μ-calpain and between 200 to 300 μM for m-calpain are much higher than the free calcium concentration found in the cytoplasm which is less then 1 μM, 2) in most tissues calpastatin concentration is sufficient to block the activity of both calpains and 3) the calcium concentration needed to bind calpastatin to calpains is less than that required for the proteolytic activity of calpains themselves.

Calpains are able to degrade three classes of substrate proteins in vitro; cytoskeletal proteins, hormone receptors and kinases and phosphatases. In order to fully understand the various roles that these two proteinases play, either

singly or jointly, in calcium mediated processes, several possible distinctions between their functions and chronology are apparent in muscle cells. Other factors such as hormones and growth factors are also known to influence the rate of muscle cell differentiation by having significant effects on the expression of the calcium dependent proteolytic system (Goll et al., 1992; Cottin et al., 1994; Kakkar et al., 1997).

XII. CALPAIN AND CALPASTATIN

This proteolytic system plays an important role in many essential cellular events that are mediated by calcium, such as activation of kinases, turnover of myofibrillar proteins (maturation and degradation), organization of cytoskeletal proteins and myoblast differentiation and fusion (Kwak et al., 1993; Barnoy et al.,1997; OU and Forsberg, 1991).

Increased levels of calcium in lesions may activate the neutral proteinase calpain, selectively degrading cytoskeletal and myelin proteins, which in turn play a direct role in spinal cord injuries in regard to tissue damage. The losses of proteins catalyzed by calpain are associated with neuronal death, degeneration of axons and myelin vesiculation. This closely linked series of events suggest an extremely important role of calpains in the destruction of spinal cord tissue following injury. Consequently, the use of enzyme inhibitors may possibly play a productive role in spinal cord trauma (Banik et al., 1997).

Analyzing sequences of appearance for both calpains during muscle cell differentiation has allowed determination that these two enzymes are very

different in biological roles. For instance, m-calpain appears to be dominant in events such as calcium mediated membrane alterations in conjunction with myoblast fusion (Cottin et al., 1994). In comparison, μ -calpain, due to its later appearance (concentration level of activity), and being predominantly cytoplasmic in distribution, is probably involved in other calcium dependent activities signaling degradative roles on cytoskeletal proteins.

During maturation OU and Forsberg (1991) found that calpain and calpastatin activities declined rapidly between birth and 1 month of age and remained unchanged thereafter. Several factors can account for these losses such as muscle protein concentration is increased between birth and 1 month of age, thus diluting the calpain and calpastatin. Furthermore, a marked reduction of muscle RNA concentration between birth and 1 month of age occurs indicating that protein synthesis declines with age.

In rat skeletal muscle there is a mechanism for the interconversion of the single calpastatin isozyme into two different forms, each characterized by a different inhibitory specificity, versus the two homologous calpain isozymes. Interconversion is operated by a kinase and phosphatase reaction. Researchers demonstrated that following phosphorylation, calpastatin I undergoes a modification in its inhibitory specificity characterized by an increase in its efficiency versus m-calpain, and a decrease in inhibition versus μ-calpain. Thus, phosphorlated calpastatin I has similar characteristics of native skeletal muscle calpastatin II. In addition, during dephosphorlization, calpastatin II undergoes conversion into a highly active form on μ-calpain and much less active on m-

calpain. The predominant enzyme (μ-calpain) isolated from rat muscle, acquires inhibitory specificity identical to those of the less represented enzyme (m-calpain) following phosphorylation by a protein kinase. The demonstration of this cyclic mechanism of interconversion provides an explanation of how a single calpastatin isozyme can efficiently regulate the activity of two different calpain isoforms (Pontremoli et al., 1992).

In regard to mechanism of action (Mechetti et al., 1991), rat skeletal muscle contains a calpain activator protein characterized by a high specificity for m-calpain. The activator protein will interact with m-calpain forming a 1:1 complex. After the complex is immobilized to membranes the activator will resist degradation by m-calpain but the rate at which calpastatin is degraded by proteinase will increase. These results would suggest that translocation and association of the native proteinase to the activator within the cytoskeletal framework controls the expression of m-calpain activity. Furthermore, the modulation of the calcium dependent neutral proteinases is based on the presence of distinct protein molecules localized in different cell compartments and represented by a natural inhibitor and by a natural activator.

β-agonist induced effects on the calpain system are associated with corresponding changes in specific mRNA. Administration of β-adrenergic agonists to mammals can produce skeletal muscle hypertrophy in some species and muscle types. Parr et al., (1992) fed β-agonist to steers and caused significant longissimus dorsi hypertrophy, muscle mass was significantly increased as well as m-calpain and calpastatin activity in treated animals.

Treated animals also showed a 96% increase in calpastatin mRNA and a 30% increase in m-calpain large subunit mRNA in muscles. Therefore, both m-calpain and calpastatin expression may be stimulated by β-agonist action at the level of transcription. Among the many potential regulatory effects of calpains the relationship with hypertrophy may be indirect since calpains also interact with hormone and growth factor receptors, protein kinase C, and transcription factors, in addition to a range of membrane cytoskeletal and nuclear proteins. Therefore, the differential changes in messages induced by β-agonist suggest the expression of alternative mRNA may be a factor in calpastatin regulation (Parr et al., 1992; Higgins et al., 1988; Morgan et al., 1993).

XIII. ROLE OF CALPAIN AND CALPASTATIN IN MEAT TENDERIZATION

The role of calpain and calpastatin in postmortem tenderization has been suspected for around 20 years. The importance of calcium ions was first demonstrated by Davey and Gilbert (1969) who showed that the binding of calcium ions by the addition of ethylenediaminetetraacetic acid (EDTA) prevented the breakdown of muscle fibers. The first demonstration that in situ levels of calpains could be implicated indicated degradation of troponin T (an indicator of tenderness) was lower in psoas major than in semitendinosus or longissimus dorsi and was correlated to its lower content of calpains. Direct evidence for the involvement of calpains and calpastatin to tenderness was later shown with the addition of calpains and/or calcium ions to raw meat resulting in enhanced tenderness of the cooked meat (Koohmaraie, 1988).

Skeletal muscle is composed of three general classes of proteins: sarcoplasmic, connective tissue and myofibrillar. Of these three classes, the principle mechanism involved in postmortem tenderization is limited to the proteolysis of the myofibrillar protein fraction. To bring about a change in meat tenderness involves the actions of proteases. To be considered a protease they need to have the following characteristics: (1) located within the skeletal muscle (2) have access to the substrate and 3) have the ability to hydrolyze the same proteins in an in vitro system that are degraded during postmortem storage (Koohmaraie, 1988; Koohmaraie, 1992a).

There are many proteases in skeletal muscle however, only calpains and certain lysosomal enzymes thus far have been shown to degrade myofibrillar proteins (Koohmaraie, 1988; Thomson et al., 1997; Goll et al., 1992). Consequently, the focus of the scientific community has been geared towards these two proteolytic systems in attempting to sort out their role in postmortem tenderness. However, it is assumed that during postmortem storage, lysosomes are ruptured and cathepsins are released into the cytosol but little research is able to support this theory. Thus, it is questionable what role lysosomal proteases have in postmortem tenderness. Contrarily, considerable experimental evidence will support the theory that calpains are the primary enzymes involved in postmortem proteolysis and tenderization (Koohmaraie, 1988; Goll et al., 1992).

Several changes occur in skeletal muscle during postmortem storage of carcasses leading to loss of integrity of those tissues. This loss of integrity, which

leads to the changes in skeletal muscle causing disruption of the muscle cells and meat tenderization, are the result of proteolysis. Key changes occur in postmortem muscle to cause tenderization such as: (1) Z-disk weakening leading to fragmentation of myofybrils, (2) disappearance of troponin-T, (3) degradation of desmin leads to fragmentation, (4) degradation of titin and nebulin whose effects on meat tenderness are not fully understood, and (5) the contractile proteins actin and myosin are not affected, even after 50 plus days of postmortem storage (Koohmarie, 1988; Koohmaraie, 1992a).

Conversion of muscle to meat is complex and involves metabolic, physical and structural changes. The oxygen supply is ceased when blood to the tissues is ceased. The products of metabolism (glycolysis) cannot be removed resulting in lactic acid accumulation. This in turn causes a gradual decline in pH of the tissue from 7.0 to 5.5 over a 24-h period. In conjunction, temperature of the carcass falls and elevation of the free Ca concentration due to its release from the mitochondria and sarcoplasmic reticulum will occur. These three changes alone (gradual decline in pH, temperature, and an increase in calcium/calpains concentration) have a dramatic effect on the endogenous proteolytic systems in regards to postmortem tenderization (Koohmaraie et al., 1992b).

The activity of tenderization in meat due to the proteolytic activity is correlated to the amount of free unbound μ-calpain and m-calpain. This activity of proteolytic enzymes was modeled (Dransfield, 1993) to illustrate the importance of calpastatin and calcium ions in modifying the tenderization by calpains. For instance, as pH declines free calcium concentration rises and activates μ -calpain

with most of it being bound to calpastatin. As pH further declines the binding of activated μ-calpain to calpastatin is decreases and the level of free activated μ-calpain increases causing increase tenderization (Dransfield, 1993). The same type of process occurs with calpain m only at higher free calcium ion concentrations, which occur as pH further declines. The level of free activated calpains is determined by the balance between their decay and their release from calpastatin as well as their inactivation in the presence of a sufficient concentration of calcium ions. This model identifies the steps, which takes place in the presence of calcium ions.

Step 1. Initiation – the inert calpains are activated by the increase in calcium ion concentration thus initiating the tenderization process.
Step 2. Binding – equilibrium of the binding of calpains to calpastatin determines the level of free activated calpains, which increase as the pH declines.
Step 3. Inactivation of free activated calpains. Decay of free activated calpains by autolysis.
Step 4. Inactivation of calpastatin.

Step 5. Tenderization – proteolysis of structural components by remaining calpains causes tenderization.

This balance between inhibition, inactivation and activity of calpains and their decrease as the pH declines maintains the proteolytic activity of calpains and gradually produces the process of tenderization (Koohmaraie, 1988; Dransfield, 1993).

The work reported thus far indicates that the proteolytic roles of calpain and calpastatin in relation to muscle have a wide array of activity from degradation to maturation. More research is needed in determining what the exact mechanism of action is as far as regulation of calpastatin and calpains.

Determining this regulation mechanism may allow a better understanding of a number of questions ranging from meat tenderization to human spinal cord injury treatments, all of which have a great deal to offer economically and socially.

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EFFECTS OF SUPPLEMENTAL VITAMIN D_3 LEVELS ON FEED INTAKE AND BLOOD MINERALS OF YEARLING STEERS[1]

K. Karges, J.B. Morgan, D.R. Gill, J.E. Breazile, and F.N. Owens

ABSTRACT

Two trials were conducted to evaluate the effect of VITD on feed intake of a 80% concentrate diet fed to yearling steers (371 kg). Although supplemental Vitamin D_3 (VITD) improves tenderness of beef cuts, it may depress feed intake. In trial 1, VITD was top-dressed onto the ration daily at a rate of 0, 5, 7.5, 15 or 75 million IU per steer daily. Four steers were allocated to each diet and fed individually once daily; daily dry matter consumption was recorded. Significant depressions ($P<.05$) in dry matter intake were first observed on d 2 with 75 million IU, d 4 with 15 million IU, d 5 with 7.5 million IU, and d 6 with 5 million IU added VITD. This decreased feed consumption was due to a dose effect of VITD and was significant ($P<.05$) on d 3. Starting on d 2, the depression in feed intake was linearly ($P<.05$) related to concentration of VITD in the diet. In trial 2, five steers were allocated to each of four dietary concentrations with VITD pelleted into a corn-based supplement. VITD was added as percentage of the total daily diet so that at a 11 kg base line daily feed intake, each steer received 0, 2.5, 5 or 7.5 MIU daily. The amount of VITD supplement fed remained constant throughout the trial, while the total amount of feed fed was allowed to fluctuate for the 25-d trial. Significant ($P<.05$) depressions in dry matter intake were first detected on d 12 for both the 5 and 7.5 million IU supplements and at d 20 when 2.5

million IU added VITD was fed. Feed intake was first depressed on d 12 with the response being linear ($P < .05$). Results of the present study confirm that feed intake is depressed when high concentrations of VITD are fed, and to minimize the detrimental effects of VITD on dry matter intake, VITD should be fed as percentage of the total ration and incorporated in the supplement as a pellet.

(Key Words: Feedlot, Vitamin D, Cattle, Intake.)

INTRODUCTION

Vitamin D is commonly referred to as the "sunshine vitamin" due to its production in the skin by the conversion of 7-dehydrocholesterol by ultraviolet irradiation to cholecalciferol (vitamin D_3, VITD). The rapidly expanding knowledge of the metabolism and actions of VITD has recently led to investigations on the effectiveness of VITD as a possible means to improve tenderness of beef. Tenderness was recently ranked by the National Beef Quality Audit (NCBA, 1995) as the second most important beef quality problem that the cattle industry should address in order to prevent further decline in market share to other food protein sources. Smith et al. (1995) suggested that the lack of beef tenderness costs the industry $250 million annually. Consequently, it is of no surprise that National Cattlemen's Beef Association (NCBA, 1995) placed high emphasis on the development of an instrument or procedure that could be used to improve or predict beef tenderness.

Recently, Oklahoma State University has been investigating the use of VITD fed at high levels for a short period prior to harvest to improve tenderness. Swanek et al. (1997) reported that feeding high amounts of VITD for 5 to 10 d prior to harvest would increase tenderness of beef cuts. The mechanism of action for VITD is not clear, although elevations in plasma calcium concentrations and increased pH levels appear to be associated with supplemental VITD. However, the effects supplementing high levels of VITD on feed intake are not clear. Supplementation with high levels of VITD will elevate blood calcium (Swanek et al., 1997; Hibbs and Pounden, 1955; Horst and Littledike, 1979), but little research has addressed the effects of different levels of VITD on feed intake and other blood mineral concentrations. Therefore, the present experiments were conducted to evaluate the effects of different levels of VITD on feed intake plus blood mineral concentrations of yearling cattle.

MATERIALS AND METHODS

Trial 1: Twenty Angus x Hereford crossbred steers (371 kg) were stratified by weight and assigned randomly to one of five treatments: 0, 5, 7.5, 15 or 75 million IU (MIU) of VITD per steer daily. Steers were housed in individual 3.65 x 3.65 meter slated floor concrete pens equipped with automatic waterers at Oklahoma State University Nutrition Physiology Research Barn. The diet was fed individually each morning after orts from the previous feedings were removed and weighed. Dry matter intakes were monitored daily with the amount of fresh feed added being based on intake of

the previous day. Steers were fed an 80% concentrate diet (Table 1) ad libitum with fresh feed provided once per day. VITD, in a powder form and containing 500,000 IU VITD per gram, was top dressed onto the ration and mixed into each daily diet. The amount of VITD fed was held constant throughout the trial for each treatment regardless of fluctuations in intake. Steers were adapted to the diet for 7 d prior to VITD addition. All steers received VITD for a 12 d period. Blood samples were taken by veni-puncture from the tail using heparinized collection tubes. Blood collection was performed three times each week through-out the trial starting 4 d prior to the beginning of supplementation with VITD. Blood was centrifuged following collection and the plasma separated and frozen (-17°C). Plasma was analyzed for total calcium, phosphorus and magnesium. Total mineral analysis was conducted using a Vitros 750 x RC (Ortho-Clinical Diagnostics Systems, Raritan, NJ).

Trial 2: The same cattle that were utilized in trial 1 were also utilized in trial 2 following a 120 d rest period. Twenty Angus x Hereford crossbred steers (445 kg) were stratified by weight and assigned randomly to one of four treatments with five steers per treatment: 0, 2.5, 5 or 7.5 MIU of VITD per steer daily. Cattle were housed with one or two steers per pen in partially covered 3.65 x 15.85 pens equipped with automatic waterers and fence-line cement bunks with 96.5 cm of linear bunk space per steer at OSU Nutrition Physiology Barn. The diet (Table 1) was fed twice daily with orts removed and weighed each morning prior to adding fresh feed. Daily dry matter intake

was monitored with the amount of feed added adjusted based on the previous days intake. Steers were adapted to the diet for 8 d prior to VITD addition. In this trial, VITD was included as a part of a pelleted supplement with corn serving as the base. VITD was added as percentage of the total daily diet so that at a 11 kg base line daily feed intake, each steer received 0, 2.5, 5 or 7.5 MIU daily. The amount of VITD supplement fed remained constant throughout the trial, while the total amount of feed fed was allowed to fluctuate for the 25-d trial. Blood samples were collected using the same procedures and analyzed as described for Trial 1.

Statistical Analysis: For both trials, the general linear model procedure of SAS (1985) was used to analyze DMI and blood parameters within each sampling day. Orthogonal contrasts were used to determine 1) the effect of VITD supplementation and 2) the response to VITD concentration in the diet (Trial 1) or VITD intake (Trial 2).

RESULTS AND DISCUSSION

Trial 1: The primary objective of Trial 1 was to determine if VITD supplementation altered dry matter intake (DMI) and blood mineral concentrations compared to non-supplemented steers. Mean DMI for each treatment (Figure 1) indicated supplemental VITD depressed DMI. Depressions in DMI first became significant ($P < .05$) after 2 d with 75 MIU were fed, 4 d with 15 MIU, 5 d with 7.5 MIU, 6 d with 5 MIU of added VITD. DMI difference between those cattle fed the control diet and those supplemented with VITD was significant ($P < .05$) after d 3, and the response

to VITD level was first detected as linear ($P < .05$) on d 2. The decrease in DMI in the present study is in contrast to that observed by Swanek et al. (1999), although DMI was restricted to 1.5% of BW (Swanek et al. 1999), which possibly accounted for no further decrease in DMI. The observations from the current trial with excessive intakes of VITD produced some common observations of VITD toxicity such as loss of appetite, weight loss, depression and elevated blood calcium (Hoffman-LaRoche Inc. 1994; NRC, 1987). However, the effects of VITD on DMI for those steers fed the lower levels of VITD were not as pronounced as was observed for those animals receiving higher VITD concentrations. Parenteral administration of 15 million IU of vitamin D_3 in a single dose caused toxicity and death in pregnant dairy cows (Littledike and Horst, 1982). Whereas, an oral dose of 20 to 30 million IU of vitamin D_2 daily for 7 days caused little or no toxicity in pregnant dairy cows (Hibbs and Pounden, 1955). This difference between oral and parenteral dose probably relates to the fact that the D_3 form of VITD is much more potent than the D_2 form. In addition, the rumen microbes are able to metabolize vitamin D to the inactive form of 10-keto-19-nor vitamin D, thus explaining some of the differences observed between oral and parenteral dosing (Hoffman-LaRoche Inc. 1994). The toxic dose of vitamin D is variable and greatly depends on the duration of intake and level of dose, as VITD appears to be a cumulative toxicity.

Interestingly, some of the cattle that stopped receiving supplemental VITD and were switched from VITD diets to the control diet on d 13 continued

to have low intakes that increased slowly, only reaching DMI equal to that of control cattle on d 25. Cattle maintained on the VITD supplemented diets continued to have low DMI intakes throughout the duration of the experiment. These data indicate that VITD will alter intake patterns and reduce feed intake when the total amount of VITD intake on previous days exceeds about 30 MIU. For cattle that have been fed high levels of VITD for an extended period of time feed intake will continue to be low even after supplementation has stopped.

Blood plasma concentrations (Figure 2) for total calcium and phosphorus (Figure 3) were significantly increased with VITD supplementation and became linearly related to VITD levels on d 2 ($P<.05$). It is well established that VITD increases plasma calcium concentrations by the stimulation of intestinal absorption (DeLuca, 1986; Wasserman et al., 1961; Behar and Kerstein, 1976), mobilization of calcium from bones (DeLuca, 1982) and increases renal absorption of calcium from the kidney (DeLuca, 1979). However, bone resorption of calcium is thought only to slightly influence the increase of calcium concentration observed after VITD supplementation (Hove et al., 1983; Goff et al., 1986). Thus, increases that are typically observed for plasma calcium are primarily the result of increased intestinal or renal absorption.

The blood plasma parameters in the current trial indicate total calcium as well as total phosphorus levels increased while magnesium levels were decreased. Thus, the results of the present trial support the concept of

Swanek et al. (1999) that supplementation with VITD alters the blood profile allowing more calcium to be available in the post-mortem tenderization process. The hypercalcemic, hyperphosphatemic, and hypomagnesemic activity that occurred after supplementation of VITD is also in agreement with Hove et al. (1983) who gave intramuscular injection of 1,25-dihydroxyvitamin D_3 to dairy cows and observed a 30% increase in plasma calcium, 25% increase in phosphorus and a 20% reduction in magnesium concentrations. In trial 1, total plasma concentrations for calcium and phosphorus first peaked at d 7 (35%) and d 4 (34%), respectively for 75 MIU of VITD while magnesium levels by d 7 had decreased by 47% when compared to controls. Regarding increased levels of phosphorus, high levels of VITD will cause suppression of the parathyroid hormone resulting in the stimulation of phosphate mobilization from bone (Chen et al., 1972) and absorption of phosphorus from the small intestine (Castillo et al., 1975). Thus, an increase in blood plasma phosphate is also observed when high levels of VITD are supplemented.

A significant decrease in magnesium (Figure 4) attributable to a dose effect of VITD was first detected on d 2 and became linearly related to VITD dose level on d 4. These results disagree with previous research that has demonstrated that in VITD replete animals, large doses of VITD appear to increase magnesium absorption (Hardwick et al., 1991). This is contrary to other research that has shown with large doses of VITD, a decrease in fecal magnesium and a rise in urinary magnesium will occur without changing the magnesium balance (Brickman et al., 1975). Other investigators have

reported high doses of VITD greatly enhance urinary magnesium, leading to a decrease in magnesium retention in animals (Hanna, 1961; Lifshitz et al., 1967). Furthermore, it has been postulated that calcium may have an indirect effect on magnesium absorption through changes in membrane permeability (Hardwick et al., 1991). Thus, an interaction between calcium and magnesium is probable since research has shown that high levels of calcium in the diet result in a significant decrease in magnesium absorption in the ileum (Norman et al., 1981).

Trial 2. Significant depressions in DMI (Figure 5) were first detected on d 12 with both 5 and 7.5 MIU and on d 20 with 2.5 MIU added VITD. As compared to results from trial 1, steers in this trial tolerated higher VITD intakes (50 to 60 MIU compared to about 30 MIU) before DMI was depressed. The mean DMI of vitamin D supplemented cattle became different ($P<.05$) from the DMI of control cattle on d 12 with linearity of the DMI response to VITD intake becoming significant on d 12. These results could be interpreted to suggest that it is possible to avoid, or at least postpone the depression in feed intake noted with supplemental VITD by including VITD into the total diet by incorporating VITD into a pellet rather than top-dressing VITD on the diet as was done in trial 1.

Blood analysis indicated that total calcium levels on d 25 was increased for cattle receiving VITD supplementation (Table 2); this difference was significant ($P<.05$) when tested for linearity but was not significant ($P=.09$) when comparison of control to VITD supplemented cattle. Plasma

phosphorus was elevated but was not found to be significant supporting results of Trial 1. Note that these samples in trial 2 were taken on d 1 and d 25 after VITD supplementation began. As noted with dairy cows (Hibbs and Conrad, 1983), calcium responses to high doses of VITD are transient and may disappear as homeostatic systems readjust their calcium metabolism. It appears though that even after long term supplementation with VITD calcium levels will be elevated. If elevated blood calcium concentrations are desired, supplementing with VITD for longer than 8 days would not be desirable due to the probability of depressing DMI. VITD assays of the supplement fed indicated that VITD concentration of the pellet was within 96.5% of the concentration in the mixture before pelleting.

IMPLICATIONS

Cumulative intake of 25 to 50 MIU of VITD over a period of 15 days will depress dry matter intake by market-ready cattle. This intake depression was postponed by feeding VITD as a percentage of the total ration rather than top-dressing it onto the feed and incorporating the VITD into a pellet. Blood plasma concentrations of calcium were increased by 2 days and reached a plateau, possibly associated with decreased VITD intake, after about 6 days. After prolonged feeding of VITD (25 days), the response in blood calcium was not lost. Feeding VITD for a short period of time to possibly buffer feed intake depressions but still improve beef tenderness may be a possible.

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Table 1. Diet and calculated nutrient composition (% of DM)[a]

Ingredients	% of diet DM
Cracked corn	62.7
Cottonseed hulls	14.3
Alfalfa pellets	6.1
Soybean meal 44%	10.2
Cane molasses	4.25
Dicalcium phosphate	.55
Limestone	.56
Salt	.55
Urea	.11
Potassium chloride	.58
Rumesin-80	.0187
Tylan-40	.0125
Pellet supplement	
Cracked corn	68.0
Soybean hulls	25.7
Cane molasses	3.0
Vitamin D	3.3
Diet composition, calculated	
NEm, Mcal/cwt	87.06
NEg Mcal/cwt	53.27
Crude protein	12.95
Calcium	.52
Phosphorus	.41
Potassium	1.24
Magnesium	.17
Pellet composition, calculated	
NEm, Mcal/cwt	89.15
NEg, Mcal/cwt	57.06
Crude Protein	9.54
Calcium	.17
Phosphorus	.25
Potassium	.71
Magnesium	.10

[a]Monensin supplied at 30 g/ton.

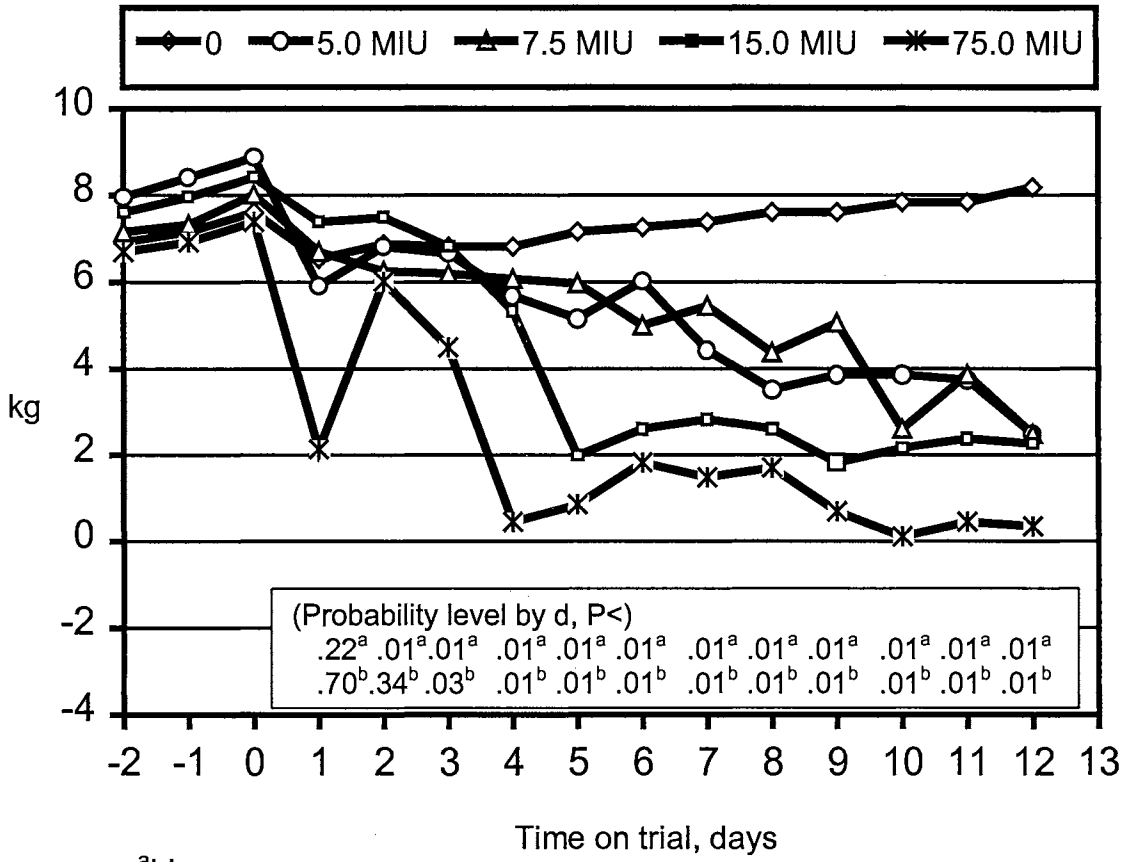
Tylosin supplied at 10 g/ton.

Table 2. Least squares means for blood plasma samples from steers in trial 2

Item	Day	0	2.5 MIU	5.0 MIU	7.5 MIU
Ca, mg/dl	1	9.60	9.80	10.25	9.80
Ca, mg/dl	25	10.20	11.60	12.00	12.20
P, mg/dl	1	8.20	8.40	7.25	7.60
P, mg/dl	25	10.00	10.80	10.20	9.80

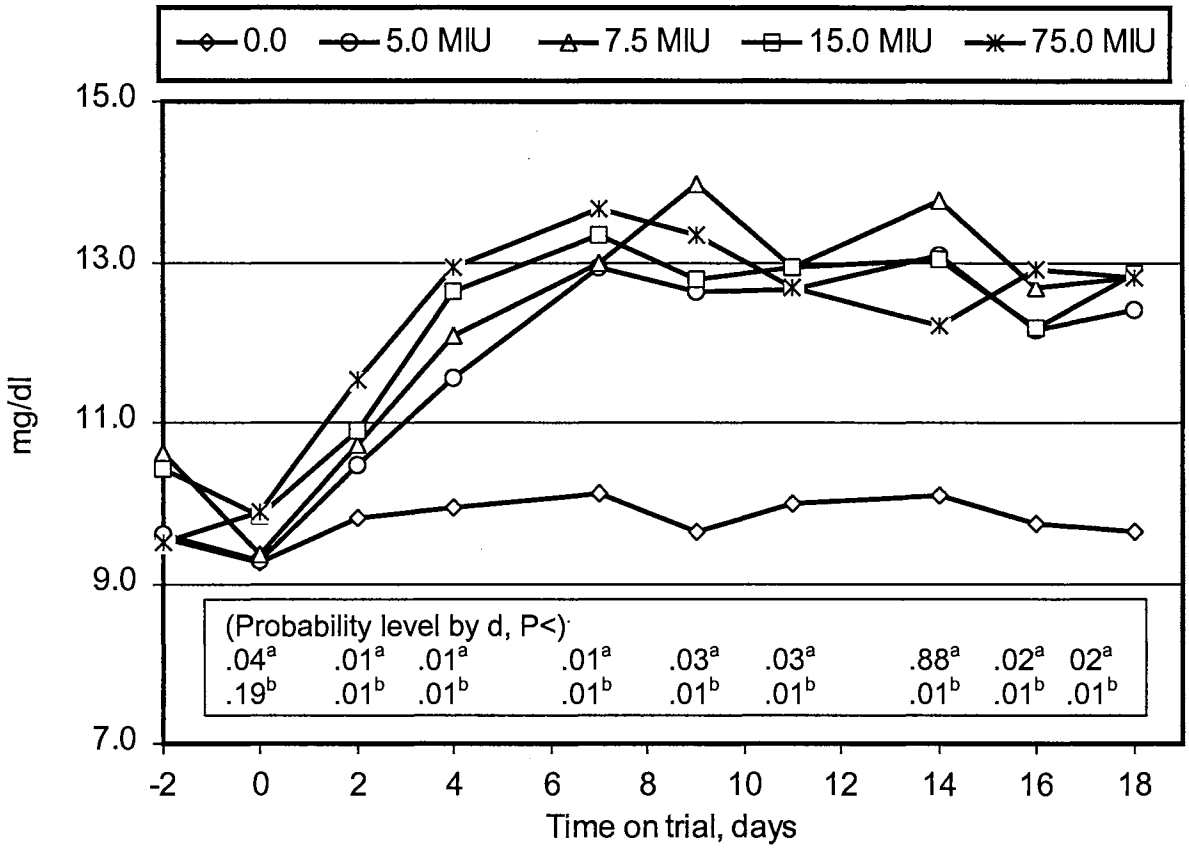
[a]Linear response (P<.008)
[b]Dose effect (P<.04)

Figure 1. Least squares means for DMI of steers supplemented different levels of vitamin D (Trial 1)



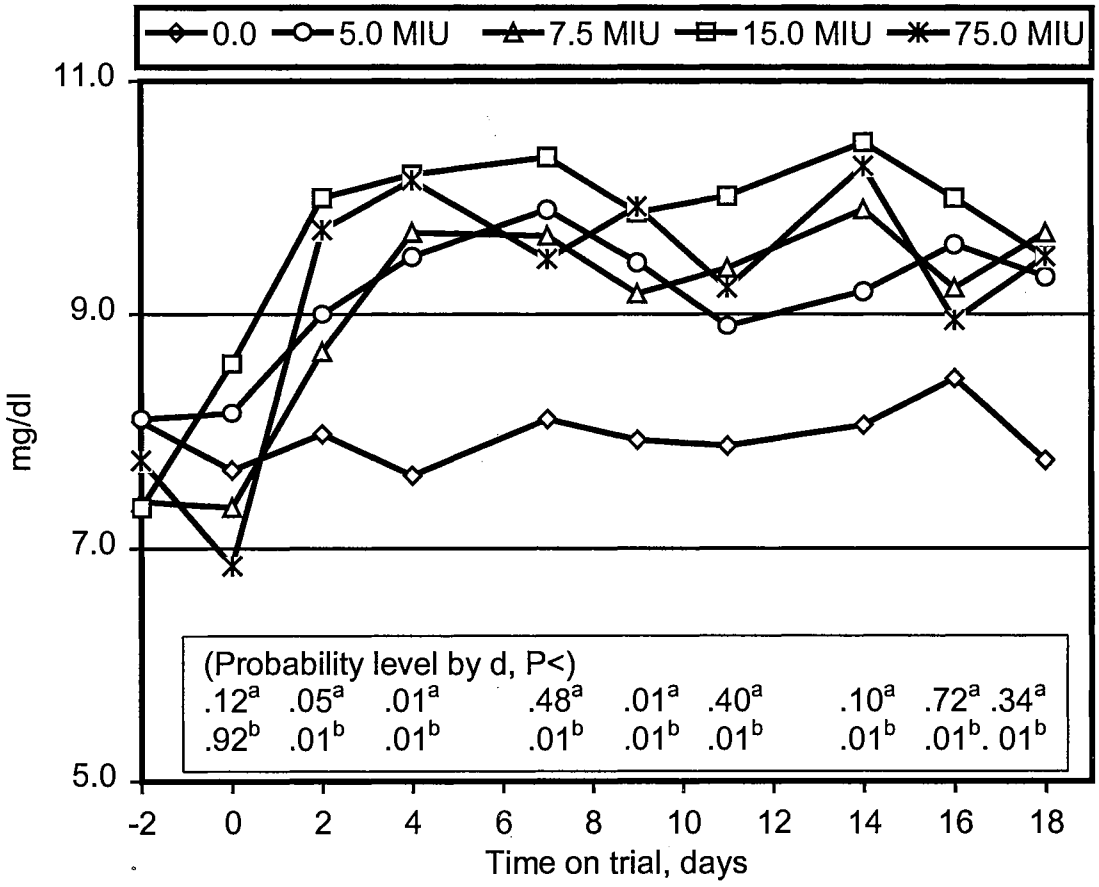
[a]Linear response
[b]Dose effect

Figure 2. Least squares means of blood plasma calcium concentrations of steers supplemented different levels of vitamin D



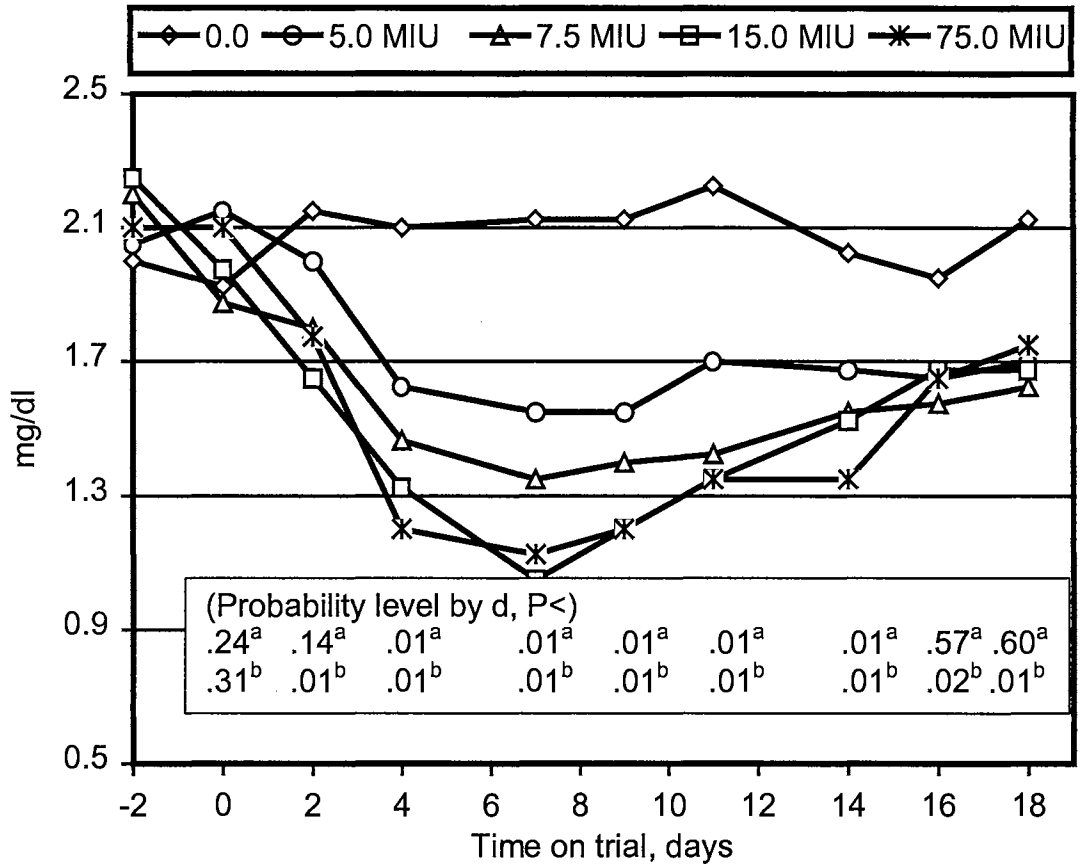
[a]Linear response
[b]Dose effect

Figure 3. Least squares means of blood plasma phosphorus concentrations of steers supplemented different levels of vitamin D



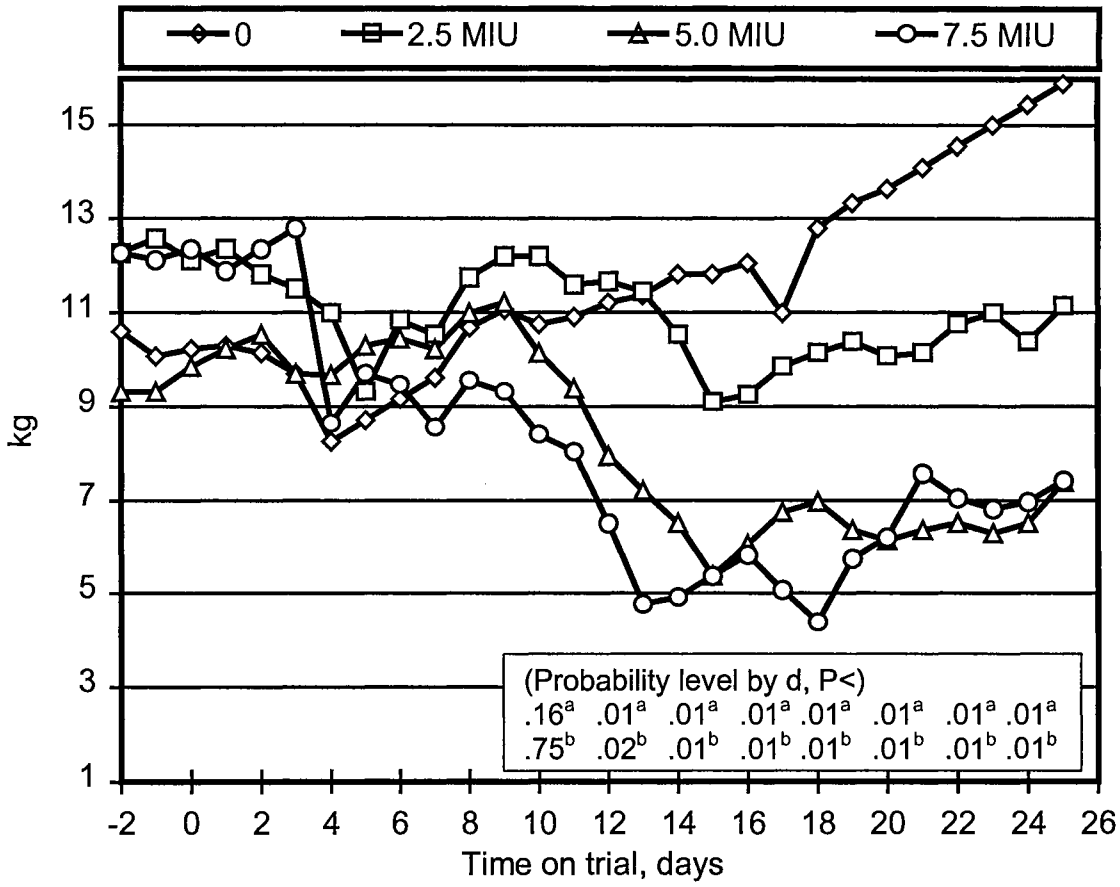
[a]Linear response
[b]Dose effect

Figure 4. Least squares means of blood plasma magnesium concentrations of steers supplemented different levels of vitamin D



[a]Linear response
[b]Dose effect

Figure 5. Least squares means for DMI of steers supplemented different levels of vitamin D (Trial 2).



[a]Linear response
[b]Dose effect

EFFECTS OF SUPPLEMENTAL VITAMIN D_3 ON CARCASS CHARACTERISTICS OF BEEF STEERS.

K. Karges, J. B. Morgan, D. R. Gill, J.E. Breazile, and F. N. Owens

ABSTRACT

Supplementing Vitamin D_3 (VITD) to market-ready cattle pre-harvest has been shown to improve beef tenderness. The current trial was conducted to determine effects of supplemental VITD on blood calcium (Ca), carcass traits, Warner Bratzler Shear (WBS) force values, pH (0, 3, 12, and 24 h post-harvest), water holding capacity and calpastatin activity of three different muscles: Longissimus dorsi (LD), Gluteus medius (GM) and Biceps femoris (BF). Market-ready steers (n=24; 545 kg) were allocated in a completely randomized design to one of four treatments: no supplemental VITD or 6 million IU (MIU) VITD daily for either 4 or 6 d pre-harvest. Steers were fed a 90% concentrate ration (12.35% CP; 1.34 Mcal Neg/kg) twice daily while VITD at 6 MIU was supplemented once daily in a pellet at 3.8 percent of the total ration. Cattle were harvested and carcasses were chilled prior to steak removal. Three steaks (2.54 cm) were fabricated from each of the LD, GM, and BF and aged 7, 14 or 21 d. Steaks were cooked at 185°C to a final internal temperature of 70°C and Warner-Bratzler shear force values (WBS) were obtained. Supplementing VITD resulted in lower Warner-Bratzler shear force values among LD and GM steaks ($P< .02$) aged 14 or 21 d postmortem compared to control steaks. LD and GM steaks from steers supplemented VITD for 6 d had lower ($P < .01$) shear force values than their steak counterparts from steers fed VITD for 4 d at d 7 or d 14

postmortem, respectively. BF steaks from steers receiving VITD had lower shear force values than BF steaks from control steers at d 7 postmortem. As a percentage of total steaks, supplementing VITD decreased the percentage of steaks that had WBS values of 3.86 kg or greater by 68, 45, and 56% of the controls for LD, GM, and BF steaks, respectively. Feeding VITD for 6 d vs 4 d resulted in a delay in pH decline for all muscle types as evidenced by higher pH values after 0, 3, or 12 h postmortem but lower ($P<.05$) pH values 24 h postmortem. Feeding VITD for greater than 4 d had no effect on calpastatin activity. Water holding capacity was improved ($P < .02$) after 0 h, 24 h and 21 d postmortem when VITD was fed and was greater at time 0 or 24 h if VITD was fed for 6 d vs 4 d. Blood calcium concentrations were greater ($P<.05$) when VITD was fed and with increased feeding time of VITD. These data suggest that supplementing 6 MIU VITD will improve beef tenderness, as well as increase blood plasma Ca^{++} concentrations and water holding capacity.

(Key words: Vitamin D, Carcass, Beef, Tenderness, pH, Calpastatin, Calcium)

INTRODUCTION

Consumers today consider tenderness to be the single most important determinant of meat quality. This is reflected by the positive relationship between the price of a beef cut and its perceived tenderness (e.g., psoas major). Smith et al. (1995) estimated that lack of tenderness in beef cuts costs the industry $250 million annually. The 1995 National Beef Quality Audit stated that lack of uniformity, excessive fatness, and inadequate tenderness were all ranked in the top ten quality concerns; tenderness was ranked as the second most important

beef quality problem. Consumers have demonstrated that they are able to discern between categories of tenderness and are willing to pay more for meat known to be tender (Boleman et al., 1997; Shackelford et al., 1999). Because consumers consider tenderness to be a major factor determining the eating quality of meat, there is an economic incentive for producing tender beef. With this in mind, scientists have focused their attention on developing nutrition/management programs addressing the tenderness issue. There have been advances in the areas of the calcium-mediated process (Wheeler et al., 1994) as well as the development of a classification system that allows carcasses to be grouped based on tenderness ratings (Shackelford et al., 1997).

One means of improving tenderness in beef cuts is to add supplemental Vitamin D (VITD) to the diet shortly before cattle are harvested. Previously, Swanek et al., (1997) reported that VITD supplementation increased tenderness in beef cuts when fed to steers from 5 to 10 d prior to harvest. Early studies on the prevention of milk fever indicated that either oral dosing (Hibbs and Pounden, 1955) or injecting (Horst and Littledike, 1979) VITD resulted in increased blood calcium concentrations. Increased calcium levels have been shown to have an important role in postmortem tenderization because of the importance of Ca^{++} in activating the calpain proteases system (Koohmaraie et al., 1988). However, the mechanism by which VITD may increase tenderness is not known. The objective of this trial was to examine the effects of feeding VITD and the length of time VITD was fed on Longissimus dorsi (LD), Gluteus medius (GM), and Biceps femoris (BF) characteristics.

MATERIALS AND METHODS

Twenty-four Angus-cross steers (550 kg) that had been on feed for approximately 150 d were transported to the Oklahoma State University progeny barn, where upon arrival at the research facilities steers were allotted into eight pens (three head per pen) which were assigned randomly to one of three dietary treatments: no VITD supplementation (12 steers) or 6 million IU (MIU) of VITD daily for either 4 or 6 d pre-harvest (6 steers per time interval). Housing consisted of partially covered pens with slatted floors and cement fenceline feedbunks. Automatic waterers were shared by adjacent pens. Steers were fed a 88% concentrate ration (Table 1) twice daily with VITD fed once daily in a pellet as a percentage (3.8%) of the total diet. Cattle were allowed a 12-d adaptation period to the diet before supplementation of VITD began. To ensure that all steers consumed the amount of VITD offered in the diet, DMI during the VITD supplementation period was restricted to 90% of the previous 5 d mean DMI.

All steers were harvested using an approved humane technique at the Oklahoma State University Food and Agriculture Processing Center. Following a 48-h postmortem chill (2°C), carcasses were ribbed and USDA quality and yield grades were recorded for each carcass. Seven d postmortem, the LD, GM, and BF muscles were fabricated from the left side of each carcass. Five, 2.54 cm thick steaks were fabricated from each of the three subprimals and vacuum packaged individually. These steaks were aged at 2°C for 7, 14 or 21 d after steaks were frozen at –29°C. Steaks from these subprimals were used later for

Warner Bratzler Shear (WBS) force determination (7, 14, or 21 d) and sensory panel evaluation (7 or 14 d).

Steaks were thawed for 24 h at 2°C and then broiled on a impingement oven (model 1132, Lincoln Foodservice Products, Fort Wayne, IN) to an internal temperature of 70°C. After cooling to room temperature (22°C), six to eight 1.27-cm diameter cores were obtained from each steak parallel to the muscle fiber orientation and shear force values were determined following the procedure of Wheeler et al., (1997); these values were averaged within steak groups and used as a single measurement. Cores were sheared once each on an Instron Universal Testing Machine model 1135 (Instron, Canton, MA) with a standard Warner-Bratzler attachment and 5 cm/min crosshead speed.

Steaks cooked for sensory panel evaluation were held at 70°C for up to 10 min before they were cut into 1 cm x 1 cm x 1 cm cubes and served warm to an eight-member panel trained according to Cross et al. (1978). Panelists independently evaluated two cubes from each sample for juiciness, beef flavor, tenderness, off flavors, flavor intensity, and connective tissue amount utilizing an 8-point scales (8 = extremely juicy, intense, tender, none: 1= extremely dry, bland, tough, abundant) and off-flavor on a 4-point scale (4 = none, 1 = intense) and beef flavor on a 3-point scale (0 = none, 2 = very strong).

Calpastatin activity was determined utilizing a slightly modified procedure of Shackelford et al. (1994); a 5 g tissue sample from 0 and 24 h of LD and GM muscles was extracted in 20 mL of extraction buffer (150 mM TRIS, 10 mM EDTA, and 7 mM MCE, pH 8.3) by homogenizing for 3 x 30 s with a polytron and

allowing a 30 s rest between each burst. The homogenate was centrifuged for 1 h at 30,000 x g and the supernant was filtered through cheesecloth. The supernant was transferred into 13 x 100-mm borosilicate test tubes and heated in a water bath (95°C) for 50 min to denature calpains. Following heating, samples were chilled on ice for 15 min. Final centrifugation was accomplished by allowing tubes to centrifuge for 30 min at 6,000 x g. Following centrifugation, the volume of supernant was determined and recorded for subsequent calculation of calpastatin. One unit of calpastatin activity was defined as the amount of inhibitor necessary to inhibit one unit of DEAE-purified m-calpain activity.

Water holding capacity was determined after 0-h, 24-h, 7-d, 14-d, and 21-d postmortem using a slightly modified Carver Press procedure. Briefly, ash-less Whatman filter paper was equilibrated to a constant humidity using a desiccator. Approximately .50 mg of tissue from each subprimal was placed on a single sheet of filter paper and placed between two Plexiglas plates. Using the Carver Press, 5000 p.s.i. was applied to samples for one min, then pressure was removed and both the moisture and meat ring were traced using a red pencil. Papers were dried at ambient temperature for 24-h prior to determining the area of the moisture and meat rings. Calculation of free water as % of the total water followed the procedure of Wierbicki and Deatherage (1958).

At the time of exsanguination, plasma blood samples were obtained using heparinized blood collection tubes. Blood was centrifuged immediately and the plasma separated and frozen (-17°C). Plasma was analyzed for total calcium, phosphorus, and magnesium concentrations. Blood was analyzed using a Vitros

750 x RC (Ortho-Clinical Diagnostics, Raritan, NJ). Measurements of pH were performed at 0, 3, 12 and 24-h postmortem by sampling 5 g of muscle tissue from the left side of the carcass at the LD, GM and BF locations. These samples were homogenized with 50 ml of distilled H_2O and pH measured.

Statistical Analysis: Shear force values, DMI, carcass traits, blood plasma measurements, pH values, calpastatin activity, water holding capacity, and palatability were analyzed statistically as a completely randomized design using the general linear model procedure of SAS (1985). Treatment x cut interactions were tested for palatability, water holding capacity, and pH while treatment x age interactions were tested for shear force values. Treatment sums of squares were separated using orthogonal contrasts that compared control vs VITD fed animals and duration of feeding of VITD (4 vs 6 d).

RESULTS AND DISCUSION

Although DMI was restricted (90% of ad libitum) during the time of VITD supplementation, DMI was still lower for those steers supplemented with VITD. Lower DMI was detected on d 4 for cattle receiving 6 MIU for 4 d of VITD (11.7% decrease) and for cattle receiving 6 MIU for 6 d on d 5 (13.9% decrease) as shown in Figure 1. These results support previous findings of Karges et al. (1999) in which DMI was significantly reduced when high levels of VITD were supplemented to yearling cattle.

Simple statistics of carcass characteristics as impacted by VITD supplementation are presented in Table 2. Carcass weights and live weights were lighter ($P < .05$ and .07, respectively) for those cattle that received VITD

compared to the control. Although these results are significant live weight data from the start of the trial was not obtained making it difficult to determine if this effect was due to VITD supplementation. However, a decrease in body weight is commonly observed in rats that have been subject to high doses of VITD (Thys-Jacobs et al., 1997). Fat thickness was lower (P<.05) for cattle receiving vitamin D and for those that received VITD longer (6 vs 4 d) as compared to controls. Although the decreased DMI associated with feeding VITD might explain these differences in carcass weight and yield grade, feed withdrawal for 24 h has not been associated with such drastic effects on carcass measurements (Janloo et al., 1999). These carcass differences can be attributed to VITD feeding or to random chance is not yet certain. No such effects were noted previously by Swanek et al. (1999).

Warner-Bratzler shear force (WBS) values (Table 3) after 7 d of aging of LD steaks were improved more (P<.0001) when VITD was fed for 6 d than when fed for 4 d. For meat aged 14 or 21 d, WBS was improved (P<.02) for those steaks from steers receiving VITD supplements; however, no differences (P>.05) were noted between VITD treatments (4 vs 6 d). For GM steaks, no differences were detected after 7 d of aging, but at 14 and 21 d, shear force was reduced (P<.04) for steaks from steers receiving VITD supplements. After 14 d of aging, tenderness was improved more (P<.03) by feeding VITD for 6 d than for 4 d. For BF steaks aged 7 d, WBS was reduced (P<.03) by VITD supplementation but no responses were detected with longer aging times. In regard to sensory panel ratings, steaks were grouped due to the lack of treatment x cut interaction. No

detectable differences (P>.05) for any of the categories evaluated of the three subprimals at 7 or 14 d of age were found (Table 5). Ideally, steaks should have shear force values below 3.86 kg to be considered "very tender" (Shackelford et al., 1994). Percentage of steaks within subprimals by treatment group >3.86 kg after 7, 14, or 21 d of postmortem aging is indicated in Table 4. After 7 d of postmortem aging, approximately 50% of all control cuts failed to meet the shear force threshold of < 3.86 kg compared with 17, 26, and 23% for steaks from steers supplemented with 6 MIU for 6 d for the LD, GM, and BF steaks, respectively. Extending the postmortem aging time for either 14 or 21 d for LD and BF steaks negated any advantages observed from VITD supplementation; only for GM did extending aging time increase tenderness. These results indicate that improvements in mean shear force can be attributed primarily to reduction in shear force of the toughest steaks. Contradictory to previous studies (Swanek et al., 1999), aging beef steaks did not improve WBS values in the present study. Perhaps the low number of head per treatment in this trial is a possible explanation. The fact that response differed among cuts suggests that response to VITD in tenderness, like the response to aging, will differ with the specific muscle group being tested.

No differences were detected (P<.05) in calpastatin activity between tissue samples of carcasses from control vs VITD supplemented cattle (Table 6). This does not support previous results of Swanek et al. (1999) even though the calpastatin activity values are comparable to previous reported values (Wheeler and Koohmaraie, 1992; Morgan et al., 1993). These results are possibly

confounded by the fact that the samples were stored for a period of 4 months (–29°C) prior to assays be conducted. Previous research has demonstrated that long-term freezer storage over 40 d reduced calpastatin activity up to 44% (Duckett et al., 1998). Koohmaraie (1990) suggested that although calpastatin activity is decreased with long-term freezer storage that the activity of the calcium dependent proteases is not altered. With sufficient calcium ions present it has been demonstrated that both calpain proteases (i.e., μ- and m-calpain) are active in postmortem proteolysis with a rapid loss of μ-calpain occurring while m-calpain activity remains near constant throughout postmortem storage. This suggests that μ-calpain is the primary protease involved in postmortem aging (Koohmaraie, 1987). With elevated plasma calcium levels due to VITD supplementation observed in the current study as well as others (Karges et al.,1999; Swanek et al., 1999) it would be unlikely that the calpain protease system is not involved in the postmortem tenderization process.

Blood calcium concentrations are normally between 8-10 mg/dl in cattle and are closely regulated by the parathyroid hormone, calcitonin and VITD. The effects of feeding VITD on prevention of parturient paresis in lactating dairy cows has long been established. Hibbs and Pounden (1955) demonstrated that oral dosing of VITD at 5, 10, 20, and 30 MIU for 3 to 8 d prepartum resulted in increased blood calcium by 1.9, 1.0, 1.9, and 2.3 mg/dl, respectively. Sachs et al., (1987) gave a single injection to dairy cows for the prevention of parturient paresis. A single injection of 1,25-hyroxyvitamin D_3 resulted in increasing blood calcium by 33% 4 days post-injection. The concentration of 1,25-hyroxyvitamin

D_3 peaked 24 to 48 h post-injection then dropped to the initial concentrations at 5 d post-injection. This decline in vitamin D concentration post-injection is supportive of what Bar et al. (1985) determined; the biological half-life of vitamin D is approximately 2.1 days. In addition to mobilizing calcium, VITD will increase the transport of calcium into skeletal muscle cells via activation of calcium channels. Recently, it was determined that calcium channels actually are involved in calcium entry into muscle cells by 1,25-hyroxyvitamin D_3 (Vazquez et al., 1998).

Blood plasma calcium concentrations (Table 7) in this investigation were significantly greater ($P<.03$) for animals supplemented with VITD and for those supplemented for 6 vs 4 d prior to harvest, which supports previous findings (Montgomery et al., 1999; Karges et al., 1999; Swanek et al., 1999). Concentrations of blood plasma magnesium (Table 7) were decreased numerically with the lowest concentration occurring with 6 MIU VITD for 6 d. This trended towards significance for a feeding effect ($P<.09$) but no duration of feeding VITD effect was detected ($P>.05$). Administration of $1,25(OH)_2D$ to cattle generally results in a reduction of plasma magnesium concentration, although the exact mechanism for this response is currently not clear (Fontenot et al., 1989). Previous research has determined that administration of $1,25(OH)_2D$ results in increased renal excretion of magnesium (Schneider et al., 1985), probably due to induced hypercalcemia resulting from high levels of VITD more so than being related to a direct effect of VITD. In another study, $1,25(OH)_2D$ administration to nonpregnant, nonlactating dairy cows caused a decline in plasma magnesium

concentrations although this was not the result of increased urinary excretion of magnesium (Goff et al., 1986).

Plasma phosphorus concentrations (Table 7) numerically increased slightly with VITD supplementation, which supports the findings of Karges et al. (1999) who observed a significant increase in phosphorus concentrations by d 2 of VITD supplementation. Chen et al. (1974) demonstrated that $1,25(OH)_2D$ will stimulate the mobilization of phosphorus from bone and Castillo et al. (1975) indicated that absorption of phosphorus from the small intestine is enhanced with increased levels of $1,25(OH)_2D$.

Typically, beef muscle pH taken 15 min after harvest will be within the range of 6.9 to 7.0 and declines to an ultimate pH of 5.3 to 5.5 by 48 h postmortem (Pearson and Young, 1989). In the present study, there was no treatment by cut interaction so data were pooled by time. Carcasses from cattle that had not been supplemented with VITD displayed a typical decline in pH over a 24-h period (Table 8) reaching a final pH of 5.28. In contrast, carcasses from cattle that had been supplemented with VITD for 4 d had lower pH values at 3 and 12 h post-harvest compared to controls. pH value for those carcasses from steers that had been supplemented with VITD for 6 d were consistently higher than those that had been supplemented for only 4 d; however, ultimate pH (24 h) was lower for those carcasses from steers fed VITD 6 d. An effect of feeding VITD occurred at 3 h ($P<.007$) and a duration effect of feeding VITD occurred at 0, 3, and 12 h ($P<.003$) and trended towards significance at 24 h ($P<.06$). These results suggest that possibly higher pH levels are conducive to increasing the activity of

the calcium activated proteases, specifically μ-calpain (Koohmaraie, 1992; Goll et al., 1992; Pearson and Young, 1989), which in turn would enhance postmortem proteolysis. These proteases have been repeatedly shown to play an enormous role in the postmortem proteolysis and meat tenderization process (For review see Koohmaraie, 1992).

In terms of water holding capacity (Table 9), no treatment by cut interaction was detected and data were pooled by time. A significant effect occurred for feeding VITD at 0 and 24 h and 21 d ($P<.02$), while a duration of feeding VITD occurred at 0 and 24 h ($P<.02$). Enright et al. (1999) observed significant improvement in objective color scores as well as a decrease in percentage drip loss indicating an increased water holding capacity for pork that had been supplemented with VITD 10 d prior to harvest. In the current investigation, there was an increase in water holding capacity for carcasses that had been supplemented with VITD at 0 and 24 h with a minimum of 6.5% and 3.5%, respectively.

In the present experiment, dietary supplementation of feedlot steers with 6 MIU of VITD daily for 4 or 6 d prior to harvest numerically increased tenderness of all three subprimals investigated. Supplementing VITD also reduced the percentage of tough steaks at 7 d of postmortem aging for all subprimals. However, including VITD in the diet tended to decrease feed intake and hot carcass weights. It appears that VITD will improve the postmortem tenderness of muscles investigated but the optimum dosage rate and duration of feeding VITD remains to be pinpointed to reduce or avoid effects on feed intake and carcass

weights. Improved tenderness of the expensive cuts would be of greatest interest in the hotel and restaurant trade whereas tenderness of lower-priced beef cuts could make beef that currently must be cooked with moist heat more suitable for grilling. In addition, dietary supplementation of feedlot steers with VITD for 4 or 6 d at 6 MIU prior to harvest increased blood Ca^{++} and decreased Mg^{++} concentrations. Calpastatin activity was not affected by VITD supplementation. Water holding capacity was improved for those carcasses from steers that had VITD supplementation. Muscle pH values were significantly increased with the longer duration of VITD supplementation suggesting that both increased blood Ca^{++}, higher pH and increased water holding capacity may be influencing the postmortem tenderization process.

IMPLICATIONS

The importance of meat tenderness to the U.S. cattle industry cannot be overstated. In 1995 the National Beef Cattlemen's Association identified tenderness as the second most beef quality problem and concluded that a procedure must be developed addressing this concern. It appears that supplementation with VITD for a short period prior to harvest is one possible procedure that can be used to address this concern.

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Table 1. Diet and calculated nutrient composition (% of DM)[a].

Ingredients	% of diet DM
Whole corn	79.2
Cottonseed hulls	5.0
Cane molasses	4.0
Supplement	8.0
Vitamin D supplement	3.8
Supplement	
Cottonseed meal	62.9
Soybean hulls	10.0
Urea	7.5
Salt	3.75
Limestone 38%	13.75
Potassium chloride	1.9
Zinc sulfate	.06
Manganous oxide	.05
Rumensin-80	.0187
Tylan-40	.0125
Vitamin D supplement	
Cracked corn	68.0
Soybean hulls	25.7
Cane molasses	3.0
Vitamin D	3.3
Diet composition, calculated	
NEm, Mcal/cwt	94.53
NEg, Mcal/cwt	60.33
Crude Protein	12.37
Calcium	.57
Phosphorus	.31
Potassium	.71
Magnesium	.16

[a]Monensin provided at the rate to supply 30 g/ton

Tylosin provided at the rate to supply 10 g/ton

Table 2. Least squares means for live weight and carcass traits of steers supplemented vitamin D at 6 MIU 4 or 6 d prior to harvest

	Vitamin D				Contrasts[a]	
Item	0	6 MIU/4 d	6 MIU/6 d	RSD	C vs D	4 vs 6 d
Steers, n	12	6	6			
Live weight, kg	597.00	573.50	563.40	81.89	.07	.64
HCW, kg	370.65	355.15	346.82	51.91	.05	.54
Dressing %	62.01	61.92	61.55	1.14	.45	.58
Lean maturity[b]	153.33	146.66	153.33	17.91	.65	.52
Marbling score[c]	415.83	406.66	390.00	37.69	.26	.45
FT[d]	3.24	2.80	3.05	.34	.03	.21
FYG[e]	3.52	2.61	2.98	1.13	.13	.58
KPH fat, %	3.95	2.08	2.33	4.02	.29	.91
Ribeye area, cm^2	83.55	85.35	81.68	1.33	.98	.47

[a]Contrasts:
C vs D = control vs all with vitamin D.
4 vs 6 = Duration of feeding vitamin D for 4 d vs 6 d.
[b]Lean maturity: 100 to 199 = "A" maturity (USDA, 1997) .
[c]Marbling scores: 300 to 399 = Slight (U.S. Select).
400 to 499 = Small (U.S. Choice).
[d]FT = Fat Thickness.
[e]Final Yield Grade.

Table 3. Least squares means for Longissimus, Gluteus medius and Biceps femoris steaks with Warner-Bratzler shear force values of steers supplemented vitamin D at 6 MIU for either 4 or 6 d

		Vitamin D			Contrasts[a]	
Item	0	6 MIU/4 d	6 MIU/6 d	RSD	C vs D	4 vs 6 d
Steer, n	12	6	6			
LD shear force, kg						
Aged 7 d	4.15	4.67	3.66	.95	.90	.0001
Aged 14 d	4.12	3.79	3.58	.88	.001	.28
Aged 21 d	4.30	4.00	4.02	.79	.02	.96
GM shear force, kg						
Aged 7 d	4.20	4.41	4.22	.70	.31	.22
Aged 14 d	4.37	4.31	3.95	.78	.04	.03
Aged 21 d	4.03	3.74	3.62	.57	.0002	.35
BF shear force, kg						
Aged 7 d	5.06	4.74	4.63	1.09	.03	.66
Aged 14 d	4.95	5.18	5.00	1.42	.57	.59
Aged 21 d	5.11	5.29	4.87	1.24	.90	.14

[a]Contrasts:
C vs D = control vs all with vitamin D
4 vs 6 = duration of feeding vitamin D for 4 d vs 6 d

Table 4. Percentage of Longissimus, Gluteus medius and Biceps femoris steaks with Warner-Bratzler shear force values greater then 3.86 kg

		Vitamin D	
Item	0	6 MIU/4 d	6 MIU/6 d
Steer, n	12	6	6
LD shear force values > 3.86 kg %			
Aged 7 d	52.48	30.69	16.83
Aged 14 d	58.24	20.88	20.88
Aged 21 d	58.00	24.00	18.00
GM shear force values > 3.86 kg %			
Aged 7 d	46.67	27.50	25.83
Aged 14 d	54.46	26.79	18.75
Aged 21 d	61.73	20.99	17.28
BF shear force values > 3.86 kg %			
Aged 7 d	51.81	26.61	22.58
Aged 14 d	49.58	26.05	24.37
Aged 21 d	50.39	26.77	22.83

Table 5. Least squares means for sensory evaluation for Longissimus and Gluteus medius steaks aged 7 or 14 d from steers supplemented with vitamin D at 6 MIU for either 4 or 6 d

	Vitamin D			Contrasts[a]	
Item	0	6 MIU/4 d	6 MIU/6 d	C vs D	4 vs 6 d
7-d age					
Juiciness	4.88	4.77	4.96	.91	.42
Beef flavor	.42	.40	.43	.97	.76
Tenderness	4.71	4.39	4.64	.38	.42
CTA	4.74	4.40	4.76	.46	.24
Off flavor	3.86	3.81	3.88	.85	.42
Flavor intensity	5.14	5.41	5.18	.48	.47
14-d age					
Juiciness	4.58	4.33	4.40	.22	.75
Beef flavor	.32	.29	.28	.52	.92
Tenderness	4.75	4.50	4.79	.61	.29
CTA[b]	4.90	4.63	5.03	.71	.13
Off flavor	3.80	3.79	3.89	.36	.15
Flavor intensity	5.09	4.96	4.97	.17	.98

[a]Contrasts:
C vs D = control vs all with vitamin D
4 vs 6 = Duration of feeding vitamin D for 4 vs 6 d
[b]Connective tissue amount

Table 6. Least squares means for calpastatin activity of muscle supplemented vitamin D for 4 or 6 d with 6 MIU

	Vitamin D				Contrasts[a]	
Item	0	6 MIU/4 d	6 MIU/6 d	RSD	C vs D	4 vs 6 d
LD[b]	2.91	2.18	2.74	.75	.26	.38
LD[c]	2.21	2.35	1.45	.87	.48	.19
GM[b]	2.16	1.71	2.07	.78	.31	.33
GM[c]	1.68	1.40	1.86	.75	.87	.35

[a]Contrasts:
C vs D = control vs all with vitamin D
4 vs 6 = Duration of feeding vitamin D for 4 vs 6 d
[b]Calpastatin activity at 0-h
[c]Calpastatin activity at 24-h

Table 7. Least squares means of blood plasma and ionized calcium from steers supplemented vitamin D for 4 or 6 d with 6 MIU

	Vitamin D				Contrasts[a]	
Item	0	6 MIU/4 d	6 MIU/6 d	RSD	C vs D	4 vs 6 d
Ca, mg/dl	9.91	11.33	12.16	.66	.0001	.03
P, mg/dl	7.08	7.33	8.33	1.53	.24	.27
Mg, mg/dl	2.16	2.00	1.83	.34	.09	.41

[a]Contrasts:
C vs D = control vs all with vitamin D
4 vs 6 = Duration of feeding vitamin D for 4 vs 6 d

Table 8. Least squares means for pH of carcasses supplemented vitamin D for 4 or 6 d with 6 MIU

	Vitamin D				Contrasts[a]	
Item	0	6 MIU/4 d	6 MIU/6 d	RSD	C vs D	4 vs 6 d
0 h	6.18	6.12	6.34	.21	.15	.0001
3 h	5.65	5.44	5.62	.27	.007	.003
12 h	5.38	5.29	5.40	.13	.12	.0008
24 h	5.28	5.33	5.28	.01	.25	.06

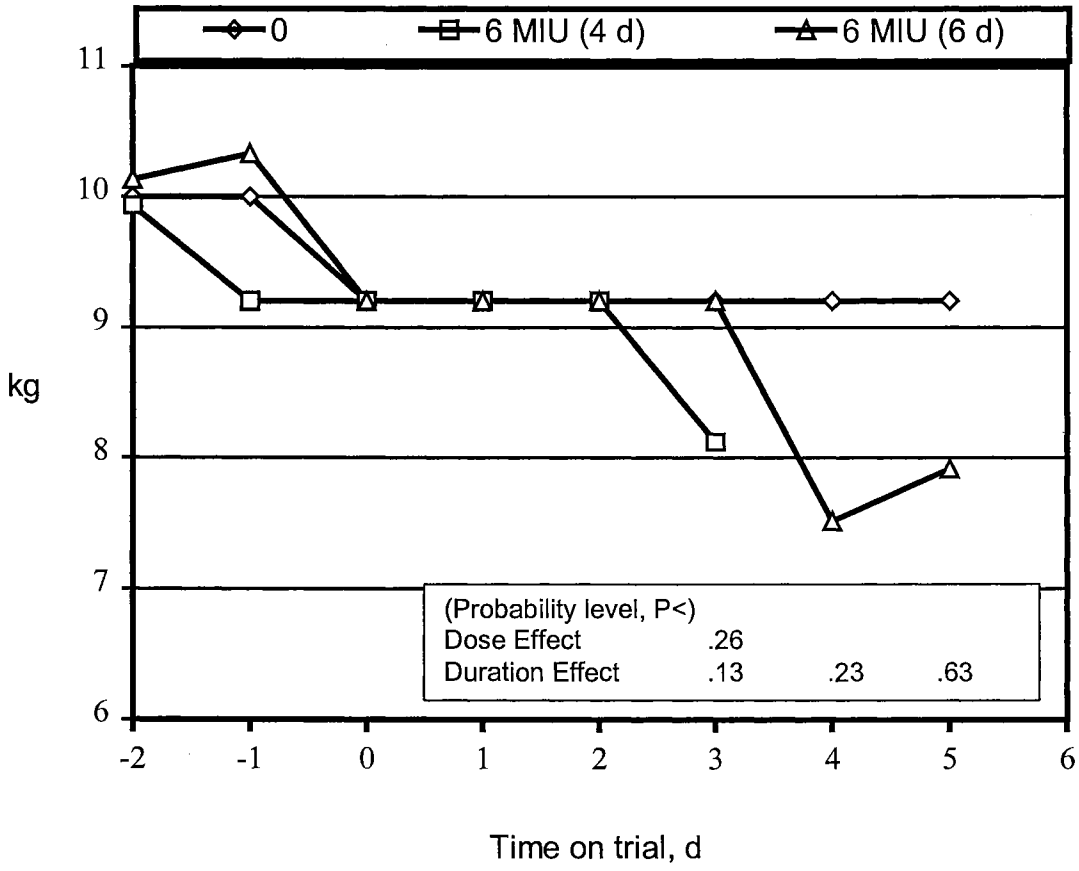
[a]Contrasts:
C vs D = control vs all with vitamin D
4 vs 6 = Duration of feeding vitamin D for 4 vs 6 d

Table 9. Least squares means for free water as percent of total water of muscle supplemented with either 0 or 6 MIU vitamin D for 4 or 6 d

	Vitamin D				Contrasts[a]	
Item	0	6 MIU/4 d	6 MIU/6 d	RSD	C vs D	4 vs 6 d
0-h	27.22	33.48	28.99	5.84	.005	.02
24-h	30.31	35.81	31.39	5.31	.01	.01
7-d	29.60	30.01	28.55	3.21	.68	.18
14-d	28.27	27.55	27.81	4.27	.56	.85
21-d	26.01	27.71	28.31	3.81	.02	.63

[a]Contrasts:
C vs D = control vs all with vitamin D
4 vs 6 = Duration of feeding vitamin D for 4 vs 6 d

Figure 1. Least squares means for DMI of steers supplemented with 0 or 6 MIU of vitamin D for either 4 or 6 d



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