

EFFECT OF PREPARTUM SUPPLEMENTATION WITH
VITAMINS A AND E AND SELENIUM ON SUBSEQUENT
PERFORMANCE OF HOLSTEIN COWS

by

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INTRODUCTION

The performance capability of the dairy cow has increased substantially in the past 40 years. Milk production per cow is higher than ever before and confinement housing (no pasture), plus more animals per unit of space have evolved as trends in the dairy industry. These circumstances contribute more stress and affect the dairy cow's rate of exposure to environmental pathogens which, collectively, may lessen the cow's ability to respond to an adverse situation.

The most common and costly disease affecting the dairy cow is mastitis. Antibiotic treatment, improved hygiene, and proper nutrition have all contributed to reducing the occurrence and severity of the disease. However, high rates of new intramammary infections occur in the early stages of the prepartum period and near calving time. If the pathogen is able to breach the cow's teat canal, it initiates an inflammatory response which results in the recruitment of polymorphonuclear leukocytes and macrophages, and the activation of complementary systems. Improper nutrition during the prepartum period, including deficiencies of vitamins and minerals, can have a negative effect on this self-defense system. Thus, this research was conducted to observe the results of feeding various combinations of vitamins A and E, and selenium during the prepartum period on

disease resistance and total performance of the dairy cow in the subsequent lactation.

LITERATURE REVIEW

VITAMIN A, VITAMIN E, AND SELENIUM: SOURCES, INTERACTIONS,
AND PHYSIOLOGICAL IMPORTANCE FOR DAIRY CATTLE - A REVIEWVitamin A

Requirements. The current U. S. requirement for lactating and dry dairy cattle is 19 mg of carotene per 100 kg of live weight (National Research Council, 1978). The requirement is expressed in units of carotene as this is the main dietary source of provitamin A for dairy cattle.

At this concentration, Ronning et al. (1959, as cited by NRC, 1978) reported successful reproduction in Guernsey cattle for extended periods of time. Swanson et al. (1968) investigated lactational responses to vitamin A supplementation for dairy cattle fed diets high in concentrate and low in roughage. They used 10 pairs of identical twins in two groups, with one of the groups fed a vitamin A-deficient diet prior to calving. After calving, both groups received 88% of the NRC requirements for carotene, and, in addition, one group received 50,000 I.U. of vitamin A/cow/d. The supplemented groups attained much higher liver and plasma vitamin A concentrations, but milk yield was not significantly different between the two groups. They concluded that providing adequate vitamin A for successful reproduction will

provide ample intakes for normal lactation.

Plasma Values. The measurement of cerebrospinal fluid pressure (CSF) is a sensitive indicator of vitamin A adequacy. Moore et al. (1948) fed differing levels of β -carotene to Jersey and Guernsey calves and evaluated CSF for each calf. The CSF values correlated well with β -carotene intake, but the plasma vitamin A concentrations were in poor agreement with vitamin A and β -carotene intakes. They concluded that a CSF pressure above 120 mm saline was abnormal. Eaton et al. (1970) were able to group vitamin A-deficiency symptoms within a range of plasma values that were in agreement with CSF values. Holstein calves were fed vitamin A-depletion rations to the 6th or 10th week of age, then supplemented with varying levels of vitamin A. The occurrence of parotid duct metaplasia and ocular papilledema were the deficiency indicators. They determined that a plasma vitamin A concentration of less than approximately 20 $\mu\text{g}/100\text{ ml}$ suggested the presence of a deficiency in growing Holstein calves.

Plasma concentrations of vitamin A in two groups of lactating Holsteins, one receiving no supplemental vitamin A and the other 50,000 I.U./d/cow, ranged from 6 to 25 $\mu\text{g}/100\text{ ml}$ and 10 to 35 $\mu\text{g}/100\text{ ml}$, respectively (Swanson et al., 1968). Milk yields were not significantly different between the two groups at each stage of the 24-week sampling period.

They reported that milk yield comparisons were possible to only 24 weeks of lactation because of health disturbances. Abortions and mastitis were the main causes for halting the comparisons. The correlation between low plasma vitamin A values and occurrence of mastitis strengthens conclusions from recent trials regarding this interrelationship. Several recent studies have indicated lower somatic cell count (SCC) and California Mastitis Test (CMT) scores that correlate with higher plasma vitamin A concentrations in dairy cows (Chew and Johnston, 1984; 1985; Chew et al., 1982). The plasma vitamin A values averaged 85 $\mu\text{g}/100\text{ ml}$ for the cows with the lowest CMT scores (SCC < 500,000) and 67 $\mu\text{g}/100\text{ ml}$ for those with the highest CMT scores (severe infection).

Thus, plasma vitamin A concentrations that correspond with dietary intakes adequate for maintaining milk production may be inadequate for overall health.

Sources and Availability. There has been no proof of retinol in plants, but over 400 carotenoids have been isolated from plants and about 50 to 60 have demonstrated provitamin A activity (Bauernfeind et al., 1981). Carotenoids are found in some higher animals, but they are of plant origin. Thus, all vitamin A in nature originates from carotenoids.

Carotenoids are present in tissues of green plants and are usually associated with chlorophyll in the chloroplasts, probably as protein complexes, and in lipid formations

(Goodwin 1954). Crampton and Harris (1969) compiled an extensive list of feedstuffs with analytical data that displayed protein, energy, vitamins, minerals, plus plant maturity and method of harvesting, among other traits. From these data it was determined that green grass, immature and early-bloom legumes, and grasses cut and quickly dried into hay provide the major sources of provitamin A. Alfalfa is a preferred forage for dairy cattle. Carotene contents of dehydrated alfalfa and direct-cut alfalfa haylage are consistently higher than the carotene content of sun-cured alfalfa hay (Charkey et al., 1961; Gorden et al., 1960; Livingston et al., 1970). Losses of carotene during storage of alfalfa are substantial. Over a 12-week period, losses ranged from 40 to 75% of the carotene content of alfalfa processed in various forms (Charkey et al., 1961; Gorden et al., 1960; Livingston et al., 1970). Further, destruction of carotene and vitamin A in the digestive tract of ruminants has been observed by King et al. (1962) and Klatte et al. (1964) who used ruminal fluid to evaluate recovery rates of β -carotene and vitamin A. Losses ranged from 33 to 40%. King et al. (1962) also fed vitamin A and carotene with a chromic oxide marker and reported losses of approximately 40%. Use of a commercial, stabilized vitamin A preparation with antioxidants (tocopherol, santokuin) reduced losses in vitro (King et al., 1962). Attempts to stabilize vitamin A in the rumen environment by adding α -tocopherol or cottonseed oil

proved ineffective, with losses averaging 40-60% (Long et al., 1971; Mitchell et al., 1968).

Warner et al. (1970) determined if higher proportions of grain in a diet might influence vitamin A destruction. Losses of vitamin A at the 60 and 80% grain intakes were higher ($P < .01$) than at the 20 and 40% grain consumptions. They suggested that elevated ruminal microbial populations, which coincided with high concentrations of available carbohydrates in the diet, accounted for the increased destruction of vitamin A.

The high producing dairy cow requires feeds with an energy density above what forages can provide. Feed grains fill this role. However, with lower forage amounts and higher grain intakes, the supply and availability of vitamin A and its precursors will be lower. Thus, supplementation is of the utmost importance for the dairy cow.

Interactions. The relationship of vitamin A and vitamin E in vivo has been recognized and disputed for many years. Davies and Moore (1941) showed vitamin E had a protective effect on vitamin A stored in the liver. Another benefit was improved growth in rats when a minimal amount of vitamin E was added to a diet that contained a marginal level of vitamin A (Hickman et al., 1944). McGillivray (1951) evaluated the carotene content of bovine butterfat. During the summer period the carotene content of butterfat dropped substantially

unless 1 gram of tocopherol was supplemented in the diet. McGillivray attributed the benefits derived from the tocopherol to its antioxidant properties, which prevented peroxidation of the fat-soluble vitamin A.

Green et al. (1967) disagreed with the antioxidant theory on the basis that in previous experiments in rats and chicks they were unable to detect lipid peroxidation in vitamin E or selenium deficiencies. They theorized that vitamin E had other effects on vitamin A metabolism, such as modifying the absorption process of the gut, the storage mechanism of the liver, or the utilization of vitamin A in specific tissues. However, subsequent trials proved conclusively that vitamin E is directly involved with prevention of lipid peroxidation at the cellular level (Noguchi et al., 1973; Rotruck et al., 1972).

The protection that vitamin E or *santoquin* (an antioxidant) afforded vitamin A in the rumen could only be demonstrated in vitro (King et al., 1962). Sklan and Donoghue (1982) and Yang and Desai (1977), using rats and chicks, have shown that vitamin A is protected intraluminally by vitamin E. They found that dietary vitamin A reduced the absorption of tocopherol as Green et al. (1967) had reported, but it was mainly due to increased oxidation of the tocopherol before it reached the duodenum, thus sparing the vitamin A. Robinson et al. (1979, 1980) used diets varying in vitamin E and A concentrations to determine the effects of these diets on

photoreceptor membranes of rats. The degeneration of photoreceptor cells was significantly higher in the -E -A diet (92%), compared to the -E +A (20%), or +E -A (34%) diets. Vitamin A availability at the tissue level appears to be enhanced through the antioxidant properties of vitamin E.

Vitamin E

Requirements. The National Research Council (NRC) (1978) recommends adding 300 ppm of vitamin E to calf milk replacers but has given no other requirements for vitamin E in the diets of dry or lactating dairy cows. The NRC concluded that, under most conditions, natural feedstuffs appear to supply adequate quantities of vitamin E, but recognized that prolonged storage of feedstuffs decreases vitamin E content and may cause production of milk that is susceptible to oxidized flavor. The conclusion of the NRC was based on a study by Gullickson et al. (1949) in which 30 animals were fed throughout their lives on diets providing adequate amounts of all nutritive factors known to be essential except vitamin E. Milk production and reproductive performance were considered adequate. However, the milk-producing capability of the dairy cow today is much higher than cows of 40 years ago, and other management changes have contributed to a much higher degree of stress for the cow. Further, several of the animals died from apparent heart failure (Gullickson et al., 1949). They

did not attribute this to a vitamin E deficiency. However, muscular degeneration of the heart could have been a possibility with the low vitamin E intake. Thus, the amount of vitamin E required by the high-producing dairy cow should be re-evaluated.

Plasma Values. Several studies have demonstrated that dietary tocopherol provided to animals appears rapidly in tissues and plasma. Schingoethe et al. (1982) reported that serum vitamin E concentrations doubled within a day after cows received an injection of vitamin E and Se (1.5 I.U., .11 mg per kg body weight, respectively). Oral supplementation of α -tocopherol acetate at 500 I.U./cow/d beginning at parturition rapidly increased the serum vitamin E concentrations within the first month of lactation in dairy cows (Stowe et al., 1988). Rousseau et al. (1957) provided d- α -tocopherol acetate at 0, 1.0, 3.0, or 9.0 mg./lb. of live weight/day (0, .45, 1.36, or 4.1 mg./kg in the diet), to calves, lambs and pigs. They reported that plasma vitamin E concentrations increased as the supplementation rate of tocopherol increased at a decreasing rate, whereas liver tocopherol increased at a constant rate with actual tocopherol intake. Several other researchers, using various species, also have reported plasma vitamin E increasing at a decreasing rate when higher amounts were supplemented (Combs and Scott, 1974; Reddy et al., 1987; Yang and Desai, 1977).

Reddy et al. (1986) reported higher ($P < .05$) lymphocyte stimulation indexes for calves with the highest serum vitamin E concentrations. Cipriano et al. (1982) supplemented 1 gm α -tocopherol/d/calf and observed plasma tocopherol values of approximately 650 $\mu\text{g}/100\text{ ml}$ at 6 weeks of age. The lymphocyte stimulation indexes were higher for these supplemented calves, but not significantly different from the calves receiving no vitamin E. Other results relating higher plasma vitamin E concentrations to various animal performance traits have been reported. Among them are reductions in the occurrence of intramammary infections, retained placenta, and oxidized flavor in milk (Harrison et al., 1984; Schingoethe et al., 1978; Smith et al., 1985). Vitamin E is deposited in several tissues; however, plasma concentrations of vitamin E appear to be a good indicator of the vitamin E nutritional status for various species.

Sources and Availability. There is a wide range in the vitamin E content of grains and forages. Bunnell et al. (1968) compiled extensive tables displaying the means and ranges of the α -tocopherol contents for a number of foods and feedstuffs. Two important feed grains for dairy cattle in the U. S. are corn and milo. The average tocopherol content of milo (1.22 $\mu\text{g}/100\text{g}$) is much lower than that of corn (1.99 $\mu\text{g}/100\text{g}$). Numerous by-products fed to dairy cattle, such as wheat middlings, distiller's grains, corn gluten feed, and

others, are also good sources of α -tocopherol (2.59 to 3.05 $\mu\text{g}/100\text{g}$). Potentially the richest sources of α -tocopherol for dairy cattle are green forages that are provided in the form of pasture, green chop, dehydrated meals or pellets, and freshly baled hay (Bunnell et al., 1968; Charkey et al., 1961; Kivimae and Carpena, 1973; Livingston et al., 1968). The data indicate alfalfa is potentially the most abundant source of α -tocopherol of the common feedstuffs.

The time of year, stage of plant maturity, methods of processing, and length of storage directly affect α -tocopherol content of forages and grain. Bruhn and Oliver (1978) reported highly significant differences between α -tocopherol content of first (May 22) and fifth (September 17) cuttings of alfalfa hay. The α -tocopherol content of alfalfa attained a maximum value when 50-75% of the plants had matured to bud stage (Burrows and King, 1968). These workers also reported that α -tocopherol declined in corn after the full dent stage. When forage crops were exposed to natural sunlight there was a rapid decline in the tocopherol content (Bunnell et al., 1968; Charkey et al., 1961; Thafvelin and Oksanen, 1966). Artificial drying was superior to normal haymaking in the preservation of tocopherols of grasses (Bunnell et al., 1968; Charkey et al., 1961; Livingston et al., 1970). Artificial drying of corn containing approximately 75% dry matter to a dry matter content of approximately 87.5% caused only a slight reduction in the α -tocopherol content (9.30 vs. 8.99 $\mu\text{g}/\text{g DM}$).

However, substantial losses of α -tocopherol from high moisture corn which was ensiled occurred throughout the 230 d storage period (Young et al., 1975). Dehydrated alfalfa meals were stored at 32°C for 12 wk and the tocopherol losses ranged from 48 to 80% for various samples (Livingston et al., 1970). When grain and forage samples were compared after storage to determine α -tocopherol losses, those stored at higher moisture and heat had the largest reductions (Bruhn and Oliver, 1978; Kodicecek et al., 1959; Livingston et al., 1968, 1970).

Differences of bioavailability for various α -tocopherol compounds were reported by Hidiroglou et al. (1988a,b). In two separate studies equal amounts of dl- α -tocopherol, d- α -tocopherol, dl- α -tocopheryl acetate, and d- α -tocopheryl acetate were fed to sheep (400 I.U./d) and to cattle (1,000 I.U./d) for a 28 day period. Blood samples were taken during the feeding period and tissue samples collected at slaughter. Higher concentrations of plasma α -tocopherol were observed in sheep and cattle receiving d- α -tocopherol, compared to all other forms. Tissue samples also contained higher concentrations of α -tocopherol when d- α -tocopherol was fed. Concentrations of α -tocopherol in tissue samples were higher when d- α -tocopherol and d- α -tocopheryl acetate were fed than when the l-isomeric forms were supplemented.

The preintestinal environment of the ruminant exerts a major influence on the availability of vitamin E. Hidiroglou and Jenkins (1974) administered radiotocopherol into the

rumen, abomasum, and duodenum of sheep and found that the preintestinal losses of α -tocopherol were substantial. Alderson et al. (1971) reported increasing amounts of vitamin E disappearance as higher amounts of corn were fed to steers in a nutritionally balanced diet. Though no direct evidence was given, they concluded that with higher amounts of available carbohydrates from corn the number of micro-organisms in the rumen increased and exerted a destructive influence on the fat-soluble vitamin E.

Interactions. Studies with poultry, rabbits, and cattle have shown that increased quantities of poly-unsaturated fatty acids (PUFA) lead to increased requirements for vitamin E (Dam, 1943; Hove and Harris, 1947; Blaxter et al., 1953). Chicks fed purified diets low in PUFA and free of vitamin E or other antioxidants grew and developed normally (Bieri et al., 1960). By comparison, the addition of cod-liver oil to the diet of calves raised the vitamin E requirement 100-fold (Blaxter, 1962). Thus, a major biological function of α -tocopherol in vivo is to prevent lipid peroxidation in cellular membranes by terminating oxidative chain reactions among unsaturated fatty acid molecules (Noguchi et al., 1973; Tappel, 1962).

Mertin and Hughes (1975) reported a non-specific suppressive action by PUFA on lymphocyte transformation. Arachidonic (C20:4) and linoleic acid (C18:2) were responsible

for the largest decreases in lymphocyte transformation. Erickson et al. (1980) also reported that mice fed diets high in PUFA exhibited significantly lower lymphocyte blastogenesis. Linoleic and arachidonic acid are known to be precursors of prostaglandins, and prostaglandins play a significant role in the pathogenesis of bovine mastitis (Giri et al., 1984). Regulation of prostaglandin synthesis can be affected by vitamin E (Lawrence et al., 1985) and in this way vitamin E might alter the severity of inflammatory responses.

The metabolic interrelationship of vitamin E and selenium (Se) is particularly evident in deficiency diseases that can be prevented either by vitamin E or Se. The singular and additive effects of these nutrients were first revealed by Schwarz and Foltz (1957), using rats. Necrotic liver degeneration was prevented by the addition of Se or vitamin E to the semipurified diet. Thompson and Scott (1968) demonstrated a relationship between the Se requirement and the amount of vitamin E in the diet for chicks. The Se requirement was lowered to 0.01 ppm when 100 ppm of dl- α -tocopherol acetate was included in the semipurified diet, but with no added vitamin E, Se requirements were increased to 0.05 ppm for maintenance of overall health.

The most prominent condition in ruminants which indicates a Se deficiency is nutritional muscular dystrophy. Naturally occurring myopathies of sheep and cattle are preventable by low concentrations of Se, whereas large doses of vitamin E are

either ineffective or afford only partial protection (Blaxter, 1962). However, the experimental dystrophy induced in livestock by feeding of unsaturated fats is preventable by vitamin E, but not by supplementation with Se (Blaxter, 1962).

Se is known to be a component of glutathione peroxidase (GSHpx), an important enzyme in the destruction of H_2O_2 (Rotruck et al., 1973). Hoekstra (1975) proposed a scheme concerning the maintenance of cellular integrity in which GSHpx was responsible for destroying H_2O_2 and organic hydroperoxides within the cytosol, and vitamin E prevented the formation of lipid hydroperoxides in the cellular membranes. These additive but separate effects at the cellular stage have been demonstrated adequately in chicks and rats (Noguchi et al., 1973; Rotruck et al., 1972).

Selenium

Requirements. The discovery by Schwarz and Foltz (1957) that Se was an integral part of "factor 3" which protected rats against necrotic liver degeneration initiated the era when Se would not be considered as a toxic element only, but also as an essential mineral. Se deficiency in calves results in white muscle disease (Ammerman and Miller, 1975), which causes degeneration and necrosis in cardiac and skeletal muscles. Also, heart failure, paralysis (usually of the hind legs), a dystrophic tongue and elevated serum glutamic oxaloacetate

transaminase (SGOT) values may be evident (National Research Council, 1978). Deficiencies in Se have also been linked to exudative diathesis in chicks (Thompson and Scott, 1968), hemoglobin oxidation in rats (Rotruck et al., 1972), and impaired neutrophil function in rats and cattle (Arthur et al., 1981; Serfass and Ganther, 1975). The role of Se as an essential element in glutathione peroxidase established a unique function for this mineral apart from its well known relation to vitamin E (Rotruck et al., 1973). Erskine et al. (1987) proposed that a decrease in bactericidal activity by GSHpx-deficient leukocytes of dairy cattle may result in a significant increase in somatic cell counts. They reported significantly higher plasma Se values and GSHpx activity in cows from low SCC herds, as compared to high SCC herds.

The present dietary requirement of dairy cows for Se is 0.1 ppm, and the lowest toxic concentration is 3-5 ppm (National Research Council, 1978). The elevated plasma Se values in the blood of cows within dairy herds that exhibited fewer cases of clinical mastitis and lower SCC (Erskine et al., 1987; Smith et al., 1985) may indicate a need for higher dietary intakes of Se.

Sources. The occurrence of Se has been determined in a wide variety of rocks, minerals, soils, plant materials and waters (Lakin, 1972). Soils that produce plants which are toxic because of their Se content are confined to small areas but

occur throughout the world.

The Se content of forages and grains in the United States is highly variable (Kubota et al., 1967). As cited by Ammerman and Miller (1975) the northwest, southeast and northeast regions of the U.S. are locations where over 80% of the forage and grain contain $< .05$ ppm Se. The Se content of plants in areas which border these regions is variable ($50\% > .1$ ppm). Throughout the central plains the average Se content is $.1$ ppm for 80% of the forages and grains. The Dakotas, Nebraska, Wyoming, Montana, Colorado, and Kansas are states which have the majority of locations where Se accumulator plants grow which contain > 50 ppm Se. A high correlation exists between the Se content of grains and forages and serum Se concentrations of animals consuming those feedstuffs (Stevens et al., 1985). Increasing the Se intake of animals can be accomplished by supplying feed to them from regions where the Se content of the crops is known to be higher (Ku et al., 1973; Ullrey et al., 1977). Transporting feed can be costly and supplementation with inorganic sources, mainly sodium selenite and sodium selenate, have proven very effective in supplying the Se in animal diets necessary for the maintenance of health and optimal performance (Hidiroglou et al., 1965; Julien et al., 1976; Smith et al., 1984, 1985).

Availability. There are many factors that determine the availability of Se for use by the host animal. The method of

supplementation has a direct influence on availability. Whanger et al. (1968) indicated that much of the dietary Se is reduced to insoluble forms by rumen microbes. They also reported that a high-carbohydrate diet was more favorable for converting Se to the insoluble form than was a high-roughage diet. Waite et al. (1975) reported a 66% loss of ruminally administered Se in the feces compared to 3.6% for the intramuscular dose. Julien et al. (1976) were not able to detect an increase in plasma Se concentration after administration of a single 6 mg oral bolus of sodium selenite to cows. A second group of cows received a 12.5 mg bolus of sodium selenite for the initial 5 days and weekly thereafter in a 60 day prepartum period (Total = 165 mg). A third group received a single injection of 50 mg of sodium selenite at 20 days prepartum. The plasma Se concentrations were virtually identical (.084 vs. .085 ppm) respectively for the second and third groups at day of parturition.

Vitamin E and elements such as sulfur, copper, mercury, cadmium, and arsenic also influence the availability of Se. Groce et al. (1968) reported a significant decrease in the amount of Se excreted in the feces of pigs fed seleniferous corn when vitamin E was added to the diet. However, this group of pigs exhibited higher fecal and urinary excretion of Se compared to those pigs fed a selenite premix diet. Hill (1974) fed toxic amounts of Se dioxide to chicks and found that additions of mercuric chloride, cupric sulfate, or

cadmium sulfate partially alleviated the selenium toxicity. Arsenic has the ability to reduce Se toxicity by increasing the biliary excretion of Se and thus lowering the tissue content of Se (Levander, 1977). The effect of sulfur on Se absorption is inconclusive. Paulson et al., (1966) reported that supplementary sulfate had no significant effect on the fate of selenate in the lactating ewe, whereas Pope et al. (1980) reported the amount of Se retained was inversely related to increasing amounts of sulfur added to the diet. Whanger et al. (1978) reported that when a sulfur deficiency occurred intraruminally, it caused a significant decrease in the Se content of ruminal micro-organisms. However, the availability to the animal of the Se that is incorporated into micro-organisms is not known.

Differences between absorption rates for organic Se vs. inorganic Se in animals are quite apparent. Ullrey et al. (1977) used growing lambs in two experiments and growing beef cattle in another to compare net Se absorption rates. The Se content of diets was adjusted by use of seleniferous corn or sodium selenite. They reported that Se from the seleniferous corn had a much more pronounced effect on increasing tissue Se concentration. Similar increases in the tissue Se content for pigs were reported by Ku et al., (1973) who compared sodium selenite and seleniferous corn in diets. Selenomethionine exhibited a higher retention rate in chicks than sodium selenite, fish meal, or fish solubles (Miller et

al., 1972). Cantor et al. (1975) evaluated the availability of Se according to the protection it provided against exudative diathesis in chicks. They reported a low correlation between GSHpx activity and plasma Se concentrations when the Se source in the diet was selenomethionine, compared to a high correlation when sodium selenite was the Se source. Two distinctly different methods were used to measure biological availability, but the results may indicate an important basis for evaluating the net benefit derived from various sources of Se. Ku et al. (1973) reported higher tissue retention rates of Se with seleniferous corn, but no differences in performance were recorded. Smith et al. (1985) reported higher plasma GSHpx values with supplementation of sodium selenite, and Stevens et al. (1985) observed higher GSHpx activity in dairy cows that received Se from forage and grain, but a direct comparison of GSHpx activities between cows which received the same amount of Se from different sources (organic vs. inorganic) has not been reported. This type of trial may determine the true availability for various sources of Se.

Nutritional Status. The concentration of Se in animal tissue progresses from the highest concentration in the kidney, followed by the liver, spleen, pancreas, intestine, and lungs. Cardiac muscle contains appreciably more than skeletal muscle, and wool or hair may have relatively high amounts. The Se

concentrations in these tissues and in plasma accurately reflect dietary Se intake (National Research Council, 1983). Selenium plasma concentrations of less than .05 $\mu\text{g}/\text{ml}$ significantly increases the risk of retained placenta (NRC, 1983). Julien et al. (1976) reported the overall incidence of retained placenta was reduced from 38 to 0% when Se was supplemented at .92 mg/cow/d beginning 3 days prepartum. Harrison et al. (1984) also reported higher plasma Se and GSHpx values in cows that received a single intramuscular injection 3 wk prior to calving. The dosage of Se was .1 mg/kg of body weight, and the Se plasma values at calving were approximately .057 $\mu\text{g}/\text{ml}$ (supplemented) and .0306 $\mu\text{g}/\text{ml}$ (unsupplemented).

The activity of GSHpx and its direct relationship to blood Se concentrations have been proven in a number of animal species. Scholz and Hutchinson (1979) examined several components of blood from dairy cows and reported that 98% of the GSHpx activity in peripheral blood was associated with erythrocytes. The GSHpx activity and blood selenium concentrations exhibited a positive linear relationship ($r = 0.958$). Stevens et al. (1985) evaluated GSHpx activity, plasma Se concentrations, and aspartate amino-transferase values of dairy cattle, and the Se content of grains and forages grown on those dairies. The herds were grouped according to predicted Se content of the crops. The groupings were labeled as deficient (non-detectable), variable (an

average of $.118 \mu\text{g/g}$ feed) and toxic (an average of $1.14 \mu\text{g/g}$ feed). There was a positive correlation ($r = 0.9493$) for all 3 groups between GSHpx and serum Se concentration. Both GSHpx activity and plasma Se concentrations are acceptable methods to determine the Se status of dairy cattle.

Humoral Immune Response

Vitamin A. Antibody forming cells (AFC) were five times higher in mice supplemented with 3,000 I.U./d of vitamin A palmitate than in mice receiving no vitamin A palmitate (Cohen and Cohen, 1973). Mice were supplemented four consecutive days with 0, 1,000, 3,000, or 9,000 I.U. of vitamin A palmitate. On the 4th day the mice were immunized with sheep red blood cells (SRBC), and 4 days later the number of AFC/spleen were measured. The highest dose of vitamin A (9,000 I.U.) proved to be toxic, while supplementation of 3,000 I.U. resulted in the highest AFC response. Cohen and Cohen (1973) also reported that vitamin A was effective for improving antibody response to immunization with dinitrophenylated ovalbumin. Higher AFC quantities were reported when supplementations of hydrocortisone were given with vitamin A than when given without vitamin A. Cohen and Cohen (1973) attributed this higher value to the ability of vitamin A to increase permeability of lysosomal membranes. This conclusion was based on the theory that lysosomal

contents can initiate cell division. Jurin and Tannock (1971) reported an increase in hemagglutinin antibodies in vitamin A-supplemented rats after they had been sensitized with SRBC. The titres were measured at 10 and 15 d after sensitization and the titre values were highest at 15 d. Krishnan et al., (1974) reported that incorporation of [^3H -methyl] thymidine into DNA of cells derived from the thymus and spleen of vitamin A-deficient rats was six times lower than in vitamin A-adequate littermates. AFC capabilities were nearly two times higher for the rats receiving adequate vitamin A.

Tengerdy and Brown (1977) evaluated the effects of supplementing vitamins E and A for the protection of chicks against *E. coli* infection. Either vitamin when supplemented (vitamin A 60,000 I.U./kg diet, vitamin E 300 mg/kg diet) by itself reduced mortality among the chicks, but in combination the vitamins were less effective than when given by themselves or not supplemented at all. The higher clearance rates (removal of antigens over time) of *E. coli* observed in the supplemented chicks was attributed to improved humoral immunity and its positive effects upon phagocytosis.

Barnett and Bryant (1980) reported a suppressed primary response to ovalbumin in mice supplemented with retinol at day 1 after sensitization, but a much higher antibody concentration following a second injection of ovalbumin at day 30. The variability in results from these tests may be a result of the antibody measuring system used, the form or

route of administration of the vitamin A, or amounts of vitamin A used. However, it appears that vitamin A does significantly influence humoral immune responsiveness.

Vitamin E and Se. Significant improvement of the humoral immune response system has been reported as a result of vitamin E or Se supplementation to various animals. Spallholz et al. (1973) observed that Se supplemented at rates of 10-30 times nutrient requirements enhanced the primary immune response, measured by the number of AFC present in mice challenged with SRBC. This stimulation by Se was independent of vitamin E. Sheffy and Schultz (1978) isolated a suppressor substance in the serum of dogs that was removed by the addition of vitamin E, but Se had no effect. The dogs were vaccinated with a canine distemper-infectious hepatitis virus and the vitamin E deficient group exhibited low antibody titres for the 28 d following immunization. Sheffy and Schultz (1978) used dithrotritol, ethoxyquinone and other synthetic antioxidants in vitro and were able to eliminate the suppressive factor in the serum. This does not agree with earlier work by Tengerdy et al. (1973), who reported improved humoral response with the addition of vitamin E to diets of mice, but the use of synthetic antioxidant did not restore the immune response. Differences between species and antioxidants may explain this discrepancy.

Vitamin E and Se concentrations in diets of various

animals have a variable effect on the subclasses of immunoglobulins (Ig) that the animal produces. Reffet et al. (1988) observed increased IgM concentrations with Se supplementation but neither vitamin E or Se affected IgG concentrations in sheep challenged with parainfluenza virus. Spallholz et al. (1973) reported that mice supplemented with Se and challenged with SRBC had enhanced IgM and IgG titres at day 14 post-sensitization. Mice immunized with SRBC and supplemented with vitamin E had increased IgM and IgG hemagglutinin titres compared to deficient mice and those receiving a synthetic antioxidant (Nockels, 1979).

Variations among individual classes of Ig are important because certain qualities are inherent to each in their role of fighting disease. The different antigens, species of animal, and method of analysis for Ig influenced the conclusions drawn from these experiments. However, an improvement in total AFC capability was a typical response for vitamin E or Se supplementation.

Cell Mediated Immune Response

Vitamin A. The literature regarding the effect of vitamin A and carotenoids on cell-mediated immune (CMI) response is quite extensive. The most common methods for measuring CMI responses are blastogenic responses, transplant rejection, and in vitro cell-mediated cytotoxicity against tumor cells.

Jurrien and Tannock (1971) used mice to evaluate mean rejection times of skin grafts. Three groups of mice were injected with different amounts of vitamin A from 5 days before to 10 days after grafting. A 4th group was given no supplemental vitamin A. The three supplemented groups averaged 23-24 d for total rejection of graft to occur while the unsupplemented group required 35 d. Seifter et al. (1981) reported a similar decrease in rejection time when β -carotene was the vitamin A source.

Vitamin A deficiencies in rats have resulted in lowered splenocyte and thymocyte blastogenesis. Nauss et al. (1979) reported that lymphocytes of vitamin A-deficient rats exhibited only one-third the transformation response to the mitogens concanavalin A (Con-A), phytohemagglutinin (PHA), and lipopolysaccharide (LPS), compared to rats receiving adequate vitamin A. Vitamin A deficiency depressed splenocyte response to poke-weed mitogen (PWM), LPS, Con-A, PHA, and lentil (Butera and Krakowka, 1986). Shapiro and Bendich (1985) observed that carotenoids were an immunoenhancing factor against T and B cell mitogens and that the effect was separate from that exhibited by vitamin A.

Saffiotti et al. (1967) reported that reductions of squamous metaplasia and malignant squamous tumors occurred in golden hamsters that received high doses of vitamin A but they were unable to determine the mechanism involved. Retinoic acid supposedly enhances natural killer cell activity, which

is antagonistic to tumor promoters (Goldfarb and Herberman, 1981). However, Seifter et al. (1981) reported that mice supplemented with retinoic acid required significantly more days for complete tumor regression, compared to mice receiving other carotenoids. Vitamin A influences reactions to different antigens and the mechanism by which it accomplishes this is not completely understood.

Vitamin E and Se. The ability of vitamin E to improve CMI response was perhaps first noted by Tengerdy and Brown (1977). They assigned day-old broiler chicks to one of nine dietary groups. Various concentrations of vitamins A and E were fed in the diets. Half of the chicks were immunized with a virulent strain of *E. coli* at 7 d of age and survivors were challenged again at 21 d. Those chicks receiving E (300 mg/kg diet) or A (60,000 I.U./kg diet) had improved phagocytotic capabilities aided by increased production of humoral antibody. They reported higher amounts of vitamin E in the spleen of chicks that were supplemented, and proposed that the additional protection against *E. coli* infections was a lymphopoietic function or that vitamin E adversely affected prostaglandin (PG) synthesis. Likoff et al. (1978) supplemented aspirin and vitamin E to evaluate immune response in chicks and observed decreased PG synthesis in the bursa and spleen when vitamin E or aspirin were fed.

There is a positive correlation between the vitamin E and

Se status of animals and their lymphocyte proliferation capabilities. Lymphocytes from vitamin E-deficient dogs responded poorly to Con-A, PHA, PWM, and Streptolysin O (Sheffy and Schultz, 1978). Eskew et al. (1985) fed a torula yeast-based diet with various combinations of vitamin E and Se to rats. Rats fed a diet deficient in vitamin E or Se exhibited decreased blastogenesis to mitogens and those deficient in E and Se displayed a greatly reduced mitogenic response. Reddy et al. (1986) supplemented 4 groups of calves weekly with α -tocopherol at 0, 1400, 2800 mg orally or 1400 mg injected intramuscularly. At the 8th wk of the trial, the lymphocytes of the calves receiving the injection had the highest lymphocyte stimulation index of the 4 groups. Cipriano et al. (1982) reported higher lymphocyte stimulation indexes for calves receiving supplementary vitamin E. However, the differences were not significant, compared to the groups receiving no vitamin E supplementation.

Mastitis and the Immune Response. Once an antigen has breached the teat and entered the lumina of the udder, there is a host of cellular and humoral defense systems that may take part in its destruction. An essential component of intramammary defense against disease is the leukocyte family, of which polymorphonuclear neutrophils (PMN) and macrophages (MO) are the major participants (Paape, 1979). Opsonization of antigens via Ig or Ig plus complement is considered an

essential function of the humoral immune system (Targowski, 1983). Phagocytosis by the PMN and MO depends partially on the concentration of Ig present in the mammary system and the interaction of cell surface receptors. The amounts of the different classes of Ig are: IgG1, 0.59; IgG2, 0.02; IgA, 0.14; and IgM, 0.05 mg/ml of milk (Butler, 1974). These low amounts, plus the inability to sustain higher concentrations of opsonizing factors in vivo, can adversely affect phagocytosis (Lascelles, 1979). However, bovine PMN and MO have been reported to have multiple receptor sites which would accommodate a variety of opsonizing factors (Grewal et al., 1978).

Studies of the CMI response in vivo for dairy cattle are limited and much of the information used to define bovine lymphocyte response has been derived through experiments with rodents. The roles defined at this point for CMI are: synthesis of chemoattractants (lymphokines), production of interferon (antiviral agent), activation of phagocytic cells, and providing cytotoxic T-lymphocytes (killer cells). Perhaps the most important is the ability T-lymphocytes have to attract and activate PMN and MO for phagocytosis (Schultz, 1981). The importance of neutrophils in fighting an infection was demonstrated by injecting cows with equine anti-bovine leukocyte serum to establish neutropenia (Jain et al., 1968). Subsequent exposure of the mammary gland to *Aerobacter aerogenes* resulted in unrestricted growth of the coliform

bacteria in the udder.

Concha et al. (1978) reported that the total lymphocyte population (T and B-cells) in normal milk is only 160-300 lymphocytes/ml milk (1% of total cell population), and their proportion in milk and blood of bovines (20% B cells, 45% T cells, 35% unidentified) is approximately the same. Lymphocyte cells in bovine milk exhibit an adequate blastogenic response in vitro, but the mitogenic response of the blood lymphocyte cells is much higher than those in the milk of the same animal (Smith and Schultz, 1977; Concha et al., 1980). Also, the ability of these lymphocytes to perform is different throughout the lactation. Kashiwazaki (1984) examined the peripheral blood lymphocyte activities of primiparous, multiparous, and mastitic cows using PHA, Con A, and PWM as mitogens. He concluded that cows most susceptible to infection were those with low blastogenic responses and that for older cows this vulnerability usually occurred within the first 10 d of lactation.

The total number of lymphocytes in the mammary gland is relatively small and perhaps their activity is restricted by the internal environment of the mammary gland. However, the potential benefits they can provide to fight infections are substantial.

Antioxidants

Tappel (1962) discussed the function of antioxidants in terminating the chain reaction which occurs in lipid peroxidation. He concluded that animals which contain high amounts of polyunsaturated lipids have a great need for an antioxidant and that vitamin E fills this role. Free radicals that arise from either the cleavage of covalent bonds in organic compounds or electron capture by a molecule can initiate the formation of additional free radicals by reacting with PUFA (McCay and King, 1980). This peroxidation can be interrupted by α -tocopherol which donates a phenolic hydrogen atom to the free radical, thereby quenching the unpaired electron of the radical.

Membranes are composed mainly of proteins and lipids (Molenaar et al., 1980). These constituents, especially the lipids which form a nonaqueous compartment within the cell, would require a fat-soluble antioxidant such as α -tocopherol to prevent lipid peroxidation in the membrane (McCay and King, 1980).

Rotruck et al. (1973) identified Se as a component of GSHpx, and this discovery became the basis for explaining the complimentary role that Se and vitamin E share in nutrition. They noted in previous studies that concentrations of reduced glutathione (GSH) were higher in Se-deficient erythrocytes. Using purified GSHpx from erythrocytes of Se-adequate rats

which had been injected with Se 2 or 4 weeks earlier, they were able to isolate 70% of the Se in the GSHpx fraction. Thus, the importance of Se in the enzyme GSHpx, which aids in the destruction H_2O_2 and lipid hydroperoxides, was established. The activity of GSHpx is associated mainly with the cytosolic portion of the cell while vitamin E exhibits stabilizing effects predominately in the membrane (Noguchi et al., 1973; Rotruck et al., 1972).

Antioxidants and Phagocytosis. Phagocytosis is an important and necessary step in the successful elimination of antigenic particles by cells (Paape, 1979). The intracellular killing of the antigen has been linked to a cellular response called the respiratory burst. Iyer et al. (1961) linked this respiratory burst to intracellular killing of *E. Coli*. They reported a marked increase in oxygen consumption during the first 80 minutes of the exposure of leukocytes to *E. coli*, plus a large increase in activity for the hexosmonophosphate shunt pathway. Previous researchers concluded that the increased oxygen consumed during phagocytosis was for glucose oxidation to provide energy. Iyer et al. (1961) provided evidence that H_2O_2 was formed during phagocytosis and that the addition of catalase with H_2O_2 present increased the rate of formate oxidation. The identification of O_2^- , which is the main end product of the respiratory burst, as a precursor for potent microbicidal agents eventually determined the method

of oxidative killing by phagocytes (Babior et al., 1973).

The importance of oxygen-dependent microbial killing is obvious, and the need to regulate this function to prevent oxidative damage to friendly cells is supported by observations of Capuco et al. (1986). They observed significant epithelial cell damage of mammary tissue in vitro when it was exposed to PMN concentrations of 10^6 or 10^7 /ml. Though tissue damage was significant using lysed phagocytosing PMN, the greatest amount of damage was recorded using intact phagocytosing PMN. The role of vitamin E as an antioxidant, and Se as a component of GSHpx to maintain phagocyte effectiveness, has been demonstrated in several studies. Serfass and Ganther (1975) observed a decrease in fungicidal activity for yeast cells by PMN cells of rats fed a Se-deficient diet. They correlated this decrease with lower GSHpx activity, which at normal rates of action would eliminate lipid hydroperoxides, specifically linoleic acid hydroperoxide, which is reported to have a negative effect on normal phagocytosis and bacterial killing. Boyne and Arthur (1979) isolated neutrophils from the blood of 2 groups of young calves. One group of calves received adequate selenium in their diet, and the other group was Se-deficient. Analysis of neutrophils from the two groups showed equal ability to phagocytize *C. albicans* but there was a 3 fold increase of intracellular killing capabilities for the neutrophils of Se-adequate calves compared to the neutrophils of Se-deficient

calves. Boyne and Arthur (1979) also observed a decrease in nitroblue tetrazolium reduction for the Se-deficient calves, possibly indicating a decreased ability to form superoxide (O_2^-). Gyang et al., (1984) compared differences between the PMN killing ability of dairy cows that were Se-deficient and those receiving a Se-vitamin E injection. After 20 minutes of exposure to the bacteria, the PMN of the Se-deficient cows had 29.4% viable bacteria remaining while the supplemented group retained 20.3%. It is critical to supply the proper amounts of Se and vitamin E to facilitate oxidative killing by phagocytes but there is a potential for inhibiting this and other beneficial mechanisms that promote good health by over-supplementing a source of free radical scavengers and antioxidants. Masataka and Johnston (1980) observed substances that were able to scavenge hydroxy radicals or singlet oxygen which inhibited the killing of *Candida* by mouse MO. Thus, there appear to be various complex mechanisms which regulate the capability of the phagocytic system.

Arachidonic Acid Metabolism and Prostaglandins

The PGs are a complex group of oxygenated fatty acids that have been detected in virtually all mammalian tissues (Kuehl and Egan, 1980). The PGs do not exist in free form, but, as a result of inflammation, they are rapidly biosynthesized from arachidonic acid or other 20-carbon fatty

acids (Samuelsson et al., 1978). Fatty acids released from disrupted cell membranes are considered to be the major source of these precursors of PG. The availability of these fatty acids is the limiting factor in the biosynthesis of PG. Once the arachidonic acid is available an enzymatic oxygenation occurs, producing the endoperoxides PGG and PGH, which then proceed through various pathways with the major one being the PGE₂ isomerase (Kuehl and Egan, 1980).

Bourne et al., (1974) hypothesized that the character and intensity of inflammatory and immune responses is mediated by hormones which elevate the intracellular content of adenosine 3', 5'-monophosphate (cAMP). They observed from previous studies that cAMP inhibits immunologic or inflammatory action of certain leukocytes and that in each trial β -adrenergic catecholamines, histamine, and E-series PGs stimulated cAMP production. The manipulation of PG synthesis by indomethacin, aspirin, vitamin E and other factors has shown this hypothesis of Bourne et al., (1974) to be feasible. Muscoplat et al. (1978) used *Mycobacterium bovis*-sensitized lymphocytes of bovine peripheral blood, and Con-A or pure protein derivative (PPD) for mitogens. Indomethacin, a known inhibitor of the PG synthetase enzyme system, was added to alternate lymphocyte-mitogen preparations. Samples containing the indomethacin exhibited a much higher blastogenic response but the addition of indomethacin had to be within 4 hours of the lymphocyte exposure to Con-A for this response to occur. A

negative correlation between vitamin E concentrations in the spleen and liver of chicks and PG synthesis was reported for the immune response in chicks (Tengerdy and Brown, 1977). Likoff et al. (1978) reported decreased PG synthesis for *E. coli*-challenged chicks receiving aspirin or vitamin E.

A conflicting report regarding the effect of vitamin E on PG concentrations was given by Chan et al. (1980). They fed rabbits a vitamin E-deficient diet for 5 wk and observed a significant reduction in the cyclooxygenase activity. Oral supplementation of vitamin E restored the cyclooxygenase activity to control values within 48 hours. They were not able to quantify whether decreased cyclooxygenase activity or increased synthesis of arachidonate caused accumulation of arachidonic acid in the vitamin E-deficient rabbits. They concluded that lower concentrations of PGE_2 and PGF_2 in vitamin E-deficient rabbits indicated depressed cyclooxygenase activity. While this conclusion does not agree with previous work cited, differences in species, tissue sources, nutritional status, and whether the host is antigenically challenged could all influence PG synthesis.

Halevy and Sklan (1987) examined the effects of β -carotene, retinol, and α -tocopherol on inhibition of arachidonic acid oxidation in bovine seminal vesicles and kidney. β -carotene, retinol, and α -tocopherol depressed prostaglandin formation at all concentrations tested. However, when Tenderdy and Brown (1977) supplemented vitamin

A to chicks, PGE and PGE₂ concentrations were higher than vitamin E-supplemented or control animals. Halevy and Sklan (1987) hypothesized that β -carotene was effective in lowering PG concentrations because of its ability to trap free radicals, and that retinol affects oxidative changes in enzymes which protect against oxidative damage.

These conclusions, plus the role of vitamin E as an antioxidant and free radical scavenger, fit well into a hypotheses put forth by Molenaar et al. (1980). They recognized that unsaturated phospholipids are susceptible to peroxidation when located near integral proteins which form part of an electron transport chain that is capable of generating free radicals. Thus, when unsaturated phospholipids are located near these electron transport systems, the potential release of arachidonic acyl chains is greater. Through calculations they determined that there is approximately 1 molecule of tocopherol per 50 arachidonic acyl chains in the inner mitochondrial membrane which contains electron transport systems, compared to 1 molecule of tocopherol per 500 arachidonic acyl chains in plasma membranes of erythrocytes which are devoid of mitochondria. Thus, the higher concentration of α -tocopherol in certain membranes may act as a built-in regulator of arachidonic acid metabolism.

Prostaglandins and Mastitis. The diverse properties which PGs exhibit are demonstrated in the dairy cow during a

mastitic infection. Jain et al. (1971) reported that cows having a normal blood neutrophil content exhibited rapid inflammation and leukocytosis of the udder within 4 to 6 hours when exposed to *Aerobacter aerogenes*, and bacterial growth was immediately restricted. However, cows made neutropenic showed little inflammation or restriction of bacterial growth for 24 hours. At 30 hours postinoculation, the large amounts of endotoxin present initiated an overwhelming inflammatory response which resulted in irreversible damage to mammary parenchyma. They concluded that the delayed inflammatory response of neutropenic cows was caused by an unknown system operating independently of the leukocytes. However, in a subsequent study using various tissue factors and leukocytes they reported that there was a very close relationship, timewise, between inflammation and leukocytosis (Jain et al., 1972). Histamine caused extreme inflammation but no leukocytosis. The data which indicate that PMN and MO are producers of PGs and that some products of the lipoxigenase pathway, in particular leukotrienes, are chemotactic factors for PMN may help explain the correlation between inflammatory response and PG concentrations (Kuehl and Egan, 1980). Recent studies have reported a positive correlation between the concentrations of various arachidonic acid metabolites and the intensity of the inflammatory response (Giri et al., 1984; Anderson et al., 1985).

Atroschi et al. (1986a,b) examined the relationship

between PG concentrations and GSHpx or vitamin E during periods of intramammary infection or non-infection. In each study the GSHpx and vitamin E concentrations were lower ($P < .05$) for the mastitic cows. The mastitic cows also had higher PGE_2 ($P < .05$) and PGF_2 ($P < .01$) concentrations in their milk during the first study. The thromboxane B_2 (TXB_2) concentrations were higher in mastitic cows but differences were not significant. The PG values in the second report were also higher for infected animals but this time PGE_2 ($P < .05$) and TXB_2 ($P < .01$) exhibited substantial differences. Atroschi et al. (1986a,b) concluded that the benefits that vitamin E and GSHpx may have for reducing mastitis are their antioxidant capabilities that can potentially inhibit PG synthesis and the subsequent effect of PGs on PMN and MO.

EFFECT OF PREPARTUM SUPPLEMENTATION WITH VITAMINS A AND E
AND SELENIUM ON SUBSEQUENT PERFORMANCE OF HOLSTEIN COWS

ABSTRACT

The objectives of this research were to evaluate the effects of supplementation of vitamins A and E, and selenium during the prepartum period on overall health and immune response of cows in early lactation, and to determine the effect of treatments on concentrations of vitamins A and E in plasma. Fifty-six cows and 34 heifers were assigned to one of eight groups in a 2x2x2 factorial design at 60 d prior to expected calving date. Treatments were: (1) Se, (2) E, (3) E Se, (4) A, (5) A Se, (6) A E, (7) A E Se, and (8) control. Blood samples were taken for 12 wk at 4-wk intervals, starting 60 d prepartum, to determine concentration of serum vitamin A (VA) and vitamin E (VE). Serum glutamic oxaloacetate transaminase (SGOT), lactic dehydrogenase (LDH) and lymphocyte blastogenesis (LB) were determined at 7 d and 21 d postpartum.

The VE values were significantly higher in the 4 groups receiving E than in those not receiving E. Group E Se maintained the highest VE concentration throughout the trial. The VA amounts were generally higher in the A supplemented groups, with the A treatment group attaining the highest value. The LDH concentrations, measured at 7 d and 21 d, tended to be lower for all the supplemented groups than in the control group, with the E, E Se, A Se, and A E values being significantly different when compared to those in the control group. However, the SGOT concentrations were not

significantly lower in any of the supplemented groups than in the control group. The LB response for poke weed mitogen was significantly higher for the A E group, than for the control group at 7 d. There were no significant differences in SCC, incidence and duration of clinical mastitis, summit milk yield, or reproductive responses between supplemented groups and controls. Improvements in cellular integrity and immune system responsiveness in dairy cattle during early lactation may be promoted through dietary supplementation of vitamins A and E and Se during the prepartum period. However, the effect will depend on various nutrient concentrations and interactions prior to and during the prepartum period. Proper hygiene and stress management during this time also will influence the effectiveness of supplementation for the dairy cow.

INTRODUCTION

The appropriate management techniques utilized during the dry period regarding hygiene (Macmillan et al., 1983; Neave et al., 1950), duration of dry period (Coppock et al., 1974; Swanson, 1965), and nutrition (Harrison et al., 1986; Julien et al., 1976; Nocek et al., 1986; Smith et al., 1985) have a significant effect upon the performance of the dairy cow in the subsequent lactation. Adequate amounts of vitamins A and E and of selenium are required by the dairy cow for

maintenance of health and milk production (National Research Council, 1978). The functions of these nutrients are quite extensive and well documented. Prepartum supplementation of vitamin A and β -carotene have been effective in reducing somatic cell counts in early lactation (Chew and Johnston, 1985). Supplementing vitamin E and selenium prior to calving and during lactation has proven useful to reduce incidence and duration of clinical mastitis (Smith et al., 1984, 1985).

Research involving supplementation of these nutrients for dairy animals evolved from the realization that feedstuffs can be deficient as a result of location (Ammerman and Miller, 1975) or method of harvesting and storage (Bruhn and Oliver, 1978; Schingoethe et al., 1978). Preintestinal destruction of vitamins A and E can be substantial (King et al., 1962; Alderson et al., 1962).

High milk yields, increase in animals per unit of space, and decrease in man-hours used per cow impose greater stress on the present day dairy cow. Protection from this stress and from infectious agents, such as those which cause mastitis, require a defense system within the cow, including adequate physical barriers (skin, mucus, cilia) and a functional immune system. Vitamins A and E and selenium play important roles in blastogenic responses (Butera and Krakowka, 1986; Nockels, 1979), microbicidal activity (Arthur et al., 1981), and cellular integrity (Noguchi et al., 1973).

This study was undertaken to measure the effectiveness

imparted by various combinations of vitamin A, vitamin E, and selenium, given prepartum, on the overall health and immune response of dairy cattle during early lactation.

MATERIALS AND METHODS

Cows and Rations

Thirty-four primiparous and fifty-six multiparous Holstein-Friesian cows were allotted to blocks of eight animals each as they became available at 60 d prepartum. Cows and heifers in each block were allocated randomly to one of eight treatments in a 2x2x2 factorial experiment with vitamins A, E, and selenium (Se) being the main effects. Supplementations during the prepartum period consisted of: vitamin A (propionate)¹, 1,000,000 I.U., injected intramuscularly at time of allotment; vitamin E (dl- α -tocopheryl acetate)², 2500 I.U. per cow per day, given orally; Se (sodium selenite)³, 0.1 mg/kg body weight (BW), given by injection 21 d prior to expected calving date.

The remaining management procedures were the same for all cows. Prairie hay (Se<.10 ppm; α -tocopherol-110 I.U./kg DM) was provided for ad libitum consumption and supplemented with

1 Injacom, Hoffman-La Roche Inc., Nutley, NJ 07100.

2 Rovimix E125, Hoffman-La Roche Inc., Nutley, NJ 07100.

3 Mu-Se, Schering Corp., Kenilworth, NJ 07033.

4.54 kg of concentrate (Table 1) per cow per d through day of calving. In addition, beginning 21 d prepartum, 2.25 kg/per head (DM basis) of corn silage (α -tocopherol-25 I.U./kg DM) was fed each day, and concentrate intakes were increased daily so the consumption would equal 1% of BW by projected day of calving. During lactation all cows were fed a total mixed ration consisting of alfalfa hay (Se<.10 ppm; α -tocopherol 32 I.U./kg DM), corn silage and a corn-soybean meal based concentrate (α -tocopherol-25 I.U./kg DM). The hay and silage were in a ratio of 60% hay, 40% silage (DM basis) and were fed at 1.75% of BW. All cows received 6.8 kg of concentrate per day in the total mixed ration and additional concentrate as needed to meet production requirements through a computer feeder.

Prepartum Care and Milking

All quarters of all multiparous animals were dry-treated with a commercially available product⁴ at drying off. Housing consisted of free stalls bedded with sand until 21 d prepartum at which time cows and heifers were moved to an open concrete lot bedded with wheat straw. At the first indication that calving was imminent, they were placed in individual maternity stalls with fresh bedding. The housing for the milking herd was total confinement with freestalls bedded with sand as needed.

Immediately after calving the cows were milked with a

⁴ Cefa-Dri, Bristol-Meyers Co., Dewitt, NY 13214.

bucket milker. If no post calving complications occurred, they were returned to the milking herd within 12 h. Cows were milked daily at 0950 and 2150h. In the parlor, the pre-milking procedure included washing udders with water and drying the udders with individual paper towels. After milking, an iodine-based teat dip was applied.

Mastitis Detection and Therapy

Incidence and duration of clinical mastitis were recorded for the first 90 d of lactation. Foremilk samples were removed onto black strip plates for examination at each milking. Occurrences of clots, flakes, or obvious abnormal secretions were recorded. No antibiotic therapy was initiated unless clinical signs persisted beyond six milkings. Comparison of spontaneous recovery capabilities was accomplished in this manner. If the symptoms persisted beyond six milkings, treatment of infected quarters by intramammary infusion with a commercial intramammary infusion product⁵ was initiated. The initial treatment consisted of infusing two tubes of the product per infected quarter, followed by one tube per quarter at the two subsequent milkings. No additional therapy was initiated for 3 d following the final day of the previous treatment period. If clinical symptoms persisted beyond the 3 d, the same regimen was repeated. Recurring clinical symptoms by the same cow did not constitute a new or additional occurrence. However, the total days a cow

5 Today, Bristol-Meyers Co., Dewitt, NY 13214.

exhibited clinical mastitis, regardless of the number of separate occurrences, were used in tabulating the duration of clinical symptoms. The SCC values of the milk samples were determined by a Coulter Counter⁶ at the Kansas DHIA lab on the first four sampling periods postpartum for which each animal was eligible under DHIA regulations. There was an average of 25 d between samplings.

Milk Production and Reproductive Performance

Summit milk yields were tabulated for each cow and used to determine milk production differences between treatment groups. Calving difficulty scores (CDS), incidence of retained placenta, and status of uterine involution at approximately 20 days after calving were evaluated to assess reproductive efficiency. The CDS were on a scale of 1 to 5, with 1 representing no assistance at calving and 5 for extreme difficulty. If the fetal membranes were retained for more than 24 h, the cow was recorded as having a retained placenta. Adequacy of uterine involution was assessed by the same theriogenologist and each cow was scored as normal or abnormal, according to uterine involution or symptoms of infection.

Blood Sampling

Blood was drawn from the coccygeal vein into sterile nonheparinized blood collection tubes on the day of assignment and at 4-wk intervals for 12 wk. Serum concentrations of

⁶ Coulter Counter, Coulter Electronics, Inc., Hialeah, FL.

vitamins A and E were determined. Heparinized tubes were used at 7 and 21 d postpartum to collect blood from the coccygeal vein to determine glutamic oxaloacetate transaminase (SGOT), lactic dehydrogenase (LDH), and phytohemagglutinin (PHA)- and poke weed mitogen (PWM)-induced lymphocyte blastogenesis (LB).

Analytical Procedures

Se and Vitamin E Content of Feeds: Samples of hay, concentrate mixture, and corn silage were collected throughout the experimental period and frozen. After the completion of the experiment, the samples were pooled, and sub-samples of hay and concentrate were analyzed for vitamin E⁷ and selenium⁸ content.

Serum α -Tocopherol: Blood samples taken at 0, 4, 8, and 12 wk of the trial were used to determine serum α -tocopherol by high performance liquid chromatography (HPLC) (Reddy et al. 1985). The following is a brief description of the procedure. Sample tubes were centrifuged and the supernatant was frozen in screw cap vials. After thawing, 1 ml of serum was mixed with 1.2 ml 95% ethanol and extracted into 1 ml iso-octane by stirring for 1 min. The extractions were done within 1 h before the serum was used in the HPLC procedure.

Apparatus consisted of a normal phase 5 μ , 150 x 4.6 mm,

7 TPD Product Development Analytical Services, Hoffman-La Roche Inc., Nutley, NJ 07100.

8 Harris Laboratories Inc., Lincoln, NE 68501.

chromegasphere SI 60 column,⁹ a mobile phase consisting of 8% tetrahydrofuran in iso-octane delivered by an Altex 110A pump, and an Altex 210 injector¹⁰ with a 20 μ l fixed loop. Flow rate was 1.5 ml/min and α -tocopherol was detected using a Kratos FS 970 spectrofluorometer¹¹. Chromatographs were recorded on a Fisher Recordall¹². Serum α -tocopherol identification and quantitation was by comparison of retention time and peak areas with 1 μ g/ml α -tocopherol¹³ standard.

Serum Vitamin A: Serum vitamin A (retinol) concentrations were determined by HPLC. Extraction of serum and equipment used was similar to that described for vitamin E, except that the solvent phase was 100% methanol and excitation was at 380 nm, and an emission filter of 320 nm was used. The standards were prepared by first solubilizing 10 mg of retinol¹⁴ in 10 ml of iso-octane. Further dilutions (10-50 μ g/ml) were in 100% methanol.

Lymphocyte Blastogenesis: The PHA- and PWM-induced lymphocyte blastogenesis was determined by the method described by Reddy et al. (1986). Briefly, lymphocytes were separated from blood by density centrifugation, counted and resuspended in RPMI-1640 medium at a concentration of 2.5×10^5 /ml. The cells

⁹ E. S. Industries, Fullerton, CA.

¹⁰ Beckman Instruments, Fullerton, CA.

¹¹ Kratos Analytical Instruments, Ramsey, NJ 07446.

¹² Fisher Scientific Co., St. Louis, MO 63178.

¹³ dl- α -Tocopherol, Sigma Chemical Co., St. Louis, MO.

¹⁴ Retinol, Sigma Chemical Co., St. Louis, MO.

were then cultured for 48 h in 96-microwell plates (0.2 ml/well) in the presence or absence of PHA and PWM in quadruplicate wells. The cells were then labeled with ^3H [methyl]-thymidine and 24 h later they were harvested on an automatic cell harvester and counted in a scintillation counter.

SGOT and LDH: Serum samples obtained at 7 and 21 d postpartum were analyzed for glutamic oxaloacetic transaminase (SGOT) and lactic dehydrogenase (LDH) by using ready-to-use kits.¹⁵

Statistical Analysis

Data were analyzed as a randomized block design according to the General Linear Model procedure of Statistical Analysis System (SAS, 1985). The model was:

Observation = overall mean + block effect + vitamin A effect (A) + vitamin E effect (E) + Se effect (S) + A*E + A*S + E*S + A*E*S + residual.

The treatment means were compared by calculating least significant differences.

RESULTS

Mean VE values for each sampling period are shown in Table 2. The treatment groups receiving supplemental α -tocopherol maintained higher VE concentrations than the unsupplemented groups in each of the final three sampling

¹⁵ Abbot Diagnostics, North Chicago, IL 60064.

periods. However, only the E Se and A E Se groups were significantly higher for each sampling after supplementation had begun. For most treatment groups, the highest VE concentration usually was recorded at the third sampling, whereas the initial VE concentration tended to be lowest. Excluding the A treatment group, the VE values declined between the 3rd and 4th sampling period.

Mean VA values for each sampling period are in Table 3. The A and A Se groups were higher ($P < .05$) than the control group in period 2, and the A group was higher than the E group, whereas all other groups had similar VA values. VA concentrations were lowest at the third sampling period. Six of the eight treatment groups had their highest VA value at the 4th sampling period. The A group VA concentration was significantly higher than other treatment groups, except group A E, at sampling period 4.

The LB values at 7 and 21 d postpartum are in Figures 1 and 2, respectively. At 7 d the LB response to PHA was similar for all groups. There was a significant difference between the A E treatment and the control group for the PWM response. LB values at 21 d were similar for all treatments.

Mean LDH values are shown in Figure 3. The control group had significantly higher concentrations than the E Se, A Se, or A E groups at the 7-d sampling. At the 21-d sampling, the control group was significantly higher than the E, E Se, and A Se groups.

Mean concentrations of SGOT are shown in Figure 4. The A group was higher than the E and A E groups at 7 d, and at 21 d the A group was significantly higher than the Se, E, A Se, and A E groups.

Least square means for SCC, incidences of mastitis requiring therapy, duration of clinical mastitis, summit milk yield, and reproductive performance traits are presented in Tables 4 - 6. There were no significant differences between supplemented groups and controls regarding these measures.

DISCUSSION

The VE concentrations for the initial sampling period ranged from 2.52 to 3.07 $\mu\text{g/ml}$, which are similar to the values reported by Stowe et al. (1988). They found VE values of 2.18 to 3.17 $\mu\text{g/ml}$ at dry-off. In the present study, VE amounts increased rapidly in the vitamin E supplemented groups throughout the prepartum period, then declined after parturition. Small increases in VE values during the prepartum period and a decline after calving was a trend for the unsupplemented group, which agrees with a similarly reported decline occurring from day of parturition through d 7 of lactation (Stowe et al., 1988).

The E Se group had the highest VE concentrations throughout the final three sampling periods. Erskine et al. (1987) reported higher VE values in cows that also had higher

blood Se contents. Combs and Scott (1974) provided 100 I.U. of dl- α -tocopheryl acetate/kg of diet for chicks, and added different amounts of Se (.01, .04, .06, .08, and .10 ppm). Each increase in Se resulted in higher plasma content of α -tocopherol.

The supplementation of dl- α -tocopheryl acetate at 2500 I.U./d/head significantly increased the VE values, but values were similar or lower than those reported by Smith et al. (1985) who supplemented a much smaller amount (2 mg dl- α -tocopherol acetate/d/kg BW) and 2 μ g Se/d/kg BW. The lower response observed for VE concentrations, when compared to concentrations in their study, may be related to several factors. The complimentary role of Se previously mentioned and the initial VE values of over 5 μ g/ml reported by Smith et al. (1985), may partially explain the comparatively low response for VE values that we observed, using a higher rate of vitamin E supplementation.

Hidiroglou et al. (1988a,b) reported much higher plasma and tissue α -tocopherol values for sheep and cattle when the α -tocopherol source was d- α -tocopherol as compared to d- α -tocopherol acetate, dl- α -tocopherol, and dl- α -tocopherol acetate. Thus, the amount of vitamin E that is retained when supplemented may be affected by the α -tocopherol source.

Animals receiving 1,000,000 I.U. of vitamin A at day of assignment tended to have higher VA concentrations at the second sampling period. The lowest amount for all groups

occurred at the third sampling. Johnston and Chew (1984) reported a similar decline in VA concentrations for cows during the prepartum period. They also observed a marked increase in VA concentrations after parturition, which agrees with results of this study.

The A group maintained the highest VA concentration throughout the study. The significant differences between the A group and other treatment groups may indicate a displacement effect for the storage of vitamin A in the liver when large amounts of vitamin E are supplemented. Research concerning this relationship has given conflicting results. Rousseau et al. (1959) observed that increasing amounts of α -tocopherol fed to calves, lambs, and pigs increased the liver tocopherol concentrations while decreasing the amount of vitamin A in the liver. Yang and Desai (1977) reported that increasing dietary vitamin E improved liver vitamin A stores in rats, and that the highest rate of vitamin E supplementation did not significantly increase or decrease liver vitamin A stores. There appears to be a particular concentration of vitamin E in the diet that facilitates maximum vitamin A storage, which is species dependent. In the present study the 500 I.U./day/head of α -tocopherol in the basal ration and the 2500 I.U./day/head in the supplemented diet were both higher than originally intended. It appears that the lower concentration may be more appropriate than higher supplementation for vitamin A retention.

The improved mitogenic response in the A E supplemented group on d 7 supports previous data that indicate positive roles for these nutrients in cell-mediated immune responses. Others have also reported that Se is important for an adequate lymphocyte response. Eskew et al. (1985) observed that rats fed a diet deficient in vitamin E or Se exhibited decreased blastogenesis to mitogens, whereas the diet deficient in both E and Se caused a greatly reduced mitogenic response. Reddy et al. (1986) reported improved lymphocyte stimulation indexes for calves that had the highest serum α -tocopherol concentrations. Retinol or β -carotene increased bovine blood lymphocyte transformation when exposed to Con-A in vitro, but suppressed blastogenesis when lipopolysaccharide was used as the mitogen (Daniels et al., 1986).

Improvement in the blastogenic response for all groups between the 7 and 21-d samplings agrees well with previous research. Kashiwazaki (1984) found that lymphocyte activity in blood from dairy cows at various stages of lactation (10 d prepartum through sixth week of lactation) was quite variable. Periods of low mitogenic response for multiparous cows were recorded at the beginning of lactation and at 30 to 40 d of lactation, whereas primiparous cow's lymphocytes showed a decrease in activity at 20 to 30 d postpartum.

The increased mitogenic response to PHA and PWM reported for the A E treatment group may be a result of suppressing PG synthesis. PG concentrations were not measured in this trial,

but previous research has indicated that vitamin A and vitamin E may inhibit PG production in vivo. Bourne et al. (1974) concluded that the E-series PGs were part of a group of hormones which increase the intracellular content of adenosine 3', 5' monophosphate (cAMP), and as a result, inhibit the immune response of certain leukocytes. Atroschi et al. (1986b) found a negative correlation between the vitamin E in bovine milk and PGE₂ concentrations when mastitis was present. Likoff et al. (1978) supplemented vitamin E to chicks and observed decreased PG synthesis in the bursa and spleen. β -carotene, retinol, and α -tocopherol all suppressed PG formation in bovine seminal vesicles and kidney (Halevy and Sklan, 1987). However, other researchers have found conflicting results. Tengerdy and Brown (1977) reported that PGE₁ and PGE₂ production was decreased in chicks supplemented with vitamin E but was increased when vitamin A was given. They also reported that when A and E were given in combination, PGE₂ production was the highest. Chan et al. (1980) observed increased activity for the cyclooxygenase pathway when rabbits were fed adequate vitamin E. It is difficult to explain the elevated LB value for our A E group without PG concentration data, considering the conflicting results given by other researchers.

LDH activities were significantly elevated for the control group at both samplings. The supplementation of A, E, and Se alone or in combination in the treatment groups gave

significantly lower values at d 7 and d 21 postpartum and would seem to indicate that all of them are part of a complex system which maintains cellular integrity. The protection provided by vitamin E and Se against lipid peroxidation of inner and outer cellular membranes has been reported in several studies. Rotruck et al. (1972) found that dietary selenite reduced oxidative damage of rat erythrocytes provided glucose was in the medium. Inclusion of glucose was not necessary for vitamin E to prevent hemolysis. However, vitamin E did not prevent hemoglobin oxidation, thus complimentary but distinct roles for vitamin E and Se for the maintenance of cellular integrity were proposed. Noguchi et al. (1973) provided further support for this conclusion by determining that exudative diathesis was prevented through a combined effort of GSHpx destroying peroxides that may form in the cytosol and vitamin E maintaining the lipid membrane by neutralizing free radicals. The importance of vitamin A in maintaining cellularity was reported by Butera and Krakowka (1986). Vitamin A-deficient rats exhibited a marked decrease in cellularity of the B-cell germinal centers, the T-cell sheaths in the spleen, and a reduction of thymic cortical cellularity. Several hypotheses have been proposed to explain the role of vitamin A in maintaining cellularity, but no single theory prevails.

The responses observed at 7 d for SGOT do not indicate that any of the treatments provided more cellular protection

than the control treatment. However, at d 21 the responses of the E group were significantly lower than the control group. Whereas SGOT and LDH are both indicators of cellular integrity, they are dissimilar in some ways. If some type of trauma occurs, SGOT and LDH values will both increase, but clearance values are quite different. SGOT concentrations will return to normal by the fifth day in the case of myocardial infarction, whereas LDH requires 10 to 14 d (Zimmerman and Henry, 1984). Thus, the values observed for LDH and SGOT are time-dependent from when the disturbance occurred.

The values reported for SCC, clinical mastitis observations, summit milk yield, and reproductive performance traits failed to indicate a significant advantage for the supplemented groups. Others have observed dramatic responses for some of these traits when supplementing minerals and vitamins, but in most instances the positive response was due to the alleviation of a deficiency (Julien et al., 1976; Nicholson and Cunningham, 1965; Smith et al., 1984, 1985). The improvements in cellular integrity and lymphocyte proliferation that we observed in this trial may be important contributions to the positive responses previously observed in some studies. Some of the results in this trial indicate that adequate nutrition was maintained in all groups throughout the prepartum and postpartum period to ensure satisfactory performance. Similar responses have been

reported by others. Ishak et al. (1983) found no advantage for the addition of vitamins A, D, and E, or Se when adequate concentrations of those nutrients were present in the diet. However, many factors such as location, timing and method of harvesting, storage, or feeding methods can cause deficiencies of these nutrients in feedstuffs (Alderson et al., 1971; Burrows and King, 1968; Klatte et al., 1964; Kubota et al., 1967; Schingoethe et al., 1978).

Distinct advantages for the addition of vitamins A and E, or selenium were not evident by the results observed in these production data. However, the significant improvements for LB, SGOT, and LDH that were reported for the supplemented groups should not be discounted. The relatively low occurrence of clinical mastitis and reproductive disorders for all groups may indicate that an adequate immune response was present for each treatment group under these conditions.

TABLE 1. Ingredient composition of dry cow concentrate.

| Ingredient | Basal (%) | E-Supplemented (%) |
|------------------------------------|-----------|--------------------|
| Sorghum grain, rolled | 76.7 | 76.5 |
| Soybean meal | 20.0 | 20.0 |
| Monosodium phosphate | 1.5 | 1.5 |
| Salt, trace mineral | 1.0 | 1.0 |
| Vitamin A,D,E, premix ¹ | .8 | .8 |
| Vitamin E supplement ² | ... | .2 |

¹4,400 I.U. of vitamin A, 2,200 I.U. of vitamin D, 13 I.U. of vitamin E/g premix.

²276 I.U. of vitamin E/g supplement.

TABLE 2. Effect of supplementation of vitamin A, vitamin E, and selenium on vitamin E concentrations at 4 sampling periods¹ [least square means ($\mu\text{g/ml}$) \pm SE].

| Treatment group ² | Sampling period | | | |
|------------------------------|-----------------|---------------------------|----------------------------|----------------------------|
| | 1 | 2 | 3 | 4 |
| Control | 3.0 \pm .2 | 3.1 \pm .2 ^a | 3.4 \pm .2 ^b | 3.0 \pm .2 ^{ab} |
| Se | 2.7 \pm .2 | 3.0 \pm .2 ^a | 3.3 \pm .2 ^{bc} | 2.8 \pm .2 ^{ab} |
| E | 2.8 \pm .2 | 3.6 \pm .2 ^b | 4.0 \pm .2 ^{de} | 3.2 \pm .2 ^{bc} |
| E Se | 2.9 \pm .2 | 4.1 \pm .2 ^b | 4.2 \pm .2 ^e | 3.7 \pm .2 ^c |
| A | 2.9 \pm .2 | 2.8 \pm .2 ^a | 2.5 \pm .2 ^a | 2.7 \pm .2 ^a |
| A Se | 2.8 \pm .2 | 3.0 \pm .2 ^a | 2.9 \pm .2 ^{ac} | 2.6 \pm .2 ^a |
| A E | 3.1 \pm .2 | 3.8 \pm .2 ^b | 3.6 \pm .2 ^{bd} | 3.2 \pm .2 ^{bc} |
| A E Se | 2.5 \pm .2 | 3.8 \pm .2 ^b | 4.1 \pm .2 ^{de} | 3.5 \pm .2 ^c |

a,b,c,d,e Means within columns with different superscripts differ ($P < .05$).

¹ First sampling period at 60 d prepartum followed by samplings at 4-wk intervals.

²Se indicates that group was supplemented with .1mg/kg bodyweight of sodium selenite at approximately 21 d prepartum by intramuscular injection; E indicates that group was supplemented 2500 I.U./head/d orally throughout prepartum period; A indicates that group was injected 60 d prepartum with 1 million I.U. of vitamin A propionate.

TABLE 3. Effect of supplementation of vitamin A, vitamin E, and selenium on serum vitamin A concentrations at 4 sampling periods¹ [least square means ($\mu\text{g}/100\text{ ml}$) \pm SE].

| Treatment group ² | Sampling period | | | |
|------------------------------|-----------------|------------------------------|------------------------------|------------------------------|
| | 1 | 2 | 3 | 4 |
| Control | 29.3 \pm 2.3 | 22.6 \pm 2.2 ^a | 21.3 \pm 1.8 ^{bc} | 32.1 \pm 2.5 ^a |
| Se | 30.0 \pm 2.5 | 25.9 \pm 2.4 ^{ac} | 24.1 \pm 2.1 ^{bd} | 31.5 \pm 2.6 ^a |
| E | 29.3 \pm 2.4 | 23.6 \pm 2.3 ^{ab} | 19.1 \pm 1.9 ^{ab} | 32.0 \pm 2.5 ^a |
| E Se | 26.1 \pm 2.5 | 24.7 \pm 2.4 ^{ac} | 18.4 \pm 2.0 ^{ac} | 26.9 \pm 2.6 ^a |
| A | 29.9 \pm 2.4 | 30.3 \pm 2.3 ^c | 28.5 \pm 1.9 ^d | 39.5 \pm 2.5 ^b |
| A Se | 30.8 \pm 2.3 | 29.2 \pm 2.2 ^{bc} | 21.2 \pm 1.9 ^{bc} | 28.9 \pm 2.4 ^a |
| A E | 28.3 \pm 2.4 | 27.9 \pm 2.2 ^{ac} | 22.8 \pm 1.9 ^{bc} | 32.5 \pm 2.4 ^{ab} |
| A E Se | 30.2 \pm 2.6 | 24.2 \pm 2.4 ^{ac} | 24.2 \pm 2.0 ^{bd} | 27.0 \pm 2.6 ^a |

a,b,c,d Means within columns with different superscripts differ ($P < 0.05$).

^{1,2}Refer to Table 2.

TABLE 4. Effect of supplementation of vitamin A, vitamin E, and selenium on somatic cell counts $\times 10^3$ at the first 4 sampling periods¹ (least square means \pm SE).

| Treatment group ² | Sampling period | | | |
|------------------------------|-----------------|-----------------------------|--------------|--------------|
| | 1 | 2 | 3 | 4 |
| Control | 11 \pm 348 | 96 \pm 102 ^a | 96 \pm 79 | 126 \pm 82 |
| Se | 948 \pm 398 | 92 \pm 111 ^{ab} | 172 \pm 88 | 293 \pm 91 |
| E | 176 \pm 397 | 388 \pm 116 ^b | 195 \pm 91 | 171 \pm 90 |
| E Se | 244 \pm 368 | 161 \pm 104 ^{ab} | 118 \pm 82 | 233 \pm 82 |
| A | 659 \pm 344 | 128 \pm 103 ^{ab} | 212 \pm 79 | 127 \pm 81 |
| A Se | 376 \pm 362 | 162 \pm 106 ^{ab} | 117 \pm 83 | 146 \pm 82 |
| A E | 216 \pm 380 | 134 \pm 111 ^{ab} | 116 \pm 87 | 84 \pm 90 |
| A E Se | 80 \pm 383 | 109 \pm 112 ^{ab} | 121 \pm 88 | 296 \pm 87 |

^{a,b}Means within column with different superscripts differ ($P < .05$).

¹First four sampling periods postpartum that each animal was eligible for under DHIA regulations.

²Refer to Table 2.

TABLE 5. Effect of supplementation of vitamin A, vitamin E, and selenium on mastitis cases that required antibiotic treatment¹ and the duration of clinical infection² (least square means \pm SE).

| Treatment groups ³ | Infection rate requiring treatment, % | Clinical mastitis (d) |
|-------------------------------|---------------------------------------|---------------------------|
| Control | 4 ^a | 3 \pm 18 ^{ab} |
| Se | 32 ^b | 21 \pm 10 ^{ab} |
| E | 5 ^a | 60 \pm 20 ^b |
| E Se | 3 ^a | 7 \pm 12 ^{ab} |
| A | 18 ^{ab} | 16 \pm 13 ^{ab} |
| A Se | 10 ^{ab} | 17 \pm 20 ^{ab} |
| A E | 12 ^{ab} | 6 \pm 11 ^a |
| A E Se | 11 ^{ab} | 38 \pm 17 ^{ab} |

^{a,b}Means within columns with different superscripts differ significantly ($P < .05$).

^{1,2}Refer to text.

³Refer to Table 2.

TABLE 6. Effect of supplementation of vitamin A, vitamin E, and selenium on retained placenta, services per conception, and summit milk yield (least square means \pm SE).

| Treatment groups ¹ | Retained placenta ² , % | Services per conception | Summit milk yield, kg |
|-------------------------------|------------------------------------|-------------------------|-----------------------|
| Control | 1 | 2.5 \pm .4 | 30.2 \pm 1.7 |
| Se | 0 | 2.7 \pm .4 | 31.4 \pm 1.8 |
| E | 2 | 2.2 \pm .4 | 32.2 \pm 1.9 |
| E Se | 5 | 2.9 \pm .4 | 32.9 \pm 1.7 |
| A | 7 | 2.6 \pm .4 | 30.7 \pm 1.6 |
| A Se | 8 | 2.7 \pm .4 | 33.1 \pm 1.8 |
| A E | 6 | 2.4 \pm .4 | 30.6 \pm 1.8 |
| A E Se | 0 | 2.9 \pm .4 | 32.2 \pm 1.8 |

¹Refer to Table 2.

²Refer to text.

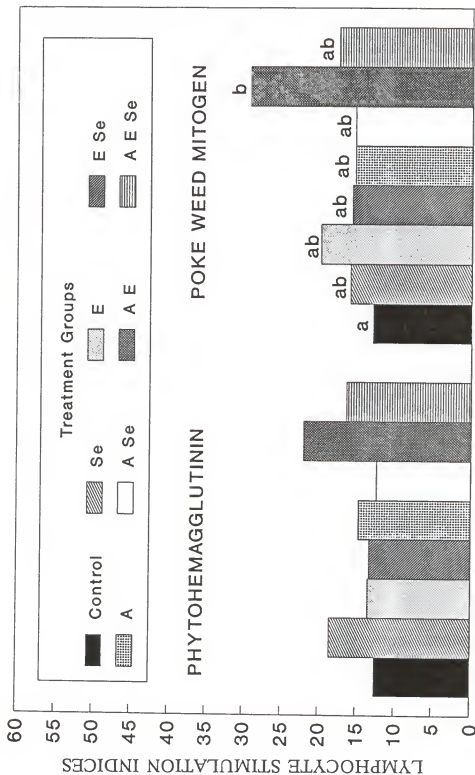


FIGURE 1. Effect of supplementations of vitamin A (1 million I.U. injected 60 d prepartum), vitamin E (2500 I.U./head/d), and selenium (.1 mg/kg BW 21 d prepartum) on lymphocyte blastogenesis values on d 7 postpartum using phytohemagglutinin and poke-weed mitogen. ^{a,b}Columns with differing superscripts differ ($P < .05$).

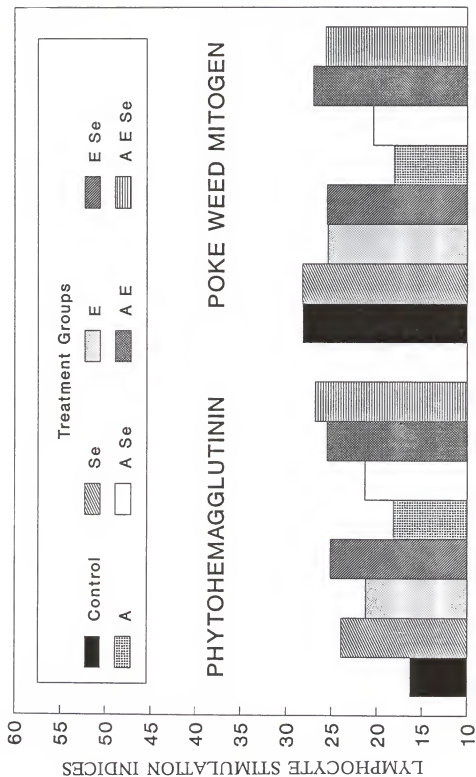


FIGURE 2. Effect of supplementations of vitamin A (1 million I.U. injected 60 d prepartum), vitamin E (2500 I.U./head/d), and selenium (.1 mg/kg BW 21 d prepartum) on lymphocyte blastogenesis values on d 21 postpartum using phytohemagglutinin and poke-weed mitogen.

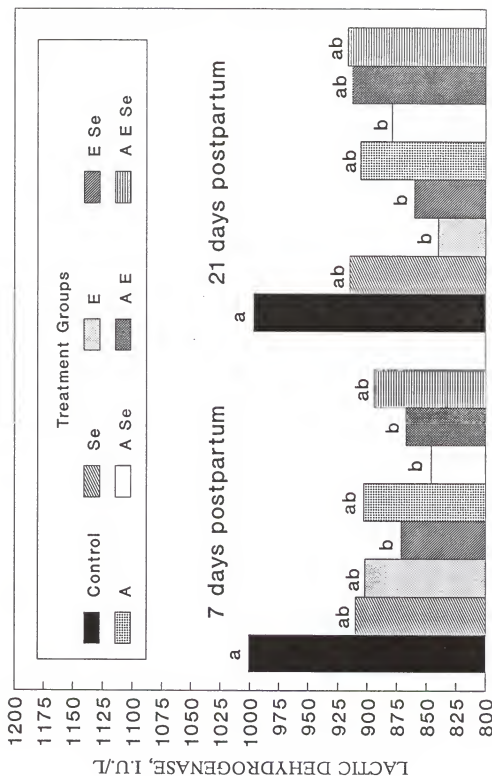


FIGURE 3. Effect of supplementations of vitamin A (1 million I.U. injected 60 d prepartum), vitamin E (2500 I.U./head/d), and selenium (.1 mg/kg BW 21 d prepartum) on lactic dehydrogenase values at 7 and 21 d postpartum. ^{a,b}Columns with differing superscripts differ ($P < .05$).

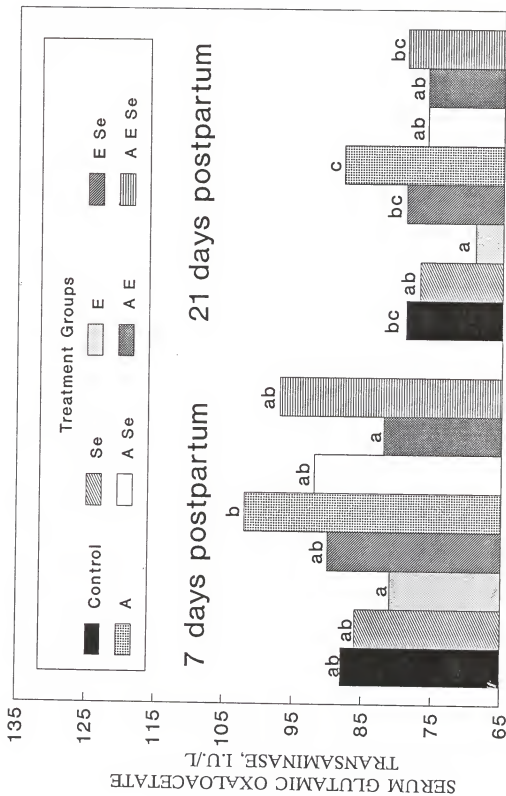


FIGURE 4. Effect of supplementations of vitamin A (1 million I.U. injected 60 d prepartum), vitamin E (2500 I.U./head/d), and selenium (.1 mg/kg BW 21 d prepartum) on serum glutamic oxaloacetate transaminase values at 7 and 21 d postpartum. _{a,b,c}Columns with differing superscripts differ ($P < .05$).

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EFFECT OF PREPARTUM SUPPLEMENTATION WITH
VITAMINS A AND E AND SELENIUM ON SUBSEQUENT
PERFORMANCE OF HOLSTEIN COWS

by

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The objectives of this research were to evaluate the effects of supplementation of vitamins A and E, and selenium during the prepartum period on overall health and immune response of cows in early lactation, and to determine the effect of treatments on concentrations of vitamins A and E in plasma. Fifty-six cows and 34 heifers were assigned to one of eight groups in a 2x2x2 factorial design at 60 d prior to expected calving date. Treatments were: (1) Se, (2) E, (3) E Se, (4) A, (5) A Se, (6) A E, (7) A E Se, and (8) control. Blood samples were taken for 12 wk at 4-wk intervals, starting 60 d prepartum, to determine concentration of serum vitamin A (VA) and vitamin E (VE). Serum glutamic oxaloacetate transaminase (SGOT), lactic dehydrogenase (LDH) and lymphocyte blastogenesis (LB) were determined at 7 d and 21 d postpartum.

The VE values were significantly higher in the 4 groups receiving E than in those not receiving E. Group E Se maintained the highest VE concentration throughout the trial. The VA amounts were generally higher in the A supplemented groups, with the A treatment group attaining the highest value. The LDH concentrations, measured at 7 d and 21 d, tended to be lower for all the supplemented groups than in the control group, with the E, E Se, A Se, and A E values being significantly different when compared to those in the control group. However, the SGOT concentrations were not significantly lower in any of the supplemented groups than in the control group. The LB response for poke weed mitogen was significantly higher for the A E group, than for the control

group at 7 d. There were no significant differences in SCC, incidence and duration of clinical mastitis, summit milk yield, or reproductive responses between supplemented groups and controls. Improvements in cellular integrity and immune system responsiveness in dairy cattle during early lactation may be promoted through dietary supplementation of vitamins A and E and Se during the prepartum period. However, the effect will depend on various nutrient concentrations and interactions prior to and during the prepartum period. Proper hygiene and stress management during this time also will influence the effectiveness of supplementation for the dairy cow.