DSM in Animal Nutrition & Health

Vitamin E

Properties and Metabolism

Vitamin E activity in foods and feedstuffs is derived from a series of compounds of plant origin. Natural vitamin E is the mixture of two classes of compounds, tocopherols andtocotrienols. The term vitamin E, according to the International Union of Pure and Applied Chemistry-International Union of Biochemistry (IUPAC-IUB) Commission on Biochemical Nomenclature, is used as a generic descriptor for all tocol and tocotrienol derivatives that qualitatively exhibit the biologic activity of alpha-tocopherol (IUPAC-IUB, 1973). Both the tocopherols and tocotrienols consist of a hydroquinone nucleus and an isoprenoid side chain. Characteristically, tocopherols have a saturated side chain, whereas 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 vitamin E active compound in feedstuffs and the form used commercially for supplementation of animal diets. The biological activity of the other tocols 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

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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

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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)			

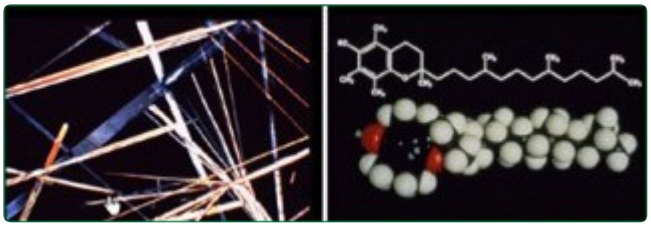
There are three asymmetric carbon atoms in the tocopherol molecule located at the 2, 4′, and 8′ positions. The d-form of alpha-tocopherol has all of the methyl groups in these positions facing in one direction and is referred to as the RRR-form. This is the form found in plants. The dl- or chemically synthesized form of alpha-tocopherol has an equal mixture of the R and S configurations at each of the three positions, (i.e., it contains eight

stereoisomers) and is referred to as the all-rac (for racemic) form of the compound. Commercially there is no truly "natural" tocopherol product available since thed-alpha-tocopherol commercial products are obtained from the original raw material only after several chemical processing steps. Hence, it should be referred to as "natural-derived" and not natural. In addition, the international unit (IU) is the standard of vitamin E activity, and consequently it is the same regardless of the source. However for some studies in several species the natural derived RRR compared to the synthetic dl-alpha-tocopherol has been shown to be more effective in elevating plasma and tissue alpha-tocopheral concentrations when administered on an equal IU basis (Jensen et al, 2006). Nevertheless, there have been no performance differences found between the d- and dl-form in poultry studies. Typically, deodorizer distillates produced during the purification and manufacture of vegetable oils are utilized in the production of d-alpha-tocopherol or tocopheryl acetate. These deodorizer distillates contain a mixture of alpha-, beta-, gamma- and delta-tocopherols, and this mixture is extracted and purified. In the final process, ultra-vacuum molecular distillation is performed and the end material methylated to produce an alpha-tocopherol concentrate. This material may then be acetylated. The acetate ester is used because it is more stable in processing and storage of foods and feeds than the alcohol (tocopherol) form.

All-rac-alpha-tocopherol acetate is the most common vitamin E form used to supplement animal feeds. This form of vitamin E is manufactured by condensing trimethyl hydroquinone and isophytol and conducting ultra-vacuum molecular distillation, producing a highly purified form of alpha-tocopherol. This material may then be acetylated. As previously stated, all-rac-alpha-tocopherol is a mixture of eight stereoisomers (four enantiomeric pairs) of alpha-tocopherol acetate. The enantiomeric pairs, racemates, have been shown to be present in equimolar amounts (Cohen et al., 1981; Weiser and Vecchi, 1981, 1982; Scott et al., 1982). This finding indicates that the manufacturing processes employed leads to all-rac-alpha-tocopherol acetate with a similar proportion of all eight stereoisomers (Weiser and Vecchi, 1982).

Alpha-tocopherol is a yellow oil that is insoluble in water but soluble in organic solvents (Illus. 4-1). Tocopherols are extremely resistant to heat but readily oxidized. Natural vitamin E is subject to destruction by oxidation, which is accelerated by heat, moisture, rancid fat, copper, and iron. Alpha-tocopherol is an excellent naturally occurring antioxidant that protects carotene and other oxidizable materials in the feed and in the body. However, in the process of acting as an anti-oxidant, it is oxidized and becomes biologically inactive.

Illustration 4-1



The naturally occurring tocopherol form is subject to destruction in the digestive tract to some extent, while the acetate ester is not. Much of the acetate is readily split off in the intestinal wall and the alcohol is absorbed, thereby permitting the vitamin to function as a biological antioxidant. Any acetate form absorbed into the body is converted to the alcohol form. Vitamin E absorption is related to fat digestion and is facilitated by bile and pancreatic lipase (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). Whether presented as free alcohol or as esters, most vitamin E is absorbed as the alcohol. Esters are largely hydrolyzed in the intestinal wall, and the free alcohol enters the intestinal lacteals and is transported via the lymph to the general circulation. An alpha-tocopherol transfer protein has been identified (Traber, 2006). For ruminants, apparently there is little or no preintestinal absorption of dietary tocopherol. It has been found that vitamin E absorption is related to vitamin E status with vitamin E-deficient ruminants absorbing 50% to 75% of the dietary tocopherol intake. Vitamin E-adequate animals absorb 20% to 30% and the animals receiving excess dietary vitamin E only 1% to 5%. However, Hidiroglou et al. (1988) reported no correlation between vitamin E status and tocopherol absorption. Vitamin E absorption may be impaired by a variety of disorders associated with fat malabsorption. The animal appears to have preference for 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 best, with gamma-tocopherol absorption slightly less than that of alpha-forms but with a more rapid excretion. It can be generally assumed that most of the vitamin E activity within plasma and other animal tissues is alpha-tocopherol (Ullrey, 1981). Vitamin E in plasma is attached mainly to lipoproteins in the globulin fraction within cells and occurs mainly in mitochondria and microsomes. The vitamin is taken up by the liver and is released in combination with low-density lipoprotein (LDL) cholesterol.

Vitamin E does not cross the placenta in any appreciable amounts; however, it is concentrated in colostrum (Van Saunet al., 1989). With respect to neonatal ruminants (Hidiroglou et al., 1969; Van Saun et al., 1989; Njeru et al., 1994) and baby pigs (Mahan, 1991), several investigators have reported limited placental transport of alpha-tocopherol, making neonates highly susceptible to vitamin E deficiency. This may be related to either a decreasing efficiency in placental vitamin E transfer as gestation proceeds, a dilution effect as a result of rapid fetal growth or possibly a decrease in available maternal vitamin E. With limited placental transfer of vitamin E, newborns must rely heavily on ingestion of colostrum as a source of vitamin E. Van Saun et al. (1989) reported decreased fetal serum vitamin E concentrations with increasing fetal age. Additionally, these authors reported less of a decline in fetal serum vitamin E concentration during gestation from vitamin E-adequate dams.

Table 4-2: Effect of Supplemental Vitamin E in Prepartum Ewes on α-tocopherol Concentration in Serum and Colostrum (µg/ml)

Supplemental Vitamin E (IU per day)	Ewes		Lambs	
	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

Njeru et al. (1994b)
Treatments administered as dl-α-tocopherol

There is inefficient placental transfer of vitamin E, but high levels of the vitamin have been shown in calves (Nockels, 1991) and lambs (Njeru et al., 1994) after consumption of colostrum. Nockels (1991) reported alpha-tocopherol levels in plasma from beef calves prior to colostrum consumption, and for several days thereafter. Precolostral plasma vitamin E levels averaged 0.2 µg per ml and increased to 3.3 µg per ml at five to eight days of age. Njeru et al. (1994) fed ewes dl-alpha-tocopherol acetate at graded levels (0, 15, 30 and 60 IU per head daily) to study placental and mammary gland transfer. Supplemental vitamin E had no effect on serum alpha-tocopherol of lambs prior to nursing, averaging 0.35 µg per ml. By day 3, lamb serum tocopherol increased to 1.41, 1.84, 2.43 and 4.46 µg per ml, respectively, for the four supplemental dietary levels of vitamin E (Table 4-2). Vitamin E at the given levels of supplementation increased colostral alpha-tocopherol at a linear rate of 3.3, 6.8, 8.0, and 9.6 µg per ml, respectively. The importance of providing colostrum rich in vitamin E is quite apparent, as both calves and lambs are born with low levels of the vitamin (Nockels, 1991; Njeru et al., 1994). Low blood vitamin E may lead to diminished disease resistance and immune response in the neonate (Nockels, 1991).

Less than 1% of the dam's tocopherol intake is secreted in the milk (Miller et al., 1973), however, colostrum tocopherol concentration is directly affected by maternal intake (Whitting and Loosi, 1948). Levels of vitamin E in the colostrum are considerably higher than in milk.

Vitamin E is stored throughout all body tissues, with highest storage in the liver. However, liver contains only a small fraction of total body stores, in contrast to vitamin A, for which about 95% of the body reserves are in the liver. Small amounts of vitamin E will persist tenaciously in the body for a long time. However, stores are

exhausted rapidly by polyunsaturated fatty acids (PUFA) in the tissues, the rate of disappearance being proportional to the intake of PUFA. A major excretion route of absorbed vitamin E is bile, in which tocopherol appears mostly in the free form (McDowell, 2000).

Vitamin E is transferred to the egg. Scott et al. (1982) reported 3 IU of vitamin E in 100 g of whole eggs. Providing optimum vitamin nutrition to laying hens significantly increased egg vitamin E concentration (Pérez-Vendrell et al., 2003b). Maternal supplementation with high levels of vitamin E (120 to 160 mg per kg or 54.5 to 72.7 mg per lb) enhanced antioxidant capability and depressed oxidative stress in chicks (Lin et al., 2005b).

Tocopherol entering the circulatory system becomes distributed throughout the body with the majority localizing in the fatty tissues. Subcellular fractions from different tissues vary considerably in their tocopherol content, with the highest levels found in membranous organelles, such as microsomes and mitochondria, that contain highly active oxidation-reduction systems (McCay et al., 1981; Taylor et al., 1976).

Increased antioxidative stability in the skeletal muscle of poultry is beneficial to avoid or delay the development of rancid products or warmed-over flavor in raw products (Ruiz et al., 2001). Supplemental vitamin E will increase alpha-tocopherol in tissue (Lanari et al., 2004; Bou et al., 2006) and alleviate oxidative stress and rancidity levels in chicken meat (Ruiz et al., 2001; Fellenberg and Speisky, 2006; Gao et al., 2010; Singh et al., 2010). The increased oxidative stability of lipids from the Musculus pectoralis major was observed after dietary treatment of alpha-tocopherol supplemented solely (Goñi et al., 2007) or with ascorbic acid (Young et al., 2003).

Functions

Vitamin E has been shown to be essential for integrity and optimum function of reproductive, muscular, circulatory, nervous, and immune systems (Hoekstra, 1975; Sheffy and Schultz, 1979; Bendich, 1987; McDowell, 2000). It is well established that some functions of vitamin E, however, can be fulfilled in part or entirely by traces of selenium or by certain synthetic antioxidants. Even sulfur bearing amino acids, cystine and methionine, affect certain vitamin E functions. Considerable evidence indicates there may be undiscovered metabolic roles for vitamin E, which may be paralleled biologically by roles of selenium and possible other substances. The most widely accepted functions of vitamin E are discussed in this section.

A. Vitamin E as a Biological Antioxidant

Vitamin E has several different but related functions. One of the most important functions is its role as an intercellular and intracellular antioxidant. Vitamin E is part of the body’s intracellular defense against the adverse effects of reactive oxygen and free radicals that initiate oxidation of unsaturated phospholipids (Chow, 1979) and critical sulfhydryl groups (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 (Dunnett, 2003). Lipids, especially phospholipids present in cell membranes are particularly susceptible to oxidative damage, being positively correlated with the degree of unsaturation of its fatty acids. Vitamin E functions as a quenching agent for free radical molecules with single, highly reactive electrons in their outer shells. Free radicals attract a hydrogen atom, along with its electron, away from the chain structure, satisfying the electron needs of the original free radical, but leaving the PUFA short one electron. Thus, a fatty acid free radical is formed that joins with molecular oxygen to form a peroxyl radical that steals a hydrogen-electron unit from yet another PUFA. This reaction can continue in a chain, resulting in the destruction of thousands of PUFA molecules (Gardner, 1989; Herdt and Stowe, 1991). Free radicals can be extremely damaging to biological systems (Padh, 1991). Highly reactive oxygen species such as the superoxide anion radical (O2-), hydroxyl radical (•OH), hydrogen peroxide (H2O2) and singlet oxygen (O2) are continuously produced in the course of normal aerobic cellular metabolism. Also, phagocytic granulocytes undergo respiratory burst to produce oxygen radicals to destroy the intracellular pathogens. However, these oxidative products can, in turn, damage healthy cells if they are not eliminated. Antioxidants serve to stabilize these highly reactive free radicals, thereby maintaining the structural and functional integrity of cells (Chew, 1995). Therefore, antioxidants are very important to immune defense and health of humans and animals.

In order to improve the oxidative stability, and thus increase the shelf life of meat, antioxidants have been successfully added to animal feeds. In the last few years, different compounds such as vitamin E, carotenoids, vitamin C, selenium and plant extracts have been tested in different experiments in order to verify their potential antioxidant effect on poultry meat (Bou et al., 2001; Bou et al., 2005; Surai, 2002; Surai et al 2002; Açikgöz et al., 2011; Halici et al., 2011; among others). Of all of them, alpha tocopherol has demonstrated the highest biological efficiency in preventing the lipid oxidation in vivo (Barroeta, 2007).

The antioxidant function of vitamin E is closely related to and synergistic with the role of selenium. 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 has been shown to act in aqueous cell media (cytosol and mitochondrial matrix) by destroying hydrogen peroxide and hydroperoxides via the enzyme glutathione peroxidase (GSH-Px), of which it is a co-factor. In this capacity, it prevents oxidation of unsaturated lipid materials within cells, thus protecting fats within the cell membrane from breaking down. 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. It is the oxidation of vitamin E that prevents oxidation of other lipid materials to free radicals and peroxides within cells, thus protecting the cell membrane from damage (Drouchner, 1976). If lipid hydroperoxides are allowed to form in the absence of adequate tocopherols, direct cellular tissue damage can result, in which peroxidation of lipids destroys structural integrity of the cell and causes metabolic derangement.

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 Summers, 2001). These reactions are enhanced by the presence of metal ions, especially iron. Broilers fed additional alpha-tocopherol of between 30 and 50 mg per kg (13.6 and 22.7 mg per lb) had reduction in lipid peroxidation as evidenced by the decrease in malondialdehyde (Zhang et al., 2009).

Vitamin E reacts or functions as a chain-breaking antioxidant, thereby neutralizing free radicals and preventing oxidation of lipids within membranes. Free radicals may damage not only their cell of origin but migrate and damage adjacent cells in which more free radicals are produced in a chain reaction leading to tissue destruction (Nockels, 1991). At least one important function of vitamin E is to interrupt production of free radicals at the initial stage.

Myodystrophic tissue is common in cases of vitamin E-selenium deficiency, with leakage of cellular compounds such as creatinine and various transaminases through affected membranes into plasma. The more active the cell (e.g., the cells of skeletal and involuntary muscles), the greater is the inflow of lipids for energy supply and the greater is the risk of tissue damage if vitamin E is limiting. This antioxidant property also ensures erythrocyte stability and maintenance of capillary blood vessel integrity.

Interruption of fat peroxidation by tocopherol explains the well-established observation that dietary tocopherols protect or spare body supplies of such oxidizable materials as vitamin A, vitamin C and the carotenes. Certain deficiency signs of vitamin E (i.e., muscular dystrophy) can be prevented by diet supplementation with other antioxidant nutrients, which helps validate the antioxidant role of tocopherols. Semen quality of boars was improved with selenium and vitamin E supplementation, in which vitamin E helped maintain sperm integrity in combination with selenium (Marin-Guzman et al., 1989). A blend of synthetic antioxidants was shown to have a sparing effect on vitamin E for broilers based on performance and tissue vitamin E concentrations (Zhao et al., 2010). However, chemical antioxidants are stored at very low levels, and thus are not as effective as tocopherol. It is clear that highly unsaturated fatty acids in the diet increase vitamin E requirements (McDowell, 2000). When acting as an antioxidant, vitamin E supplies become depleted, which explains the frequent observation that the presence of dietary unsaturated fats (susceptible to peroxidation) increases or precipitates 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). It is reported that alpha-tocopherol stimulated the incorporation of 14C from linoleic acid into arachidonic acid in fibroblast phospholipids. Also, it was found that alpha-tocopherol exerted a pronounced stimulatory influence on formation of prostaglandin E from arachidonic acid, while a chemical antioxidant had no effect.

C. Blood Clotting

Vitamin E is an inhibitor of platelet aggregation in pigs (McIntosh et al., 1985), and may play a role by inhibiting peroxidation of arachidonic acid, which is required for formation of prostaglandins involved in platelet aggregation (Panganamala and Cornwell, 1982; Machlin, 1991).

D. 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 the beneficial effect of certain nutrients, such as vitamin E reducing disease risk, can be through its effect on the immune response. Higher concentrations of d-tocopherol (50 and 100 mg per kg or 22.7 and 45.5 mg per lb) in feed reduced lipid peroxidation activity and enhanced activities of anti-oxidative enzymes and also improved cell-mediated immune response in commercial broilers (Ram Rao et al., 2011).

Considerable attention is presently being directed to the roles that vitamin E and selenium play in protecting leukocytes and macrophages during phagocytosis, the mechanism whereby animals immunologically kill invading bacteria. Both vitamin E and selenium may help these cells to survive the toxic products that are produced in order to effectively kill ingested bacteria (Badwey and Karnovsky, 1980). Macrophages and neutrophils from vitamin E-deficient animals have decreased phagocytic activity (Burkholder and Swecker, 1990).

Since vitamin E acts as a tissue antioxidant and aids in quenching free radicals produced in the body, any infection or other stress factor may exacerbate depletion of the limited vitamin E stores from various tissues. With respect to immunocompetency, dietary requirements may be adequate for normal growth and production; however, higher levels have been shown to influence positively both cellular and humoral immune status of ruminant species. The former two responses are generally used as criteria for determining the requirement of a nutrient. During stress and disease, there is an increase in production of glucocorticoids, epinephrine, eicosanoids and phagocytic activity. Eicosanoid and corticoid synthesis and phagocytic respiratory bursts are prominent producers of free radicals, which challenge an animals antioxidant systems. Vitamin E has been implicated in stimulation of serum antibody synthesis, particularly IgG antibodies (Tengerdy, 1980). The protective effects of vitamin E on animal health may be involved with its role in reduction of glucocorticoids, which are known to be immunosuppressive (Golub and Gershwin, 1985). In rats an in vivoinflammatory challenge decreased vitamin E blood and liver concentrations (Fritsche and McGuire, 1996). Vitamin E also most likely has an immunoenhancing effect by virtue of altering arachidonic acid metabolism and the subsequent synthesis of prostaglandin, thromboxanes and leukotrienes. Under increased stress conditions, levels of these compounds by endogenous synthesis or exogenous entry may adversely affect immune cell function (Hadden, 1987).

The effects of vitamin E and selenium supplementation on protection against infection by several types of pathogenic organisms, as well as antibody titers and phagocytosis of the pathogens, have been reported for calves (Cipriano et al., 1982; Reddy et al., 1985, 1987b) and lambs (Reffett et al., 1988; Finch and Turner, 1989; Turner and Finch, 1990). As an example, calves receiving 125 IU of vitamin E daily were able to maximize their immune responses compared to calves receiving low dietary vitamin E (Reddy et al., 1987a). In sows, vitamin E restriction depressed lymphocytes and polymorphonuclear cells for immune function (Wuryastuti et al., 1993). Dogs with vitamin E deficiency had a depressed proliferative lymphocyte responsiveness (Langweiler et al., 1983).

Vitamin E at high supplementation levels has a strong immune response with enhance resistance of poultry to infectious diseases (Silva et al., 2009). 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.

Large doses of Vitamin E protected chicks and poults against Escherichia coli with increased phagocytosis and antibody production (Tengerdy and Brown, 1977). In studies, vitamin E supplementation of the feed, at levels of 150 to 300 IU per kg (68 to 136 IU per lb), decreased chick mortality due to E. coli challenge from 40% in unsupplemented birds to 5% in supplemented birds (Tengerdy and Nockels, 1975; Nockels, 1979). Chicks fed 100 IU per kg diet (45 IU per lb) had increased weight gains and reduced mortality during coccidiosis challenge (Colnago et al., 1984). Heat stress severely reduced growth performance and immune response of broilers, whereas the immune response of broilers was improved by vitamin E (Niu et al., 2009). Broiler chicks fed 80 IU vitamin E per kg (36.4 IU per lb) had increased innate and humoral immune response against coccidiosis vaccine and an Eimeria (protozoan parasites) challenge (Perez-Carbajoal et al., 2010).

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 levels of vitamin E. A selenium or vitamin E deficiency leads to a change in viral phenotype, such that an avirulent 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.

Mastitis incidence has been shown to be related to vitamin E and selenium status in dairy herds. Mastitis is an extremely prevalent and costly disease. Surveys have found that in well-managed dairy herds, approximately 50 cases of mastitis can be expected per 100 cows annually (Weiss et al., 1998). Each case of clinical mastitis costs between $100 and $140 (Hoblet et al., 1991). 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).

Weiss et al. (1990; 1998) reviewed trials where dairy cows were fed three levels of vitamin E during the dry period. The prevalence of mastitis during the first week of lactation was 37%, 14% and 0% of quarters for first-lactation cows fed the low, intermediate and high concentrations of vitamin E. For multiparous cows, the prevalence was 18%, 18%, and 4% for the three treatments, respectively. Compared with the low vitamin E treatment, the 1,000 IU per day treatment reduced clinical mastitis by 30% and the 4,000 IU per day treatment reduced clinical mastitis by 80%. In agreement for sheep, Morgante et al. (1999) reported that administration of vitamin E and selenium to ewes during the dry period appeared to have influenced mammary gland status during the subsequent lactation and particularly the total and differential milk cell counts.

Vitamin E and selenium appear to enhance host defenses against infections by improving phagocytic cell function. Both vitamin E and GSH-Px are antioxidants that protect phagocytic cells and surrounding tissues from oxidative attack by free radicals produced by the respiratory burst of neutrophils and macrophages during phagocytosis (Baboir, 1984; Baker and Cohen, 1983). Hogan et al. (1990, 1992) reported that vitamin E supplementation of diets increased intracellular kill of Staphylococcus aureus and Esherichia coli by neutrophils.

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

There is limited evidence that vitamin E is involved in biological oxidation-reduction reactions (Hoffmann-La Roche, 1972). Vitamin E also appears to regulate the biosynthesis of DNA within cells. Vitamin E appears to be of particular importance in cellular respiration of heart and skeletal muscles (Leeson and Summers, 2001).

F. Relationship to Toxic Elements or Substances

Both vitamin E and selenium provide protection against toxicity of various heavy metals (Whanger, 1981). Vitamin E is highly effective in reducing toxicity of metals such as silver, arsenic and lead, and shows slight effects against cadmium and mercury toxicity.

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., 1987). Recent research has shown a beneficial response for vitamin E supplementation on male reproduction for bulls fed high concentrations of gossypol. Velasquez-Pereira et al. (1998) reported that bulls which received 14 mg free gossypol per kg body weight had a lower ($P < 0.05$) percentage of normal sperm than those which also received supplemental vitamin E, 30% versus 55%, respectively (Table 4-3).

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, %	68.3±6.7[h]	29.7±7.0[i]	55.1±6.4[h]
Abnormal (DIC)[e]	4.1±1.4[h]	12.2±1.4[i]	4.8±1.0[h]
DSPG[f] ($x10^8$/g)	14.6±1.0[h]	10.2±1.0[i]	17.6±1.0[h]
DSP[g] ($x10^8$)	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 superscript differ ($P<0.05$).
Velasquez-Pereira et al. (1998)

Likewise, sperm production per gram of parenchyma and total daily sperm production were higher ($P < 0.05$) when gossypol-treated animals also received vitamin E. Bulls receiving gossypol exhibited more sexual inactivity ($P < 0.05$) than bulls in other treatments (Table 4-4). Vitamin E supplementation to bulls receiving gossypol improved number of mounts in the first test and time of first service in the second test. The final conclusion of the Florida data is that vitamin E is effective in reducing or eliminating important gossypol toxicity effects for male cattle.

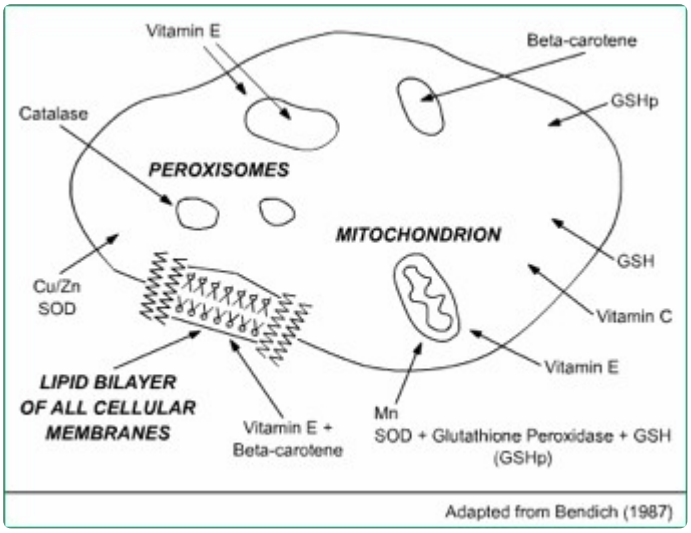
Table 4-4: Effects of Gossypol and Vitamin E on Sex Drive of Holstein Bulls

Item	Treatment			
	Mo.	Control	+Gossypol[1]	+Gossypol + Vitamin E[2]
Libido Score	16	10.4±0.7	8.9±0.5	10.0±6
	12	5.7±1.2	3.2[b]±1.1	6.3[a]±1.2
No. of Mounts	16	9.4±1.3	9.5±0.9	7.9±1.1
No. of Services	16	2.4±0.5	1.7±0.4	2.3±0.4
Time of Mounts (sec)	16	38[a]±7	29[a],b±5	21[b]±5
	12	223±94	232±69	232±69
Time of First Service (sec)	16	154±71	213±52	213[a]±52
	12	1.2[d]±0.8	3.9[c]±0.7	3.9[c]±0.7
Sexual Inactivity (min)	16	0.1±0.2	0.1±0.1	0.1±0.1

[a,b]Means with different superscripts in the same row differ (P<0.01).
[c,d]Means with different superscripts in the same row differ (P<0.05).
[1]Animals received 15 mg free gossypol per body weight.
[2]Recieved 4,000 IU vitamin E per day as *dl*-alpha-tocopheryl acetate.
Velasquez-Pereira et al. (1998)

G. Relationship with Selenium in Tissue Protection

There is a close working relationship between vitamin E and selenium within tissues. 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. Tissue breakdown occurs in most species receiving diets deficient in both vitamin E and selenium, mainly through peroxidation. Peroxides and hydroperoxides are highly destructive to tissue integrity and lead to disease development. It now appears that vitamin E in cellular and subcellular membranes is the first line of defense against peroxidation of vital phospholipids (Figure 4-2), but even with adequate vitamin E, some peroxides are formed. Selenium, as part of the enzyme GSH-Px, is a second line of defense that destroys these peroxides before they have an opportunity to cause damage to membranes. Therefore, selenium, vitamin E and sulfur-containing amino acids, through different biochemical mechanisms, are capable of preventing some of the same nutritional diseases. Vitamin E prevents fatty acid hydroperoxide formation, sulfur amino acids are precursors of GSH-Px, and selenium is a component of GSH-Px (Smith et al., 1974).



H. Non-Alpha Tocopheral 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., 2001; 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 alpha-tocopheral undoubtedly arise from observations that gamma-tochopherol 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 an anti-atherogenic and anti-cancer 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.

I. Other Functions

Additional functions of vitamin E that have been reported (Scott et al., 1982) include (1) normal phosphorylation reactions, especially of high-energy phosphate compounds, such as creatine phosphate and adenosine triphosphate (ATP); (2) a role in synthesis of vitamin C (ascorbic acid); (3) a role in synthesis of ubiquinone; and (4) a role in sulfur amino acid metabolism. Pappu et al. (1978) have reported vitamin E to play a role in vitamin B12 metabolism. A deficiency of vitamin E interfered with conversion of vitamin B12 to its coenzyme 5’ deoxyadenosylcobalamin and concomitantly, metabolism of methylmalonyl-CoA to succinyl-CoA. For humans, Turley and Brewster (1993) suggest that cellular deficiency of adenosylcobalamin may be one mechanism by which vitamin E deficiency leads to neurologic injury.

In rats, vitamin E deficiency has been reported to inhibit vitamin D metabolism in the liver and kidneys with the formation of active metabolites and decreases in the concentration of the hormone-receptor complexes in the target tissue. Liver vitamin D hydroxylase activity decreased by 39%, 25-(OH)D3 1-alpha hydroxylase activity in the kidneys decreased by 22% and 24-hydroxylase activity by 52% (Sergeev et al., 1990).

Requirements

The NRC (1994) requirement for vitamin E for poultry species varies from 5 to 25 IU per kg (2.3 to 11.4 IU per lb) of diet. Growing and laying chickens have the lowest requirement at 5 IU per kg (2.3 IU per lb) while breeding turkeys and Japanese quail have the highest requirement at 25 IU per kg (11.4 IU per lb). Vitamin E requirements are exceedingly difficult to determine because of the interrelationships with other dietary factors, therefore its requirement is dependent on dietary levels of polyunsaturated fatty acids (PUFA), antioxidants, sulfur

amino acids and selenium and variations within strains and breeds. Surai et al, (2002) compared antioxident status in two lines of breeder broiler genotypes: fast growing and slow growing. A difference in antioxident requirement was found; the fast growing genotype had a higher vitamin E requirement. The requirement may be increased with increasing levels of PUFA, oxidizing agents, vitamin A, carotenoids and trace minerals and decreased with increasing levels of fat-soluble antioxidants, sulfur amino acids and selenium (McDowell et al.,1996; McDowell, 2000a).The levels of PUFA found in unsaturated oils such as cod liver oil, corn oil, soybean oil, sunflower seed oil, linseed oil and blended commercial feed grade fat all increase the vitamin E requirements. This is especially true if these oils are allowed to undergo oxidative rancidity in the diet or are in the process of peroxidation when consumed by the animal. If they become completely rancid before ingestion, the only damage is the destruction of the vitamin E present in the oil and in the feed containing the rancidifying oil. But if they are undergoing active oxidative rancidity at the time of consumption, they apparently cause destruction of body stores of vitamin E as well (Scott et al., 1982). As previously noted, the vitamin E requirement depends very much on the level and kind of PUFA in the diet (Barroeta, 2007). For example, PUFA generate an additional vitamin E requirement in the order of 0.6, 0.9, 1.2, 1.5, and 1.8 mg of vitamin E (RRR-alpha-tocopherol-equivalents), respectively, for 1 g of dienoic, trienoic, tetraenoic, pentaenoic, and hexaenoic fatty acids (Bassler, 1991; Surai, 1999). In this respect, some vitamin E-containing oils have a positive vitamin E balance (sunflower and wheat germ oils), corn oil has zero balance, and fish oil and lard have negative vitamin E balance. Inclusion of such oils and fat into the diet increases the vitamin E requirement. Fish oil PUFA that contains arachidonic acid (20:4), docosapentaenoic acid (22:5) and docosahexaenoic acid (22:6) require much more vitamin E for stabilization than typical plant PUFA such as linoleic acid (18:2) and linolenic acid (18:3). The longer the carbon chain of a fatty acid and the more double bonds, the greater the vitamin E requirement.

Requirements of both vitamin E and selenium are greatly dependent on the dietary concentrations of each other. As noted earlier, they are mutually replaceable within certain limits. Chicks consuming a diet containing 100 mg per kg (45 mg 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). For broilers, diet supplementation with 200 IU per kg (90.9 IU per lb) of alpha-tocopherol or 100 IU per kg (45.4 IU per lb) of alpha-tocopherol plus 0.3 ppm selenium were most effective in increasing lipid oxidation stability and delaying microbial growth (Kim et al, 2010). The combination of supplemental vitamin E and selenium in broiler diets increased the meat quality of raw and marinated breast fillets (Quant et al.,2010). Determination of vitamin E requirements is further complicated because the body has an ability to store both vitamin E and selenium. Studies to establish requirements for both nutrients have often underestimated the requirements by failing to account for both body stores as well as experimental dietary concentrations.

There is a delicate balance between pro-oxidants and antioxidants in the organism, and dietary vitamin E supplementation is considered to be an effective means of antioxidant system enhancement in stress conditions. The risk of lipid peroxidation is related to both dietary and environmental factors, which can greatly vary (Surai, 1999). Dietary factors include balance of antioxidant nutrients, such as selenium, vitamin E, carotenoids, ascorbic acid and synthetic antioxidants, with pro-oxidant nutrients, such as PUFA, copper and iron. Environmental factors include atmospheric pollutants, toxins, infectious agents and other stressors of various types (Herdt and Stowe, 1991).

The amount of vitamin E needed to maintain adequate growth, egg production, and reproduction would not necessarily be enough to ensure optimal immune function as noted previously. For improving immunocompetence, vitamin E supplementation is needed at levels beyond those needed to support optimal growth. For example, chicks hatched from hens supplemented with very high levels of vitamin E had significantly higher antibody titers at one and seven days of age than chicks from the control group (Haq et al., 1996). Chicks receiving supplements of 200 mg vitamin E (90.9 mg per lb) and selenium produced higher antibody titers; this was associated with increased serum immunoglobulins and circulating immune complexes (Singh et al., 2006). Vitamin E supplementation has been shown to increase resistance to infection in farm and laboratory animals (Tengerdy, 1989). Vitamin E increases immune response against infections by improving phagocytic cell function. Phagocytic macrophages in young poults were increased when vitamin E in the diet was at 120 IU per kg (54.5 IU per lb), while a higher level at 300 IU per kg (136.4 IU per lb) was shown to optimize humoral immunity when poults were challenged with sheep red blood cells. Up to 115 IU per kg (52.3 IU per lb) of vitamin E was required to minimize red blood cell destruction in poults that were challenged with sheep red blood cells (Soto-Salanova, 1997). Reduced mortality and increased humoral immune titers were reported when chicks infected with Escherichia coli were supplemented with 150 or 300 mg dl-alpha-tocopherol per kg (68.2 to 136.4 mg per lb)

diet (Heinzerling et al., 1974). Supplementation of vitamin E may enhance the immune status of layers during heat stress and potentially during other stress periods such as transport, vaccination and molt.

Increasing vitamin E allowances beyond NRC (1994) is required for optimum performance. Supplementation of vitamin E during the laying period improved the reproductive performance of breeder pullets (Lin et al., 2004). The addition of 80 mg per kg (36.4 mg per lb) vitamin E obtained the best performance in egg production, egg mass, feed efficiency, hatchability and fertility. In order to enhance the antioxidant capability and to depress the oxidative stress of hatching chicks, hens received high levels (120-160 mg per kg or 54.5-72.7 mg per lb) of vitamin E (Lin et al., 2005b). For maximum duration of fertility for chicken cockerels, Lin et al. (2006) suggested supplemental vitamin E at 160 mg per kg (72.7 mg per lb) at 49 weeks of age.

The criteria is changing in relation to determining poultry nutritional requirements (Leeson, 2008). In the NRC (1994) poultry requirements edition virtually all nutrient needs for broilers were determined in terms of growth rate and perhaps feed utilization. For layers the criteria were egg production and egg weight. In more recent years the criteria also includes egg quality, breast meat yield, carcass quality, antioxident protection, and for general health an optimum immune response.

Sources

Many vitamin E analyses of foods and feedstuffs have been reported using a variety of analytical techniques; however, there is a lack of characterization of individual tocopherols in the majority of analyses. Total tocopherol analysis of a food or feedstuff is of limited value in providing a reliable estimate of the biological vitamin E values.

Because alpha-tocopherol is the most active form of vitamin E, many nutritionists prefer listing this form in feeds versus the unreliable total tocopherol values. Some of the less active tocopherois, particularly gamma-tocopherol, are present in mixed diets in amounts two to four times greater than that of alpha-tocopherol. Cort el al. (1983), utilizing HPLC (high pressure liquid chromatography) assay procedures, which allow 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-tocopherol. If only alpha-tocopherol in a mixed diet is reported, the value in mg can be increased by 20% to account for other tocopherols that are present, thus giving an approximation of total vitamin E activity as mg of alpha-tocopherol equivalents.

Vitamin E is widespread in nature, with the richest sources being vegetable oils, cereal products containing these oils, eggs, liver, legumes and, in general, green plants. In nature, the synthesis of vitamin E is a function of plants and thus their products are by far the principal sources. It is abundant in whole cereal grains, particularly in germ, and thus in by-products containing the germ (McDowell, 2000; Traber, 2006). 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). There is wide variation in vitamin content of particular feeds having a threefold to tenfold range in reported alpha-tocopherol values. Naturally occurring vitamin E activity of a feedstuff cannot be accurately estimated from feed tables or content of various feedstuffs compared to previously published assay values. Alpha-tocopherol is especially high in wheat germ oil, safflower oil and sunflower oil. Corn and soybean oils contain predominantly gamma-tocopherol, as well as some tocotrienols (McDowell, 2000; Traber, 2006). Cottonseed oil contains both alpha and gamma-tocopherols in equal proportions. 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 Values

Feedstuff	Assay Values (IU/lb, as fed)				
	Recent Assay Values[a]			Previously Published 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
Barley	2	6.0	5.4-6.6	24.6	14.7-28.9
Barley, 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]Adapted from Bunnell et al. (1968); NAS (1969)

Milk is highly variable in vitamin E content. As a source of vitamin E there can be a fivefold seasonal difference in the alpha-tocopherol content of cow's milk (McDowell, 2004). The vitamin E content of colostrum is of special importance for the newborn, because at birth many species have very small amounts of vitamin E in their tissues. Colostrum from dairy cows has been reported to have a mean value of 1.9 µg alpha-tocopherol per ml, declining at least 30 days to 0.3 µg alpha-tocopherol per ml (Hidiroglou, 1989). In this study, milk tocopherol was raised from 0.3 µg per ml to 1.6 µg ml 12 hours after an intraperitoneal injection of dl-alpha-tocopherol acetate. Supplemental vitamin E to ewes at graded levels of 0, 15, 30 and 60 IU (as dl-alpha-tocopherol acetate) increased colostral alpha-tocopherol at a linear rate of 3.3, 6.8, 8.1 and 9.6 µg per ml, respectively (Njeru et al., 1994). In sows, colostrum alpha-tocopherol concentration can be elevated by increasing the gestation dietary level of vitamin E or via injection during the last four days of pregnancy. Colostrum alpha-tocopherol concentration was approximately fivefold higher than milk (Mahan and Vallet, 1997).

Animal by-products supply only small amounts of vitamin E, and milk and dairy products are poor sources. Eggs, particularly the yolks, make a significant contribution, depending on the diet of the hen. Wheat germ oils are the most concentrated natural source, and various other oils, such as soybean and peanut, and particularly cottonseed, are also rich sources of vitamin E. Unfortunately, most of the oilseed meals now marketed are also devoid of these oils because of their removal by solvent extraction (Maynard et al., 1979). Green forage and other leafy materials, including good quality leaf meals, are very good sources, with alfalfa being especially rich.

Concentration of tocopherols per unit dry matter in fresh herbage is between 5 and 10 times greater than that in some cereals or their by-products (Hardy and Frape, 1983). A more available form of vitamin E is present in pastures and green forages, containing ample quantities of alpha-tocopherol versus lower bioavailable forms in grains (McDowell, 2004). Variability in forage vitamin E content is so great, both between and within farms, that one must have current results of representative samples to ensure proper vitamin E fortification programs (Harvey and Bieber-Wlaschny, 1988). These authors indicated that previously published values on vitamin E content of forages are unacceptable for use in feed formulation.

Stability of all naturally occurring tocopherols is poor and substantial losses of vitamin E activity occur in feedstuffs when processed and stored, as well as in manufacturing and storage of finished feeds (Dove and Ewan, 1991; McDowell et al., 1996). Vitamin E sources in these ingredients are unstable under conditions that promote oxidation of feedstuffs– heat, oxygen, moisture, oxidizing fats and trace minerals. Vegetable oils that normally are excellent sources of vitamin E can be extremely low in the vitamin if oxidation has been promoted. Not only does oxidized oil have little or no vitamin E, but it will destroy the vitamin E in other feed ingredients and deplete animal tissue stores of vitamin E. Rats fed a diet containing 15% oxidized frying oils had significantly lower alpha-tocopherol in plasma and tissues (Liu and Huang, 1995; 1996).

For concentrates, oxidation increases following grinding, mixing with minerals, the addition of fat, and pelleting. When feeds are pelleted, destruction of both vitamins E and A may occur if the diet does not contain sufficient antioxidants to prevent their accelerated oxidation under conditions of moisture and high temperature. Iron salts (i.e., ferric chloride) can completely destroy vitamin E. Vitamin E content in forage is affected by stage of maturity at time of forage cutting and the period of time from cutting to dehydration. Storage losses can reach 50% in one month and losses during drying in the swath can amount to as much as 50% within four days. Vitamin E losses of 54% to 73% have been observed in alfalfa stored at 33°C for 12 weeks, and 5% to 33% losses have been obtained with commercial dehydration of alfalfa.

In a study testing vitamin E stability, Orstadius et al, (1963) reported that vitamin E content of corn was reduced from 30 to 50 mg per kg (14 to 23 mg per lb) to about 5 mg per kg (2.2 mg per lb) of dry weight as a result of artificial drying at 100°C for 24 hours under a continuous flow of air. Similarly, it has been reported that artificial drying of corn for 40 minutes at 88°C produced an average 19% loss of alpha-tocopherol and 12% loss of other tocopherols (Adams, 1973). When corn was dried for 54 minutes at 107°C, the alpha-tocopherol loss averaged 41%. Artificial drying of corn results in a much lower vitamin E content. Young et al. (1975) reported a concentration of 9.3 or 20 mg per kg (4 or 9 mg per lb) alpha-tocopherol in artificially dried corn versus undried, respectively. Preservation of grains by ensiling caused almost complete loss of vitamin E activity. Corn stored as acid-treated (propionic or acetic-propionic mixture) high moisture corn contained approximately 1 mg per kg (0.45 mg per lb) dry matter of alpha-tocopherol, whereas similar corn artificially dried following harvesting contained approximately 5.7 mg per kg (2.6 mg per lb) alpha-tocopherol (Young et al., 1978). Apparently damage is not due to moisture alone, but to the combined propionic acid/moisture effect (McMurray et al., 1980). Further decomposition of alpha-tocopherol occurs over a more extended period of time until the grain eventually has alpha-tocopherol levels of less than 1 mg per kg (0.45 mg per lb), which is commonly found in propionic acid-treated barley.

The most active form of vitamin E, as previously stated, found in feed ingredients is d-alpha-tocopherol. For many years, the primary source of vitamin E in animal feed was the natural tocopherols found in green plant materials and seeds. Commercially available sources of vitamin E activity are shown in Table 4-6. Differences in biopotency of the stereoisomers of alpha-tocopherol can be seen from the definition of International Unit (IU). According to The United States Pharmacopeia (1980), dl-alpha-tocopherol acetate (also called dl-rac-tocopherol acetate) is the International Standard of vitamin E activity with an IU equivalent to 1 milligram of dl-alpha-tocopherol acetate (Table 4-6). This is the most widely available source of vitamin E activity for the supplementation of animal feeds. The acetate ester of d- or dl-alpha-tocopherol is synthesized to stabilize the compound from oxidation and maintain vitamin E activity. During commercial synthesis of dl-alpha-tocopherol acetate, all-rac-alpha-tocopherol is esterified to the acetate form to stabilize it, with the ester extremely resistant to oxidation. Thus, dl-alpha-tocopherol acetate does not act as an antioxidant in the feed. However, it has antioxidant activity after is is hydrolyzed in the intestine and free dl-alpha-tocopherol is released and absorbed.

Table 4-6: Commercially Available Sources of Vitamin E Activity	
Source	**Vitamin E Activity (IU/mg)**
dl-alpha tocopheryl acid succinate (all-rac)	0.89
dl-alpha tocopheryl acetate (all-rac)	1.00
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
Adapted from United States Pharmacopeia (1980)	

The acetate forms of alpha-tocopherol are commercially available from two basic sources: (1) d-alpha-tocopherol derived from vegetable oil refining, molecular distillation to obtain the alpha-form, and then acetylation to form the acetate ester, and (2) dl-alpha-tocopherol acetate made by complete chemical synthesis, producing a racemic mixture of equal proportions of eight stereoisomers.

Deficiency

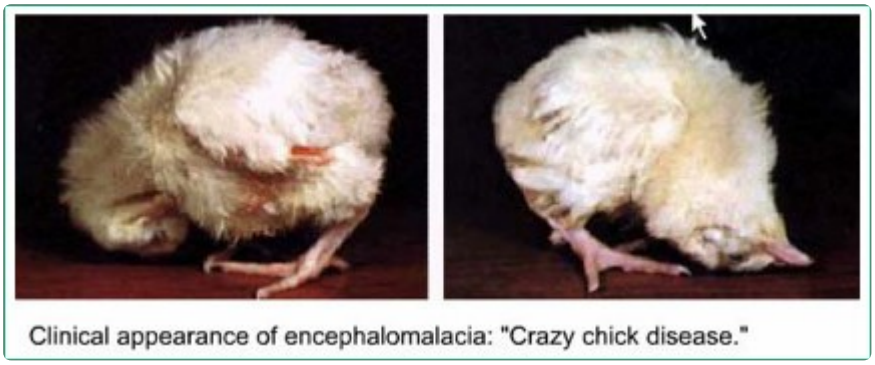
Vitamin E deficiency in poultry can result in at least three conditions: exudative diathesis (Illus. 4-2) with signs of subcutaneous edema and, in severe cases, blackening of the affected parts, apathy and inappetence; encephalomalacia (crazy chick disease) (Illus. 4-3) characterized by ataxia, head retraction and cycling of legs; and muscular dystrophy (Illus. 4-4) (Scott et al., 1982).

Illustration 4-2: Vitamin E Deficiency, Exudative Diathesis

Chicks with Vitamin E-selenium deficiency exhibited exudative diathesis with profuse subcutaneous edema.

Courtesy of L.E. Krook, Cornell University

Illustration 4-3: Vitamin E Deficiency in the Chicken: Torticollis, Encephalomalicia



Clinical appearance of encephalomalacia: "Crazy chick disease."

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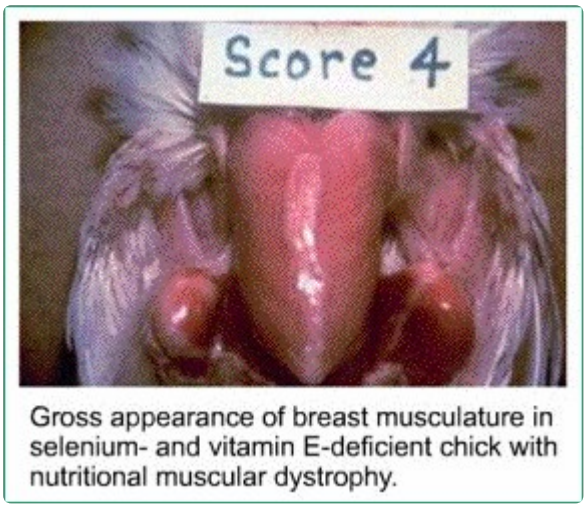
Illustration 4-4: Vitamin E Deficiency, Muscular Dystrophy



Gross appearance of breast musculature in selenium- and vitamin E-deficient chick with nutritional muscular dystrophy.

Courtesy of M.L. Scott, Cornell University

A. Chickens

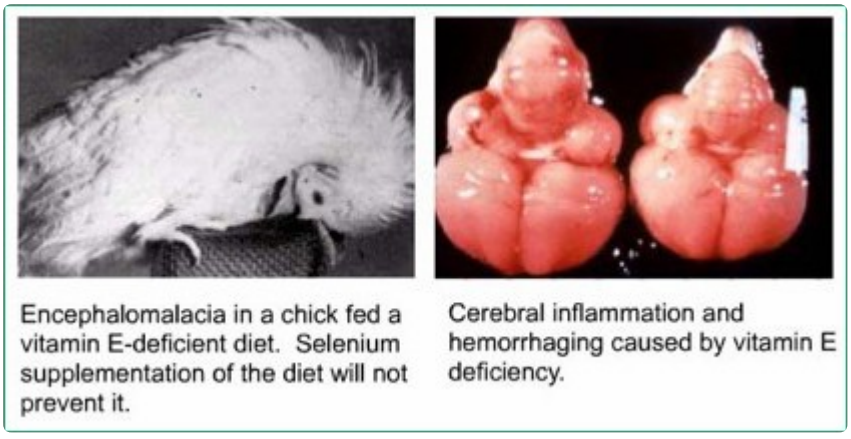
Exudative diathesis in chicks is a severe edema produced by a marked increase in capillary permeability. The subcutaneous edema soon progresses to a hemorrhagic stage, producing a blue-green discoloration of the skin (Illus. 4-5). Affected chicks show reduced spontaneous activity and food intake. If not treated with vitamin E or selenium, they survive usually no more than two to six days. Both vitamin E and selenium are involved in prevention of exudative diathesis and nutritional muscular dystrophy. In diets severely deficient in selenium, however, vitamin E does not prevent or cure exudative diathesis, whereas addition of as little as 0.05 ppm of dietary selenium completely prevents this disease.

Illustration 4-5: Vitamin E Deficiency in the Chicken: Exudative Diathesis

Blue-green discoloration of ventral skin.

Encephalomalacia generally affects chicks from two to six weeks of age and results from hemorrhages and edema within the cerebellum (Illus. 4-6). At least one important function of vitamin E is to interrupt the production of free radicals at the initial stage of encephalomalacia. The quantitative need for vitamin E for this function depends on the amount of linoleic acid in the diet. However, Verice et al. (1991) fed diets high in linoleic acid with or without vitamin E supplementation. Chicks receiving the diets high in linoleic acid or vitamin E did not develop encephalomalacia pathology. Furthermore, Fuhrmann and Sallman (1995) reported little influence of fatty acid type on development of encephalomalacia. These authors also stated that the brain cerebellum is the most susceptible to changes in vitamin E status because of its low vitamin E content. Selenium is ineffective in preventing encephalomalacia, while synthetic antioxidants are partially effective. The fact that low concentrations of antioxidants are capable of preventing encephalomalacia in chicks, but fail to prevent exudative diathesis or muscular dystrophy in the same chicks strongly suggests that in preventing encephalomalacia vitamin E acts as an antioxidant.

Illustration 4-6: Vitamin E Deficiency, Encephalomalacia



Courtesy of M.L. Scott, Cornell University

When vitamin E deficiency is accompanied by a sulfur amino acid deficiency, chicks show a severe nutritional muscular dystrophy, especially of breast muscle, at about four weeks of age. Cystine is effective in preventing nutritional muscular dystrophy in vitamin E-deficient chicks. Cystine, however, is apparently ineffective in preventing the dystrophic condition in other animals. Although vitamin E and selenium are generally both highly effective in preventing exudative diathesis, selenium is only partially effective in protecting against muscular

dystrophy in chicks when added in the presence of a low level of dietary vitamin E. Much larger quantities of selenium are required to reduce the incidence of dystrophy in chicks receiving a vitamin E-deficient diet low in methionine and cystine (Scott et al., 1982). Prolonged vitamin E deficiency can result in reproductive failure and permanent sterility. Vitamin E has been shown to be essential for normal hatchability (Wilson, 1997). Hatchability of eggs from vitamin E-deficient hens is reduced (NRC, 1994), and embryonic mortality may be high during the first four days of incubation and during later stages as a result of circulatory failure. A vitamin E inadequacy caused an increase in embryo mortality during the last week of incubation in White Leghorns and during the second and third weeks of incubation in Rhode Island Reds (Leeson et al., 1979). Vitamin E showed a protective effect against a decrease in hatchability, and dietary vitamin E concentration was positively correlated with the hatching percentage (r = 0.74) in the first week of an experiment (Tobias et al., 1992). Supplemental vitamin E (80 mg per kg or 36.4 mg per lb) during the laying period improved reproductive performance of breeder pullets (Lin et al., 2004). Reproduction of Taiwan native chicken cockerels was improved with supplemental vitamin E (Lin et al., 2005a). Providing high levels of vitamin E (120 to 160 mg per kg or 54.5 to 72.7 mg per lb) to hens resulted in hatched chicks with enhanced antioxidant capability, with a lower level of oxidative stress (Lin et al., 2005b).The effect of vitamin E supplementation on egg production in physiologic conditions is often negligible (Hossain et al., 1998). Nevertheless, in some stress conditions, vitamin E is considered to have a protective effect against an egg production decline (Puthpongsiriporn et al., 2001; Lind et al., 2004). The depression in egg production in laying hens brought about by heat stress was partially prevented by dietary supplementation with vitamin E (Utomo et al., 1994; Balnave and Brake, 2005). Evidence was also obtained that the mechanism might involve a restoration of the supply in the circulation of egg yolk precursors, particularly vitellogenin. A larger-scale experiment was carried out in which hens were housed in two climatically controlled houses and exposed to chronic heat stress of 32°C (90°F) from 24 to 28 or 32 to 36 weeks. They were fed diets containing 10, 125 or 500 mg vitamin E per kg (4.5, 56.8 or 227.3 mg per lb) from point of lay and egg production characteristics and feed intake were measured up to 40 weeks. Egg production was severely depressed by the stress, but over both periods the diet containing 500 mg per kg (227.3 mg per lb) gave 7% better production than the diet with 10 mg per kg (4.5 mg per lb). The diet containing 125 mg per kg (56.8 mg per lb) gave immediate results (Bollengier-Lee et al., 1998). When flaxseed was fed to laying hens, vitamin E at 50 IU per kg (22.7 IU per lb) significantly improved egg production compared to 27 IU per kg (12.3 IU per lb) (Scheideler and Froning, 1996). The association between vitamin E deficiency and decreased fertilizing capacity of cockerel's spermatozoa was established more than 30 years ago (Surai, 1999). Vitamin E, by acting as a lipid-soluble antioxidant within membranes and hence preventing chain reactive oxidation (Niki, 1993), plays a key role in the protection of spermatozoan lipids against peroxidation. For Japanese quail higher levels of corn oil increased sperm peroxidation, which was controlled by dietary vitamin E (150 mg per kg or 68.2 mg per lb) (Golzar adabi et al., 2011). For chickens, vitamin E tended to improve semen quality traits by increasing concentrations of spermatozoa and cell viability (Franchini et al., 2001).

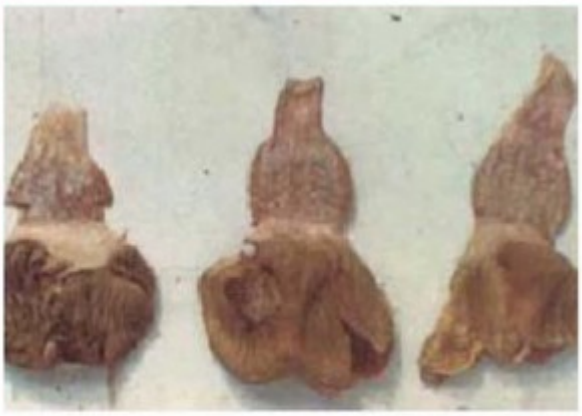
Mortality in broiler chickens associated with fluid accumulation in the abdominal cavity (ascites) is the ultimate consequence of an excessively high blood pressure in the pulmonary circulation known as pulmonary hypertension syndrome (PHS). High levels of vitamin E and C have been shown to have a protective affect against ascites. The ascites is caused by an imbalance between oxygen supply and its requirement to sustain fast growth and high feed efficiency. Increased dietary vitamin E (250 mg per kg or 113.6 mg per lb) plus selenium (Roch et al. 2000) or high levels of vitamins E and C (Broz and Ward, 2007) were able to reduce acites-related mortality in broiler chickens.

B. Turkeys and Other Poultry Species

The combined deficiency of selenium and vitamin E in poults was found to produce a mild type of exudative diathesis (Creech et al., 1957). This condition was characterized by hemorrhaging on the inner margins of the thighs and caudal breast muscles; in contrast to the exudative diathesis of the selenium- and vitamin E-deficient chick, it involved only a mild edema. For ducklings, exudative diathesis would appear to be more similar to that of the chick, i.e., green-colored edema of the subcutaneous tissues can be seen most frequently on the thigh with associated petechial hemorrhages of the thigh musculature (Combs and Combs, 1986). The appearance of exudative diathesis is infrequent and occurs in association with only the more severe cases of nutritional muscular dystrophy in deficient ducklings (Jager, 1977). For Japanese quail the combined deficiency of selenium and vitamin E has only produced exudative diathesis in some animals.

Degeneration of the smooth muscle of the gizzard is the most characteristic sign of selenium deficiency in the young turkey poult. In marked contrast to the skeletal myopathy of the vitamin E-deficient chick, gizzard myopathy in the selenium- and vitamin E-deficient poult is not prevented by dietary sulfur-containing amino acids but is completely prevented by supplements of selenium (Walter and Jensen, 1963). However, the dietary level of vitamin E affects the amount of selenium required for the prevention of the disorder. It was necessary to use a basal diet low in methionine and vitamin E, as well as selenium, to produce gizzard myopathy experimentally. Muscular dystrophy in ducklings is characterized by degeneration of the sarcoplasmic reticulum and mitochondria of the smooth muscle of the duodenum and gizzard, and is prevented with either vitamin E or selenium (Illus. 4-7).

Illustration 4-7: Vitamin E Deficiency in the Chicken: Gizzard Erosion



Different stages of gizzard erosion associated with nutritional muscular dystrophy.

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Vitamin E deficiency is also known to reduce hatchability in turkey eggs (Jensen and McGinnis, 1957). Turkey embryos deficient in vitamin E may have protruding eyes with a bulging cornea. In Japanese quail, embryonic survival (i.e., egg hatchability) was markedly depressed among females reared to maturity with vitamin E- and selenium-deficient diets. Many of the surviving progeny of selenium- and vitamin E-deficient females showed extreme generalized muscular weakness and prostration after hatching (Jensen, 1968). Similarly, sterility in quail was caused by vitamin E deficiency (Price, 1968). Hooda et al. (2007) concluded that 75 IU of vitamin E per kg (34.1 mg per lb) was best for male breeder quail, with lower supplementation amounts affecting testicular activity. Turkeys suffer from a pale, soft and exudative syndrome (PSE)-like condition that is characterized by breast meat that is light colored, loses moisture rapidly and falls apart when sliced. Ferket et al. (1993) reported that vitamin E, when fed at 331 IU per kg (150 IU per lb) for the last three weeks before processing reduced the incidence of PSE from 30% to essentially 0%. Soto-Salanova et al. (1993) demonstrated that alpha-tocopherol concentrations in plasma and the liver declined significantly in poults during three weeks post-hatch. In this regard, Sell (1996) reported that within a week of hatching, poults showed classic signs of vitamin E deficiency. It was determined that, post-hatch, the serum and liver tocopherol levels of these poults declined in an exponential fashion. Supplementation of the diet with levels of vitamin E from 80 to 120 IU per kg (36.4 to 54.5 IU per lb) appeared to reverse this condition.

Fortification Considerations

To offset losses of vitamin E activity in feedstuffs, rations should be adequately fortified using dl-alpha-tocopheryl acetate, the most stable source of vitamin E activity available for feed use. Methods of providing supplemental

vitamin E are: (1) as part of a concentrate or liquid supplement, (2) as an injectable product and (3) in drinking water preparations. Commercially, the dl- and d-alpha-tocopheryl acetates are available in purified form or in various dilutions and include (1) a highly concentrated oil, for further processing; (2) emulsions incorporated in powders for use in dry premixes or water-dispersible preparations; and (3) adsorbates or absorbates of the tocopheryl acetate oil on selected carriers, in free flowing, "dry" powders, or granules. The dry type is for use in feeds only. Vitamin E in the acetate form is highly stable in vitamin premixes, with 98% retention after six months, but the alcohol form is completely destroyed during that time period. Vitamin E acetate is stable in feeds with neutral or slightly acidic pH. However, even slightly alkaline conditions may affect the stability, such as when limestone carrier is used or in the presence of large quantities of magnesium oxide. Rate of oxidation of natural tocopherols is increased in diets containing increased levels of copper, iron, zinc or manganese (Dove and Ewan, 1991). The need for supplementation of vitamin E is dependent on the requirement of individual species, conditions of production and in relation to available vitamin E in food or feed sources. The primary factors that influence the need for vitamin E supplementation in poultry include: (1) vitamin E- and (or) selenium-deficient concentrates; (2) diets that contain predominantly non-alpha-tocopherols and thereby are less biologically active; (3) diets that include ingredients that increase vitamin E requirements (e.g., unsaturated fats, waters high in nitrates); (4) harvesting, drying or storage conditions of feeds that result in destruction of vitamin E and (or) selenium; (5) accelerated rates of gain, production and feed efficiency that increase metabolic demands for vitamin E; and (6) intensified production that also indirectly increases vitamin E needs of animals by elevating stress, which often increases susceptibility to various diseases. For breeding birds, consideration may be given to the effect of vitamin E on progeny. Haq et al. (1996) fed broiler breeders a diet containing 300 IU per kg (136.4 IU per lb) of vitamin E. Other antioxidants were fed as well. Only vitamin E appeared to improve the immune status of the chicks hatched when measured by seven-day post-hatch antibody titers. Three levels of vitamin E (0, 40, 100 IU per kg or 0, 18.2, 45.5 IU per lb) of diet were fed to hens under stress. For the offspring of vitamin treated birds, there was a positive effect on the immune response that appeared to be dose-dependent (Zhao et al., 2011). Likewise, in turkey poults, McKnight et al. (1996) reported that when hens were fed 140 IU per kg (63.6 IU per lb) of vitamin E, their progeny had superior performance vs. poults from hens fed 40 IU per kg (18.2 IU per lb) of vitamin E. Both these levels may seem excessive when compared to the NRC (1994) level of 25 IU per kg (11.4 IU per lb). However, the study this level was based upon (Jensen and McGinnis, 1958) has little resemblance to today's turkey industry as breeders were raised on the range and allowed to mate naturally. Thus, one can only assume that the actual need of the turkey breeder for vitamin E has increased over the years. The same may be said for commercial laying hens. Miles et al.(1994) gave evidence showing that pullets responded to levels of vitamin E from 25 to 50 IU per kg (11.4 to 22.7 IU per lb) diet in terms of improved feed efficiency and reduced tracheal lesions due to infectious bronchitis challenge. High levels of vitamin E supplementation to broiler flocks with subclinical infectious bursal disease (IBD) have proved beneficial (McIlroy et al., 1993). The birds were fed either 48 or 178 mg vitamin E per kg of diet (21.8 or 89.9 mg per lb) throughout the life cycle. Flocks with subclinical IBD that were fed the higher level of vitamin E showed a 1.45% improvement in feed efficiency and a 2.2% increase in weight gain. These flocks produced 10.3% greater net income than flocks receiving the lower level of supplemental vitamin E. These researchers concluded that the increased performance in the vitamin E supplemented flocks was due to the increased immunocompetence and increased disease resistance. Gore and Quereshi (1997) injected turkey and chicken embryos with vitamin E three days prior to hatching. The results from this study demonstrated an enhanced antibody and macrophage response and suggest that in-ovo exposure with vitamin E may improve post-hatch poult and broiler performance. The effect of vitamin E supplementation to the parent hen on peroxidation susceptibility was examined and it was concluded that the high peroxidative susceptibility of the chick's brain can be normalized by supplementation of the parent hen with vitamin E (Surai et al., 1999).

Supplementing the diets of turkeys with vitamin E can reduce the chance of transmission of Listeria monocytogenes, a major human bacterial food borne pathogen found in poultry (Zhu et al., 2003). Turkeys supplemented daily with 100 to 200 IU per kg (45.4 to 90.9 IU per lb) vitamin E had elevated levels of several types of lymphocytes (T-cells) when infected with Listeria monocytogenes. Therefore, vitamin E stimulates the turkeys' immune response (via lymphocytes) and enhances clearance of the microorganism from the gut.

Chronic heat stress is well known to have adverse effects on laying hens, depressing feed intake, egg number and weight and shell thickness (Balnave and Brake, 2005). Heat stress stimulates the release of corticosterone and catecholamines and initiates lipid peroxidation in cell membranes including membranes of T and B lymphocytes. Vitamin E has been shown to reduce the negative effects of corticosterone induced stress. After heat stress, poultry may have reductions in alpha-tocopherol concentrations in certain tissues. Supplemental vitamin E may be

required after stress to restore alpha-tocopherol in tissues (Nockels et al., 1996). Whitehead (1998) reported that yolk precursors were elevated by vitamin E in hens subjected to heat stress. Both during and following heat stress, the circulating levels of vitellogenin were higher in hens fed 500 IU per kg (227.3 IU per lb) of vitamin E. Similarly, Scheideler (1998) reported that hens fed 45 or 65 IU per kg (20.5 to 29.5 IU per lb) of vitamin E held their production levels through a period of heat stress while hens fed a lower level of vitamin E (25 IU per kg or 11.4 IU per lb) declined in egg production. Bollengier-Lee et al. (1998; 1999); Lin et al., 2006) studied the effects of different dietary concentrations of vitamin E (tocopherol acetate) on laying hens exposed to chronic heat stress at 32°C (90°F) from 26 to 30 weeks of age. Egg production and egg weight were significantly higher for vitamin E-supplemented birds. It was concluded that a dietary supplement of 250 mg per kg (113.6 mg per lb) of vitamin E provided before, during and after heat stress is optimum for alleviating, at least in part, the adverse effects of chronic heat stress in laying hens.

The immune system of poultry is challenged by the modern intensive production by various disorders, infections, heat stress, and stress caused by management, which can all result in economic losses. Enhancing the immune response and resistance to pathogens of birds through nutrients has been considered to be both a practical and efficient means of improving performance in modern poultry production (Abdukalykova et al, 2008; Zhang et al., 2009).

High levels of vitamin E (250 ppm) were found to optimize performance and was considered a protective management practice for reducing the negative effects of heat stress (32°C) in broilers (Sahin et al., 2002a). Increased supplemental vitamin E decreased MDA concentrations (Sahin et al, 2001b).

Both dietary vitamin E (65 IU per kg or 29.5 IU per lb) and vitamin C at 1,000 ppm enhanced in vitro lymphocyte proliferative responses of hens during heat stress (Puthpongsiriporn et al., 2001). Supplementing hens with 60 IU vitamin E per kg (27.3 IU per lb) feed improved feed intake, egg production, vitelline membrane strength, yolk and albumen solids, and foam Note: please check stability. However, egg weight, emulsfication capacity, yolk color and yolk viscosity was not improved by vitamin E supplementation.

A survey of the literature suggests a positive effect of vitamin E on performance and immune functions in poultry. Although the National Research Council (NRC) guidelines recommend 5 to 25 IU of vitamin E per kg (2.3 to 11.4 IU per lb) of diet (NRC) 1994, poultry producers are supplementing vitamins up to 10 times the NRC requirements (Ward, 1993). Supplemental levels as high as 25 times NRC have increased antibody response in turkeys (Ferket et al., 1993). In addition, it is generally observed that nutrient levels considered adequate for growth may not be adequate for optimal immune response and disease resistance (Nockels, 1988).

Field trials have shown a positive influence of vitamin E supplementation on immunity. Improving the health of broiler flocks produces a quantifiable positive economic benefit for broiler producers. On the basis of field trials, Chung and Boren (1999) recommend vitamin E at 240 mg per kg (109.1 mg per lb) of diet in broiler starter diets to achieve optimum health, production and processing performance. Vitamin E has proved beneficial for inclusion in inactivated vaccines (Franchini et al., 1991; 1995). Results show that vaccines with vitamin E, especially when vitamin E replaced 20% or 30% of mineral oil, induced a more rapid and higher antibody response in broilers than control vaccines.

In a study with turkeys, poults were supplemented with either 40 or 400 IU per kg (18.2 or 181.8 IU per lb) vitamin E during the initial 6 weeks of age (Heffels-Redmann et al, 2003). The higher level of vitamin E was beneficial for the development and maturation of the thymus and bursa fabricii at an earlier age, indicating a more rapid onset of immunocompetence. The higher level of vitamin E also led to a significantly higher lymphocyte proliferation rate. Although the 400 IU vitamin E level was fed for only 6 weeks of age, significant increases in body weights of toms and hens was still present at processing (Heffels-Redmann et al., 2003).

Kennedy et al. (1992) reported net income as a comparative index of broiler performance. He reported that broiler flocks fed a 180 mg per kg (81.8 mg per lb) vitamin E diet had a 1.3% significantly heavier ($P < 0.05$) weight bird and a 0.84% significantly better ($P < 0.05$) feed efficiency than controls fed 44 mg per kg (20 mg per lb) vitamin E. Net income for the flock on high vitamin E was 2.7% more than for the control flocks.

In a commercial broiler trial, 1,524,000 birds received either 33 or 240 mg vitamin E per kg (15 or 109 mg per lb) in a starter diet for only the first 20 days of the life cycle (Boren and Bond, 1996). At slaughter the feed:gain ratio

for the higher vitamin E supplementation level was 2.3% higher. Whole-bird disease condemnations, septicemia/toxemia, air sacculitis and inflammatory process (cellulitis) were 34%, 25%, 25% and 61% lower for the group that originally had received the higher vitamin E supplementation.

Vitamin E/selenium deficiencies are found in specific world regions and are characterized by low concentrations of vitamin E and selenium in feedstuffs. Regions that rely on concentrate importations from these deficient in selenium and (or) vitamin E (e.g., the Midwest and eastern coastal United States) must provide these nutrients to poultry. Adverse conditions such as poor weather (drought and early frost), molds and insect infestation will reduce the vitamin E value of feedstuffs. The vitamin E activity in blighted corn was 59% lower than that in sound corn, and the vitamin E activity in lightweight corn averaged 21% below that in sound corn (Adams et al., 1975). Feed spoilage will also promote vitamin E/selenium deficiencies. To prevent loss of vitamin E in diets, the producer should use fresh feed at all times because the vitamin is rapidly destroyed under hot, humid conditions. Also, the producer should use an antioxidant in the diet to prevent the destruction of the vitamin E. Losses during storage increase as the duration and temperature of storage increase.

Results of numerous studies have shown that feeding high supplemental vitamin E levels prior to slaughter increased the shelf life and delayed rancidity development in chicken and turkey meat, thus preventing off-odors and off-flavors (Marusich, 1984; 1978a,b; Ruiz et al., 2001; Goñi et al., 2007; Gao et al., 2010; Quant et al., 2010). Supplemental vitamin E extended the shelf life of fresh and frozen whole carcasses, as well as further-processed meat products. These high supplemental vitamin E levels provided allowances greater than those needed for adequate growth, feed conversion or reproduction.

Increased antioxidative stability in skeletal muscle is beneficial to avoid or delay the development of rancid product, or warmed-over flavor in raw products (Sheldon et al., 1997; Ruiz et al., 2001). Vitamin E supplementation was shown to suppress the formation of lipid peroxidation in both plasma and skeletal muscle tissues (Gao et al., 2010). Diet supplementation with vitamin E increases tissue vitamin E (Pérez-Vendrell et al., 2003a; Lanari et al., 2004; Bou et al., 2006) with the result of reduced rancidity levels in meat. The increased oxidative stability of lipids from musculus pectoralis major was observed after dietary treatment of alpha-tocopherol supplemented solely (Goñi et al., 2007) or with ascorbic acid (Young et al., 2003). Ahn et al. (1998) reported that the combination of dietary vitamin E and vacuum packaging of cooked turkey meat immediately after cooking is a good strategy to minimize oxidation and volatiles production in cooked meat.

For broilers, dietary vitamin E supplementation at levels > 120 IU per kg (>54.5 IU per lb) significantly reduced the rate of lipid oxidation in cooked ground breast and thigh meat as compared with the basal level. In this study, microbial and oxidative changes that occur during refrigerated storage of ground cooked broiler meat appeared to correlate and were positively influenced by dietary vitamin E supplementation (Saenmahayak et al., 2010). Supplementation of 200 mg per kg (90.9 mg per lb) of vitamin E to broilers significantly increased the shelf-life of refrigerated breast and thigh meat (Narciso-Gaytán et al., 2010).

Sheldon et al. (1997) reported that vitamin E supplementation at 20 and 25 times NRC levels produced the most typical and acceptable turkey meat flavors with the fewest oxidized off-flavor notes for both fresh and frozen samples as opposed to the more oxidized flavor notes detected in the control samples. Mean color scores increased, indicative of less pale meat, as the level and duration of feeding dietary vitamin E increased. Morrissey et al. (1997) found that feeding a 200 mg alpha-tocopheryl acetate per kg (90.9 mg per lb) diet to broiler chicks for at least four weeks prior to slaughter is necessary to optimize muscle content and stability against lipid peroxidation. When feeding oxidized sunflower oil to broiler chicks, Galvin et al. (1997) concluded that supplementation with 200 to 400 mg alpha-tocopheryl acetate per kg (90.9 to 181.8 mg per lb) may be necessary to achieve an optimum muscle alpha-tocopherol concentration.

Vitamin E supplementation to hens will be of benefit to consumers of eggs and to the hatching offspring. Vitamin levels provided to poultry need to be increased if consumers are to receive at least the same nutritional value as in the past. More desirable, of course, is to elevate vitamin E concentrations in poultry diets to optimum levels and thereby increase vitamin consumption for humans consuming egg and poultry meat with higher concentrations of dietary vitamin E. Vitamin E was dramatically increased in meat (e.g in fillets 12 vs 5.4 mg per kg (5.5 vs 2.5 mg per lb) (Castain et al., 2003).

Incorporation of vitamin E to the egg will increase oxidative stability and be a source of vitamin E (Franchini et al., 2002). The vitamin E status of the laying hen is essential for the efficiency of the antioxident system throughout embryonic and early postnatal development of the offspring (Surai, 2000; Surai and Sparks, 2001; Lin et al., 2005b).

Vitamin E enrichment of eggs for Japanese quail showed a linear relationship to the quantity of dietary vitamin E (Marques et al., 2010). In treatments using diets supplemented with 600 IU vitamin E per kg (272.7 IU per lb) feed, alpha-tocopherol reached a value of 479% above control valves.

Vitamin E and other vitamin requirements (e.g. NRC) established decades ago have changed little and do not reflect greatly improved genetic selection and changes in management procedures of modern poultry operations. Vitamin supplementation allowances, including vitamin E, need to be set at levels that reflect different management systems. They must be 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). To allow poultry to express their genetic potential and to account for not always ideal farm management conditions, optimum levels of vitamins are necessary. Top poultry industry leaders recognize the need for optimum vitamin nutrition.

Vitamin Safety

Compared with vitamin A and vitamin D, in both acute and chronic studies with animals vitamin E has been shown to be relatively nontoxic, but not entirely devoid of 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). For chickens, the effects of vitamin E toxicity are depressed growth rate, reduced hematocrit, reticulocytosis, increased prothrombin time (corrected by injecting vitamin K) and reduced calcium and phosophorus in dry, fat-free bone ash (NRC, 1987). Excess dietary vitamin E was found to lower the activities of antioxidant enzymes in red blood cells of rats fed salmon oil (Eder et al., 2002). Lowered egg production resulted when hens were fed 30,000 IU per kg (13,636 IU per lb) of vitamin E (Mori et al., 2003). Previous studies indicated that extremely high dosages (20,000 mg per kg or 9,091 mg per lb) of vitamin E might affect the thyroid hormone concentrations in hatching chicks and therefore, the chicks might be inhibited in piping the eggshell (Engelmann et al., 2001) An adverse effect of excessive intake of vitamin E is its interference with vitamin D utilization, particularly when the vitamin D level is marginal. In broiler chickens, increasing dietary vitamin E adversely affected ($P < 0.01$) bone ash, plasma calcium, and plasma and liver vitamin A concentrations (Aburto and Britton, 1998a, b). Bartov (1997) concluded that vitamin E, at a concentration of 150 mg per kg (68.2 mg per lb) of diet, did not aggravate a mild vitamin D deficiency. It is important to formulate feed with the proper ratios of vitamins A, D3 and E.

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 Appel, 2003; Wolf, 2006). For livestock, the effects of high supplemental levels of alpha-tocopherol on other forms of vitamin E are unknown.