EFFECTS OF VITAMIN D_3 SUPPLEMENTATION OF BEEF STEERS ON LONGISSIMUS MUSCLE TENDERNESS

By

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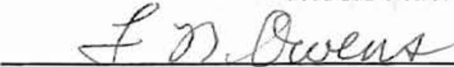

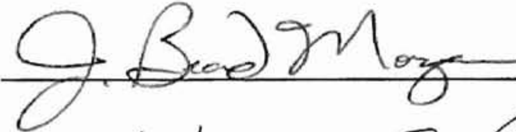

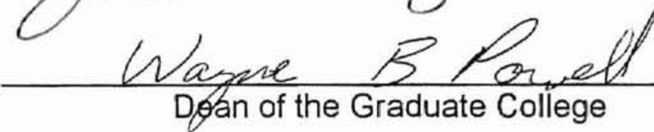

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NOMENCLATURE

1α-OH-D_3	1α-hydroxyvitamin D_3
1,24,25-$(OH)_3D_3$	1,24,25-trihydroxyvitamin D_3
1,25-$(OH)_2D_3$	1,25-dihydroxyvitamin D_3
1,25,26-$(OH)_3D_3$	1,25,26-trihydroxyvitamin D_3
24,25-$(OH)_2D_3$	24,25-dihydroxyvitamin D_3
24,25-F_2-1,25-$(OH)_2D_3$	24,25-difluro-1,25-dihydroxyvitamin D_3
25-OH-D_3	25-hydroxyvitamin D_3
25,26-$(OH)_2D_3$	25,26-dihydroxyvitamin D_3
ATP	Adenosine Triphosphate
°C	degrees Celsius
Ca	Calcium
CaBP	Calcium Binding Protein
cAMP	cyclic AMP
CDP	Calcium dependent protease
Cl	Chlorine
cm	centimeters
d	days
Exp.	Experiment
GFR	Glomerular Filtration Rate

h	hour
i.m.	intramuscular
IU	international unit
K	Potassium
kg	kilograms
meq/l	milliequivalants per liter
Mg	Magnesium
mg	milligrams
mg/dl	milligrams per deciliter
mM	millimoles
MW	Molecular weight
Na	Sodium
ng/ml	nanograms per milliliter
P	Phosphorus
PTH	Parathyroid Hormone
μg	microgram
Vitamin D_2	Ergocalciferol
Vitamin D_3	Cholecalciferol
WBS	Warner-Bratzler shear value

CHAPTER I

INTRODUCTION

Inadequate meat tenderness is a major problem for the beef industry. The 1995 National Beef Quality Audit ranked inadequate tenderness as the second most important beef quality problem (Smith et al., 1995). The annual economic loss associated with beef toughness equals $7.64 per animal or $217.0 million to the beef industry (Smith et al., 1995). In order to compete with other food protein sources, the beef industry must provide the consumer with a consistently high quality, palatable product.

Postmortem tenderization of meat is attributed largely to the calcium dependent proteolytic enzymes (CDP), μ- and m- calpain (Koohmaraie et al., 1988a), and the endogenous calpain inhibitor, calpastatin (Koohmaraie et al., 1988b). Intracellular calcium concentrations usually are not high enough during normal postmortem aging to activate m-calpain; therefore, most postmortem tenderization is via μ-calpain (Vidalenc et al., 1983; Koohmaraie et al., 1987). Koohmaraie et al. (1988a, 1990), Koohmaraie and Shackelford (1991), Morgan et al. (1991), and Wheeler et al. (1992) have shown that injecting meat with calcium chloride increases tenderness through activation of the calpain proteolytic system. Wheeler et al. (1992) concluded that exogenous calcium applied to prerigor muscles activates both μ- and m- calpain, accelerates postmortem aging, and enhances meat tenderness.

Early studies of preventing parturient paresis in lactating dairy cows showed that orally administered vitamin D at 5 million IU daily for two weeks prepartum increased serum calcium 2.1 mg/dl (Hibbs et al., 1951). Hibbs and Pounden (1955) observed that oral supplementation of 5, 10, 20 and 30 million IU of vitamin D for 3 to 8 d prepartum increased serum calcium by 1.9, 1.0, 1,9 and 2.3 mg/dl, respectively. Single injections of 1α-Hydroxyvitamin D_3 (500 or 700 μg) alone or in combination with 25-hydroxyvitamin D_3 (4 mg) will increase serum calcium concentrations by 1.8 to 2.4 mg/dl at 3 to 8 days post injection (Bar et al., 1985, 1988; Sachs et al., 1987; Hodnett et al., 1992). Therefore, we tested the impact of supplemental dietary vitamin D_3 on ionized blood calcium concentration and tenderness of aged longissimus muscle steaks from beef steers.

CHAPTER II

REVIEW OF LITERATURE

Introduction

There are two major sources of dietary vitamin D; cholecalciferol (vitamin D_3), derived primarily from animal sources, and ergocalciferol (vitamin D_2), produced by ultraviolet irradiation of the plant sterol ergosterol. Vitamin D is known as the "sunshine vitamin" because cholecalciferol is synthesized by the skin when exposed to ultraviolet light. Vitamin D has effects on intestinal absorption, bone deposition and mobilization, and renal retention of calcium and phosphorus. Vitamin D was recognized and used to treat rickets over 70 years ago (Howland and Kramer, 1921).

Early studies comparing the biological activity of ergocalciferol and cholecalciferol in mammals indicated that biological activity of the two was equal (Bethke et al., 1946). More recently, cholecalciferol has been shown to have a higher biological activity than ergocalciferol in New World monkeys (Hunt et al., 1967a,b, 1969), rats and pigs (Horst et al., 1982), and horses (Harrington and Page, 1983). Vitamin D_3 has been known for many years to be substantially

more active than vitamin D_2 in birds (Chen and Bosmann, 1964) and also appears to be two to three times more active in ruminants (Sommerfeldt et al., 1983).

Absorption

Vitamin D like other fat soluble vitamins requires bile salt emulsification for efficient absorption into the lymphatic system. Orally administered vitamin D is absorbed in the small intestine (Norman, 1980). Although absorption is most active in the duodenum (Schachter et al., 1964), the greatest total amount is absorbed in the ileum because of the longer time that food spends there (Norman and DeLuca, 1963). Norman and DeLuca (1963) demonstrated that up to 80% of orally administered radioactive vitamin D in oil was absorbed. Norman (1980) reported that 50% of orally administered vitamin D passed through into the lymph duct before entering the blood stream and then was transported to the liver. Vitamin D injected as an alcoholic solution, is rapidly taken up by the liver. After vitamin D_3 is absorbed, large amounts accumulate in the liver so that the blood does not contain high concentrations (Neville and DeLuca, 1966). Ponchon and DeLuca (1969) demonstrated that within 60 minutes, plasma vitamin D is cleared from the blood into the liver. Vitamin D_3, transported in blood bound to an α-globulin (52,000 MW; Rikkers et al., 1969), circulates in the plasma at about 1-2 ng/ml (DeLuca, 1979), well below the concentration of its major metabolite, 25-hydroxyvitamin D_3.

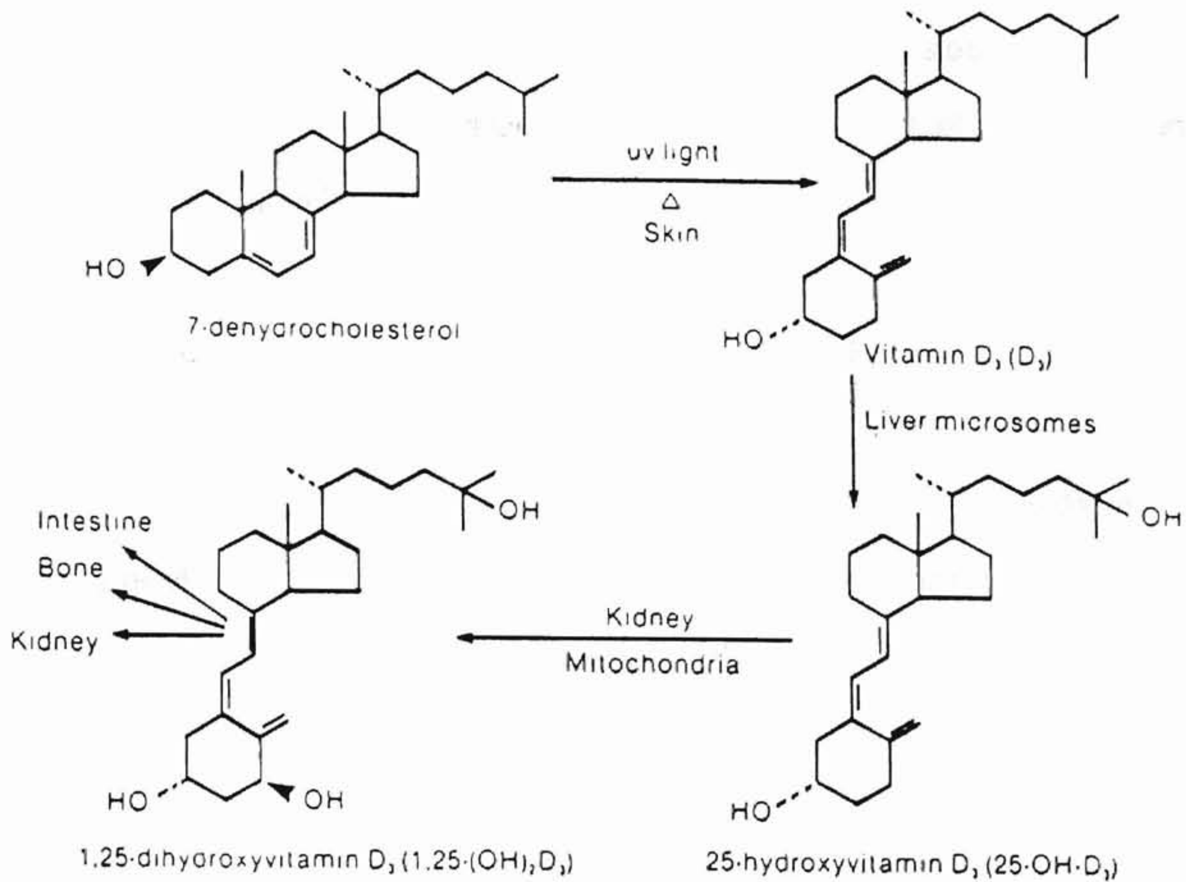
Storage of vitamin D

Ruminants do not store large amounts of vitamin D. In the rat, stored vitamin D is primarily found in fat deposits and not in the liver as was once believed (Rosenstreich et al., 1971). Depletion of vitamin D in animals and man is a tedious process, likely to take years, primarily because it depends on turnover of lipid deposits (DeLuca, 1979). In contrast, vitamin D in plasma has a very short lifetime, estimated at 22 h (Avioli et al., 1967). 1α,25-dihydroxyvitamin D_3 has a plasma lifetime of only 2 to 4 h in humans (Gaynor et al., 1989).

Activation of vitamin D

Vitamin D_3 must be activated metabolically to be functional physiologically. Vitamin D_3 taken up by the liver is converted to 25-hydroxyvitamin D_3 (25-OH-D_3). 25-hydroxyvitamin D_3 does not accumulate in the liver (DeLuca, 1979), but instead is the major circulating metabolite of Vitamin D_3 in the blood. Likewise, 25-OH-D_3 does not act directly at physiological concentrations (DeLuca and Schnoes, 1976) but must be transported to the kidney were it undergoes 1α-hydroxylation to form 1α,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$); this is the form of vitamin D_3 that is metabolically active; as shown in Figure 1.

Figure 1. Activation of vitamin D_3



(Hoffmann-La Roche, 1994)

25-hydroxylation

Identified chemically by Blunt et al. (1968), 25-OH-D_3 is hydroxylated from vitamin D_3 primarily in the liver at the 25 position of the carbon side chain (Ponchon and DeLuca, 1969). Some 25-hydroxylation may occur in other organs as Tucker et al. (1973) demonstrated that it also occurs in the kidney and

intestine of chicks. Although DeLuca (1974) detected no 25-hydroxylation in the kidney of the chick, Bhattacharyya and DeLuca (1974a) revealed that 25-hydroxylation can occur in intestinal tissue from birds. Holick et al. (1976) found no 25-hydroxylation in the intestine of rats. Olsen et al. (1976) concluded that the liver is the major site of 25-hydroxylation.

The 25-hydroxylation system is located primarily in the microsomal fraction of the liver (Bhattacharyya and DeLuca, 1974b). A microsomal enzyme with high affinity and low capacity hydroxylates the 25 carbon of the side chain (McDowell, 1992). 25-hydroxylation requires NADPH, molecular oxygen, magnesium ions, and the cytosolic fraction (DeLuca, 1979). Cytochrome P_{450} is not involved in this reaction (Bhattacharyya and DeLuca, 1974a). The system is stimulated by a protein from the supernatant fraction and requires reduced pyridine nucleotide and molecular oxygen (DeLuca, 1974).

1,25-hydroxylation

25-hydroxyvitamin D_3 is transported to the kidney via the vitamin D_3 transport binding protein transcalciferin that has a molecular weight of 52,000 (DeLuca, 1979), where it is hydroxylated in the mitochondria at carbon 1 to yield $1,25\text{-}(OH)_2D_3$. 1,25-hydroxylation is controlled directly by parathyroid hormone (PTH); PTH increases the production of $1,25\text{-}(OH)_2D_3$ (Horiuchi et al., 1976; Booth et al., 1977). Parathyroid hormone's action is mediated by cyclic AMP

and plasma calcium; cAMP can increase 1,25-hydroxylation just as if PTH had been provided (Suda et al., 1977).

Mitochondria swollen with calcium ions use NADPH as their reducing equivalent. NADPH is the correct electron donor (DeLuca, 1974); reduction is independent of the electron transport chain and oxidative phosphorlation (DeLuca and Schnoes, 1976). The 1α-hydroxylation of 25-OH-D_3 to 1,25-$(OH)_2D_3$ involves the insertion of molecular oxygen using NADPH, flavoprotein, ferredoxin, and cytocrome P_{450} as the cofactors (Ghazarian et al., 1974).

1α-hydroxylase is located in the proximal convoluted tubule cells of the kidney cortex (Norman, 1974). Intact mitochondria use malate, succinate, or other Krebs cycle substrates as the oxidizeable substrates to generate internally reduced pyridine nucleotides (DeLuca, 1974). These substrates along with molecular oxygen generate reducing equivalents by reversal of the electron transport chain for the hydroxylation reaction (DeLuca, 1974).

The kidney is the exclusive site of 1,25-$(OH)_2D_3$ production in non-pregnant mammals (DeLuca, 1988). One exception to this is the placenta of pregnant mammals; it has the ability to synthesize 1,25-$(OH)_2D_3$ (DeLuca, 1988).

1,25-dihydroxyvitamin D_3

1,25-dihydroxyvitamin D_3 clearly is the active form of vitamin D_3 for intestinal calcium (Boyle et al., 1972) and phosphate (Chen et al., 1974) absorption, and bone calcium mobilization (Holick et al., 1972). Nephrectomized

animals do not respond to vitamin D_3 or 25-OH-D_3 when given in physiological amounts, but 1,25-$(OH)_2D_3$ produces these responses either with or without the kidney present (DeLuca and Schnoes, 1976). Because vitamin D_3 and 25-OH-D_3 are not metabolically active in physiological amounts, 1,25-$(OH)D_3$ must be the active form of vitamin D_3. Garabedian et al. (1974a) and Pechet and Hesse (1974) demonstrated that vitamin D_3 and 25-OH-D_3 can produce a response by binding to the 1,25-$(OH)_2D_3$ receptor in nephrectomized animals if doses are large enough. Large doses of 25-OH-D_3 also cause mobilization of bone calcium (Reynolds et al., 1973), calcium transport across the intestinal membrane (Olson and DeLuca, 1969), and production of calcium binding protein (Corradino, 1973). However, none of these responses occur with doses of 25-(OH)-D_3 at physiological levels.

Other metabolites of vitamin D_3

Besides the production of 1,25-$(OH)_2D_3$, the kidney converts 25-(OH)-D_3 to 23(S),25-dihydroxyvitamin D_3 (23(S),25-$(OH)_2D_3$), 24,25-dihydroxyvitamin D_3 (24,25-$(OH)_2D_3$), 25,26-dihydroxyvitamin D_3 (25,26-$(OH)_2D_3$), 1,25,26-trihydroxyvitamin D_3 (1,25,26-$(OH)_3D_3$), and 1,24,25-trihydroxyvitamin D_3 (1,24,25-$(OH)_3D_3$). Hove et al. (1983) concluded that 1,24,25-$(OH)_3D_3$ and 1,25,26-$(OH)_3D_3$ were only one-tenth as potent as 1,25-$(OH)_2D_3$ in causing hypercalcemia. The role of these compounds is somewhat controversial and yet to be determined.

23- and 24- hydroxylation of 25-OH-D_3 or 1,25-$(OH)_2D_3$ to 23(S),25-$(OH)_2D_3$, 24,25-$(OH)_2D_3$ or 1,24,25-$(OH)_3D_3$ is a regulated process; when 1α-hydroxylation of 1,25-$(OH)_2D_3$ is suppressed, the 23- and 24- hydroxylation systems are elevated; see Figure 2. 23-hydroxylation produces a biologically inactive form of vitamin D (DeLuca, 1988). 24-hydroxylase is a mitochondrial enzyme (Knutson and DeLuca, 1974), but the kidney is not the sole site of 24-hydroxylation; the existence of 24-hydroxylase has been demonstrated in intestinal tissue (Kumar et al., 1978) and in cartilage tissue (Garabedian et al., 1977). Although more research is needed, 24-hydroxylation presumably is an initial event in the inactivation of 1,25-$(OH)_2D_3$ (DeLuca, 1979).

Vitamin D deficient animals show the same level of 1α-hydroxylation activity regardless of calcium or phosphorus status (Boyle et al., 1971). Supplementing a low calcium diet with vitamin D_3 stimulates production of 1,25-$(OH)_2D_3$,whereas supplementation of a high calcium diet with vitamin D_3 turned

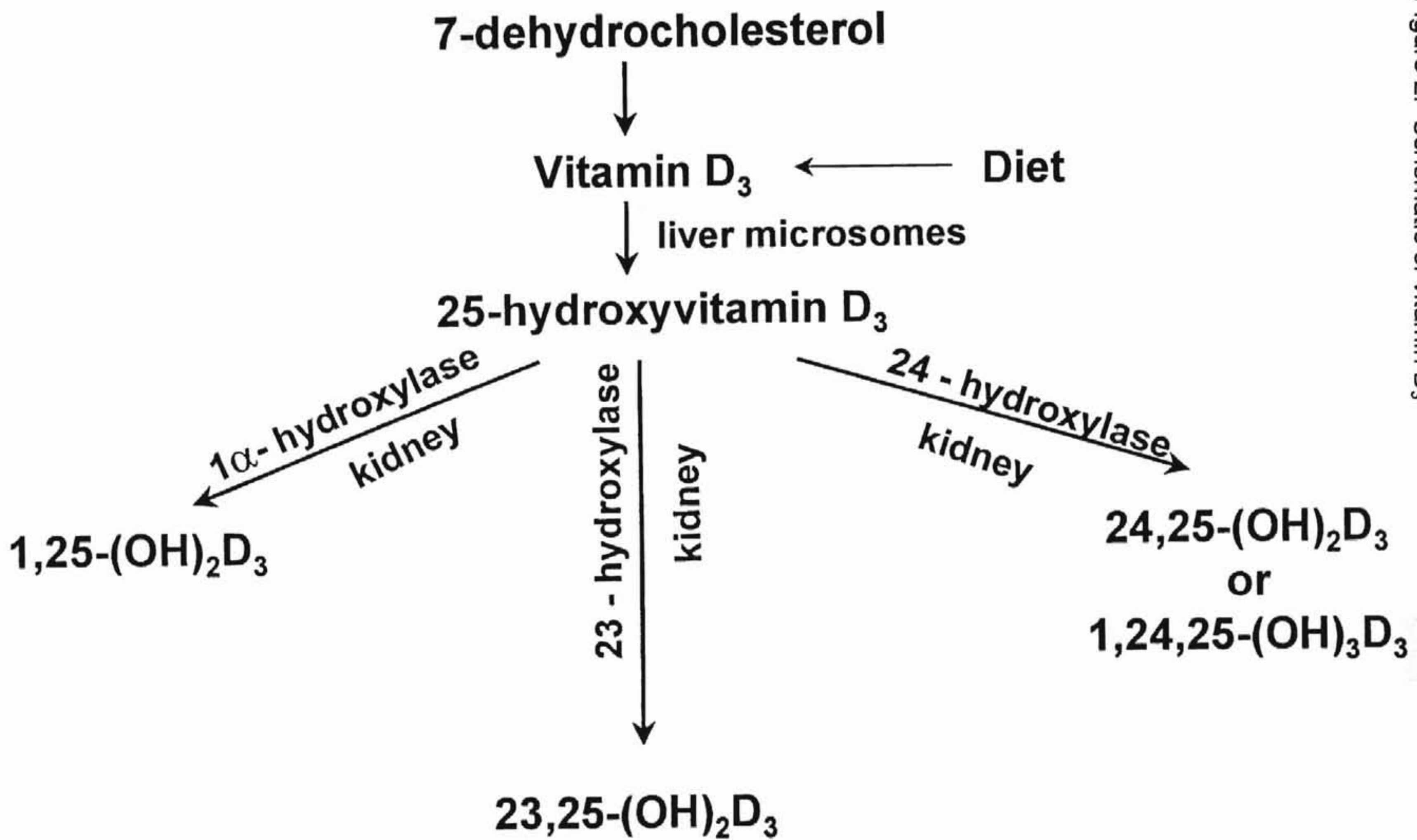


Figure 2. Schematic of vitamin D_3

off the production of 1,25-$(OH)_2D_3$ and stimulated the production of 24,25-$(OH)_2D_3$. 1α-hydroxylase is not regulated by parathyroid hormone or calcium. Administering 1,25$(OH)_2D_3$ to vitamin D deficient rats will suppress 1α-hydroxylase and stimulate 24-hydroxylase to produce 24,25-$(OH)_2D_3$ (DeLuca and Schnoes, 1976).

Vitamin D_3 as a hormone

Vitamin D_3 is considered a hormone (Norman, 1979). This is because 1,25-$(OH)_2D_3$ is produced in the kidney while it functions at other target organs (intestine and bone), and its production is regulated by serum inorganic phosphorus and serum calcium (DeLuca and Schnoes, 1976). The International Union of Pure and Applied Chemistry (1960) defines cholecalciferol (vitamin D_3) as a steroid hormone. Chemically, its name is 9,10-secocholesta-5,7,10(19)-trien-3β-ol.

Vitamin D regulation of calcium and phosphorus

Boyle et al. (1971) clearly demonstrated with rats that feeding low calcium diets stimulated and high-calcium diets suppressed the production and appearance of vitamin D_3. How does the system differentiate between calcium or phosphorus mobilization? Low serum calcium signals the secretion of PTH. Parathyroid hormone activates renal mitochondria to convert 25-OH-D_3 to 1,25-$(OH)_2D_3$; both PTH and 1,25-$(OH)_2D_3$ mobilize bone calcium and increase renal

calcium reabsorption. Low serum phosphorus signal the secretion of 1,25-$(OH)_2D_3$ without increasing PTH; without PTH, then, bone calcium is not readily mobilized and calcium is not completely reabsorbed from the kidney (DeLuca and Schnoes, 1976). In this manner, 1,25$(OH)_2D_3$ can regulate both serum calcium and phosphorus by having different effects at the kidney due to low or high levels of PTH. Ribovich and DeLuca (1975) demonstrated that rats maintained on a phosphorus deprived diet and fed exogenous 1,25-$(OH)_2D_3$ as their only source of vitamin D_3, had elevated intestinal calcium transport. Hence these different signals can regulate the bone and renal systems, but their differential effects on intestinal transport needs more study.

Animals that are hypocalcemic synthesize 1,25-$(OH)_2D_3$ and suppress 24-23- hydroxylase, while animals that do not need calcium suppress 1,25-$(OH)_2D_3$ *production*, except in the case where phosphorus is low which in turn can stimulate 1α-hydroxylase production of 1,25-$(OH)_2D_3$ (DeLuca and Schnoes, 1976). When 1,25-$(OH)_2D_3$ is suppressed, 24- and 23- hydroxylase production are stimulated. Garabedian et al. (1972) demonstrated that removal of the parathyroid gland in hypocalcemic animals eliminated 1,25-$(OH)_2D_3$ production and stimulated 24,25-$(OH)_2D_3$ production. Administering PTH restored the ability to produce 1,25-$(OH)_2D_3$ and suppressed 24,25-$(OH)_2D_3$ synthesis. Fraser and Kodicek (1973) confirmed that PTH in chicks is the primary regulator of 1,25-$(OH)_2D_3$ synthesis in response to low blood calcium.

These hormones regulate calcium both independent and interdependently via modifications of calcium and phosphorus absorption by the intestine, mobilization from bone and reabsorption by the kidney.

Vitamin D_3 increases blood calcium

Blood calcium homeostasis is regulated very closely; normal plasma calcium concentrations continuously remains between 8 and 12 mg/dl in cattle. Three factors regulate calcium homeostasis; two peptide hormones, PTH and calcitonin, and the steroid hormone 1,25-$(OH)_2D_3$. Calcitonin regulates hypercalcemia by depressing calcium absorption from the gut, reducing bone mineral resorption, and reducing renal calcium reabsorption (McDowell, 1992). Parathyroid hormone stimulates 1,25-$(OH)_2D_3$ production in response to low blood calcium (Garabedian et al., 1974b). Mobilization of calcium from bones and renal reabsorption of calcium requires both 1,25-$(OH)_2D_3$ and PTH (DeLuca, 1979; Sutton et al., 1977; Kleeman et al., 1961). Increased intestinal absorption of calcium responds only to 1,25-$(OH)_2D_3$ and does not respond to PTH (Garabedian et al., 1974b). Parathyroid hormone increases intestinal calcium absorption indirectly by stimulating 1,25-$(OH)_2D_3$ production. Although the intestine normally has an incredible ability to adapt to dietary calcium (Nicolaysen, 1943), Ribovich and DeLuca (1975) demonstrated that 1,25-$(OH)_2D_3$ eliminates the ability of the intestine to adjust to calcium absorption in relation to dietary calcium levels.

The effects of vitamin D_3 on plasma calcium concentrations

For prevention of parturient paresis in lactating dairy cows oral administration of vitamin D_2 at 5 million IU daily for two weeks has been used; this increased prepartum serum calcium by 2.1 mg/dl (Hibbs et al., 1951). Hibbs and Pounden (1955) observed that oral supplementation of 5, 10 , 20 and 30 million IU of vitamin D for 3 to 8 d prepartum increased serum calcium by 1.9, 1.0, 1.9, and 2.3 mg/dl, respectively.

Intramuscular injections of vitamin D_3 have effects similar to oral administration. Horst and Littledike (1979) demonstrated that four weekly injections of 15 million IU of vitamin D_3 increased blood calcium by approximately 3.5 mg/dl. Elevated blood calcium levels began 8 to 10 d after the first injection of vitamin D_3 and continued to increase after each injection, peaking 3 d after the fourth injection. 1,25-dihydroxyvitamin D_3 levels continued to increase after each injection, but levels declined between injections, demonstrating that 1,25-$(OH)_2D_3$ has a short half life.

In studies of parturient paresis, 1α-hydroxyvitamin D_3 (1α-OH-D_3) causes even faster increases in serum calcium concentrations. 1α-hydroxyvitamin D_3 is hydroxylated rapidly to 1,25-$(OH)_2D_3$ by the liver (Fukushima et al., 1978). A single 700 μg injection of 1α-OH-D_3 increased blood calcium 1.8 mg/dl to 2.4 mg/dl by 4 to 8 d postinjection; blood calcium remained high for 14 days (Bar et al., 1985, 1988; Sachs et al., 1987). 1α-hydroxyvitamin D_3 concentrations peaked at 24 to 48 h post injection but dropped to initial concentration 5 to 7 d

postinjection. Bar et al. (1985) estimated that the biological half life of 1α-hydroxyvitamin D_3 was 2.1 d

Hodnett et al. (1992) utilized 500 μg of 1α-hydroxyvitamin D_3 to quickly increase plasma calcium; 4 mg of 25-OH-D_3 administered every 5 d maintained an increased plasma calcium. Plasma calcium was increased by 2 mg/dl at 5 to 7 d postinjection. Calcium and 1,25$(OH)_2D_3$ remained high until parturition, 15 d after the first injection.

The active metabolite of vitamin D_3 also can increase blood calcium concentration and may be beneficial because 1,25-$(OH)_2D_3$ may have a direct effect on the gut, increasing intestinal calcium absorption. Goff et al. (1989) demonstrated that 1,25-$(OH)_2D_3$ injected daily for 7 d increased blood calcium by 1.9 mg/dl on d 3 and continued to elevate blood calcium 8 d after the last injection, with a peak increase at approximately 2.8 mg/dl some 7 to 9 d after the first injection.

Intramuscular injections of 1α-OH-D_3 increased 1,25-$(OH)_2D_3$ concentrations longer than equivalent amounts of 1,25-$(OH)_2D_3$ (Carstairs et al., 1981; Hove et al., 1981). Intramuscular injections of either 1α-OH-D_3 or 1,25-$(OH)_2D_3$ resulted in 5 times greater blood 1,25-$(OH)_2D_3$ levels than orally administered 1α-OH-D_3 or 1,25-$(OH)_2D_3$, but the decline after peak concentration occurred more slowly after oral administration than after i.m. administration (Hove et al., 1983). Although blood 1,25-$(OH)_2D_3$ levels vary with type of administration of 1,25-$(OH)_2D_3$, degree of hypercalcemia was comparable

with oral and i.m. administration of 1,25-$(OH)_2D_3$; both types of administration increased blood calcium by 20 to 30% above pretreatment levels for 5 d (Hove et al., 1983). This suggests that orally administered 1,25-$(OH)_2D_3$ may have a direct effect on the gut to increase intestinal calcium absorption. If 1,25-$(OH)_2D_3$ is used to increase blood calcium, oral administration may be more advantageous because although equivalent plasma calcium concentrations can be achieved, the lower plasma 1,25-$(OH)_2D_3$ concentration could cause less feedback inhibition of renal 1α-hydroxylase enzyme by 1,25-$(OH)_2D_3$ circulating in the blood. With cattle mean plasma calcium concentrations with 1α-OH-D_3 injected i.m. was similar to that of 1,25-$(OH)_2D_3$, but orally administered 1α-OH-D_3 only increased plasma calcium by 10% indicating that 1α-OH-D_3 may be degraded in the rumen.

Another metabolite of vitamin D_3, has recently been the subject of interest. Goff et al. (1986) demonstrated that cattle when injected intramuscularly, 24,24-difluoro-1,25-dihydroxyvitamin D_3 (24,24-F_2-1,25-$(OH)_2D_3$) was approximately four times more active than equivalent dose of 1,25-$(OH)_2D_3$. Similar studies in the rat (Okamoto et al., 1983) and the chick (Corradino et al., 1980) confirmed that 24,24-F_2-1,25-$(OH)_2D_3$ has a biological activity higher than 1,25-$(OH)_2D_3$.

Intravenous administration of 2 million IU of vitamin D_3 increased serum calcium 0.9 mg/dl on d 2 and 3; intramuscular injecting the same dose of vitamin D_3 resulted in a smaller, but more prolonged increase of serum calcium from d 2 to 25 (Boling and Evans, 1979).

Mechanisms

Vitamin D increases plasma calcium concentration by stimulating intestinal calcium absorption (Nicolaysen, 1937), mobilizing calcium from previously formed bone mineral (Carlsson, 1952), and increasing renal reabsorption of calcium from the kidney (Sutton and Dirks, 1978).

Urinary hydroxyproline excretion has been used as an index of bone reabsorption in cattle (Black and Capen, 1971). Goff et al. (1986) demonstrated that glomerular filtration rate is reduced during hypercalcemia; hence, plasma hydroxyproline levels may be a better index of bone resorption during periods of hypercalcemia. Bone resorption of calcium probably only contributes slightly to the increase calcium concentration seen after vitamin D_3 supplementation because plasma hydroxyproline concentrations are either reduced (Hove et al., 1983) or remain unchanged (Goff et al., 1986) during the hypercalcemic period. Bar et al. (1988) demonstrated that prepartum PTH levels did not increase in cows injected with 1α-hydroxyvitamin D_3; PTH and 1,25-$(OH)_2D_3$ are both needed to increase bone resorption and renal reabsorption of calcium, but PTH is not needed for intestinal calcium absorption. Hove et al. (1984) and Braithwaite (1980) concluded that hypercalcemia caused by pharmacological doses of vitamin D or its metabolites probably is the result of stimulation of transcellular calcium transport in the intestinal tract.

Intestinal calcium absorption

Calcium absorption from the small intestine occurs via two independent processes. The first is a saturable transcellular process that is regulated by vitamin D and its product vitamin D-dependent calcium-binding protein (CaBP, MW = 8800). Absorption takes place in the duodenum and upper jejunum (Pansu et al., 1983a). The second process is nonsaturable, it is concentration dependent, and it moves calcium paracellulary along the entire length of the small intestine (Behar and Kerstein, 1976; Pansu et al., 1983a). The Nernst equation predicts net flux of calcium into extracellular fluid (Goff et al., 1991), when ionized calcium concentration over the epithelial cells exceeds 6mM. In the rat, 16% of the luminal calcium concentration is transferred to the body fluids per hour (Pansu et al., 1981, 1983a, 1983b).

When dietary calcium is low or calcium is in demand most intestinal calcium absorption occurs via the transcellular active transport system. When adequate dietary calcium is provided, most intestinal calcium absorption occurs through the non-saturable concentration dependent process.

Transcellular intestinal calcium absorption

The saturable process of transcellular calcium movement involves three steps. In the first step, calcium enters the cell via the brush border membranes on the luminal side of the intestinal cell. In the second step, calcium diffuses across the cell cytoplasm to the basal lateral side of the cell. In the third step,

calcium is extruded into the extracellular fluid at the basolateral pole of the cell. These three steps must occur in dynamic equilibrium (Bronner, 1987).

Step 1

Calcium entry into the cell occurs rapidly without the expenditure of metabolic energy because calcium concentration is 1000-fold greater in the gut than in the interior of the cell. The capacity of calcium to enter the brush-border membrane is not the rate limiting step for transcellular calcium transport (Bronner, 1987).

Step 2

Feher (1983,1984) demonstrated that vitamin D-dependent calcium-binding protein acts as a intracellular ferry to transfer calcium across the cell to the basal later side. This is the rate limiting step in transcellular calcium transport (Bronner, 1987). Calcium-binding protein increases the intracellular calcium gradient between the brush border and the basolateral poles of the cell, providing the driving force needed for calcium diffusion (Bronner, 1987). There is a close linear relationship between the rate (Bronner et al., 1986) and amount of calcium transported (Roche et al., 1986) to the intestinal content of CaBP. 1,25-dihydroxyvitamin D_3 promotes the synthesis of intestinal CaBP (Hoffmann-La Roche, 1994).

Step 3

Extrusion of calcium from the cell against a 1000-fold concentration gradient requires metabolically driven Ca:Mg-ATPase-dependent pumps

(Bronner, 1987). Calcium-binding protein does not stimulate the Ca:Mg-ATPase-dependent pump to extrude calcium (Ghijsen et al., 1986); these Ca:Mg-ATPase pumps normally operate at much less than their maximum velocity but they are able to adequately handle the calcium flux across the cell when CaBP is transporting calcium at its maximum rate (Bronner, 1987). Although it does not stimulate calcium transport, 1,25-$(OH)_2D_3$ increases Ca:Mg-ATPase activity by 2 or 3 fold (Bronner et al., 1986), although they still pump at much less than maximum velocity.

Rasmussen et al. (1982) postulated that 1,25-$(OH)_2D_3$ can cause a change in the fluidity of the plasma membrane of the intestinal target cell that allows more calcium to enter, but that it did not alter the magnitude of CaBP. This change in calcium permeability is independent of vitamin D induced CaBP synthesis. Intracellular calcium and 1,25-$(OH)_2D_3$ levels may play a role in the posttranscriptional regulation of CaBP expression (Bronner and Buckley, 1982).

Calcium intake greatly effects the route of calcium transport. An increase in dietary calcium leads to down-regulation of the vitamin D dependent saturable process (Pansu et al., 1981). The amount of calcium moved by the nonsaturable route will increase in direct proportion to calcium intake. Decreasing dietary calcium will have the opposite effect.

Calcium dependent proteases

Calcium dependent proteases (CDP) are major contributors to the tenderization process during postmortem aging (Koohmaraie et al., 1986, 1987, 1988a, 1988b). Calcium dependent proteases degrade myosin (Ishiura et al., 1979), Z-disks (Dayton et al., 1976a,b), troponin-T and desmin (Koohmaraie et al., 1986). Two distinct forms of CDPs exist; μ- (CDP-I) and m- (CDP-II) calpain. Activation of μ-calpain requires 50 to 70 μM calcium while activation of m-calpain requires 1 to 5 mM calcium (Mellgren, 1980; Dayton et al., 1981; Szpacenko et al., 1981; Goll et al., 1983; Inomata et al., 1985; Karlsson et al., 1985). Intracellular calcium concentrations usually are not high enough during normal postmortem aging to activate m-calpain; therefore, most postmortem tenderization is via μ-calpain (Vidalenc et al., 1983; Koohmaraie et al., 1987, 1988a). Koohmaraie et al. (1988a,b, 1990), Koohmaraie and Shackelford (1991), Morgan et al. (1991), and Wheeler et al. (1991, 1992) have shown that injecting meat with calcium chloride increases tenderness through activation of the calpain proteolytic system. Wheeler et al. (1992) concluded that exogenous calcium applied to prerigor muscles increased intracellular calcium concentration, activating both μ- and m- calpain, accelerating postmortem aging, and enhancing meat tenderness.

Vitamin D toxicity

The maximum tolerable level for vitamin D in the diet has not been established. Factors such as chemical form (vitamin D_2 or vitamin D_3), potency

of the metabolites and their biological half lives, animal species, dietary calcium and phosphorus, route of administration, stage of production, and length of treatment all can influence the upper safe level of vitamin D.

Chemical form

Vitamin D_3 is more potent than vitamin D_2 in most species but the difference in the degree of potency is species dependent. Cholecalciferol has a higher biological activity than ergocalciferol in New World monkeys (Hunt et al., 1967a,b, 1969), rats and pigs (Horst et al., 1982), and horses (Harrington and Page, 1983). Vitamin D_3 is substantially more active than vitamin D_2 in birds (Chen and Bosmann, 1964), and two to three times more active in ruminants (Sommerfeldt et al., 1983). NRC (1987) indicates that vitamin D_3 is 10 to 20 times more toxic than vitamin D_3 in ruminants when given in excessive amounts.

Potency

Various metabolites differ in potency. The relative potency of vitamin D and its metabolites are: $24,24\text{-}F_2\text{-}1,25\text{-}(OH)_2D_3 > 1,25\text{-}(OH)_2D_3 = 1\alpha\text{-}OH\text{-}D_3 > 25\text{-}OH\text{-}D_3 >$ Vitamin $D_3 > 24,25\text{-}(OH)_2D_3 > 1,24,25\text{-}(OH)_3D_3 = 1,25,26\text{-}(OH)_3D_3$. Biological half life of each metabolite also affects the degree of toxicity: $25\text{-}OH\text{-}D_3 >$ vitamin $D_3 > 1,25\text{-}(OH)_2D_3 > 1\alpha\text{-}OH\text{-}_{D3}$ (NRC, 1987).

Mineral Status

Availability of dietary calcium for absorption can increase the degree of vitamin D toxicosis when pharmacological doses of vitamin D are given (Hines et

al., 1985). Feeding a low phosphorus diet increases calcium absorption both with or without large doses of vitamin D.

Route of Administration

The potential for vitamin D toxicity is influenced by route of administration: intravenous > intramuscular > oral. This is not necessarily true for 1,25-$(OH)_2D_3$ or 25-OH-D_3 because 1,25-$(OH)_2D_3$ does not need to be absorbed but can have a local effect on the gut increasing calcium absorption; 25-OH-D_3 when given in large enough doses can have the same local effect as 1,25-$(OH)_2D_3$ (Hove et al., 1983). Rumen microbes can partially metabolize and deactivate vitamin D in the rumen (Napoli et al., 1983).

Stage of Production

Pregnant cows are much more likely to develop vitamin D toxicity than dry cows (Littledike and Horst, 1980). Toxic effects of vitamin D are greater if vitamin D_3 treatment precedes parturition by several weeks (Littledike and Horst, 1982). 1α,25-dihydroxyvitamin D_3 receptors increase during pregnancy and lactation in the cow (Goff et al., 1991). The number of 1,25-$(OH)_2D_3$ receptors decline with age in both the rat and cow (Horst et al., 1990).

Duration of Supplementaion

Duration of vitamin D treatment also can affect the onset of vitamin D toxicosis. Most species of animals appear to be able to tolerate 10 times (2,200 IU per kg of body weight for ruminants) their dietary vitamin D requirements for long periods of treatment (greater than 60 d); for short periods of treatment (less

than 60 d) most species are able to tolerate up to 100 times (22,000 IU per kg of body weight for ruminants) dietary requirements (NRC, 1987).

Mechanisms

Increasing blood calcium levels beyond its normal boundaries due to excessive vitamin D supplementation can produce a variety of effects (McDowell, 1992). The kidney can handle a 10 to 20% increase in serum calcium without effecting GFR (NRC, 1987) but further increases in serum calcium will cause GFR to decrease because of nephrolithiasis caused by hypercalciuria. This reduction in renal function causes a loss of control of calcium homeostasis resulting in hypercalcemia which in turn causes extensive calcification of soft tissue (NRC, 1987).

Signs of toxicity

The first sign of vitamin D toxicity in cattle is inappetence; severe anorexia lasts several weeks and may cause death (Littledike and Horst, 1982). Other signs of vitamin D toxicity are reduced rumination, depression, pasty discharge around the eyes, rough dry hair coat, delayed shedding, reduced milk production, stiffness and inflammation of muscles and joints, excessive water intake, air bubbles under the skin around injection sites, crepitation of the skin in the injection area, thinning of bones, decreased blood Mg, premature ventricular systoles, and bradycardia (Littledike and Horst, 1982; NRC, 1987; McDowell, 1992; Hoffmann-La Roche, 1994).

Result of toxicity

Widespread mineralization of the kidney, cardiovascular system, respiratory tract and the salivary glands is evident during postmortem examination of vitamin D intoxicated animals; lesions in these areas usually are a result of mineralization. The kidney usually is the first place to find soft tissue mineralization, with the collecting tubules of the medulla being the major site of mineralization with less mineralization of the cortex (Capen et al., 1966; Simesen et al., 1978). Cardiovascular lesions caused by extensive mineralization are located primarily in the aorta, aorta valves, aortic arch, arteriole wall, stomach mucosa, large arterial bifurcations, and small vessels (NRC, 1987). Calcification of the small bronchi, alveolar ducts, alveolar septa, and bronchial cartilage and submucosa (Kent et al., 1958; Chineme, 1976) also is evident during postmortem examination. Calcification of the submaxillary and parotid salivary glands are the next most frequently seen lesions that result from vitamin D toxicity (Kent et al., 1958).

CHAPTER III

MATERIALS AND METHODS

In Exp. 1, 118 steers (528 kg mean live weight), consisting of 20 Angus-Hereford crossbred steers in Trial 1 and 98 Saler or Charolais-sired steers with Brangus dams in Trial 2, were supplemented with either 0 or 5 million IU of vitamin D_3 per day for 5 d immediately prior to slaughter. Steers in Trial 1 were assigned randomly to 8 pens, with either 2 or 3 steers per pen. Steers in Trial 2 were divided into groups based on breed of sire (Charolais or Saler) and weight class (heavy, medium and light). Steers in Trial 2 were assigned randomly to 20 pens with 4 or 5 steers per pen. In Exp. 2, 44 crossbred steers (570 kg mean weight) slaughtered on two different dates, designated as trial 3 and 4, were supplemented with either 0 or 7.5 million IU of vitamin D_3 per day for 10 d immediately prior to slaughter. In Trial 3, 20 steers were fed individually; in Trial 4, 24 steers were fed in 4 pens with 6 steers per pen. To ensure that all of the vitamin D_3 included in the diet was consumed, steers in both experiment 1 and 2 were limit fed their high concentrate diet (1.5 % of their body weight daily) while vitamin D_3 was supplemented. All steers were slaughtered at commercial packing plants. Following a 48 hour postmortem chill (2°C), yield and quality grade of each carcass was determined by a USDA grader and a rib section was removed from the left side of each carcass. Longissimus muscle steaks (2.5 cm thick) were prepared, vacuum packaged, aged at 2°C for either 7, 14 or 21 d (except for Trial 1 when only 1 steak was prepared and aged 7 d) and then

frozen at -30°C. Steaks later were thawed for 24 hours at 2°C and broiled on a impingement oven to an internal temperature of 70°C. After cooling to 20°C, eight 1.3 cm diameter core samples were obtained from each steak; shear force values were measured using a Warner-Bratzler attachment to an Instron Universal Testing Machine. The maximum shear force value determined for each of the eight core samples, was averaged and used as a single measurement.

Plasma from blood samples obtained during exsagination in Trial 1 were analyzed for calcium, magnesium, triglyceride, glucose, chlorine, phosphorus, sodium and potassium using a Roche Cobas Mira (Roche Diagnostic Systems, Inc., New Jersey).

In Exp. 3, 20 crossbred steers (550 kg mean weight) were assigned randomly to one of four treatments. Steers were individually fed 1.5 % of their body weight daily and supplemented with either 0, 2.5, 5.0 or 7.5 million IU of vitamin D_3 per day for 10 d with 5 steers per dose level. Blood samples were obtained daily during this supplementation period and 5 d thereafter. Blood plasma samples were analyzed for ionized calcium using a Orion calcium ion selective electrode (Orion Research Incorporated, Boston, MA) . Ionized blood calcium concentrations for treatment groups were calculated.

For statistical analysis, samples from Exp. 1 were considered to be blocked by Trial (1 and 2) and for Trial 2 by breed of sire and weight class. Levene's test was performed to test for heterogeneity of variances among the 12 group by treatment combinations. Variance was not heterogeneous according to that test. Shear force and carcass data were analyzed using the general linear model procedure of SAS (1985) using pen(rep*trt) as the error term. For Exp. 2, Trial 3 and Trial 4 were considered as two blocks based on feeding system (individual vs pen fed) and slaughter date. The mixed model procedure of SAS

(1985) was used to analyze shear force and carcass data for Exp. 2. In Exp. 3, contrasts were used to determine effects of providing vitamin D_3 and whether the response was linearly related to dosage.

CHAPTER IV

RESULTS AND DISCUSSION

In Trial 1 (Table 1), vitamin D_3 supplementation resulted in 26.6% lower (P<.001) blood plasma magnesium concentration, while blood plasma calcium was 12.6% greater (P<.03).

Least square means for carcass characteristics for Exp. 1 are reported in Table 2. Carcass weight (P<.08) and ribeye area (P<.08) both tended to be greater for control steers, presumably due to chance. Least square means for shear force values for Exp. 1 are reported in Table 3. WBS values were 6.6% lower (P<.01) (more tender) and the number of tough steaks was 21.8% lower (to P<.01) for steers supplemented vitamin D_3 at 7 d postmortem aging.

Least square means for carcass characteristics for Exp. 2 are reported in Table 4. Marbling score (P<.06) and final yield grade (P<.09) both tended to be greater for steers supplemented with vitamin D_3, presumably due to chance. In Exp. 2 (Table 5), vitamin D_3 supplementation resulted in 18.0% lower (P<.02)

WBS values at 7d postmortem aging and tended to reduce the number of tough steaks by 23.3% ($P<.09$), and by 22.5% ($P<.07$) at 14 and 21 d of postmortem aging.

In Exp. 3 (Figure 1), mean concentrations of ionized blood calcium are plotted as a percentage of those of control steers. Supplemental vitamin D_3 increased ionized blood calcium concentrations ($P<.01$ $P<.04$) on d 6 through 13 and d 15, and tended to increase concentrations on d 5 ($P<.07$) and d 14 ($P<.09$). Response to dosage of vitamin D_3 was linear on d 6 through 13 and 15 ($P<.01$ to $P<.03$) and tended to be linear on d 5 ($P<.07$).

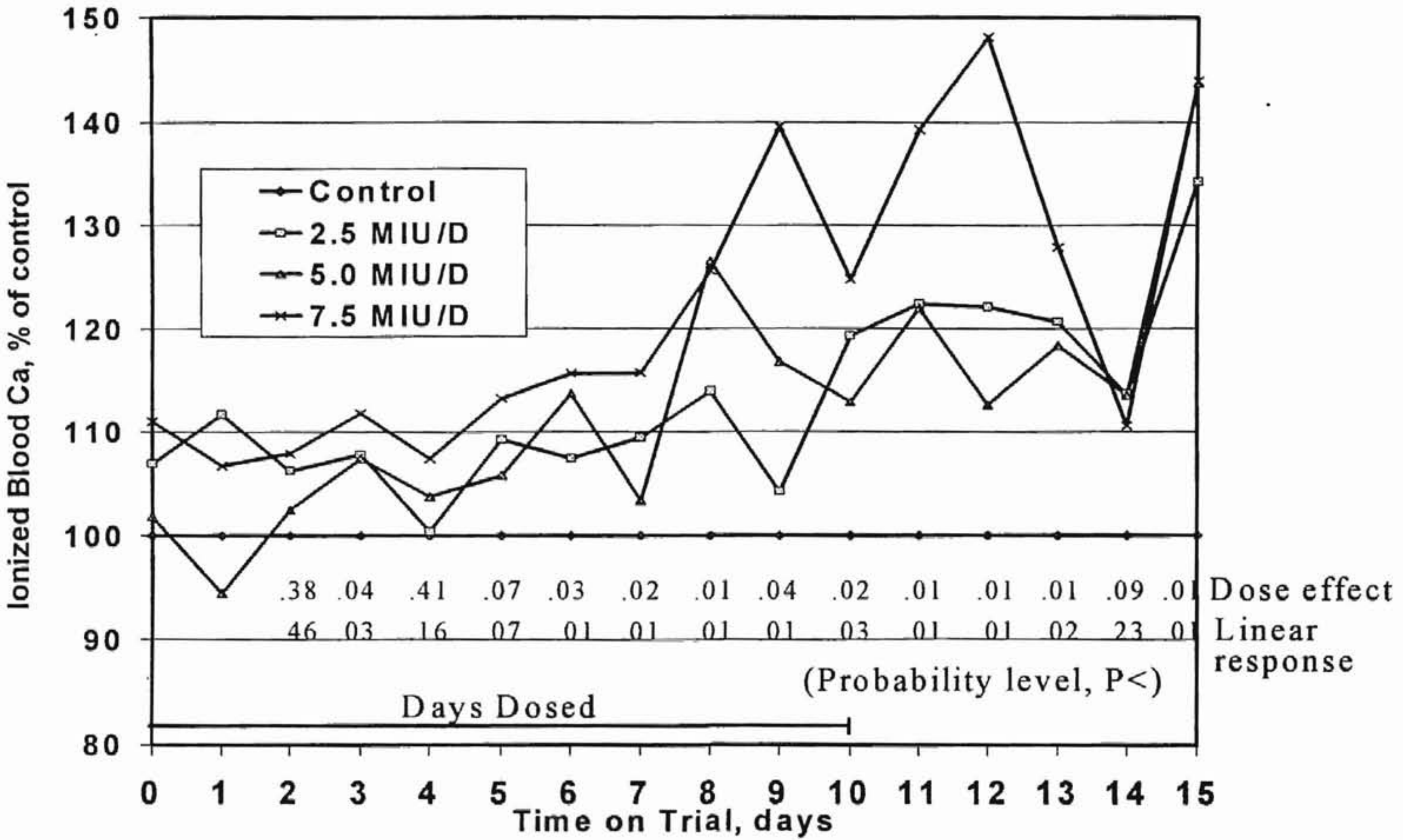


Figure 3. Temporal effects of vitamin D_3 supplementation on ionized blood calcium concentration of steers in Exp. 3.

Table 1. Least squares means for blood plasma samples for steers supplemented with either 0 or 5 million IU of vitamin D_3 for 5 days prior to slaughter (Trial 1)

	Vitamin D_3, IU/d			
Item	0	$5 * 10^6$	SE	Probability level (P<)
Steers, n	10	10		
Ca, mg/dl	9.23	10.39	0.29	.03
Mg, meq/l	1.99	1.46	0.06	.001
Triglyceride, mg/dl	22.5	26.9	2.20	.21
Glucose, mg/dl	153.6	200.2	38.01	.42
Cl, mM	103.6	104.0	1.08	.79
P, mg/dl	6.66	6.36	0.15	.20
Na, mM	149.2	148.0	1.51	.58
K, mM	7.22	6.77	0.44	.50

Table 2. Least squares means for carcass traits for steers supplemented with either 0 or 5 million IU of vitamin D_3 per day for 5 days prior to slaughter (Trials 1 and 2)

	Vitamin D_3, IU/d			
Item	0	$5 * 10^6$	SE	Probability level (P<)
Steers, n	59	59		
Carcass weight, kg	336.2	326.4	3.53	.08
Marbling score [a]	300.4	294.5	9.00	.70
Preliminary Yield Grade	2.85	2.80	0.03	.35
Final Yield Grade	2.40	2.37	0.08	.91
Ribeye area, cm^2	86.2	84.1	0.80	.08
Internal (KPH) fat, %	1.96	1.87	0.06	.39
Skeletal Maturity [b]	148.8	145.9	2.44	.43
Lean Maturity [c]	136.1	139.9	2.43	.30

[a] Marbling score: 200 to 299 = "traces" (U.S. Standard); 300 to 399 = "slight" (U.S. Select).

[b] Skeletal maturity: 100 to 199 = A maturity: approximately 9 to 30 months chronological age at slaughter.

[c] Lean maturity: 100 to 199 = light cherry red, fine in texture.

Table 3. Least squares means for Warner-Bratzler shear force values for steers supplemented with either 0 or 5 million IU of vitamin D_3 per day for 5 days prior to slaughter (Trials 1 and 2)

	Vitamin D_3, IU/d			
Item	0	$5 * 10^6$	SE	Probability level (P<)
Steers, n	59	59		
Shear force, kg				
Aged 7 day	4.70	4.39	0.08	.01
Aged 14 day	4.03	3.87	0.09	.25
Aged 21 day	3.58	3.60	0.11	.90
Steaks with shear force values > 4.5 kg, %				
Aged 7 day	56.0	34.2	5.53	.01
Aged 14 day	20.0	17.0	7.22	.78
Aged 21 day	6.0	8.0	4.75	.77

Table 4. Least squares means for carcass traits for steers supplemented with either 0 or 7.5 million IU of vitamin D_3 per day for 10 prior to slaughter (Trials 3 and 4)

	Vitamin D_3, IU/d			
Item	0	$7.5 * 10^6$	SE	Probability level (P<)
Steers, n	22	22		
Carcass weight, kg	358.5	368.4	7.56	.38
Marbling score [a]	384.3	434.0	18.0	.06
Preliminary Yield Grade	2.81	3.00	0.10	.15
Final Yield Grade	2.16	2.63	0.19	.09
Ribeye area, cm^2	92.5	90.0	2.45	.50
Internal (KPH) fat, %	1.99	2.30	0.17	.20
Skeletal Maturity [b]	130.0	124.7	3.95	.35
Lean Maturity [c]	185.3	177.7	10.92	.62

[a] Marbling score: 300 to 399 = "slight" (U.S. Select); 400 to 499 = "small" (U.S. Choice).

[b] Skeletal maturity: 100 to 199 = A maturity: approximately 9 to 30 months chronological age at slaughter.

[c] Lean maturity: 100 to 199 = light cherry red, fine in texture.

Table 5. Least squares means for Warner-Bratzler shear force values for steers supplemented with either 0 or 7.5 million IU of vitamin D_3 per day for 10 prior to slaughter (Trials 3 and 4)

	Vitamin D_3, IU/d			
Item	0	$7.5 * 10^6$	SE	Probability level (P<)
Steers, n	22	22		
Shear force, kg				
Aged 7 day	5.13	4.21	0.28	.02
Aged 14 day	4.40	3.81	0.25	.13
Aged 21 day	4.04	3.44	0.24	.13
Steaks with shear force values > 4.5 kg, %				
Aged 7 day	55.8	39.2	9.59	.23
Aged 14 day	47.5	24.2	9.37	.09
Aged 21 day	32.5	10.0	8.46	.07

CHAPTER V

SUMMARY AND CONCLUSIONS

Dietary supplementation with vitamin D_3 for 5 or 10 d prior to slaughter increased tenderness of longissimus steaks at 7 d postmortem aging. Supplementation with vitamin D_3 for 5 d also reduced the percentage of tough steaks at 7 day of postmortem aging whereas supplementation with 7.5 million IU for 10 d prior to slaughter tended to decrease the percentage of tough steaks at the longer aging periods. Optimum dosage levels and times for vitamin D_3 to reduce shear force of the longissimus and other muscle groups remain to be pinpointed.

CHAPTER IV

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