Vitamins and minerals functioning as antioxidants with supplementation considerations

Lee R. McDowell[1], Nancy Wilkinson, Rachel Madison and Tara Felix
Department of Animal Sciences
University of Florida

Introduction

Vitamin E, vitamin C, carotenoids, Se and other trace minerals are important antioxidant components of animal diets and their roles in animal health and immune function are indispensable. The present paper will discuss these nutrients in relation to antioxidant and health considerations and draw conclusions as to vitamin and mineral supplementation needs.

Free radicals and antioxidants

Free radicals are atoms or molecules containing one or more unpaired electrons, making them very "reactive" (Surai, 2003). Biologically relevant free radicals are activated atoms or groups of atoms (usually containing oxygen or nitrogen) with an odd (unpaired) number of electrons. In a non-radical compound, all orbits are occupied by two electrons. When a chemical reaction breaks the bonds that hold paired electrons together, free radicals are produced. Therefore in a 'free radical' compound, there is a single unpaired electron in the outer orbit.

A single excited electron is searching to become part of a paired set and will steal an electron from another, nearby atom to accomplish this pairing. During this theft, the original free radical becomes stable while the neighboring atom, by losing an electron, becomes a free radical itself. This new free radical will then seek out another atom to steal from, creating a chain reaction.

The extreme reactivity driven by a desire to acquire another electron underlies their ability to interact with and ultimately damage tissue. Biologically relevant molecules such as DNA, proteins, lipids and carbohydrates are damaged. Free radical collective terms are reactive oxygen species (ROS) and reactive nitrogen species (RNS) and include not only the oxygen or nitrogen radicals, but some non-radical reactive derivatives of oxygen and nitrogen.

Free radicals (ROS/RNS) are constantly produced during normal physiological metabolism in tissues. Oxygen is utilized within the mitochondria to generate ATP. The majority of oxygen is reduced to form water however a small quantity (2-5%) is not

---

[1] Contact at: P.O. Box 110910, Gainesville, FL 32611, (352) 392-7561, FAX (352)392-7652, Email: mcdowell@animal.ufl.edu

completely reduced with the result of oxygen intermediates formed. The super oxide radical is the main free radical produced in living cells and the electron transport chain in the mitochondria is considered to be responsible for it. Free radicals generated naturally during oxidative metabolism if present in excess react with fatty acids to form fatty acid hydroperoxides which can then induce a chain reaction forming further free radicals and hydroperoxides: if the chain reaction is not terminated cell damage will occur. The activation of macrophages in stress conditions is another important source of free radical generation. Immune cells produce ROS/RNS and use them as an important weapon to destroy pathogens (Schwarz, 1996).

Under normal conditions the deleterious effects of ROS/RNS are counteracted by the body's antioxidant defenses, which are contributed to through dietary intake of key nutrients (e.g. vitamins and trace minerals). Antioxidants serve to stabilize these highly reactive free radicals, thereby maintaining the structural and functional integrity of cells. Therefore, antioxidants are very important to immune defense and health of humans and animals. Oxidative stress occurs when the production of reactive oxygen metabolites exceeds the capacity of the antioxidant system of the cell, tissue or body.

Because free radicals are toxic to cells, the body has developed a sophisticated antioxidant system that depends on antioxidant nutrients (Table 1). Tissue defense mechanisms against free-radical damage generally includes vitamin C, vitamin E, and β-carotene (and other carotenoids) as the major vitamin antioxidant sources. In addition, several metalloenzymes which include glutathione peroxidase (Se), catalase (Fe), and superoxide dismutase (Cu, Zn, and Mn) are also critical in protecting the internal cellular constituents from oxidative damage. Only when these metals are delivered in the diet in sufficient amounts can the animal body synthesize these antioxidant enzymes. In contrast, deficiency of those elements causes oxidative stress and damage to biological molecules and membranes.

There is a delicate balance between the amount of free radicals generated in the body and the antioxidants needed to provide protection against them. An excess of free radicals, or lack of antioxidant protection, can shift this balance resulting in oxidative stress. The dietary and tissue balance of all these nutrients (vitamins and trace elements) are important in protecting tissues against free-radical damage as well as participating in immune function.

Antioxidant Vitamins and Selenium

The principal antioxidant vitamins for tissue defense against free-radical damage includes vitamins E, C and β-carotene. Selenium would be the mineral most specifically related to antioxidant function. There are at least 35 antioxidant Se proteins and include selenoprotein P, five glutathione peroxidases and three thioredoxin reductases (TrXR1). Most recent research indicates TrXR1 reduces harmful ROS and facilitates gene expression of other cytoprotective antioxidants (Nakamura, 2005).

Vitamin E functions as a membrane-bound antioxidant, trapping lipid peroxyl free radicals produced from unsaturated fatty acids under conditions of 'oxidative stress'.

Table 1. Vitamins and minerals in antioxidant systems[a]		
Nutrients	Component (location in cell)	Function
Vitamin C	Ascorbic acid (cytosol)	Reacts with several types of ROS/RNS
Vitamin E	α-tocopherol (membranes)	Breaks fatty acid peroxidation chain reactions
β-carotene	β-carotene (membranes)	Prevents initiation of fatty acids peroxidation chain reaction
Selenium	Glutathione peroxidase (cytosol)	An enzyme that converts hydrogen peroxide to water
Copper and zinc	Superoxide dismutase (cytosol)	An enzyme that converts superoxide to hydrogen peroxide
Manganese and zinc	Superoxide dismutase (mitochondria)	An enzyme that converts superoxide to hydrogen peroxide
Copper	Ceruloplasmin (water phase)	An antioxidant protein, may prevent copper and iron from participating in oxidation reactions
Iron	Catalase (cytosol)	An enzyme (primarily in liver) that converts hydrogen peroxide to water
[a]Modified from Weiss (2005)		

Orientation of vitamin E within cell membranes appears to be critical to its functionality (Dunnett, 2003). Vitamin E functions as a chain-breaking antioxidant, neutralizing free radicals and preventing oxidation of lipids within membranes (McDowell, 2000). Vitamin E serves as the 1st line of defense against peroxidation of phospholipids. Selenium as part of glutathione peroxidase (GSH-Px) is the 2nd line of defense as the enzyme destroys peroxides and hydroperoxides. 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.

The principal vitamin E form with antioxidant and immune functions is α-tocopherol. However, although studies are limited, non α-tocopherol and tocotrienols have important functions. γ-tocopherol has been shown to be a more effective inhibitor of peroxy nitrite-induced lipid peroxidation (McCormick and Parker, 2004). Also, γ-tocopherol is more effective at inhibiting inflammatory reactions. Tocotrienols possess excellent antioxidant activity in vitro and have been suggested to suppress ROS more efficiently than tocopherols (Schaffer et al., 2005). Tocotrienols were found to be more effective for reducing the ageing process and age-related diseases.

In the lipid environment of biological membranes a combination of carotenoids and other antioxidants, especially tocopherols, may provide better antioxidant protection than tocopherols alone. Antioxidant properties of carotenoids include scavenging singlet oxygen and peroxyl radicals, sulfur, thiyl, sulfonyl and NO_2 radicals and provide protection of lipids from superoxide and hydroxyl radical attack (Surai, 2002). Therefore, carotenoids can actively quench singlet oxygen (O_2) and prevent lipid peroxidation caused by O_2 and they can intercept the propagation step of lipid peroxidation. One molecule of β-carotene is able to quench 1000 molecules of O_2 before it reacts chemically and forms products. Although the principal antioxidant carotenoid is β-carotene, other carotenoids (e.g. lutein, lycopene and zeaxanthin) have strong antioxidant activities.

Vitamin C is the most important antioxidant in extracellular fluids and can protect biomembranes against lipid peroxidation damage by eliminating peroxyl radicals in the aqueous phase before the latter can initiate peroxidation. Vitamin C is located in the aqueous phase of cells, where it contributes to radical scavenging. Vitamin C is a potent antioxidant, it easily gives up electrons to provide stability to reactive species such as ROS.

Mechanisms of Antioxidant Functions

Antioxidants are effective via various mechanisms including: 1) preventive antioxidants, 2) free radical scavengers, 3) sequestration of elements by chelation and 4) quenching active oxygen species (Dunnet, 2003).

- Preventive antioxidants – These antioxidants suppress formation of free radicals; for example catalase (Fe containing) and glutathione peroxidase (Se containing), two antioxidant enzymes, decompose hydrogen peroxide, preventing the formation of oxygen radicals.

- Free radical scavengers: These antioxidants confer stability to the 'reactive' species by donation of an electron and become oxidized themselves to form a more stable radical. For example α-tocopherol (vitamin E) scavenges peroxyl radicals and is converted to a tocopherol radical. Illustrating antioxidant interactions, the vitamin E becomes "re-activated" by ascorbic acid donating an electron which in turn forms an ascorbate radical in the process.

- Sequestration of metal by chelation - Although trace minerals are important dietary constituents, they can act as pro-oxidants (promote free radical formation). Since trace minerals such as Fe and Cu can propagate the formation of more reactive radicals they are kept bound to transport proteins such as transferrin or ceruloplasmin, as this renders them less available to contribute to radical or pro-oxidant formation.

- Quenching of active oxygen species - Antioxidants can convert active oxygen species to more stable forms, for example, carotenoids and vitamin E stabilize singlet oxygen radicals, forming less reactive hydrogen peroxide.

Antioxidant-pro-oxidant Balance in the Body and Stress Conditions

In equilibrium, free radical generation is neutralized by the antioxidant system. Natural and synthetic antioxidants in the feed as well as optimal levels of Mn, Cu, Zn, Fe and Se help to maintain the efficient levels of endogenous antioxidants in the tissues. Optimal nutrient composition allows the food antioxidants to be efficiently absorbed and metabolized. Optimal temperature, humidity and other environmental conditions are also needed for effective protection against free radical production. Prevention of different diseases by antibiotics and other drugs is an integral part of the efficient antioxidant defense as well.

Different stress conditions are associated with overproduction of free radicals and cause oxidative stress i.e., a disturbance in the pro-oxidant-antioxidant balance leading to potential tissue damage. Stress conditions can be generally divided into three main categories (Table 2). The most important part is nutritional stress conditions including high levels of PUFAs, deficiencies of vitamin E, Se, Zn or Mn, Fe-overload, hypervitaminosis A and presence of different toxins and toxic compounds. Other stress factors include environmental conditions: increased temperature or humidity, radiation, etc.

The third group of stress factors is internal and includes various bacterial or viral diseases as well as allergy. All animals protect themselves from invasion of microorganisms, parasites, fungi, viruses and any foreign molecules. Immunity phagocytosis is the major mechanism by which microbes are removed from the body and is especially important for defense against extracellular microbes. The mechanism of the phagocytic process is the killing of microbes by bombarding them with oxidants (superoxide and hydroxyl radicals, hydrogen peroxide, nitric oxide, etc.). Without adequate antioxidant nutrient reserves, cellular machinery will be damaged by the free radicals, thereby reducing the effectiveness of the immune cell. When antioxidant capacity is limited, the lifespan of immune cells is reduced and an infection can become established or severity of an infection can increase (Weiss, 2005).

Intensified production increases stress and subclinical disease level conditions because of higher densities of animals in confined areas. Stress and disease

conditions in ruminants may increase the basic requirement for certain vitamins and minerals. A number of studies indicate that nutrient levels that are adequate for growth,

Table 2. Stress conditions that generate free radical production[a]		
Nutritional	Environmental	Internal
Toxins, high PUFA Deficiencies-Vitamins A and E Carotenes and Trace Minerals Iron or Copper overload	Temperature Humidity Radiation	Diseases-bacterial, viral and fungal Allergy Mycotoxins
[a]Adapted from Surai (2002).		

feed efficiency, gestation and lactation may not be adequate for normal immunity and for maximizing the animal's resistance to disease (Cunha, 1985). Diseases or parasites affecting the gastrointestinal tract will reduce intestinal absorption of vitamins, both from dietary sources and those synthesized by microorganisms. If they cause diarrhea or vomiting this will also decrease intestinal absorption and increase needs. Vitamin A deficiency is often seen in heavily parasitized animals that supposedly were receiving an adequate amount of the vitamin.

Mycotoxins in feed can substantially decrease antioxidant nutrient assimilation from the feed and increase their requirements to prevent damaging effects of free radicals and toxic products of their metabolism. It is now increasingly recognized that at least 25% of the world's grains are contaminated with mycotoxins (Surai, 2002). Mycotoxins are known to cause digestive disturbances such as vomiting and diarrhea as well as internal bleeding, and interfere with absorption of dietary vitamins A, D, E and K. Environmental pollutants (e.g. heavy metals, pesticides, fungicides, herbicides, etc.) can cause oxidative stress.

Animal Studies of Antioxidant and the Immunity Role of Vitamins and Trace Minerals

Antioxidant vitamins generally enhance different aspects of cellular and non-cellular immunity. The antioxidant function of these vitamins could, at least in part, enhance immunity by maintaining the functional and structural integrity of important immune cells. A compromised immune system will result in reduced animal production efficiency through increased susceptibility to diseases, thereby leading to increased animal morbidity and mortality.

One of the protective effects of vitamin C may partly be mediated through its ability to reduce circulating glucocorticoids. The suppressive effect of corticoids on neutrophil function in cattle has been alleviated with vitamin C supplementation. Vitamin C and E supplementation resulted in a 78% decrease in the susceptibility of lipoproteins to mononuclear cell-mediated oxidation (Rifici and Khachadurian, 1993). Ascorbic acid is reported to have a stimulating effect on phagocytic activity of leukocytes, on function of the reticuloendothelial system, and on formation of antibodies. Ascorbic acid levels are very high in phagocytic cells with these cells using free radicals and other highly reactive oxygen containing molecules to help kill

pathogens that invade the body. In the process, however, cells and tissues may be damaged by these reactive species. Ascorbic acid helps to protect these cells from oxidative damage.

Considerable attention is directed to the role vitamin E and Se play in protecting leukocytes and macrophages during phagocytosis, the mechanism whereby animals immunologically kill invading bacteria. Both vitamin E and Se 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.

Since vitamin E acts as a tissue antioxidant and aids in quenching free-radicals produced in the body, any infection or other stress factors 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 positively influence 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 the animal antioxidant systems. Vitamin E has been implicated in stimulation of serum antibody synthesis, particularly IgG antibodies (Tengerdy, 1980). Vitamin E supplementation increased α-tocopherol status and immune response of weaned piglets (Lauridsen and Jensen, 2005).

The effects of vitamin E and Se 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 and lambs (Reffett et al., 1988). 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 (Ready et al., 1987).

Bovine respiratory disease (BRD) is the most economically important disease affecting feedlot cattle, causing approximately 75% of morbidity and over 50% of mortality. Rivera et al. (2002) suggest that vitamin E supplementation may enhance recovery from BRD. Interestingly when given as an adjuvant at vaccination, vitamin E is highly effective in enhancing antibody titers, implying that this may be an effective way of obtaining immunological response following vaccination. Cows given a single subcutaneous injection of 3,000 IU of vitamin E one week before expected calving tended to have reduced risk of retained placenta (LeBlanc et al., 2002)

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 vitamin E adequacy. A Se 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).

Carotenoids have been shown to have biological actions independent of vitamin A (Koutsos, 2003). Recent animal studies indicate that certain carotenoids with antioxidant capacities, but without vitamin A activity, can enhance many aspects of immune functions, can act directly as antimutagens and anticarcinogens, can protect against radiation damage, and can block the damaging effects of photosensitizers. Also, carotenoids can directly affect gene expression and this mechanism may enable carotenoids to modulate the interaction between B cells and T cells, thus regulating humoral and cell-mediated immunity (Koutsos, 2003).

Vitamin A and β-carotene have important roles in protection against numerous infections including mastitis. Potential pathogens are regularly present in the teat orifice, and under suitable circumstances can invade and initiate clinical mastitis. Any unhealthy state of the epithelium would increase susceptibility of a mammary gland to invasion by pathogens.   There are reports of improved mammary health in dairy cows supplemented with β-carotene and vitamin A during the dry and lactating periods (Chew and Johnston, 1985).

Polymorphonuclear neutrophils (PMN) are the major line of defense against bacteria in the mammary gland. β-carotene supplementation seems to exert a stabilizing effect on PMN and lymphocyte function during the period around dry off (Tjoelker et al., 1990). Daniel et al. (1991) reported that β-carotene enhanced the bactericidal activity of blood and milk PMN, against *S. aureus* but did not affect phagocytosis. Vitamin A either had no effect or suppressed bactericidal activity and phagocytosis. Control of free radicals is important for bactericidal activity but not for phagocytosis. The antioxidant activity of vitamin A is not important; it does not quench or remove free radicals. β-carotene, on the other hand, does have significant antioxidant properties and effectively quenches singlet oxygen free radicals (Mascio et al., 1991).

Supplemental levels of vitamin E higher than recommended by the dairy cattle NRC (2001) have been beneficial in the control of mastitis. Smith and Conrad (1987) reported that intramammary infection was reduced 42.2 % in vitamin E-Se supplemented versus unsupplemented controls. The duration of all intramammary infections in lactation was reduced 40 to 50% in supplemented heifers. Clinical mastitis was negatively related to plasma Se concentration and concentration of vitamin E in the diet.

Somatic cell counts (SCC) are a primary indicator of mastitis and milk quality in dairy herds. The PMN are a major defensive mechanism against infection in the bovine mammary gland. A known consequence of vitamin E and Se deficiency is impaired PMN activity and postpartum vitamin E deficiencies are frequently observed in dairy cows. Dietary supplementation of cows with Se and vitamin E results in a more rapid PMN influx into milk following intramammary bacterial challenge and increased intracellular kill of ingested bacteria by PMN. Subcutaneous injections of vitamin E approximately 10 and 5 d before calving successfully elevated PMN α-tocopherol

concentrations during the periparturient period and negated the suppressed intracellular kill of bacteria by PMN that commonly is observed around calving (Smith et al., 1987).

Plasma α-tocopherol decreased at calving for cows fed dietary treatments with low or intermediate concentrations of vitamin E, but not for cows fed high vitamin E treatment (Weiss et al., 1997). High dietary vitamin E increased concentration of α-tocopherol in blood neutrophils at parturition. The high vitamin E treatment was 1,000 IU/d of vitamin E during the first 46 d of the dry period, 4,000 IU/d during the last 14 d of the dry period, and 2,000 IU/d during lactation.

The percentage of quarters with new infections at calving was not different (32.0%) between cows receiving treatments that contained low and intermediate concentrations of vitamin E but was reduced (11.8%) in cows receiving the high vitamin E treatment. Clinical mastitis affected 25.0, 16.7 and 2.6% of quarters during the first 7 d of lactation for cows receiving the low, intermediate, and high vitamin E treatments, respectively. Cows with plasma concentrations of α-tocopherol <3.0 µg/ml at calving were 9.4 times more likely to have clinical mastitis during the first 7 d of lactation than were cows with plasma concentrations of α-tocopherol >3.0 µg/ml (Weiss et al., 1997). Based on neutrophil function and clinical mastitis, the minimal plasma vitamin E level for peripartum dairy cows is 3.0 to 3.5 µg/mL.

Baldi et al. (2000) reported significantly lower SCC (log 10) in cows fed 2,000 IU/d supplemental vitamin E versus 1,000 IU/d from 14 d pre-calving through 7 d post-calving in diets with 0.3 ppm added Se. Plasma and milk vitamin E levels were increased in cows fed 2,000 vs 1,000 IU/d. Taken together, these studies substantiate the cooperative roles of vitamin E and Se in maintaining mammary immune function and suggest that 2,000 to 4,000 IU vitamin E/d during the peripartum period can be beneficial to dairy cows in terms of udder health and milk quality (Seymour, 2001).

Additional Benefits of Vitamin E Supplementation

Supplementing vitamin E in well balanced diets has been shown to increase humoral immunity for ruminant (Hoffmann-LaRoche, 1994) and monogastric species (Wuryastuti et al., 1993). These results suggest that the criteria for establishing requirements based on overt deficiencies or growth do not consider optimal health.

Gill et al. (1986) supplemented newly received feedlot cattle with 1,600 IU vitamin E (as dl-α-tocopheryl acetate) per head per day for the first 21 days and 800 IU vitamin E for the remaining 7 days of a 28 day trial. Average daily gain and gain to feed ratios were improved by 23.2 and 28.6%, respectively, for vitamin E supplemented stressed cattle. The number of sick pen days per head was reduced by 15.6%, and morbidity was reduced by 13.4% with vitamin E supplementation.

Baldi et al. (2000) supplemented cows with 1,000 or 2,000 IU vitamin E starting 14 d prepartum through 7 d postpartum and reported significant reduction in days open in cows fed 2,000 IU/d (111 vs 84 d) and fewer services per conception (1.3 vs. 2.2).

Kim et al. (1997) used 120 Holstein cows to test the effects of injecting 500 IU vitamin E and 40 mg Se at 20 d prepartum on reproductive performance. The combination of vitamin E and Se significantly reduced the incidence of retained placenta (30 vs 13.3%) and days to first service (103 vs 60 d). Olson and Seidel (2000) reported that vitamin E enhanced the development of bovine embryos both *in vitro* and after their transfer to recipient cows.

Selenium source for heat stressed dairy cows (Florida) were fed supplemental Se as yeast Se or as selenite for 81 days (beginning 26 d prepartum) (Thatcher, 2006). The benefits of organic Se were: 1) elevated plasma Se, 2) phagocytosis and killing activity of neutrophils increased, immunocompetence increased at parturition, milk yield increased, uterine health improved and 2nd service pregnancy rate increased.

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/kg body weight had a lower (P < 0.05) percentage of normal sperm than those which also received supplemental vitamin E, 31 vs 55%, respectively (Table 2). 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. 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 3. Relationship of gossypol and vitamin E on semen characteristics of dairy bulls[a]

Item	Treatment		
	TRT1[b]	TRT2[c]	TRT3[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 (x 10^6/g)[e]	14.5 ± 1.0^h	10.2 ± 1.0^i	17.6 ± 1.0^h
DSP (x 10^9)[f]	3.2 ± 3.0^h	2.2 ± 3.0^i	4.1 ± 3.0^h

[a]Least square means + SEM.
[b]Diet based on SMB, 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]Daily sperm production per gram of parenchyma.
[f]Daily sperm production total.
[h,i]Means in row with different superscript differ P < 0.05.

Many attempts have been made to control lipid oxidation in meats through the use of antioxidants. Dietary supplementation of vitamin E, and intravenous infusion of vitamin C immediately before harvest, are efficacious techniques for increasing the concentration of these vitamins in beef skeletal muscle (Schaefer et al., 1995). Meat

with elevated levels of either and probably both of these antioxidant vitamins possesses greater stability of oxymyoglobin and lipid, which results in less discoloration and rancidity. Vitamin E would seem to be the most practical since it is administered dietetically. Increased dietary levels of vitamin E result in higher tissue α-tocopherol concentrations and greater stability of these tissues toward lipid oxidation (Roeber et al., 2001).

Dramatic effects of vitamin E supplementation (500 IU per head daily) to finishing steers on the stability of beef color have been observed (Faustman et al., 1989). 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. This was most likely due to the increased α-tocopherol content of the loin tissue of the supplemented animals, which was approximately 4-fold greater than controls (Faustman et al., 1989). Vitamin E supplementation of feedlot cattle at 1,000 IU daily for at least 100 d increased color of beef products, increased α-tocopherol concentration in beef cuts and subsequently increased its retail case life (Roeber et al., 2001).

Color is an extremely critical component of fresh red meat appearance and greatly influences the customer perception of meat quality. Steaks from cattle supplemented with vitamin E were preferred over control steaks by 91% of Japanese survey participants (n=10,941), and 58% of all participants identified muscle color as the most important factor in selecting beef products (Sanders et al., 1997).

Optimum Vitamin and Mineral Allowances

The National Research Council (NRC) requirements for vitamins and minerals are usually close to minimum levels required to prevent deficiency signs and for conditions of health and adequate performance, provided sufficient amounts of all other nutrients are supplied. Most nutritionists usually consider NRC requirements for vitamins and minerals to be close to minimum requirements sufficient to prevent clinical deficiency signs and they may be adjusted upward according to experience within industry in situations where higher levels are needed. The concept of optimum vitamin nutrition under commercial production conditions is illustrated in Figure 1 (Roche, 1979).

Allowances of vitamins and minerals are those total levels from all sources fed to compensate for factors influencing the needs of animals. These "influencing factors" (Figure 2) include those that may lead to inadequate levels of dietary vitamins and minerals.   Influencing factors can include stress, infectious diseases, parasites, biological variations, diet composition, bioavailability of sources, stability and nutrient interrelationships. Mineral levels vary in feed ingredients because of crop location, fertilization, plant genetics, plant disease and weather. For example, corn and other grains will vary in their concentration of vitamins and minerals depending on varieties, soils, levels of fertilization, maturity at harvest, processing methods and storage. Intensive cropping practices and use of new crop varieties may result in reduced levels of these nutrients in many feedstuffs. In forage crops, factors that favor production of

lush, green plants also favor production of higher levels of vitamins and minerals, as most plants decline in their vitamin and mineral content as they mature. Harvesting conditions, processing and storage will affect the vitamin and mineral content of both feedstuffs and supplements.

Vitamin Supplementation Most Needed By Ruminants

Supplementation allowances need to be set at levels that reflect different management systems and that are high enough to take care of fluctuations in environmental temperatures, energy content of feed, or other factors that might influence feed consumption or the vitamin requirements in other ways. Grazing ruminants generally only need supplemental vitamin A, if pastures are low in carotene, and possibly vitamin E (influenced by Se status). Vitamin D is provided by ultraviolet light activity on the skin, while all other vitamins can be provided by ruminal or intestinal microbial synthesis.

Ruminants housed under more strict confinement conditions generally require vitamins A and E and may require vitamin D if deprived of sunlight. Additional supplemental vitamin E would be needed to stabilize the meat color of finishing animals. Under specific conditions, relating to stress and high productivity, ruminants may be benefitted by supplemental B vitamins, particularly thiamin and niacin. Biotin deficiency has been linked to lameness in cattle (Budras et al., 1997). Increased plasma biotin levels have been associated with hardness and positive confirmational changes in bovine hooves. Future research may find a need for carnitine supplementation. Adding a complete B-vitamin mixture to cattle diets entering the feedlot during the first month can reduce stress and increase gains. In one study, supplemental B vitamins given feedlot calves tended to reduce morbidity of animals (Zinn et al., 1987). Apparently under stress conditions of feedlots, the microbial population in the rumen is not synthesizing certain B vitamins at adequate levels.

Mineral Supplementation Most Needed by Ruminants

Grazing Ruminants

Ruminants that depend primarily on pasture have different mineral supplementation needs than those receiving concentrates. Phosphorus is generally most limiting, with free-choice mineral supplements containing Ca, P, common salt and selected trace minerals (McDowell and Arthington, 2005). The trace elements most likely to be deficient for grazing livestock are Cu, Co, I, Se and Zn. Iron is generally not required unless animals are losing blood due to parasites; Mn deficiency is restricted to few regions of the world. Animals in some tropical regions may benefit from S supplementation particularly if non-protein nitrogen (e.g., urea) supplements are fed. Magnesium supplementation is warranted when grass tetany is a problem. Potassium may be needed in winter or the dry season when forage K is less than required.

Ruminants Receiving Concentrates

The best means of providing minerals to ruminants receiving concentrates would be to combine the minerals with the concentrate diets, including Ca, P and a trace mineralized salt (NaCl plus, Co, Cu, I, Fe, Mn, Se and Zn). Chromium has been shown to be beneficial to stressed calves (e.g., transport stress). The less dietary forage, the higher the level of Ca required. Some studies would recommend K supplementation for high-producing dairy cows and finishing ruminants when high quality K-rich forages are limited; Mg is often added to high concentrate diets. Sulfur often is needed when non-protein nitrogen (e.g., urea) is used. To prevent milk fever, dairy cattle need special diets that regulate the Ca and P concentration and increase acidity. Normally the Ca:P ratio is less important than that of monogastric diets, assuming the P concentration is adequate.

Diets that limit forage in relation to concentrates need some mineral salts as buffers as well as individual elements. High-producing dairy cows, finishing cattle and sheep often receive high concentrate diets. Feeding large amounts of concentrate with small amounts of forage or with forages that have been fermented or finely chopped will decrease ruminant saliva output and increase the acid load of these ruminants.

Conclusion/Implications

Oxidative stress occurs when production of free radicals exceeds the capacity of the antioxidant system of body cells. Certain nutrients serve as antioxidants or are components of antioxidant enzymes. Antioxidants include vitamins C and E, carotenoids, and antioxidant enzymes containing Se, Cu, Mn, Fe or Zn. Stress conditions that generate free radical production can be classified as nutritional (e.g., high PUFA and or excesses of certain minerals and vitamins), environmental (e.g., temperature and humidity) and internal (e.g., bacterial, viral and fungal diseases). Antioxidant nutrients play important roles in animal health by inactivating harmful free radicals produced through normal cellular activity and from various stressors. A compromised immune system will result in reduced animal production efficiency through increased susceptibility to disease, thereby leading to increased animal morbidity and mortality. A number of factors influence vitamin and mineral requirements and utilization and include: physiological make-up and production function; confinement rearing without pasture; stress, disease and adverse environmental conditions; vitamin antagonists, use of antimicrobial drugs and body vitamin reserves. Under commercial ruminant production conditions, vitamin and mineral allowances higher than NRC requirements may be needed to allow optimum performance. Generally, the optimum vitamin and mineral supplementation level is the quantity that achieves the best growth rate, feed utilization, health (including immune competency), and provides adequate body reserves.

Reference

Badwey, J.A and M.L. Karnovsky. 1980. Active oxygen species and the functions of
        phagocytic leukocytes. Ann. Rev. Biochem. 49:695.

Baldi, A., G. Savoini, L. Pinotti, E. Monfardini, F. Cheli and V. Dellorto. 2000. Effects of
        vitamin E and different energy sources on vitamin E status, milk quality and
        reproduction in transition cows. J. Vet. Med. Assoc. 47:599.

Beck, M.A. 1997. Increased virulence of coxsackievirus B3 in mice due to vitamin E or
        selenium deficiency. J. Nutr. 127:966S.

Beck, M.A., P.C. Kolbeck, L.H. Kohr, Q. Shi, V.C. Morris and O.A. Lavander. 1994.
        Vitamin E deficiency intensifies the myocardial injury of coxsackievirus B3
        infection in mice. J. Nutr. 124:345.

Budras, K.D., T. Hochsetter, Ch. Muelling and W. Natterman. 1997. Structure, function
        and horn quality in the bovine hoof: The influence of nutritional and
        environmental factors. J. Dairy Science 80(suppl.1); 192(abstract)

Chew, B.P. and L.A. Johnston. 1985. Effects of supplemental vitamin A and β-
        carotene on mastitis in dairy cows. J. Dairy Sci. 68(suppl. I):l9l(Abstr.).

Daniel, L.R., B.P. Chew, T.S. Tanaka and L.W. Tjoelker. 1991. β-carotene and vitamin
        A effects on bovine phagocyte function in vitro during the peripartum period. J.
        Dairy Sci. 74:124.

Faustman, C., R.G. Cassens, D.M. Schaefer, D.R. Buege and K.K. Scheller. 1989.
        Vitamin E supplementation of Holstein steer diets improves sirloin steak color. J.
        Food Sci. 54(2):485.

Gill, D.R., R.A. Smith, R.B. Hicks and R.L. Ball. 1986. The effect of vitamin E
        supplementation on the health and performance of newly arrived stocker cattle.
        Ok. Agr. Exp. Sta. Res. Rep. MP 118:240.

Hoffmann-La Roche. 1994. Vitamin Nutrition for Ruminants. RCD 87751/894.
        Hoffmann-La Roche, Inc., Nutley, NJ.

Kim, H.S., J.M. Lee, S.B. Park, S.G. Jeong, J.K. Jung and K.S. Im. 1997. Effects of
        vitamin E and selenium administration on the reproductive performance of dairy
        cows. Asian J. Anim. Sci. 10:308.

Koutses, E. 2003, Carotenoids in avian immunity. Proc. California Animal Nutrition
        Confer. p. 179, Fresno, CA.

Lauridsen, C. and S.K. Jensen. 2005. Influence of supplementation of all-rac-α-
        tocopheryl acetate preweaning and vitamin C postweaning on α-tocopherol and
        immune responses of pigs. J. Anim. Sci. 83:1274.

LeBlanc, S.J., T.F. Duffield, K.E. Leslie, K.G. Bateman, J. TenHag, J.S. Walton and
        W.H. Johnson., 2002. The effect of prepartum injection of vitamin E on health in
        transition dairy cows. J. Dairy Sci. 85:1416.

Mascio, P.D., M.E. Murphy and H. Sies. 1991. Antioxidant defense system: The role of
        carotenoids, tocopherols and thiols. Am. J. Clin. Nutr. 53: 194S.

McCormick, C.C., and R.S. Parker. 2004. The cytotoxicity of vitamin E is both vitamer-
        and cell specific and involves a selectable trait. J. Nutr. 134:3335.

McDowell, L.R. 2000. Vitamins in Animal and Human Nutrition, 2nd ed., Iowa State
        University Press, Ames, IA.

McDowell, L.R. 2003. Minerals in Animal and Human Nutrition. 2nd ed., Elsevier, Amsterdam.

McDowell, L.R. and J. Arthington. 2005. Minerals for grazing ruminants in tropical regions. 4th ed., 86p. Department of Animal Sciences, University of Florida, Gainesville, FL.

NRC. 2001. Nutrient Requirements of Dairy Cattle. (7th rev. ed.) Natl. Academy Press, Washington, D.C.

Nakamura, H. 2005. Thioredoxin and its related molecules: Updated 2005. Antioxidants and Redox Singaling 7(5-6):823.

Olson, S.E. and G.E. Seidel, Jr. 2000. Culture of in vitro-produced bovine embryos with vitamin E improves development in vitro and after transfer to recipients. Biol. Reprod. 62:248.

Reddy. P.G., J.L. Morrill, H.C. Minocha and J.S. Stevenson. 1987. Vitamin E is immunostimulatory in calves. J. Dairy Sci. 70:993.

Reffett, J.K., J.W. Spears and T.T Brown, Jr. 1988.   Effect of dietary selenium and vitamin E on the primary and secondary immune response in lambs challenged with parainfluenza virus. J. Anim. Sci. 66:1520

Rifici, V.A. and A.K. Khachadurian. 1993. Dietary supplementation with vitamins C and E inhibits in vitro oxidation of lipoproteins. Am. J. Coll. Nutr. 12:631.

Rivera, J.D., G.C. Duff, M.L. Galyean, D.A. Walker and G.A. Nunnery. 2002. Effects of supplemental vitamin E on performance, health, and humoral immune response of beef cattle. J. Anim. Sci. 80:933.

Roche. 1979. Optimum Vitamin Nutrition. Hoffmann-La Roche, Nutley, NJ.

Roeber, D.L, K.E. Belk, J.D. Tatum, J.W. Wilson and G.C. Smith. 2001. Effects of three levels of α-tocopheryl acetate supplementation to feedlot cattle on performance of beef cuts during retail display. J. Anim. Sci. 79:1814.

Sanders, S.K., J.B. Morgan, D.M. Wulf, J.D. Tatum, S. N. Williams and G.C. Smith. 1997. Vitamin E supplementation of cattle and shelf-life of beef for the Japanese market. J. Anim. Sci. 75:2634.

Schaefer, D.M., Q. Liu, C. Faustman and M. Yin. 1995. Supranutritional administration of vitamin E and C improves oxidative stability of beef. J. Nutr. 125:1792S.

Schaffer, S., W.E. Müller and G.P. Eckert. 2005. Tocotrienols: Constitutional effects in aging and disease. J. Nutr. 135:151.

Schwarz, K.B. 1996. Oxidative stress during viral infection: A Review: Free Radical Biology and Medicine. 21:641.

Seymour, W. 2001. Review: Update on vitamin nutrition and fortification in dairy cattle. The Professional Anim. Sci. 17:227.

Smith, K.L. and H.R. Conrad. 1987. Vitamin E and selenium supplementation for dairy cows. RCD 7442, Proc. Roche Technical Symposium, The role of Vitamins on Animal Performance and Immune Response, p.47. Daytona Beach, FL.

Surai, P.F. 2002. Natural Antioxidants in Avian Nutrition and Reproduction. Nottingham University Press, Nottingham, UK.

Surai, P.F. 2003. Selenium-vitamin E interactions: Does 1 + 1 equal more than 2? In: Nutritional Biotechnology in the Feed and Food Industries (T.P. Lyons and K.A. Jacques, eds.) Nottingham University Press, Nottingham, UK.

Tengerdy, R.P. 1980. Disease resistance: Immune response. In Vitamin E: A Comprehensive Treatise (L.J. Machlin, ed.) Marcel Dekker, NY.

Thatcher, W.W. 2006. Selenium source may aid heat-stressed dairy cows. Feedstuffs 78(42):14-15.

Tjoelker, L.W., B.P. Chew, T.S. Tanaka and L.R. Daniel. 1990. Effect of dietary vitamin A and β-carotene on polymorphonuclear leukocyte and lymphocyte function in dairy cows during the early dry period. J. Dairy Sci. 73:1017.

Velasquez-Pereira, J., C.A. Risco, P.J. Chenoweth, L.R. McDowell, D. Prichard, F.G. Martin, N.S. Wilkinson, S.N. Williams and C.R. Staples. 1998. Reproductive effects of feeding gossypol and vitamin E to bulls. J. Anim. Sci. 76:2894.

Weiss, W.P. 2005. Antioxidants nutrients, cow health and milk quality. Dairy Cattle Nutrition Workshop, Department of Dairy and Animal Sciences, Penn State, p. 11-18.

Weiss, W.P, J.S. Hogan, D.A. Todhunter and K.L. Smith. 1997. Effect of vitamin E supplementation in diets with a low concentration of selenium on mammary gland health of dairy cows. J. Dairy. Sci. 80: 1728.

Wuryastuti, H., H.D. Stowe, R.W. Bull and E.R. Miller. 1993. Effects of vitamin E and selenium on immune responses of peripheral blood, colostrum and milk leukocytes of sows. J. Anim. Sci. 71:2464.

Zinn, R.A, F.N. Owens, R.L. Stuart, J.R. Dunbar and B.B. Norman. 1987. B-vitamin supplementation of diets for feedlot calves. J. Anim. Sci. 65-267.
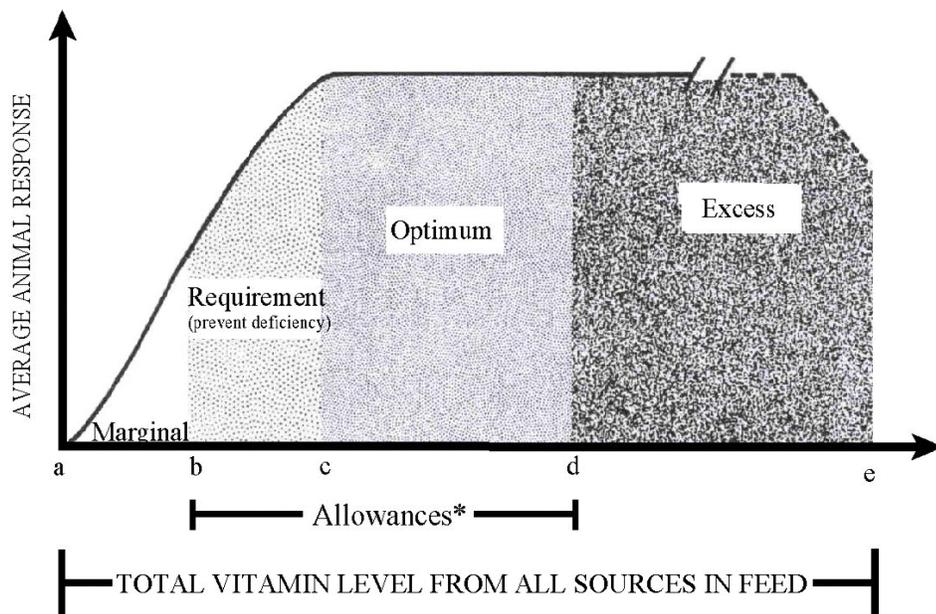
*Those total vitamin levels from all sources fed to compensate for factors affecting animals' vitamin needs.
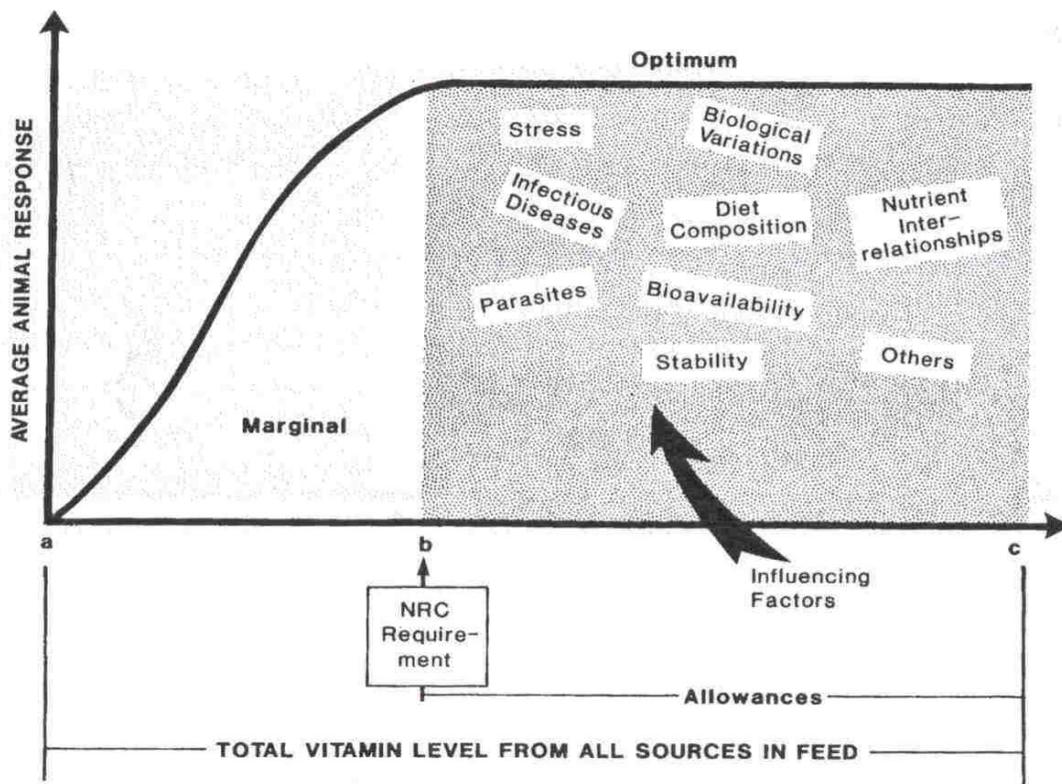
Figure 1

Figure 2