Vitamin E

Properties and Metabolism

A. Chemistry and Synthesis of Vitamin E Compounds

Vitamin E activity in plants is derived from eight naturally occurring plant compounds, the tocopherols and tocotrienols. In1922, Evans and Bishop at the University of California, Berkeley first recognized the presence of a nutritional factor in vegetable oils essential for reproduction in the rat (Scott et al., 1982). In 1936, Evans et al. isolated and named the compound alpha-tocopherol, deriving the name from the Greek "tokos-pherein," meaning "offspring-to bear" and adding the -ol suffix to denote the presence of an alcohol group (Scott et al., 1982). According to the International Union of Pure and Applied Chemistry-International Union of Biochemistry (IUPAC-IUB) Commission on Biochemical Nomenclature, vitamin E is used as a generic descriptor for all tocol and tocotrienol derivatives which qualitatively exhibit the biologic activity of alpha-tocopherol (IUPAC-IUB, 1973). Both the tocopherols (tocols) and tocotrienols consist of a hydroquinone nucleus and an isoprenoid side chain (Scherf et al., 1996; Machlin, 1991). Characteristically, tocopherols have a saturated side chain, while the tocotrienols have an unsaturated side chain containing three double bonds. There are four principal compounds of each of these two sources of vitamin E activity (alpha, beta, gamma, delta), differentiated by the presence of methyl (-CH3) groups at positions 5, 7 or 8 of the chroman ring (Figure 4-1). Alpha-tocopherol, the most biologically active of these compounds, is the predominant active form of vitamin E in feedstuffs and the form used commercially for supplementation of animal diets (Scherf et al., 1996). The biological activity of the other tocols and the tocotrienols is limited (Table 4-1), but some new functions have recently been found for non-alpha-tocopherol forms of vitamin E (Schaffer et al., 2005; Freiser and Jiang, 2009).

Figure 4-1: Structural Differences Among Various Vitamin E Forms				
Vitamin E Form	**R, (5)**	**R, (7)**	**R, (8)**	**Side chain double bonds (3^1, 7^1, 11^1 positions)**
alpha-tocopherol	CH_3	CH_3	CH_3	-
beta-tocopherol	CH_3	H	CH_3	-
gamma-tocopherol	H	CH_3	CH_3	-
delta-tocopherol	H	H	CH_3	-
alpha-tocotrienol	CH_3	CH_3	CH_3	+
beta-tocotrienol	CH_3	H	CH_3	+
gamma-tocotrienol	H	CH_3	CH_3	+
delta-tocotrienol	H	H	CH_3	+
Adapted from McDowell (2000)				

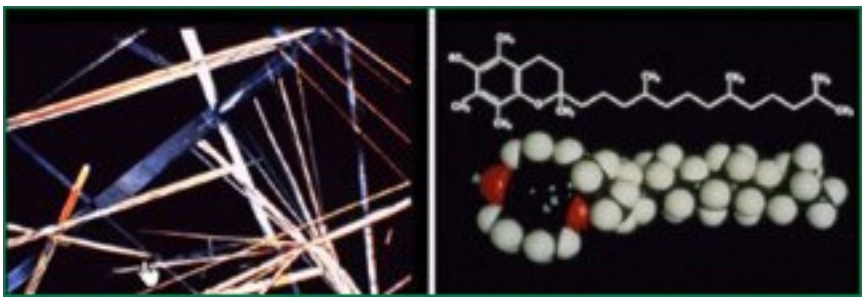
Table 4-1: Relative Biological Activities of various Tocopherols and Tocotrienols			
Item	**Fetal Resorption (Rat)**	**Hemolysis (Rat)**	**Muscle Dystrohy (Chicken)**
Alpha-tocopherol (5,7,8-trimethyl tocol)	100	100	100
Beta-tocopherol (5,8-dimethyl tocol)	25-40	15-27	12
Gamma-tocopherol (7,8-dimethyl tocol)	1-11	3-20	5
Delta-tocopherol (8-methyl tocol)	1	0.3-2	-
Alpha-tocotrienol-3	29	17-25	-
Beta-tocotrienol-3 (5,8-dimethyl tocotrienol)	5	1-5	-
Adapted from Machlin (1984)			

The tocopherol molecule contains three asymmetric (chiral) carbon atoms located at the 2′, 4′ and 8′ positions (Figure 4-1). In the d- form of alpha-tocopherol, the methyl groups at all three of the asymmetric carbon atoms have identical spatial arrangement, facing below the plane of the tocopherol molecule. This is designated as the RRR form and is the form of alpha-

tocopherol found in plants (Scherf et al., 1996). The dl- form of alpha-tocopherol consists of an equal proportion of the R and S configurations at each of the three chiral carbons, which results in an equimolar mixture (12.5% each) of the eight possible stereoisomers of alpha-tocopherol (Weiser et al., 1992). This is designated the all-racemic or the "all-rac" form of alpha-tocopherol, in addition to being called dl-alpha-tocopherol. Despite claims to the contrary, there is no truly "natural" vitamin E commercially available (Scherf et al., 1996). Natural vegetable oils contain varying mixtures of tocopherols and tocotrienols, each occurring in the alpha, beta, gamma and delta forms. These oils must be processed by vacuum distillation and chemically methylated in order to produce d-alpha-tocopherol specifically and in quantity. Thus, d-alpha-tocopherol is only obtained from natural sources after several chemical processing steps, and the product should be referred to as "natural-derived" and not "natural" vitamin E. More importantly, the International Unit (IU) remains the recognized standard for measuring and comparing the vitamin E activity of various sources. One IU of vitamin E is defined as 1.0 mg of dl-alpha-tocopheryl acetate. In most cases, natural-derived vitamin E is esterified to acetate forming the more stable dl-alpha-tocopheryl acetate ester. The mixed tocopherols derived as a secondary product of dl-alpha-tocopherol production are used as natural-source antioxidants in the feed and food industries. All-rac-alpha-tocopheryl acetate is the most common form of vitamin E used in animal feeds and supplements. This form of vitamin E is manufactured by chemical synthesis, condensing trimethyl hydroquinone and isophytol and conducting ultra-vacuum molecular distillation, producing a highly purified form of alpha-tocopherol. This material is then acetylated for stability. As discussed previously, all-rac-alpha-tocopherol is an equimolar mixture of eight stereoisomers (four enantiomeric, R,S, pairs) of alpha-tocopheryl acetate. The enantiomeric pairs, called racemates, have been shown to be present in equimolar proportions (Cohen et al., 1981; Weiser and Vecchi, 1981, 1982: Scott et al., 1982). Synthetic alpha-tocopherol (dl-or all-rac-alpha-tocopherol) is not identical to the naturally derived tocopherol (d- or RRR-alpha-tocopherol). On the basis, principally, of rat bioassay work and using dl-alpha-tocopheryl acetate as a standard (1 mg=1 IU) dl-alpha-tocopheol equals 1.1 IU, 1 mg d-alpha-tocopheryl acetate equals 1.36 IU, and d-alpha-tocopherol equals 1.49 IU of vitamin E. However, some studies in several species including cattle and sheep, show the naturally derived d-alpha-tocopheryl acetate compared to the synthetic dl-alpha-tocopheryl acetate is more effective in elevating milk, colostrum, plasma and alpha-tocopherol concentrations when administered on an equal IU basis. Cattle and sheep discriminate between RRR and all-rac vitamin E with a preference for RRR-alpha-tocopheryl acetate, thus the official bioequivalence ratio of 1.36:1 RRR-alpha-tocopheryl acetate to all-rac-alpha-tocopheryl acetate is

underestimated (Hidiroglou and McDowell, 1987; Hidiroglou et al., 1988a, b; Meglia et al., 2006; Waller et al., 2007; Dersjant et al., 2009; Weiss et al; 2009; de Ondarza et al., 2011). Feeding a higher dietary level of dl-alpha-tocopheryl acetatae could circumvent the lower bioavailability of the dl-form.It is not just naturally derived versus synthetic that is important for vitamin E biopotency but also the ester and carrier used. In sheep, Hidiroglou and Singh (1991) reported that with equivalent IU dosage, the natural form of d-alpha-tocopheryl succinate had only one-third the biopotency of the syntheticdl-alpha-tocopheryl acetate, indicating that the ester succinate has less value than the acetate. Jensen et al. (1999) also found the acetate to be a better form of vitamin E than succinate.Alpha-tocopherol is a viscous, yellow oil, insoluble in water but soluble in most organic solvents (Illus. 4-1). Tocopherols are extremely heat resistant but are readily oxidized. Naturally occurring tocopherols and tocotrienols are subject to oxidative destruction, which is accelerated by heat, moisture, rancid fats, copper and iron. As a result, native tocopherols and tocotrienols are excellent antioxidants that can protect carotenes, retinol, biotin and other oxidizable substrates in feed. However, in the process of acting as antioxidants, both the tocoperhols and tocotrienols are rendered biologically inactive as a source of vitamin E. For this reason, the acetylated form of alpha-tocopherol, tocopheryl acetate, is used as a source of dietary vitamin E. In some applications, the alcohol form of tocopherol or mixed tocopherols are used specifically as antioxidants.

Illustration 4-1



B. Absorption

Vitamin E absorption takes place in concert with fat digestion and absorption, requiring bile salts and pancreatic lipase and esterase enzymes (Sitrin et al., 1987). The efficiency of digestion and absorption of vitamin E varies with dietary inclusion level. At 10 IU per kg (4.5 IU per lb), there is about 98% uptake of vitamin E, while at 100 and 1,000 IU per kg (45.45 and 454.34 IU per lb), efficiency declines to 80% and 70%, respectively (Leeson and Summers, 2001). Most vitamin E is absorbed in the upper two-thirds of the small intestine (Bjorneboe et al., 1990). Whether presented as free alcohol or as esters, most vitamin E is absorbed as the alcohol, and

unlike vitamin A, is not re-esterified during absorption. Tocopherol esters are largely hydrolyzed in the intestinal lumen. Intestinal cells (enterocytes) absorb vitamin E in association with mixed lipid micelles. Within the enterocytes, vitamin E is incorporated into chylomicrons, which are then absorbed into the lymphatic system. Chylomicrons are in turn partially hydrolyzed and absorbed by the tissues, primarily the liver. All body tissues and organs accumulate vitamin E, but the largest accumulation is in adipose tissue, skeletal muscle and liver (Bjorneboe et al., 1990). Immune cells (neutrophils, macrophages and lymphocytes) contain very high concentrations of vitamin E, as do erythrocytes. The liver accumulates vitamin E, although not to the extent of vitamin A, and exports vitamin E in combination with very low density lipoproteins (VLDL) for use by other tissues. In general, vitamin E is disseminated to body tissues by mass action and in proportion to intake. A continuous intake of vitamin E is required in order to maintain vitamin E concentrations in cellular membranes throughout the body.In ruminants, there is little or no pre-intestinal absorption of dietary tocopherol. Rumen microbial destruction of tocopherol has been reported (McMurray and Rice, 1982; Weiss, 1998). However, the most recent studies, using the stabilized form of vitamin E (dl-alpha-tocopheryl acetate), have reported little if any degradation of vitamin E in the rumen (Weiss, 1998). Using high producing dairy cows, Hymøller and Jensen (2010) also showed no degradation of dl-alpha-tocopheryl acetate in the rumen. The vitamin E was added to the rumen contents through a rumen fistula and later was found at a constant level, indicating no degradation in the rumen. In most species, including ruminants, vitamin E absorption is proportional to the vitamin E status and requirement of the animal (Scherf et al., 1996; Traber and Sies, 1996; Hidiroglou et al., 1992b). Vitamin E absorption ranges from 50% to 75% of intake in deficient animals, from 20% to 30% in animals with adequate vitamin E status, and from 1% to 5% in animals fed large excesses of vitamin E. However, this may not always be the case. Hidiroglou et al. (1988b) reported no correlation between vitamin E status and tocopherol absorption. Other dietary and animal factors—in particular fat intake, digestion and liver function—affect absorption of vitamin E and the other fat-soluble vitamins. Vitamin E absorption can be impaired by a variety of disorders associated with fat malabsorption (Combs, 1991). Either a deficiency (Kim et al., 1998) or an excess of dietary zinc (Lu and Combs, 1988) impairs absorption of vitamin E. Earlier studies with poultry reported that vitamin E accumulation in plasma and liver was proportional to the log of vitamin E intake (Hidiroglou et al., 1992b). An interesting contrast noted in that work was that vitamin E accumulated in liver more slowly and persisted longer than the synthetic antioxidant ethoxyquin, which declined rapidly beginning 30 minutes after an oral dose. This indicates a specific cellular role of tocopherol, which was concentrated in sites of free-radical

generation, the mitochondria and microsomes, of liver cells. Mammals and birds preferentially absorb tocopherol versus other tocols. Rates and amounts of absorption of the various tocopherols and tocotrienols are in the same general order of magnitude as their biological potencies. Alpha-tocopherol is absorbed most efficiently. Gamma-tocopherol is absorbed less efficiently and more rapidly excreted than the alpha form. In general, it can be assumed that most vitamin E activity in plasma, erythrocytes and other tissues is alpha-tocopherol (Ullrey, 1981; McDowell, 2000). Vitamin E is transported in plasma by lipoproteins, and is delivered to tissues in association with lipids. Red blood cells contain significant amounts of vitamin E, which protects the erythrocytes from hemolysis. In ruminants, vitamin E does not cross the placenta in any appreciable amounts, with levels in the fetus generally lower than in the dam (Malone, 1975), making the neonate highly susceptible to vitamin E deficiency (Hidiroglou et al., 1969; Van Saun et al., 1989). Placental vitamin E transfer may decrease as gestation proceeds, possibly a dilution effect resulting from rapid fetal growth or a decrease in maternal vitamin E supply. Neonatal calves, lambs and kids are dependent on colostrum as a source of vitamin E. Less than 1% of the dam's tocopherol intake is secreted in milk (Millar and Dawe, 1973). However, vitamin E concentration in colostrum is high (Table 4-2), and is directly affected by maternal vitamin E intake during gestation (Whitting and Loosli, 1948; Quigley and Drewry, 1998; Njeru et al., 1994).

Table 4-2: Levels of Various Vitamins in Colostrum and Whole Milk Colostrum (Milking After Calving)

Vitamin	Units	1st	2nd	3rd	4th	5th	6th	Milk
A	IU/100 m	982	633	376	253	246*	—	113
D	IU/g fat	0.89-1.81**	—	—	—	—	—	0.41
E	IU/g fat	0.125	0.113	0.083	0.066	0.046*	—	0.022
Thiamin (B1)	µg/ml	0.58	—	0.59	—	0.59	—	0.38
Riboflavin (B2)	µg/ml	4.83	2.71	1.85	1.80	1.76	1.73	1.47
Nicotinic Acid (niacin)	µg/ml	0.74-0.097**	—	—	—	—	—	0.80
d-Pantothenic acid	µg/ml	1.73	—	3.20	—	3.96	—	3.82
d-Biotin	µg/100 ml	1.0-2.7**	—	—	—	—	—	2.0
B12	µg/100 ml	4.9	—	2.5	—	2.4	—	0.6
Folic acid	µg/100 ml	0.8	—	0.2	—	0.1	—	0.2
Choline	mg/ml	0.70	0.34	0.23	0.19	0.16	0.15	0.13
C (ascorbic Acid)	mg/100 ml	2.5	—	2.3	—	2.0	—	2.2

*Composite of fifth and sixth milkings after calving.
**Composite of first through sixth milkings.
Modified from Foley and Otterby (1968).Analyses of colostrum and milk primarily from Holstein cows.

Relatively little storage of vitamin E occurs in the body. The liver is not a true storage organ for vitamin E. It contains only a small fraction of total body vitamin E stores, in contrast to vitamin A, for which about 95% of the body reserves are contained in the liver. Unlike vitamin A, there is no known plasma transport protein for vitamin E, which is transported to and absorbed by tissues in association with lipoproteins (Traber, 2006). The liver preferentially secretes alpha-tocopherol into plasma under the control of haptic-alpha-transfer protein (Traber, 2006). Small amounts of vitamin E will persist tenaciously in the body during deficiency, in particular in neural tissues (Bjorneboe et al., 1990). However, tissue stores are exhausted rapidly by polyunsaturated fatty acids (PUFA), the rate of disappearance being proportional to the intake of PUFA (McDowell, 2000). Other sources of oxidative stress, including disease and inflammation deplete vitamin E. The major excretory route of absorbed vitamin E is bile, in which tocopherol appears mostly in the free alcohol form. Several oxidation products of vitamin E are also excreted in bile and urine, with tocopheryl quinone being the primary catabolite (Traber and Sies, 1996).

Functions

Vitamin E has been shown to be essential for the integrity and optimum function of reproductive, muscular, circulatory, nervous and immune systems in animals and humans (Hoekstra, 1975; Sheffy and Schultz, 1979; Bendich, 1993; Traber and Sies, 1996; McDowell, 2000). It is well established that some functions of vitamin E can be fulfilled in part or entirely by selenium or by other antioxidants. The vitamin E requirement is affected by the sulfur-bearing amino acids, cystine and methionine. Vitamin C (ascorbic acid) has been shown to spare vitamin E in tissues by regenerating alpha-tocopherol from its oxidation products. Considerable evidence indicates there may be undiscovered metabolic roles for vitamin E, which may be paralleled biologically by roles of selenium and possibly other substances. For example, vitamin E has been shown to potentiate the action of insulin in humans (Cabalero, 1994). The most widely accepted functions of vitamin E are discussed in this section.

A. Vitamin E as a Biological Antioxidant

Aerobic metabolism generates reactive oxygen species (superoxide anion, hydroxyl radical, hydrogen peroxide, etc.). These species damage cellular proteins, nucleic acids and membrane lipids (Traber, 2006). Living cells possess multiple defenses against oxidative damage, including the antioxidant enzymes, vitamin E and vitamin C (Sies et al., 1992). These defenses work synergistically to protect the cell from oxidative damage. Vitamin E has several interrelated functions, most of which can be traced to

its role as the primary lipid-soluble antioxidant in cell membranes (Traber, 2006). Vitamin E is part of the body's intracellular defense against the adverse effects of reactive oxygen species and free radicals that initiate oxidation of unsaturated phospholipids (Chow, 1979; Sies et al., 1992) and critical sulfhydryl groups of proteins and DNA (Brownlee et al., 1977). Vitamin E functions as a membrane-bound antioxidant, trapping lipid peroxyl free radicals produced from unsaturated fatty acids under conditions of "oxidative stress." Orientation of vitamin E within cell membranes appears to be critical to its functionality (Dunnet, 2003). Lipids, especially phospholipids present in cell membranes are particularly susceptible to oxidative damage. This is positively correlated with the lipid's degree of unsaturation. The antioxidant function of vitamin E is closely related to and synergistic with the role of selenium and other antioxidants. Selenium has been found to be part of 25 selenoproteins, with most of the functions unknown, although these selenoproteins generally participate in antioxidant and anabolic processes (Hatfield and Gladyshev, 2002). Selenium is a cofactor of the enzyme glutathione peroxidase (GSH-Px) that neutralizes hydrogen peroxide and hydroperoxides in the aqueous phase (cytosol and mitochondrial matrix). The various GSH-Px enzymes are characterized by different tissue specificities and are expressed from different genes. In general, different forms of GSH-Px perform their protective functions in concert, with each providing antioxidant protection at different sites of the body. Also involved in these defenses are the copper-zinc-manganese superoxide dismutase (SOD) enzymes and the iron (heme) containing enzyme, catalase. These enzymes indirectly prevent oxidation of unsaturated lipids within cell membranes. Lipid membranes have an abundance of unsaturated fatty acids and thus are very susceptible to peroxide formation. The peroxidation of membrane lipids can, in turn, cause oxidation of membrane proteins (Leeson and Summer, 2001). These reactions are enhanced by the presence of metal ions, especially iron. Vitamin E supplementation in semen extender reduced the lipid peroxidation potential and improved bovine semen quality during freezing-thawing (Hu et al., 2011). Vitamin E provides direct protection of membrane lipids by itself reacting with peroxyl radicals formed during oxidation of PUFA. By doing so, vitamin E breaks the self-propagating chain of oxidative damage to the cell membrane and related structures (Drouchner, 1976; Sies et al., 1992; Traber, 2006). When lipid hydroperoxides form in the absence of adequate vitamin E, direct cellular damage can result, in which peroxidation of lipids destroys the structural integrity of the cell and eventually cell function. Vitamin E also offers antioxidant protection to aqueous-phase cell components. Vitamin E increased the concentration of reduced glutathione in red blood cells and the activity of hepatic SOD in rats (Lii et al., 1998). Supplemental vitamin E was also shown to reduce to concentration of water-soluble oxidation products in

mice challenged with dietary pro-oxidants (fish oil and excess iron). By neutralizing free radicals and preventing oxidation of lipids within membranes, vitamin E reacts or functions as a chain-breaking antioxidant. Lipid peroxyl radicals are formed when reactive oxygen species abstract a hydrogen atom from an unsaturated fatty acid molecule. The fatty acid-peroxyl radical becomes self-propagating as double bonds of adjacent fatty acids are attacked. This "chain reaction" of membrane oxidation can produce widespread tissue damage if left unchecked. One of the unique properties of vitamin E is its direct incorporation into cell membranes where it interrupts free radical damage at the initiation stage, thus preventing the chain reaction of cell damage. Muscle damage and muscular dystrophy are common signs of both vitamin E and selenium deficiency (McDowell, 2000). This results in leakage of cellular metabolites such as creatinine and enzymes (i.e., transaminases, dehydrogenases) through damaged membranes into plasma. The more metabolically active tissues (such as skeletal and smooth muscles and liver) have a greater potential for oxidative tissue damage if vitamin E supply is limiting. Erythrocytes and capillary walls are also susceptible to damage in animals with marginal vitamin E status. The antioxidant properties of vitamin E explain the well-established observation that dietary tocopherols protect or spare oxidizable nutrients such as vitamin A, vitamin C and the carotenes. Certain deficiency signs of vitamin E (i.e., muscular dystrophy) can be prevented by dietary supplementation with other antioxidant nutrients, such as selenium, which helps validate the antioxidant role of tocopherols. Other deficiency signs respond only to vitamin E (Maynard et al., 1979). Synthetic antioxidants such as ethoxyquin exhibit limited tissue storage, as well as rapid clearance from the body, and thus cannot replace tocopherol. It is clear that diets high in PUFA increase the vitamin E requirement. Vitamin E is depleted during its action as an antioxidant, which explains the frequent observation that the presence of dietary unsaturated fat, especially PUFA, increases the vitamin E requirement and can precipitate a vitamin E deficiency.

B. Membrane Structure and Prostaglandin Synthesis

Alpha-tocopherol may be involved in the formation of structural components of biological membranes, thus exerting a unique influence on architecture of membrane phospholipids (Ullrey, 1981, Chen et al., 1998). It is reported that alpha-tocopherol stimulates the incorporation of 14C from linoleic acid into arachidonic acid in fibroblast phospholipids (Machlin, 1991). Additionally, alpha-tocopherol exerted a marked stimulatory effect on the formation of prostaglandin E from arachidonic acid, while a synthetic antioxidant had no effect. Supplemental vitamin E increased the proportion of linoleic and eicosapentaenoic acids in liver phospholipids of the rat (Chen et al., 1998). These changes in membrane composition may induce changes in

functionality or stability. Supplemental vitamin E has been reported to reverse the age-related decline in T-lymphocyte function, in part by reducing prostaglandin production (Beharka et al., 1997).

C. Blood Clotting

Vitamin E is an inhibitor of platelet aggregation in pigs and humans (McIntosh et al., 1985; Traber and Sies, 1996). Vitamin E inhibits peroxidation of arachidonic acid, which is required for formation of prostaglandins involved in platelet aggregation (Panganamala and Cornwell, 1982; Machlin, 1991). Vitamin E-deficient animals exhibit an elevation in plasma thromboxane that is relieved by supplemental vitamin E (Chen et al., 1998). The authors state that vitamin E deficiency may promote clot formation.

D. Stress, Immune Function and Disease Resistance

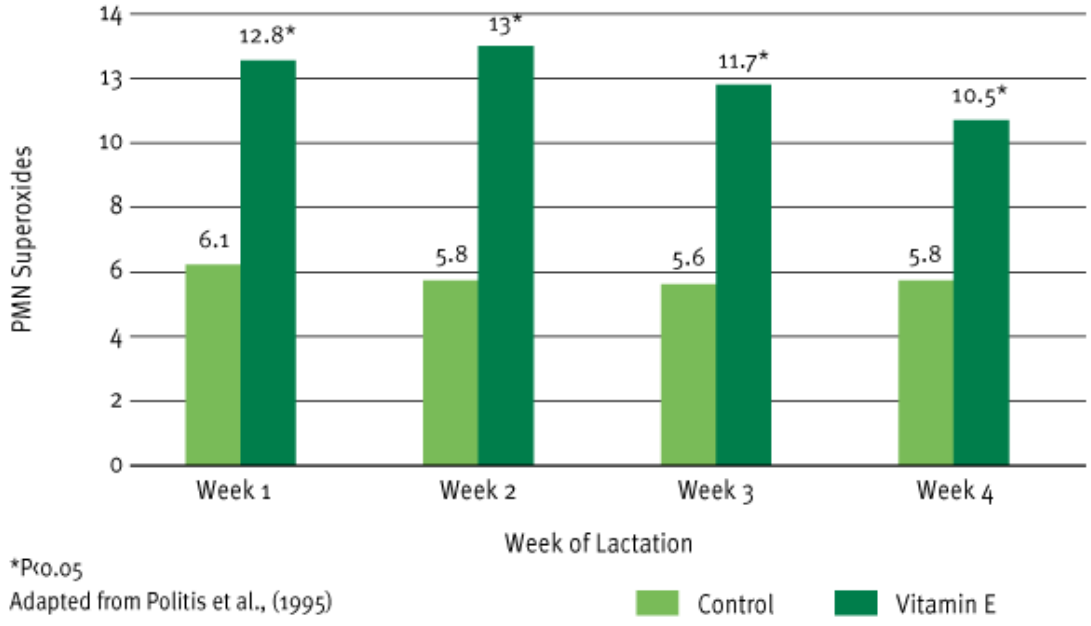
Vitamin E is perhaps the most studied nutrient related to the immune response (Meydani and Han, 2006). Evidence accumulated over the years and in many species indicates that vitamin E is an essential nutrient for the normal function of the immune system. Furthermore, studies suggest that beneficial effects of certain nutrients, such as vitamin E reducing disease risk, can be through their effects on the immune response.

Vitamin E clearly enhances immune function (Bendich, 1993; Machlin, 1991; Traber and Sies, 1996; McDowell, 2000). Furthermore, the dietary levels of vitamin E that result in optimum immune function are well above those required to prevent the classical vitamin E deficiency symptoms in humans and animals (Beharka et al., 1997; McDowell, 2000; Traber, 2006). Lymphocytes and mononuclear cells have the highest vitamin concentration of any circulating cells (Traber and Sies, 1996). Considerable attention is presently being directed to the role that vitamin E, selenium and other antioxidant nutrients (vitamin C, beta-carotene, zinc, manganese and copper) play in protecting leukocytes and macrophages during phagocytosis, the mechanism whereby immune cells engulf and kill bacteria and other pathogens. Vitamin E and selenium each play a specific role in protecting immune cells from damage by the oxygen radicals that these cells produce to kill ingested microorganisms (Badwey and Karnovsky, 1980; Machlin, 1991; Sies et al., 1992). For example, lung alveolar macrophages accumulate vitamin E, which enhances their defense against oxygen-free radicals generated during phagocytosis (Pathania et al., 1998). During stress and disease, there is an increase in production of glucocorticoids, epinephrine, eicosanoids, oxygen radicals, nitric oxide and phagocytic activity of immune cells (Gross and Siegel, 1997; McDowell, 2000). Eicosanoid and corticoid synthesis and phagocytic respiratory bursts of

leukocytes are prominent producers of free radicals. Supplemental vitamin E has been shown to enhance immune cell function under these conditions in a variety of species, including humans (Traber and Sies, 1996; McDowell, 2000). The adrenal glucocorticoids, cortisol and corticosterone, and thyroid hormone (T3) are secreted in response to stress, including adverse environmental conditions, disease, food or water deprivation or social competition (Gross and Siegel, 1997). As these hormones are absorbed from plasma, there is an increase in the ratio of "polymorphic" leukocytes (i.e., neutrophils and related cells) to lymphocytes (T- and B-lymphocytes) in plasma (Gross and Siegel, 1997). The change in this "P/L" ratio is a measure of the severity of the stress. Corticosterone treatment, a model of stress response, increases lipid peroxidation, decreases the activity of antioxidant enzymes and decreases growth rate and feed efficiency in laboratory rats (Ohtsuka et al., 1998). In the same study, feeding high levels of supplemental vitamin E significantly reduced lipid peroxidation, increased antioxidant enzyme activity and largely reversed the depression in growth rate and feed efficiency caused by corticosterone (Ohtsuka et al., 1998). Vitamin E has been implicated in stimulation of serum antibody synthesis, particularly immunoglobulin G (IgG) (Tengerdy, 1980; Machlin, 1991). As discussed previously, the protective effects of vitamin E on animal health appear to involve reversal of the oxidative and immunosuppressive effects of glucocorticoids (Golub and Gershwin, 1985). Vitamin E also supports immune function by effects on arachidonic acid metabolism and subsequent synthesis of prostaglandins, thromboxanes and leukotrienes. The production of these compounds increases under stress conditions. Thromboxane and interleukin II appear to exert a negative feedback effect on leukocyte function (Hadden, 1987). Supplemental vitamin E and selenium enhance the immune response against several types of pathogenic organisms. Increasing dietary vitamin E increases both antibody titers and phagocytosis of pathogens in calves (Cipriano et al., 1982; Reddy et al., 1985b, 1987b); lambs (Reffett et al., 1988; Finch and Turner, 1989; Turner and Finch, 1990); and dairy cows (Politis et al., 1995, 1996; Weiss et al., 1997; Weiss, 1998). For example, immune response was maximized in calves receiving 125 IU per day of vitamin E compared to calves receiving lower levels of dietary vitamin E (Reddy et al., 1987a). Garber et al. (1995) reported that mitogen-stimulated lymphocyte proliferation of dairy steers was maximized by feeding 1,000 IU of vitamin E daily as compared to either lower or higher levels of supplementation. Vitamin E and selenium deficiency reduced in vitro lymphocyte proliferation of calves, while repletion restored both numbers and function of lymphocytes (Pollock et al., 1994). Vitamin E and selenium exerted specific and joint effects on immune cell function. Stabel et al. (1992) reported that vitamin E increased immunoglobulin M (IgM) production by blood monocytes in vitro, and increased interleukin-1 gene

expression by monocytes isolated from vitamin E-supplemented steers. Vitamin E at high supplementation levels provides a strong immune response in livestock with enhancement of resistance to infectious diseases. Vitamin E affects both cellular and humoral immune function; T-lympocytes were increased (Abdukalykova et al., 2008). Previously, Moriguchi and Muraga (2000) observed that vitamin E improved the immune system by enhancing host antiviral activity and the production of the antiviral cytokine interferon, which is produced by activated T-cells.Vitamin E and selenium supplementation of dairy cows resulted in reduced rates and duration of intramammary infections and incidence of clinical mastitis (Smith et al., 1984, 1985, 1997). Vitamin E and selenium enhance host defenses by improving phagocytic cell function (Erskine et al., 1989; Hogan et al., 1992; Politis et al., 1995, 1996). Both vitamin E and the selenium-dependent glutathione peroxidase (GSH-Px) protect phagocytic cells and surrounding tissues from oxidative attack by free radicals produced by the respiratory burst of neutrophils and macrophages during phagocytosis (Baker and Cohen, 1983; Machlin, 1991; Baboir, 1984). Hogan et al. (1990, 1992) reported that vitamin E supplementation of diets increased intracellular kill of Staphylococcus aureus and Escherichia coli bacteria by neutrophils. Cows supplemented starting four weeks prior to calving through eight weeks postpartum with 3,000 IU vitamin E per day, in the presence of adequate selenium (0.3 ppm), had increased neutrophil and macrophage function and reduced somatic cell count compared to controls (Politis et al., 1995, 1996). (Figure 4-2 & 4-3). Supplemental vitamin E was shown to specifically stimulate phagocytosis of S. aureus by bovine neutrophils (Ndiweni and Finch, 1995). Both vitamin E and selenium increased neutrophil chemotaxis and superoxide production in these studies.

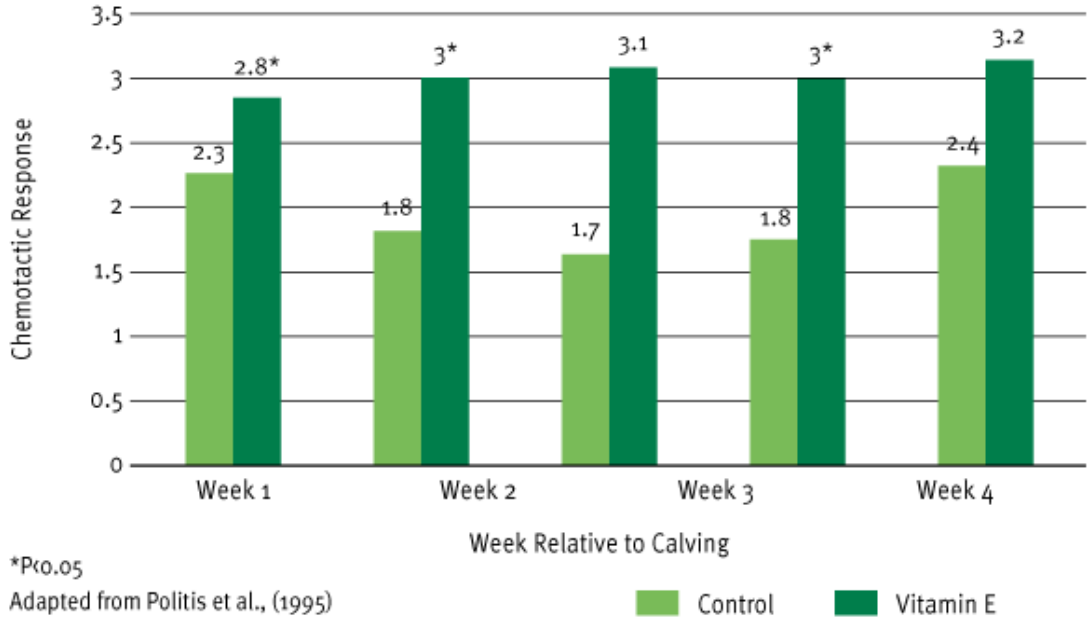
Figure 4-2: Effects of Vitamin E on Neutrophil Superoxide Production



*P<0.05

Adapted from Politis et al., (1995)

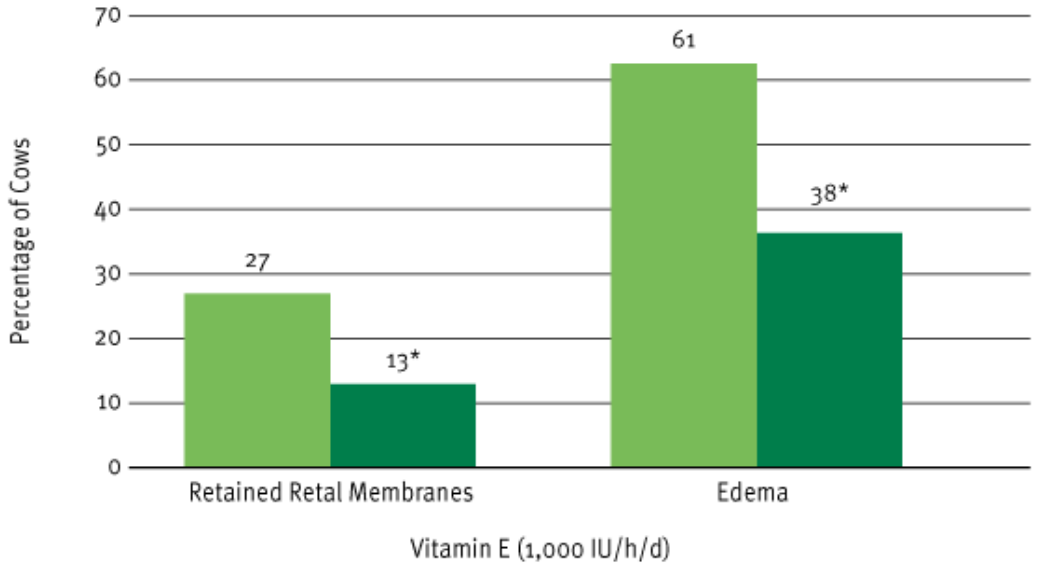
Figure 4-3: Effects of Vitamin E on Blood Neutrophil Chemotaxis



*P<0.05

Adapted from Politis et al., (1995)

Figure 4-4: Effect of Vitamin E on Retained Fetal Membranes and Udder Edema



Neutrophil function in dairy cattle is suppressed around the time of calving (Guidry et al., 1976; Kehrli et al., 1989; Gilbert et al., 1993a; Politis et al., 2004), as are several other indices of immune function (Mallard et al., 1998). High levels of vitamin E (3,000 IU per day) essentially prevented suppression of blood neutrophil and macrophage function in dairy cows in the study by Politis et al. (1996). Gilbert et al. (1993b) found that impaired neutrophil function was associated with retained fetal membranes in dairy cows. This link is of interest in light of the studies showing that supplemental vitamin E during the dry period results in a decreased incidence of retained placenta; (Julienet al., 1976a, b; Harrison et al. 1984; Aréchiga et al., 1994; Miller et al., 1997; Erskine et al., 1997; Kim et al., 1997; LeBlanc et al., 2002). The immune response in sheep has been improved with supplemental vitamin E. Vitamin E improved disease resistance in lambs challenged with chlamydia (Stephens et al., 1979). Reffett et al. (1988) reported that vitamin E and selenium independently increased the immune response of lambs challenged with a viral pathogen. Myopathic lambs exhibit low lymphocyte responses when deficient in vitamin E and selenium (Finch and Turner, 1989; Turner and Finch 1990). The poor lymphocyte responses of the lambs with nutritional myopathy were rapidly reversed by intramuscular administration of vitamin E-selenium, with the prophylaxis most effective during the first five weeks of life (Finch and Turner, 1989). In a study of 1,300 lambings, supplementing 330 IU of vitamin E daily for 21 days prior to lambing significantly reduced lamb mortality for ewes lambing early in the

season and increased the total weight of lambs weaned per ewe (Hatfield et al., 1999). Late-lambing ewes did not show a significant response to vitamin E. Supplementing ewes for 28 days prior to and 28 days after lambing has been shown to significantly increase vitamin E concentration in colostrum in the serum of their nursing lambs (Njeru et al., 1994). Dairy ewes injected twice during the dry period with vitamin E and selenium (5 mg and 0.1 mg/kg or 2.3 and .045 mg/lb body weight) had significantly lower somatic cell counts, increased erythrocyte glutathione peroxidase activity and enhanced neutrophil function compared to controls (Morgante et al., 1999). Injection of 900 IU of vitamin E per week to ewes in late pregnancy increased the survival and growth rate of lambs from birth through weaning (Ali et al., 1999). Antioxidants, including vitamin E, play a role in resistance to viral infection. Vitamin E deficiency allows a normally benign virus to cause disease (Beck et al., 1994). In mice, enhanced virulence of a virus resulted in myocardial injury that was prevented with adequate vitamin E. A selenium or vitamin E deficiency leads to a change in viral phenotype, such that a non-virulent strain of a virus becomes virulent and a virulent strain becomes more virulent (Beck, 1997; 2007; Sheridan and Beck, 2008). Thus host nutritional status should be considered a driving force for the emergence of new viral strains or newly pathogenic strains of known viruses.

E. Reproduction

Early attempts to establish a practical role for vitamin E in ruminant reproduction were inconclusive (NRC, 2000). In one experiment, four generations of male and female dairy cattle were fed low-vitamin E diets (Guilickson et al., 1949). Although growth, reproduction and milk production were normal, several cattle died suddenly of apparent heart failure between 21 months and 5 years of age. In the bull, supplemental vitamin E did not affect sperm or semen characteristics or fertility (Salisbury, 1944). However, large doses of vitamins A, D, E, and C have been reported to favorably affect some characteristics of semen and sperm (Kozicki, 1981). Velásquez-Pereira et al. (1998a) reported that feeding 4,000 IU per day of supplemental vitamin E to Holstein bulls reversed the negative effects of feeding gossypol (14 mg free gossypol per day as cottonseed meal) on semen quality and reproductive performance (Table 4-3). Supplemental vitamin E enhanced semen characteristics, plasma testosterone and breeding performance of gossypol-fed bulls above that of the control group, which was not fed gossypol. This suggests a potential benefit of vitamin E for breeding bulls regardless of diet. LaFlamme and Hidiroglou (1991) reported that pregnancy rate was improved (70% versus 33%) in beef heifers that had been fed supplemental vitamin E and selenium starting from eight months of age (weaning) and continuing for six months until breeding.

Table 4-3: Relationship of Gossypol and Vitamin E on Semen Characteristics of Dairy Bulls[a]			
Item	**Treatment**		
	Control[b]	**+Gossypol[c]**	**+Gossypol + Vitamin E[d]**
Normal	64.7±6.4[h]	31.4±7.4[i]	54.6±6.4[h]
% Abnormal	4.4±1.3[h]	13.4±1.5[i]	4.8±1.2[h]
DSPG[f] (x10⁶/g)	14.6±1.0[h]	10.2±1.0[i]	17.6±1.0[h]
DSP[g] (x10⁹)	3.2±3.0[h]	2.2±3.0[i]	4.1±3.0[h]

[a]Least square means ± SEM.
[b]Diet based on SBM, corn and 30 IU vitamin E/kg of supplement.
[c]Diet containing 14 mg free gossypol/kg BW/d and 30 IU vitamin E/kg of supplement.
[d]Diet containing 14 mg free gossypol/kg BW/d and 4,000 IU vitamin E/bull/d.
[e]Midpiece abnormalities evaluated in isotonic formal saline using DIC.
[f]Daily sperm production per gram of parenchyma.
[g]Daily sperm production total.
[h,i]Means in a row with different superscripts differ (P<0.05).
Velasquez-Pereira et al., (1998).

In dairy cattle, supplementation with selenium or both selenium and vitamin E reduced the incidence of retained placenta in herds where prevalence of retained placenta was high, or when selenium or vitamin E were marginal in the diet (Hurley and Doane, 1989). Supplemental vitamin E-selenium has also been reported to reduce metritis, cystic ovaries (Harrison et al., 1984) and time of uterine involution in cows with metritis (Harrison et al., 1986). Miller et al. (1997) summarized seven years of experimental data (n = 602 cows) in which comparisons were made between cows fed either 200 or 1,000 IU of vitamin E per day, d for the last 42 days of the dry period. Statistical analysis revealed a highly significant effect of supplemental vitamin E in reducing the incidence of retained placenta (27.4% in controls versus 12.6% in vitamin E-supplemented; $P < 0.0001$) (Figure 4-3. Cows fed 1,000 IU of supplemental vitamin E per day were 2.6 times less likely to develop retained placenta. In a more recent trial with 126 cows, feeding 1,000 IU per day of supplemental vitamin E during the dry period significantly reduced the interval to first estrus (Miller et al., 1997). Vitamin E increased plasma fast-acting antioxidant capacity and may increase plasma estradiol through protection of the cytochrome P-450 dependent enzyme system required for estradiol synthesis (Miller et al., 1993, 1997). These authors hypothesize that the ratio of corticosterone to estradiol in plasma is an indicator of stress level and predisposition toward reproductive disorders in dairy cattle.

Campbell and Miller (1998) fed 144 cows (64 primigravid heifers and 80 cows) either 0 or 1,000 IU of supplemental vitamin E per day in the presence or absence of excess iron and 800 mg added zinc for the last 42 days prepartum. Supplementation was discontinued after calving. Plasma vitamin E levels were low for all treatments (1.0 to 1.5 µg/ml), compared to levels considered minimal for periparturient cows (3.0 to 3.5 µg/ml), based on neutrophil function and udder health (Weiss, 1998). Despite the low levels of plasma vitamin E, cows supplemented with 1,000 IU vitamin E before calving had a significant reduction in days to first estrus, days to first breeding and days open. Days open were reduced by 32 overall. Retained placenta was not affected by any treatment. Therefore, 1,000 IU of supplemental vitamin E fed for 42 days prior to calving had significant beneficial effects on reproduction after calving. In Ohio research, incidence of retained placenta was reduced from a mean of 51.2% in control cows to 8.8% in cows injected with a combination of selenium and vitamin E (Julien et al., 1976b). Harrison et al. (1984) reported 17.5% retained placenta for control dairy cows, with no incidence for cows receiving both selenium and vitamin E. (Neither vitamin E or selenium was as effective alone.) From the same study, control versus selenium administration reduced cystic ovaries (47% versus 19%) and incidence of metritis (84% versus 60%). Other research found no effect of a prepartum injection of selenium and vitamin E on the incidence of retained placenta (Gwazdauskas et al. 1979; Schingoethe et al., 1982; Kappel et al., 1984; Hidiroglou et al., 1987). However, more recent studies have reported positive effects of vitamin E or vitamin E-selenium injection on reproduction in dairy and beef cattle. Aréchiga et al. (1994), using 198 Holstein cows in Florida, found that a single injection of 50 mg selenium and 680 IU vitamin E at 21 days prepartum significantly reduced retained placenta (10.1% versus 3%), increased first service pregnancy rate (41% versus 25%), reduced services per conception and reduced days open (141 versus 121 days). Erskine et al. (1997), reported results of a trial with 420 Holstein cows, in which a single injection of approximately 4,000 IU vitamin E at 14 days prepartum significantly reduced retained placenta (6.4% versus 12.5%), metritis (3.9% versus 8.8%) and increased serum vitamin E up to 14 days after injection. Kim et al. (1997) compared cows injected 20 days prior to calving with: placebo, selenium (40 mg), vitamin E (500 IU), or both selenium and vitamin E. They reported significant reductions in retained placenta (13.3% versus 30%) and days to first service (59.5 versus 102.7) in cows injected with both selenium and vitamin E. Table 4-4 summarizes results of studies on the effect of vitamin E and selenium on reproduction in dairy cattle.

Table 4-4: Effect of Vitamin E and Selenium on Retained Placenta in Cattle

Cow Description	Diet	Supplementation	Retained Placenta (%)				Observations	Reference
			Control		Supplemented			
			Prime	Multiple	Prime	Multiple		
Primiparous and multi-parous Holstein cows	0.16-0.20 ppm Se	**IM Injections**					This high producing Israeli Holstein herd had history of retaining placenta (RP) in 17% of primiparous and 25% of multiparous cows.	Egar et al., (1985)
		1-2.3 mg Se	16	35	4.5	16		
		2-4.6 mg Se+140 IU vit. E	6	27	3	9		
		3-9.2 mg Se+280 IU vit. E	6	44	5	14		
		4-23 mg Se+700 IU vit. E	22	14	4.5	14		
Pregnant Holstein cows	0.19-0.26 ppm Se	**IM Injections**					No known toxicity or deficiency of Se existed, and no common disease problem was identified.	Ishak et al., (1983)
		1-50 mg Se+680 IU vit. E	27		32			
		2-3,000,000 IUvit. A; 450,000IU vit. D;300 IU vit. E	27		30			
Multiparous dry cows	1.0-3.5 mg Se/ day	**IM Injections**					60% metritis (M) and 19% cystic ovaries (CO) in cows injected with Se. Untreated cows had 84% M and 47% CO.	Harrison et al., (1984)
		1-0.1 mg Se/ kgBW + 1,000IU vit. E	-		0			
		2-1,000 IU vit. E	-		20			
		3-0.1 mg Se/ kgBW	-		17			
		4-Control	16		-			
Multiparous dairy cows	1.0-3.5 mg Se/ day	**IM Injections**					Injection of vit. E and Se in cows already consuming adequate amounts of Se; no reduction in incidence of RP.	Schingoethe et al., (1982)
		68 IU vit. E+0.11 mg Se/kgBW at 21 days prepartum	20		22			
Holstein + Jersey cows 3 yrs of age or older	0.02-0.07 ppm Se	1-5.75 mg Sein oral bolus/ day for 5 days beginning 60 days prepartum	50		0		Cows were deficient in Se but control group for vit. E+ Se-treated cows had more Se (0.07ppm) in diet than other control groups. In both Se treatments, overall incidence of RP was reduced from 38% to 0%.	Julien et al., (1976a)
		2-IM injection of23 mg Se +680 IU vit. E at 20 days prepartum	20		0			

Holstein + Jersey cows	0.02-0.07 ppm Se	**IM Injections** 1-Control 2-23 mg Se+680 IU vit. E at 40 & 20 days prepartum 3-23 mg Se+680 IU vit. E at 20 days prepartum	 51 - -	 - 9 10	No difference in response to two injections was detected. A single injection of 50 mg Se + 680 IU vit. E was effective for prevention of RP.	Julien et al., (1976b)
Holstein + Jersey cows	N/A	1- Control 2- 1 mg Se/day 3- IM injection of 50 mg Se+680 IU vit. E	- - -	- - -	Calves from cows receiving Se or Se+ vit. E were stronger at birth, although 11% of cows had RP.	Daniels et al., (1987)
Friesian, Friesian x Ayshire cows	0.017-0.026 ppm Se	**IM Injections** 1- None 2- 15 mg Se+ 680 IU vit. E 3- 15 mg Se	 47 - -	 - 4 17	Treatments administered 28 days prepartum.	Trinder et al., (1973)
Four Holstein herds	N/A	**Herd** A BC D	 21 25 7	 16 15 22	IM injections of 50 mg Se + 680 IU vit. A was administered 20 days prepartum in herds in Se-deficient area.RP was reduced in cows considered to deficient is SE but not reduced in cows with adequate Se or extreme Se deficiency.	Segerson et al., (1981)
Holstein, Jersey, Guernsey, Ayshire	N/A	IM injections of 10 mg Se+ 680 IU vit. E at 4 wks prepartum for 3 years	10	10	Se-vit. E treatment neither affected time for placenta to pass nor reduced calving difficulty, days open or service per conception.	Gwazadauskas et al., (1979)
Dairy heifers	N/A	1- Control 2- 1 mg Se/day from breeding 3- IM injection	- - -	- - -	Calves born to heifers receiving Se were stronger at birth.	Daniels et al., (1987)

Holstein cows and heifers		IM injection of 50 mg Se + 680 IU vit. E injection 21 days prior to calving	-		-		Treatment had no effect on days to first estrus, service or conception or number of uterine infusions.	Kappel et al., (1987)
Holstein cows	-	Injection 50 mg Se+ 750 IU vit. E at 21 days		10		3	Supplemented cows had higher rates of conception (41.2 vs. 25.3) fewer days open (121 vs. 141), fewer services (2.3 vs. 2.8)	Aréchiga et al., 1994
Holstein cows (n=25)	-	Injection Se at 5 mg/100 kg BW + vit. E at 25 IU/100 kg	-		-		Treated cows had higher glutathione peroxidase activity and produced more colostrum and more milk	Lacetera et al., 1997
Holstein cows (n=420)	-	Injected 3,000 IU vit. E 14 days prepartum		12.5		6.4	Supplemented cows had reduced incidence of metritis (3.9% vs. 8.8%)	Eskine et al., 1997
Holstein cows (n=120)	-	20 days prepartum:1- Control2- 500 IU vit. E3- 40 mg Se4- 500 IU vit. E+ 40 mg Se	31.6	27.3	7.7	17.7	Significant reduction in retained placentas and days to first service in vit. E + Se group	Kim et al., 1997
All parities 640 obs 7 years	0.3 ppm Se+ 100 IU vit. E	1,000 IU vit. E per day		27.4		12.6	Supplemented cows had reduced retained placenta and udder edema	Miller et al., 1997
Holstein cows- 64 heifers- 56 Jersey cows-16 heifers- 8	0.3 ppm Se+ 100 IU vit. E	1,000 IU vit. E per day 800 mg supplemental zinc	16.6		16.0		Significant reduction in days to first service and days open in vit. E supplemented groups.	Campbell and Miller, 1998

Miller et al. (1993, 1997) reported that udder edema of dairy heifers was significantly reduced by supplementing diets with 1,000 IU of vitamin E daily for the last 42 days of gestation.

F. Cellular Respiration, Electron Transport and Deoxyribonucleic Acid (DNA)

Vitamin E appears to be of particular importance in cellular respiration of heart and skeletal muscles (Leeson and Summers, 2001). There is limited evidence that vitamin E is involved in biological oxidation-reduction reactions and may influence the biosynthesis of DNA within cells. Vitamin E appears to enhance the activity of microsomal cytochrome P-450 (Chen et al., 1998), which has multiple roles in detoxification and cell biosynthesis (Lehninger, 1982). Conflicting data exist on the role of vitamin E and DNA stability (Umegaki et al., 1997; Antunes and Takahashi, 1998; Pincheira et al., 1999).

G. Relationship to Toxic Elements or Substances

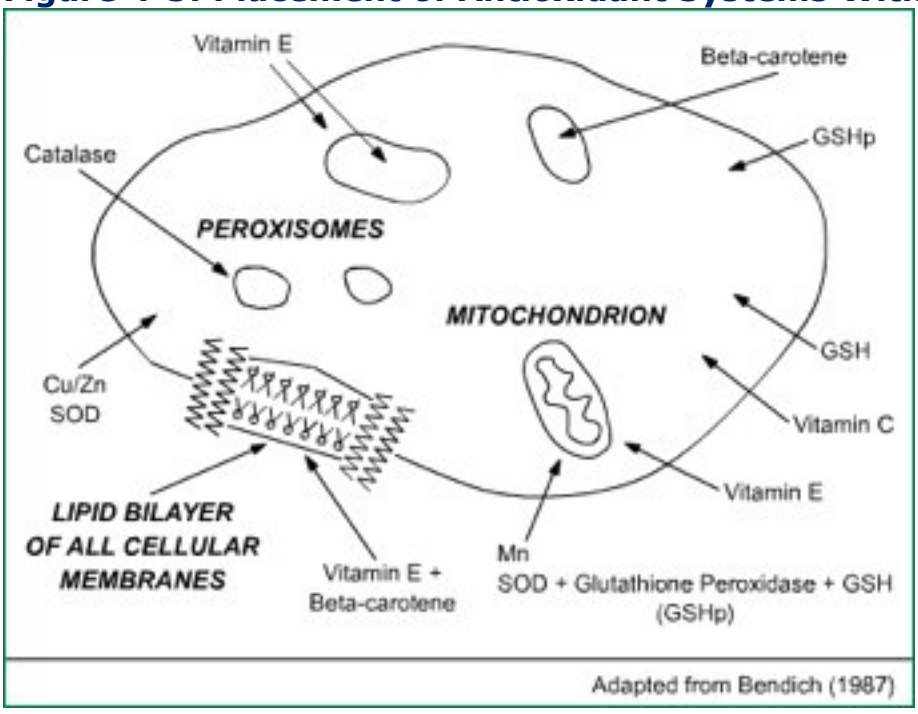
Both vitamin E and selenium provide protection against toxicity of certain heavy metals (Whanger, 1981). Vitamin E is highly effective in reducing toxicity of silver, arsenic, nickel and lead, and shows slight effects against cadmium and mercury toxicity. Heavy metals produce oxidative damage to tissues, and thus vitamin E can exert a protective antioxidant effect. Vitamin E can be effective against other toxic substances. For example, treatment with vitamin E gave protection to weanling pigs against monensin-induced skeletal muscle damage (Van Vleet et al., 1977). Mycotoxins must be detoxified by the cytochrome P-450 system, the activity of which appears related to vitamin E status. Adsorbants (bentonites, calcium aluminosilicates) used in research to alleviate symptoms of mycotoxicosis have been shown to reduce plasma vitamin E concentrations (Plank et al., 1990), suggested that vitamin E levels may need to be increased if these products are fed. Velásquez-Pereira et al. (1998a) found that 4,000 IU of supplemental vitamin E per day significantly reduced bull sperm abnormalities caused by feeding 14 mg per day free gossypol occurring naturally in cottonseed meal. Likewise, Velásquez-Pereira et al. (1999) reported that feeding 4,000 IU vitamin E per day alleviated negative effects of gossypol on the growth and health of dairy calves, and increased calf performance compared to the positive control ration.

H. Relationship with Selenium in Tissue Protection

Vitamin E and selenium have synergistic roles in protecting cells and tissues from oxidative damage. Selenium has a sparing effect on vitamin E and delays onset of deficiency signs. Likewise, vitamin E and sulfur amino acids partially protect against or delay onset of several forms of selenium deficiency syndromes. Vitamin E prevents fatty acid hydro-peroxide formation, sulfur amino acids are precursors of glutathione and selenium is an essential component of glutathione peroxidase (Smith et al., 1974). Tissue necrosis occurs in most species as a result of vitamin E-selenium

deficiency, primarily due to oxidative damage. Peroxides and related oxygen radicals are highly destructive to tissues and can increase susceptibility to disease. It now appears that vitamin E in cellular and subcellular membranes is the first line of defense against peroxidation of vital membrane phospholipids (Figure 4-5). Selenium, as part of the enzyme glutathione peroxidase (GSH-Px), is a second line of defense against oxygen radicals and their oxidation products. Vitamin C, beta-carotene, copper, iron, manganese and zinc also play important roles in the cellular antioxidant system, either directly or as cofactors in the antioxidant enzymes (e.g., superoxide dismutase and catalase).

Figure 4-5: Placement of Antioxidant Systems Within the Cell



I. Other Functions

Other functions attributed to vitamin E (Scott et al., 1982; Traber, 2006) include:

- phosphorylation reactions, especially of high-energy phosphate compounds, such as creatinine phosphate and adenosine triphosphate (ATP)
- support of the cytochrome P-450 system;
- a role in synthesis of vitamin C (ascorbic acid);
- a role in synthesis of ubiquinone;
- a role in sulfur amino acid metabolism; and
- potentiation of insulin action (Cabalero, 1994).

Pappu et al. (1978) reported that vitamin E plays a role in vitamin B12 metabolism. A deficiency of vitamin E interfered with conversion of vitamin B12 to its coenzyme 5'-deoxyadenosylcobalamin and therefore reduced conversion of methylmalonyl-CoA to succinyl-CoA. Turley and Brewster (1993) reported that vitamin E deficiency increases urinary excretion of methylmalonic acid, which is symptomatic of vitamin B12 deficiency. They also reported that conversion of cyanocobalamin to its active form was reduced by vitamin E deficiency.
In rats, vitamin E deficiency has been reported to inhibit vitamin D metabolism in the liver and kidneys with decreased formation of active vitamin D metabolites and decreases in the concentration of the hormone-receptor complexes in the target tissue. Liver vitamin D 25-hydroxylase activity decreased by 39%, 25-(OH)D-1-alpha-hydroxylase activity in the kidneys decreased by 22%, and 24-hydroxylase activity by 52% (Sergeev et al., 1990).

J. Non-Alpha Tocopherol Functions of Vitamin E

Although alpha-tocopherol has been the most widely studied form of vitamin E, other tocopherols and tocotrienols have recently been shown to have biological significance (Qureshi et al., 2004; Eder et al., 2002; McCormick and Parker, 2004; Schaffer et al., 2005; Nakagawa et al., 2007; Sun et al., 2008; Freiser and Jiang, 2009). The greater emphasis on the alpha-tocopherol undoubtedly arises from observations that gamma-tochpherol and delta-tocopherol are only 10% and 1% as effective as alpha-tocopherol, respectively, in experimental animal models of vitamin E deficiency. Tocotrienols have been shown to possess excellent antioxidant activity in vitro and have been suggested to suppress reactive oxygen substances more efficiently than tocopherols (Schaffer et al., 2005). Studies have shown that tocotrienols exert more significant neuroprotective, anti-cancer and cholesterol-lowering properties than do tocopherols (Qureshi et al., 2001; Sun et al., 2008). Gamma-tocopherol has beneficial properties as an anti-inflammatory and possibly anti-atherogenic and anticancer agent (Wolf, 2006). Research with tocotrienols and non alpha-tocopherols has been carried out with laboratory animals and in vitro studies; the significance for farm animals is unknown.

Requirements

Dietary vitamin E requirements of ruminants have not been clearly defined. Minimum requirements for beef cattle (NRC, 2000), dairy cattle (NRC, 2001) and sheep (NRC, 1985) are estimated to range from 10 to 60 IU per kg (4.5 to 27 IU per lb) of diet dry matter. The dairy NRC (2001) vitamin E requirement for pregnant heifers and dry pregnant cows ranges from 80 to

120 IU per kg (36.4 to 54.5 IU per lb) and for lactating cows it is 16 to 27 IU per kg (7.3 to 12.3 IU per lb). The vitamin E requirement for all sheep is 15 IU per kg (6.8 I.U. per lb). The vitamin E requirement for beef cattle (NRC, 2000) and goats (NRC, 1981) has not been established, however for beef cattle it is estimated to be between 15 and 60 IU per kg (6.8 to 27.3 IU per lb) of diet (NRC, 2000). The NRC (2007b) has updated the sheep NRC (1985) and the goat NRC (1981). This NRC is designated as requirements of small ruminants including sheep, goats, cervids and new world camelids. The NRC (2007b) nutrient requirements for small ruminants expresses requirements for vitamins not in concentrations of feed, but as per kg of body weight or on a day basis. The suggested vitamin E minimum daily requirement of growing small ruminants is 5.3 IU per kg (2.4 IU per lb) of body weight. For all small ruminants, it is suggested that 5.3 mg of dietary vitamin E per kg (2.4 IU per lb) of body weight would maintain blood alpha-tocopherol concentrations above critical levels Absolute vitamin E requirements are difficult to determine due to interrelationships with other nutrients. Dietary levels of PUFA directly affect the vitamin E requirement. The PUFA found in unsaturated oils such as cod liver oil, corn oil, cottonseed oil, soybean oil, sunflower seed oil and linseed oil all increase vitamin E requirements (McDowell, 2000). This is especially true if these oils undergo oxidative rancidification or are in the process of oxidizing when consumed by the animal. Rancidification of dietary fats prior to consumption destroys vitamins E, A and biotin. Fats ingested during the process of oxidation can cause damage to body tissues during absorption and metabolism and reduce vitamin E stores (Scott et al., 1982). Ruminants are partly protected from the effects of dietary PUFA as a result of rumen biohydrogenation. However, investigations have shown that PUFA, when fed in large quantities, can escape rumen hydrogenation (McMurray et al., 1980). McMurray et al. (1980) and Rice et al. (1981) showed that nutritional muscular dystrophy (white muscle disease) could be induced by rapid introduction of calves to lush pasture, which is high in PUFA content. The pathogenesis was associated with and reproducible by elevations in plasma linolenic acid, which increased three-fold within three days of turnout. In addition to these effects, dietary PUFA can cause destruction of vitamin E in feed prior to its contact with rumen microorganisms. Various forms of stress, including weather extremes, social dominance interactions, feed or water deprivation, hauling, handling, disease exposure, trauma and toxins, all contribute to increased vitamin E requirements. For improving immunocompetence, vitamin E supplementation is needed at levels beyond those needed to support optimal growth. Due to feedlot stress, the latest NRC beef cattle publication (NRC, 2000) recommends that 400 to 500 IU of vitamin E be fed to receiving and starting feedlot cattle. There is a growing distinction between minimum requirements and optimal fortification with vitamins in

the scientific literature. Beef stocker cattle previously fed a low vitamin E ration or kept on low quality pasture exhibit a stress syndrome commonly referred to as "buckling." Affected calves come off the truck or out of the processing chute with weakness in the rear quarters, buckling of the hocks and fetlocks and generalized shaking and quivering of skeletal muscles (McDowell, 2000). Necropsy revealed pale, white streaks in striated muscles of the hamstring and lower back and sometimes in the heart, diaphragm and intercostal muscles of the ribcage. This disease condition was confirmed as a combined selenium-vitamin E deficiency. Handling and bleeding heifers periodically over a 10-day period resulted in a large decrease in the vitamin E content of red blood cells and a 62% decrease in neutrophil vitamin E levels (Nockels, 1996). Vitamin E supplementation increased immune response in stressed calves (Golub and Gershwin, 1985). Cottonseed meal is commonly fed to cattle upon arrival at feedlots. The combination of shipping stress and gossypol consumption may reduce tissue vitamin E levels, further reducing disease resistance and increasing susceptibility to muscle damage (myopathy). Vitamin E and selenium requirements are interdependent. The relationship has been quantified to a certain degree for poultry. Chicks consuming a diet containing 100 IU per kg (45.5 IU per lb) vitamin E required 0.01 ppm selenium, while those receiving no added vitamin E required 0.05 ppm selenium (Thompson and Scott, 1969). Hogan et al. (1990) reported that both vitamin E and selenium improved bactericidal ability of bovine neutrophils of cows previously fed vitamin E and selenium-deficient diets, but that their effects were not strictly additive. Therefore, these nutrients appear to exert a sparing effect on each other's requirement. Politis et al. (1995, 1996) found that high levels of vitamin E supplementation (3,000 IU per day) just prior to calving improved white blood cell function in cows fed diets that were adequate in selenium. Factors such as the level of disease challenge and the overall stress load on cattle are likely to influence this relationship. Tissue storage of vitamin E and selenium further complicate the determination of minimum requirements of these nutrients. Short-term studies may fail to account for the effects of tissue nutrient stores and thereby underestimate dietary requirements for both nutrients. Data summarized by Agricultural Research Council (1980) indicated that the minimum requirements for vitamin E in the diet of growing or pregnant sheep were between 10 and 15 IU per kg (4.5 to 6.8 IU per lb) of dry matter. However, if dietary selenium levels are below 0.05 mg per kg (0.023 mg per lb), even 15 to 30 IU per kg (6.8 to 13.6 IU per lb) may prove inadequate.

Sources

Published values of vitamin E analyses of foods and feeds are based on a wide variety of analytical techniques. As a result, there is a lack of

characterization of individual tocopherols in the majority of analyses. Total tocopherol analysis of a food or feed is of limited value in providing a reliable estimate of the biological vitamin E content in IU equivalents, due to the inclusion of beta-, gamma- and delta-tocopherols.

Alpha-tocopherol is the most active form of vitamin E, and therefore most nutritionists prefer a listing of alpha-tocopherol equivalents or IU of vitamin E activity instead of the less reliable total tocopherol value. The less active tocopherols, particularly gamma-tocopherol, are present in mixed diets in amounts two to four times greater than alpha-tocopherol. Cort (1983), utilizing high-pressure liquid chromatography (HPLC) assay procedures, which allows separation of alpha and non-alpha forms of both tocopherol and tocotrienols, determined that corn, corn gluten meal, oats, barley and wheat contained significant amounts of alpha-tocotrienol. If feed analysis is based solely on alpha-tocopherol content, the total vitamin E activity of the feed can be approximated by increasing this value by 20% to account for the activity of the other vitamin E-active compounds present. However, all the naturally occurring vitamin E compounds are chemically unstable and variable in concentration, and are therefore not normally relied on as the sole source of vitamin E in animal diets.

Vitamin E is widespread in nature, with the richest sources being vegetable oils, oilseeds and grains containing these oils; eggs; liver; legumes; and, in general, green plants and pastures. In nature, plants synthesize vitamin E (mixtures of tocopherols and tocotrienols), and thus plant products are the principal sources of vitamin E activity. Vitamin E is abundant in whole cereal grains, particularly in germ, and in byproducts containing the germ (McDowell, 2000; Traber, 2006). For example, wheat germ oil was long used as a vitamin E supplement. Alpha-tocopherol is especially high in wheat germ oil, safflower oil and sunflower oil. Corn and soybean oils contain predominatly gamma-tocopherol, as well as some tocotrienols (McDowell, 2000; Traber, 2006). Cottonseed oil contains both alpha- and gamma-tocopherols in equal proportions.

There is a three- to ten-fold range of variation in vitamin E content in common feeds. As a result, the naturally occurring vitamin E activity of feedstuffs cannot be accurately estimated from most published vitamin E or tocopherol values. Feed table averages are often of little value in predicting individual content of feedstuffs or bioavailability of vitamins. Vitamin E content of 42 varieties of corn varied from 11.1 to 36.4 IU per kg, (5.0 to 16.5 IU per lb), a 3.3-fold difference (McDowell and Ward, 2008). Table 4-5 presents the alpha-tocopherol content of various feedstuffs compared to previously published assay values.

Table 4-5: Comparison of Recent Assay Values of Vitamin E Content of Feedstuffs to Previously Published Assay Value

Feedstuffs	Vitamin E Activity (IU/lb, as fed)				
	Previously Published Recent Assay Values[a]			Assay Values[b]	
	No. of samples	Average	Range	Average	Range
Corn	11	6.2	4.5-10.0	13.5	7.7-23.6
Soybean meal	15	1.0	0.6-1.9	2.0	1.0-3.3
Cottonseed meal	7	5.2	0.7-12.4	6.2	1.7-10.9
Corn gluten meal	5	6.0	3.4-9.8	17.5	8.5-26.4
Oats	3	4.8	3.0-5.1	13.8	12.0-15.9
Rolled Oats	1	5.4	-	-	-
Alfalfa, dehydrated	4	37.8	24.4-52.2	54.7	22.2-88.6
Alfalfa, sun cured	1	35.8	-	35.6	35.5-41.3
Alfalfa meal	3	32.3	18.6-56.6	49.2	18.9-81.8
Alfalfa pellets	1	20.4	-	-	-
Milo	2	3.7	2.8-4.5	10.1	6.9-10.7
Barely	2	6.0	5.4-6.6	24.6	14.7-28.9
Barely, crimped	2	4.0	3.3-4.8	-	-
Wheat	4	5.3	3.4-8.2	7.5	2.2-9.9
Animal fat	4	3.9	1.8-7.6	5.3	1.6-10.7
Poultry fat	1	13.9	-	12.8	9.4-16.2

[a]Adapted from Cort et al., (1983).
[b]Adpapted from Bunnell et al., (1968); NAS (1969).

Vitamin E status is reduced in cows fed stored forages for extended periods of time, while pasture improves vitamin E status (Schingoethe et al., 1982; Lynch, 1983). A more available form of vitamin E is present in pastures and green forages, containing ample quantities of alpha-tocopherol versus the less bioavailable forms in grains (McDowell, 2004). Serum vitamin E levels are higher in summer and fall than in the winter and spring seasons (Miller et al., 1995), which likely reflects the vitamin E level of forages consumed in the months prior to sampling. Besides seasonal effects, herd and time since parturition affected serum vitamin E and selenium concentrations (Miller et al., 1995).

There can be a five-fold seasonal variation in the alpha-tocopherol content of cow's milk. Colostrum is the primary source of vitamin E for the neonate, whose tissue vitamin E levels at birth are low (Whitting and Loosli, 1948;

Hidiroglou et al., 1996; Van Saun et al., 1989; Njeru et al., 1994a). Colostrum from dairy cows has been reported to have a mean value of 1.9 µg alpha-tocopherol per gram, declining to 0.3 µg/g at 30 days postpartum (Hidiroglou, 1989). In this study, milk tocopherol was increased from 0.3 µg/g to 1.6 µg/g 12 hours after an intraperitoneal injection of dl-alpha-tocopheryl acetate. Four experiments conducted at Ohio State University (Weisset al., 1990b, 1992, 1994, 1997) reported higher overall values for colostral vitamin E (Figure 4-6). The mean alpha-tocopherol concentration in colostrum from unsupplemented cows was 4.7 µg/g, while colostrum from vitamin E-supplemented cows (fed 900 to 1,000 IU per day during dry period) averaged 6.7 µg/g alpha-tocopherol. In one study, cows were also injected twice with 3,000 IU supplemental vitamin E at five and 10 days prepartum. Injectable vitamin E also elevated colostral vitamin E to 8.9 µg/g in cows not fed supplemental vitamin E and to 11.6 µg/g in cows fed 1000 IU supplemental vitamin E. Using a colostrol vitamin E content of 6.7 µg/g and an intake of 3.63 kg colostrum per calf per day, newborn calves would receive approximately 24 mg (24 IU) alpha-tocopherol per day while fed colostrum (Figure 4-7). Most commercial milk replacers supply a minimum of 20 IU vitamin E per pound of powder, and therefore per calf per day. Higher levels have been shown to impact health benefits to calves, as will be discussed in later sections.

Figure 4-6: Effect of Dry-Period Vitamin E Intake on Vitamin E in Colostrum and Total Vitamin E Output of Dairy Cows

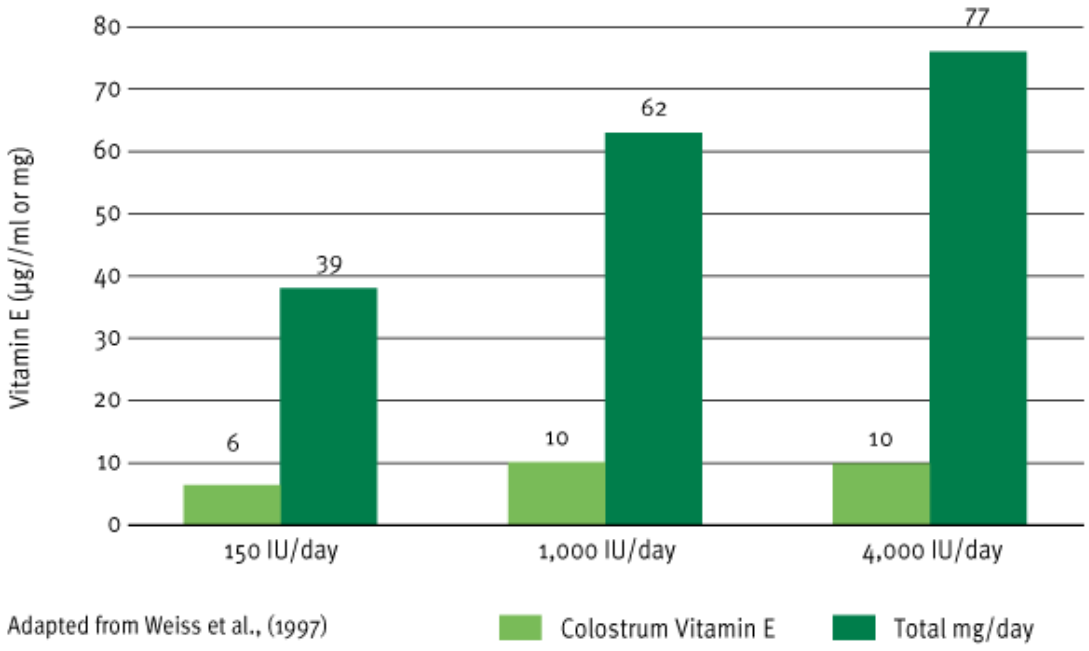


Figure 4-7: Average Nutrients in 8 lbs Colostrum	
Protein, grams	381
IgG, grams	163
Fat, grams	182
Vitamin A, IU	26,733
Vitamin D, IU	246
Vitamin E, IU	24
Vitamin C, mg	45

Animal byproducts supply only small amounts of vitamin E. Milk and dairy products are also poor sources. Eggs, particularly the yolk, make a significant contribution, depending on the diet of the hen. Wheat germ oil is the most concentrated natural source of vitamin E. Soybean, cottonseed and peanut oil are also good sources of vitamin E activity, but also contain significant levels of polyunsaturated fatty acids that can undergo peroxidation and destroy tocopherols. The majority of oilseed meals are solvent extracted and therefore contain little vitamin E activity (Maynard et al., 1979). Green forage and good quality leaf meals, such as alfalfa meal, are normally good sources. Concentration of tocopherols per unit dry matter in fresh herbage is between five and 10 times as great as that in most cereals or their byproducts (Hardy and Frape, 1983). Cattle grazed on green-lush pastures commonly had 4 to 6 µg alpha-tocopherol per g muscle compared with 1 to 2 µg alpha-tocopherol per g muscle for standard grain-fed feedlot cattle (Yang et al., 2002). It was reported that variability in forage vitamin E content is so great, both between and within farms, that ongoing vitamin E analysis of forages and feeds must be conducted in order to ensure proper vitamin E fortification levels (Hardy and Bieber-Wiaschny, 1988). These authors conclude that previously published forage vitamin E concentrations are not suitable for feed formulation.

Stability of all naturally occurring topcopherols and tocotrienols is poor, and substantial losses of vitamin E activity occurs in feedstuffs during processing and storage, as well as in manufacturing and storage of finished feeds, supplements and mineral mixes, (Dove and Ewan, 1991; McDowell, 2000).

Vitamin E in typical feeds and forages is unstable under conditions that promote oxidation (heat, oxygen, moisture, oxidizing fats and trace minerals). For concentrates, oxidation increases following grinding, mixing with minerals, addition of fat and pelleting. Iron salts (i.e., ferric chloride) can completely destroy vitamin E.

Artificial drying of corn results in a much lower vitamin E content. In a stability study, Orstadius et al. (1963) reported that vitamin E content of corn was reduced from 40 mg per kg (range of 30 to 50 mg/kg) to 5 mg per kg (18.1 to 2.27 mg/lb) of dry weight by drying at 212°F (100°C) for 24 hours under a continuous air flow. Similarly, it has been reported that artificial drying of corn for 40 minutes at 190°F (88°C) produces an average 19% loss of alpha-tocopherol and a 12% loss of other topcopherols (Adams, 1973). Corn dried for 54 minutes at 199°F (93°C) had an average vitamin E loss of 41%. Young et al. (1975) reported alpha-tocopherol concentrations of 9.3 and 20 mg per kg (4.2 and 9.1 mg per lb) dry weight in artificially dried corn and field-dried corn, respectively.

Preservation of grain by ensiling causes almost complete loss of vitamin E activity. Corn stores as acid-treated (propionic or acetic-propionic mixture) high-moisture corn contains approximately 1 mg per kg (0.45 mg per lb) of dry matter alpha-tocopherol, while artificially dried corn contains approximately 5.7 mg per kg (2.6 mg per lb) alpha-tocopherol (Young et al., 1978). Destruction of tocopherol under these conditions is apparently due to the combination of moisture and acid (McMurray et al., 1980). Tocopherol concentration of acid-treated, high moisture grain continues to decline during storage to levels less than 1 mg/kg (0.45 per lb) dry matter.

Drying or processing of forages can reduce tocopherol content. For example, in one study 80% of the vitamin E was lost in hay making (King et al., 1967). Ensiled or rapidly dehydrated forages retain a greater proportion of tocopherol content. Vitamin E content in forage is affected by stage of maturity at harvest and by drying time. Frost dramatically reduced both alpha-tocopherol and beta-carotene concentrations in four tropical grasses (Arizmendi-Maldonado et al., 2003). Losses during field drying can amount to as much as 60% within four days. Storage losses can reach 50% in one month. Vitamin E losses of 54% to 73% have been observed in alfalfa stored at 91°F (33°C) for 12 weeks and 5% to 33% losses have been obtained within commercial dehydration of alfalfa.

A. Commercial Sources of Vitamin E

The most active naturally occurring form of vitamin E, as previously stated, is d-alpha-tocopherol. For many years the primary source of supplemental vitamin E for animal feed were tocopherols found in green plant products, oilseeds and vegetable oils. Commercially available sources of vitamin E activity are shown in Table 4-6. Biopotency of the stereoisomers of alpha-

tocopherol is expressed in terms of the International Unit (IU). According to The United States Pharmacopeia (1980), dl-alpha-tocopheryl acetate, also called all-rac-tocopheryl acetate, is the International Standard of vitamin E activity, with one IU equivalent to one milligram of dl-alpha-tocopheryl acetate (Table 4-6). This form of vitamin E is the most widely available and cost-effictive source of vitamin E activity for the supplementation of animal feeds. The acetate ester of d- or dl-alpha-tocopherol is synthesized in order to stabilize the compound from oxidation and maintain vitamin E activity. Vitamin E as the acetate form is highly stable in vitamin premixes with 98% retention after 6 months but in the alcohol form is completely destroyed during this time period (Coelho, 1991). The succinate ester is synthesized for some food applications. Both esters are resistant to oxidation. Thus, dl-alpha tocopheryl acetate does not act as an antioxidant in feed. Synthetic antioxidants are often added to unstable feed ingredients such as liquid fat in order to prevent oxidation and rancidification.

Table 4-6: Commercially Available Sources Of Vitamin E Activity[a]

Source	I.U. of Vitamin E Activity per mg
dl- alpha-tocopheryl acid succinate (all-rac)	0.89
dl- alpha-tocopheryl acetate (all-rac)	1.0
dl- alpha-tocopherol (all-rac)	1.10
d- alpha-tocopheryl acid succinate (RRR)	1.21
d- alpha-tocopheryl acetate (RRR)	1.36
d- alpha-tocopherol (RRR)	1.49

[a]Adapted from United States Pharmacopeia (1980).

Tocopheryl acetates are commercially available in two basic forms: (1) d-alpha-tocopheryl acetate is produced by extraction of natural tocopherols from byproducts of vegetable oil refining, methylation to increase the yield of the alpha-isomer, molecular distillation to separate the alpha-isomer, and acetylation to form d-alpha-tocopheryl acetate; and (2) dl-(all-rac)-alpha-tocopheryl acetate is the product of complete chemical synthesis, which yields a racemic mixture of equal proportions of eight stereoisomers. Although there is typical biological variation in the results, the majority of well-controlled scientific studies of vitamin E biopotency have affirmed the USP standards of vitamin E activity for the two primary isomeric forms (Scherf et al., 1996). Weiser et al., (1992) determined that biodiscrimination between stereoisomers of alpha-tocopherol is based almost entirely on the three-dimensional structure of the 2-carbon of the hydroquinone ring system. The 2R isomers have approximately twice the activity of the 2S

isomers (Scherf et al., 1996). Given the fact that the eight possible stereoisomers of alpha-tocopherol occur in equal proportions in commercial vitamin E, and that half of the isomers are 2S in conformation, the all-rac (dl) form of tocopherol should have 75% the activity by weight of the RRR (d) form. This is basically the value assigned (74%) by the USP (Scherf et al., 1996).

Hidiroglou et al., (1997b) compared the effects of feeding 1,000 IU of vitamin E activity from either dl- or d-alpha-tocopheryl acetate to dairy cows from eight weeks prior to calving through eight weeks postpartum. Although plasma and red blood cell alpha-tocopherol concentrations of plasma and red blood cells were elevated by both sources, the dl- form resulted in higher values than the d-form. However, tocopherol concentrations of neutrophils and the production of both superoxide anion and hydrogen peroxide by blood neutrophils were not significantly different between the two sources, suggesting similar bioactivity of the two sources for neutrophil function.

Eicher et al., (1997) compared single oral doses of either d- or dl-alpha tocopherol in dairy calves and found higher vitamin E concentration in plasma and the kidney with the d-isomer, while levels in red blood cells, spleen, liver, adipose tissue, muscle, gut and heart were not significantly different between the two vitamin E sources.

Bioavailability of three physical forms of dl-(all-rac)-alpha-tocopheryl acetate were compared in dairy cows (Baldi et al., 1997). The forms compared were: adsorbate on silica; microencapsulated in stearic and palmitic acid; and vitamin E oil. Italian Friesian dairy cows were fitted with rumen cannulas and fed a balanced lactation ration consisting of 62% forage (corn silage, alfalfa and grass hays) and 38% grain (corn, barley and soybean meal). Cows were administered 5,000 IU dl-alpha-tocopheryl acetate gelatin capsules in a 4×4 Latin Square experiment. An intraperitoneal injection of 5,000 IU vitamin E was used as a standard of comparison to the intraruminal route. Overall kinetics of vitamin E absorption and decay were similar to published studies. Vitamin E adsorbate and microencapules had a long half-life in plasma compared to vitamin E oil. Bioavailability tended to be higher for the adsorbate and microencapsulated forms of vitamin E.

Deficiency

Vitamin E displays a wide variety of deficiency signs, more than any other vitamin. Deficiency signs differ among species and even within species. Blaxter (1962) reported that muscle degeneration and muscular dystrophy appear to be the one vitamin E deficiency syndrome common to all species. White muscle disease (WMD), also known as nutritional muscular dystrophy or nutritional myodegeneration (NMD), is the major clinical manifestation of a vitamin E or selenium deficiency in newborn calves, lambs and kids. Cardiac white muscle disease, the equivalent of Mulberry heart disease in

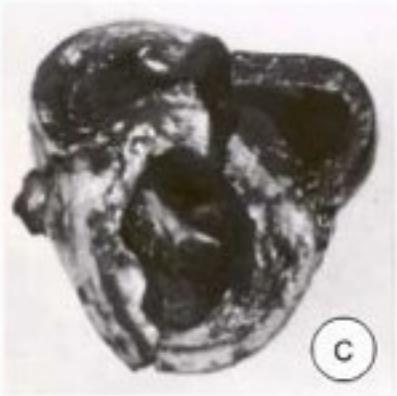
pigs, is a common deficiency lesion in calves and lambs born to vitamin E-deficient dams. The young also tend to lack a normal suckling reflex and may be unable to stand or walk. White muscle disease occurs in all laboratory and farm animals, as well as camels, buffalo, rhinos and kangaroos. Muscular dystrophy is reported in a number of wild animals, the condition in antelope, for example, being indistinguishable from WMD in cattle or sheep (NRC, 1983). Fundamentally, WMD is a Zenker's degeneration of both skeletal and cardiac muscle fibers. Damaged muscle is replaced by connective tissue that is observable as gross white striations in the muscle fiber bundles (Smith, 1970). Lesions are usually symmetrical and bilateral (Blaxter, 1962), and affected muscles may tear easily and appear edematous. Necropsy may reveal generalized edema of the abdominal cavity and the lungs (Morrill and Reddy, 1987). Serum glutamic-oxaloacetic transaminase (SGOT), lactic dehydrogenase (LDH) and creatinine phosphokinase (CPK) are elevated in vitamin E-deficient calves (Cipriano et al., 1982; Reddy et al., 1987b) and yearling cattle (Allen et al., 1975). Osmotic fragility of erythrocytes has been reported to increase in some instances of vitamin E deficiency (McDowell, 2000). Nutritional muscular dystrophy of ruminants occurs worldwide, but its incidence or at least diagnosis, particularly in a mild or subclinical form, varies widely between and even within countries (McDowell et al., 1985). The incidence of WMD in certain world regions is sporadic, with less than 1% of livestock herds affected. In other areas, such as Turkey and New Zealand, a 20% to 30% incidence of WMD may occur regularly. Considerable research has revealed an inverse relationship between the selenium content of soil and the geographic occurrence of vitamin E-selenium responsive muscular dystrophy. Similarly, signs of vitamin E deficiency have been observed in zoo animals fed diets devoid of their natural forage or browse. White muscle disease occurs with two clinical patterns. The first is a congenital type of muscular dystrophy in which calves, lambs or kids may be born dead or die within a few days of birth, following sudden physical exertion such as nursing or running. The second clinical pattern ("delayed white muscle disease") develops after birth; it is observed most frequently in lambs within three to six weeks of birth but may occur as late as four months after birth. The condition in calves is generally manifested at one to four months of age. A vitamin E-responsive white muscle disease has been observed in four- to six-month-old lambs that are kept on dry, poor quality, late-season pasture. Godwin (1975) reported that the electrocardiogram of WMD-affected animals shows progressive development of a characteristic abnormality accompanied by a fall in blood pressure. Therefore, a fundamental change occurring in vitamin E-selenium deficiency is circulatory failure, linked to cardiac muscle degeneration and possibly also to loss of blood vessel integrity. Muscle damage resulting from vitamin E and (or) selenium deficiencies causes

leakage of cell contents into the bloodstream. Thus, elevated levels of selected enzymes, above normal ranges, serve as diagnostic aids in detecting tissue degeneration. Serum enzyme concentrations used to monitor the incidence of nutritional muscular dystrophy include SGOT, LDH, CPK, aspartate amino transferase (AST) and malic dehydrogenase (MDH). These enzymes may also be elevated by liver damage. An elevation of enzyme activity in serum usually precedes any gross pathological changes or clinical signs (Tollersrud, 1973). In addition to elevation of selected enzymes, serum and tissue concentrations of vitamin E and selenium decrease as a result of deficiencies and may be used to monitor the nutritional status of livestock at high risk of developing WMD (McDowell, 1992, 2000).

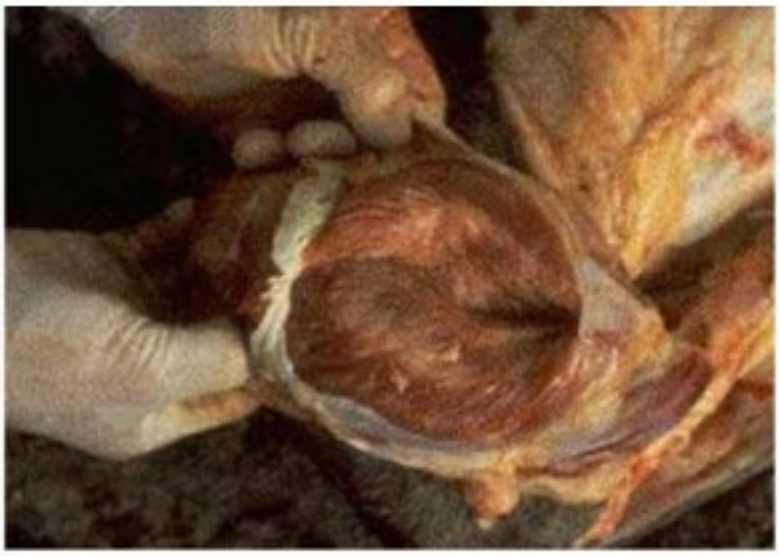
Vitamin E Deficiency in Cattle

Typically, WMD in calves is characterized by generalized leg weakness, stiffness of gait and myodegeneration (Illus. 4-2). Affected animals have difficulty standing, exhibit crossover walking and have impaired suckling reflex and ability (Muth, 1955). In calves, the tongue musculature may be affected, explaining the impaired suckling response (NRC, 2000). Calves may display a repeated extension and curling of the tongue (Morrill and Reddy, 1987). Death often occurs suddenly during exertion, from heart failure as a result of severe damage to heart muscle. Calves with WMD have chalky white striations, degeneration, and necrosis in the skeletal muscles and heart (Illus. 4-3). In milder cases with calves, where the chief clinical signs are stiffness and difficulty standing, dramatic, rapid recovery can be achieved with vitamin E-selenium injection followed by dietary fortification with vitamin E and selenium.

Illustration 4-2: Vitamin E- Selenium Deficiency in Cattle, White Muscle Disease



Calf (A) about three months old; lameness and generalized weakness of muscles can be seen. B and C: Abnormal white areas in heart muscles.



Necrosis of gastrocnemius muscle, evidenced by chalky white streaks in belly of the muscle.

Acute, chronic and peracute forms of the disease can be distinguished in older calves, usually during the latter growth or early finishing period. Sudden stressors such as transport, regrouping, disease exposure, multiple vaccinations, severe weather or abrupt changes in feed composition are generally considered precipitating factors. Sudden death without previous clinical signs of WMD is the main feature of the peracute condition. The cause is usually found by necropsy as advanced degeneration of the myocardium, with possible lesions of skeletal muscle. In acute cases, motor disturbances, such as an unsteady gait or stiff-calf disease, stiffened muscles in the lumbar region, neck, and forelimb muscles, muscle tremors, perspiration and sudden collapse known as "buckling," are encountered. Chronic marginal vitamin E deficiency is characterized by reduced disease resistance, reduced feed-to-gain ratio and generally poor performance, especially under stress conditions. Feeder calves also display the vitamin E deficiency symptom known as buckling, in which a stress, such as unloading at the feedlot or passage through the processing chute, triggers weakness of rear legs, buckling of fetlocks and, frequently, shaking or quivering of muscles (Illus. 4-4). In many cases, calves become progressively worse until they are unable to rise and may appear to be paralyzed. Frequently, affected calves will be down or continue to buckle for extended periods, and death loss is high in severe cases. Calve breeds with excitable temperaments appear to be most affected. Postmortem examination reveals pale, chalky streaks in muscles of the hamstring and back, often with damage to the heart, rib (intercostal) muscles and diaphragm (McDowell, 1985).

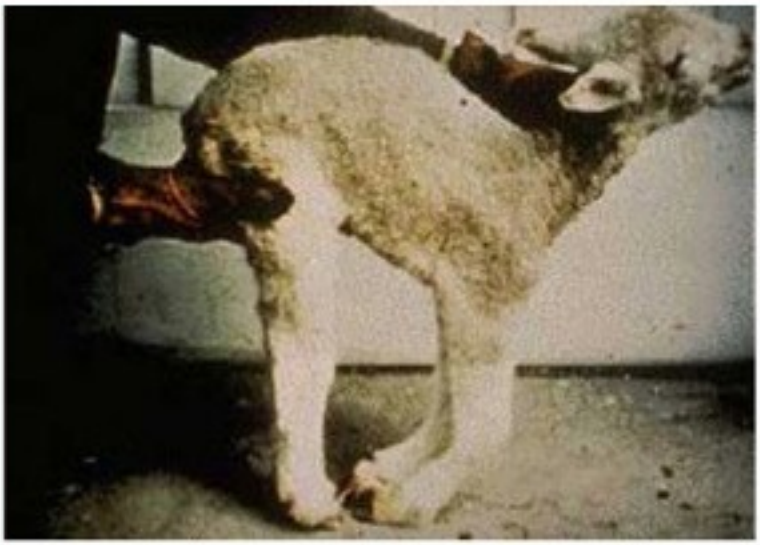
Illustration 4-4: Vitamin E-Selenium Deficiency in Cattle, White Muscle Disease

Flexion of hock and fetlock joints. Flexion is due to decreased support of gastrocnemius muscle severely affected by myodegeneration.

Calves with experimentally induced vitamin E deficiencies have exhibited clinical signs of nutritional muscular dystrophy similar to those observed in calves under field conditions (Safford et al., 1954). The same symptoms also appeared in calves fed milk containing high levels of polyunsaturated oils, but not in calves fed milk containing high levels of hydrogenated (saturated) oils (Adams et al., 1959). WMD can be easily induced in preruminant calves by feeding polyunsaturated oils. Originally, it was thought that ruminating calves would be protected from the vitamin E-depleting effect of PUFA because of the apparent near 100% hydrogenation of all unsaturated fatty acids by the rumen microflora (Noble et al., 1974). However, more recent research indicates that unsaturated fatty acids in grasses (e.g., linolenic acid) can produce oxidative damage and nutritional muscular dystrophy (NMD) in ruminating calves (McMurray and Rice, 1984). Nutritional muscular dystrophy (degenerative myopathy) in older calves occurs most frequently at turnout to spring pasture (Anderson et al., 1976). McMurray et al. (1980) showed that polyunsaturated fatty acids escape ruminal hydrogenation, resulting in a threefold increase of plasma linolenic acid within three days after turnout. Rice et al. (1981) showed that linolenic acid, if protected from ruminal hydrogenation, rapidly reaches high levels in blood and is associated with a rise in plasma creatine phosphokinase, indicating degenerative myopathy (muscle damage). Likewise, Walsh et al. (1993a) reported that ruminating calves fed diets deficient in either vitamin E or both vitamin E and selenium had increased lipid peroxidation products in muscle tissue. Feeding rumen-protected linseed oil to vitamin E-selenium deficient calves further increased the level of lipid peroxidation in muscle. Although most cases of WMD involve younger animals, degenerative myopathy has been reported in adult cattle (Van Vleet et al., 1977; Gitter and Bradley, 1978; Hutchinson et al., 1982). Yearling Chianina heifers exhibited abortion, stillbirth and periparturient recumbency (downer cow syndrome) (Hutchinson et al., 1982). Necropsy and tissue analysis revealed myodegeneration and a combined deficiency of vitamin E and selenium. Rapid growth in these heifers, coupled with the stresses of late pregnancy and parturition, may have contributed to this form of vitamin E deficiency. Marginal selenium status would be a predisposing factor. A myopathic condition affecting yearling cattle was reported by Barton and Allen (1973) and was associated with animals fed grains treated with propionic acid, which is known to destroy vitamin E. Depletion/repletion studies indicate that feedlot cattle require 50 to 100 IU per day supplemental vitamin E (Hutcheson and Cole, 1985). The NRC (2000) states that receiving and starting feedlot cattle should be supplemented with 400 to 500 IU of vitamin E per day to optimize performance and health. The common factors in development of vitamin E-related WMD appear to be marginal vitamin E and selenium status; sudden change of diet to one high in polyunsaturated fatty

acids; and sudden stress of the animal. These factors in combination can precipitate vitamin E responsive myopathy. Vitamin E should be supplemented at levels that ensure protection against marginal or outright deficiency.B. Vitamin E Deficiency in Sheep and Goats In lambs, white muscle disease (WMD, also known as stiff-lamb disease) takes a course similar to that observed in calves. Motor disturbances such as unsteady gait (Illus. 4-5), stiffness of the muscles of the hindquarters, neck and forelimbs, arched back, muscle tremors and perspiration are encountered in the acute form. On necropsy, white striations in cardiac muscle and bilateral lesions in skeletal muscles characterize the disease. A gradual but progressive swelling of the muscles, particularly in the lumbar region and rear legs, gives the erroneous impression of muscular development. Like the peracute deficiency encountered in calves (changes occur primarily in the myocardium), chronic cardiac muscle degeneration also occurs in the lamb. Affected lambs appear normal at birth, but quickly lose weight after the third week of life. They also show aversion to social stress and may stand apart from the flock. Cardiac arrhythmia and increased heart rate can result even after slight exercise. In the advanced stage, animals consume little if any feed and rapid wasting occurs. Symptoms can be reversed by prompt administration of vitamin E and selenium.

Illustration 4-5: Vitamin E- Selenium Deficiency in Sheep, White Muscle Disease



White Muscle disease (WMD), also known as stiff lamb disease. Lamb unable to stand as a result of tissue degeneration.

Courtesy of O.H. Muth, Oregon State University

For dystrophic lambs, an oral therapeutic dose of 500 IU dl-alpha-tocopheryl acetate followed by 100 IU on alternate days until recovery is successful (Rumsey, 1975). Vitamin E-selenium responsive conditions are not restricted to young animals and are manifested as lack of thrift, occurring in lambs at pasture (Underwood, 1981). Marginal vitamin E deficiency of yearling sheep can progress to WMD. In sheep of nine to 12 months of age, the disease is frequently observed following driving of the flock with the rapid onset of listlessness, muscle stiffness, inability to stand, prostration and, in severe acute cases, death within 24 hours (Andrews et al., 1968). Hartley and Grant (1961) reported the incidence of WMD in barren ewes was reduced from over 30% to 5% with selenium administration. Farms in New Zealand have had lamb losses as high as 40% to 50%. In these regions, the syndrome may respond to vitamin E, selenium or both. Maas et al. (1984) described nutritional myodegeneration in lambs and yearling ewes with normal selenium status but deficiency of vitamin E.Deficiency of vitamin E and (or) selenium in the goat, as in other ruminants, results mainly in WMD. Goat kids are born with little or no reserves of the fat-soluble vitamins A, D, and E. Sudden death of young kids under two weeks of age may reveal postmortem evidence of muscle disease and degeneration in the heart muscle or the diaphragm. In older kids and mature animals, deficiency can occur after sudden exertion and stress. Affected animals exhibit bilateral

stiffness, usually in the hind legs. In high-producing dairy goats, deficiency manifests itself in poor involution of the uterus, accompanied by retained placenta and metritis following kidding (Guss, 1977). Goat kids four to five weeks of age that were diagnosed with nutritional muscular dystrophy (myodegeneration, NMD) had lower concentrations of both vitamin E and selenium in the liver, skeletal muscle and myocardium (Rammell et al., 1989). Vitamin E concentrations in the liver, skeletal muscle and myocardium in NMD cases averaged 40%, 43% and 30% of those in healthy goat kids, clearly indicating vitamin E depletion.

Fortification Considerations

A. General Considerations

The two primary reasons for fortification of ruminant diets with supplemental vitamin E are to prevent marginal deficiency and to optimize animal performance. The levels required to optimize performance are, in most cases, considerably higher than those needed to prevent deficiency symptoms. Vitamin E and other vitamin requirements established decades ago have changed little in recent NRC publications and do not reflect greatly improved genetic selection and changes in management procedures of modern ruminant operations. Vitamin supplementation allowances, including vitamin E, need to be set at levels that reflect different management systems and are high enough to take care of fluctuations in environmental temperatures, energy content of feed and influencing factors (e.g., infectious diseases, stress, parasites, biological variations, diet composition, bioavailability, nutrient interrelationships, etc.) that might influence feed composition or vitamin requirements (McDowell and Ward, 2008). When the value obtained through feeding higher levels of vitamin E outweighs the cost, the higher level of supplementation becomes a sound nutritional management practice. Factors of primary importance in determining vitamin E supplementation include:

- vitamin E levels in the basal diet;
- selenium levels in soils and feedstuffs;
- selenium availability to the animal, which is reduced by high sulfate or calcium levels in feed or water;
- presence of compounds in the diet that increase selenium and vitamin E requirements, (i.e., heavy metals, unsaturated fats, nitrates, mycotoxins);
- excessively dry or poor quality ranges or pastures for grazing livestock;
- harvesting, drying or storage conditions of forages that destroy vitamin E;
- accelerated rates of gain or milk production;

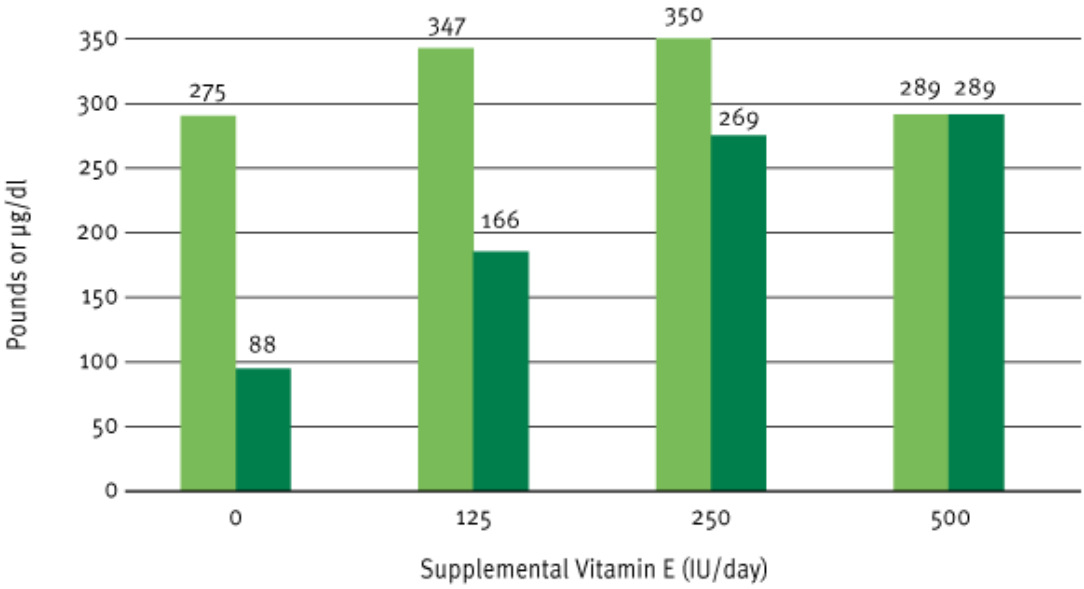
- intensified, confinement production systems that increase overall stress load; and
- disease exposure (McDowell and Williams, 1991; McDowell, 1992, 2000).

To protect against loss of vitamin E activity during processing and storage, feeds should be fortified using alpha-tocopheryl acetate, the most stable source of vitamin E activity available for feed use. Methods of providing supplemental vitamin E are: (a) in dry feeds, mineral supplements or liquid supplements; (b) in drinking water; and (c) parentally as intramuscular injection.
Dispersible liquid concentrates of vitamin E are available for liquid feeds and water supplementation. Parental vitamin E is usually given as an intramuscular injection in an oil base for highest absorption (Machlin, 1991). Combination products containing vitamin E and selenium are often given intramuscularly to animals exhibiting clinical signs of muscular degeneration, or prophylactically, just prior to or just after stress, as in dairy cows prior to calving or receiving cattle upon arrival at the feedlot.

B. Dairy Calves

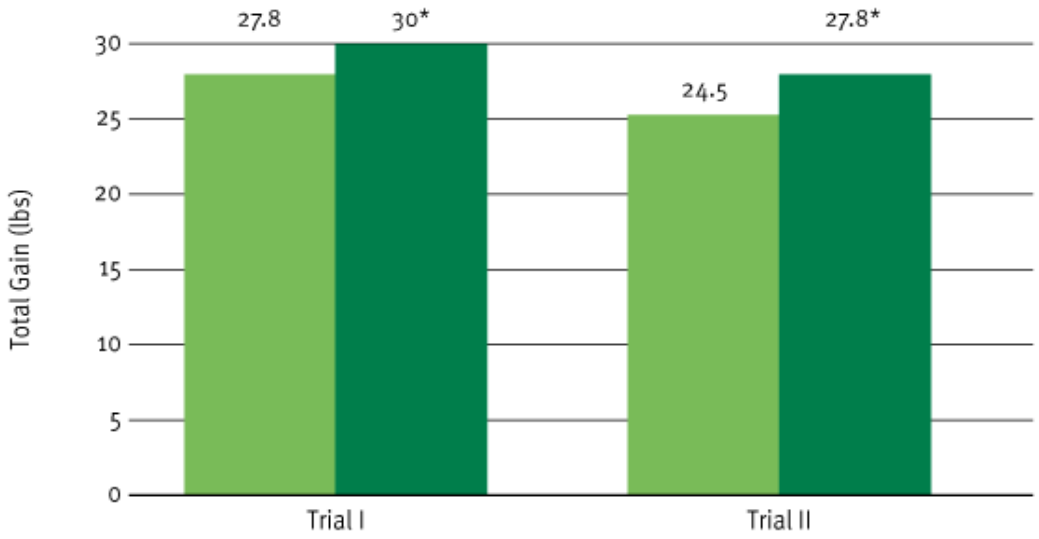
Based on a series of studies, Reddy et al., (1987a) concluded that the addition of 125 to 250 IU of vitamin E per head per day to conventional dairy calf diets optimized calf performance through 24 weeks of age (Fig. 4-6). The same authors recommended that cattle from six to 12 months of age receive 200 to 400 IU vitamin E daily, depending on previous dietary history in relation to vitamin E and selenium. Luhman et al. (1993) conducted two experiments with calves from birth to four weeks of age, and fed either 20 or 100 IU vitamin E per day in milk replacer. Calves fed 100 IU vitamin E per day gained 8% to 13% more weight and had fewer scour days than calves supplemented with 20 IU per day vitamin E (Fig. 4-7). Another study from the same research group (Johnson et al., 1997) compared feeding either 100 or 200 IU vitamin E daily in milk replacer to Holstein bull calves from 0 to 28 days of age. Although weight gain and feed efficiency were similar between the two groups, calves fed 200 IU vitamin E had numerically fewer scour days (2.9 versus 3.8) and lower medication costs ($4.46 versus $5.29) than calves fed 100 IU vitamin E daily (Fig. 4-8). Based on these results, the optimum level of supplemental vitamin E in calf diets appears to be 100 to 200 IU per day. Both milk replacers and calf starter feeds should be fortified to provide these levels of vitamin E in order to ensure optimum vitamin E intakes from early life through weaning and into the early phases of postweaning growth.

Figure 4-6: Effect of Supplemental Vitamin E on Growth and Serum Vitamin E in Dairy Calves from 0-24 Weeks of Age



Adapted from Reddy et al., (1987)

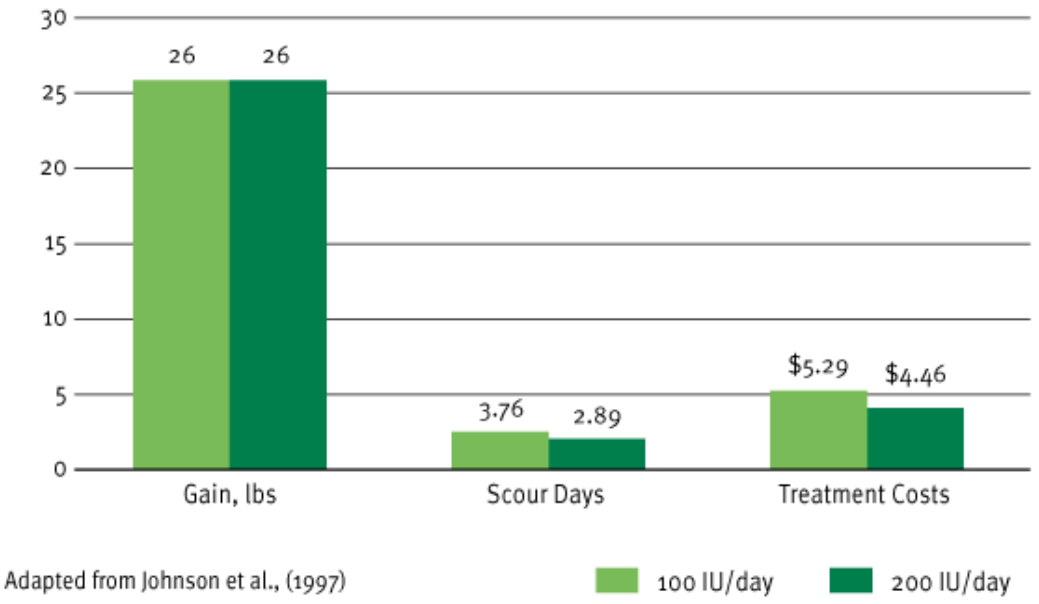
Figure 4-7: Effect of Vitamin E Level in Milk Replacer on Calf Perfomance



*P<0.05

Adapted from Luhman et al., (1993)

Figure 4-8: Effect of Vitamin E Level in Milk Replacer on Calf Performance



Adapted from Johnson et al., (1997)

Cellular effects of vitamin E supplementation in calves include: 1) maintenance of cell membrane stability, as evidenced by lower activity of certain cytoplasmic enzymes in serum (Hidiroglou et al., 1973; Cipriano et al., 1982; Lynch, 1983; Reddy et al., 1985a); 2) decreased serum cortisol concentrations (Reddy et al., 1987b); and 3) potentiation of the immune system (Cipriano et al., 1982; Reddy et al., 1986; Hidiroglou et al., 1992; Eicher-Pruiett et al., 1992; Eicher et al., 1994). A University of Florida study (Velásquez-Pereira et al., 1999) tested the effects of supplemental vitamin E on young dairy calves fed gossypol. Calves were started at either two or four weeks of age and fed starters containing either soybean meal or cottonseed meal for one to two months. All calf starters contained 30 IU per kg (13.6 IU/lb) supplemental vitamin E. Calves fed cottonseed meal received an additional 0, 2,000 or 4,000 IU per day vitamin E either through milk replacer (calves started at two weeks of age) or calf starter. Supplemental vitamin E reversed several indices of gossypol toxicity and increased overall growth performance compared to unsupplemented calves. Calf mortality was 0 percent for the soybean meal treatment and 20.7, 9.1 and 8.7 percent for the cottonseed meal treatment groups with 0, 2,000 or 4,000 IU vitamin E per day, respectively.

C. Dairy Heifers

There is little data on vitamin E supplementation of dairy heifers beyond the

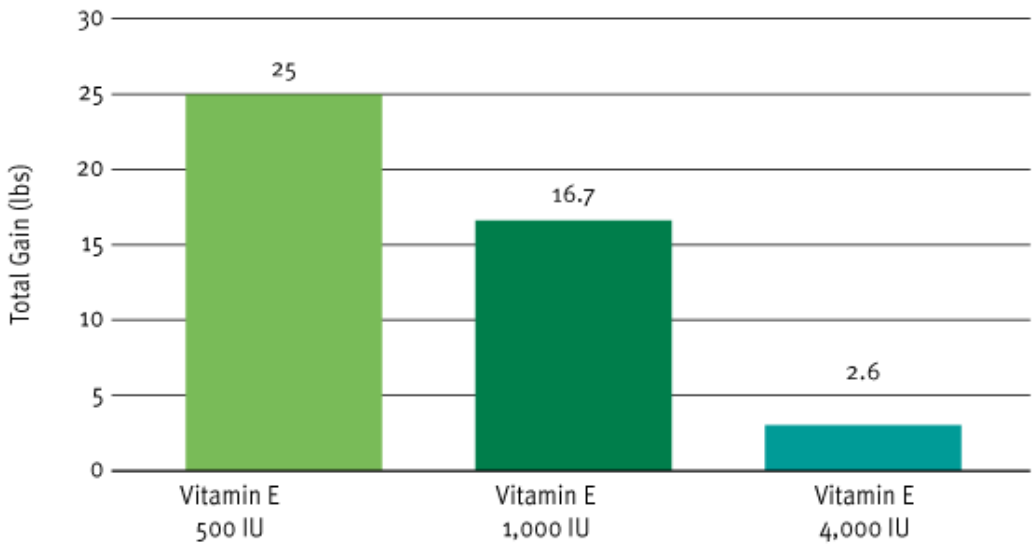
young-calf stage of development. Reddyet al. (1987a) suggested that dairy cattle six to 12 months of age be supplied with 200 to 400 IU vitamin E per head per day. Cattle of this age range are often kept on pasture during part of the year. Good quality pasture contains ample vitamin E, but pasture quality often declines markedly during summer and fall, during which time supplementation may be needed. Ohio State research (Hogan et al., 1992) showed that increased vitamin E supplementation prepartum reduced the incidence of mastitis in dairy heifers. Data from beef cattle, discussed later in this section, indicate that feeding 400 to 500 IU per day vitamin E results in improved overall performance during stress periods, such as handling and shipping. Supplemental vitamin E has been shown to increase pregnancy rate in beef heifers (LaFlamme and Hidiroglou, 1991). Based on the available data, vitamin E supplementation (1,000 IU per day) is warranted for dairy heifers during the last four to six weeks prior to first calving, and may be beneficial at 400 to 500 IU per day during periods of high stress such as shipping, regrouping or mass vaccination.

D. Dairy Cows

Beneficial effects of supplemental vitamin E, in conjunction with adequate dietary selenium, for dairy cows include: 1) reduced incidence of reproductive disorders (Harrison et al., 1984; Miller et al., 1997); 2) reduced clinical mastitis and somatic cell count (Smith et al., 1997; Weiss, 1998; Politis et al., 2004) 3) protection of milk from oxidation (King, 1968; Charmley and Nicholson, 1994; Focant et al., 1998; Al-Mabruk et al., 2004; Politis et al., 2004; Löhrke et al., 2005; Wicheanson et al., 2007; Bouwstra et al., 2008; Gobert et al., 2009). These findings support the concept that optimal vitamin E supplementation for a dairy cow is 1,000 IU per day during the dry period and 500 IU per day during lactation for optimum reproduction and udder health.Feeding supplemental vitamin E at levels of 1,000 to 2,000 mg of naturally occurring mixed tocopherols per cow per day increased the vitamin E content of milk and its stability against oxidized flavor (Krukovsky and Loosli, 1952; Neilsen et al., 1953). The vitamin E content of milk from cows fed stored feeds was lower than that of milk from cows on pasture and their milk was more susceptible to development of oxidized flavor (Krukovsky et al., 1950). The increased oxidative deterioration of milk produced from cows fed red clover silage was avoided by vitamin E supplementation (Al-Mabruk et al., 2004). Feeding supplemental vitamin E as dl-alpha-tocopheryl acetate, providing an equivalent of 500 IU per cow per day, increased the vitamin E content and oxidative stability of milk (Dunkley et al., 1967). Nicholson et al. (1991) provided evidence that adequate selenium status improves the transfer of dietary tocopherol to milk. Higher levels of vitamin E (9,000 IU per day) are required to prevent milk oxidation and off-flavor caused by unsaturated

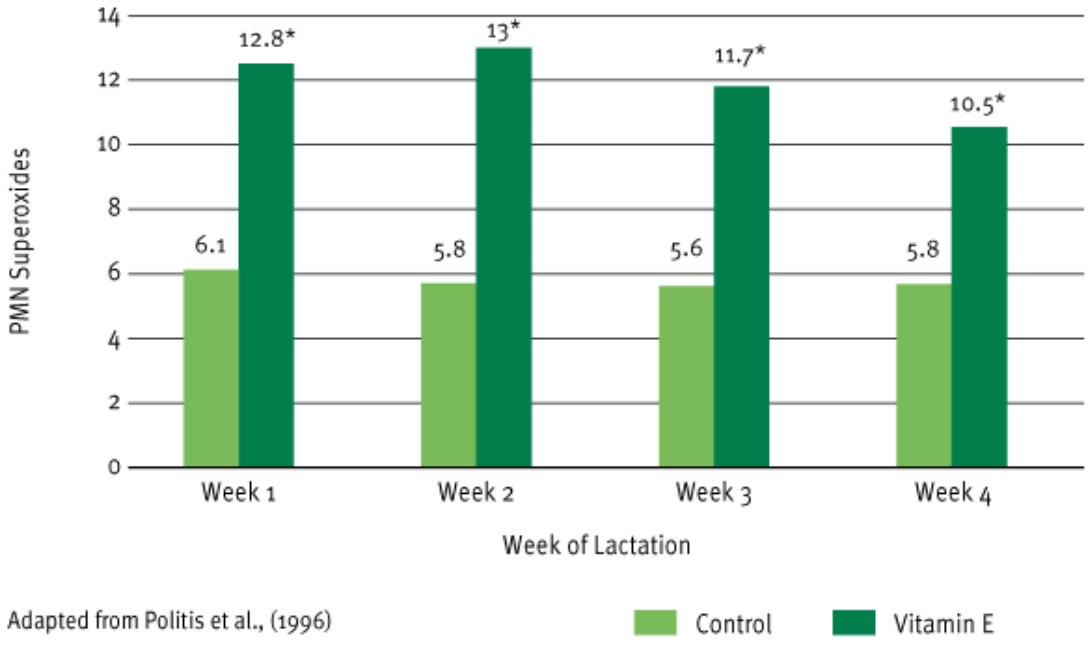
lipids in the diet or other factors, such as high dietary copper (Charmley and Nicholson, 1994; Focant et al., 1998). More recent studies have shown that vitamin E levels higher than 1,000 IU per day may be optimal during the late dry period and early lactation, the time when the highest rate of new intramammary infections occur. Feeding 4,000 IU of vitamin E for 14 days prepartum, followed by 2,000 IU per day for 30 days postpartum, resulted in a significant reduction in new mammary infections and clinical mastitis compared to levels of 1,000 IU and 500 IU per day vitamin E during the same periods (Weiss et al., 1997) (Fig. 4-9). Both groups were fed 1,000 IU vitamin E per day for the first 46 days of the dry period. Dietary selenium concentration was 0.15 ppm (dry matter), and plasma selenium concentrations (>50 ng/ml) were within the adequate range. Politis et al. (1995,1996) provided evidence that feeding 3,000 IU per day of supplemental vitamin E, in diets containing 0.3 ppm selenium, for 28 days prior to calving and continuing for 56 days after calving significantly improved neutrophil and macrophage function and reduced somatic cell count in dairy cows (Fig. 4-10, 4-11).

Figure 4-9: Effect of Vitamin E Supplementation on Clinical Mastitis of Dairy Cows and Heifers During Peripartum Period



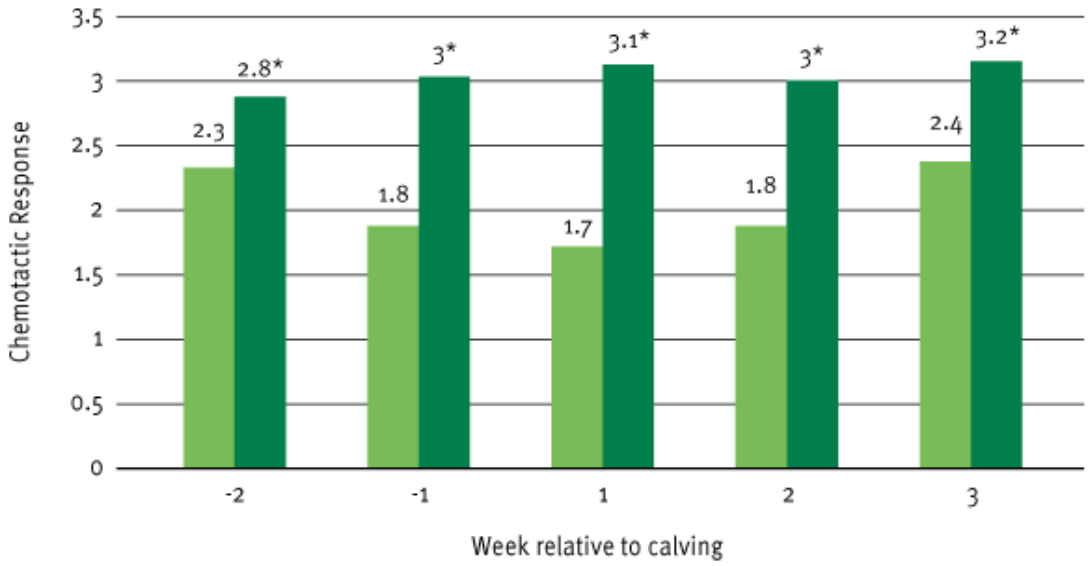
Adapted from Weiss et al., (1997)

Figure 4-10: Effects of Vitamin E on Neutrophil Superoxide Production



Adapted from Politis et al., (1996)

Figure 4-11: Effect of Vitamin E on Blood Neutrophil Chemotaxis



*P<0.05

Adapted from Politis et al., (1995)

Treated cows also received a single intramuscular injection of 5,000 IU vitamin E seven days prior to expected calving date. Plasma selenium levels

were 86 to 90 ng/ml. The higher level of vitamin E supplementation maintained plasma vitamin E levels above the 3.0 µg/ml threshold identified by Weiss et al. (1997) as minimal for optimum udder health in dairy cows. The authors concluded that supplementing the higher level of vitamin E (3,000 IU per day for 84 days) prevented suppression of white blood cell function during the parturient period (Politis et al., 1995). Based on this data, it has been suggested that 2,000 IU vitamin E per day may be optimal during the late dry period and early lactation (Goff and Horst, 1998). Assessing vitamin E status of dairy cows has been discussed by Weiss (1998). Plasma levels of 3.0 to 3.5 µg/ml are suggested as minimal for optimal neutrophil function and reduction of clinical mastitis. A study of 50 U.S. dairy herds (Miller et al., 1995) reported a mean plasma vitamin E value of 2.55 µg/ml, with significantly higher levels in summer and fall (June through November) than in winter and spring (December through May), which reflects forage quality in the months preceding each period. An epidemiological study of Holstein cows (Barnouin and Chassagne, 1998) identified vitamin A, D and E supplementation during the dry period as the most discriminating factor associated with a reduced incidence of clinical mastitis. Politis et al., (1995) reported that 3,000 IU of vitamin E per day were required to maintain plasma vitamin E above 3.0 µg/ml at parturition. Given these findings, and those discussed earlier, levels of vitamin E greater than 1,000 IU per day may be economically justified during the last 28 days of the dry period and the first four to eight weeks of lactation.

E. Breeding Bulls

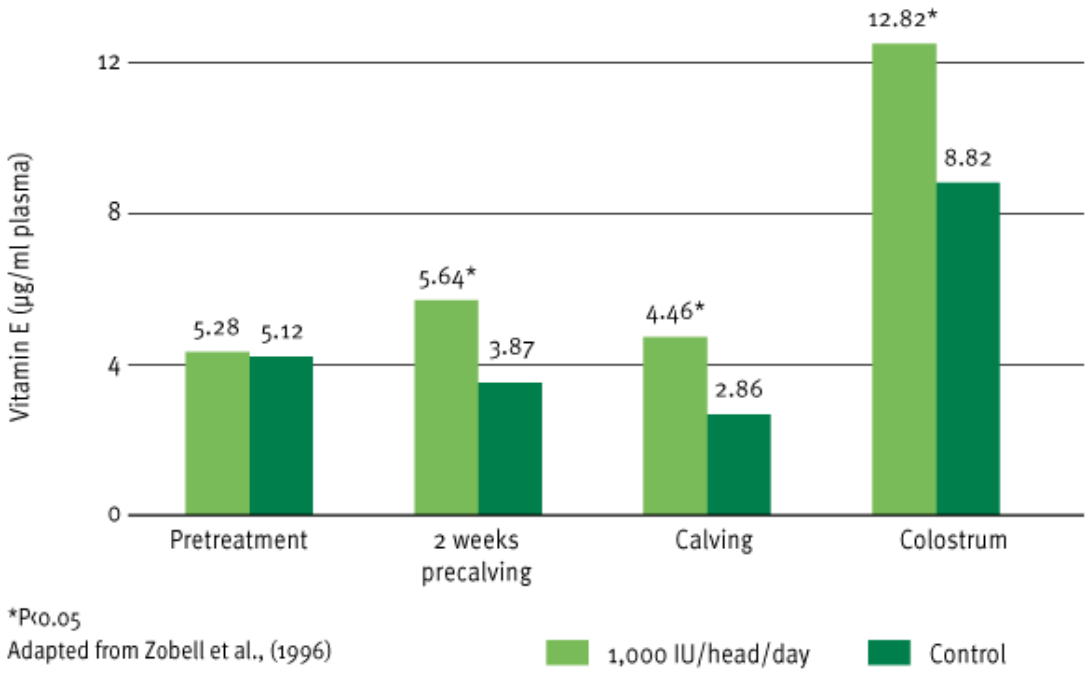
Effects of supplemental vitamin E above minimum requirements on breeding performance of dairy or beef bulls are largely unknown. A recent, long-term study of gossypol toxicity in dairy bulls (Velásquez-Pereira et al., 1998a) found that feeding bulls 4,000 IU per day of supplemental vitamin E reversed the negative effects of gossypol on several reproductive parameters, compared to bulls fed gossypol plus approximately 240 IU per day. Compared to the soybean meal-based control diet (average vitamin E intake of 240 IU per day), bulls fed gossypol plus 4,000 IU of vitamin E per day had numerically higher sperm production, earlier age at puberty and higher plasma testosterone, suggesting that vitamin E supplementation may exert positive effects on male reproduction in cattle. However, this has not yet been rigorously tested experimentally.

F. Beef Cows and Calves

Recent studies have examined the benefits of feeding supplemental vitamin E to beef brood cows during the last trimester of pregnancy. Zobell et al.

(1995) conducted a trial using 134 crossbred beef cows during the last 60 to 100 days of gestation. Treatment groups were balanced by age, weight, breed, sire and body condition. The control group was fed 80 IU per day vitamin E and the treatment group 1,000 IU per day supplemental vitamin E. Feeding 1,000 IU per day vitamin E significantly increased vitamin E concentrations in the plasma of both cows and calves, increased vitamin E in colostrum, numerically increased the plasma immunoglobulin G (IgG) concentration of calves and significantly reduced the incidence of calf scours (Fig. 4-12, 4-13). In this study, spring-calving beef cows fed 1,000 IU of vitamin E per day conveyed improved vitamin E status and health status to their calves compared to cows fed 80 IU of supplemental vitamin E daily.

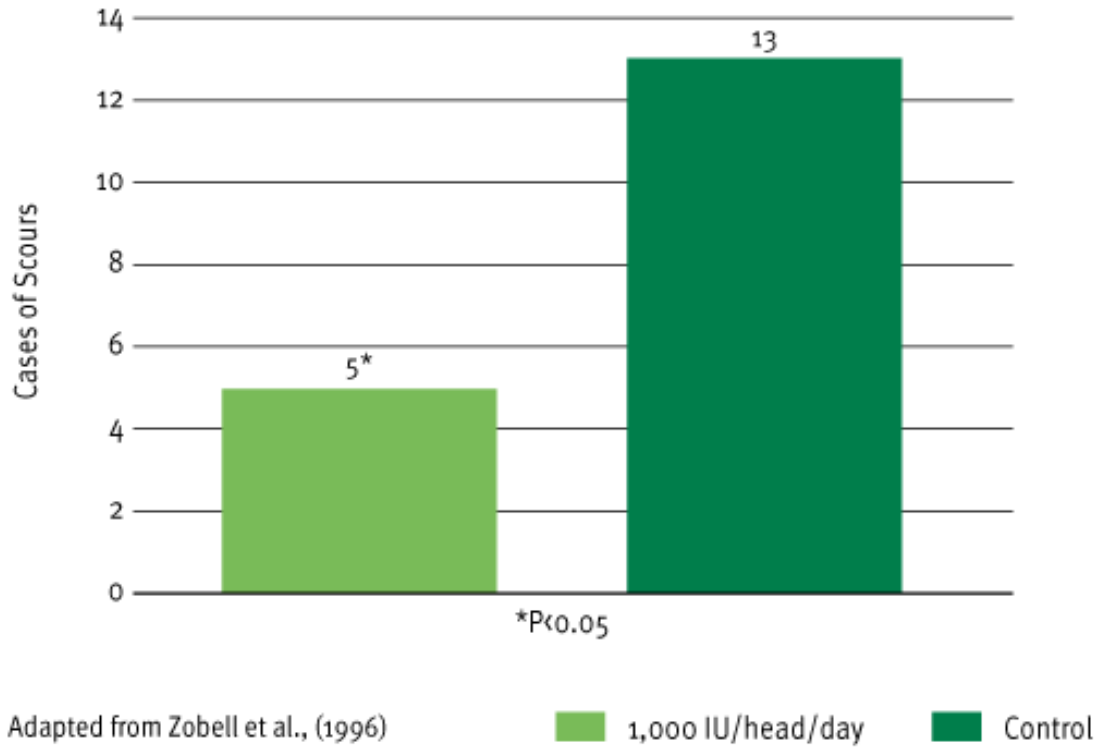
Figure 4-12: Effect of Supplemental Vitamin E on Plasma Concentration in Beef Cows



*P<0.05

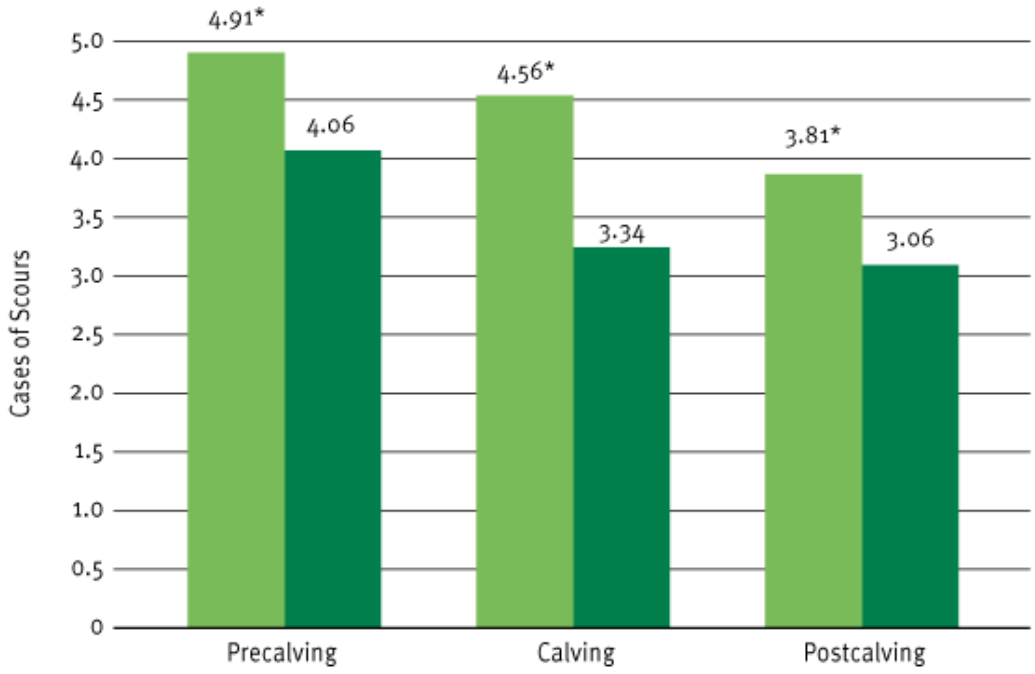
Adapted from Zobell et al., (1996)

Figure 4-13: Vitamin E Supplementation and Scours Incidence in Beef Calves



Bass et al. (1996) conducted two experiments using 90 beef cows calving in late winter and 42 cows calving in early fall. Cows were allotted to treatment groups by breed, age and initial serum vitamin E concentration. All cows were fed a free-choice complete vitamin-mineral mix, with the treatment group receiving either 1,000 IU per day (winter-calving) or 600 IU per day (fall-calving) of supplemental vitamin E mixed with the free-choice supplement. Vitamin E was provided starting one month before the start of the 65-day calving season and continued until calving. In the winter-calving cows, feeding 1,000 IU per day of supplemental vitamin E significantly increased serum vitamin E levels in cows and calves, serum IgG levels of calves and average daily gain (ADG) of calves measured from birth to weaning (average of 204 days) (Fig. 4-14, 4-15, 4-16).

Figure 4-14: Vitamin E Supplementation and Vitamin E Status of Beef Cows

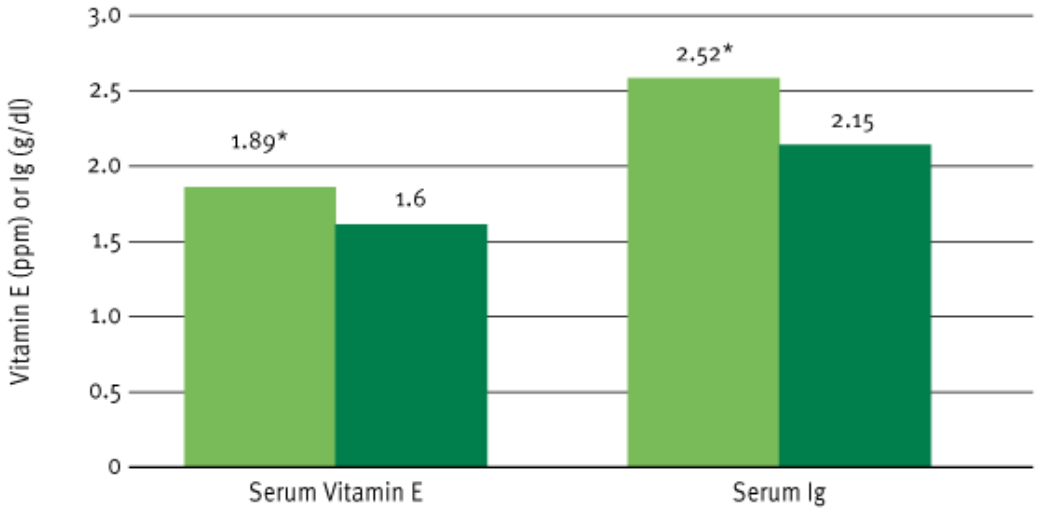


*P<0.05

Adapted from Bass et al., (1996)

1,000 IU/head/day Control

Figure 4-15: Vitamin E Supplementation of Dam and Serum Vitamin E Levels of Calves

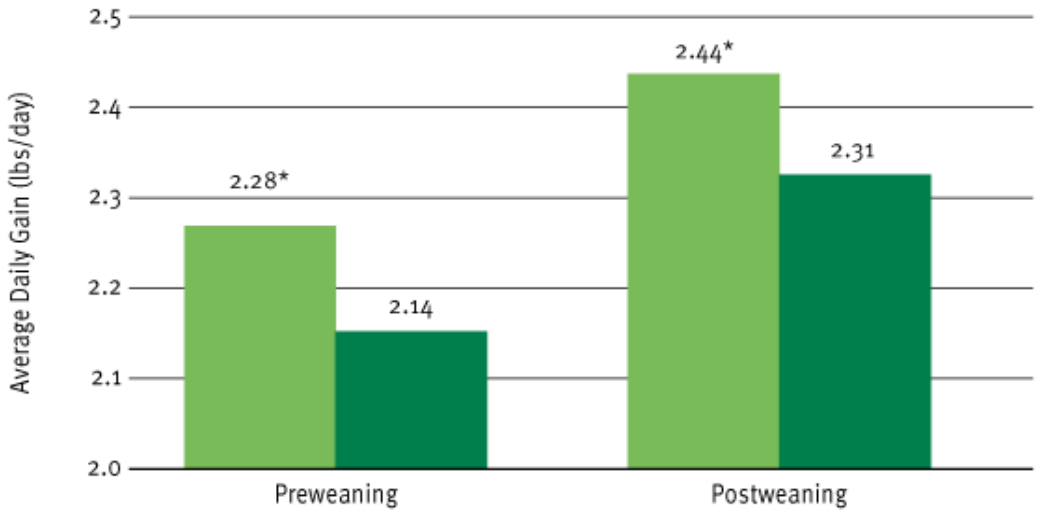


*P<0.05

Adapted from Bass et al., (1996)

1,000 IU/head/day Control

Figure 4-16: Effect of Vitamin E on Beef Calf Gain



*P<0.05

Adapted from Bass et al., (1996)

1,000 IU/head/day Control

This study indicates that cows calving in winter/spring benefited from 1,000 IU per day of supplemental vitamin E. Cows calving in early fall and supplemented with 600 IU per day of vitamin E exhibited similar levels of serum and colostrol vitamin E and similar average daily gains for calves. Fall-calving cows were expected to have higher vitamin E status due to recent grazing of pasture and as a result, were supplemented with less vitamin E. However, serum vitamin E levels were similar to those in the first study. Several factors may explain the smaller response to supplemental vitamin E in this group, including better tissue vitamin E status due to pasture grazing, the lower level of vitamin E supplementation or fewer cows per treatment. Overall, the results confirm those of Zobell et al. (1995) that winter- and spring-calving beef cows and their calves benefit from 1,000 IU per day of supplemental vitamin E during the last 60 to 90 days of gestation. The response of fall-calving cows to vitamin E supplementation will depend on the quality and quantity of pasture available during gestation. A study of pregnant beef heifers (Coalson et al., 1997) found no significant effect of feeding supplemental vitamin E (1,000 IU per day, fed for 50 days precalving) on calf immune status or breeding performance after calving. However, the vitamin E concentration in serum and colostrum were not affected by treatment, leaving open the question of actual intake of supplements by heifers in the study. Feeding beef calves 500 IU per day of supplemental vitamin E starting at 165 days of age tended to increase rate of gain of calves in one of two experiments (Wright et al., 1997). The

response of growing calves to vitamin E will likely depend on pasture and forage quality and exposure to stress. Based on available data, the optimal level of vitamin E supplementation of winter- and spring-calving beef cattle appears to be 1,000 IU per day. The rationale for supplementing fall-calving cows and growing beef calves is open to interpretation by the nutritionist, veterinarian and producer, in consideration of pasture and forage quality and overall stress level of the cattle.

G. Background and Receiving Cattle

An excellent compilation of research trials on the effects of supplemental vitamin E on receiving and feedlot cattle has been published by Secrist et al. (1997). A summary of five trials involving transport-stressed receiving cattle (average body weight of 550 lbs, or 250 kg) found that supplemental vitamin E at 400 to 1,400 IU daily tended to increase average daily gain (ADG) and improved feed efficiency when fed for the first 28 days after shipment. Morbidity was numerically less for the vitamin E supplemented animals.In a series of 28-day feedlot receiving trials, Lee et al. (1985) observed an improvement in early performance of newly arrived growing cattle supplemented with 450 IU of vitamin E (as dl-alpha-tocopheryl acetate) per head per day that were stressed by long distance shipment and changes from green forages to high grain feedlot diets. Gill et al. (1986) supplemented newly received feedlot cattle with 1,600 IU vitamin E (as dl-alpha-tocopheryl acetate) per head per day for the first 21 days and 800 IU vitamin E for the remaining seven days of a 28-day trial. Average daily gain and feed-to-gain ratios were improved by 23.2% and 28.6%, respectively, for vitamin E-supplemented stressed cattle (Table 4-7). The number of sick pen days per head was reduced by 15.6%, and morbidity was reduced by 13.4% with vitamin E supplementation.

Table 4-7: Effect of Vitamin E Supplementation on PerformanceMorbidity and Mortality of Stressed Cattle

Item	Control	Vitamin E[a]
Number of Head	252	250
Average Daily Gain (kg)	0.43[b]	0.53[c]
Feed Conversion	18.56	15.06
Number of Sick Days	3.2	2.7
Morbidity (%)	43.2	37.5
Mortality (%)	1.8	1.6

[a]1,600 IU vitamin E per head per day for first 21 days and 800 IU for the last seven days
[b,c]Means with different superscripts differ (P<0.01)
From Gill et al., (1986).

The NRC (2000) beef cattle publication recommends feeding 400 to 500 IU of vitamin E per day during the receiving and starting period. Based on available data, optimal vitamin E levels for receiving cattle appear to fall in the range of 500 to 1,400 IU per day, depending on stress load and previous nutritional history (Table 4-8). Cattle received from grazing good quality pasture will have higher vitamin E status.

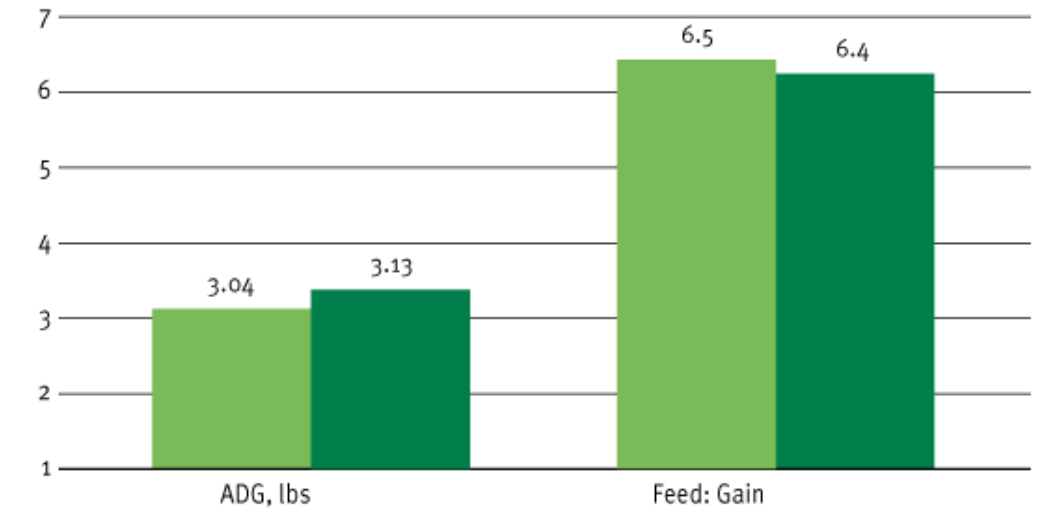
Table 4-8: DSM Guidelines for Optimum Vitamin Nutrition			
Ruminants[1]	**A(IU)**	**D3(IU)**	**E(mg)**
Calves/milk-replacer, 0-3 mo.	20,000-32,000	1,400-1,800	100-150[2]
Cattle, rearing and receiving	20,000-40,000	2,500-4,000	500-2000[3]
Cattle, growing and feedlot	50,000-70,000	6,000-9,000	200-300
Cattle, fattening and finishing	50,000-70,000	5,000-7,000	500-2,000[4]
Dairy cows, dry	75,000-100,000	25,000-35,000	1,000-3,000[5]
Dairy cows, lactating	100,000-150,000	30,000-50,000	5,000-1,000[6]
Bulls/nursing cows	50,000-120,000	5,000-10,000	300-500
Sheep/goats	5,000-10,000	400-600	100-200

[1]Added per animal per day
[2]For optimum immune function; additional 50 mg/day
[3]Upper level for receiving cattle in dry lot, first 28 days
[4]For improved color shelf life, feed for the last 50-100 days pre-marketing
[5]Upper level for -21 to +28 days postpartum6Upper level for optimum udder health.

H. Feedlot Performance

A summary and analysis of 21 controlled beef feedlot studies (Secrist et al., 1997) (Fig. 4-17) on the effects of feeding supplemental vitamin E revealed an overall significant improvement in ADG and feed efficiency in cattle supplemented with vitamin E (200 to 2,000 IU daily) during the finishing period (38–298 days). In most of the studies, cattle were on feed for 84 to 145 days and fed 85% to 90% concentrate. The authors concluded that supplementing 500 IU of vitamin E daily during the finishing period may be justified based on improved cattle performance alone, although these levels are also adequate to significantly improve the retail value of beef based on visual appearance (Faustman et al., 1998).

Figure 4-17: Feedlot Data Summary: Vitamin E



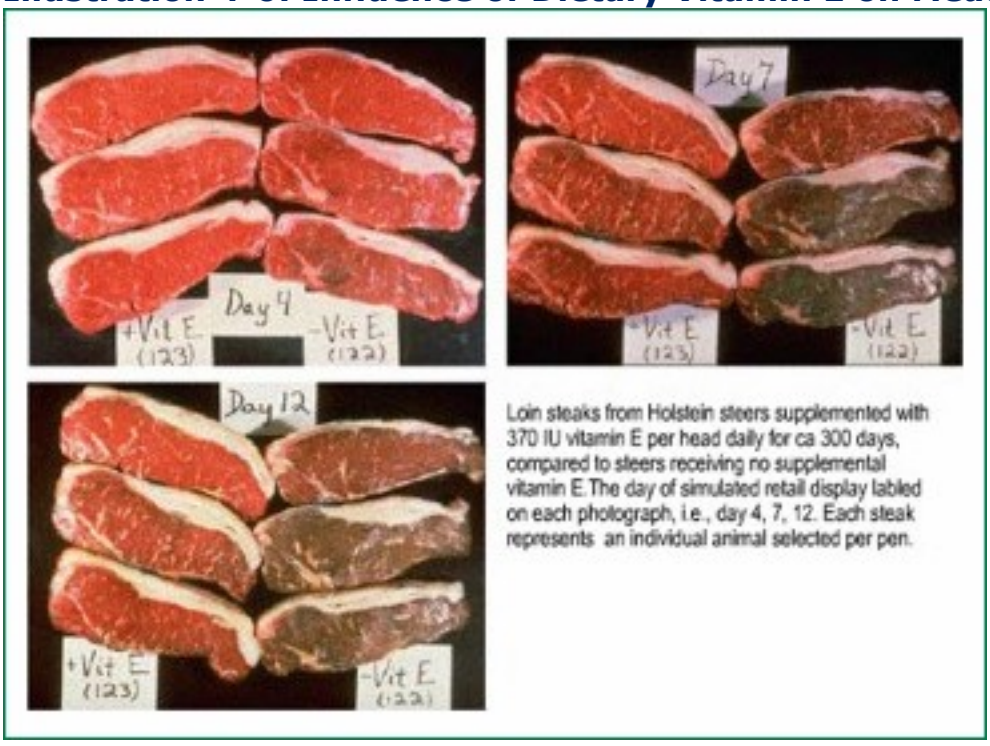
Adapted from Secrist et al., (1997)

I. Beef Meat Quality

Color is an extremely critical component of fresh red meat appearance and greatly influences the customer's perception of meat quality. Discoloration of meat results in retail price discounts and early discards from the meat case display (Liuet al., 1995; Smith et al., 1997). The cost of discounting or discarding meat prior to its normal expiration date is high. Many attempts have been made to control lipid oxidation in meats through the use of antioxidants. The most successful approach is through dietary supplementation of vitamin E, which functions as a lipid-soluble antioxidant in cell membranes (Linder, 1985), thus protecting phospholipids and even cholesterol against oxidation. Increased dietary levels of vitamin E result in higher tissue levels of alpha-tocopherol and greater oxidative stability of these tissues. (Löhrke et al., 2005; Bouwstra et al., 2008; Gobert et al., 2009). Faustman et al. (1998) observed that increasing tissue levels of alpha-tocopherol by supplementing finishing cattle with 500 IU of vitamin E daily stabilized and reduced oxidation of muscle myoglobin, thus preserving the cherry-red color of beef. Dramatic effects (Illus. 4-6) of vitamin E supplementation (500 IU per head daily) to finishing steers on the stability of beef color have been observed (Faustman et al., 1989a). Loin steaks of control steers discolored two to three days sooner than those supplemented with vitamin E. Supplemental dietary vitamin E extended the color shelf-life of loin steaks from 3.7 to 6.3 days. The alpha-tocopherol content of loin tissue of the supplemented animals was approximately four times greater

than controls (Faustman et al., 1989a). Vitamin E supplementation of finishing steers increased the stability of beef color in several other studies (Hill et al., 1989; 1992; Arnold et al; 1992; 1993; Zerby et al., 1999; Roeber et al., 2001; Yang et al., 2002; Formanek et al., 2003; Shin et al., 2007). Vitamin E also plays a role in controlling the color of veal calf meat. Combined feeding of monosodium phosphate and 100 IU of vitamin E per calf daily produced a light colored veal without producing anemia (Agboola et al., 1990). These results are consistent with the role of vitamin E as primary biological antioxidant of cellular membranes.

Illustration 4-6: Influence of Dietary Vitamin E on Meat Quality



Feeding 1,000 or 2,000 IU of supplemental vitamin E daily for the last 100 days of the finishing period was found to improve color retention of ground beef (Cabedo et al., 1998). Likewise Roeber et al., (2001) had improved color of ground beef by feeding 1,000 IU of vitamin E daily for the last 100 days of the finishing period. Crossbred (Angus x Hereford x Salers) cattle were brought from grass pasture to the feedlot for the 100-day finishing trial. Growth of bacterial populations on ground beef was not influenced by supplemental vitamin E. Sherbeck et al. (1995) reported that meat from steers fed 500 IU per day of vitamin E for 123 days prior to slaughter had significantly better color retention and less lipid oxidation than meat from unsupplemented cattle. Wulf et al. (1995) found improved appearance and extended caselife of meat harvested from lambs fed 500 IU of supplemental

vitamin E daily for a 56-day feeding period. Sanders et al. (1997) studied the effects of feeding 0, 1,000 or 2,000 IU of vitamin E daily for 100 days prior to slaughter in beef for the Japanese export market. Meat from cattle fed supplemental vitamin E during the finishing period displayed significantly superior lean color, less discoloration, less lipid oxidation and more desirable overall appearance. Steaks from vitamin E-fed cattle were preferred by 91% of 10,941 Japanese survey participants. This study clearly demonstrated the benefit of increased vitamin E supplementation to the export value of beef, as well as confirming the results of previous studies. A large project evaluated the effect of supplementing 500 IU of vitamin E daily to 235,000 feedlot cattle for the last 100 days of the finishing period on retail beef case-life in four retail grocery store chains (Westcott et al., 1997). Meat from vitamin E-supplemented cattle had a significant increase in display life and reduced discounts compared to meat from control cattle. The net economic retail value of beef harvested from cattle fed 500 to 1,000 IU of vitamin E daily for 100 days prior to slaughter is $20 to $60 (U.S.), with an average value of approximately $30 per carcass (Smith et al., 1997). The normal cost of vitamin E supplementation is $1.50 to $3.00 per head for 100 days of supplementation. This significant increase in retail value and consumer appeal of the final product, along with improved animal performance from feeding 500 IU per day or more vitamin E to finishing cattle (Secrist et al., 1997), provides a strong rationale for supplementation of vitamin E at 500 to 1000 IU per day in beef-cattle finishing rations. Vitamin E may have an additional effect on meat quality related to tenderness. Intramuscular collagen is responsible for the background toughness in cattle. Vitamins E and C may increase collagen turnover, but handling of cattle may reduce vitamin concentrations in muscles, impeding the removal of reactive oxygen species (ROS) and leading to oxidative stress. Vitamin E may reduce intramuscular collagen maturity, while vitamin C has been reported to improve meat texture in beef. Collagen turnover was increased by vitamins E and C, with a higher rate of turnover increasing meat tenderness (Aranda-Osorio et al., 2010; Archile et al., 2010). Providing vitamin E at pharmacological levels (i.e., >1,000 IU per animal per day) to stressed newly received feedlot cattle was beneficial for decreasing bovine respiratory disease (Duff and Galyean, 2007). Providing supplemental vitamin E (1,259 IU/calf/day) did not significantly influence performance or health status of newly arrived feedlot calves (Carter et al., 2005). However, there were higher serum alpha-tocopherol concentrations in calves and the trend toward decreasing medical treatment costs, and the numeric decrease in morbidity and treatments per calf support previous literature, and suggests that the positive effects of vitamin E on health status might be time-dependent.

J. Sheep and Goats

Lamb mortality was decreased by 50% when ewes were supplemented with 330 IU per day of vitamin E for the last 21 days prior to lambing (Thomas et al., 1995). Total lamb production per ewe was 2.3 kg (5 lb) higher for vitamin E-supplemented ewes. In a subsequent study (Kott et al., 1998), 1,302 ewes were used in a three-year study in which ewes were supplemented with either 0 or 330 IU per day of vitamin E during the last 21 days prior to lambing. Lamb mortality was reduced significantly, from 17% to 12%, in early-lambing ewes fed 330 IU of vitamin E daily. Effects of vitamin E were not significant for late-lambing ewes, probably due to their access to good quality pasture. Supplementing ewes with safflowerseed (226 g per ewe) without additional vitamin E compromised the ability of newborn lambs to adapt to cold environmental conditions (Dafoe et al., 2008). In two experiments, safflowerseed lambs had less body weight and survival at weaning. Providing 350 IU daily of vitamin E prevented the detrimental affects of safflower seeds. Williamson et al. (1995) reported that injecting ewes with 4,800 IU of vitamin E two weeks prior to lambing and again at lambing significantly improved lamb vigor and increased average daily gain of lambs through weaning. Danielset al. (1998) tested effects of oral vitamin E supplementation in 580 twin lambs. Lambs were administered either no vitamin E, a single oral dose of 391 IU vitamin E within six hours of birth, or two oral doses of 391 IU vitamin E at six hours after birth and between 10 and 18 hours after birth. Body weight of male lambs given two doses of vitamin E was greater than the control at 120 days. Death loss was lowest for male lambs given two oral doses of vitamin E.

Ewes were supplemented with 0, 15, 30 or 60 IU of vitamin E 28 days prepartum. This had no effect on lamb serum alpha-tocopherol at parturition, but colostrom concentrations for ewes increased linearly according to supplement received (Table 4-9). Dairy ewes given two subcutaneous injections of a vitamin E–selenium preparation (5 mg and 0.1 mg per kg body weight) during the dry period had significantly lower somatic cell counts and increased neutrophil activity compared to controls (Morgante et al., 1999). Incidence of clinical mastitis (4% versus 6%) and intramammary infection (9% versus 11.3%) were numerically, but not significantly lower for the vitamin E treatment.Gladinis et al. (2011) reported that sheep with mastitis had lower serum concentrations of vitamin E, selenium and vitamin A compared to healthy animals.

Table 4-9: Effects of Supplemental Vitamin E to Prepartum Ewes on a-Tocopherol Concentration in Serum and Colostrum (µg/ml)

Supplemental Vitamin E (IU per day)	Serum at Parturition	Colostrum Day 1	Serum Prior To Nursing	Serum Day 3
0	0.94	3.3	0.40	1.41
15	1.94	6.8	0.40	1.84
30	2.53	8.0	0.38	2.43
60	4.07	9.6	0.23	4.46

Source: Njeru et al. (1994).
Note: Treatments administered as dl-a-tocopheryl acetate 28 days prepartum through 28 days postpartum.
Linear (P<0.05) treatment effects for a-tocopherol in ewe serum and colostrum and lamb serum at day 3.

Available data indicate that gestating ewes fed stored forages or poor quality pasture should be supplemented with vitamin E at the rate of 330 IU per day in order to reduce lamb mortality and increase lamb weight at weaning. To date, intermediate levels of supplementation have not been tested.
As was true for beef, supplementation of diets with vitamin E in excess of NRC requirements increases the alpha-tocopherol concentration of muscle and improves color stability in lamb meat (Wulf et al., 1995; Turner et al., 2002). During a 6 day display period, semimembranosus steaks from lambs fed 300 IU of supplemental vitamin E per kg (136 IU per lb) for either 7 or 21 days had higher color readings than steaks from lambs fed 15 IU per kg (6.8 IU per lb) of supplemental vitamin E (Turner et al., 2002).

Vitamin Safety

Vitamin E has a wide margin of safety in animals, and toxicity of this vitamin has not been demonstrated in ruminants. Compared with vitamin A and vitamn D, both acute and chronic studies in animals have shown that with vitamin E, the toxicity risk is relatively lower, but excessively high levels can result in undesirable effects. Hypervitaminosis E studies in rats, chicks and humans indicate maximum tolerable levels in the range of 1,000 to 2,000 IU per kg (455 to 910 IU per lb) of diet (NRC, 1987). Excess dietary vitamin E was found to low the activities of antioxidant enzymes in red blood cells of rats fed salmon oil (Eder et al., 2002). Although alpha-tocopherol has been the most widely studied, the other three tocopherols and four tocotrienols have recently been shown to have functions apart from alpha-tocopherol. Excess supplementation of alpha-tocopherol could be detrimental to the other vitamin E forms. In humans, excess supplementation of diets with alpha-tocopherol reduced serum concentrations of gamma and delta tocopherols (Haung, and Apple, 2003; Wolf, 2006). For livestock, the effects

of high supplemental levels of alpha-tocopherol on the other forms of vitamin E are unknown.

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