7 Vitamins

Vitamins are classified as either fat-soluble or water-soluble. Vitamins A, D, E, and K are fat-soluble and the B-vitamins and vitamin C are water-soluble. Vitamins have diverse functions including involvement in many metabolic pathways, immune cell function, and gene regulation. A clinical deficiency of a vitamin results in a specific deficiency disease such as rickets when vitamin D is deficient. Subclinical deficiencies may occur in which clinical signs of the deficiency are not evident but performance or overall animal health is less than optimal.

FAT-SOLUBLE VITAMINS

Dairy cattle require vitamins A, D, E, and K; however, vitamins A and E are the only ones with an absolute dietary requirement. Vitamin K is synthesized by ruminal and intestinal bacteria. Vitamin D is synthesized by ultraviolet radiation of the skin. Many natural feedstuffs contain vitamin A precursors and vitamin E, and under certain situations these will not need to be supplemented. However, relying solely on vitamins contained within feedstuffs and on synthesis of vitamin D via exposure to sunlight has risk because of the large variability in vitamin concentrations in feeds and exposure to sunlight. As management systems for dairy cattle trend toward more confinement with less exposure to sunlight and fresh forages, there is an increased need to add supplemental sources of vitamins A, D, and E.

Vitamin A

SOURCES

Vitamin A activity is defined in retinol equivalents. An IU of vitamin A corresponds to 0.3 μg of all-*trans* retinol (0.344 μg of all-*trans* retinyl acetate or 0.550 μg of all-*trans* palmitate). Retinol is not found in plants, but many feeds contain β-carotene (provitamin A). Other carotenoids can be converted to vitamin A by animals, but conversion efficiency appears to be poor and most common feeds do not contain substantial amounts of those carotenoids. Most of the β-carotene in plants is found in vegetative material; therefore, forages can contain substantial amounts of β-carotene but most grains and grain byproducts are practically void of β-carotene (corn gluten meal contains moderate concentrations of β-carotene). Beta-carotene concentrations decrease as forages mature (Park et al., 1983). Beta-carotene is easily oxidized and once plants are cut, concentrations decrease quickly so that stored forages (silage and hay) have significantly lower concentrations of β-carotene than do fresh forage (Bruhn and Oliver, 1978; Park et al., 1983). The length of time forages are stored is negatively correlated with β-carotene concentrations (Bruhn and Oliver, 1978). Even when known sources of variation are considered, the β-carotene concentrations in feedstuffs are highly variable.

The common forms of supplemental vitamin A used in the United States are all-*trans* retinyl acetate and all-*trans* retinyl palmitate. When these forms of vitamin A are stored properly, vitamin A activity is relatively stable with losses of about 1 percent/month. When these retinyl esters are stored in combination with minerals or other feedstuffs or are pelleted, storage losses increase to 5 to 9 percent/month (Coelho, 1991).

BIOAVAILABILITY

Studies on the bioavailability of various forms of vitamin A and β-carotene for dairy cattle are extremely limited. Bioavailability of vitamin A is dependent upon the degree of ruminal destruction and on absorption efficiency by the small intestine. In addition to those factors, the bioavailability of β-carotene also depends on the efficiency of converting it to retinol. Beta-carotene is converted to retinol by enzymes located in intestinal mucosal cells. Dairy cattle also absorb and store β-carotene. Blood and milk of Guernsey and Jersey cattle contain more β-carotene than that from other breeds because they are either more efficient

at absorbing β-carotene or less efficient at converting β-carotene to retinol. The vitamin A activity of β-carotene for cattle is defined as 1 mg of β-carotene = 400 IU of vitamin A (equivalent to 120 μg of retinol), and is much lower for cattle than for rats (1 mg β-carotene = 1800 IU of vitamin A). The defined activity of β-carotene for cattle is based largely on experiments using lambs fed corn silage (Martin et al., 1968).

Ruminal destruction of vitamin A can be extensive. Approximately 60 percent of supplemental vitamin A was destroyed in the rumen of steers fed hay and corn grain diets (Warner et al., 1970). Similar values have been obtained using in vitro rumen systems (Rode et al., 1990; Weiss et al., 1995). In vitro data suggest that ruminal destruction of vitamin A was approximately 20 percent when cattle were fed high forage diets, but it increased to about 70 percent when cattle were fed diets with 50 to 70 percent concentrate. Limited studies with β-carotene suggest that between 0 and 35 percent of dietary β-carotene is destroyed in the rumen (Potanski et al., 1974). Essentially no reliable data are available on the intestinal absorption of retinyl esters in cattle. Data collected from humans and rats suggest 20 to 60 percent of dietary retinyl esters are absorbed (Blomhoff et al., 1991). Absorption in those species is dependent on the amount and type of dietary fat. Apparent digestibility of β-carotene from a variety of forages averaged 77 percent in dairy steers (Wing, 1969), but Cohen-Fernandez et al. (1976) reported that fecal recovery (indigestibility) of radiolabeled β-carotene was about 90 percent in sheep.

FUNCTIONS AND ANIMAL RESPONSES

Vitamin A (retinaldehyde) is necessary for the production of rhodopsin (a vision pigment) that is necessary for low light vision. Vitamin A also is needed for normal growth and development (including fetal growth), spermatogenesis, and for maintenance of skeletal tissue and epithelial tissue. Abortions, increased prevalence of retained fetal membranes, and increased calf morbidity and mortality are indicators of vitamin A deficiency in gestating cows. Ross and Ternus (1993) reported that retinoic acid indirectly regulates gene expression which may explain the many diverse functions of vitamin A. Vitamin A also increases disease resistance and has stimulatory effects on cell-mediated immunity (Chew, 1987; Bendich, 1993). A deficiency of vitamin A often results in increased prevalence of infectious diseases. Beta-carotene, independent of its provitamin A function, is an antioxidant and can enhance the killing ability of neutrophils (Chew, 1993). In some (Chew, 1987) but not all (Michal et al., 1994) studies, supplementing between 150,000 and 250,000 IU/day of vitamin A or feeding 300 to 600 mg of β-carotene/day reduced the incidence of intramammary gland infections and mastitis. These studies were conducted with cows at dry-off or peripartum cows.

Vitamin A is clearly needed for good reproduction and some data suggests that β-carotene also may be involved with reproduction. In a review, Hurley and Doane (1989) reported that supplemental β-carotene (usually at 300 to 400 mg/day) improved some measure of reproductive efficiency in 12 of 22 studies. When studies conducted only in North America were summarized, β-carotene had no effect on reproduction in 4 of 5 studies.

FACTORS THAT AFFECT REQUIREMENTS

Since the actual β-carotene content of diets is highly variable and almost never known in commercial situations, the vitamin A requirements presented in this publication are for supplemental vitamin A, not total dietary vitamin A. Fresh forage (e.g., pasture) has relatively high concentrations of β-carotene. Therefore the amount of supplemental vitamin A needed when fresh forage is fed will be less than for cattle consuming conserved forages. The requirements presented below assume conserved forages are fed and are probably in excess of requirements for grazing cattle.

Based on a reevaluation of older data, the vitamin A requirement for growing dairy animals was increased to 80 IU/kg of body weight (BW). In the previous *Nutrient Requirements of Dairy Cattle* (National Research Council, 1989), the requirement for vitamin A of growing dairy animals was 42 IU/kg of BW. That requirement for growing cattle was based on the amount of vitamin A needed to maintain cerebrospinal fluid pressure below 120 mm Hg in calves (Rousseau et al., 1954). Other data (Rousseau et al., 1954; Eaton et al., 1972) using different criteria (i.e., a statistically significant increase in cerebrospinal fluid pressure or the presence of papillary edema of the eye) suggests that the vitamin A requirement for growing dairy animals was between 60 and 100 IU/kg of BW. The subcommittee decided that rather than discounting these studies, a compromise using all the data was appropriate.

The vitamin A requirement for adult dairy cattle has been increased to 110 IU/kg of BW. In *Nutrient Requirements of Dairy Cattle* (National Research Council, 1989), the vitamin A requirement for adult animals (76 IU/kg of BW) was based largely on a long-term reproduction study (Ronning et al., 1959). The sole source of vitamin A in that study was β-carotene, and cows were fed low concentrate diets. The previous requirement (National Research Council, 1989) also was based on data from a study (Swanson et al., 1968) that indicated the amount of vitamin A deemed adequate by Ronning et al. (1959) was adequate to maintain milk production. In that study, cows produced approximately 3500 kg of milk during a 40-week lactation. Mean milk production is currently about twice as high and many

herds have mean milk production that is more than four times higher. Furthermore, in a more recent study, milk production increased from about 35 kg/day to 40 kg/day when cows in early lactation were fed diets that provided approximately 280 IU of vitamin A/kg of BW compared with cows fed approximately 75 IU/kg of BW (Oldham et al., 1991). The new requirement for lactating cows (110 IU/kg of BW) was based on data used by the previous *Nutrient Requirements of Dairy Cattle* (National Research Council, 1989) and on data showing that the bioavailability of vitamin A (retinyl esters) may be as much as 50 percent less than that of β-carotene when fed in high concentrate diets because of ruminal destruction. Dry cows are typically fed diets with lower amounts of concentrate and bioavailability of vitamin A should be higher than for lactating cows. The previous National Research Council requirement for dry cows (76 IU/kg of BW) may be adequate, but in light of potential improvements in mammary gland health and data showing increased milk production after dry cows were supplemented with vitamin A in amounts greater than National Research Council (1989) requirements, the vitamin A requirement for dry cows was kept the same as that for lactating cows (110 IU/kg of BW).

Presently available data are not adequate to define a specific requirement for β-carotene for any class of dairy cattle. Conditions that may warrant additional supplementation of vitamin A include:

- low forage diets (more ruminal destruction and less consumption of β-carotene);
- diets that contain larger amounts of corn silage and smaller amounts of haycrops (lower concentrations of β-carotene and potentially lower bioavailability of basal β-carotene);
- diets that contain lower quality forages (lower basal concentrations of β-carotene);
- increased exposure to infectious pathogens (increased demands on the immune system); and
- times when immunocompetence maybe reduced (peripartum period).

Vitamin A toxicosis should not be a problem under most practical situations. The presumed safe limit for vitamin A is 66,000 IU/kg of diet for both lactating and nonlactating cattle (National Research Council, 1987).

Vitamin D

PHYSIOLOGY

Vitamin D is a pro-hormone, a necessary precursor for the production of the calcium regulating hormone 1,25-dihydroxyvitamin D. Vitamin D can be produced within the skin of most mammals, including cattle, as a result of the photochemical conversion of 7-dehydrocholesterol to vitamin D_3. In plants, ultraviolet irradiation causes photochemical conversion of ergosterol to vitamin D_2. Vitamin D, supplied by the skin or the diet, is rapidly transported to and sequestered by the liver. The rapid removal of vitamin D from circulation prevents concentrations of vitamin D in blood from becoming very high; the normal concentration is 1 to 2 ng vitamin D/ml plasma (Horst and Littledike, 1982). Within the liver, vitamin D can be converted to 25-hydroxyvitamin D by vitamin D 25-hydroxylase and released into the blood. The production of 25-hydroxyvitamin D within the liver is dependent on the vitamin D content of the diet. Thus plasma 25-hydroxyvitamin D concentration is the best indicator of vitamin D status of an animal (Horst et al., 1994).

The 25-hydroxyvitamin D then circulates to the kidney where it can be converted to the hormone 1,25-dihydroxyvitamin D. This hormone acts to increase the active transport of calcium and phosphorus across the intestinal epithelial cells, and potentiates the action of parathyroid hormone to increase bone calcium resorption. Both functions are vital for calcium and phosphorus homeostasis. In addition to calcium and phosphorus homeostasis, 1,25-dihydroxyvitamin D is involved in maintaining immune function (Reinhardt and Hustmyer, 1987); generally it promotes Th_2 (humoral) immunity while inhibiting Th_1 (cell mediated) immunity (Daynes et al., 1995).

Renal production of 1,25-dihydroxyvitamin D is tightly regulated. The 25-hydroxyvitamin D-1-α-hydroxylase activity of the kidney is stimulated by parathyroid hormone, which is released in response to declining concentrations of calcium in blood (DeLuca, 1979). In the absence of parathyroid hormone, when an animal is in positive Ca balance, 25-hydroxyvitamin D can be hydroxylated in the kidney to 24,25-dihydroxyvitamin D as a primary step in the inactivation and catabolism of vitamin D. The vitamin D catabolic enzymes also function to deactivate 1,25-dihydroxyvitamin D. These catabolic enzymes exist in tissues throughout the body. In these tissues the catabolic pathway is generally stimulated by 1,25-dihydroxyvitamin D as a negative feedback to reduce high concentrations of 1,25-dihydroxyvitamin D in plasma (Goff et al., 1992; Reinhardt and Horst, 1989).

A low concentration of phosphorus in blood also can enhance renal production of 1,25-dihydroxyvitamin D, even when the concentration of calcium in plasma is normal or above normal (Tanaka and DeLuca, 1973; Gray and Napoli, 1983). Also, higher than normal concentrations of phosphorus in blood can inhibit renal production of 1,25-dihydroxyvitamin D, which can be a factor contributing to milk fever in the periparturient cow (Barton et al., 1987). Pharmacologic doses of vitamin D have been utilized with limited success to prevent milk fever. This is discussed in the section on milk fever (Chapter 9).

Vitamin D_2, the form associated with plants, and vitamin D_3, the form associated with vertebrates are both used for supplementation of diets. The biologic activity of the two forms is generally considered equal in cattle; however Horst and Littledike (1982) demonstrated an apparent discrimination against the vitamin D_2 form in cattle. Presumably this discrimination is the result of reduced binding of vitamin D_2 metabolites to vitamin D-binding proteins in blood leading to more rapid clearance of vitamin D_2 metabolites from plasma. However, the subcommittee does not recommend adjusting the vitamin D requirement based on the form of vitamin D used as a supplement.

Vitamin D deficiency reduces the ability to maintain calcium and phosphorus homeostasis, resulting in a decline for phosphorus and less often a decrease for calcium in plasma. This eventually causes rickets in young animals and osteomalacia in adults; both are bone diseases in which the primary lesion is failure to mineralize the organic matrix of bone. In young animals rickets causes enlarged and painful joints; the costochondral joints of the ribs are often readily palpated. In adults, lameness and pelvic fracture are a common sequelae of vitamin D deficiency.

REQUIREMENT

The amount of dietary vitamin D required to provide adequate substrate for production of 1,25-dihydroxyvitamin D is difficult to define. Animals exposed to sunlight at the lower latitudes may not require any dietary vitamin D. Sun-cured hay also may provide enough vitamin D to prevent symptoms of vitamin D deficiency (Thomas and Moore, 1951).

The movement away from pasture feeding systems and toward confinement and feeding of stored feeds and by-products has increased the need for dietary supplementation of vitamin D for dairy cows. As a general rule, the contribution of sunlight and forage to the supply of vitamin D for the cow is not considered when describing the vitamin D requirement. The vitamin D requirement in this publication will consider the ''requirement'' to be the amount of supplemental vitamin D that should be added to the diet.

Horst et al. (1994) determined that plasma 25-hydroxyvitamin D concentrations below 5 ng/ml are indicative of vitamin D deficiency and concentrations of 200 to 300 ng/ml would indicate vitamin D toxicosis. Normal cows have concentrations of 25-hydroxyvitamin D in plasma between 20 and 50 ng/ml.

Dry, pregnant cows housed indoors and fed a corn silage based diet had plasma concentrations of 25-hydroxyvitamin D in plasma of 19 ng/ml at 14 days prior to parturition and 10.5 ng/ml at 35 days into lactation. Supplementation of the diet with 5,000 (7.5 IU vitamin D/kg BW) or 10,000 IU vitamin D (15 IU vitamin D/kg BW) maintained plasma concentrations between 25 and 31 ng/ml throughout the dry period and early lactation (Vinet et al., 1985).

Ward et al. (1971) reported that cows fed an alfalfa hay-concentrate diet receiving 300,000 IU vitamin D_3 once each week ($\approx$ 43,000 IU/day) returned to estrus 16 days earlier than cows given no supplement. Ward et al. (1972) also demonstrated that cows receiving 300,000 IU vitamin D_3/week had improved absorption of dietary calcium. Hibbs and Conrad (1983) summarized the results of several Ohio State University trials and concluded that cows supplemented with 40,000 IU vitamin D_2/day (50 to 70 IU vitamin D/kg BW) produced more milk and generally ate more than cows fed the same diets with no vitamin D supplementation or supplemented with 80,000 or more IU vitamin D/day. Reduced milk production, which could be interpreted as the beginning of vitamin D intoxicosis, was observed when cows were fed 80,000 IU vitamin D/day (120–140 IU/kg BW).

McDermott et al. (1985) fed an orchard grass-corn silage based ration supplemented with 0, 10,000, 50,000, or 250,000 IU vitamin D_3/day to Holstein cows in late gestation and for the first 12 weeks of lactation. Cows had no access to sunlight from 2 weeks before calving until 4 days postpartum. Thereafter they were outside and exposed to sunlight 1 to 2 h/day. Plasma 25-hydroxyvitamin D concentrations in unsupplemented cows were below 20 ng/ml during late gestation and the first 4 weeks of lactation. Plasma 25-hydroxyvitamin D concentrations in cows receiving 10,000 or 50,000 IU vitamin D_3/day (16–80 IU/kg) were similar (between 30 and 45 ng 25-hydroxyvitamin D/ml). Cows receiving 250,000 IU vitamin D/day had elevated plasma 25-hydroxyvitamin D concentrations (60–80 ng/ml). The rapid changes in plasma concentrations of 25-hydroxyvitamin D, 24,25-dihydroxyvitamin D, and vitamin D suggested that at 250,000 IU/day the capacity of the liver to store vitamin D had been exceeded, which was interpreted as excessive vitamin D supplementation though no outward clinical signs of vitamin D intoxication were noted.

Under most circumstances 10,000 IU/day (16 IU vitamin D/kg BW) should provide adequate vitamin D for dairy cows during late gestation. Astrup and Nedkvitne (1987) reported that lactating cows producing about 20 kg of milk/day required about 10 IU vitamin D/kg body weight to maintain normal concentrations of calcium and phosphorus in blood. These studies were conducted in Norway in winter and spring when effective sunlight exposure should have been minimal.

The 1989 *Nutrient Requirements of Dairy Cattle* (National Research Council, 1989) requirement for vitamin D for adult dairy cows was set at 30 IU/kg body weight. This is more vitamin D than many studies suggest is necessary for maintenance of normal plasma concentrations of 25-hydroxyvitamin D (17 IU/kg BW) (McDermott et al.,

1985) or calcium and phosphorus (10 IU/kg BW) (Astrup and Nedkvitne, 1987) in plasma. However, Ward et al. (1971, 1972) and Hibbs and Conrad (1983) suggested that milk production and reproductive and health benefits were potentially improved when diets were supplemented with as much as 70 IU/kg BW. Based on all available data, the requirement of 30 IU/kg BW established previously (National Research Council, 1989) seems justified.

TOXICITY

The maximum tolerable amount of vitamin D is inversely related to dietary concentrations of calcium and phosphorus. The studies of McDermott et al. (1985) suggest that 50,000 IU D_3/day (80 IU/kg BW) is well tolerated while 250,000 IU vitamin D_3/day (400 IU/kg BW) is not. Hibbs and Conrad (1983) reported a slight decline in milk production when cows were fed 80,000 IU D_2/day ($\approx$ 160 IU/kg BW). The 1987 National Research Council committee on vitamin tolerance of animals (National Research Council, 1987) suggested the maximal tolerable level of vitamin D is 2,200 IU/kg diet when fed for long periods (more than 60 days) and 25,000 IU/kg diet when fed for short periods of time. Vitamin D intoxication is associated with reduced feed intake, polyuria initially followed by anuria, dry feces, and reduced milk production. Upon necropsy calcification of kidneys, aorta, abomasum, and bronchioles is evident (Littledike and Horst, 1980).

Some of the dietary vitamin D is degraded in the rumen by bacteria to inactive metabolites (Sommerfeldt et al., 1983; Gardner et al., 1988). Injection of vitamin D avoids this problem; however, the maximal tolerable dose of parenterally administered vitamin D is at least 100-fold lower than the maximal tolerable oral dose and repeated injections can be especially toxic (Littledike and Horst, 1980).

Vitamin E

SOURCES

Vitamin E is a generic name for a series of lipid-soluble compounds called tocopherols and tocotrienols. The most biologically active form of vitamin E is ∝-tocopherol; it is also the most common form of vitamin E found in feedstuffs. Eight different stereoisomers of ∝-tocopherol can exist, and the isomer that has the highest biologic activity is *RRR*-∝-tocopherol. The vitamin E content of feedstuffs is highly variable (coefficients of variation are often 50 percent). Depending on species and maturity (Tramontano et al., 1993; Jukola et al., 1996), fresh forage plants contain between 80 and 200 IU of vitamin E/kg of DM. Alpha-tocopherol concentrations in forages decrease quickly after the plant is cut; prolonged exposure to oxygen and sunlight exacerbates the loss in vitamin E activity (Thafvelin and Oksanen, 1966). Silage and hay contain 20 to 80 percent less ∝-tocopherol than does fresh forage. In general, concentrations of vitamin E in concentrates are low; possible exceptions are raw whole soybeans and whole cottonseeds. Heat treatment of whole soybeans destroys essentially all the ∝-tocopherol (Weiss, unpublished). Alpha-tocopherol concentrations in feeds generally decrease as storage time increases.

The commercial form of supplemental vitamin E usually fed to dairy cows is all-*rac*-∝-tocopheryl acetate (previously designated DL-∝-tocopheryl acetate). The esterified form of the vitamin is more stable than the alcohol form; expected losses in biologic activity from premixes containing all-*rac*-∝-tocopheryl acetate are less than 1 percent per month under most storage conditions, but extruded products containing all-*rac*-∝-tocopheryl acetate may have storage losses of 6 percent per month (Coelho, 1991). *RRR*-∝-tocopherol is available commercially but is not commonly fed to ruminants.

BIOAVAILABILITY

Early data (Alderson et al., 1971) suggested that significant amounts of supplemental vitamin E were destroyed in the rumen and that destruction increased as the amount of concentrate in the diet increased. More recent studies (Leedle et al., 1993; Weiss et al., 1995) found that vitamin E (all-*rac*-∝-tocopheryl acetate) was not destroyed during in vitro fermentation. The authors of these studies suggested that poor extraction of tocopherol from digesta was the reason early reports indicated that vitamin E was destroyed by ruminal fermentation. Based on the new data using better analytic techniques, ruminal metabolism of vitamin E appears minimal.

The USP defines 1 IU of vitamin as equal to 1 mg of all-*rac*-∝-tocopheryl acetate, and 1.49 IU of vitamin E is equal to 1 mg of *RRR*-∝-tocopherol. Those conversion factors are based largely on research with laboratory animals. Data from cows comparing bioavailability of various tocopherol stereoisomers is contradictory. Hidiroglou et al. (1988, 1989) reported no or only slight differences in concentrations of ∝-tocopherol in plasma and tissue between cows and heifers fed similar IU amounts of vitamin E as all-*rac*-∝-tocopherol or all-*rac*-∝-tocopheryl acetate. Concentrations of ∝-tocopherol in tissue and plasma were 20 to 60 percent higher in beef cows fed *RRR*-∝-tocopherol than those fed all-*rac*-∝-tocopheryl acetate (Hidiroglou et al., 1988). Based on that study 1 mg of *RRR*-∝-tocopherol would be equal to 1.8 to 2.4 IU of vitamin E. Different formulations of all-*rac*-∝-tocopheryl acetate (silica adsorbate, oil, or a microencapsulated form) did not greatly affect concentrations of ∝-tocopherol in plasma suggesting equivalent bioavailability (Baldi et al., 1997). Insufficient consistent data are available currently to war-

rant changing IU conversion factors for vitamin E for ruminants. The current USP factors will be used for describing vitamin E requirements in this publication.

FUNCTIONS AND ANIMAL RESPONSES

The best understood function of vitamin E is as a lipid-soluble cellular antioxidant (Hogan et al., 1993). Via this function and perhaps other functions, vitamin E is involved in maintenance of cellular membranes, arachadonic acid metabolism, immunity, and reproductive function.

White muscle disease is a classic sign of a clinical deficiency of vitamin E. White muscle disease was prevented in preweaned calves when 50 IU of vitamin E/day (all-*rac*-∝-tocopheryl acetate) were supplemented to a vitamin E-free diet based on skim milk (Blaxter et al., 1952). Presumably those data were used to formulate the vitamin E requirements for all classes of dairy cattle in the last *Nutrient Requirements of Dairy Cattle* (National Research Council, 1989). More recent experiments with vitamin E have focused on its relationship with reproductive disorders, mastitis, and immune function.

Dietary or parenteral supplementation of vitamin E to dairy cows during the peripartum period has consistently improved the function of neutrophils and macrophages (Hogan et al., 1990, 1992; Politis et al., 1995, 1996). In those studies, the amount of supplemental vitamin E fed per day during the prepartum period was either 1000 IU/day or 3000 IU/day. In three of those studies (Hogan et al., 1992; Politis et al., 1995, 1996) vitamin E also was injected (3000 or 6000 IU on approximately day 7 prepartum). During the postpartum phase, cows were fed either 500 or 3000 IU/day of supplemental vitamin E. Cows in all studies were fed stored forages.

Clinical studies have been conducted to evaluate the effect of supplemental vitamin E on prevalence of retained fetal membranes, intramammary infections, and clinical mastitis. Feeding approximately 1000 IU/day of supplemental vitamin E (usually all-*rac*-∝-tocopheryl acetate) to dry cows when adequate selenium was supplemented reduced the prevalence of retained fetal membranes in some (Harrison et al., 1984; Miller et al., 1993) but not all (Wichtel et al., 1996) studies. When vitamin E was injected (usually in combination with selenium) rather than fed, about half the time there was no effect for prevalence of retained fetal membranes and about half the time there was a positive response (Miller et al., 1993). The typical treatment was a single injection of approximately 700 IU of vitamin E and 50 mg of selenium given about 3 weeks before calving. Relative to the amount of vitamin E normally consumed, 700 IU of vitamin E over 21 days is trivial. Most likely, selenium, not vitamin E, was the nutrient responsible for the positive effect.

Two clinical studies conducted in Ohio (Smith et al., 1984; Hogan et al., 1993) reported that feeding supplemental vitamin E significantly reduced the incidence and duration of intramammary gland infections and clinical mastitis. In those studies, approximately 1000 IU/day of supplemental vitamin E was fed during the 60-day prepartum period and 500 IU/day was fed during lactation. Conversely, a study conducted in Canada (Batra et al., 1992) found that similar amounts of supplemental vitamin E did not reduce the incidence of clinical mastitis. Based on the concentrations of selenium in the plasma (<35 ng/ml), cows in that study (Batra et al., 1992) were deficient in selenium. Another study (Weiss et al., 1997) using diets low in total selenium (0.15 ppm) but with animals in better selenium status (plasma selenium >50 ng/ml) than cows in the Batra et al. (1992) study found that feeding 1000 IU/day of vitamin E during the dry period reduced clinical mastitis at calving by 30 percent but did not affect prevalence of intramammary gland infections. In that same study, feeding 4000 IU of supplemental vitamin E/day during the last 2 weeks of the dry period resulted in an 80 percent reduction in clinical mastitis at calving and a 60 percent reduction in intramammary gland infections (Weiss et al., 1997).

REQUIREMENTS AND FACTORS THAT AFFECT REQUIREMENTS

The vitamin E requirement (15 IU/kg of DMI) in the previous *Nutrient Requirements of Dairy Cattle* (National Research Council, 1989) was for total, not supplemental, vitamin E and the basis for that requirement was not given. The previous vitamin E requirement should prevent classic signs of vitamin E deficiency. The vitamin E content of the basal diet is highly variable and will not be known in most situations; therefore, vitamin E requirements in this edition are presented for supplemental vitamin E. The requirements presented assume that cattle are consuming conserved forages. Because fresh forage is an excellent source of vitamin E the requirements for supplemental vitamin E for grazing cattle are probably substantially less than those presented for cattle fed conserved forages. Because titration studies are lacking, a specific requirement cannot be defined. The subcommittee concluded that there were adequate data available on the effect of vitamin E on mastitis and reproductive disorders to justify an increase in the vitamin E requirement. Based on health and immune function in cows, plasma concentrations of ∝-tocopherol in peripartum cows should be approximately 3 μg/ml (Weiss et al., 1994, 1997). To maintain these blood values, dry cows and heifers fed stored forages during the last 60 days of gestation require approximately 1.6 IU of supplemental vitamin E/kg of body weight (approximately 80 IU/kg of DMI). An additional benefit on calf health may be observed by increasing vitamin E intake by cows and heif-

ers in late gestation. Only minor amounts of vitamin E can pass the placenta (Van Saun et al., 1989); hence newborn calves rely on colostrum for vitamin E. Increased intake of vitamin E during the prepartum period significantly elevates vitamin E in colostrum. For lactating cows, the recommended amount of vitamin E (supplemental) was changed to 0.8 IU/kg of body weight (approximately 20 IU/kg of DMI) when stored forages are fed. This recommendation is based on a reduction in mastitis. The difference between the recommendations for vitamin E for the two classes of cattle is mainly caused by expected differences in intake of vitamin E from basal feedstuffs and perhaps reduced absorption of vitamin E by cows fed conventional dry cow diets. Based on typical feed intakes and average vitamin E concentrations in feedstuffs, the recommended amount of total vitamin E (supplemental plus vitamin provided by feedstuffs) is approximately 2.6 IU/kg of body weight during late gestation and for lactating dairy cows. Of that amount, the basal diet will provide on average about 1.8 IU/kg of body weight for lactating cows (ranges from about 0.8 for cows fed diets based on severely weathered hay to about 2.8 IU/kg of body weight for cows fed diets based on pasture) and about 1 IU/kg body weight (ranges from 0.5 to about 2.3 IU/kg of body weight) for dry cows.

Although several factors are known to influence vitamin E requirements, limited data make quantifying the necessary adjustments difficult. The amount of supplemental vitamin E fed may need to be changed during the following situations:

- When fresh forage is fed there should be less need for supplemental vitamin E. A diet based on fresh forage (ca. 50 percent of dietary DM) would require about 67 percent less supplemental vitamin E to meet the cows requirements compared with a diet that contained a similar amount of stored forage. Requirements for supplemental vitamin E is reduced 67 percent in the accompanying software when animals are fed pasture.
- The amount of supplemental vitamin E probably should be increased when low forage diets are fed (forages typically have more vitamin E than do concentrates). The requirements listed above were generated from studies using diets with 50 to 60 percent forage (lactating cows) and 60 to 80 percent forage (animals in late gestation).
- Cows in suboptimal selenium status probably require more vitamin E.
- Milk is not a major excretion route for ∝-tocopherol (0.4 to 0.6 μg/ml) but colostrum contains high concentrations of ∝-tocopherol (3 to 6 μg/ml). Additional vitamin E may be useful during colostralgenesis.
- Intake of polyunsaturated fatty acids increases the vitamin E requirement of nonruminants. As methods for protecting fats from biohydrogenation in the rumen improve, additional vitamin E may be required when protected unsaturated fats are fed.
- Additional vitamin E may be useful during periods of immunosuppression (peripartum period).
- Large amounts of supplemental vitamin E (>1000 IU/day) can reduce oxidative flavors in milk (St.-Laurent et al., 1990).

TOXICITY

Vitamin E is one of the least toxic vitamins due in part to its relatively low absorption. Toxicity studies have not been conducted with ruminants but data from rats suggest an upper limit of approximately 75 IU/kg of body weight per day (National Research Council, 1987).

Vitamin K

Vitamin K is a generic term used to describe a group of quinone compounds exhibiting antihemorrhagic effects. The basic form of vitamin K is 2-methyl-1,4-naphthoquinone. Isomers of vitamin K differ in the length and nature of the side chain (Frye et al., 1991). The three most common isomers or vitamers of K are: phylloquinone (vitamin K_1), menaquinones (vitamin K_2) and menadione (vitamin K_3). The phylloquinones are commonly found in the chloroplast of green plants and have side chains consisting of several isoprenoid units. Menaquinones are synthesized by bacteria flora and have isoprene side chains containing double bonds. Menadione (2-methyl-1,4-napthoquinone without a side chain) does not exist naturally. Menadione and its derivatives are the synthetic forms of vitamin K used in feed supplements (Combs, 1992).

Cattle require vitamin K for the synthesis of at least a dozen proteins. Among these are four blood clotting factors; prothrombin (factor II), and factors VII, IX and X. These vitamin K dependent protein factors are components of a complex system that functions to prevent hemorrhage by activation of thrombin and ultimately clot formation (Combs, 1992).

Because large quantities of menaquinones are synthesized by ruminal bacteria and ruminant diets generally contain green forages and/or pasture plants high in phylloquinones, a deficiency of vitamin K rarely occurs. The only reported deficiencies have occurred when moldy sweet clover hay was fed (National Research Council, 1989). Dicoumarol is a fungal metabolite produced from substances in sweet clover that inhibits the synthesis of clotting factors. Holstein calves were shown to develop dicoumarol toxicosis when fed sweet clover hay containing 18 mg/kg of dicoumarol for two weeks or longer (Yamini et al., 1995). Early signs of vitamin K deficiency include stiffness and/or lameness and hematoma of tissues. Prolonged feeding of dicoumarol leads to uncontrolled bleeding. Dicoumarol

can pass placental barriers resulting in the fetus or newborn animals being affected (Frye et al., 1991). Vitamin K_3 was found to be ineffective in preventing the anticoagulant effects of dicoumarol (Casper et al., 1989).

Toxicity data for either naturally occurring or synthetic forms of vitamin K are extremely limited. For humans and laboratory animals the presumed upper safe level for oral ingestion of menadione (K_3) is 1,000 times the dietary requirement (National Research Council, 1987), but toxicity data for cattle are not available.

WATER-SOLUBLE VITAMINS

Ruminal microorganisms synthesize most water-soluble vitamins (biotin, folic acid, niacin, pantothenic acid, pyridoxine, riboflavin, thiamin, and vitamin B_{12}) and common feedstuffs generally contain high concentrations of most of those vitamins. Vitamin C is synthesized by ruminant animals. True deficiencies of these vitamins are rare in animals with a functional rumen. To date, a limited amount of research has been conducted on most water-soluble vitamins (niacin is the exception) for adult cattle; however, research in this area has increased during the past few years. Adequate data to quantify bioavailability, ruminal synthesis, and requirements for most water-soluble vitamins are not available. Deficiency diseases for most B vitamins can be induced when preruminant calves are fed synthetic diets but are rare when calves are fed milk. Milk replacers should be supplemented with B vitamins as described in Chapter 10.

B-VITAMINS

Biotin

Biotin acts as a cofactor for many enzymes involved in carboxylation reactions. Ruminal bacteria normally synthesize biotin and concentrations of the vitamin may exceed 9 μg/L of strained ruminal fluid (Briggs et al., 1964). Biotin is not extensively metabolized in the rumen and increased intake of dietary biotin results in elevated concentrations of biotin in serum and milk (Frigg et al., 1993; Midla et al., 1998). Unpublished epidemiologic data suggest a negative relationship between serum concentrations of biotin and the incidence of clinical lameness in dairy cattle. In controlled long-term field studies, feeding approximately 20 mg/day of supplemental biotin statistically improved measures of hoof health (Bergsten et al., 1999; Fitzgerald et al., 2000; Midla et al., 1998). However, insufficient data are available at this time to quantify the requirement for biotin of dairy cattle.

Folic Acid

Folic acid containing coenzymes are involved in movement of one-carbon units in biochemical pathways. Methionine also serves as a methyl donor; therefore, folic acid may spare methionine. Folic acid is necessary for the synthesis of nucleic acids. Growth rate and hematologic responses have been used to access adequacy in animal studies. Microbial degradation of supplemental folic acid can be extensive (Zinn, 1987). Consequently, parenteral administration of folic acid is usually used to examine responses to supplemental folic acid.

Weekly intramuscular injections of 40 mg folic acid from 45 days after mating until 6 weeks after parturition did not alter blood parameters or influence calf birth weight, therefore, dietary folic acid and microbial synthesis of this vitamin appear to supply sufficient amounts to prevent deficiency symptoms in adult dairy cattle (Girard et al., 1995). Young calves that do not have a completely developed ruminal microflora may be most susceptible to folic acid deficiency. Calves given weekly intramuscular injections of 40 mg of folic acid from 10 days of age until 16 weeks of age increased average daily gain by 8 percent during the 5 weeks following weaning (approximately 7 to 12 weeks of age; Dumoulin et al., 1991). Treatment also increased serum folates, blood hemoglobin, and packed cell volume suggesting that folic acid may be deficient in young calves.

Parenteral supplementation of 160 mg of folic acid each week from 45 days of gestation until 6 weeks postpartum tended to increase milk and milk protein production during mid to late lactation of primi- and multiparous cows. After calving, milk protein percentage was increased in multiparous cows only during the first 6 weeks of lactation (Girard et al., 1995). Milk production during days 1 to 200 of lactation was increased linearly for multiparous cows but not for primiparous cows when 0, 2, or 4 mg folate/kg BW were fed (Girard and Matte, 1998). Blood folates were increased indicating that some dietary folic acid escaped ruminal degradation. Deficiency symptoms for folate have not been observed in lactating dairy cattle. The increased milk production observed when supplemental folate was fed may be a direct response to the vitamin or may be an indirect response caused by sparing methionine. Insufficient data are currently available to quantify the folic acid requirement of cattle.

Inositol

Inositol is an important nutrient in the metabolism and transport of lipids, and is a constituent of phospholipids, and has lipotropic activity. Myo-inositol is found in feeds as a component of phytic acid (Gerloff et al., 1984). Because phytic acid can be degraded in the rumen, deficiencies of

inositol are not likely to occur. However, during periods of hepatic lipidosis or fatty liver syndrome where feed intakes may be low, supplementation of inositol has been investigated as an aid to help minimize triglyceride accumulation in the liver. Gerloff et al. (1984) in a field study involving 80 multiparious cows reported that the lipid content of liver was not decreased by feeding 17 grams of nonphytate myo-inositol for one month pre- and postpartum. Similarly, Grummer et al. (1987) indicated that neither milk production nor milk fat percentage were increased with abomasal infusion of 37 grams of myo-inositol.

Dietary requirements for inositol have not been demonstrated in dairy animals with normal rumen activity. Bacterial synthesis in the rumen and/or the amounts in feeds apparently supply adequate amounts to meet metabolic requirements. The previous edition of *Nutrient Requirements of Dairy Cattle,* (National Research Council, 1989) cited research from 1940 and 1950 showing deficiencies in calves after several weeks when fed purified or semipurified diets. Because dairy products are generally good sources of these vitamins (Combs, 1992) and milk replacers are fortified with additional amounts (Tomkins and Jaster, 1991), deficiencies are unlikely to occur under typical calf raising practices.

Niacin

Niacin is a generic name for pyridine 3-carboxylic acids and their derivatives that demonstrate activity similar to the amide form (i.e., nicotinamide). Niacin functions as a coenzyme for the pyridine nucleotide electron carriers NAD(H) and NADP(H). Consequently, niacin plays a critical role in mitochondrial respiration and the metabolism of carbohydrates, lipids, and amino acids.

Net synthesis of niacin in the rumen is likely because supply of niacin to the intestine exceeds intake when unsupplemented diets are fed to cattle (Zinn et al., 1987). Rate of niacin synthesis may be inversely related to level of supplementation (Abdouli and Schaefer, 1986b). During supplementation, the amount of niacin reaching the intestine may be less than that fed, indicating niacin degradation or absorption from the rumen (Zinn et al., 1987). Niacin absorption from the rumen appears to be low, particularly for nicotinic acid (Erickson et al., 1991). Feeding supplemental niacin increases concentrations in ruminal and duodenal fluid, which suggests that some supplemental niacin reaches the small intestine (Zinn et al., 1987; Campbell et. al., 1994). Estimates are that 17 to 30 percent of supplemental niacin reaches the small intestine (Harmeyer and Kollenkirchen, 1989; Campbell et al., 1994). Nicotinamide is rapidly converted to nicotinic acid in the reticulorumen (Harmeyer and Kollenkirchen, 1989; Campbell et al., 1994).

Niacin may increase microbial protein synthesis (Shields et al., 1983; Riddell et al., 1980, 1981); however, several studies indicate no effects (Hannah and Stern, 1985; Abdouli and Schaefer, 1986a; Doreau and Ottou, 1996). Differences between these studies, all of which utilized in vitro systems, may reflect the amount and availability of niacin in the unsupplemented diet, the niacin status of the microbes, or both. When niacin was fed in combination with other B-vitamins to feedlot calves (Zinn et al., 1987), or nicotinic acid or nicotinamide was fed to lactating cows (Doreau and Ottou, 1996), there were no treatment effects on microbial flow to the intestine.

Niacin is required in the diet of preweaned calves. Calves fed synthetic milk that was deficient in niacin developed scours within 48 hours (Hopper and Johnson, 1955). Immediate improvement was observed on the day following oral (6 mg/head/day) or intramuscular (10 mg/head/day) nicotinic acid administration. Niacin supplementation did not improve growth rates of heifers that began on trial at approximately 110 or 370 kg (Riddell et al., 1981).

A total of 30 treatment comparisons from peer reviewed literature (Fronk et al., 1980; Kung et al., 1980; Riddell et al., 1981; Dufva et al., 1983; Jaster et al., 1983a,b; Horner et al., 1986, 1988; Muller et al., 1986; Skaar et al., 1989; Driver et al., 1990; Erickson et al., 1990, 1992; Martinez et al., 1991; Lanham et al., 1992; Zimmerman et al., 1992; Bernard et al., 1995; Ottou et al., 1995; Madison-Anderson et al., 1997; Minor et al., 1998) were summarized to examine niacin effects on lactation; a significant increase or decrease was declared if $P < 0.05$. One comparison indicated a significant increase in milk production and 29 comparisons indicated no significant change in milk production. For the fourteen comparisons in which niacin administration began prepartum or prior to two weeks postpartum, none indicated a positive response. The absence of a response in many trials may be the consequence of inadequate replication. The only positive milk yield response was from one of the two field trials that have utilized large animal numbers (Muller et al., 1986). The numbers of significant positive, neutral, or significant negative responses were 3, 26, and 1 for milk fat percentage and 5, 20, and 2 for milk protein percentage.

A similar summary by Erdman (1992) indicated that average milk yield response was 0.3 kg/day; 0.4 kg/day if studies were restricted to those in which niacin supplementation began prepartum. A summary of 23 to 30 treatment comparisons by Drackley (1992) indicated that average milk production was increased by 0.62 kg/day and milk fat and protein were increased by 0.033 and 0.002 percentage units when supplemental niacin was fed. Summaries by Erdman (1992) or Drackley (1992) indicated that milk production was decreased 1.1 or 0.42 kg/day when niacin was added to diets that contained supplemental fat. However, there have been no significant ($P < 0.05$) interactions

between fat and niacin for milk yield in the eight studies that have tested for interactions (Horner et al., 1986; Skaar et al., 1989; Driver et al., 1990; Martinez et al., 1991; Erickson et al., 1992; Lanham et al., 1992; Ottou et al., 1995; Madison-Anderson et al., 1997).

Niacin is antilipolytic and has been examined closely as a feed additive to prevent or treat fatty liver and ketosis. Early studies indicated that small (12 g/day until negative milk acetone test; Fronk and Schultz, 1979) or large (160 grams over 8 hours; Waterman et al., 1972) pharmacologic doses of niacin reduced blood ketones in ketotic cows. In these studies, there were no ketotic cows assigned to a control (no niacin) treatment. Consequently, niacin effects were confounded with time. A slug dose of 12 or 120 grams of niacin decreased plasma concentrations of nonesterified fatty acids but not beta-hydroxybutyrate (Jaster et al., 1983a). A summary of 14 treatment comparisons in which niacin was fed (Fronk et al., 1980; Dufva et al., 1983; Jaster et al., 1983a; Skaar et al., 1989; Driver et al., 1990; Erickson et al., 1990; Martinez et al., 1991; Erickson et al., 1992; Zimmerman et al., 1992; Bernard et al., 1995; Chilliard and Ottou, 1995; Ottou et al., 1995; Minor et al., 1998) indicated plasma nonesterified fatty acids were significantly reduced once, increased twice, and not altered 11 times. If restricted to studies in which niacin was fed prepartum or within two weeks postpartum, plasma nonesterified fatty acids were significantly reduced once, increased twice, and not altered 8 times. In 10 comparisons (9 of which niacin treatment began prepartum or prior to two weeks postpartum) plasma ketones were significantly reduced 4 times and not affected 6 times. However, three of the four comparisons in which significant reductions were observed were from a single experiment and corresponded to contrasts between three different doses of niacin to a control treatment (Dufva et al., 1983). Initiating the feeding of niacin prepartum did not reduce the amount of fat in liver of cows at 1 to 2 days or 28 to 35 days postpartum (Skaar et al., 1989; Minor et al., 1998).

Niacin requirements for dairy cattle are not known. Supplemental niacin may be required by calves fed milk replacer (Hopper and Johnson, 1955) but not by post weaned growing heifers (Riddell et al., 1981). Data summarized from more than 25 trials does not support routine use of niacin to enhance lactation performance of dairy cattle. Data also do not support the routine use of niacin to minimize the risk of lipid-related metabolic disorders such as ketosis and fatty liver.

Pantothenic Acid

Pantothenic acid is a constituent of coenzyme A and is therefore essential for several fundamental reactions in metabolism including fatty acid oxidation, amino acid catabolism and acetylcholine synthesis (Smith and Song, 1996). No dietary requirement for pantothenic acid has been established as synthesis of pantothenic acid by ruminal microorganisms appears to be 20 to 30 times more than dietary amounts. Net microbial synthesis of pantothenic acid in the rumen of steer calves has been estimated to be 2.2 mg/kg of digestible organic matter consumed per day and degradation of dietary pantothenic acid in the rumen is estimated to be 78 percent (Zinn et al., 1987). Supplementation of pantothenic acid at five to 10 times theoretic requirements did not improve performance of feedlot cattle (Cole et al., 1982; Zinn et al., 1987). Deficiency symptoms are very diverse and nonspecific. In nonruminants, some generally reported symptoms include: disorders of the nervous, gastrointestinal, and immune systems, reduced growth rate, decreased food intake, skin lesions and changes in hair coat, alterations in lipid and carbohydrate metabolism and death (Smith and Song, 1996).

Riboflavin (B_2)

Riboflavin is a constituent of several enzyme systems associated with intermediary metabolism. No dietary requirement for ruminants has been established. Tissue requirements are apparently met through microbial synthesis of the vitamin in the rumen as destruction of dietary riboflavin in the rumen is nearly 100 percent (Zinn et al., 1987). Miller et al. (1986) reported ruminal synthesis of riboflavin to be 148 percent of intake with apparent absorption from the small intestine averaging 23 percent. Synthesis of riboflavin in the rumen and flow to the small intestine was unaffected by concentrate content of the diet fed to steers. Zinn et al. (1987) estimated the flow of riboflavin from the rumen at 15.2 mg/kg of digestible organic matter consumed per day and a net absorption from the small intestine of 25 percent.

Thiamin (B_1)

Thiamin is a water-soluble vitamin, which in pure form is white in color, and has a sulfurous odor. It functions as an important coenzyme in several energy metabolism pathways and has a role, although not well defined, in nerve and brain function (Combs, 1992). Sources of thiamin include grains, grain by-products, soybean meal, and brewers yeast. Amounts of thiamin synthesized daily in the rumen (28 to 72 mg) have been reported to equal or exceed dietary intake (Breves et al., 1981).

A dietary requirement for thiamin has not been established for healthy animals with a functional rumen. The combination of thiamin in feeds and synthesis of thiamin in the rumen meet or exceed metabolic requirements even with an estimated 48 percent destruction of dietary thiamin in the rumen (Zinn et al., 1987). Thiamin is generally

nontoxic as the upper safe feeding level for most nonruminants is 1,000 times the requirement (National Research Council, 1987). An upper safe feeding level has not been established for ruminants.

Deficiencies of thiamin have been found when thiaminases associated with either feeds or produced from altered ruminal fermentation destroy thiamin or produce an antimetabolite of thiamin, which blocks thiamin dependent reactions. Bracken ferns and some raw fish products have been found to contain thiaminases. Feeding diets high in sulfate (Gould et al., 1991) or those which cause a sudden drop in ruminal pH (Zinn et al., 1987) can result in a thiamin deficiency. Because thiamin is intricately involved in several of the energy producing Krebs cycle reactions and because of the importance of glucose as an energy supply for the brain, any deficiency of thiamin results in a central nervous system disorder. Polioencephalomalacia (PEM), is the most common thiamin deficiency disorder. Symptoms of PEM include a profuse, but transient diarrhea, listlessness, circling movements, opisthotonus (head drawn back over neck), and muscle tremors. If treated promptly by parenteral injection of thiamin (2.2 mg/kg of body weight), the condition can be reversed (National Research Council, 1996).

Vitamin B_{12}

Vitamin B_{12} is a cofactor for two major enzymes; methylmalonyl coenzyme A mutase necessary for conversion of propionate to succinate, and tetrahydrofolate methyl transferase which catalyzes transfer of methyl groups from 5-methyltetrahydrofolate to homocysteine to form methionine and tetrahydrofolate. Vitamin B_{12} is not found in the tissues of plants. Microbes are the only natural source of vitamin B_{12}. Ruminal microbes can produce all of the vitamin B_{12} required by the cow provided adequate available cobalt is in the diet (see section on cobalt, Chapter 6).

Vitamin B_{12} deficiency has been demonstrated in calves when fed diets devoid of animal protein (Lassiter et al., 1953) demonstrating that vitamin B_{12} is a required nutrient in dairy cattle. Based on this work, it was suggested that the vitamin B_{12} requirement for dairy cattle was between 0.34 and 0.68 μg/kg of live weight. Vitamin B_{12} deficiency is the principle manifestation of cobalt deficiency (See section on cobalt).

Significant quantities of vitamin B_{12} are synthesized in the rumen. Vitamin B_{12} activity in the rumen tends to be greater in animals either grazing or fed high forage diets compared with animals fed high concentrate diets (Sutton and Elliot, 1972; Walker and Elliot, 1972). Data from beef cattle (Zinn et al., 1987) suggest more than adequate synthesis of vitamin B_{12} to meet expected requirements for lactating dairy cows (Erdman, 1992), although exact requirements have not been established.

In the mature ruminant, vitamin B_{12} is of interest because of its roles in propionate metabolism (gluconeogenesis) and in methionine synthesis. It was suggested that inadequate B_{12} may be related to the low-milk fat syndrome in cows fed high grain diets (Frobish and Davis, 1977). Studies using both supplemental (Elliot et al., 1979) and injected (Croom et al., 1981) vitamin B_{12} failed to show any response in fat test from cows fed high grain diets. There is no evidence that lactating dairy cows fed adequate amounts of cobalt will respond to dietary or intramuscular injections of vitamin B_{12}.

Vitamin B_{12} also is required as part of the enzyme complex methionine synthase in which methionine is synthesized from S-adenosylhomocysteine and 5-methyl tetrahydrofolate. Methionine is used as a methyl donor for synthesis of choline , carnitine, and others compounds; therefore, a deficiency of vitamin B_{12} is likely to affect methionine and methyl donor metabolism. Methyl donor requirements are not defined in ruminants and again it is unlikely that vitamin B_{12} deficiency is of practical significance except during cobalt deficiency.

B-Vitamins-General

In general, B-vitamin requirements can be met through synthesis by ruminal microorganisms and escape of dietary sources from the rumen. Table 7-1 illustrates potential requirements extrapolated from swine requirements and average vitamin concentrations found in milk. Based on these estimated requirements and limited research on B-vitamins of Miller et al. (1986) and Zinn et al. (1987) only folic acid and pantothenic acid appear to be limiting based on ruminal synthesis and escape of these vitamins occurring naturally in feeds. In contrast, some studies have demonstrated production and/or health benefits to dairy cows when diets have been supplemented with B-vitamins, most notably niacin, biotin, and folic acid. At the present time, almost no research is available on requirements of B complex vitamins for gestation, health, and milk production of high producing dairy cows.

VITAMIN C

Vitamin C or ascorbic acid is synthesized from L-gulonic acid within the cells of ruminants. Calves cannot synthesize ascorbic acid until approximately 3 weeks of age (Cummins and Brunner, 1991). Therefore, vitamin C is not considered an essential nutrient for healthy cattle that are older than about 3 weeks. Some studies, however, have reported beneficial responses when supplemental vitamin C is administered to cattle, particularly calves. Ascorbic acid functions as a water-soluble cellular antioxidant. Specifically, ascorbic acid is thought to be involved in regulation of steroid synthesis and the concentration of ascorbic acid is high in

TABLE 7-1 Estimated Absorption of Selected B-vitamins From the Small Intestine Compared with Estimated Requirements for Tissue and Milk Synthesis of a 650-kg Cow Producing 35 kg of 4 Percent Fat-Corrected Milk/Day

Vitamin	Daily Estimated Requirement			Ruminal Synthesis[c,d] (mg/day)	Ruminal Escape[d] from diet (%)
	Tissue[a] (mg/day)	Milk[b] (mg/day)	Total (mg/day)		
Biotin	5	1	6	14	100
Folic acid	33	2	35	7	3
Niacin	256	33	289	1804	6
Pantothenic acid	304	121	425	38	22
Riboflavin	95	61	156	261	1
Thiamin	26	15	41	143	52
B_6	26	22	48	96	100
B_{12}	0.4	0.2	0.6	70	10

[a] Based on lactating sow (175 kg) requirements (NRC, 1998) adjusted to 650 kg lactating cow weight.
[b] Adapted from Jenness (1985) and adjusted to 35 kg milk production.
[c,d] Adapted from Miller et al. (1986)[c] and Zinn et al. (1987)[d] and adjusted to digestible organic matter intake of 17.2 kg/day (total DM intake 22.9 kg/day).

steroid secreting cells. Plasma concentrations of ascorbic acid were lower in calves (Cummins and Brunner, 1991) and growing steers (Hidiroglou et al., 1977) reared under stressful (i.e., slatted floors, cold stress) conditions than animals housed in better environments. That effect may be mediated by cortisol. Oral supplementation of 1 or 2 grams of vitamin C/day to preruminant calves elevated plasma concentrations of ascorbic acid compared with no supplemental vitamin C (Hidiroglou et al., 1995). The 2 grams supplementation rate tended to increase plasma concentrations of ascorbic acid above the 1 gram rate but the difference was not statistically consistent during the 35 day experiment. Data are lacking on the effect of oral supplementation of vitamin C with cattle. Most orally ingested ascorbic acid is destroyed in the rumen, but newer formulations of vitamin C may provide some protection from ruminal metabolism. With sheep, oral supplementation of 4 g/day of various forms of vitamin C for 28 days significantly increased plasma ascorbic acid concentrations (Hidiroglou et al., 1997).

No growth response has been reported when calves were supplemented with vitamin C. Because of its antioxidant function, most research has concentrated on the effects of vitamin C on immune function. Immunoglobulin titers in calves were generally not affected by vitamin C supplementation (Cummins and Brunner, 1989; Hidiroglou et al., 1995). Steers injected subcutaneously with 20 mg of ascorbic acid/kg of BW had improved neutrophil function compared with uninjected controls (Roth and Kaeberle, 1985). In the same study, an injection of 40 mg of ascorbic acid/kg of BW counteracted the negative effects on neutrophil function induced by dexamethasone. Current data do not support routine supplementation of vitamin C to calves or adult cattle.

CHOLINE

Choline is not a vitamin in a traditional sense because it is not a part of an enzyme system, and is required in gram rather than milligram amounts as for true vitamins. Johnson et al. (1951) produced a choline deficiency in week-old dairy calves using synthetic milk replacer diets containing 15 percent casein. Choline requirements estimated from that experiment were 260 mg/L of milk replacer (1733 mg/kg DM). Current estimates of requirements for the calf are 1000 mg/kg dry matter (DM) (Chapter 10). The predominant sign of choline deficiency in most animals is fatty liver. In calves, reported deficiency signs included muscular weakness, fatty infiltration of the liver, and renal hemorrhage; similar to those observed in other species.

Both naturally occurring choline in feeds, predominantly found in phospholipids (lecithin) and dietary choline from supplements such as choline chloride have been shown to be extensively degraded in the rumen (Neil et al., 1979; Sharma and Erdman, 1988a,b, 1989b). Microbial degradation of choline in the rumen results in the production of acetaldehyde and trimethylamine. Methyl group carbon from trimethylamine is subsequently degraded to methane (Neil et al., 1978). Supplementation of dietary choline in an unprotected form is useless because of extensive ruminal degradation (Erdman, 1992).

Because of extensive degradation of dietary choline, methyl group requirements for synthesis of methyl-containing metabolites in the dairy cow are presumably produced via methylation pathways involving methionine and the enzyme, S-adenosylmethionine methyl transferase. Sources of methyl groups for ruminants would include dietary methionine, betaine resulting from degradation of choline, and de novo synthesized methyl groups produced through 5-methyl tetrahydrofolate. Approximately one-third of the methionine methyl groups were transferred to choline in studies with lactating dairy goats (Emmanuel and Kennelly, 1984). Intravenous infusion of choline and carnitine reduced the irreversible loss of methionine by 18 to 25 percent in sheep suggesting that methionine could be spared with the addition of methyl-group-containing metabolites (Lobley et al., 1996).

Choline content of whole milk varies substantially (43 to 285 mg/L; Hartman and Dryden, 1974) with about 25 mg/L in the form of phospholipids. More recently, Deuchler et al. (1998) found that the concentration of choline in milk ranged from 70 to 90 mg/L with an average secretion rate of choline into milk of between 2 to 3 g/day. Secretion of choline into milk was increased by either postruminal infusion of choline chloride (Aliev and Burkova, 1987; Deuchler et al., 1998) or by dietary supplementation of rumen-protected choline. This suggests that secretion of choline into milk could be used as a qualitative indicator of postruminal choline supply.

Choline requirements for lactating dairy cows have not been established. As a ruminant animal, the dairy cow has evolved under circumstances where intestinally absorbed choline is almost nonexistent because of extensive ruminal degradation of dietary choline. Experiments where choline has been supplemented either by feeding in a rumen-protected form or by postruminal infusion of choline chloride have produced variable results. Milk production increased 0 to 3 kg/day in experiments where 15 to as much as 90 grams of choline chloride were infused postruminally (Grummer et al., 1987; Erdman and Sharma, 1991; Sharma and Erdman, 1989a). In an experiment where methyl transfer from methionine was inhibited but choline was provided, 4 percent FCM production was increased by 3.4 kg/day suggesting the importance of methionine in methyl group metabolism in the dairy cow (Sharma and Erdman, 1988b).

Lactational responses to choline are likely to be affected by methionine supply. Dairy cows that are fed diets that supply adequate amounts of intestinally absorbed methionine are less likely to respond to supplemental choline than when methionine is limiting. Because of the relationship between fatty liver and ketosis, it has been speculated that choline could play a role in ketosis treatment and prevention, but there is no direct evidence to date to support this theory (Erdman, 1992). The establishment of a choline requirement, either for the lactating dairy cow, or for the transition cow in the late dry period and in early lactation, will require more extensive feeding experiments than were available at the time of this publication.

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