VITAMIN E AND THE IMMUNE SYSTEM IN CALVES

by

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INTRODUCTION

The vitamin E requirement of the calf is not well established. The quantity of vitamin E required to protect calves against vitamin E deficiency varies depending not only on the amount of vitamin E in the diet or body reserves but also on other factors, especially the amounts of unsaturated fat and selenium in the diet. Vitamin E deficiency has been observed in calves under research and field conditions. It is difficult to produce vitamin E deficiency in calves when selenium is present in the diet and unsaturated fat is absent. Under such conditions the vitamin E requirement of calves appears to be very low.

The function of vitamin E as a lipid antioxidant has been well established. Research implicates the need for vitamin E in more specific metabolic functions in the animal body. One of the lesser known functions of vitamin E is its role in the function and development of the immune system. Vitamin E enhances the immune system of many species but this relationship has not been adequately studied in the bovine.

The neonatal calf is born essentially devoid of serum immunoglobulins and therefore depends upon the absorption of immunoglobulins from colostrum for passive immune protection as soon as possible after birth. As passive immunity declines, the active immune system of the calf is initiated. Calves are more susceptible to disease during the first few months of life before their active immune system is fully developed. Low levels of serum immunoglobulins in young calves are related to increased incidence of disease. Vitamin E supplementation may be a means of reducing the incidence of disease among calves by increasing the protection provided by the immune system.

This research was conducted to study the effect of vitamin E on the humoral and cell-mediated immune responses in calves.

PHYSIOLOGICAL IMPORTANCE OF VITAMIN E - A REVIEW

Physical and Chemical Properties of Vitamin E

Vitamin E is a fat soluble vitamin which was first discovered in 1922 as a factor in vegetable oils required for normal reproduction in rats. Later studies have led to the recognition of vitamin E deficiency as the cause of many different pathological conditions in numerous animal species. The deficiency symptoms tend to be degenerative in nature including fetal degeneration, testicular and liver necrosis, kidney and muscular degeneration, erythrocyte destruction and others (Hoekstra, 1975; Scott et al., 1969).

Vitamin E was isolated by Evans et al. (1936) from wheat germ oil. Several different chemically-similar compounds having vitamin E activity have been isolated from plants and named the "tocopherols" (Hjarde et al., 1973; Lehninger, 1975). The name tocopherol comes from the Greek words tokos (offspring) and pherein (to bear). All of the tocopherols are biologically active but show very different degrees of potency (Hjarde et al., 1973).

Tocopherols are methyl-substituted hydroxychromans with an isoprenoid side chain (Parrish, 1980). They are composed of two homologous series: the tocopherols, with a saturated side chain; and the tocotrienols, with an unsaturated side chain. In each series four members have been identified in plant sources and are designated as alpha, beta, gamma, and delta, depending on the methyl group substitutions in the chroman ring (Hjarde et al., 1973; Parrish, 1980). These eight naturally occurring compounds are the dextro-rotatory isomers called the "d forms" (Hjarde et al., 1973).

The best known member of the group is d-alpha-tocopherol since it is the most abundant form found in nature (Lehninger, 1975) and the most biologically active within the animal (Hjarde et al., 1973; Lehninger, 1975; Parrish, 1980). Its structural name is 2,5,7,8,-tetramethyl-2-(4',8',12'-trimethyltri-

decyl)-6-chromanol, or 2-methyl-2(4',8',12'-trimethyl-tridecyl)chroman-6-ol (Figure 1) (Parrish, 1980).

The tocopherols are pale yellow, fat soluble, slightly viscous oils. They are more stable to heat and alkalies in the absence of oxygen and more stable to acid than alkali (Parrish, 1980; Stecher, 1968). They are slowly oxidized by atmospheric oxygen (Stecher, 1968) and readily oxidized by a number of ions (Parrish, 1980). The tocopherols are much more stable to visible light than to ultraviolet (Parrish, 1980). They have antioxidant activity stemming from the presence of the 6-OH group on the chroman ring (Diplock, 1973; Parrish, 1980).

Esters and ethers of tocopherols can be synthetically prepared. The esters have no antioxidant activity until hydrolyzed (Parrish, 1980). The commonly used supplementary form of vitamin E in animal diets is the synthetic racemic d,l-alpha-tocopherol acetate. It is practically unaffected by the oxidizing influence of air, light and ultraviolet light (Stecher, 1968). The International Unit for expressing "vitamin E activity" of various tocopherols is defined as the activity of 1 mg of d,l-alpha-tocopherol acetate (Hjarde et al., 1973; Parrish, 1980).

Selenium and its Interrelationship with Vitamin E

Selenium (Se) is a metallic element with properties similar to those of sulfur (S), both belonging to group VI of the periodic table. Se is often found associated with S in organic and inorganic compounds (Scott et al., 1969). Several Se analogues of S compounds of biological significance have been identified including selenomethionine, selenocystine, and seleno-coenzyme A. Inorganic selenite seems to be well incorporated into organic S compounds, possibly by reduction to H_2Se and then -SH:SeH exchange (Olson, 1965).

The initial concern with Se in animal feeds was due to toxicities resulting from excess Se. It was found that certain soils in certain areas of the

U.S. contain excess Se, while others may be deficient (Scott et al., 1969). The essentiality of Se as a nutrient was first established through its interrelation with vitamin E. In the 1950's an unidentified factor called Factor 3 was discovered in dried brewers yeast that was as effective as vitamin E in preventing liver necrosis in rats (Schwartz, 1951) and exudative diathesis in chicks (Patterson et al., 1957; Scott et al., 1969). Schwartz and Foltz in 1957 reported Se to be the active component of Factor 3 (Hoekstra, 1975; Schwartz, 1972; Scott, 1979). Se was also shown to be effective in preventing other vitamin E related defects such as white muscle disease in farm animals under some conditions (Hartley and Grant; 1961; Muth et al., 1958; Proctor et al., 1958; Schubert et al., 1961). By 1969, work clearly demonstrated that Se is required at low levels (around 0.1 ppm) in animal diets even in the presence of large amounts of vitamin E (McCoy and Weswig, 1969; Thompson and Scott, 1969). Although these findings led to intensive and widespread study on the metabolic interaction between vitamin E and Se, there is still no unifying concept acceptable to all (Leach, 1975). The interaction is complicated due to their further interrelationships with S amino acids, synthetic antioxidants, polyunsaturated fatty acids (PUFA), and other nutrients (Hoekstra, 1975).

Functions of Vitamin E and Se

There are two main hypotheses regarding the mechanisms of action of vitamin E and Se. The biological antioxidant theory, elaborated by Tappel (1962, 1972) holds that both substances act simply as nonspecific biological antioxidants. The metabolic function theory (Schwartz, 1965, 1972; Schwartz and Baumgartner, 1970; Scott et al., 1974) proposes that the functions of vitamin E and Se are entirely different, and that vitamin E is needed in specific metabolic roles in the animal body in addition to its role as a lipid antioxidant.

Biological Antioxidant Theory. The antioxidant theory of the function of vitamin E, although not accepted by all investigators (Diplock, 1973; Green, 1962, 1972; Lucy, 1972; Nair, 1972; Schwartz, 1951, 1965, 1972; Schwartz and Baumgartner, 1970; Scott, 1970, 1979) is the only attempt to unite vitamin E into a basic concept (Hoekstra, 1975; Sheffy and Schultz, 1979). The theory relates vitamin E to the stabilization of PUFA that are in danger of destructive peroxidation. Vitamin E inhibits the formation of destructive peroxides by reacting as a chain breaking antioxidant with free radical intermediates of lipid peroxidation (Tappel, 1962, 1972). The hydrogen of the 6-OH group, which is the group responsible for the antioxidant function of alpha-tocopherol, combines with a peroxide free radical and forms an inactive product which breaks the peroxide chain. Thus, alpha-tocopherol is oxidized while PUFA are protected. The oxidation products of alpha-tocopherol are complex. In the simplest reaction mechanism, alpha-tocopherol is oxidized to alpha-tocopherolquinone (Tappel, 1962, 1972) with a loss in its biological activity and the animal is unable to reconvert it into alpha-tocopherol (Scott et al., 1969).

Because all biological membranes contain phospholipids that are subject to oxidative degradation, which leads to structural damage of cells (Combs et al., 1975), vitamin E appears to be essential for the integrity and optimum function of many systems, including the reproductive, muscular-skeletal, nervous, circulatory, and hematopoietic systems (Hoekstra, 1975; Sheffy and Schultz, 1979). The antioxidant theory serves to explain the great diversity of vitamin E deficiency lesions tending to be degenerative in nature and the many factors that can influence them (Hoekstra, 1975).

Se also functions as an antioxidant. However, studies have demonstrated that the mechanism of Se is dissimilar to that of vitamin E (Hoekstra, 1974; Noguchi et al., 1973; Rotruck et al., 1972, 1973). Se is a component of the enzyme glutathione peroxidase, a non-heme iron protein (Hoekstra, 1975). Highly purified glutathione peroxidase contains 4 g of Se per mole of enzyme

(Rotruck et al., 1972, 1973). This selenoenzyme protects cellular and subcellular membranes from peroxidative damage by converting fatty acid hydroperoxides to the less damaging alcohols before they can undergo chain reactions and cause malfunctions in the membranes (Hoekstra, 1974; Scott, 1979; Tappel, 1972). The selenoenzyme is associated primarily with the aqueous phase of the cytosol and plasma, working synergistically with vitamin E in the membrane itself (Noguchi et al., 1973; Lucy, 1972). Dietary Se has been shown in vitro to protect both the erythrocyte membrane and its contents, hemoglobin, against oxidative damage while vitamin E protects only the erythrocyte membrane. The effects of dietary Se were dependent on the presence of glucose while the effects of vitamin E were not. Glucose metabolism is necessary for the generation of reduced glutathione within the cell (Hoekstra, 1974; Rotruck et al., 1972, 1973). The selenoenzyme utilizes glutathione, a tripeptide containing cysteine, to reduce damaging hydroperoxides (Hoekstra, 1974; Rotruck et al., 1972, 1973; Tappel, 1972).

The selenoenzyme has the dual role of destroying hydrogen peroxide (H_2O_2) by this reaction: $2\ GSH + H_2O_2 \xrightarrow{\text{glutathione peroxidase}} GSSG + 2\ H_2O$, where GSH is reduced glutathione and GSSG is oxidized glutathione (Hoekstra, 1974, 1975; Rotruck et al., 1972, 1973; Scott, 1979). Hydrogen peroxide may react with superoxide ion in the tissues to form the highly membrane-destructive hydroxyl free radical (Scott, 1979; Scott et al., 1969). The superoxide ion and hydrogen peroxide are the most important free radical intermediates of lipid peroxidation as they are extremely reactive and capable of irreversible damage to cell membranes. They are formed along with other toxic parital reduction products of oxygen during electron transport to molecular oxygen and in various hydroxylation and oxygenation reactions (Lehninger, 1975).

The antioxidant theory ties together the roles of vitamin E, Se, other antioxidants, and the S amino acids into the protection of cell membranes as well as proteins and enzymes from lipid peroxidation. Free radical inter-

mediates of lipid peroxidation react with proteins and enzymes, especially those with reactive sulfhydryl groups, to cause inactivation. Sulfhydryl compounds such as GSH and cysteine react in small amounts as free radical scavengers and peroxide decomposers. Small amounts of methionine can also do this. Selenoamino acids react similarly and are powerful catalysts of sulfhydryl-disulfide exchange (Tappel, 1970).

The biological antioxidant theory of vitamin E function was challenged by those who had difficulty detecting lipid peroxides in vitamin E deficient animals (Green, 1972). However, lipoperoxides have been found in adipose tissues of vitamin E deficient animals but not in phospholipids of muscle, kidney, or testes (Glavind, 1973). Increased rates of *in vitro* peroxidation have been demonstrated in homogenates of several tissues of Se- and vitamin E-deficient animals (Combs *et al.*, 1975). Also, lipid peroxidation was found *in vivo* in vitamin E- and Se-deficient rats by ethane evolution. The peroxidation process greatly accelerated during the terminal phase of the fatal disease caused by vitamin E and Se deficiency (Hafeman and Hoekstra, 1977). Furthermore, the studies which demonstrated a role for Se in glutathione peroxidase (Rotruck *et al.*, 1972, 1973) and two distinctly separate antioxidant functions of vitamin E and Se (Noguchi *et al.*, 1973), provided additional evidence for the antioxidant theory.

Metabolic Function Theory. In a review of the evidence for and against the biological antioxidant theory as an explanation of all functions of vitamin E, Green (1972) stated that the theory cannot possibly explain all functions of the vitamin. Schwartz (1972) stated that it seems unlikely an organism should depend on vitamin E, a structurally highly specific, essential dietary agent, for the simple prevention of random peroxidation in tissues. In the process each free radical would destroy a vitamin E molecule by converting it into a useless inactive byproduct. However, there is no doubt that tocopherol has antioxidant properties (Schwartz, 1972). Scott (1970) believes vitamin E,

in addition to its role as an antioxidant, is related to the control of oxidation-reduction potentials in one or more metabolic systems. The nature of these systems is unknown. Green (1972) suggested vitamin E may act as a mediator of specific hydrogen transfer or electron transfer reactions at particular membrane sites, possibly involving free radicals or ions.

The most definitive function for Se is its essential role as a component of glutathione peroxidase, although the specific role of Se in the enzyme remains to be elucidated. It is postulated to have a redox or electron-transferring role (Hoekstra, 1974). Glutathione peroxidase may have a biosynthetic role, such as in steroid synthesis or other hydroxylation reactions (Hoekstra, 1975). The role of Se in the enzyme does not exclude other possible roles for this element in animal tissues (Hoekstra, 1974).

Vitamin E may function as a membrane-bound substance directed specifically towards oxidation-sensitive proteins that contain S or Se or both (Diplock, 1973; Lucy, 1972). Experimental observations have demonstrated that Se is present in the mitochondria and microsomes obtained from normal rat liver as protein-bound selenide, a reduced form of Se, and its occurrence is directly related to the vitamin E status of the animal. Therefore, in a Se deficiency, liver necrosis may develop because of a metabolic failure caused by the inadequacy of a selenide-depleted protein in the respiratory chain. The active form of Se as selenide may form a part of the active center of non-heme iron proteins, and vitamin E may function by protecting the selenide from oxidation. The redox function of vitamin E is located in the chromanol ring and is associated with polar glycerol moieties of the membrane phospholipids. It is then able to interact with polar regions of membrane-associated proteins, specifically oxidation-sensitive proteins containing Se and/or S (Diplock, 1973). The results of Noguchi et al. (1973) do not appear to support a redox function of vitamin E for prevention of exudative diathesis since this disease appears to be prevented by either glutathione peroxidase, which destroys peroxides, or by

vitamin E which prevents peroxide formation.

Lucy (1972) suggested the hydrophobic chain of vitamin E may form a stable complex with arachidonic acid residues of phospholipids in the membrane bilayer, with interactions specifically between methyl groups of the phytol side chain and the cis double bonds of the fatty acid. Such a complex could stabilize membranes by preventing peroxidative destruction of the PUFA in cell membranes and reducing the permeability of biological membranes containing high levels of PUFA, particularly arachidonic acid. Results presented by Scott et al. (1974) lend support to this suggestion.

An attempt has been made to define the function of vitamin E and its relation to Se through the study of livers from animals undergoing the latent phase of liver degeneration of which respiratory decline is the characteristic phenomenon (Schwartz, 1965, 1972; Schwartz and Baumgartner, 1970). Vitamin E deficient liver tissue was unable to maintain normal respiratory activity in vitro. This respiratory decline was not related to the rate of peroxide formation. Vitamin E protected against respiratory decline when supplemented in the diet, injected, or added to in vitro systems. Se protected against respiratory decline only when applied in vivo (Schwartz, 1972).

Studies with inhibitors of individual electron transport reactions and other data showed that the initial impairment leading to respiratory decline in the mitochondria during liver necrosis does not involve oxidative phosphorylation or the cytochrome chain but those dehydrogenase systems which connect the citric acid cycle to the cytochrome chain (Schwartz, 1965). Vitamin E supplementation to deficient liver homogenate prevented the rapid decline of oxygen uptake with alpha-ketoglutarate and oxalacetic acid as substrates. Using the alpha-ketoglutarate oxidase system, which involves intermediate steps and cofactors in the conversion of alpha-ketoglutarate to succinyl CoA, to delineate the positions at which vitamin E, Se, and S amino acids may be effective, Schwartz (1965) proposed that vitamin E may interact with the sulfhydryl sites

on lipoyl dehydrogenase; Se may be involved with the decarboxylation reaction considering that Se is a potent inorganic catalyst of carbonic anhydrase which releases carbon dioxide from carbonic acid; and S amino acids may be involved in the overall supply of coenzyme A and lipoic acid or on the total amount of available sulfhydryl-containing enzyme protein in one or the other of these steps.

NADH oxidase, which catalyzes the transfer of reducing equivalents from the citric acid cycle to the cytochrome chain, declined markedly preceding respiratory breakdown in vitamin E deficient liver homogenates *in vitro* suggesting that vitamin E may affect respiration directly by involvement with this enzyme (Schwartz and Baumgartner, 1970). Cytochromes, heme-containing enzymes of the electron transport chain, were increased by vitamin E or by methionine supplementation. Vitamin E appears to have a direct effect on mitochondrial oxygen consumption *in vitro* and an indirect effect on cytochrome levels *in vivo* (Schwartz, 1972).

Shapiro *et al.* (1981) found about a 30% decrease in the enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) binding to the erythrocyte membrane in vitamin E deficient monkeys. Since no differences were found in the activity or binding affinity for substrate of GAPDH from vitamin E supplemented or deficient animals, a modification of the GAPDH binding site may have resulted from vitamin E deficiency. GAPDH catalyzes the first redox reaction in glycolysis, has sulfhydryl groups that must be reduced for catalytic activity, and requires NAD^+ specifically as oxidant (Lehninger, 1975). It appears that the proper function of this dehydrogenase system requires the protective action of vitamin E on the erythrocyte membrane.

An active site of Se in preventing respiratory decline during liver necrosis has been proposed by Levander *et al.* (1973). Dietary Se, unlike dietary vitamin E, had no protective effect against reagents (ascorbate, iron, or GSH plus GSSG) which caused mitochondrial swelling associated with lipoperoxidation.

It was observed that the swelling of Se-deficient mitochondria induced by GSH could be accelerated by the addition of selenite *in vitro*. This finding could be related to a role for Se in catalyzing the transfer of electrons from GSH to cytochrome c *in vivo*. In a vitamin E deficient animal receiving Se, the Se could permit the transfer of electrons directly from GSH to cytochrome c. Thus, the potentially damaging hydrogen peroxide-producing step of the respiratory chain could be bypassed and the animal would not succomb to liver necrosis (Levander *et al.*, 1973).

Coenzyme Q_3 or ubiquinone, participates in the transport of electrons from organic substrates to oxygen in the mitochondrial respiratory chain. This coenzyme is structurally similar to vitamin E, consisting of a reversibly reducible quinone with a long isoprenoid side chain, and has vitamin E-like activity (Lehninger, 1975). Vitamin E has been reported to control coenzyme Q biosynthesis, although this role has not been widely confirmed (Green, 1962; Frost and Poucke, 1972; Moore, 1962; Olson, 1965). Se has been shown, without a doubt, to be needed for coenzyme Q biosynthesis but the part of coenzyme Q biosynthesis catalyzed by Se is not known. The role of vitamin E may be indirect and lie in its ability to maintain Se in its reduced functional state (Frost and Poucke, 1972).

The association of vitamin E deficiency with anemia in pigs, rats, and primates involving a reduced synthesis of porphyrins and heme by the erythroid cells of the bone marrow led to the study of a role for vitamin E in heme biosynthesis. Studies by Nair (1972) led to the observation that vitamin E functions as a regulator of heme biosynthesis in an inducer-repressor type system in liver at the levels of two important enzymes, delta-aminolevulinic acid (ALA) synthetase and ALA dehydratase. These enzymes are necessary for the synthesis of protoporphyrin IX which accepts iron to form heme proteins, including cytochromes, hemoglobin, catalase, microsomal mixed function oxidases, and many more, that have a diversity of functions. Heme proteins are fundamen-

tal to all living cells. In vitamin E deficient animals the activity of hepatic ALA dehydratase was significantly lower than controls (Caasi et al., 1972; Nair, 1972), heme proteins were significantly decreased (Caasi et al., 1972) and bone-marrow ALA synthetase was depressed (Nair, 1972). Allylisoprophylacetamide, a porphyrinogenic agent that affects ALA synthetase, induced RNA synthesis specific for ALA synthetase and also stimulated vitamin E uptake into rat liver nuclei. Vitamin E blocked this induced RNA synthesis suggesting that vitamin E regulates the synthesis of this RNA (Hauswirth and Nair, 1972), thus regulating synthesis of heme.

Vitamin E has been suggested to be involved in the regulation of other enzyme systems (Carpenter, 1972; Catignani et al., 1974). In vitamin E deficient rats the liver microsomal drug hydroxylation system has been shown to be depressed. This enzyme system requires NADPH, oxygen, and a heme protein, cytochrome P-450. Oral administration of alpha-tocopherol to deficient rats reversed the response of liver microsomes within 12 hr. Actinomycin D prevented the increase in drug hydroxylation when administered to vitamin E deficient rats 15 min before feeding vitamin E. The effect of actinomycin D on the response of rats to alpha-tocopherol appeared to be a specific inhibition. A mechanism involving RNA synthesis and/or turnover was implied in which vitamin E may regulate the activity of a repressor (Carpenter, 1972). In the liver of vitamin E deficient rabbits, it was shown that xanthine oxidase activity increased due to an elevated synthesis of the enzyme rather than loss of an inhibitor or presence of an activator. Therefore, it was suggested that vitamin E might regulate the synthesis of the enzyme (Catignani et al., 1974).

In summary, the role of vitamin E in membrane structure and integrity may be related both to its role as an antioxidant in preventing PUFA peroxidation in the inner hydrophobic part of the membrane and to its redox function in the hydrophilic portions of the membrane. Evidence exists for the implication of both vitamin E and Se at several key steps in respiration. For vitamin

E these are: NADH oxidase; heme-containing enzymes of the cytochromes; and both coenzyme Q and non-heme iron proteins where vitamin E may prevent the oxidation of selenide. Se may have a role as an active center of non-heme iron proteins in both the mitochondrial and microsomal systems of the liver, in catalyzing cytochrome c reduction by glutathione, and in coenzyme Q biosynthesis. Vitamin E may be involved in the regulation of RNA synthesis/turnover of several enzyme systems. These include the enzymes of heme biosynthesis, the liver microsomal drug hydroxylation system, and xanthine oxidase.

VITAMIN E, SELENIUM, AND THE IMMUNE SYSTEM - A REVIEW

A lesser known function of vitamin E and selenium (Se) is their role in the function and development of the immune system. The fact that vitamin E and Se prevent peroxidative damage to cells and subcellular elements could be a mechanism by which they aid to maintain the body's normal defense mechanism against disease and stress (Scott, 1979).

The subcellular organelles whose membranes are protected by vitamin E and Se from peroxidative damage include mitochondria, microsomes, and lysosomes. Lysosomes are less labile to lipid peroxidation than mitochondria and microsomes because their membranes contain less lipid and do not contain hemoprotein prooxidants of lipid peroxidation (Combs et al., 1975). Since mitochondria, microsomes, and ribosomes act to produce antibodies and other defense mechanisms, it is clear that adequate vitamin E and Se are necessary for preservation of the organelles responsible for building defense mechanisms against disease and other stresses (Scott, 1979).

It has been well established that vitamin E and Se function to maintain erythrocyte membrane integrity. Erythrocyte membranes are quite labile to lipid peroxidation due to their high polyunsaturated fatty acid (PUFA) content and to their direct exposure to molecular oxygen. Vitamin E protects the erthrocyte plasma membrane from peroxidative hemolysis (Combs et al., 1975). Since erythrocytes and lymphoid cells originate from common pluripotential stem cells, there is a basis provided for the concept that vitamin E and Se may be associated with membrane fluidity of lymphoid cells as well as immune response mechanisms (Sheffy and Schultz, 1979).

Effect on Humoral Immunity

Studies have demonstrated in vivo (Tengerdy et al., 1972, 1973; Tengerdy and Heinzerling, 1972; Tengerdy and Nockels, 1972) and in vitro (Campbell et al., 1974) that the humoral immune response (HIR) of chickens and mice immunized

with sheep red blood cells (SRBC) increased when their rations were supplemented with vitamin E. Seven-day-old chicks were immunized with SRBC and fed a normal diet containing the recommended level of vitamin E, approximately 22 mg/kg, plus a vitamin E supplement of 132 mg/kg diet. The HIR of these chicks, compared to the controls without supplement, was increased 20-25%, as measured by the antibody (AB) plaque forming cell (PFC) and hemagglutination tests (Tengerdy et al., 1972; Tengerdy and Heinzerling, 1972; Tengerdy and Nockels, 1972). Similar results were obtained when the same experiment was repeated using one year old laying hens (Tengerdy and Heinzerling, 1972).

To determine if vitamin E would stimulate immune response in another animal species, Tengerdy et al. (1973) conducted similar studies with mice. Two different antigens (AG) were used in these studies: SRBC, a good particulate AG which does not stimulate a true primary response since it is not completely foreign for mice; and tetanus toxoid which does stimulate a true primary response. The two antigenic stimuli were used as a means of comparison to distinguish whether vitamin E supplementation affects IgG and IgM production differently. Results supported their earlier finding with chicks that vitamin E supplementation stimulates the HIR. Eight to 10 week old mice were fed semisynthetic or natural commercial diets containing 60-180 mg vitamin E/kg. In the primary immunization with either AG, the enhancing effect was the same, that being a 30-40% increase in the HIR measured by PFC or hemagglutination tests. Vitamin E particularly affected the IgG response. When SRBC was used, a significant increase in the spleen weights of immunized and non-immunized vitamin E supplemented mice was noted. This indicates, perhaps, the general cell differentiation effect of vitamin E which is in addition to that caused by SRBC in this lymphopoietic organ. Following reimmunization with tetanus toxin 30 days later, the effect of vitamin E was much stronger on the primary response than the secondary. Since IgG production is more pronounced than IgM, the main effect of vitamin E on the primary response apparently is

the regulation of IgG biosynthesis, probably at an early stage of immunocyte development and proliferation.

Tengerdy et al. (1973) also studied the specificity of the vitamin E effect by replacing the vitamin with the antioxidant N,N-diphenylenediamine (DPPD) in the diet of the mice. Their results clearly showed that the enhancing effect of vitamin E cannot be explained by a simple antioxidant action. DPPD added in the amount of 222 or 240 mg/kg feed was ineffective in restoring the IgG response to vitamin E deficient mice, and was only partially effective in enhancing the immune response of mice fed normal vitamin E-containing diets of 60 mg/kg feed.

The findings which demonstrated that dietary supplementation of vitamin E enhanced the in vivo immune response were confirmed by an in vitro study by Campbell et al. (1974). AB production by normal mouse spleen cells in vitro required the presence of both an adherent and nonadherent cell population. The addition of alpha-tocopherol clearly enhanced the immune response of nonadherent mouse spleen cells when the number of adherent spleen cells was markedly depleted, apparently by providing some function normally provided by normal numbers of adherent cells. In addition, soluble alpha-tocopherol enhanced the in vitro immune response of normal mouse spleen cells to SRBC.

Similar stimulatory immune responses in mice have been reported when evaluating the effects of Se. Experiments have indicated that supplementation of dietary Se as selenite enhanced the primary immune response in mice (Martin and Spallholz, 1976; Spallholz et al., 1973c) characterized by increased anti-SRBC hemagglutinating titers of IgM and IgG (Spallholz et al., 1974b). Unlike vitamin E which promoted IgG synthesis (Tengerdy et al., 1973), dietary selenite promoted IgM synthesis (Martin and Spallholz, 1976; Spallholz et al., 1973c). The most effective dietary levels, which would be considered toxic levels for several other species of mammals, were 10-30 times the dietary requirement of 0.1 ppm for mice (Martin and Spallholz, 1976; Spallholz et al.,

1973a,b,c, 1974b).

Injectible selenite also enhanced the primary immune response in mice sensitized with SRBC. Mice injected intraperitoneally with either 3 or 5 μg of selenite had significantly higher IgM titers on day five post SRBC sensitization than control mice. Enhancement of the primary immune response is greatest when Se is administered prior to or simultaneously with SRBC (Martin and Spallholz, 1976; Spallholz et al., 1973a, 1974b, 1975). Similar results have been reported in rabbits injected subcutaneously with 0.05 mg Na selenite/kg before or during immunization with typhoid vaccine (Berenshtein, 1978).

Of the selenicals tested by injection, selenite and selenide were the two most effective in enhancing AB titers, while selenate and diphenylselenide, as well as the sulfur compounds, sulfate and sulfite, exhibited little or no biological activity (Martin and Spallholz, 1976; Spallholz et al., 1974b).

The effect of administering selenite, vitamin E or a combination of the two on the HIR has been studied in rabbits, mice and swine. Rabbits given a combination of Na selenite (0.05 mg/kg subcutaneously) and vitamin E (0.03 mg/kg) or Na selenite alone before or during immunization with typhoid vaccine showed higher AB titers than did rabbits given the vaccine alone. However, the combination was more effective in stimulating AB formation. Vitamin E given alone had no effect on AB formation (Berenshtein, 1978). Mice administered tetanus toxoid vaccine or SRBC were simultaneously injected either subcutaneously or intramuscularly with 0, 3, 5, 10, 20, 60 or 100 μg selenite or 0, 0.15, 0.25, 0.50, 1.0, 3.0 or 5.0 mg alpha-tocopherol acetate or a mixture of the two. Some mice were sacrificed to determine effects on the primary immune response and some were resensitized and sacrificed to determine effects on the secondary immune response. Anti-tetanus toxoid IgM and IgG were not significantly enhanced in the primary immune response but were in the secondary immune response while anti-SRBC IgM and IgG were significantly enhanced

in both the primary and secondary immune response. Differences in AB titers were dependent upon the amount of selenite, alpha-tocopherol acetate or a mixture of the two administered and the route of administration (Martin and Spallholz, 1976; Spallholz et al., 1974a). Weanling swine, antigenically challenged with SRBC, received supplemental vitamin E and/or Se provided either in the diet or by intramuscular injection. The supplemental levels fed were 0 or 0.5 ppm Se, 0 or 220 IU vitamin E/kg diet, or 0.5 ppm Se and 220 IU vitamin E/kg diet. Injected quantities totaled 0 or 6 mg Se, 0 or 220 mg alpha-tocopherol, or 6 mg Se and 220 mg alpha-tocopherol. Hemagglutination assays indicated that anti-SRBC AB production was similar for the two supplementation methods. Vitamin E and/or Se administration increased hemagglutination titers over those of the negative control group, particularly during the latter weeks of the experiment. The combination of both nutrients resulted in higher titers than when each was supplied independently suggesting an additive response (Peplowski et al., 1980).

The effect of vitamin E on the HIR of swine was also tested using an E. coli bacterin. Six to 8 week old pigs were fed a nutritionally complete ration with an additional 0, 20,000, or 100,000 IU of vitamin E/ton feed and given an intramuscular injection of an E. coli bacterin. Pigs fed the diet containing 100,000 IU of vitamin E/ton feed had serum AB titers two to three times higher than pigs fed the normal ration. Pigs fed the diet containing 20,000 IU of vitamin E/ton feed, which is the recommended level of vitamin E supplementation, had serum AB titers intermediate to those of pigs in the other two groups (Ellis and Vorhies, 1976).

Using a non-living antigenic stimulus, vitamin E nutrition has also been shown to influence the HIR of dogs. Secondary immune responses to SRBC were reduced in vitamin E-deficient dogs while primary immune responses did not appear to be affected (Sheffy and Schultz, 1979).

The effect of supplementary vitamin E fed to hens on the passive transfer

of AB from immunized hens to chicks was studied (Jackson et al., 1978). In chickens, AB are transferred from the hen into the yolk sac of the egg and are then taken up by the embryo (Nockels, 1979, 1980). Hens were fed diets to which 0, 90, 150, 300, 450, or 900 ppm of d,l-alpha-tocopherol acetate was added. After 2 weeks on these diets the hens were immunized intramuscularly with Brucella abortus. Passively transferred AB levels were significantly increased in plasma of 2 and 7 day old chicks when the hens were fed 150 and 450 ppm vitamin E prior to immunization with Brucella abortus. The chicks from hens fed 90, 300, and 900 ppm vitamin E before immunization evidenced no increase in AB titers relative to controls. Seven-day-old chicks also had significantly lower titers than when 2 days old (Jackson et al., 1978).

A trial was also conducted to determine if feeding the same vitamin E supplemented rations to non-immunized hens would alter primary immune response of chicks. At 2 weeks of age chicks were immunized with Brucella abortus. Non-immunized chicks did not have detectable AB titers. Only immunized chicks from hens fed 450 IU vitamin E/kg feed had significantly higher AB titers compared to immunized controls (Nockels, 1979, 1980). The results of these studies show a non-linear AB response reflected by the chicks (Jackson et al., 1978). Similar results involving a biphasic dose response have been previously reported in mice by Heinzerling et al (1974b). Vitamin E was used as the stimulant and percent survivors to Diplococcus pneumoniae type I infection in mice as the immune parameter. The survival rate of both immunized and non-immunized mice was increased 15-80% by dietary supplementation of 180 and 360 mg vitamin E/kg feed while doses in between were less effective. A biphasic dose response was also observed with phagocytic activity. Increased protection by vitamin E was associated with increased phagocytosis activity measured by the carbon clearance test. However, effective phagocytosis was found only in immunized mice. The data indicated that increased macrophage activity, probably aided

by increased AB production, was the principal reason for increased protection.

Although vitamin E has been shown to stimulate AB response, this does not necessarily mean that the animal has improved disease resistance. Therefore studies were designed, in which animals were given pathogens, to determine if increased AB production resulting from vitamin E supplementation had a direct role in protecting the animal from infection (Nockels, 1979, 1980). Day-old chicks, uninfected or challenged with E. coli, were fed a normal diet containing 43.2 mg vitamin E/kg feed to which 0, 150, or 300 IU of d,l-alpha-tocopherol acetate/kg feed was added. Dietary supplementation with 150 and 300 mg vitamin E/kg feed significantly reduced mortality in E. coli infected chicks at the level of infection which caused 25-30% mortality in normal chicks. Chick survival was concluded to be in part immunologic due to a correlative two- to three-fold increase in AB titer (Heinzerling et al., 1974a). Similar results of reduced mortality in chicks to E. coli infection by vitamin E supplementation were reported by Likoff et al. (1978). Likewise, the disease protection in turkeys inoculated with E. coli was significantly improved with supplementary levels of 100 or 300 IU of vitamin E/kg diet (Julseth, 1974).

A more direct proof for the role of increased immunity in protection was provided by testing the resistance of immunized chickens against E. coli upon reinfection. Concurrantly the protective immunological role of a combination of vitamins A and E were investigated since vitamin A has also been found to enhance the immune response (Nockels, 1980). Chicks, fed a standard ration supplemented with either 0, 150, or 300 IU vitamin E/kg or 0, 30,000, or 60,000 IU vitamin A/kg or any possible combination of vitamin A and E at the levels mentioned, were immunized with E. coli. Survivors were reinfected with a massive dose of E. coli that would kill 40% of immunized chicks but 100% of normal chicks. The supplementation of 300 IU vitamin E/kg diet or 60,000 IU vitamin A/kg diet increased the protection of immunized chicks

(Tengerdy and Brown, 1977; Tengerdy and Nockels, 1975) decreasing mortality from 40% to 5% (Tengerdy and Nockels, 1975). However, all combinations of the two vitamins fed did not give as much protection as either vitamin fed alone (Tengerdy and Brown, 1977; Tengerdy and Nockels, 1975). Vitamin E or A did not protect chicks from weight loss and severe morbidity due to infection, but slightly increased the rate of recovery (Tengerdy and Nockels, 1975). Phagocytosis aided by humoral AB appeared to be a contributing factor to the increased immunity. Both vitamins E and A enhanced phagocytosis and humoral immunity, however, neither immune mechanism alone significantly correlated with mortality. Only the early stages of phagocytosis were affected by the vitamins, the maximum effect in blood clearance being found at 1 hr. The effect of vitamin E on immune performance, phagocytosis, and overall disease protection may be connected with the levels of the vitamin in the lymphopoietic and storage organs. Vitamin E content of the liver increased with each increase in the feeding level while the spleen reached an apparent saturation point at 16,000 mg/kg diet. Vitamin A in the diet significantly reduced the accumulation of vitamin E in both organs, especially the spleen. This may partially explain the decreased level of protection by the combined use of both vitamins. Based on these results the best practical use of these vitamins may be in conjunction with vaccines since absolute protection is greater in immunized than non-immunized birds (Tengerdy and Brown, 1977).

Vitamin E supplementation was also tested to determine if it improved disease resistance against Chlamydia, an organism promoting pneumonia in sheep. Vitamin E supplemented lambs received 1000 IU of d,l-alpha-tocopherol acetate orally in gelatin capsules as well as 300 IU d,l-alpha-tocopherol acetate/kg of ration. Both control and vitamin E supplemented lambs were inoculated intratracheally with Chlamydia. Vitamin E supplementation did not prevent infection with Chlamydia or development of typical lesions, but it did appear to speed recovery. The vitamin E supplemented lambs had less exten-

sive pneumonia, greater post-infection feed consumption and significantly heavier weight gains than non-supplemented lambs. From gross lung observations at slaughter, Chlamydia were isolated from 40% of the infected non-supplemented lambs whereas none were detected in the vitamin E supplemented lambs (Stephens et al., 1979).

Guinea pigs were used in the first experiment using an attenuated live-virus vaccine to determine if vitamin E would enhance their HIR. Venezualan equine encephalomyelitis attenuated live-virus vaccine was the antigenic stimulus. Several trials were run varying the dosage, form and method of vitamin E administration. In all trials guinea pigs were fed a commercial diet containing 62 IU of d,l-alpha-tocopherol acetate/kg (Barber, 1977). In the first trial guinea pigs were fed the commercial diet supplemented with d,l-alpha-tocopherol acetate at 0, 150, or 300 IU/kg ration. No effect was found on AB response. This may be related to whether guinea pigs are able to cleave the acetate ester, converting the vitamin to its biologically active form, free d,l-alpha-tocopherol, which can be absorbed. In the second trial guinea pigs were given two intramuscular injections of vitamin E in the form of d,l-alpha-tocopherol acetate in an oil emulsion at dosages of 0, 250, and 500 IU/dose. Severe localized tissue reactions occurred in every guinea pig injected. The injection sites appeared swollen, became hardened, and at necropsy were fibrous and indurated. Similar severe reactions have occurred in man and other species. The tissue reactions could have been caused by the oil emulsion or the form of vitamin E. However, guinea pigs receiving 250 IU vitamin E/dose did have a significantly higher AB response compared to vaccinated controls (Barber, 1977). Nockels (1979, 1980) suggested that the higher vitamin dosage, which did not stimulate a significant AB response, may have caused more tissue damage at the injection site, possibly impairing absorption, or the AB response may be non-linear as observed in chicks (Jackson et al., 1978) and mice (Heinzerling et al., 1974b). In the third trial guinea pigs received seven

additional intramuscular injections of d,l-alpha-tocopherol in an aquaeous emulsion at dosages of 0, 16.5, or 33 IU/kg of body weight. Significantly higher AB titers resulted in the guinea pigs given 33 IU d,l-alpha-tocopherol/kg of body weight, before and after immunization, compared to vaccinated controls. The results of these trials demonstrated that the stimulation of the immune response appears to vary more in relation to the dose requirement, effective method of administration, and effective form of vitamin E than in relation to the nature of the antigenic stimulus (Barber, 1977). The results also confirmed research indicating the effectiveness of vitamin E in enhancing the HIR of animals immunized with non-living AG and bacteria (Ellis and Vorhies, 1976; Tengerdy and Heinzerling, 1972; Tengerdy and Nockels, 1972; Tengerdy et al., 1972, 1973).

The effect of vitamin E injection on the HIR of horses vaccinated with Venequelan, eastern and western equine encephalomyelitis killed vaccine has been studied. Either 1500 IU d,l-alpha-tocopherol acetate or 440 IU d,l-alpha-tocopherol was injected simultaneously with the vaccine. Control horses received vaccine only. Two injections of vitamin E and vaccine were given 3 weeks apart. Horses were bled weekly for 17 weeks and AB was measured by hemagglutination and plaque neutralization tests. Horses injected with tocopherol acetate had higher AB titers to all three virus strains in the vaccine throughout the majority of the 16 week trial. However, the enhancement was significantly higher only at certain intervals after immunization. Tocopherol enhanced the plaque neutralizing AB response to only the western equine encephalomyelitis virus portion of the vaccine. The more pronounced enhancement with the tocopherol acetate treatment than with the tocopherol treatment could have been due to the different dosage levels and/or the rate of absorption or elimination of the two forms of vitamin E used. Tocopherol acetate was in the oil emulsion form which would be absorbed slower than the aquaeous form (Lewis, 1978).

Vaccine viruses have also been used to show that vitamin E nutrition influences the HIR of dogs. Dogs fed a diet deficient in vitamin E with 0.5% cod liver oil added had lower AB titers to distemper and infectious canine hepatitis vaccination than normal dogs. Dogs fed a diet supplemented with 20 mg alpha-tocopherol acetate per pound of diet and given a distemper vaccination produced significantly higher and earlier neutralizing AB titers after vaccination than dogs on a vitamin E deficient diet (Sheffy and Schultz, 1978).

In summary, vitamin E and Se alone or in combination have been shown to improve effectively the HIR of laboratory and domestic animals when challenged with non-living AG, bacteria and viruses. Vitamin E also significantly improved the disease resistance of animals challenged with living AG. The effective use of vitamin E and Se among different animal species may depend on the dose requirement, route of administration, and chemical form.

Effect on Cell-Mediated Immunity

A series of studies were made using beagle dogs fed vitamin E- and Se-free purified diets to assess their immune responsiveness compared to dogs fed normal diets. After feeding the test diets, vitamin E serum levels, measured by the dialuric acid hemolysis assay, were quickly reduced, particularly in rapidly growing puppies. Plasma Se was also reduced but more slowly (Sheffy and Schultz, 1978). The absolute number of lymphocytes, erythrocyte rosette forming cells and membrane immunoglobulin-containing cells were similar in vitamin E deficient and normal dogs (Schultz et al., 1979). However, lymphocytes from vitamin E deficient dogs were significantly less responsive to mitogens, including concanavalan A, phytohemagglutinin, pokeweed mitogen, and streptolysin O, than were lymphocytes from normal dogs in the lymphocyte blastogenesis test (Langweiler, 1979; Schultz et al., 1979; Sheffy and Schultz, 1978, 1979). Lymphocyte responsiveness positively correlated with serum vita-

min E levels (Langweiler, 1979; Sheffy and Schultz, 1979). An assumption that remains to be proved is that depressed helper T cell function could be the mediator affecting the measured delay and decreased AB titers in vitamin E deficient dogs postvaccination (Sheffy and Schultz, 1979). Direct effects of vitamin E on B cell function have not yet been determined (Sheffy and Schultz, 1978, 1979). Phagocytosis activity did not appear to be affected (Sheffy and Schultz, 1978).

The in vitro suppression of the lymphoproliferative response to mitogens has been shown to be associated with a serum factor. Lymphocytes from vitamin E and Se deficient dogs that were washed extensively responded normally with mitogens and the function of lymphocytes from normal dogs was inhibited when the immunosuppressive factor was added (Langweiler, 1979; Schultz et al., 1979; Sheffy and Schultz, 1978, 1979). Vitamin E deficient serum also significantly suppressed the mitogen-induced lymphoproliferation of cells from pigs, cows, cats, and humans suggesting that these species might be susceptible to similar immunosuppressive phenomena in a vitamin E deficient state. The diminished responsiveness of lymphocytes in vitamin E deficient serum was not simply due to a lack of nutrients, as it was apparent even in the presence of fetal bovine serum supplementation. The suppressive action of vitamin E deficient serum was not inactivated by heat treatment and not due to a cytotoxic effect (Langweiler, 1979).

Vitamin E deficient serum did not suppress lymphokine production of effector T lymphocytes from dogs fed normal diets and effector T lymphocytes from vitamin E deficient dogs were capable of lymphokine production, as measured by the direct leucocyte migration inhibition assay. A possible explanation for this is that lymphokines are preformed in lymphocytes. Therefore, mitogen stimulation may have resulted in the release of preformed lymphokines instead of reflecting the capability of lymphocytes to produce them (Langweiler, 1979).

Vitamin E supplementation to vitamin E deficient dogs transforms or re-

moves the suppressor substance, restoring the lymphoproliferative response to normal or above normal levels, while Se supplementation does not. Similarly, in vitro, the suppressive effects can be eliminated by vitamin E or reducing agents, such as 2-mercaptoethanol and dithrotritol (Langweiler, 1979; Schultz et al., 1979; Sheffy and Schultz, 1978, 1979).

The suppressor substance is currently being purified and characterized (Schultz et al., 1979). Analysis of suppressive serum revealed suppressive activity in the high molecular weight (about 200,000) range of the beta-globulin fraction, however, isolation of a single suppressive protein was not accomplished (Langweiler, 1979).

Possible Mechanisms of Action

Speculations have been made on the mechanisms of action of vitamin E and Se in affecting the immune response. The antioxidant action of vitamin E and Se must be considered as a possible mechanism. In a vitamin E and/or Se deficiency the loss of their antioxidant activity, resulting in lipid peroxidation leading to widespread damage of cell membranes, could be responsible for observed immunosuppression. The fact that various antioxidants including vitamin E significantly increased the ability of canine lymphocytes to respond to mitogens in vitamin E deficient serum supports this concept for vitamin E (Langweiler, 1979).

Vitamin E has been postulated to enhance lymphocyte responsiveness by either substituting or mimicking macrophages or a macrophage-elaborated factor (Langweiler, 1979). In some in vitro experimental situations it is possible to replace macrophages, for reasons which are yet unknown, by reducing agents or by vitamin E when inducing an immune response against particulate but not soluble AG (Tizard, 1977). An in vitro experiment demonstrating that alpha-tocopherol enhances nonadherent mouse spleen cells in the relative absence of adherent cells as well as normal mouse spleen cells to SRBC also supports

this hypothesis (Campbell et al., 1974). The role of macrophages in providing a suitable stimulus for the B cell response is unclear. Macrophages probably function to concentrate AG on their surface in a configuration suitable for B cell recognition and there is evidence to suggest that macrophages may also "assist" the immune response by releasing soluble helper substances called monokines (Tizard, 1977).

Macrophages have also been shown in vitro to release nutritive molecules enabling other cells to grow (Tizard, 1977). Mouse lymphoma cells have been observed to depend on the presence of macrophages for survival and growth in culture under some conditions. A number of sulfhydryl compounds and their corresponding disulfides including L-cysteine, cysteamine, 2-mercaptoethanol and dithiothreitol have been shown to promote growth of normal mouse lymphocytes in vitro (Broome and Jeng, 1973). In view of this, it has been suggested that vitamin E may enhance respiratory and metabolic activity of cells by keeping sulfhydryl groups in a reduced state. Vitamin E may create more favorable conditions for cell growth and interaction through its protective antioxidant function (Campbell et al., 1974).

The possible role of vitamin E (Tengerdy and Nockels, 1975) and Se (Frost and Poucke, 1972) in the immune system may involve the regulation of ubiquinone biosynthesis. Ubiquinones, as components of the electron transport chain, are important regulators of redox processes in cells. By using intravascular clearance rate of colloidal carbon, it has been demonstrated that ubiquinones increase the phagocytic rate of rats (Casey and Bliznakov, 1972). Increased ubiquinone synthesis by vitamin E could lead to more efficient electron transport and metabolism in phagocytic and immunocompetent cells (Heinzerling et al., 1974b) or be responsible for the enhancing effect of vitamin E on PFC and AB synthesis (Martin and Spallholz, 1976). Tissue levels of ubiquinones have been observed to be increased by vitamin E (Green, 1962; Heinzerling et al., 1974b) and Se (Green, 1962; Frost and Poucke, 1972). However, the necessity of vitamin

E for ubiquinone production has not been confirmed (Green, 1962; Frost and Pouke, 1972; Moore, 1962; Olson, 1965). There is no doubt that Se is needed, but the part of ubiquinone synthesis catalyzed by Se is not known. Both ubiquinone synthesis and its role in host defense appear to depend on Se and vitamin E. The role of vitamin E may be indirect and lie in its ability to maintain Se in its reduced functional state (Frost and Poucke, 1972).

A mechanism of how Se stimulates AB synthesis has been suggested based on the high levels of Se which promote increased AB production in mice, those being 10-30 times higher than considered to be nutritionally necessary. Marginal toxicity of Se could increase the level of inflammation thereby increasing antibody production (Martin and Spallholz, 1976). It is known that marginal toxicity of such metals as cadmium and lead promote increased antibody production (Koller et al., 1976). However, no adverse effects were observed in mice administered high levels of Se (Martin and Spallholz, 1976).

Vitamin E enhancement of the immune system has been suggested to be mediated by prostaglandins (PG) (Langweiler, 1979; Likoff et al., 1978; Martin and Spallholz, 1976; Sheffy and Schultz, 1979; Tengerdy and Brown, 1977). PG are a complex group of oxygenated fatty acids synthesized in mammalian tissues as a result of membrane peroxidations that cause the release of PG precursors, those being polyunsaturated fatty acids (PUFA) (Kuehl and Egan, 1980).

PG, particularly the E_2 series (PGE_2) derived from arachidonic acid, have been implicated as inflammatory mediators for many years (Kuehl and Egan, 1980). Evidence strongly suggests that the E_1 series of PG (PGE_1), derived from dihomogammalinolenic acid, play a major role in the regulation of thymus development and T lymphocyte function (Horrobin et al., 1979). However, there is substantial evidence that high levels of the E-type PG depress various types of immune responses (Horrobin et al., 1979; Kuehl and Egan, 1980; Lichtenstein et al., 1972; Mertin and Hughes, 1975), particularly the cell-mediated response. Some lymphocyte functions inhibited by PG include mitogen

responsiveness (Kuehl and Egan, 1980; Mertin and Hughes, 1975), lymphocyte mediated cytotoxicity (Horrobin et al., 1979; Kuehl and Egan, 1980), and lymphokine production (Kuehl and Egan, 1980). In addition, high levels of PGE_1 inhibited maturation of T lymphocytes (Horrobin et al., 1979).

Vitamin E was shown to inhibit both the conversion of arachidonic acid to PGE_2 and the direct action of PGE_2 when intraperitoneally injected into superfused rat fundus strips (Anderson and Menzel, 1975). Also, dietary vitamin E supplementation depressed the PG production in bursa of chickens (Likoff et al., 1978). Vitamin E may limit PG biosynthesis by preventing the peroxidation of PUFA which would limit the entry of precursors into the PG cascade (Sheffy and Schultz, 1979).

On the contrary, vitamin E deficient rabbit-skeletal muscle and aorta synthesized significantly less PGE_2 (Chan et al., 1980a) and prostacyclin (PGI_2) (Chan et al., 1981), respectively. PGI_2 is a potent inhibitor of platelet aggregation and a strong vasodilator (Chan et al., 1981). Observations have shown a depression of cyclooxgenase activity, involved in PG synthesis, in both rabbit-skeletal muscle (Chan et al., 1980a) and aorta (Chan et al., 1981), and an elevation of 15-hydroxyprostaglandin dehydrogenase (PGDH) activity, involved in PG catabolism, in rabbit skeletal muscle (Chan et al., 1980b). Oral supplementation of tocopherol acetate returned cyclooxygenase (Chan et al., 1980a) and PGDH (Chan et al., 1980b) activity back to control values in 48 hr and 30 days, respectively. The elevation of PGDH activity and depression of cyclooxygenase activity may indicate a higher turnover of PG during vitamin E deficiency. Vitamin E may be involved in the regulation of PGDH and cyclooxygenase (Chan et al., 1980b). Vitamin E has been suggested to be involved in the regulation of several enzyme systems (Carpenter, 1972; Catignani et al., 1972; Hauswirth and Nair, 1972).

It is believed that PG acts by stimulating adenylate cyclase in the lymphocyte membrane which converts ATP to cyclic AMP, thereby increasing intracel-

lular concentration of cyclic AMP (Kuehl and Egan, 1980; Langweiler, 1979; Mertin and Hughes, 1975). E-type PG have been shown to increase cyclic AMP levels in leukocyte subtypes and lymphocytes in vitro (Bourne et al., 1974; Lichtenstein et al., 1972). Cyclic AMP is hypothesized to regulate the expression of immediate and delayed hypersensitivity (Bourne et al., 1974; Lichtenstein et al., 1972; Mertin and Hughes, 1975). In vitro cyclic AMP had an inhibitory action on immunologic or inflammatory functions of leucocytes and lymphocytes suggesting that it serves to protect the host from the dangerous consequences of an unregulated immune response (Bourne et al., 1974).

The essential PUFA, linoleic and arachidonic, have also been shown to affect immunoregulatory mechanisms. They have PG-like activity which may be explained by the fact that they are precursors of PG (Meade and Mertin, 1976; Mertin and Hughes, 1975). Subcutaneous injection of linoleic acid prolonged the survival of skin allografts in mice and reduced both the primary and secondary cytotoxic response of isolated spleen cells (Meade and Mertin, 1976). Dogs fed diets high in PUFA, with linoleic acid intake 2.8 times the recommended dietary requirement (Sheffy and Schultz, 1979) but deficient in vitamin E and Se, rapidly developed a very poor lymphocyte response to mitogens (Sheffy and Schultz, 1978). PUFA were shown to inhibit phytohemagglutinin-induced lymphocyte transformation, with the degree of inhibition being related to the degree of unsaturation (Mertin and Hughes, 1975).

PUFA may act by impairing lymphocyte recognition of phytohemagglutinin or by interfering with the sequence of synthetic events (Mertin and Hughes, 1975). This effect could be mediated by alterations in the lipid fluidity of the plasma membrane (Langweiler, 1979; Meade and Mertin, 1976). Lymphoid cells might be especially prone to react to fatty acid level alterations since their free fatty acid levels are considerably higher than other cells. Therefore, lymphoid cells might be particularly susceptible to changes in membrane fluidity as a result of changes in PUFA levels modifying cell-AG interaction. In

this way PUFA may inhibit AG stimulus (Meade and Mertin, 1976).

The lymphocyte membrane may be affected in some way by a vitamin E deficiency, which may explain a suppressed lymphocyte response. It is possible that membrane related factors involving an interaction between vitamin E deficiency, PUFA, PG, and cyclic nucleotides are responsible (Langweiler, 1979).

In summary, a number of mechanisms have been proposed by which vitamin E exerts its effects on the immune response. These include: the protective antioxidant function of vitamin E; mimicking macrophages or factors produced by macrophages, and the regulation of ubiquinone and PG synthesis. Se, like vitamin E, is involved ubiquinone synthesis and in antioxidant functions, both of which affect the immune response. Se at marginally toxic levels may stimulate AB production.

ASPECTS OF VITAMIN E DEFICIENCY IN CALVES - A REVIEW

Vitamin E deficiency in calves is distinguished by generalized muscular dystrophic lesions (Blaxter and McGill, 1955). Muscular dystrophies of cattle, primarily of calves, have been recognized since the late 1800's and described in many countries under such names as "leg illness", "heart rheumatism", "fish flesh" or "hen flesh", and "transport disease" (Blaxter, 1953). Since early in their history, these muscular diseases were felt to be primarily of nutritional origin although the particular nutrient concerned was not fully established until 1953 (Blaxter and McGill, 1955). Many of the published accounts indicated that the diseases elicit similar clinical symptoms and similar pathological changes (Blaxter, 1962a). Therefore, the diseases have been grouped together using such collective names as "enzootic muscular dystrophy" (Blaxter and McGill, 1955), "white muscle disease" (Blaxter, 1957), and "nutritional muscular degeneration" (Oksanen, 1973). Countries in which muscular dystrophy (MD) in cattle occurs naturally include all European countries, Russia, Japan, New Zealand, Australia, Canada, and the United States (Blaxter, 1962b). However, its incidence in different countries varies widely, in some reaching epidemic proportions causing serious economic losses in cattle farming (Blaxter, 1962a; Oksanen, 1973).

Etiology

The etiology of MD in calves involves an insufficiency of vitamin E in the ration or body reserves as well as other nutritional and environmental factors. These include the amounts of polyunsaturated fatty acids (PUFA) and selenium (Se) in the diet and the incidence of stress in the animals concerned (Blaxter, 1962b). Variations in the quantity of vitamin E necessary to prevent or cure MD can be partially explained by these interrelating factors (Oksanen, 1973). Experimental dystrophies in calves have been produced mostly

by using artificial diets that are low in vitamin E and contain PUFA. Their etiology appears to differ from those which occur naturally (Blaxter, 1962b).

Naturally Occurring Muscular Dystrophy. MD is primarily seen in cattle confined during the winter in warm surroundings on poor quality feed (Blaxter and McGill, 1955; Blaxter, 1957). It appears that the feeding of poor quality rations during a long winter period can result in a serious depletion of body reserves of vitamin E (Blaxter, 1953). In Northeast (NE) Scotland the disease occurs invariably in calves born indoors in the spring to dams that were housed during the winter (Blaxter and Sharman, 1953). When the calves are turned out to pasture for the first time, unaccustomed muscular exertion rapidly precipitates the disease symptoms (Blaxter, 1957). In Ontario, severe outbreaks of MD occur in late May or early June usually within 2 weeks after the calves have been turned out to pasture with their dams, although some cases develop while they are still confined (Maplesden et al., 1960). Similarly, in Finland an obvious peak of MD occurs at the beginning of the pasture season (Oksanen, 1973).

In Switzerland and elsewhere, MD occurs in calves subjected to considerable muscular strain when transported by rail over long distances. Thus, the condition has been called "transport disease" (Blaxter and McGill, 1955). Exposure to other environmental stresses such as drafts, dampness, and scouring attacks can precipitate or aggravate symptoms of MD (Maplesden et al., 1960). Serum tocopherol decreased in calves having a severe scouring condition. Poor absorption of vitamin E due to the abnormal condition was suspected (Parrish, 1949).

Extensive experimentation in NE Scotland, which showed that an enzootic dystrophy of calves could be prevented by vitamin E, focused attention on the dietary intake of vitamin E and the tocopherol status of the animals concerned. The vitamin E content of the winter feedstuffs fed to the dams was found to be low and the serum tocopherol of the calves was less than 40 μg/100 ml in calves from dystrophic herds (Blaxter and Sharman, 1953). The amounts of vitamin E

required to prevent the disease were found to be large relative to the amounts normally consumed by calves in non-dystrophic areas (Blaxter, 1962a). The increased vitamin E required by the calves was not due to abnormally high levels of PUFA in the milk produced by the dams. Furthermore, the blood serum fats of the calves were not abnormally unsaturated. Blaxter (1957) concluded the disease was not caused by a simple dietary deficiency of vitamin E.

Se was found to be as effective as vitamin E in preventing the disease (Sharman et al., 1959). Areas where the disease occurs appear to have soils deficient in Se (Blaxter, 1962a,b).

A disease similar to that in NE Scotland occurs in Oregon, USA. It has been described as "white muscle disease (WMD) of calves". The disease occurs on irrigated farms having intensively fertilized pastures consisting mainly of legumes. The cause of the disease is unknown and there appears to be no relationship between the tocopherol content of the pastures and the incidence of disease (Muth, 1955). Supplementing the dams during gestation and through weaning with 0.1 ppm Se provided essentially complete protection against WMD, while vitamin E did not. The Oregon pastures, on which gypsum is commonly used as a fertilizer, were found to have a high sulfur content. A high incidence of WMD was correlated with excess sulfur and the addition of both sulfate and Se to a dystrophogenic diet reduced the prophylactic effect of Se. It was suggested that excess sulfur is antagonistic to the biological availability of Se (Schubert et al., 1961). MD in west Finland has also been associated with black clay soils very rich in sulfides (Blaxter, 1962a). In addition, the concentration of Se in forage crops sampled from areas in Finland has been shown to be very low suggesting the etiology of MD may be considered to be a deficiency of Se (Oksanen, 1973).

An important factor in the etiology of naturally occurring MD in calves was the introduction of synthetic milk replacers. It is believed that naturally occurring disease, like the experimental one, is due to an improper balance

of dietary PUFA and vitamin E. Treatment with d,l-alpha-tocopherol acetate was effective therapy (Lannek, 1973).

The major etiological cause of naturally occurring MD should be explored in the natural feedstuffs of ruminants since they show a wide range in quality and composition. The vitamin E content of ruminant feedstuffs can be adversely affected by such factors as weather conditions, stage of maturity, methods of drying (Oksanen, 1973), length of storage, and preservation method (Young et al., 1975). Stored feeds tend to lose vitamin E activity with time (Bruhn and Oliver, 1978; Young et al., 1975).

Experimental Muscular Dystrophy. MD has been produced in calves and shown to be prevented by alpha-tocopherol in many experiments (Adams et al., 1959a,b; Blaxter et al., 1952, 1953a,b,c; Maplesden and Loosli, 1960; Maplesden et al., 1960; Michel et al., 1972; Safford et al., 1954). A common factor besides low vitamin E content in the diets used was the presence of PUFA in the form of lard, cod liver oil (CLO), other fish oils, corn oil, or butter oil. Furthermore, the prophylactic dose of alpha-tocopherol depended on the amount of PUFA given and the time for MD to develop was variable.

In the case of CLO, the protective action of 50 mg of alpha-tocopherol acetate was neutralized when 15-18 ml of the oil was fed to calves (Blaxter et al., 1953c). It was necessary to provide 200 mg d,l-alpha-tocopherol acetate daily in order to prevent MD when 29.6 ml of CLO was fed and less tocopherol was necessary with smaller amounts of oil (Blaxter et al., 1953b). Studies indicated the more highly saturated fraction (iodine value 270) of CLO was more toxic than the less unsaturated fraction (iodine value 110) (Blaxter et al., 1953a). Severe MD was produced using CLO, the first clinical signs being observed between 21 and 43 days. Variation in the time of development of MD was attributed mainly to individual differences in body stores of vitamin E (Blaxter, 1953). Maplesden and Loosli (1960) confirmed the high levels of tocopherol necessary to prevent MD produced using CLO. They found 200 mg d-alpha-

tocopherol acetate daily prevented MD in calves fed diets with 30 ml of CLO. However, they observed no clinical signs of MD in calves (except for one) fed the dystrophogenic diet for an average of 105 days, but necropsy revealed severe muscle degeneration. The calves were kept in small pens and received minimal exercise. Since calves can be severely affected with MD but eat and grow normally, it is understandable how muscular exertion could precipitate clinical signs of MD (Maplesden and Loosli, 1960).

MD was produced in calves by feeding filled milks containing fat at 3.5% of liquid ration in the form of lard, corn oil, or butter oil prepared from oxidized butter. Symptoms of MD usually appeared after about 55 days (Adams et al., 1959a). On each of the dystrophogenic diets several animals showed no clinical symptoms but exhibited microscopic lesions upon necropsy. MD was prevented by giving 500 mg of d,l-alpha-tocopherol acetate daily or by hydrogenating the oils (Adams et al., 1959b).

In other experiments using lard at 3.38% (Safford et al., 1954) or 4.12% (Blaxter et al., 1952) of liquid rations, signs of MD in calves were evident as early as 14-24 days and 16-59 days, respectively. Diets supplemented with 148 and 200 mg of alpha-tocopherol, respectively, prevented MD. By comparison, experiments using lard at 2.85% (Maplesden et al., 1960) or 3.0% (Holter et al., unpublished work cited by Maplesden et al., 1960) of liquid ration took considerably longer periods to produce MD. Their calves were fed the dystrophogenic diets for periods of 82-105 days and 91-266 days, respectively. The addition of 200 IU d,l-alpha-tocopherol to the basal diet of Maplesden et al. (1960) effectively prevented MD. Differences in diets and husbandry may account for variations in the time required to produce MD (Maplesden et al., 1960).

Necropsy examination showed a greater incidence and severity of MD in calves fed rations containing fish protein concentrate compared to calves fed rations containing dried skim milk for 8 weeks. All rations contained lard oil at 10% of dry ration and were supplemented with 46 mg of vitamin E/kg of dry

ration. The fish protein concentrate, however, contained 1.43% fat and fish fats are highly unsaturated. Further experiments supplementing 46 or 92 mg vitamin E/kg of dry ration showed no muscle lesions but calves receiving fish protein at the higher level of supplementation had greater weight gains than calves on rations at the lower level of supplementation (Michel et al., 1972).

Production of MD by feeding calves low-fat or fat-free diets with low vitamin E content has taken a very long time (Holter et al., unpublished work cited by Maplesden et al., 1960) or has been unsuccessful (Adams et al., 1959a; Blaxter and McGill, 1955). These results suggest the daily requirement of calves for vitamin E in the absence of PUFA is very low (Blaxter, 1962b).

Se has not been demonstrated to be effective in preventing MD induced by feeding PUFA. The addition of 1 ppm Se to calf diets containing 30 ml CLO did not prevent MD (Maplesden and Loosli, 1960).

There is evidence that antioxidants will prevent MD in calves fed diets containing PUFA. The addition of 1 g methylene blue to calf diets containing 29.6 ml of CLO effectively prevented MD. The role of methylene blue in prevention was not associated with higher concentrations of tocopherol in serum, muscle, liver, or perinephric fat in supplemented calves than in controls that succumbed. Ascorbic acid and biotin, natural tissue constituents, and ethyl gallate, a fat soluble antioxidant, did not effectively prevent MD (Blaxter et al., 1953b). Oxidation products of alpha-tocopherol, such as the quinone and hydroquinone, were also found to be effective in preventing MD in calves. Methylene blue, quinone, and hydroquinone were not shown to have any vitamin E activity when assayed by the rat fertility method (Blaxter, 1957).

Analyses of the tocopherol content of dystrophic muscles of calves fed CLO showed that the tocopherol content was not diminished and the tocopherol content of livers of dystrophic calves fed CLO were not depleted (Blaxter, 1957). In contrast, Dehority et al. (1961) found a decrease in the tocopherol content of heart and skeletal muscle as well as liver of calves fed 28.4 g of CLO. The

results of Blaxter (1957) suggested that the MD induced by CLO could not be explained by the increased oxidation of tocopherol. PUFA may induce degeneration through the formation of abnormal phospholipids (toxic products) at cell interfaces capable of uncoupling phosphorylation from respiration. Vitamin E may prevent the action of the toxic products or facilitate the dissimilation or deposition of the PUFA (Blaxter, 1957; Blaxter et al., 1953b).

Clinical Signs and Diagnosis

The clinical signs of naturally occurring MD and experimental MD are essentially identical. Clinical signs involve the impairment of muscle function causing changes in behavior, stance, and gait. The muscles affected include both skeletal and cardiac muscle (Blaxter and McGill, 1955).

In cases in which the skeletal muscles of calves are affected, numerous symptoms exhibited have been described. These include symmetrical lameness, reluctance to stand, hind leg crossing and hock interference when walking, staggering gait, tremors of the fore and hind limbs while standing, relaxation of the carpal and metacarpal jounts and fetlocks, elevation of the suprascapular cartilages, spreading of the toes, inability to raise the head, inability to control movements of the tongue, and difficulty in drinking and swallowing (Blaxter and McGill, 1955; Blaxter et al., 1952, 1953c; Safford et al., 1954). If the pharyngeal and laryngeal muscles (Maplesden et al., 1960) or the intercostal and diaphramatic muscles (Blaxter and McGill, 1955) become dystrophic, feed particles may enter the lungs and cause pneumonia. Respiration is then carried out almost entirely by the use of the abdominal wall. Expiratory grunts may accompany such respiration (Blaxter et al., 1953c).

The cardiac muscle may be dystrophic without other clinical signs of MD exhibited. Usually the calf dies suddenly without warning, although sometimes cardiac seizures may occur and be recognized (Blaxter and McGill, 1955). Heart attacks have been reported to be precipitated by exercise, the calf collapsing

after walking or after being excited (Blaxter et al., 1953c). Heavy breathing, rapid heart rate and irregular heart beat are other clinical signs (Blaxter et al., 1953c; Maplesden et al., 1960). Gullickson et al. (1949) fed four generations of cattle vitamin E-free rations. He found no apparent effects on growth, reproduction, or lactation but several animals between the ages of 21 months and 5 years died suddenly from heart failure. Electrocardiograms (ECG) of their cattle reared on vitamin E-free rations revealed increased durations of the P-R, QRS, and Q-T intervals and a decrease in the overall potential indicating a decreased function of the myocardium in the final stages of the deficiency (Gullickson and Calverley, 1946). Increased durations of the P-R and Q-T intervals were also reported in dystrophic calves fed a diet low in vitamin E containing lard (Safford et al., 1954) and in calves fed diets containing 28.4 g of CLO with or without 200 mg of d,l-alpha-tocopherol acetate (Dehority et al., 1961). Adams et al. (1959b) observed marked alterations in the ECG patterns of several dystrophic calves fed corn oil filled milk without tocopherol supplementation.

Clinical signs of bloat occurred in several calves receiving 28.4 g or 56.8 g of CLO. Necropsy examination of a calf fed 113.6 g of CLO that died suddenly revealed a twist of the ileum. It could not be determined if bloating and abnormality of the intestinal tract were related to dystrophy of the smooth muscle. However, necropsy examination of two out of five calves fed 113.6 g of CLO showed a complete lack of normal elasticity and tonus of the rumen wall (Blaxter et al., 1953c).

Clinical signs of MD vary widely from hardly noticeable to obvious symptoms depending on the particular muscles affected. Since many cases are subclinical or involve mild symptoms, MD cannot always be diagnosed from the clinical symptoms alone. Chemical methods measuring changes in serum enzymes allow a more positive diagnosis. Serum aspartate aminotransferase shows a marked increase in cases of MD in ruminants caused by its release into the blood from

degenerated muscle cells (Oksanen, 1973). Serum glutamic oxalacetic transaminase (SGOT) has been shown to be sensitive indicator of early muscular damage in lambs (Swingle et al., 1959). Elevated SGOT levels have been correlated with low plasma tocopherol levels in calves (Hidiroglou et al., 1973).

The analysis of vitamin E content in serum or plasma is of limited clinical value as it does not reflect the true vitamin E status of the animal. Grifo et al. (1959) determined the relative sensitivity of various tissues in calves to graded levels of tocopherol intake. Heart and liver were found to be about twice as sensitive to tocopherol intake as perinephric fat and trapezius muscle. Rousseau et al. (1957) fed calves a basal ration with graded levels of tocopherol added and found plasma tocopherol increased at diminishing rates with tocopherol intake while the liver tocopherol increased at constant rates with tocopherol intake. From these studies, the heart and liver appear to be more suitable indicators of the vitamin E status of calves. Blaxter (1957) could not differentiate between potentially dystrophic calves and normal ones on the same farm by means of their serum tocopherol concentrations. He could only generalize after numerous analyses that if the serum tocopherol value exceeded 70 μg/100 ml then the chance of MD occurring could be considered very small. Other studies revealed no significant differences between the plasma tocopherol values of dystrophic and normal animals (Maplesden et al., 1960; Schubert et al., 1961). Likewise, a study of the tocopherol content of colostrum and milk of range cattle in Montana showed great individual variations among cows in the same herd and the attempt to correlate the tocopherol levels and the occurrence of MD in calves was unsuccessful (Swingle et al., 1956).

Pathological Changes

Gross Pathology. Widespread areas of degeneration in skeletal and cardiac muscle is the prominent lesion found in carcassess of calves afflicted with MD.

Areas of degeneration tend to be white, pale, or greyish (Blaxter and McGill, 1955; Maplesden et al., 1960). In the skeletal muscles, these areas are bilaterally symmetrical (Blaxter, 1953; Muth, 1955; Safford et al., 1954). Affected muscles tear easily (Blaxter and McGill, 1955) and are somewhat edematous (Blaxter and McGill, 1955; Maplesden et al., 1960; Muth, 1955). Small areas of hemorrhage may be present (Blaxter, 1953; Muth, 1955). In cases where cardiac abnormalities were present before death, necropsy findings included excessive fluid in the abdominal cavity, pneumonic lungs containing blood and milk, and edema of the lungs (Blaxter et al., 1952; Muth, 1955). Blaxter et al. (1952) observed fat to be a light brown color rather than the normal whitish yellow color. Safford et al. (1954) reported an abnormal grey color of the cortex in the kidney of one calf. Gross pathological findings, like the clinical signs, vary considerably among calves (Blaxter and McGill, 1955).

Histological Changes. The sequence of histological changes in MD has been identified by MacDonald et al. (1952). Briefly, the primary change appears to be the hyaline degeneration of individual muscle cells followed by coagulation of the sarcoplasm and necrosis. In some instances vacuole formation may occur. Next the sarcolemmal tube collapses and there is a massive proliferation of the sarcolemmal nuclei. The lesion is essentially non-inflammatory (Blaxter, 1953; Blaxter and McGill, 1955). Histological studies by Muth (1955) revealed a primary calcification of muscle fibers. Safford et al. (1954) reported the epithelium of the tubules in the cortex of the kidney was flattened and many leukocytes were present in the cortical capillaries.

Biochemical Changes. The biochemical changes occurring in MD have been extensively studied by Blaxter and Wood (1952). Biochemical analyses show a loss of contractile proteins, myoglobins, creatine, creatine phosphate, and potassium from the muscle cell. There is an increase in sodium and nucleic acid.

Analysis of muscle can be used to establish the occurrence of degeneration. Creatine content and determination of the ratio of potassium to sodium in the muscle are effective analytical criteria (Blaxter and McGill, 1955). Urinary excretion of creatine was found to be increased in dystrophic calves by Safford et al. (1954).

VITAMIN E AND THE IMMUNE SYSTEM IN CALVES

SUMMARY

Two experiments were conducted to determine the effect of vitamin E on the humoral and cell-mediated immune responses in calves and to study the plasma vitamin E and immunological status of calves under normal herd management. In experiment 1, 12 calves were fed skimmed colostrum the first 2 days of life and skimmed milk thereafter with vitamin E-stripped fat, polyoxyethylene glycol, lecithin, and minerals and vitamins A and D added at 3.3%, .2%, .2%, and .1% of liquid diet, respectively. Six calves per group received 0 (DEF group) or 1 g (SUP group) d,l-alpha-tocopherol acetate daily. In experiment 2, 20 calves (CON group) were fed colostrum for 3 days and whole milk thereafter, and starter containing 16% protein and 20% alfalfa hay ad libitum. At 6 weeks of age, mean plasma vitamin E levels and standard errors (SE) were 71±9.3, 639±177.2, and 155±15.8 μg/100 ml and mean serum glutamic oxalacetic transaminase (SGOT) levels and SE were 320±59.3, 61±59.3, and 43±32.5 IU/L for groups DEF, SUP, and CON, respectively. Mean serum immunoglobulin (Ig) levels (mg/100 ml) and SE were: IgG1, 1079±140.6, 1168±86.9, and 1315±113.9; IgG2, 488±101.6, 562±102.3, and 432±49.3; IgA, 37±4.5, 53±18.9, and 85±36.6; IgM, 151±26.5, 118±20.0, and 110±11.5 for groups DEF, SUP, and CON, respectively. Mean lymphocyte stimulation indexes (LSI) and SE for groups DEF, SUP, and CON were 76±15.8, 220±59.2, and 152±40.1, respectively. Plasma vitamin E was higher ($P < .009$) in group SUP than groups DEF or CON and SGOT was higher ($P < .001$) in group DEF than groups SUP or CON. Serum Ig levels and LSI among calves within groups varied widely. No differences ($P > .05$) in serum Ig levels or LSI were found among groups, although a trend toward enhanced mitogen-induced proliferation of lymphocytes from SUP calves appeared to be due to vitamin E supplementation. The emulsified-fat diet fed in experiment 1 resulted in some hair loss, soft fatty feces, and poor performance. Due to the adverse effects

caused by the diet, an enhancing effect of vitamin E on the humoral and cell-mediated immune responses of the SUP calves may have been partially masked.

INTRODUCTION

Calf morbidity and mortality is a serious problem facing dairymen today. A study in New York associated large herds with an average calf mortality of 33% (Hartman et al., 1974). Low levels of serum immunoglobulins in young calves have been shown to be related to increased incidence of disease (Boyd, 1972; Logan et al., 1972; McEwan et al., 1970). Vitamin E has been shown to increase effectively the humoral immune response of laboratory and domestic animals (Nockels, 1979; Sheffy and Schultz, 1979). The disease resistance of chicks (Heinzerling et al., 1974a) and turkeys (Julseth, 1974) inoculated with E. coli, and sheep inoculated with Chlamydia (Stephens et al., 1979) was significantly improved by vitamin E. Lymphocytes from vitamin E deficient dogs were significantly less responsive to mitogens in vitro than were lymphocytes from normal dogs, and supplementation of vitamin E to the deficient dogs restored the lymphoproliferative response to normal or above normal levels (Langweiler, 1979; Sheffy and Schultz, 1979). The effect of vitamin E on the immune system of the bovine has not been adequately studied.

The objectives of this study were to determine the effect of vitamin E on humoral and cell-mediated immune responses in calves and to study the plasma vitamin E and immunological status of calves under normal herd management.

MATERIALS AND METHODS

Experimental Procedure

Experiment 1. Twelve male Holstein calves were separated from their dams at birth prior to colostrum consumption and alternately assigned to a vitamin E deficient (DEF) or vitamin E supplemented (SUP) group. A liquid ration was fed at 10% body weight daily in two equal feedings. The liquid ration con-

sisted of skimmed colostrum the first 2 days and skimmed milk thereafter with additional ingredients as listed in Table 1. Originally the diet did not include lecithin. Lecithin was added to the diet of the first two calves at 4 weeks of age and to the diet of subsequent calves on experiment 1 for the entire 6 weeks.

Colostrum and milk were skimmed to remove vitamin E. Colostrum was skimmed by allowing it to settle in a separatory funnel for 8-12 hr after which the skimmed portion was collected. Calves in the SUP group each received 500 mg of d,l-alpha-tocopherol acetate[1] in their ration at each feeding. Rations were prepared by warming the combined ingredients in a water bath to about 40°C at which time they were vigorously hand-mixed and fed. No dry feed was provided. Jugular blood was sampled at birth, 24-48 hr after birth, at one week of age, and weekly thereafter until 6 weeks of age. The calves were exercised weekly with special attention devoted to signs of muscular weakness.

Experiment 2. This experiment was conducted simultaneously with experiment 1. Twenty neonatal male Holstein calves serving as a control (CON) group were fed colostrum for 3 days and whole milk thereafter at 8% body weight daily and received a starter (Table 2) ad libitum. These calves remained with their dams for 12-24 hr after birth. Jugular blood was sampled 24-48 hr after birth and at 4 and 6 weeks of age.

General. Calves were housed in elevated metal stalls and water was available ad libitum. Calf weights were recorded weekly and ration levels adjusted accordingly. Fecal consistency scores (Larson et al., 1977) were recorded twice daily.

Jugular blood for plasma collection or lymphocyte isolation was drawn into sterile heparinized vacutainers. Blood for serum collection was drawn into sterile non-heparinized vacutainers. Plasma and serum were separated by cen-

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[1]Hoffman-La Roche Inc., Nutley, NJ. 07110.

trifugation and stored frozen until analyzed. Blood for lymphocyte isolation was used within an hour after collection.

Analytical Procedures

Fluorometric Assay for Tocopherol. All blood samples were analyzed for plasma tocopherol by the fluorometric method described by Storer (1974) with the following modifications: 1) samples were prepared under amber lights; 2) 4 ml of hexane was used to extract tocopherol from .25 ml plasma; 3) tubes containing samples and reagents were vortexed for 1 min and centrifuged at 2500 rpm for 10 min; and 4) the fluorescence of the hexane extraction was measured at 290 nm excitation and 325 nm emission.

Radial Immunodiffusion (RID). Blood sampled at 24-48 hr and at 4 and 6 weeks was analyzed for serum immunoglobulins (Ig), IgG1, IgG2, IgA, and IgM, by RID based on the method described by Mancini et al. (1965) with the following modifications: 1) 2.5 g agarose[2] was dissolved in 100 ml 0.1 Tris(hydroxymethyl)aminomethane-hydrochloric acid (Tris-HCL) buffer (pH 7.4); 2) reconstituted anti-bovine Ig serum[3] was diluted to a suitable level using 0.1 M Tris-HCL buffer; 3) a mixture of 5 ml diluted antiserum and 5 ml agarose was poured onto an immunodiffusion plate[3] previously set on a leveling board; 4) circular wells were punched in the agarose using a template[3] and 4 mm cutter[3], 5) wells were charged with 10 μl of unknowns and known standards using standards from commercial kits[3]; 6) for best results, when analyzing bovine serum for IgG1 and IgG2, samples were diluted 1:9 and 1:2, respectively, with .9% saline in distilled water before charging wells; 7) to prevent drying, distilled water was placed in the outer channel of plates, plates were covered, and sealed in a plastic bag; 8) after 5 days ring diameters were read using an

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[2]No. A-6013. Type 1:Low EEO. Sigma Chemical Company, St. Louis, MO. 63178.

[3]Miles Laboratories, Inc., Elkhart, IN. 46515.

optical comparator[4], and 9) Ig concentrations of the unknowns were determined using the linear relation between the ring diameter squared and the concentration of the standards.

Lymphocyte Blastogenesis Test (LBT). Blood sampled at 24-48 hr and at 4 and 6 weeks was also analyzed for cell-mediated immune response by the *in vitro* LBT. Bovine lymphocytes were isolated from 10 ml heparinized whole blood by the distilled water technique described by Rossi and Kiesel (1977) and modified by Beeharry (1978). T lymphocytes were isolated from the total lymphocytes by nylon wool as described by Julius and Herzenberg (1973) and modified by Beeharry (1978) with one difference: prewashed nylon wool[5] was used. Triplicate or quadruplicate lymphocyte cultures for blastogenesis studies were prepared by the method of Beeharry (1978) with the following modifications: 1) 0 and 2.5 μg phytohemagglutinin/ml were added to control and stimulated cultures, respectively; 2) following 24 hr incubation, cultures were radioactively labelled; 3) cultures were washed twice with phosphate buffered saline; 4) after placing filters in scintillation vials, 0.5 ml solubilizer (1:3 solution of tissue solubilizer[6] in scintillation fluid[6]) was added after which vials were placed in a 50° water bath for 20-30 min; 6) after removing the vials from the water bath and maintaining them at room temperature, 0.5 ml neutralizer (3:400 solution of glacial acetic acid in scintillation fluid) and 9 ml scintillation fluid was added; 7) vials were counted in a Unilux II-A Liquid Scintillation System[7] for .1 min after 2 hr of dark adaptation and again after 20-24 hr. For comparative purposes, lymphocyte stimulation indexes (LSI) were calculated as described by Renshaw *et al.* (1977).

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[4]Finescale, Orange, CA. 92668.
[5]Fenwal Laboratories, Deerfield, IL. 60015.
[6](TS-1) in 3a70B Complete Counting Cocktail. Research Products International Corporation, Elk Grove Village, IL. 60007.
[7]Nuclear Chicago, Def Plains, IL. 60018.

Other Analyses. Six week blood samples were analyzed for serum proteins by electrophoresis using the method and materials in the Helena Electrophoresis Kit A[8]. The method involved the separation and quantification of serum proteins on cellulose acetate. Serum glutamic oxalacetic transaminase (SGOT) was determined by the Abbott VP Bichromatic Analyzer[9]. The additional serum constituents: creatinine, glucose, phosphorus, calcium, total protein, chloride, alkaline phosphatase, blood urea nitrogen, carbon dioxide, potassium, and sodium, were analyzed by the Sequential Multiple Auto-Analyzer 12/60 micro[10]. Six week samples were also analyzed for plasma selenium (Se) and the Se content of six colostrum samples was determined by the fluorometric method described by Whetter and Ullrey (1978).

Statistical Analyses

A two-way analysis of variance was done to determine the effects of ration, number of weeks on experiment (age), and ration x age interaction on plasma tocopherol values, serum Ig concentrations, LSI, and fecal scores. To determine the effects of ration alone and age alone on the same measurements, a one-way analysis of variance was done within each sampling time and within each treatment group, respectively. A one-way analysis of variance was also done on plasma Se values, serum protein values, SGOT levels, other serum constituents measured, and overall weight gain to determine the ration effect at 6 weeks of age.

RESULTS

Plasma Tocopherol. Mean plasma tocopherol values of calves in DEF, SUP, and CON groups over the 6 week period are presented in Figure 2. Analysis of variance showed a ration x age interaction ($P<.0001$). The SUP group had sig-

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[8](Cat. No. 5440). Helena Laboratories, Beaumont, TX.
[9]Abbott Diagnostics, North Chicago, IL. 60064.
[10]Technicon Instruments Corporation, Tarytown, NY. 10591.

nificantly higher plasma tocopherol than the DEF group at each sampling time except immediately after birth. Plasma tocopherol was significantly higher in the SUP group than the CON group at all sampling times that coincided. Plasma tocopherol of the CON group was higher than the DEF group at all sampling times that coincided, however, they did not differ ($P>.05$). Plasma tocopherol was not affected by age ($P>.05$) within the DEF or CON groups but was in the SUP group. Plasma tocopherol over the 6 week period for the DEF and CON groups without regard for time, expressed as mean ± standard error ($\bar{X}$ ± SE), were 64±11.7 and 138±13.5 µg/100 ml, respectively. Plasma tocopherol tended to increase with age in the SUP group after 1 week with a significant increase occurring between 2 and 3 weeks. For the SUP group, plasma tocopherol over the first 2 weeks and the last 4 weeks of the experiment ($\bar{X}$ ± SE) were 172±35.2 and 549±98.4 µg/100 ml, respectively.

Serum Immunoglobulin Levels. Mean serum Ig levels are presented for the treatment groups in Figures 3-6. All calves initially absorbed Ig of each class but there was wide individual variation. There were no differences ($P>.05$) among treatment groups due to ration and no ration x age interactions ($P>.05$). The CON group had consistently higher serum Ig levels in all Ig classes than the DEF and SUP groups at 24-48 hr. IgG1 (Figure 3) and IgM (Figure 6) levels did not vary significantly with age within any of the treatment groups. Serum IgG1 and IgM over the 6 weeks without regard for age or ration ($\bar{X}$ ± SE) were 1099±135.4 and 109.4±16.8 mg/100 ml, respectively. All three rations produced a similar pattern in IgG2 levels (Figure 4), the levels being significantly higher at 6 weeks than at 24-48 hr and 4 weeks. Serum IgG2 ($\bar{X}$ ± SE) was 185, 230, and 494 ±33.1 for all calves at 24-48 hr and 4 and 6 weeks, respectively. IgA (Figure 5) levels did not vary with age ($P>.05$) within the SUP group. However, IgA levels were significantly lower at 4 weeks for the CON group and at 4 and 6 weeks for the DEF groups than at 24-48 hr. Serum IgA ($\bar{X}$ ± SE) was 112, 21, and 58 ±23.8 mg/100 ml for all calves at 24-48 hr

and 4 and 6 weeks, respectively. There were no differences ($P>.05$) in total gamma-globulins among the treatment groups at 6 weeks of age (Table 3).

Lymphocyte Stimulation Indexes. Mean LSI of the treatment groups are presented in Figure 7. LSI among calves within treatment groups varied widely. There were no differences ($P>.05$) among treatment groups due to ration nor any ration x age interactions ($P>.05$). LSI were also not affected by age ($P>.05$) within the CON or SUP groups. In the DEF group, LSI were significantly higher at 4 weeks than at 24-48 hr and 6 weeks. LSI for all calves ($\bar{X} \pm SE$) were 41±30.2, 145±28.1, and 149±26.7 at 24-48 hr and 4 and 6 weeks, respectively.

Serum and Plasma Constituents at 6 Weeks (Table 3). Mean SGOT levels were higher ($P<.001$) in the DEF than in the SUP and CON groups at 6 weeks. There were no differences ($P>.05$) in plasma Se among treatment groups at 6 weeks. Analyses of the serum taken at 6 weeks revealed no differences ($P>.05$) among treatment groups in creatinine, glucose, calcium, phosphorus, chloride, alkaline phosphatase, carbon dioxide, potassium, sodium, alpha-, beta-, and gamma-globulins, and total protein. Blood urea nitrogen was lower ($P<.003$) in the CON than in the DEF or SUP groups and albumin was higher ($P<.02$) in the CON than the SUP group at 6 weeks.

Fecal Scores. There was a ration x age interaction ($P<.0005$) in fecal scores (Figure 8). Mean fecal scores were significantly higher for all treatment groups at 2 weeks than at 1 week. At each week, from 3-6 weeks, the DEF and SUP groups had significantly higher fecal scores than the CON group. At 5 weeks the DEF group had significantly higher fecal scores than the SUP group.

Body Weight Gain. The CON group had higher ($P<.006$) body weight gains than the DEF and SUP groups over the 6 week period. Body weight gains over the 6 weeks ($\bar{X} \pm SE$) were 4.2±2.2, 1.7±2.2, and 9.8±1.2 kg for the DEF, SUP, and CON groups, respectively.

General Observations. The first two calves placed on experiment 1, one in each treatment group, and fed the diet (Table 1) without lecithin developed se-

vere alopecia, dermatitis on denuded areas, and soft fatty feces. The calves lost hair extensively on the thighs and anal region. Additional hair was lost on the muzzle, face, ears, and limbs. After adding lecithin to their diets at 4 weeks of age, dermatitis disappeared and regrowth of hair was observed within 2 days. Dermatitis and severe alopecia were prevented in calves that received the diet plus lecithin for the entire study. However, all calves in experiment 1 lost hair on the muzzle and anal region, and some calves in both the DEF and SUP groups lost some additional hair on their face, ears, and limbs. A brown crust tended to form on the muzzle. The addition of lecithin to the diet did not totally prevent the development of soft fatty feces suggesting inefficient utilization of the lard in calves on experiment 1. In both the DEF and SUP groups, calves grew poorly and appeared unthrifty. Calves in both groups tended to lose vigor between 4 and 6 weeks of age, appearing weak when excercised. Five calves in the DEF group and 2 calves in the SUP group were treated for pneumonia during the study. One calf afflicted with pneumonia beginning at 4 weeks of age, was unsturdy when standing and preferred a recumbent position during the last week of the study. Several days after the study ended this calf bloated and died.

In the CON group 3 calves were treated for pneumonia during the study. The major problem with CON calves was scouring which occurred primarily between 1 and 3 weeks (Figure 8).

Selenium Content of Colostrum Samples. The Se content of six colostrum samples ranged from .071 to .097 μg/ml, the mean being .086 μg/ml.

Discussion

Plasma tocopherol values (Figure 2) are similar to those in earlier studies. Rousseau et al. (1957) found that plasma tocopherol in calves during a 12 week period increased at diminishing rates with a constant tocopherol intake. Mean plasma tocopherol over the 12 weeks in calves fed 0 and 9 mg

tocopherol/lb live weight/day were 62 and 478 µg/100 ml, respectively. A similar pattern was observed in the SUP group of the present study, although a higher level of tocopherol was fed over a shorter period of time. Plasma tocopherol of the SUP calves rapidly increased between 1 and 3 weeks of age after which there was a further increase but at a diminished rate. Mean plasma tocopherol values of the DEF and SUP groups over a 6 week period were 64 and 339 µg/100 ml, respectively.

The dams of the calves in the present study received no vitamin E supplement. Mean plasma tocopherol of DEF and SUP calves at birth was 42.9 µg/100 ml. Parrish (1949) reported that calves whose dams received no vitamin E supplement were born with a mean serum tocopherol level of 42 µg/100 ml. Mean serum tocopherol of the same calves fed colostrum from their dams increased to about 150-175 µg/100 ml between 24-48 hr of age. In the present study, CON calves had a mean plasma tocopherol of 116.5 µg/100 ml at 24-48 hr. Calves receiving only whole milk were reported to have blood tocopherol levels of 198 (Parrish, 1949) and 120 µg/100 ml (Thomas and Okamoto, 1955). Thomas and Okamoto (1955) found that the addition of grain or alfalfa to the whole milk diet doubled the level of 120 µg/100 ml. The consumption of starter by CON calves and their mean plasma tocopherol gradually increased during the course of the present study.

Only mild symptoms of muscular dystrophy were observed in DEF calves including some signs of muscular weakness and a greater incidence of pneumonia. Although DEF calves had consistently lower mean plasma tocopherol values than CON calves, they were not significantly different. Mean plasma tocopherol values of the DEF group do not indicate a depletion of plasma tocopherol in DEF calves. Variation in plasma tocopherol of the DEF calves could be attributed to individual differences in body storage of vitamin E as suggested by Blaxter (1953). Plasma tocopherol is not a true reflection of the vitamin E status of the calf (Rousseau et al., 1957). Studies revealed no significant

differences between blood tocopherol values of dystrophic and normal animals (Blaxter, 1957; Maplesden et al., 1960; Schubert et al., 1961). Therefore, blood tocopherol is of limited clinical value for the diagnosis of muscular dystrophy. The correlation of elevated SGOT levels with low blood tocopherol in calves is indicative of muscular dystrophy (Hirdiroglou et al., 1973). Such a correlation was found in the present study, the mean SGOT level of the DEF group being significantly higher than those of the CON and SUP groups (Table 3). SGOT is a sensitive indicator of early muscular damage as it becomes elevated in the serum before clinical symptoms of muscular dystrophy are evident (Swingle et al., 1959). Average concentration of SGOT was reported to be 43 IU/L in calves at birth (Willett et al., 1979). Bovine levels of SGOT have been reported to range from 50-150 IU/L (Durst et al., 1978). Although some of the SUP calves tended to lack vigor and appear weak when exercised by 6 weeks of age, their mean SGOT level when compared to that of the CON group was not indicative of muscular dystrophy. The confinement of the calves in elevated metal stalls may partially explain the lack of vigor exhibited by some DEF and SUP calves when exercised in the present study.

The wide variation found in serum Ig levels of calves agrees with earlier findings (Boyd, 1972; Logan et al., 1972; McEwan et al., 1970; Selman et al., 1970, 1971c; Smith et al., 1967). Serum Ig levels at 24 hr of age are typically indicative of the degree of passive immunity acquired by calves fed colostrum (Bush et al., 1971; Logan et al., 1972). Variation at that time has been attributed to such factors as Ig concentration in colostrum, amount of colostrum fed, age at first feeding, and differences in absorptive ability (Bush and Staley, 1980). Since calves in experiment 1 were bottle-fed a certain amount of colostrum per unit of body weight within 4 hr after birth, factors most likely causing variation in their post-colostral serum Ig levels could be Ig concentration in colostrum and differences in absorptive ability. Calves in experiment 2 suckled their dams early in life in addition to being

bottle-fed. Variation in their post-colostral serum Ig levels could be due to all the factors mentioned. Bush et al. (1971) found that 68% of the variation in serum Ig levels in calves could be attributed to differences in the mass of Ig consumed per unit of body weight. Stott et al. (1979a) reported that a sufficient volume of colostrum is required to completely contact and satiate potential absorptive cells in the intestine and that after 12 hr postpartum absorptive rates decreased at a progressive rate with increasing age. A significant negative relationship was found between time of first suckling and 48 hr serum Ig levels of calves which suckled during the first 8 hr of life (Selman, 1970). Smith et al. (1967), after failing to positively correlate serum Ig levels in calves with Ig concentration in colostrum, suggested that calves vary greatly in their ability to absorb Ig. Boyd (1972) reported that significant individual differences in post-colostral serum Ig levels dissappeared by 8-9 weeks of age. The consistently higher serum Ig levels in all Ig classes of CON calves at 24-48 hr (Figures 3-6) can be attributed to the method of feeding. Calves that suckle colostrum from their dams have been found to reach higher serum Ig levels than hand-fed calves (Selman et al., 1971a,b,c; Smith et al., 1967; Stott et al., 1979b). Reasons for the difference may include a mothering effect and stress due to separation (Selman et al., 1971c), and the transfer of some labile factor in the fresh colostrum to the suckling calf thus stimulating more rapid absorption of Ig (Stott et al., 1979b).

The pattern of serum Ig class levels during the course of the present study are typical of those described in earlier studies (Boyd, 1972; Logan et al., 1972; McEwan et al., 1970). The development of active immunity in calves depends on the initial level of Ig absorbed. Significant increases in serum Ig levels were observed within 14 days in calves with low post-colostral serum Ig levels (McEwan et al., 1970). Results of a study by Devery et al. (1979) indicated that the newborn calf has the capability of endogenously producing

about 1 g of IgG1/day during the first 3 weeks of life, the amount depending on the degree of antigenic stress to which the calf is exposed. Serum Ig levels progressively decreased in calves with high post-colostral serum Ig levels (Boyd, 1972; McEwan et al., 1970). Logan et al. (1972) reported that post-colostral serum Ig levels decreased at different rates, reaching a minimum between 2 and 4 weeks of age at which time IgA remained low while IgG and IgM rose slowly approaching adult levels at 12 weeks of age. In the present study, after 24-48 hr mean serum IgA levels of the DEF and CON groups significantly decreased to low levels at 4 weeks of age, after which there was an insignificant increase. Mean serum IgA of the SUP group was low during the entire study (Figure 5). Increases in the level of serum Ig of all treatment groups were observed by 6 weeks of age, although the increase in mean serum IgM of the CON group was extremely slight (Figure 6).

Mean LSI at 6 weeks of age (Figure 7) suggest a greater in vitro lymphocyte response of SUP calves and a depressed response of DEF calves compared to the response of CON calves. However, LSI varied widely among calves within treatment groups. The significant decrease in the mean LSI of the DEF group between 4 and 6 weeks suggests a depression of lymphocyte response within that group. Lymphocytes from vitamin E deficient dogs were significantly less responsive to mitogens, including phytohemagglutinin, than were lymphocytes from normal dogs. The in vitro suppression has been shown to be associated with a suppressive factor in vitamin E deficient serum (Langweiler, 1979; Sheffy and Schultz, 1979). Vitamin E deficient serum also significantly suppressed the in vitro response of lymphocytes from cows to mitogens suggesting that the bovine might be susceptible to similar immunosuppressive phenomena in a vitamin E deficient state (Langweiler, 1979). Wide variation in response of bovine lymphocytes from different individuals to phytomitogens in vitro have been reported (Renshaw et al., 1977) and attributed to undefined biological factors. The variation in response of lymphocytes from a single 10-14 month

old animal cultured at different times was substantially less than that occurring among cultures from different animals of the same age. The usefulness of linear and multiple regression equations to predict bovine fetal lymphocyte responses to phytohemagglutinin from fetal weight and length was minimal (Renshaw et al., 1977).

Few significant differences were found in serum and plasma constituents (Table 3). The higher mean plasma Se values of the DEF and SUP groups is probably due to additional supplementation of .1 ppm Se in their rations. Zintzen (1972) reported blood Se of normal calves to be 0.1-0.2 µg/ml. Blood glucose levels among calves within treatment groups were variable due to differences in times of blood sampling in relation to when the calves were fed. Huber et al. (1961) reported that about 15 min after carbohydrate ingestion blood glucose began to increase with a maximum increase of 130 mg/100 ml in 50 day old calves. In the postabsorptive state, blood glucose in preruminant calves is controlled within limits typical of other non-ruminants. Normal blood glucose levels in non-ruminants are about 100 mg/100 ml (Jørgensen, 1977). The significantly lower blood urea nitrogen values of the CON group compared to the DEF and SUP groups may be due to the development of a functioning rumen and to an increased output of saliva stimulated by the consumption of dry feed. Saliva contains 0.1-0.2% nitrogen of which 60-80% is urea nitrogen (Church, 1976). In ruminants, some of the urea produced in the liver during protein catabolism is recycled to the reticulorumen in the saliva and by diffusion from the blood to be reutilized in the protein synthesis of the microorganisms (Jørgensen, 1977). In developing ruminants, such as the CON calves, although less urea would be recycled than in adult ruminants, enough probably occurred to account for their significantly lower blood urea nitrogen values. The lower mean albumin levels of the DEF and SUP groups compared to the CON group may be due to poor utilization of the lard and protein in thier diet suggested by unaltered fat present in their feces and poor growth, respectively. Albumin is

responsible for transporting small chain fatty acids (10-12 carbons) absorbed from the intestines (Mayes, 1979b) and free fatty acids released from the adipose tissue in the blood (Mayes, 1979a). Inefficient digestion of the lard may have decreased the need for albumin as a fat transporting molecule in the blood and poor protein nutrition may have decreased the synthesis of albumin in DEF and SUP calves. Mean levels of creatinine, phosphorus, calcium, alkaline phosphatase, blood urea nitrogen, albumin, globulin, and total protein reported in the present study are comparable to literature values for the bovine (Durst et al., 1978).

Mean fecal scores did not differ ($P>.05$) between the DEF and SUP groups except at 5 weeks (Figure 8). However, a significant decrease in the mean fecal score of the SUP calves occurred between 3 and 4 weeks while no significant change occurred in the DEF group between 2 and 6 weeks. Michel et al. (1972) found a greater incidence of scours in calves fed rations containing protein concentrate with no vitamin E supplement and suggested a lack of the protective action of vitamin E against the action of lipid peroxides in the intestinal tract as an explanation for it.

The high level of vitamin E fed to the SUP calves is a factor that may have affected the results of the present study. Jackson et al. (1978) showed a non-linear antibody (AB) response reflected by chicks from hens immunized with Brucella abortus and fed diets containing graded levels of vitamin E. Chicks from hens fed 150 and 450 ppm vitamin E had significantly higher levels of passively transferred AB than chicks from hens fed 0, 90, 300, and 900 ppm vitamin E. Likewise, the survival rate of immunized and non-immunized mice against Diplococcus pneumoniae type 1 infection was increased 15-80% by dietary supplementation of 180 and 360 mg vitamin E/kg feed while doses in between were less effective (Heinzerling et al., 1974b). Guinea pigs administered a live-virus vaccine receiving 250 IU vitamin E/dose had a significantly higher AB response than vaccinated controls while vaccinated guinea pigs receiving 500 IU

vitamin E/dose did not (Barber, 1977). The immune responses in calves may be more effectively stimulated by lower levels of vitamin E supplementation.

Another factor that may have influenced the results of the present study is the diet (Table 1) fed to the calves in experiment 1. It was necessary to formulate a vitamin E-free diet for the present study. Therefore, the natural fat in colostrum and milk was replaced by a vitamin E-stripped fat. The addition of unnatural fats to skim milk for calves has been found to cause poor growth, muscular dystrophy, and high mortality (Adams et al., 1959a; Blaxter et al., 1953a). Many of the harmful effects were found to be due to unstable unsaturated fatty acids which yield toxic products upon oxidation and could be prevented by adequate supplementation of vitamin E (Blaxter et al., 1953b). In the present study harmful effects were observed in both the DEF and SUP calves.

During the first month of life calves are particularly sensitive to dietary changes. Therefore, the type of fat added to skim milk as well as the method by which it is incorporated are important considerations (Radostitis and Bell, 1970; Roy et al., 1961). In the present study the lard was emulsified using polyoxy-ethylene glycol and lecithin. Harmful effects such as those observed in the present study have been reported when calves were fed unhomogenized (Bate et al., 1946; Blaxter et al., 1953c; Roy et al., 1961), emulsified-fat (Maplesden et al., 1960), and homogenized diets (Lister and Emmons, unpublished work cited by Emmons et al., 1980; Gullickson et al., 1942; Jenkins and Emmons, 1979) containing various levels and types of fats. Maplesden et al. (1960) reported loss of hair on the muzzle, base of ears, anal region and legs of calves fed an emulsified-fat diet consisting of reconstituted skim milk containing 2.85% lard and .25% lecithin. Loss of hair in calves has also been associated with unhomogenized diets consisting of skim milk containing 3.3% soybean oil (Bate et al., 1946), reconstituted spray-dried skim milk containing 2% margarine (39% groundnut oil, 24% palm oil, and 37% coconut oil; Roy et al., 1961),

and 750 g of dried skim milk powder containing 28.4, 56.7 or 113.4 g of cod liver oil or 20 ml arachis oil (Blaxter et al., 1953c). Roy et al. (1961) suggested that hair loss was caused by direct contact with the unhomogenized diet and feces passed by calves receiving the diet, which would explain the consistent hair loss on the muzzle and anal region of calves in experiment 1 of the present study. Blaxter et al. (1953c) reported that the addition of 50 mg d,l-alpha-tocopherol acetate to the arachis oil diet did not prevent hair loss. Calves fed unhomogenized diets were reported to have poor growth (Bate et al., 1946; Roy et al., 1961), fatty feces (Roy et al., 1961), and a higher percent of fatty acids and neutral fat in their feces (Bate et al., 1946) in addition to hair loss. The addition of 1 g d,l-alpha-tocopherol acetate to the diet in the present study did not prevent hair loss, fatty feces, or poor growth.

The following homogenized diets caused harmful effects in calves similar to those previously mentioned: skim milk containing 3.5% cottonseed, soybean oil, or maize oil homogenized using a single-action type homogenizer (Gullickson et al., 1942), a high fat milk replacer containing 35-40% tallow/coconut oil (dry matter basis) homogenized using either a two-stage homogenizer or a polytron homogenizer (Lister and Emmons, unpublished work cited by Emmons et al., 1980), and a high fat milk replacer containing 34% tallow, 4% coconut oil, and 1.8% soybean lecithin (dry matter basis) homogenized using a two-stage homogenizer (Jenkins and Emmons, 1979). Lister and Emmons (unpublished work cited by Emmons et al., 1980) reported that calves fed their replacer developed severe alopecia. In addition to hair loss, Gullickson et al. (1942) reported dermatitis and a brown oily-like crust on denuded areas. In the present study a brown crust was observed on the muzzle of some calves in experiment 1 that could have been caused by contact with the emulsified-fat diet. Excretion of oil through the skin, suggested as a cause of dermatitis (Gullickson et al., 1942), could also be responsible for additional loss of hair on the face, ears,

and limbs observed in some calves in experiment 1 of the present study.

The young calf requires the formation of a firm curd in the abomasum for normal digestion, growth, and health (Lister and Emmons, 1976; Roy and Ternouth, 1972). A firm curd slowly releases nutrients into the small intestine which allows sufficient time for abomasal proteolysis (Roy and Ternouth, 1972) and prevents overloading of the relatively low levels of lipases in young calves (Jenkins and Emmons, 1979). A soft curd passes quickly out of the abomasum, resulting in poor nutrient digestion associated with reduced gastric proteolysis and gastric acid production, an increase in undigested proteins reaching the duodenum, and a higher pH of the abomasal fluid. Such conditions promote the growth of coliform organisms in the intestine and diarrhea (Roy and Ternouth, 1972; Tagari and Roy, 1969). Pancreatic enzyme secretion is also reduced (Roy and Ternouth, 1972). Diets containing incorrectly dispersed fat for calves may form a soft curd in the abomasum (Emmons et al., 1980; Jenkins and Emmons, 1979) acting like high-heat-treated milk diets (Emmons and Lister, 1976; Targari and Roy, 1969). The poor performance and harmful effects observed in calves fed the emulsified-fat diet in the present study suggest that the fat in the diet was incorrectly dispersed resulting in the formation of a soft curd in the abomasum of the calves.

Roy et al. (1961) found that emulsification of their diet using .2% lecithin largely prevented hair loss from the body, increased the dry matter content of the feces, and slightly increased growth rate. Severe alopecia was prevented in the present study when lecithin was added to the diet fed in experiment 1. In addition, Roy et al. (1961) found that homogenization of their diet using a single-action type of homogenizer significantly increased growth rate and dry matter content of feces, and increased plasma lipid content up to 12 days of age compared to the same diet emulsified using .2% lecithin. The inclusion of lecithin in the homogenized diet had no significant effect on growth rate or plasma lipid content. After 12 days of age, homogenization of

the diet appeared to be of less importance since the plasma lipid content of calves given the diet emulsified with lecithin was similar to that of the calves given an homogenized diet by 21 days of age. From these results, Roy et al. (1961) recommended the use of an homogenized diet for calves up to at least 2 weeks of age.

Contrary to the results of Roy et al. (1961), Hopkins et al. (1959) did not find an additional beneficial effect of mechanical homogenization over emulsification of fat with lecithin. The inclusion of .2% crude soybean lecithin in reconstituted spray-dried skim milk for calves improved the digestibility of coconut fat added at 1.8% from 65.4 to 95.6%. Homogenization of the coconut fat plus lecithin diet using a two-stage homogenizer resulted in 94.9% digestibility of coconut fat (Hopkins et al., 1959). The results of these studies were obtained using different types of fat and lecithin. A particular emulsifying agent may be more satisfactory with one type of fat than another (Roy et al., 1961). Hopkins et al. (1959) also used older calves. The formation of a firm curd in the abomasum did not appear to be a requirement for older calves (Lister and Emmons, 1976).

Calves fed a high fat milk replacer (Jenkins and Emmons, 1979) homogenized using a low pressure dispersion method resulted in good calf gains, feed efficiency, and digestion. These results indicated that the young calf has trouble with high levels of fat in milk replacers when the usual homogenization procedure is followed. In vitro work revealed differences in the curd-forming properties of replacers due to the fat dispersion method used. Low pressure dispersion resulted in high curd firmness values with rennet while the usual homogenization procedure resulted in low curd firmness values (Emmons et al., 1980; Jenkins and Emmons, 1979).

Calves require mechanical dispersion of fat in skim milk replacers for the first few weeks after birth (Radostitis and Bell, 1970; Roy et al., 1961). However, fat may be dispersed too efficiently (Emmons et al., 1980) or, as in the

diet of the present study, not dispersed enough (Roy et al., 1961). The size of the fat globule incorporated into the milk diets for calves is important. The fat globule in cows' milk varies from 0.1-10 μ in diameter (Radostitis and Bell, 1970; Roy et al., 1961). Roy et al. (1961) determined 3-4 μ to be the mean size of a fat globule produced by mechanical homogenization at a pressure of 200 atmospheres and 10-20 μ to be the mean size with some as large as 50 u, of a fat globule produced by emulsification with lecithin. Emmons et al. (1980) found that low pressure dispersion produced many fat globules in the 10-20 μ range but more small globules (1 μ) than in whole milk while two-stage and polytron homogenizers produced typically small globules (less than 1 μ). Low pressure dispersion of fat gave a product with curd-forming characteristics similar to that of whole milk in vitro (Emmons et al., 1980). The dispersion of fat similar to that of whole milk may be optimum for young calves (Emmons et al., 1980).

Lecithin, added to the diet in experiment 1, apparently improved the diet through its action as an emulsifying agent. However, some other action or constituent of the lecithin may have been responsible (Hopkins et al., 1959). Due to the level of fat used in experiment 1, homogenization of the diet using normal homogenization procedures may not necessarily have improved the utiliza- of the diet by the calves.

The adverse effects caused by the emulsified-fat diet fed to the calves in experiment 1 and the high level of vitamin E supplemented may have partially masked an enhancing effect of vitamin E on the humoral and cell-mediated immune responses of the SUP calves. A trend toward enhanced mitogen-induced proliferation of lymphocytes from SUP calves appears to have been due to vitamin E supplementation. Research is needed to evaluate further the effect of vitamin E on the humoral and cell-mediated immune responses in calves. The use of calves from vitamin E deficient dams and greater numbers of calves fed properly homogenized diets with and without vitamin E supplementation for at least 8 weeks

may provide more information about the effect of vitamin E on the immune responses in calves. Information collected in the present study on calves maintained on normal diets may serve useful as a baseline reference for future research. Results of additional research in this area could provide a way to increase the protection provided by the immune system and thus greatly reduce the incidence of disease among calves.

TABLE 1. CONTENT OF BASAL CALF DIET - EXPERIMENT 1

Ingredient[a]	(%)
Skimmed milk[b]	96.2
Vitamin E stripped lard[c]	3.3
Polyoxy-ethylene glycol[d]	.2
Lecithin[e]	.2
Vitamins A and D[f]	.05
Trace mineral premix[g]	.05

[a]As fed basis.

[b]Skimmed colostrum was substituted on days one and two of experiment. Skimmed milk was obtained from the Kansas State University Dairy Plant.

[c]ICN Pharmaceuticals, Inc., Cleveland, OH. 44128.

[d]Milk Specialties Inc., Dundee, IL. 60118.

[e]Purified grade granular lecithin. Fisher Scientific Company, Fair Lawn, NJ. 07410.

[f]Hoffman-La Roche Inc., Nutley, NJ. 07110. Supplied per kg diet: 800 IU of vitamin A and 120 IU of vitamin D.

[g]National Vitamin Products Company, Minneapolis, MN. Supplied per kg diet: 13 mg Fe, .13 mg Co, 1.3 mg Cu, .13 mg I, 5.2 mg Mn, 5.2 mg Zn, 9.8 mg Mg, and .013 mg Se. NRC (1978) recommended levels.

TABLE 2. COMPOSITION OF CALF STARTER - EXPERIMENT 2

Ingredient	(%)
Corn, cracked	32.8
Oats, whole	20.0
Alfalfa hay, ground	20.0
Soybean meal	13.6
Wheat bran	8.0
Molasses, dry	3.2
Animal fat	1.6
Calcium carbonate	.2
Salt, trace mineral	.2
Salt, plain	.2
Vitamins A and D[a]	.2

[a]Supplied per kg starter: 2200 IU of both vitamins A and D.

TABLE 3. SERUM AND PLASMA CONSTITUENTS FOR CALVES AT 6 WEEKS OF AGE[a,b]

Constituent	Groups		
	DEF	SUP	CON
Selenium, μg/ml	.079± .008	.072± .008	.064± .004
Glutamic oxalacetic transaminase, IU/L	320[c] ±59.3	61[d] ±59.3	43[d] ±32.5
Alkaline phosphatase, IU/L	146 ±31.5	142 ±31.5	166 ±17.3
Creatinine, mg/100 ml	1.1 ± .097	1.1 ± .097	1.2 ± .053
Glucose, mg/100 ml	73 ±12.5	109 ±12.5	90 ± 6.9
Phosphorus, mg/100 ml	6.5 ± .32	6.3 ± .32	6.8 ± .18
Calcium, mg/100 ml	9.1 ± .26	8.8 ± .26	9.4 ± .14
Urea nitrogen, mg/100 ml	15.8[c] ± 1.1	14.3[c] ± 1.1	11.4[d] ± 0.6
Carbon dioxide, mmHg	31 ± 1.3	32 ± 1.3	31 ± .72
Chloride, mEq/L	102 ± 1.0	101 ± 1.0	101 ± .56
Potassium, mEq/L	4.9 ± .19	5.0 ± .19	5.1 ± .10
Sodium, mEq/L	142 ± 1.5	142 ± 1.5	140 ± .83
Albumin, g/100 ml	2.7[cd]± .09	2.6[d] ± .09	2.9[c] ± .05
Alpha-globulin, g/100 ml	1.2 ± .07	1.2 ± .07	1.1 ± .04
Beta-globulin, g/100 ml	.72 ± .06	.75 ± .06	.74 ± .03
Gamma-globulin, g/100 ml	1.1 ± .24	1.2 ± .24	1.2 ± .13
Total protein, g/100 ml	5.7 ± .29	5.7 ± .29	5.9 ± .16

[a]Selenium in plasma, all other constituents in serum.

[b]Constituents expressed as mean ± standard error.

[c,d]Means within rows with different superscripts differ ($P < .05$).

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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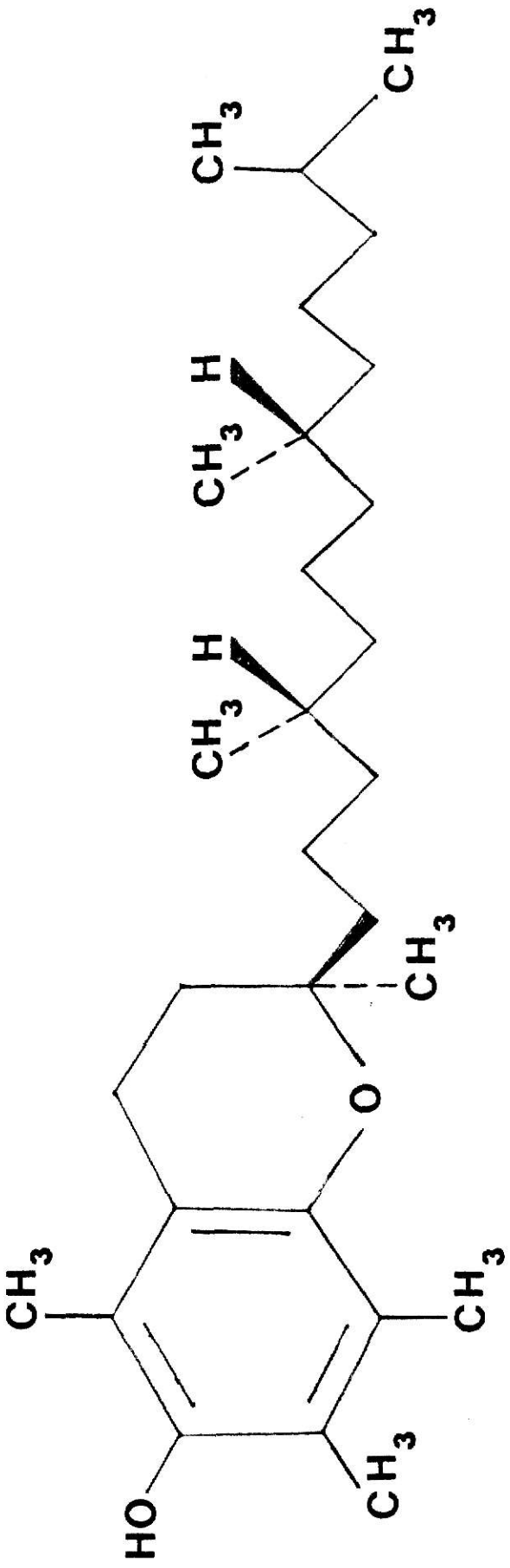

Figure 1. d-alpha-tocopherol.

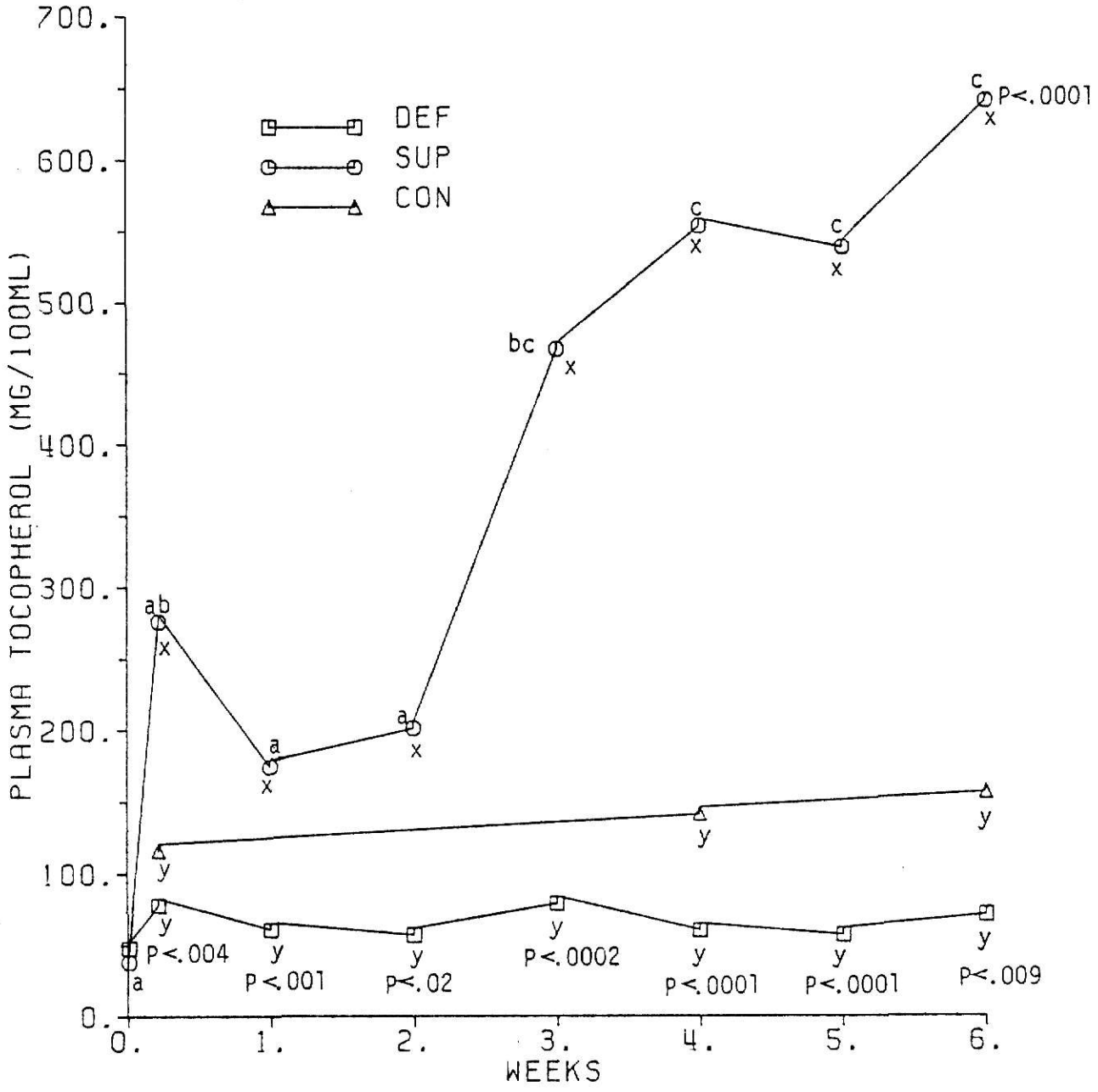

Figure 2. Mean plasma tocopherol concentrations. Points within a ration with unlike letters (a,b,c) differ at the significance indicated to the right of the given line. Points within a sampling time with unlike letters (x,y) differ at the significance indicated below the given points.

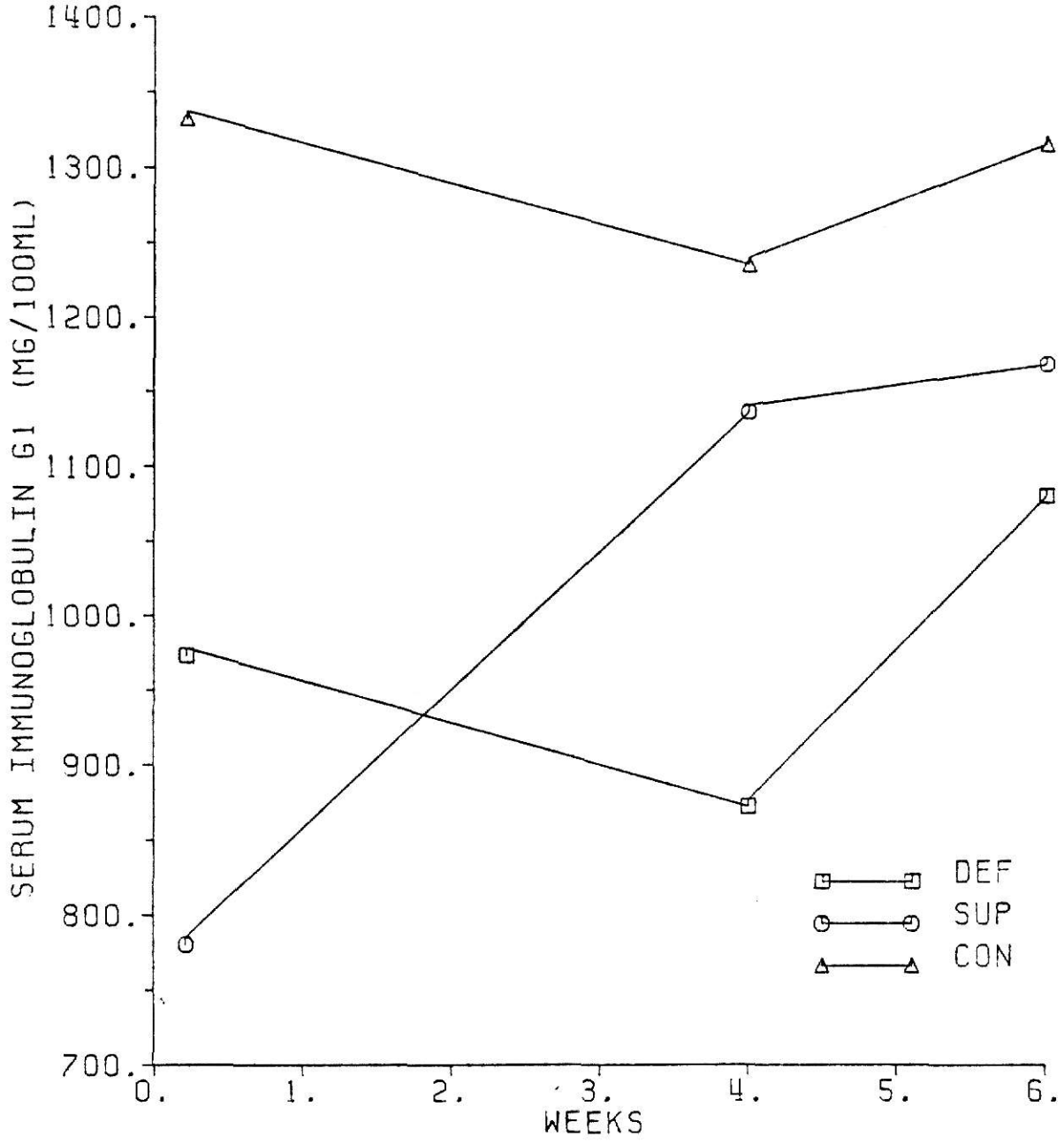

Figure 3. Mean serum IgG1 concentrations. No differences ($P > .05$) were found among means.

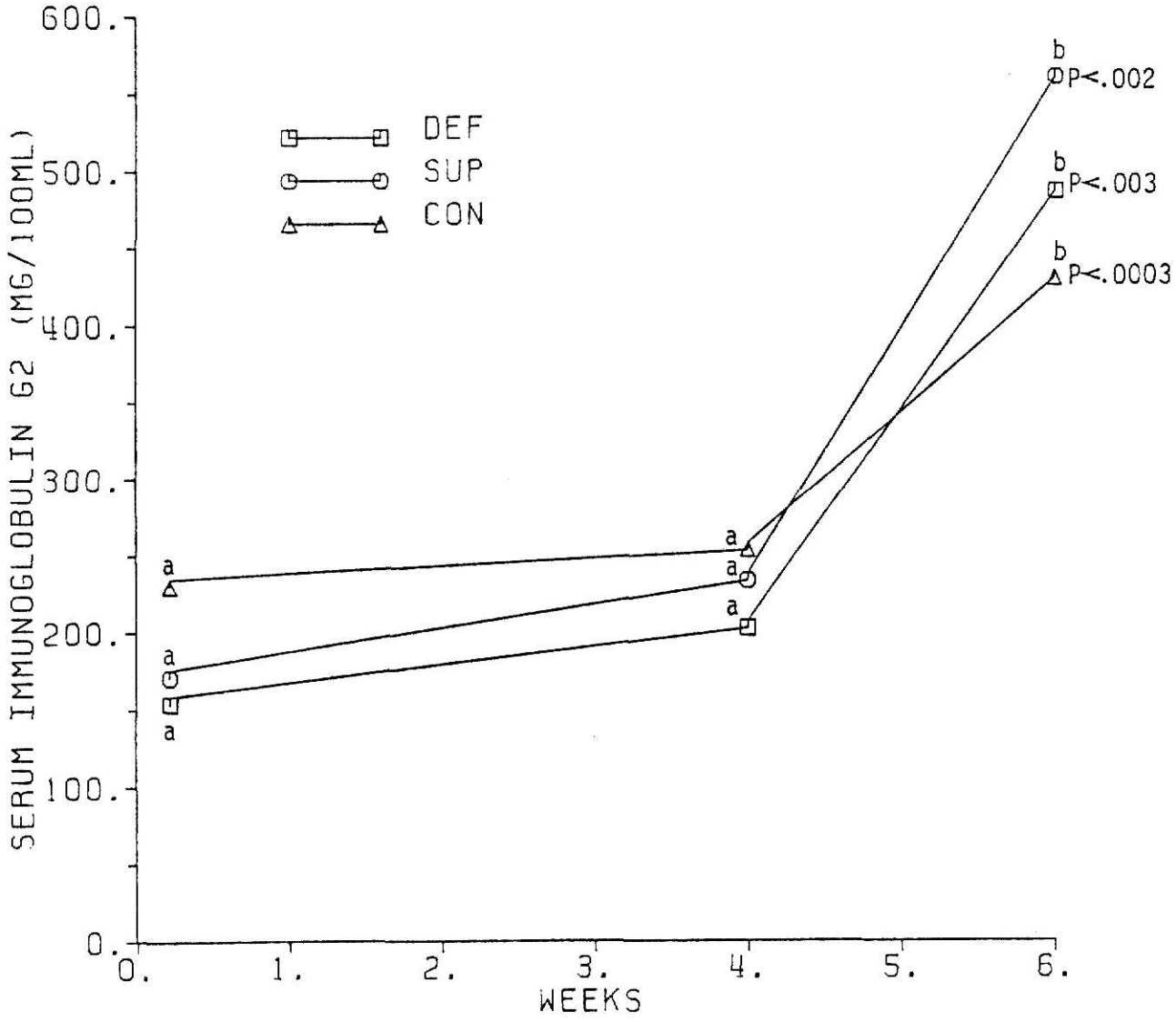

Figure 4. Mean serum IgG2 concentrations. Points within a ration with unlike letters differ at the significance indicated.

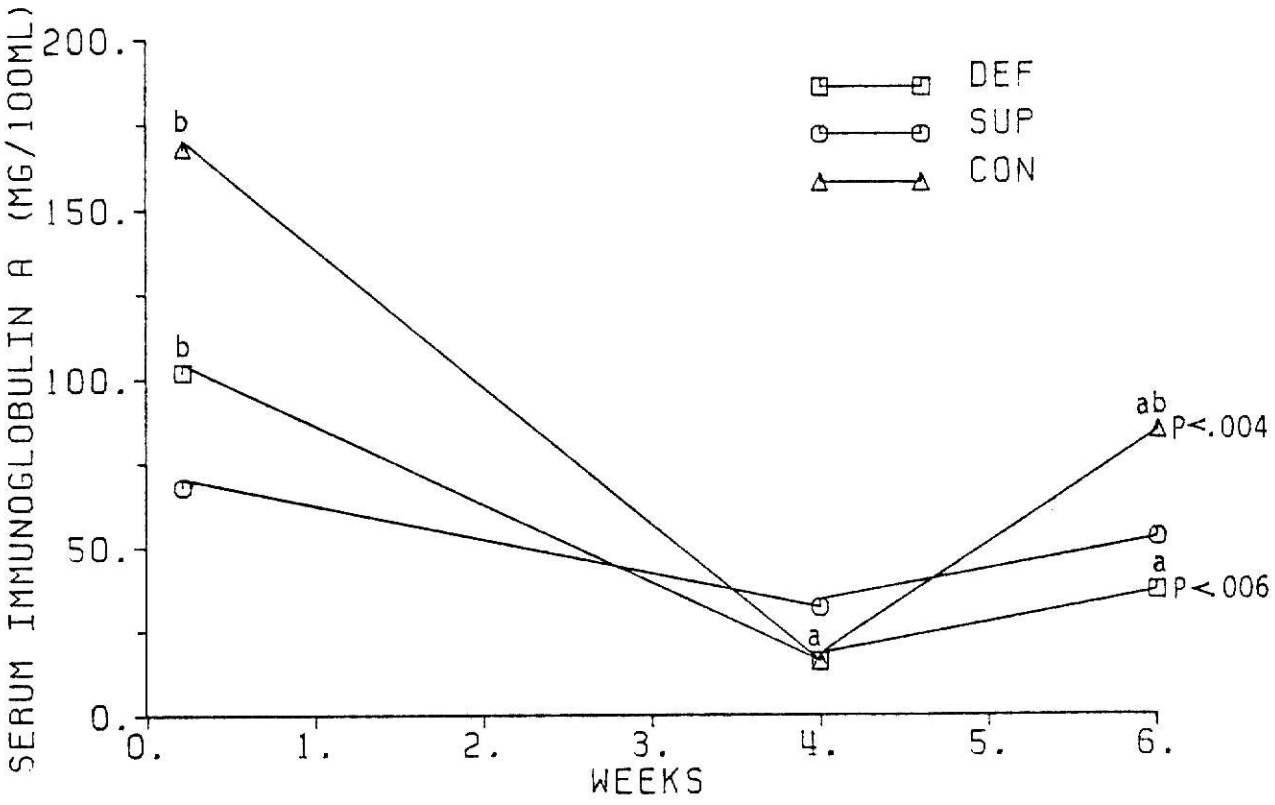

Figure 5. Mean serum IgA concentrations. Points within a ration with unlike letters differ at the significance indicated.

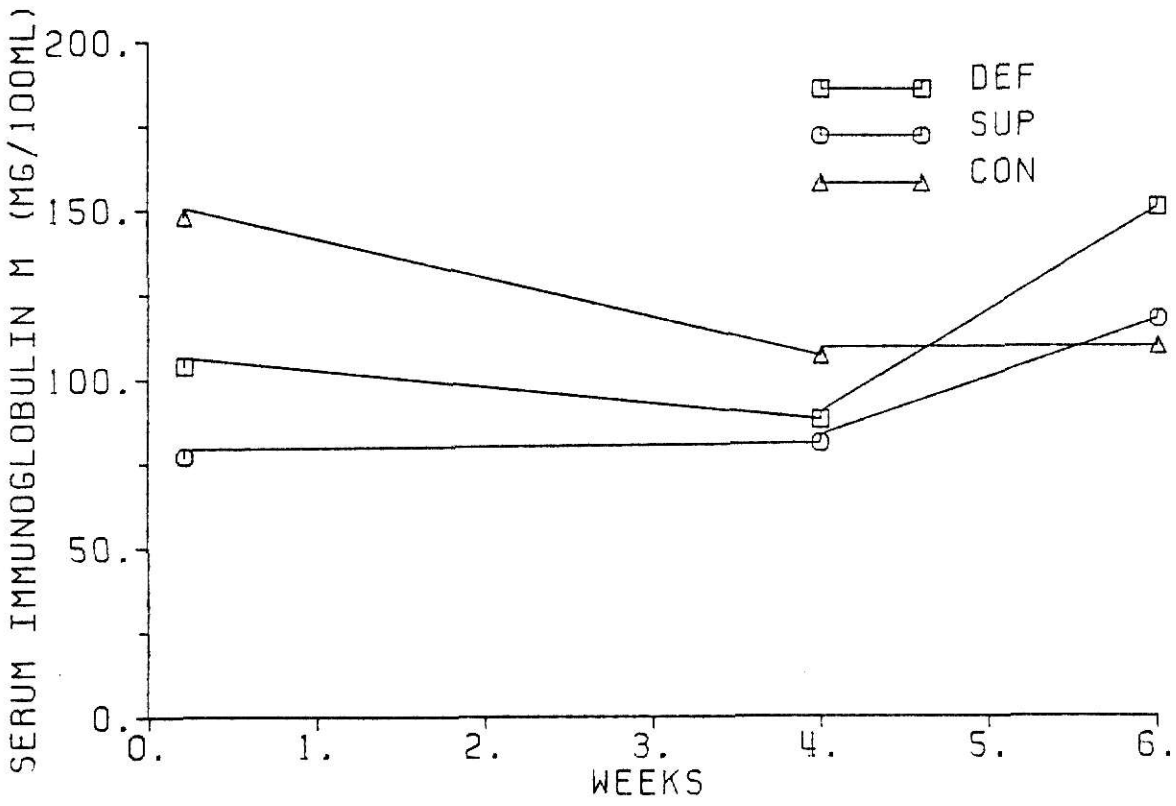

Figure 6. Mean serum IgM concentrations. No differences (P>.05) were found among means.

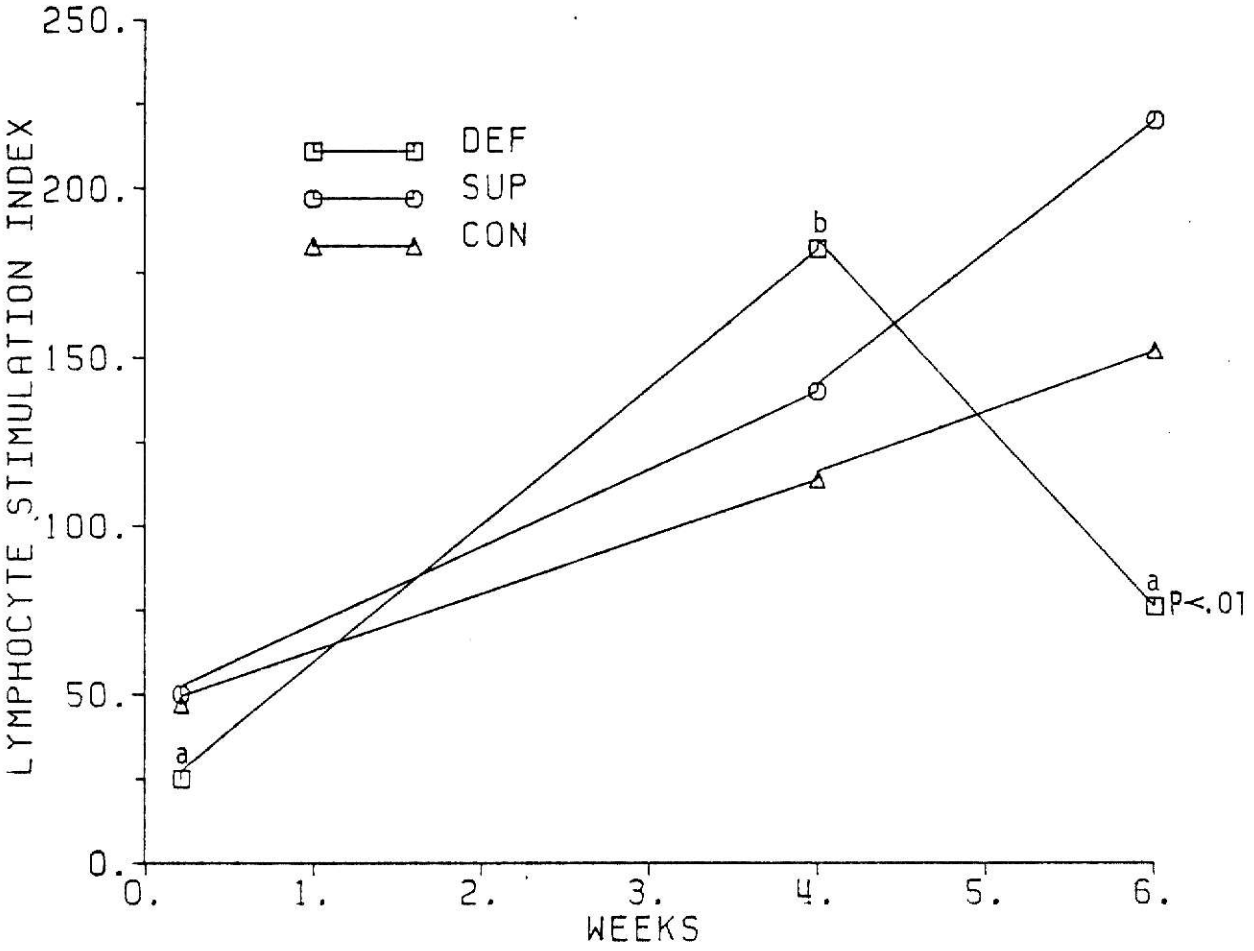

Figure 7. Mean lymphocyte stimulation indexes. Points within a ration with unlike letters differ at the significance indicated.

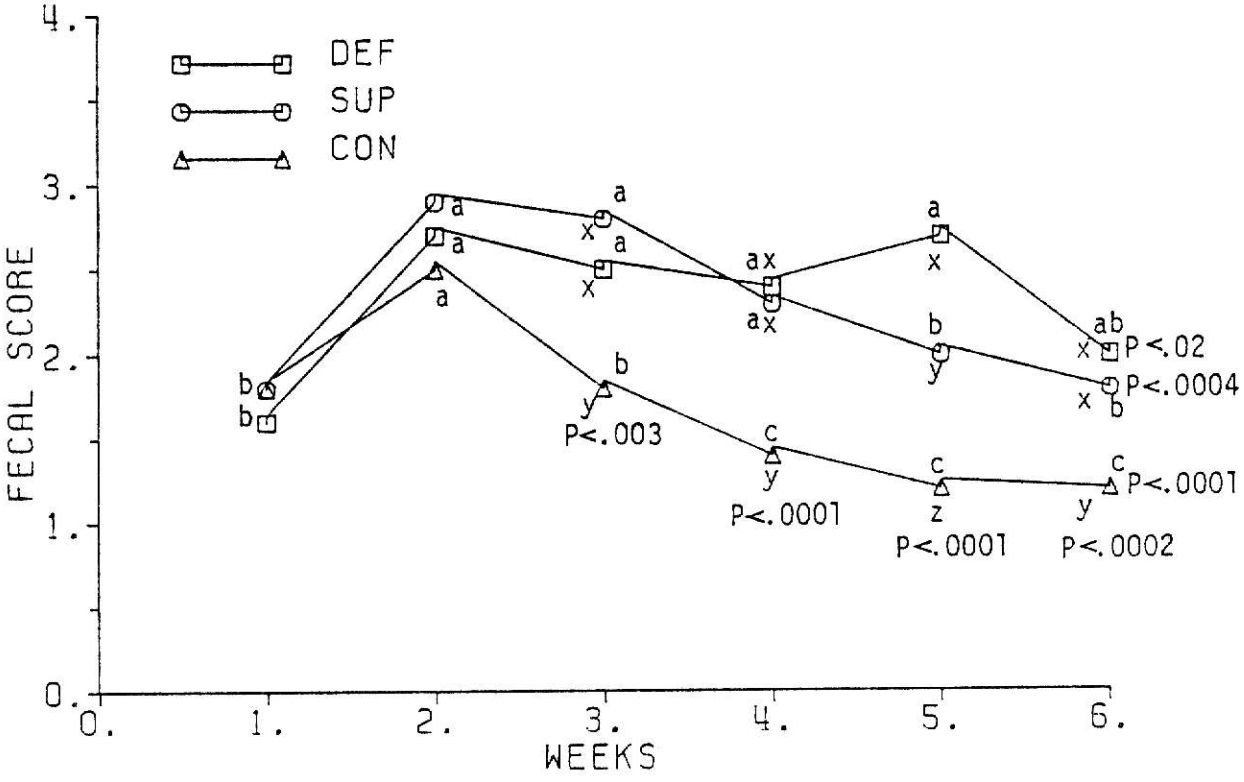

Figure 8. Mean fecal scores. Points within a ration with unlike letters (a,b,c) differ at the significance indicated to the right of the given line. Points within a week with unlike letters (x,y,z) differ at the significance indicated below the given points.

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VITAMIN E AND THE IMMUNE SYSTEM IN CALVES

by

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Two experiments were conducted to determine the effect of vitamin E on the humoral and cell-mediated immune responses in calves and to study the plasma vitamin E and immunological status of calves under normal herd management. In experiment 1, 12 calves were fed skimmed colostrum the first 2 days of life and skimmed milk thereafter with vitamin E-stripped fat, polyoxyethylene glycol, lecithin, and minerals and vitamins A and D added at 3.3%, .2%, .2%, and .1% of liquid diet, respectively. Six calves per group received 0 (DEF group) or 1 g (SUP group) d,l-alpha-tocopherol acetate daily. In experiment 2, 20 calves (CON group) were fed colostrum for 3 days and whole milk thereafter, and starter containing 16% protein and 20% alfalfa hay ad libitum. At 6 weeks of age, mean plasma vitamin E levels and standard errors (SE) were 71±9.3, 639±177.2, and 155±15.8 μg/100 ml and mean serum glutamic oxalacetic transaminase (SGOT) levels and SE were 320±59.3, 61±59.3, and 43±32.5 IU/L for groups DEF, SUP, and CON, respectively. Mean serum immunoglobulin (Ig) levels (mg/100 ml) and SE were: IgG1, 1079±140.6, 1168±86.9, and 1315±113.9; IgG2, 488±101.6, 562±102.3, and 432±49.3; IgA, 37±4.5, 53±18.9, and 85±36.6; IgM, 151±26.5, 118±20.0, and 110±11.5 for groups DEF, SUP, and CON, respectively. Mean lymphocyte stimulation indexes (LSI) and SE for groups DEF, SUP, and CON were 76±15.8, 220±59.2, and 152±40.1, respectively. Plasma vitamin E was higher ($P<.009$) in group SUP than groups DEF or CON and SGOT was higher ($P<.001$) in group DEF than groups SUP or CON. Serum Ig levels and LSI among calves within groups varied widely. No differences ($P>.05$) in serum Ig levels or LSI were found among groups, although a trend toward enhanced mitogen-induced proliferation of lymphocytes from SUP calves appeared to be due to vitamin E supplementation. The emulsified-fat diet fed in experiment 1 resulted in some hair loss, soft fatty feces, and poor performance. Due to the adverse effects caused by the diet, an enhancing effect of vitamin E on the humoral and cell-mediated immune responses of the SUP calves may have been partially masked.