

# Requirements of Fat-soluble Vitamins for Dairy Cows: A Review<sup>1,2</sup>

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## ABSTRACT

Knowledge about vitamins has evolved greatly since they were discovered <100 yr ago, but the quantitative determination of the vitamin requirements of dairy cows has been difficult. Current requirements are based on data that are at least 20 yr old and, in some cases, >50 years old. The response variables in those studies were the prevalence of overt signs of deficiency, level of milk production, reproductive performance, and vitamin concentrations in blood and tissues. **Since those studies were conducted, milk production per cow has increased substantially, and the feeding and management of cows have changed (less pasture, less forage, and more total confinement).** More refined measures of vitamin adequacy have been developed. Immunocompetence may be a useful tool for determining the requirements of some vitamins. Requirements that are based on measures of immune function are usually higher than those that are based on production or reproduction. The current requirements for vitamin D appear to be adequate, but the requirement for vitamin A should be increased by approximately 50% because of differences in bioavailability between  $\beta$ -carotene and retinyl esters. Based on health data, the vitamin E requirement should be increased at least 500% and perhaps as much as 700% for dry cows and lactating cows.

(**Key words:** vitamin A, vitamin D, vitamin E, dairy cows)

**Abbreviation key:** BC =  $\beta$ -carotene, RFM = retained fetal membranes.

## INTRODUCTION

In the late 1800s and early 1900s, the idea that animals required nutrients other than protein, fat, minerals, and carbohydrates was tested scientifically. Those studies led to the development of the concept of vitamins. Purified diets, improved analytical instrumentation, and rodent models have contributed to the tremendous progress made in vitamin nutrition. Progress in defining the vitamin requirements for functioning ruminants, however, has been slow.

In theory, the establishment of nutrient requirements for animals is straightforward. Animals are fed diets with different concentrations of the nutrient in question, and a response is measured. The point at which increased intake of the nutrient no longer affects the response variable is the requirement for that nutrient under the conditions imposed during the study. Application of this method is predicated on the ability to measure accurately and precisely both the intake of the nutrient and the response to it. Furthermore, the response variable must be sensitive to changes in the nutrient, and the nutrient in question should be first limiting. For many nutrients and for many animal species, this method works well, but attempting to define vitamin requirements of ruminants presents several challenges. Some fat-soluble vitamins are metabolized in the rumen. Purified diets are difficult to formulate for ruminants. Rodents can be depleted of many vitamins in a matter of days or weeks, but, for large ruminants, more than a year may be needed to deplete body stores of certain vitamins. Because the response variable may involve reproductive efficiency or animal health, many animals are needed, and the experiments must be of long duration. These experiments are extremely expensive to conduct.

This review concentrates on papers published from 1986 to 1997. This time span encompasses data published since the last NRC (37) requirements were published. Experiments that used adult cattle (mostly dairy cows) and whole animal studies, rather than in vitro experiments, are emphasized. The discussion of each vitamin is not based on its overall

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importance but on the amount of new data published on the vitamin.

### Current Practices

A nonscientific survey of feed companies and nutrition consultants from the US and Canada was conducted to determine the current recommendations for supplemental vitamins A, D, and E. Forty surveys were sent out, and 32 were returned. Statistical results from the survey are presented in Table 1. The mean amount of supplemental vitamin A recommended for dry and lactating cows was 2 to 2.5 times more than the NRC (37) requirement for total (basal plus supplemental) vitamin A. The amount of supplemental vitamin D recommended was 1.5 to 1.8 times the current NRC (37) requirements for lactating and dry cows. Based on typical DMI, the recommendation for supplemental vitamin E averaged about 6.7 and 1.7 times the current NRC (37) requirement for total vitamin E for dry and lactating cows, respectively. Based on the mean concentrations of  $\alpha$ -tocopherol in feedstuffs, the total amount of vitamin E that is consumed when supplemented according to the survey results would be about 12 and 4 times the NRC (37) requirements for dry and lactating cows, respectively.

### Requirements Versus Recommendations

A requirement is the amount of a nutrient needed to maintain the animal in a healthy state, to allow for successful reproduction, and to allow for a certain amount of milk production or BW gain when the animal is reared under specific environmental conditions. A recommendation is the amount of a nutrient that will meet the requirements for animals under less defined environmental conditions and will include a margin of safety to account for variations in intake, production, and availability of the nutrient. Analytical costs and the extreme variability in the concentrations of fat-soluble vitamins in feeds preclude the routine analysis of feeds for vitamins; therefore, a margin of safety should be included because the actual intake of fat-soluble vitamins (basal plus supplemental) is almost never known. A recommendation also should consider the cost of the nutrient, the cost of a nutrient deficiency, and the potential for toxicity.

Based on current prices (Wooster, OH, 1997) and NRC (37) requirements, the annual (305-d lactation and 60-d dry period) cost per cow for vitamins A, D, and E are approximately \$1.3, \$0.6, and \$0 (basal

TABLE 1. Survey<sup>1</sup> results for amounts of supplemental vitamins A, D, and E currently recommended for dairy cows.

Item	Type of cow <sup>2</sup>			
	Dry	Peripartum dry	High yielding	Low yielding
IU/d				
<b>Vitamin A</b>				
NRC (37) <sup>3</sup>	40,000	40,000	80,000	51,600
Mean	104,000	121,000	158,000	121,000
Median	100,000	100,000	155,000	102,000
SD	42,600	55,000	48,000	37,000
<b>Vitamin D</b>				
NRC (37) <sup>3</sup>	10,000	10,000	25,000	16,000
Mean	26,200	31,500	40,000	32,500
Median	25,000	30,000	40,000	35,000
SD	10,000	12,500	10,700	8200
<b>Vitamin E</b>				
NRC (37) <sup>3</sup>	150	150	375	240
Mean	760	1080	590	450
Median	900	1000	590	400
SD	280	170	160	160

<sup>1</sup>Nonscientific survey of 40 feed companies and nutritional consultants. Thirty-two surveys were returned.

<sup>2</sup>All cows weighed 600 kg. The peripartum dry cow was within 21 d of parturition. The high yielding cow produced 45 kg/d of milk (3.7% fat). The low yielding cow produced 22 kg/d of milk (3.7% fat).

<sup>3</sup>The NRC (37) requirements for vitamins A and E reflect total vitamin intake (basal plus supplemental); the values for vitamin D are for supplemental vitamin intake.

diet should provide enough vitamin E to meet NRC requirements), respectively. If the price of milk is \$0.30/kg, approximately 7 kg/yr of milk per cow are needed to pay for the vitamin costs per NRC (37) requirements. Based on the recommendations obtained from the survey (Table 1), the annual mean costs per cow for vitamins A, D, and E are approximately \$3, \$1, and \$9, respectively, or approximately the value of 45 kg of milk per cow. Economic benefits to proper vitamin supplementation include increased milk production, improved reproduction, reduced prevalence of mastitis, reduced occurrence of milk fever, and fewer reproductive disorders. The economic impact of changes in reproductive efficiency is difficult to quantify, but improved reproductive efficiency should increase net return. A case of clinical mastitis costs about \$125 in 1991 dollars (19), a case of retained fetal membranes (RFM) costs about \$100 in 1988 dollars (27), and a case of milk fever costs between \$50 and \$100 in 1994 dollars (26).

No new data on vitamin tolerances have been published since the NRC (36) review. Tolerances in that publication were 66,000, 10,000, and 2000 IU/kg of DMI for vitamins A, D, and E, respectively. Based on 20 kg of DMI, a lactating cow can probably tolerate

1.3 million IU/d of vitamin A, 200,000 IU/d of vitamin D, and 40,000 IU/d of vitamin E for long periods of time (months). In the short term (days), the vitamin tolerances may be 10 times greater than those for longer term supplementation.

### VITAMIN A

Vitamin A has many functions, including maintenance of epithelial cells, vision, gene regulation, and immune cell function (8). The current NRC (37) requirement for vitamin A for adult cows (76 IU/kg of BW) is based on reproductive efficiency (48). The requirement for growing calves (42 IU/kg of BW) is based on the amount of vitamin A that is needed to maintain cerebrospinal fluid pressure <120 mm of saline in calves that weighed 100 to 200 kg (49). If the presence of papillary edema of the eye were the determining criterion, the vitamin A requirement would be between 60 and 75 IU/kg of BW (49). If a statistically significant increase in cerebrospinal fluid pressure was the determinant, the requirement would be about 100 IU/kg of BW (10).

The current requirement for vitamin A for lactating cows is based on research conducted between 1937 and 1957 (48). Reproductive efficiency of cows was maintained when diets provided 0.18 mg of  $\beta$ -carotene (BC)/kg of BW (72 IU of vitamin A/kg of BW) (48). Consumption of less BC resulted in increased abortions, increased numbers of sick calves, and a higher incidence of RFM. All of the vitamin A was provided by BC from prairie grass hay; no preformed vitamin A was fed. The specific diets were not published, but all of the ingredients were high in fiber. No silages or starchy grains were included in the diets. Milk production for the Holstein cows on the study averaged 3600 kg/292 d. That study (48) was well designed and met the criteria for establishing vitamin requirements; however, the data need to be reexamined in light of current management and feeding practices.

#### Effect of Diet Composition on Vitamin A Requirement

Basal feedstuffs contain no vitamin A but can contain substantial amounts of BC, a precursor to vitamin A. The BC concentration in hay and haycrop silages ranged from about 5 to 100 mg/kg of DM with a mean of about 37 mg/kg; in corn silage, the range was 1 to 4 mg/kg of DM, and the mean was 1.6 mg/kg (5). Concentrates typically contain little BC. Based on these numbers and 20 kg of DMI (50% forage), a

lactating cow could consume 4000 to 400,000 IU of vitamin A/d from basal ingredients.

The current NRC (37) requirement is based on a conversion factor of 1 mg of BC = 400 IU of vitamin A. That factor is based on the retinol concentrations in the livers of sheep fed corn silage (32). Data on the bioavailability to cattle of BC from different sources are extremely limited, but the bioavailability of BC and vitamin A appear to be different. Ruminal destruction of BC was 23% and was not different for sheep fed diets that were high in starch or fiber (44). In vitro rumen studies found that 67 to 72% of retinol (retinyl esters were the source of the retinol) was destroyed within 12 h of incubation when rumen fluid was obtained from cattle fed 50 (65) or 70% (47) concentrate diets. In vitro destruction was 16 to 20% when rumen fluid was obtained from cattle that had been fed high (>75%) forage diets (47, 65). Based on these data, vitamin A from retinyl esters is about 50% as available as BC when cows are fed lactation diets (50% concentrate).

#### Increased Milk Production and Vitamin A Requirement

The current NRC (37) requirement for vitamin A is not adjusted for milk production. However, because DMI usually increases as milk production increases, intake of vitamin A increases as milk production increases. The NRC did not have a specific requirement for vitamin A for lactation because Swanson et al. (53) reported that cows that consumed approximately 10 times less BC than recommended by Ronning et al. (48) produced amounts of milk (3500 kg during a 40-wk lactation) similar to those of cows fed adequate vitamin A. Mean milk production currently is about twice as high, and many herds produce three to four times as much milk as did cows in the study by Swanson et al. (53). In a more recent study (41), cows fed approximately 170,000 IU/d of vitamin A (as retinyl acetate) starting 60 d prepartum and continuing until 42 d postpartum produced more milk (40.2 vs. 35.8 kg/d) than did cows fed 50,000 IU/d [approximately the NRC (37) requirement]. Cows fed 50,000 IU of vitamin A/d plus 300 mg of BC/d produced 38.1 kg of milk/d. The differences in production were not related to differences in mammary gland health. The peak milk production in the study by Oldham et al. (41) was about twice the peak milk production in the study by Swanson et al. (53). Conversely, Michal et al. (33) reported no difference in milk production between cows fed 0 and 120,000 IU of supplemental vitamin A/d or 300 or 400 mg of BC/d starting 4 wk prepartum and continuing until 4 wk postpartum. Milk production averaged 34 kg/d.

### Increased Reproductive Demands and Vitamin A Requirements

In a study (55) in which cows were fed forage and grain separately and housed in free stalls, days to first service (63 d) and conception rates from first AI service (28%) were not different whether 100,000 (approximately 1.5 times the current requirement) or 1,000,000 IU/d of supplemental vitamin A (retinyl acetate) was fed. The rate of estrus detection following prostaglandin treatment was about twice (60 vs. 26%) as high for the high vitamin A group as for the control group. In a concurrent experiment (55) using similar treatments but with cows fed a TMR and housed in tie stalls, no effect on any reproductive measure was observed.

The corpus luteum of cows contains high concentrations of BC and that has given impetus to studying the effects of BC supplementation on reproduction in dairy cows. Many studies have been conducted on the effects of BC on reproduction and were reviewed by Hurley and Doane (24). In about 50% of the reviewed studies, BC positively affected some measure of reproductive efficiency. Among the studies that were conducted in North America, four of five studies reported no beneficial effect. Usually 300 to 600 mg of BC (equivalent to 120,000 to 240,000 IU of vitamin A) was fed, and the control diet met or exceeded NRC requirements for vitamin A.

### Effect of Stage of Lactation or Gestation on Vitamin A Requirement

Ronning et al. (48) studied cattle from birth through several lactations but did not, however, examine vitamin A requirements at specific stages of gestation or lactation. Plasma concentrations of retinol and BC are extremely low during the peripartum period (14, 28, 33, 41, 63). This observation has led some to postulate that the requirement for BC and vitamin A may be higher during this period. Oldham et al. (41) fed diets during the dry period and early lactation that provided about three times more vitamin A (preformed vitamin A and BC) than current requirements. Mammary gland infections and clinical mastitis were not affected by treatment, but milk production was increased by feeding high amounts of vitamin A. Plasma concentrations of both BC and retinol were low for all treatments during the peripartum period. Michal et al. (33) also examined vitamin A and BC supplementation during the peripartum period. Diets were 0 or 120,000 IU/d of vitamin A and 300 or 600 mg of supplemental BC/d.

Neutrophil function after parturition was increased by the addition of vitamin A or 600 mg of BC. Lymphocyte function was generally improved when 600 mg of BC was fed. Supplemental vitamin A and BC reduced the prevalence of RFM by 28%, and BC supplementation reduced the prevalence of metritis by 50%. This study showed that BC and vitamin A influenced immunity and reproductive health, but, because the control diet contained no supplemental vitamin A, the results from this study do not mean that the current NRC (37) requirements are inadequate.

Another critical period in the lactation cycle is at dry-off. Cows are extremely susceptible to IMI during this period. Tjoelker et al. (56) fed cows 53,000 IU of vitamin A/d (approximately NRC requirements), 213,000 IU/d, or 53,000 IU/d plus 400 mg of BC/d (equivalent to 213,000 IU of vitamin A) starting at 6 wk before dry-off and continuing until 2 wk after dry-off. Treatment did not affect neutrophil or lymphocyte function, suggesting that the NRC (37) requirement is adequate to maintain immune cell function at dry-off.

### Assessing Vitamin A Status of Cows

A measurable index of the vitamin A status of cows that could be used on the farm could be useful to ensure proper vitamin A supplementation. The measures (reproductive efficiency, cerebral spinal fluid pressure, and histological changes in eye tissue) used to establish vitamin A requirements are not applicable to on-farm use. For cattle that showed clinical signs of vitamin A deficiency, plasma retinol concentrations were correlated with the severity of clinical signs, but once plasma concentrations were  $>9 \mu\text{g}/\text{dl}$ , no relationship was found (6). Typical concentrations of retinol in the plasma of dairy cows fed at NRC requirements are 40 to 50  $\mu\text{g}/\text{dl}$ . Chew et al. (9) suggested that plasma concentrations of retinol  $<80 \mu\text{g}/\text{dl}$  may be related to reduced immune function in cows. Other studies have reported either no relationship or weak relationships between plasma concentrations of retinol and reproduction (5, 29), immune status (33, 56), and mammary gland health (11, 41). Another concern about the use of plasma concentration of retinol to assess vitamin A status is the poor relationship between intake of vitamin A and plasma concentrations of retinol (33, 41, 56).

The suggested minimal concentration of BC in the plasma of dairy cows is about 300  $\mu\text{g}/\text{dl}$  (50). That recommendation is based on mastitis prevalence and reproductive efficiency. Other data do not support

that value (5, 29, 33). Plasma concentrations of BC are related to BC intake, but the relationship is not strong (5, 33). Stage of gestation and dietary fat supplementation also affects the plasma concentrations of BC (33, 63). Available data are insufficient to support the use of plasma concentrations of retinol or BC as reliable indices of vitamin A status except when cows are clinically deficient.

### Recommendations

Most recent studies conducted on vitamin A have included a control diet that provided supplemental vitamin A to meet NRC (37) requirements. The diets also would have had some BC (almost never measured); therefore, the control diet could have provided substantially more total vitamin A than needed based on current NRC (37) requirements. The lack of differences between cows fed control diets and those fed diets with additional supplemental vitamin A does not necessarily mean that the NRC (37) requirement is correct. The NRC requirement is based on BC, but the data on ruminal destruction of retinyl esters suggest that bioavailability of retinyl esters in diets with 50% concentrate may be 50% lower than that of BC. The improved milk production, mammary gland health, and immune function that were found when dry cows were fed additional supplemental vitamin A strongly suggests that the NRC (37) requirement for dry cows is not adequate. These data support increasing the requirement for vitamin A about 50% to 100 IU/kg of BW. With the low cost of vitamin A, the wide margin of safety (tolerance is approximately 1.3 million IU/d), and the potential benefits, the practice of feeding approximately twice as much vitamin A as the current NRC (37) requirement appears to be justified.

### VITAMIN D

Vitamin D is involved with calcium and phosphorous homeostasis (23) and immunity (46). Hay and other sun-dried forages contain appreciable quantities of vitamin D, and cows exposed to sunlight can synthesize vitamin D. The NRC committee (37) stated that quantitative requirements for vitamin D are not well defined for dairy cows. Since that publication, the understanding of vitamin D metabolism has increased, but little new information regarding requirements for vitamin D has been published.

The current NRC (37) requirement for vitamin D for adult dairy cows is 30 IU/kg of BW (presumed to mean supplemental vitamin D), and no allowance is given for milk production. That requirement is based

on two studies. In one study (58), dry cows were fed 0, 5000, or 10,000 IU/d of vitamin D and the incidence of milk fever and the plasma concentrations of Ca, P, and 25-hydroxyvitamin D were measured; those researchers concluded that 10,000 IU/d of vitamin D (approximately 20 IU/kg of BW) was adequate for dry cows that were fed silage and housed indoors. In another study (45), lactating cows were fed 15,000, 65,000, 115,000, and 215,000 IU/d of vitamin D, and the researchers concluded that 15,000 IU (25 to 30 IU/kg of BW) of vitamin D/d were adequate. A more recent study (2) using low producing cows (20 kg of milk/d) and lactating ewes concluded that 10 IU of vitamin D/kg of BW was adequate to maintain plasma concentrations of calcium and phosphorous. Details concerning housing and diets were not presented, but, because the experiment was conducted in Norway in the winter and spring, exposure to sunlight would have been limited. Slightly improved reproductive efficiency (59) and slightly elevated milk production (17) have been reported when cows were fed approximately twice as much vitamin D (70 IU/kg of BW) as recommended.

Supplementation of diets for prepartum cows with anionic salts has become more common since the last NRC (37) requirements were issued. The dietary cation-anion difference affects calcium metabolism. Experiments that are designed specifically to examine vitamin D requirements when anionic diets are fed have not been conducted. Goff et al. (12), however, reported that an anionic diet (cation-anion difference was -228 meq/kg) increased plasma concentrations of 1,25 dihydroxyvitamin D compared with a cationic diet (+978 meq/kg). The concentrations of supplemental vitamin D in the diets were not presented. In a subsequent study (13), Jersey cows fed a diet that was supplemented with anionic salts (cation-anion difference was +134 meq/kg) had higher numbers of vitamin D receptors in the colon after parturition than did those fed a standard diet (+337 meq/kg). Those studies suggest that dietary anionic salt does not increase the vitamin D requirement of peripartum dairy cows. In vitro studies using rumen fluid that had been collected from cows that had been fed high forage diets found that about 75% of radio-labeled vitamin D<sub>3</sub> was altered after 24 h of incubation (23). Comparative data using diets with more concentrate are not available.

Vitamin D also is involved with immune function (46). Increased lymphocyte proliferation was observed when Jersey cows were infused subcutaneously with 50 µg of 1,25-dihydroxyvitamin D/d for 7 d (25). Because of the form of vitamin D used in that



study and the duration of the study, inferences about vitamin D requirements are limited. Whether additional vitamin D would result in enhanced immune function is unknown.

### Recommendations

Available data do not contradict the current NRC (37) requirement for vitamin D of 30 IU/kg of BW. Total confinement of cows, which limited exposure to sunlight, and increased reliance on silage reduced the amount of vitamin D that is synthesized by the cows and consumed in the basal diet. The data used to formulate the current NRC (37) requirement, however, were collected from confined cows fed high silage diets. Large amounts (75%) of vitamin D are metabolized in the rumen when cows are fed high forage diets; therefore, increased concentrate feeding probably would have little additional effect. The inclusion of anionic salts in diets for peripartum cows does not appear to influence greatly the requirements for vitamin D. Horst et al. (23) suggest that plasma concentrations of 25-hydroxycholecalciferol are indicative of the vitamin D status of dairy cows. Plasma concentrations <5 ng/ml indicate a vitamin D deficiency; values between 20 and 50 ng/ml indicate adequate vitamin D status. The current NRC (37) requirement for vitamin D is probably adequate, but the low cost of supplemental vitamin D and the potential for increased milk production and reproductive efficiency support the field practice of feeding about 1.8 times the NRC (37) requirement.

### VITAMIN E

The best understood function of vitamin E is as a cellular antioxidant (21). Many papers have been published during the last 15 yr on the effects of supplemental vitamin E for dairy cows, but, as with the other fat-soluble vitamins, titration studies are lacking. The current NRC (37) requirement for dairy cows is 15 IU/kg of DMI (approximately 150 and 300 IU/d for dry and lactating cows, respectively). This requirement is for total, not supplemental, vitamin E. I could not determine the basis for this requirement. Previous publications of the NRC gave no requirement for vitamin E.

The concentration of vitamin E in feedstuffs is highly variable: coefficients of variation often were >50% for fresh and stored forages (28, 54, 57, 67). Fresh green forages contain substantial amounts of  $\alpha$ -tocopherol (80 to 200 IU/kg), but vitamin E concentrations in hay and silage are 20 to 80% lower (54, 67). The longer that mowed forages are exposed to

sunlight (i.e., hay vs. wilted silage), the lower the concentrations of  $\alpha$ -tocopherol become. Except for raw oilseeds, most concentrates contain very little vitamin E. Heat treatment and grinding greatly reduce the vitamin E content in concentrate feeds. Based on the average concentrations of vitamin E in feeds and average DMI (10 kg/d), intake of vitamin E by dry cows may range from about 200 (mostly hay) to 1800 (mostly pasture) IU/d. For lactating cows with a DMI of 20 kg/d (50% forage), intake of vitamin E from a basal source would average about 400 IU/d for diets based on hay, 1500 IU/d for diets based on silage, and about 2500 IU/d for diets based on pasture. With the variation observed in vitamin E concentrations, these mean values could vary by as much as 50%. Based on these assumptions, most cows would not need any supplemental vitamin E to meet current NRC (37) requirements.

The bioavailability of vitamin E may influence requirements. Early data (1) suggested that significant amounts of supplemental vitamin E were destroyed in the rumen and that destruction increased as the concentrate in the diet increased. More recent studies (31, 65) found that vitamin E concentration did not change during in vitro ruminal fermentation. The authors of those studies suggested that poor extraction of  $\alpha$ -tocopherol from the digesta was the reason others reported ruminal destruction of vitamin E. The US Pharmacopoeia defines 1 IU of vitamin E as equal to 1 mg of all-*rac*- $\alpha$ -tocopheryl acetate; 1.49 IU of vitamin E is equal to 1 mg of *RRR*- $\alpha$ -tocopherol. Based on plasma concentrations of  $\alpha$ -tocopherol, the conversion factor of the US Pharmacopoeia may be too low for *RRR*- $\alpha$ -tocopherol relative to all-*rac*- $\alpha$ -tocopheryl acetate for cattle (18). Based on that study, 1 mg of *RRR*- $\alpha$ -tocopherol may be equivalent to about 2.6 mg of all-*rac*- $\alpha$ -tocopheryl acetate.

### Vitamin E and Cow Health

White muscle disease is a classic sign of a clinical deficiency of vitamin E. More recently, the incidence of reproductive disorders (predominantly RFM) and mastitis has been related to vitamin E intake. The supplementation of approximately 1000 IU/d of vitamin E (usually all-*rac*- $\alpha$ -tocopheryl acetate) to dry cows when adequate Se is supplemented reduces the incidence of RFM in some (15, 35) but not all (7, 66) studies. About one-half of the studies that examined the effect of injected vitamin E (usually in combination with Se) on RFM found no effect, and about one-half reported a positive response (35). One injection of approximately 700 IU of vitamin E (and about 50

mg of Se) given about 3 wk before calving was the usual treatment. Relative to the amount of vitamin E consumed normally, 700 IU of vitamin E over 21 d is trivial. Most likely, Se, not vitamin E, was the nutrient responsible for the positive effect.

In 1984, Smith et al. (51) reported that supplemental vitamin E significantly reduced the incidence and duration of IMI and clinical mastitis. A subsequent study (21) using prepartum heifers confirmed those results. Conversely, a large study conducted in Canada (4) found that supplemental vitamin E (1000 IU/d during the dry period and early lactation and 500 IU/d from 90 DIM) did not reduce the incidence of clinical mastitis. Based on the concentrations of Se in the diet (0.1 ppm) and in plasma (<35 ng/ml), the cows were deficient in Se. Another study (64) using diets low in total Se (0.15 ppm) but with cows in better Se status (plasma Se >50 ng/ml) than in the Batra et al. (4) study found that intake of 1000 IU/d of vitamin E during the dry period reduced clinical mastitis of cows at calving by 30% but did not affect the prevalence of IMI. Also in that study, 4000 IU of supplemental vitamin E/d fed during the last 2 wk of the dry period resulted in an 80% reduction in clinical mastitis at calving and a 60% reduction in IMI. The intake of total vitamin E (basal and supplemental) during the last 14 d prepartum was 940, 1900, and 4900 IU/d for cows fed 100, 1000, and 4000 IU/d of supplemental vitamin E, respectively. These data strongly suggest that the NRC should increase the vitamin E requirement for dry cows.

### Vitamin E and Immune Function

Dietary or parenteral supplementation of vitamin E to dairy cows during the peripartum period has consistently improved the function of neutrophils. Hogan et al. (20) reported that intake of 500 IU/d of supplemental vitamin E during the first 30 d of lactation increased the ability of blood neutrophils to kill bacterial pathogens compared with that of neutrophils collected from cows fed no supplemental vitamin E. Results were similar when cows were injected with 3000 IU of vitamin E at 10 and 5 d before expected parturition (22). Politis et al. (43) reported that 3000 IU of supplemental vitamin E/d fed from 8 wk prepartum to 4 wk postpartum significantly increased neutrophil chemotaxis compared with the response of neutrophils from cows fed no supplemental vitamin E. Supplemental dietary vitamin E (3000 IU/d) fed to cows from 4 wk prepartum to 8 wk postpartum and an injection of 5000 IU of vitamin E

given 1 wk before expected calving improved neutrophil function at parturition (42).

### Assessment of Vitamin E Status

Overall, plasma concentrations of  $\alpha$ -tocopherol are correlated with intake of vitamin E (39, 52, 61, 62, 64), but other factors can influence  $\alpha$ -tocopherol concentrations in plasma. Plasma concentrations of  $\alpha$ -tocopherol are low during the peripartum period (14, 61, 62, 63, 64). Otherwise,  $\alpha$ -tocopherol concentrations in serum are not greatly affected by stage of lactation (52). Plasma concentrations of  $\alpha$ -tocopherol are highly correlated with concentrations of cholesterol in plasma (62, 63) and with the cholesterol concentration in various plasma lipoproteins (16). When dry cows were fed supplemental fat, concentrations of both plasma  $\alpha$ -tocopherol and cholesterol increased (63), but plasma concentrations of  $\alpha$ -tocopherol were not affected when lactating cows were fed added fat (3). The ratio of  $\alpha$ -tocopherol to cholesterol in plasma is affected less by stage of lactation and dietary fat than is the concentration of  $\alpha$ -tocopherol, suggesting that the ratio may be a more appropriate index of vitamin E status (16, 62). Stress (excessive handling, epinephrine, or ACTH injection) reduced the concentration of  $\alpha$ -tocopherol in the plasma of beef cattle (40).

In a study of 50 different herds (544 samples), the mean serum concentration of  $\alpha$ -tocopherol for dairy cows that had recently calved was 2.4  $\mu$ g/ml (34). Based on neutrophil function (60) and prevalence of clinical mastitis (64), the suggested minimal plasma concentration of  $\alpha$ -tocopherol for peripartum dairy cows is 3 to 3.5  $\mu$ g/ml. A study using beef heifers (30) found a high correlation between the serum concentrations of  $\alpha$ -tocopherol (treatment means ranged from 0.98 to 4.09  $\mu$ g/ml) and pregnancy rate. Once serum concentrations were >3  $\mu$ g/ml, no additional improvement in the pregnancy rate was found. Jukola et al. (29) found no difference in clinical mastitis or SCC among commercial dairy herds that had mean serum concentrations of  $\alpha$ -tocopherol >4 or <4  $\mu$ g/ml. That study sampled cows throughout lactation, and the mean serum concentration of  $\alpha$ -tocopherol was 5.9  $\mu$ g/ml. Other survey studies (38) using herds with high mean concentrations of  $\alpha$ -tocopherol (6  $\mu$ g/ml) found no difference in serum or plasma concentrations of  $\alpha$ -tocopherol between herds with high or low incidence of reproductive disorders and clinical mastitis. The plasma concentration of  $\alpha$ -tocopherol in peripartum cows should be at least 3 to 3.5  $\mu$ g/ml; the minimal acceptable concentration for other stages of lactation are not known.

## Recommendations

Based on cow health and immune function, the current NRC (37) requirement for vitamin E (15 IU/kg of DMI) is not adequate. Titration studies have not been conducted, so a specific requirement cannot be stated. In most of the published studies, the control diet contained three to five times more vitamin E than the current requirement, yet additional supplemental vitamin E produced positive responses. At the least, NRC requirements should reflect the vitamin E concentrations found in typical diets (25 to 75 IU/kg of DMI), and most data suggest that diets that include 75 to 190 IU of total vitamin E/kg for dry cows and between 25 and 50 IU/kg for lactating cows are beneficial. Plasma concentrations of  $\alpha$ -tocopherol <3 to 3.5  $\mu$ g/ml in peripartum cows may indicate that additional vitamin E is necessary. The clinical value of plasma concentrations at other times during the gestation and lactation cycle is uncertain. The cost of vitamin E is more than the cost of vitamins A and D, but the current practice of feeding 500 and 1000 IU/d of supplemental vitamin E to lactating and dry cows, respectively, is economical based on expected improvements in mammary gland health and reduced incidence of RFM.

## CONCLUSIONS

Data published since the last NRC (37) report and a reinterpretation of the data used to formulate those recommendations suggest that the vitamin A requirement of dairy cows should be increased to about 100 IU/kg of BW. The current vitamin D requirement appears to be adequate. The current requirement for vitamin E is not sufficient. Most control diets contained concentrations of vitamin E greater than the current requirements, but cow health was often improved by intake of even more vitamin E. A specific requirement cannot be determined by available data, but the current practice of feeding 1000 IU of supplemental vitamin E/d to dry cows and 500 IU/d to lactating cows is justified.

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