

GROWTH AND HEALTH OF YOUNG CALVES WITH
VARIOUS ROUTES OF LASALOCID ADMINISTRATION or
ASCORBIC ACID OR ASCORBIC ACID AND VITAMIN E SUPPLEMENTATION

BY

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INTRODUCTION

The primary goal in raising dairy bull calves is to attain market weight as efficiently and economically as possible. Two major steps to reaching that goal are 1) to increase feed efficiency and therefor profitability and 2) to maintain healthy animals, thereby decreasing mortality losses. Several potential methods that may reduce feed to gain costs are to increase the feed efficiency of the calf or to decrease days to weaning. Feeding lasalocid has been shown to increase weight gain in dairy calves (Anderson et al., 1988). Lasalocid was also credited with enhancing microbial development with early weaning. The lasalocid was delivered in milk, prestarter and starter in that study. It has not been determined if administration of lasalocid in all three feeds was the most effective route for delivery of that treatment. The purpose of the first study presented here is to determine the most effective route for the administration of lasalocid to young bull calves.

The use of antibiotics in feeds to maintain health is becoming increasingly unpopular with consumers. The use of vitamins' natural abilities to promote animal health is a potential area for further investigation. Reddy et al. (1987) have demonstrated the value of vitamin E in enhancing the immune response of dairy calves. The use of vitamin C to enhance immune responses has been shown for other species and

suggested for cattle. A cooperative relationship has been established for vitamins C and E (Bendich, 1987). The value of vitamin C alone and vitamins C and E together for changing various immune functions of the young calf has yet to be established and is the second purpose of this study.

REVIEW OF THE LITERATURE

Lasalocid Effects on Rumen Development and Growth of the Young Calf

Rumen and Metabolic Development of the Young Calf

The young calf begins life essentially as a monogastric animal. As the calf ages and grows a digestive and metabolic transition occurs, resulting in the ruminant animal (Huber, 1969). The rumen of the newborn calf is relatively small compared to the other stomach compartments. When the calf is bottle- or nipple pail- fed, the esophageal groove is effectively closed and diverts the liquid past the rumen into the abomassum. However, when fed via an open bucket, milk frequently enters the rumen (Tamate, 1962, Otterby and Linn, 1981).

Rumen development is influenced by diet (Tamate, 1962). Extensive papillary development was seen with hay and grain diets compared to all-milk diets. Papillary development and absorptive capacity have been related to increased metabolic activity of the rumen epithelial tissue (Huber, 1969, Sutton, 1963a,b). Young et al. (1965) have shown that young milk-fed calves have the metabolic capacity to utilize rumen fermentation end products and that these products (volatile fatty acids) can be absorbed from the small intestine. For example, increased intraruminal acetate, via infusion,

increased blood levels of acetate, β -hydroxybutyrate, lactate and malate. Infusion of propionate increased propionate, acetate and lactate concentrations and decreased concentrations of formate and glycolate, while the infusion of butyrate caused increased concentrations of butyrate, acetate, β -hydroxybutyrate, lactate and malate.

Blood glucose decreases with age through 8 weeks of age, at which time it stabilizes (Otterby and Linn, 1981). Johnson et al. (1982) studied the changes in plasma glucose and insulin concentrations associated with propionate in preruminant calves fed an all-milk replacer diet. Increased propionate or glucose caused a strong insulin response. The increases seen in propionate-fed calves was independent of the early post-feeding plasma glucose increase. When infused with propionate, the calves' plasma insulin concentration increased and plasma glucose concentration declined. The calves that had been supplemented with glucose or propionate were less sensitive to the propionate infusion than were the control animals. The development of rumen flora and fauna of young calves with respect to diet was studied by Lengemann and Allen (1959) and Anderson et al. (1987a,b). When given the opportunity, some young calves would consume solid feed earlier than two weeks of age, but three weeks were required to produce a flora as diverse as an adult following dry feed consumption. Calves reared solely on milk had very high counts of aerobic bacteria in the rumen. Lengemann and Allen

(1959) found established protozoa populations in the rumen by one week of age and near adult levels by five weeks of age.

The benefits of early weaning of calves accrue both to the calf and to the producer (Agabawi et al., 1968; Morrill, 1984; Morrill et al., 1984, Quigley et al., 1985; Anderson et al., 1987 a,b, Jorgenson et al., 1970, Owen and Larson, 1982). Benefits for the producer include reduction in labor and feed costs. Conversely, some researchers have found no benefit or negative results for the calf due to early weaning. (DePeters et al., 1986). This discrepancy is due in part to the variety of dry feeds used as well as the age of weaning of the calf. Morrill (1984) advocated the use of a highly digestible prestarter that is introduced to the calf at day four of age. This feeding regime was used by Anderson (1988). Calves were easily weaned by four weeks of age and some as early as two weeks. Anderson et al., (1987a) found an increase of ruminal VFA concentrations associated with the increased dry feed consumption of this early weaning program. The low ruminal pH resulting from early weaning and increased dry feed consumption delayed protozoa establishment in the rumen (Anderson et al., 1987b) and may inhibit cellulolytic activity (Slyter, 1986).

Anderson et al.(1987) looked at the changes in rumen microbes and their end products with respect to early or conventional weaning, i.e. four weeks and six weeks of age respectively. Total lactate was higher for early weaned

calves. Early weaned calves also exhibited higher total VFA concentrations. At five weeks of age facultative counts were higher and lactobacillus counts tended to be higher in the early weaned calves. This group also had higher counts of amylolytic bacteria at six weeks and higher counts of lactate-utilizing bacteria at five weeks of age. The proportion of amylolytic bacteria increased with age, proteolytic bacteria increased at ten weeks of age and lactate utilizing bacteria decreased, beginning at week five. Ruminal $\text{NH}_3\text{-N}$ decreased with age. The early weaned calves had a higher number of methanogens between five and eight weeks of age. The authors concluded that the earlier dry feed was consumed by the calf, the earlier microbial development occurred, resulting in greater ruminal metabolic activity.

Young et al. (1965) described the rumen of the newborn as being nonfunctional for the newborn ruminant, however the VFAs produced in the rumen could be utilized as early as three weeks of age. In the young milk-fed calf, acetate, propionate and butyrate were metabolized by established pathways. Acetate and butyrate caused increased ketone production and propionate decreased its production. Propionate and butyrate were equal to each other but greater than acetate in contributions to glucose carbon. Young et al. (1965) infused acetate, butyrate and propionate into calf rumen, and each infusion resulted in decreased blood glucose

concentration and increased blood lactate concentration. The concentration and specific activity of malate increased with the infusion of acetate and butyrate. This suggests activity of a portion of the glyoxylate pathway.

Biochemistry of Lasalocid

Lasalocid (X-537, RO 2-2985) is a carboxylic polyether ionophore, produced by Streptomyces sp. It forms an electrically neutral zwitterionic complex with cations, promoting an electrically neutral exchange diffusion of cations (Pressman and Fahim, 1982). Lasalocid tends to dimerize, forming electrically neutral complexes with important ions such as Ca^{2+} and Mg^{2+} (Pressman and Fahim, 1982). Other divalent cations which lasalocid binds are Ba^{2+} , Cu^{2+} , Fe^{2+} , Ni^{2+} , and Zn^{2+} . Elasser (1984) cites areas of mineral metabolism that may result in altered mineral balance due to ionophores; 1) ion presence and bioavailability (Ammerman and Miller, 1972, Miller, 1975), 2) ion uptake and transport across biological membranes, 3) whole body distribution and storage, 4) interactions between the ions, and 5) homeostatic mechanisms regulating uptake and excretion. The results of ionophore-mediated changes in metabolism may result in the compromised function of tissues dependent upon appropriate release and acquisition of ions.

The exchange-diffusion transport system of carboxylic ionophores is not as disruptive to biological membranes as are

the neutral ionophores. Lasalocid has a wide range of affinities and transport capabilities, including inorganic polyvalent and alkali ions as well as primary amines such as catecholamines. The relative affinity for lasalocid is $K^+ > Rb^+ > Na^+ > Cs^+ > Li^+$. Lasalocid also increases the permeability of membranes to H^+ ions (Henderson et al., 1969; Green, 1975). Lasalocid affects transport of ions across membranes by forming soluble complexes with the hydrated form of the metal ion. It can also affect transmembrane movement of an ion that it does not chemically react with or bind to directly (Elasser, 1984).

Physiological Properties of Lasalocid

Carboxylic polyether ionophore antibiotics were originally used as anticoccidial feed additives for poultry. They are utilized in ruminant nutrition to increase ruminal propionic acid, depress methanogenesis, and depress rapid ruminal proteolysis and deamination of dietary proteins (Bergen and Bates, 1984). When these ionophores are fed with readily fermentable carbohydrates, they depress feed intake without affecting weight gains, creating improved feed conversion ratios (Zinn, 1987). When ionophores are fed with diets containing a greater concentration of roughages, the effect of ionophores is to maintain feed intake and to increase body weight gain, resulting in improved feed

conversion ratios.

Three areas of metabolism that contribute to the improved feed conversion reported by Bergen and Bates (1984) were: 1) increased efficiency of energy metabolism, 2) improved nitrogen metabolism, and 3) reduction of disorders in feedlot cattle. Dinius (1976) suggested the improved feed efficiency was due to selective alterations of rumen microflora, favoring propionate producing microorganisms, thus reducing the acetate/propionate ratio. Thornton (1976) has shown that ionophores decrease ruminal methane production as well. The mechanism for this selective process is believed to be the transport of ions into susceptible species leading to a disruption of the microbes normal physiology (Wolin, 1981).

Lasalocid effects on mineral status of the body have been investigated by several researchers. The duodenal mucosal mineral concentrations were reduced when lasalocid was fed in the diet or acutely infused (Linder and Munro, 1977). Mucosa from lasalocid-treated animals contained more copper than a control group while the copper concentrations in the livers did not differ. The iron content of mucosal tissues was higher in lasalocid-fed calves compared to controls. Van Ryssen and Barrowman (1987) observed no difference in liver copper concentration for lasalocid-fed sheep compared to controls when given copper in the diet. Beef cows fed lasalocid while grazing oat pastures did not have different Mg, K, or Zn concentrations from controls. An increase of Ca

concentrations and a decrease of Na concentrations was observed four months after initiation of lasalocid supplementation in the treated cows. After two months of supplementation with lasalocid, acetate and butyrate molar proportions decreased in those cows, which led to a decreased acetate/propionate ratio. Spears and Harvey (1982) observed decreased magnesium concentrations in steers on pasture which were fed 200-300 mg/day lasalocid. In a continuous culture experiment, lasalocid decreased the acetate to propionate ratio, and a magnesium by lasalocid interaction existed for butyrate. A lasalocid by potassium interaction was observed for propionate and butyrate. Plasma glucose was higher for steers fed the high doses of lasalocid. Chirase et al. (1988) concluded from their data that the efficacy of lasalocid altering VFA concentrations of ruminal fermentation might be enhanced by optimizing other nutrients. Armstrong and Spears (1988) studied the effect of administering lasalocid intravenously and the resulting effects on various blood metabolites. Blood insulin, glucose, free fatty acid, Mg, P, and K concentrations were not affected by lasalocid injection. Neither growth hormone or luteinizing hormone were affected by the lasalocid administration.

Starnes et al., (1984) studied the influence of lasalocid on mineral metabolism and ruminal urease activity in steers. Ca, Mg, P, K, Na, Zn and Cu serum and rumen values were measured. Apparent absorption of Na, Mg, and P were increased

by lasalocid intake. Only Zi and Cu concentrations were significantly increased from the control group. K and Ca absorption were not affected by lasalocid administration, however concentrations of soluble K and Ca in rumen fluid were lower than those of controls. Bacterial urease was decreased by 28% in steers fed lasalocid.

Volatile fatty acids have been shown to increase plasma insulin concentration in cattle and sheep (Johnson et al., 1982), as well as in pre-ruminant milk-fed lambs. Johnson et al., (1982) investigated whether preruminant calves were stimulated to secrete insulin with propionate or glucose feeding and propionate infusion. All calves exhibited a plasma insulin rise and plasma glucose decline with propionate infusions. The calves fed glucose and propionate appeared less sensitive to the infusions than the control calves.

Steers fed 220 or 330 ppm lasalocid had less complete crude protein digestion in the large intestines than did control animals. Lasalocid lowered ruminal phosphorus digestion. Apparent digestibility of Ca, P, Mg, and K in the small intestine was higher for steers fed lasalocid than for those fed control diets. Apparent total digestibility for Ca and Mg were higher for lasalocid-fed animals as well (Gado et al., 1986).

Inhibition of lactate producing microorganisms has been attributed to lasalocid (Dennis et al., 1981). Both lasalocid and monensin were efficient in preventing lactic acidosis when

cattle were engorged with grain or a glucose solution (Nagaraja et al., 1981, 1982). Lactate producers with succinate as a major end product, and none of the lactate fermenters, were inhibited by lasalocid (Dennis et al., 1981). Lasalocid added in vitro to rumen samples reduced protozoa numbers (Dennis et al., 1986); however, young calves do not have a substantial protozoa population until after six weeks of age (Anderson et al., 1987). Anderson et al. (1988) studied the influence of lasalocid-feeding on growth and ruminal flora of early weaned calves age four days to 12 weeks. Treatments were a control group and a lasalocid-fed group. The lasalocid-fed group was fed lasalocid in milk, pre-starter and starter to deliver approximately one mg/kg body weight. Calves fed lasalocid had greater intake after six weeks of age, at which time the lasalocid intake averaged 1.5 mg/kg body weight. Weight gains were higher for the group receiving lasalocid at week 5, 8, 10 and 12. Fecal scores were not different between treatments. The ruminal pH of the lasalocid-fed group was higher at one and two weeks and tended to be lower than the control group at four weeks of age. Rumen $\text{NH}_3\text{-N}$ concentrations decreased with the age of the calf. The lasalocid-fed calves reached higher total VFA concentrations at weeks 5,6, and 10. Lasalocid-fed calves had a lower acetate/propionate ratio. Amylolytic bacteria had a treatment by age interaction. Lasalocid supplementation

seemed to depress ruminal bacterial fermentation of milk and dry feed during the first two weeks. This study did not differentiate from which source, milk, prestarter, or starter, the lasalocid was effective.

Neuendorff et al. (1985) found lasalocid did not affect height, weight:height ratio, length, or heart girth of Brahman bulls during puberal development. The lasalocid fed bulls had higher propionate and lower acetate and butyrate of ruminal VFA, with no change in total VFA. Lasalocid-fed bulls tended to have a greater average daily gain and had greater feed conversion, while heifers supplemented with monensin reached puberty at a younger age than control animals but weighed less than the control calves (McCarter et al., 1979, Moseley et al., 1977). Feeding lasalocid to cows while on pasture did not affect calf gain or 205-day adjusted weaning weight (Hopman and Weber, 1986).

Benz et al. (1987), using chicks and rats, determined the effects of two ionophores, monensin and lasalocid, on internal organ mass. Chicks' liver weights did not differ with the level of ionophore intake. The heart weight of chicks differed due to ionophore consumption. Liver weight and relative liver weight both differed with ionophore consumption for rats. These data indicated that ionophores alter vital organ mass and may alter organ function.

Toxicity

Lasalocid is considerably less toxic than monensin (Galitzer, 1986a,b) when delivered orally to cattle. Lasalocid administered intravenously to steers did not alter metabolites, mineral concentrations, or behaviors (Armstrong and Spears, 1988) compared to controls, while monensin did alter all of those parameters. Galitzer et al. (1982) found that concentrations of 25-75 mg/kg body weight were toxic but not lethal. Lasalocid delivered at 100 to 125 mg/kg caused death to the steers at day 6 and 10 respectively. These dosages are 100 and 125 times the therapeutic doses. Administration of 1 and 10 mg/kg of lasalocid caused no toxic signs (Galitzer et al., 1986 a,b). Altered values of blood leukocytes, erythrocytes, hemoglobin hematocrit, total protein, albumin, creatinine, urea nitrogen, total bilirubin, creatine kinase, lactic dehydrogenase, calcium, chloride, inorganic phosphate, urine pH and specific gravity all changed one day after oral lasalocid administration.

The initial use of lasalocid with cattle was as a coccidiostat. Lasalocid has been shown effective in preventing coccidial infections from E. zuernii and E. bovis in ten to twelve week old calves and in preventing ovine coccidia (Stromberg et al., 1982; Foreyt, 1986; Foreyt et al., 1986; Foreyt, 1981). However, when lasalocid fed calves were challenged with Sarcocystis bovicanis, they were not protected from sarcocystosis (Foreyt, 1986). A recommendation of

Bergstrom and Etherton (1984) recommended performing routine fecal analysis rather than treating routinely.

Ascorbic Acid and the Immune System of Ruminants

Ascorbic acid (vitamin C) is a water soluble vitamin that is well known for the prevention of scurvy. The physiological role of vitamin C at levels above those required to prevent clinical signs of scurvy is essential to the general health of the organism. Vitamin C also plays an interactive role with other essential vitamins and minerals. How vitamin deficiencies and supplements beyond recommended daily allowances affect the organism's ability to mount immune reactions when the animal is compromised by stress or disease is currently under investigation by some researchers, and is the purpose of this study.

Physical Properties

Ascorbic acid is a carbohydrate that appears as a white crystalline compound. It has a molecular weight of 176.1 and a melting point of 190 to 192 °C (Bauernfeind, 1982). It is readily soluble in water, but is irreversibly oxidized in solution. Ascorbic acid is relatively stable in air.

Metabolism

Ascorbic acid absorption is believed to be a passive process occurring primarily in the ileum in animals that synthesize it. In ruminants, absorption efficiency is low due to ascorbic acid destruction by microbial action and other unknown factors (Hornig et al., 1984, Itze, 1984). Therefore, the amount of ascorbic acid a ruminant can reap from a feed depends on rumen bypass of the vitamin.

Ascorbic acid can be synthesized from glucose in many species. Therefore, it is not considered an essential dietary nutrient for most domestic animals, although it is physiologically essential for all of them. The rate of ascorbic acid synthesis varies between species and from animal to animal (Chatterjee, 1973). Of the farm animals, the pig has the lowest rate, 8.1 mg/kg/d. The highest rate is that of the goat which synthesizes 32.6-190.0 mg/kg/d. Cattle synthesize ascorbic acid at 15.7-18.3 mg/kg/d and the synthesis rate for sheep is 24.8 mg/kg/d (Levine, 1986). The chicken and rat, like cattle, have the ability to synthesize ascorbic acid. The chick synthesizes it in the kidney and the rat and cattle in the liver (Hornig, 1984). The synthesis rate of ascorbic acid has been determined to be controlled by the amount of free glucose available to the animal. Problems encountered in determining ascorbic acid needs include the 10-

fold difference between the ascorbic acid needed to maintain general health and the amount needed to prevent scurvy (Levine, 1986). Whether the rate of synthesis needed to maintain health is sufficient for the animal during stress is not yet completely or unquestionably determined.

During periods of stress, such as starvation, drug treatment, or environmental stress, the rat has an adaptive increase in the activity of enzymes involved in the biosynthesis of ascorbic acid, resulting in greater availability of ascorbic acid. It is not known whether this is true for other species as well. The chick and calf both experience a lag phase in the production of ascorbic acid between birth and when ascorbic acid synthesis is at adult levels of production (Hornig, 1984, Bouda et al., 1980). Current evidence suggests that at this age these animals are not ready to have the biosynthetic capacity for ascorbic acid synthesis increased by stress conditions. Consequently, the level of synthesis is not sufficient to compensate for increased ascorbic acid requirements during stress. During stress, supplemental ascorbic acid has been beneficial to the chicken affecting growth rate, egg production, egg shell strength and thickness, fertility and spermatozoan production, counteracting unfavorable environmental conditions, and cases of intoxication or disease. The relationship of stress and

ascorbate will be discussed in greater detail later.

Ascorbic acid metabolism in farm animals has been investigated for swine, ruminants and poultry. When ascorbic acid was supplemented to growing pigs, results indicated nearly an equal number of negative or equivocal reports as positive reports (Brown, 1984). The effect of stress on the concentration of ascorbic acid in the neonate was investigated by Brown (1984) and Dvorak (1984). A 14:1 ascorbic acid gradient existed across the placenta in swine. When the piglet was born a marked decrease of ascorbic acid concentration in all but the liver tissue was seen (Brown, 1984). At the same time, ascorbic acid concentrations in the liver increased over fetal concentrations. Dvorak (1984) reported that blood serum ascorbic acid concentration of piglets was highest one day after birth and then declined rapidly until it became constant between 20 days and 10 weeks. No differences were seen in ascorbic acid concentration with weaning. The urinary ascorbic acid content was several times higher than in serum. Ascorbic acid excretion rose with colostrum ingestion and was significantly lower for fattening pigs and for sows.

Researchers have studied the tissue distribution of ascorbic acid for fish, chickens, rat, mice, man and guinea pigs. Labeled ($1-^{14}\text{C}$) ascorbic acid has been used to

investigate retention capacity for ascorbic acid in guinea pigs, fish and rats. Guinea pigs highest retention was in the central nervous system, pituitary and adrenal glands, salivary glands, spleen, testes, eye lens, and bone marrow, implying a distinct role in those tissues for ascorbic acid (Hornig, 1984). The fish showed accumulation in the collagenic skin area, liver, renal and anterior kidney, bones and the eggshell membrane. The area of highest accumulation in the chicken was the liver: 10 % after intravenous injection and 5% following oral dosing. Spleen, lungs and kidneys each contained about 1% after either route of application. This suggests that the kidney is only the location for biosynthesis of ascorbate and that formed ascorbate passes quickly out of the kidney into other tissues (Caudwell, 1977, Hornig and Frigg, 1979). The highest concentrations of ascorbic acid in tissue of the chick and fish are in the liver. The white muscle ascorbic acid concentration of the chicken was not affected by differences of dietary concentrations of ascorbic acid. Rat ascorbic acid concentrations increase in glucocorticoid treated animals after injection of sodium barbital or feeding supplements containing chromium or tungsten. How much of these findings are applicable to farm animals is not known.

Physiological Role

The reactions for which ascorbic acid is needed are diverse. The ascorbic free radical can serve as a reducing or as an oxidizing agent (Bendich et al., 1987). The role of ascorbic acid may be to transfer electrons to enzymes that provide reducing equivalents in hydroxylation, amidation, or other reactions (Levine, 1986). Ascorbate is capable of reducing Cu^{2+} , Fe^{3+} , and reducing oxygen to water. Ascorbic acid acts in hydrolase enzyme reactions as a cofactor involving two substrates. It is also involved as a physiological reducing agent in the prolyl and lysyl hydroxylase system in collagen formation (Hornig et al., 1984, Levine, 1986). Ascorbic acid has a role in carnitine biosynthesis (Hughes, 1981). Carnitine is important in the transport of fatty acids into mitochondria, providing energy for the cells in retained contractions, and has two sources; 1) the diet or 2) endogenous synthesis from dietary lysine and methionine. The hydroxylation of 4-hydroxyphenyl pyruvic acid requires ascorbic acid to form homogentisic acid in tyrosine metabolism. The 21 hydroxylation system of corticosteroid synthesis is inhibited by ascorbate. Ascorbic acid promotes non-heme iron absorption from food, most effectively when ingested with the food or feed. The effect of ascorbic acid on iron absorption is dose dependent,

increasing absorption up to seven fold. Ascorbic acid has been shown to have a protective effect against toxicity of the heavy metals cadmium, nickel, lead and vanadium in quail and rats when the metals are ingested together with ascorbic acid (Suzuki and Yoshida, 1979; Fox, 1975). Ascorbate also reduced the toxic hexavalent chromium to the trivalent form which is not known to cause major adverse effects (Samitz et al., 1968). Inversely, ascorbate has been demonstrated to impair copper uptake and utilization (Van Campen and Gross, 1968, Disilvestro and Harris, 1981). The role of ascorbate in enhancing calcium uptake in the intestines and in the maintenance of egg shell quality has been demonstrated (Pardue and Thaxton, 1986, Thornton and Moreng, 1959). However, the total function of vitamin C in the biochemistry of calcium has yet to be established.

Ascorbic acid has been shown to be beneficial in fertility, especially for males. Ascorbate concentration is greater in Fresian bull semen versus buffalo bull semen. The bull sperm motility increased over that of the buffalo corresponding to the ascorbate concentration increase. Adding ascorbate to collected bull semen influenced the sex of offspring. The percentage of female offspring of semen with added ascorbate was increased compared to controls, 55.8 versus 51.3%, respectively. There is some evidence in humans

that anovulatory women were induced to ovulate with ascorbic acid supplementation (Hornig et al., 1984).

Stress effects on ascorbic acid concentration in adrenal glands is pronounced enough to be used as an indicator for stress measurement (Warris, 1984, Dvorak, 1984). The administration of ACTH and exposure to cold for 12 hours in piglets increased the excretion of ascorbic acid, suggesting some ascorbic acid depletion. Studies with chicks, using heat and ACTH as stressors, showed ascorbic acid could reduce the catatonic effects of stress. The mechanism is thought to be a reduction in corticosterone production by ascorbic acid. The synthesis of catecholamines requires ascorbic acid and the catecholamines are important to the stress responses of chickens (Thaxton and Pardue, 1984). Studies with calves have shown a reduction in plasma ascorbic acid concentration caused by lung or gastrointestinal tract infections (Kolb, 1984). Similarly, reductions in plasma ascorbic acid concentration have been observed in lambs after infection with Salmonella typhimurium or S. abortus bovis. Vaccination in cattle also caused an immediate decrease in plasma ascorbic acid concentration. When ascorbate was added with a Salmonella vaccine in pigs and rabbits, immunity development was improved. Reduced plasma ascorbic acid concentrations have

been reported under the influence of parasitosis (Kolb, 1984).

Several immunological functions have been investigated for ascorbic acid. Studies of animals and man suggest that ascorbic acid may have a direct inhibitory effect on carcinogenic N-nitrosamine formation. Vitamin C has been studied in regard to two immune parameters, namely antiviral and antibacterial effects (Briggs, 1984). This will be covered in greater detail in a subsequent section.

Ascorbate Metabolism in Ruminants

The highest blood concentration of ascorbic acid in cattle is found immediately after birth and thereafter the ascorbic acid concentration in blood slowly declines (Bouda et al., 1980, Weggert and Moustgaard, 1982). Ascorbic acid concentrations in newborns vary widely, but are reported at 1030 ug/dl (Bouda et al., 1980). Studies of monozygotic twins have shown strong uniformity in plasma ascorbic acid concentration between the twins. This points to a possible genetic influence of ascorbic acid synthesis and requirement. This aspect, together with the congenital liver store variations, are possible explanations of the extreme variability seen in young calves. Consequently, values of plasma ascorbic acid concentrations have been reported from 0.2 mg % to 0.8 mg % (Palludan and Wegger, 1984). Conflicting

data are reported regarding correlations of plasma ascorbic acid concentration and seasons (Itze, 1984). Low blood ascorbate concentrations, even in ascorbate-synthesizing animals, can be caused by metabolic disorders, improper nutrition, inadequate vitamin A or β -carotene intake and infectious diseases (Itze, 1984). The ascorbate content of liver increases late in fetal life and decreases postnatally. Since the ascorbic acid content of colostrum and milk is relatively low and ascorbate synthesis in the liver is not fully developed in young calves, the requirement for this vitamin must be covered by the calve's congenital reserves (Palludan and Wegger, 1984). Lundquist and Phillips (1942) have established that vitamin C synthesis begins 2 to 3 weeks after birth. The serum ascorbate content of calves depends on the nutrition of the dams as well as the age and health of the calves. During gastrointestinal disorders, serum ascorbate decreases due to decreased milk intake, impaired resorption and higher utilization of ascorbic acid in diseased calves. In an experiment designed to investigate ascorbic acid content of serum in cows and their calves, Itze (1984) found the lowest serum ascorbic acid concentration in cows and in their calves was in February and the highest was in April. Reported calf serum concentrations were quite variable and values were 67.2 mg/l at day 1 after birth, dropping radically

to around 16 mg/l at day two. A general decrease was observed throughout the following eight days. Ascorbic acid intake for calves has been recommended to be 200-250 mg/day (Itze, 1984); however, these recommendations did not allow for the numerous stressors placed on young calves which are placed in a calfhouse environment. In the Itze study (1984), serum ascorbate concentration was high between days 7 and 14 and dropped drastically at day 14, suggesting an increasing degradation of ascorbate in the developing proventriculi, thereby decreasing absorption from the gastrointestinal tract.

Interactions with other Vitamins and Minerals

Both positive and negative interactions occur between ascorbic acid and other vitamins and minerals. The vitamins and minerals that are affected by ascorbic acid are vitamins A and E, iron, copper and selenium. Interactions between those vitamins and minerals also occur.

Bouda et al. (1980) showed the importance of colostrum in establishing neonate concentrations of vitamin E, whereas ascorbic acid concentration was dependent upon fetal levels. A common property of ascorbic acid and vitamin E is a rapid reaction with organic free radicals. As early as 1968 the synergistic relationship of vitamins C and E was proposed

(Tappel, 1968). Packer et al. (1979), using pulse radiolysis, directly observed a free radical interaction between ascorbate and vitamin E. In vitro synergism of these two vitamins was shown in rat and guinea pig livers. The depleting effect of weaning on vitamins C and E and the ability to increase vitamins C and E by dietary means have been demonstrated for each of the vitamins for weanling pigs (Yen, 1984). Plasma values of vitamins C and E each increase with supplementation of the other vitamin in the rat and in the guinea pig a sparing effect by ascorbic acid on vitamin E during vitamin E deficiency has been seen. Vitamin E deficient chicks fed ascorbic acid had reduced dietary selenium requirements. Increased intestinal absorption of selenium (Se) but not vitamin E resulted with dietary ascorbic acid supplementation in the chick. Dietary Se or vitamin E treatment did not have an effect on plasma ascorbic acid concentrations, but mortality was reduced. Bendich et al. (1984) investigated vitamin C and E interaction and the immune response. Guinea pigs fed high concentration of vitamin C had increased plasma vitamin E and increased vitamin E concentration in the lungs.

A synergistic relationship for vitamin C and A has also been suggested (Itze, 1984). High levels of vitamin C supplementation in neonate calves resulted in increased serum vitamin A concentration. Conversely, pigs showed no effect

of ascorbic acid in liver or adrenal with different hepatic vitamin A stores (Dvorak, 1984).

There are two areas of importance in the ascorbic acid and iron (Fe) interaction; intestinal absorption and postabsorption metabolism at the tissue level. Ascorbic acid has been shown to increase the intestinal absorption of non-heme iron in rats and pigs. This is in part due to the chelating potential of ascorbic acid. Ascorbate is required for the release of Fe from the ferri-transferrin complex and its incorporation in hemoglobin (Yen, 1984). The ascorbic acid, iron and copper (Cu) interrelationship appears to be similar in all species. Ascorbic acid decreases serum and liver copper concentrations in guinea pigs, rats, and other monogastric species. Ascorbic acid increases serum and hepatic Fe concentrations, hepatic microsomal cytochrome P-450 and cytochrome P5 erythrocyte count, hemoglobin, and hematocrit in guinea pigs (Milne and Omaye, 1980). In humans, plasma Cu^{++} concentration increases during infection, contrasted to the decrease of iron. This elevation of Cu^{++} has a bacteriostatic effect.

Immunity

Ascorbic acid has been linked to improved protection from disease. In humans, this concept has been pursued most

notably by Linus Pauling (Briggs, 1984). The effects of various vitamins on immune responses has just begun to be investigated for farm animals in the last decade. Ascorbic acid has been shown to increase cellular immune response, both in vitro and in vivo, and to affect humoral immunity.

In vitro bacterial phagocytosis by macrophages has been enhanced with ascorbic acid. This is thought to occur via enhanced activity of the hexose monophosphate shunt (HMPS). During ascorbic acid deficiency macrophage mobilization and aggregation are decreased (Sherman, 1986).

Likewise, human neutrophil migration toward leucoattractants can be stimulated by ascorbate, both in vivo and in vitro (Hornig et al., 1984, Anderson, 1981;1982; Anderson and Jones, 1982). Conflicting reports regarding neutrophil phagocytosis in vitro have been reported (Anderson, 1981). Again, increased motility has been related to enhanced activity of the HMPS. However, it has also been linked to increased intracellular guanosine 5'-monophosphate (GMP), to the inhibition of the polymorphonuclear leukocyte myeloperoxidase/hydrogen peroxide/halide (MPO/H₂O₂/halide) system, or to a serum dependent event. Ascorbic acid has also been implicated in the immunomodulation of cyclic nucleotide B and T cells (Panush et al., 1982). Human T and B cells incubated with ascorbate had increased cyclic GMP and reduced

cAMP/cGMP, suggesting disturbances of cellular cyclic nucleotide levels. Other evidence points to changes in histamine and prostaglandin levels in response to plasma ascorbic acid concentration (Hornig et al., 1984). The mechanism of enhancement of the cellular immune response is yet unknown (Hornig et al., 1984). The transformation response of lymphocytes bathed in ascorbic acid in response to concanavalin A (Con A) and phytohemagglutinin (PHA) are contradictory (Anderson, 1981). Lymphocytes incubated with influenza virus were inhibited in PHA-induced transformation until ascorbate was added and counteracted the inhibition. In mice and in humans pokeweed mitogen-(PWM), PHA- and Con A-stimulation increased lymphocyte blastogenesis with ascorbic acid supplementation. Both human and mouse ascorbate-supplemented subjects had lymphocytes that increased interferon production when challenged with virus. Human lymphocytes treated with ascorbate produced the lymphokine leucocyte migration inhibitory factor when cultured with PHA. The results regarding lymphocyte motility are conflicting. The infectivity titer of respiratory syncytial virus incubated with ascorbic acid was reduced at 24 hours and inactivated at 48 hours (White et al., 1986). Pigs with a hereditary vitamin C deficiency had decreasing lymphocyte blastogenic responses to Con A and PHA coincident with decreasing ascorbic acid

concentrations. When resupplemented with ascorbic acid, the Con-A stimulated blastogenic response gradually approached the initial values (Kristensen et al., 1986).

When lymphocytes of guinea pigs supplemented with vitamins C and E were stimulated with Con A, PHA and LPS, no effect of vitamin C alone was observed. However there was some interaction of vitamin C and E. A sparing effect of vitamin E by vitamin C was shown (Bendich et al., 1984). Under similar experimental conditions with the addition of 100% oxygen, the guinea pigs fed the high levels of both vitamins had the highest overall responses (Bendich, 1987).

Chicks deficient in water soluble vitamins have been shown to have a greater % decrease in thymus, bursa, and spleen weight compared to the total % decrease in body weight. This is consistent with previous findings of impairment of cell mediated immune response in water soluble vitamin-deficient chicks (Ghoshal and Bhattacharyya, 1985).

Various effects of ascorbic acid on neutrophils have been reported. A 5 mM to 25 mM ascorbic acid concentration caused chemiluminescence of neutrophils. Antimicrobial activity to E. coli and S. aureus was not affected by ascorbic acid supplementation in vitro or in vivo dietary supplementation. Antimicrobial activity was stimulated with intravenous ascorbic acid administration. Persons with diseases caused

by abnormal neutrophil motility have been helped with ascorbic acid supplementation (Anderson, 1981).

Ascorbic acid has also been found to inhibit human natural killer cells (NK cells) in a dose dependent manner. Vitamin C did not influence effector/target binding or interferon (IFN) or IL-2 induced increase of NK activity. When cells were incubated at 4°C for 1 hour and washed, NK activity was not affected. However, when they were incubated at 37 °C for 1 hour and washed, NK activity was strongly suppressed, indicating that vitamin C had to be taken up by the cells to become inhibitory or that it influences the cellular metabolism (Hwuyler et al, 1985). Siegel and Morton (1984) found increased prostaglandin E₂ synthesis by bone marrow macrophage in mice supplemented with ascorbic acid. Previous reports had shown increased response to IFN with mice supplemented with ascorbic acid. However, with mice as with humans, NK activity decreased with ascorbic acid supplementation (Siegel and Morton, 1984).

The effect of ascorbic acid on the humoral response is a complex area since it involves elements of the cell mediated immune response already discussed. Ascorbic acid supplementation in humans, guinea pigs and catfish has increased antibody production. In the study with guinea pigs, a much higher and earlier peak antibody (Ab) titer was

observed with ascorbic acid supplementation for the primary Ab response. The secondary responses showed no differences between treatments. This suggests that ascorbic acid stimulates an increased IgM response (Prinz et al., 1980). IgA and IgM as well as the complement component C3 were increased with ascorbic acid supplementation in humans (Prinz et al., 1977). Ascorbic acid supplementation in catfish (Li and Lovell, 1985) reduced mortality rates caused by Edwardsiella ictaluri from 100% to 0%. Ascorbic acid deficiency in these fish impaired Ab production and phagocytosis. High concentrations of ascorbate (3000 mg/kg) supplementation to these fish significantly enhanced Ab production and complement activity.

Ascorbic Acid Supplementation and Immunity in Ruminants

The development of cell mediated immunity has been investigated for lambs and calves (Outtridge, 1985, Manak, 1986). Prenatal removal of the thymus in lambs created immunodeficiency in those lambs. Resulting lymphopenia possibly was due to depletion of a particular population of T-cells that have a reduced mitogenic response and decreased numbers as the lamb grows. Non-thymectomized lambs are less responsive to vaccinations than are adult sheep due to immaturity of the cell mediated immune response as well as

high and low responder differentiations among the lambs (Otterridge, 1985)

Manak (1986) studied the blastogenic responses of lymphocytes of young calves to Con A, PHA and PWM. The serum of young calves had a suppressive effect when incubated with lymphocytes from older calves. Serum cortisol was 30 mg/ml at 6 hours and decreased to 5.5 mg/ml by 10 days of age. The results indicate that the blastogenic response of the newborn calf is suppressed relative to that of the 10 day old calf and it is mediated primarily by serum factors. Other factors, present in fetal serum, that may play a suppressive role are α -fetoprotein, transferrin, fetuin or some T or non-T cell suppressor population.

Itze (1984) found poor herd health; increased still births, high early mortality, emergency slaughter and carcass rejection at slaughter associated with low serum ascorbic acid content. Several cases of hypo-gamma-globulinemia were found, but no statistically significant correlations between ascorbic acid concentrations and immunoglobulin concentrations could be derived. Simco (1983), cited by Itze (1984) has suggested that respiratory diseases in calves are aggravated by environmental factors, long lasting transportation, unfavorable microclimate and vitamin A and C deficiencies. With 10 or 5 g/day supplementation of ascorbic acid, enteric

and respiratory disease symptoms were reduced from those of control calves. Parenteral administration of ascorbic acid also resulted in lower morbidity. Vitamin C supplementation had no effect on gamma-globulin concentrations.

The relationship of ascorbate, colostrum nutrition and Ig in serum of calves was studied by Itzeova (1984). Great differences in ascorbate content have been reported in milk for dairy cows of the same age, stage of lactation, and uniform management conditions. Ascorbic acid content in cow's colostrum and milk is low compared to non-ruminant species (Wegger and Moustgaard, 1982). Ascorbate in milk after removal from the cow is easily affected by oxidants and irradiation. Heating and air can further lower the low ascorbate present in cow's milk. Up to 20% of ascorbate in milk is lost by drying. Addition of lactic acid-producing microbes can increase the ascorbic acid content of sour milk. Therefore, under most conditions it is possible that the young calf may have a marginal ascorbic acid deficiency (Wegger and Moustgaard, 1982).

Itzeova (1984) investigated the effect of the quality of colostrum and the frequency of feeding of colostrum on Ig concentrations. Treatments were: 1) fresh colostrum from dam two times per day, 2) fresh colostrum from dam three times per day, 3) fresh colostrum two times per day and frozen colostrum

from first milking one time per day, 4) one day frozen colostrum of first milking three times per day, 5) one day fresh colostrum from dam two times per day and frozen colostrum of first milking one time, day two and three frozen mixed colostrum of first through fourth milking three times per day, and 6) one day fresh colostrum from dam, day two and three frozen mixed colostrum three times per day. Feeding fresh colostrum two times per day or one time per day frozen colostrum of the first milking avoided the decrease in plasma ascorbic acid concentration typically seen after birth. No vitamin C in the colostrum was lost due to freezing. All treatments maintained normal Ig concentrations (Itzeova, 1984).

In 1984 Blair and Cummins found increased IgG concentration with ascorbic acid supplementation when calves were deprived of colostrum. A later similar investigation (Cummins and Brunner, 1989) showed lower IgG concentration at 14 and 28 days for calves fed ascorbic acid. Calves fed ascorbic acid had lower clinical scores for diarrhea. In this study dietary ascorbate was not immunostimulatory in dairy calves at 56 days and appeared to inhibit Ab synthesis, although at 14 days plasma IgG concentrations were higher in colostrum deprived calves supplemented with ascorbic acid than in colostrum deprived calves without ascorbic acid

supplementation. Roth and Kaeberle (1985) provided some evidence for the use of ascorbic acid in counteracting the immunosuppressive effect of cortisol, although in later notes they gave several reasons that the ascorbic acid treatment might be futile in stressed animals, but felt the use of ascorbic acid should be evaluated under field conditions in stressed cattle (Roth and Kaeberle, 1986). Reddy et al. (1986; 1987) and Cipriano et al. (1982) have established that vitamin E increased the immune response of calves by 6 week of age. Little research has been published regarding the effects of vitamin C on lymphocyte and neutrophil function of young calves. Based upon the uncertainty of the vitamin C content of cows milk and the possible hypovitaminosis C state of young calves, the effect of supplemental vitamin C alone and vitamins C and E together on the growth and health of young calves merits further investigation.

THE EFFECT OF ROUTE OF ADMINISTRATION OF LASALOCID
ON RESPONSE OF YOUNG DAIRY CALVES

INTRODUCTION

Feeding lasalocid to neonate calves through twelve weeks of age has resulted in increased feed intake and weight gain (Anderson et al, 1988). The greatest feed intake and weight gain differences were in the last six weeks. The lasalocid-fed group in that study received lasalocid in the milk, prestarter and starter. However, it could not be determined from which part of the diet the lasalocid delivery was having the greatest effect. Early-weaned calves have shown metabolic signs of earlier ruminal development than control calves (Anderson et al., 1987 a,b). Early weaning leads to early development of ruminal flora (Anderson et al., 1987 a,b., Lengemann and Allen, 1959). The use of ionophores to encourage early feed consumption and early weaning may have economic and logistical benefits due to decreased labor and feed costs associated with early weaning.

The objective of this study was to determine the most effective method of administration of lasalocid. To allow for differing early feed consumptions, a weaning program was used in which weaning was based upon dry feed consumption.

METHODS AND MATERIALS

Forty Holstein bull calves were removed from their dams at 24 h of age and fed colostrum until 3 d of age. The calves were blocked by date of birth and the four calves within each block randomly assigned to one of four treatment groups. Treatments were feeds with no lasalocid (None); feeds with lasalocid in starter only (S); feeds with lasalocid in prestarter and starter (PS,S); or feeds with lasalocid in milk, prestarter and starter (M,PS,S). The calves in the group receiving lasalocid in milk, prestarter and starter received untreated prestarter and starter until two wk of age, then they received lasalocid-treated prestarter and starter. The lasalocid for this group was delivered in the milk on d 4 through 14 to provide 1 mg/kg of birth weight daily. Lasalocid concentrations in the feed were 176 mg/kg in the prestarter and 29 mg/kg in the starter, with a target consumption of 1 mg/kg of body weight per day. Tables 1 and 2 show composition of prestarter and starter used.

Milk was fed at 8% of birth weight daily, divided into two equal feedings, and prestarter was available beginning on experimental day one. Milk was fed at that rate until the calf consumed 227 g of prestarter per day. The afternoon feeding of milk was discontinued at that time and as much

1

TABLE 1. Composition of prestarter.

Ingredient	Percent
Whey, dried	46
Fat Supplement ²	23
Skim milk, dried	19
Sodium Caseinate	12
Additives ³	+

¹ Calfwenna, ® Merricks, Union Center, Wi.

² A mixture of milk solids and fat containing 7% milk protein and 60% animal fat.

³ Includes chlortetracycline (200g/ton), preservatives, vitamins, minerals, flavoring compounds, and lasalocid if added.

TABLE 2. Composition of starter.^{1,2.}

Ingredient	Percent
Alfalfa, ground	25
Oats	20
Corn, cracked	32
Soybean meal	14.4
Molasses, liquid	5
Dicalcium phosphate	0.7
Limestone,ground	0.7
Salt	0.3
Trace Mineral Salt	0.3
Vitamins ³	+
Selenium (0.02%)	+
Lasalocid (15% Active) ⁴	+

¹ As-fed basis.

² Pellet, 4.8 mm diameter.

³ 2,200 IU/kg vitamin A, 330 IU/kg vitamin D, 77 IU/kg vitamin E, and 1.0 mg/kg selenium.

⁴ Starter w/lt lasalocid.

starter as the calf would consume was added to 227 g of prestarter each day. When daily dry feed (prestarter plus starter) consumption was at 1.3% of birthweight the calf was weaned. Prestarter was discontinued at 5 weeks of age and only starter was fed. Calves were housed in individual hutches with straw bedding and given free access to water.

Sample Collection and Analysis. Daily feed intake and weekly weight gains were recorded. Fecal scores were recorded twice daily using the scale described by Larson et al. (1977). Serum samples were taken at 4, 8, and 12 weeks for metabolic evaluation using the SMA-12 analysis. Constituents determined by this analysis were Na, K, Cl, CO^2 , glucose, blood urea nitrogen (BUN), creatinine, alkaline phosphatase (ALP), total protein, albumin, globulin, albumin to globulin ratio (A/G), Ca, and P.

Statistical Analysis. Data were analyzed as a randomized complete block design, with treatment as the main source of variation, using the General Linear Model Procedure (SAS, 1985). Means were tested for treatment and treatment by time effects. LS means were separated using a protected least significant difference test when significant treatment or treatment by time effects were detected.

RESULTS AND DISCUSSION

Table 3 shows daily lasalocid intake by week for each treatment. The lasalocid intake varied by treatments across time as was intended by the design of the experiment. The greatest intake (mg/kg/day) occurred in calves receiving lasalocid in M,PS,S at week 5. Calves with lasalocid administered in all three feeds reached 1.00 mg/kg \pm .05 by the second week, those receiving lasalocid in PS,S reached the same point by week 5 and calves with S only administered lasalocid reached target lasalocid intake by week 10. At week 7, calves on all three treatments were consuming similar amounts of lasalocid. The M,PS,S lasalocid-fed group showed a decline in lasalocid intake when lasalocid was discontinued in the milk and again when the prestarter, containing higher levels of lasalocid than the starter, was discontinued. Calves with lasalocid in PS,S also showed the decrease in daily lasalocid intake after week 5 when lasalocid prestarter was discontinued.

Cumulative dry feed intake for all treatments can be seen in Table 4. The intake of calves with M,PS,S lasalocid delivery was greater ($P < .10$) than for calves on the other two lasalocid treatments at weeks 4, 5, and 6 and greater in wk 3 than those with lasalocid delivery in PS,S. There were no

TABLE 3. Daily lasalocid intake (mg/kg of body weight/day) and body weight (BW, kg) of calves.

Wk	Lasalocid Administration							
	None		M, PS, S ¹		PS, S		S	
	BW	Intake	BW	Intake	BW	Intake	BW	
1	45	.59	45	.21	45	0	47	
2	44	.97	46	.35	46	.08	47	
3	46	.91	48	.62	48	.07	50	
4	49	1.14	50	.82	50	.19	51	
5	54	1.32	56	1.11	54	.38	55	
6	59	.85	63	.73	59	.73	62	
7	66	.84	68	.84	64	.83	68	
8	72	.94	74	.90	71	.91	76	
9	77	.95	80	.95	77	.92	81	
10	82	.92	87	.97	84	.97	87	
11	91	.88	93	.95	91	.97	96	
12	98	.94	102	.97	100	1.03	106	

¹M=milk, PS=prestarter, S=starter.

TABLE 4. Cumulative dry feed intake (kg).

AGE WK	Lasalocid Administration				Pooled SE
	None	M, PS, S ¹	PS, S	S	
1	.5	.5	.4	.3	.13
2	1.5	1.4	1.0	1.0	.30
3	4.4 ^a	4.4 ^a	2.8 ^b	3.0 ^{ab}	.63
4	9.4 ^{ab}	10.2 ^a	6.4 ^b	6.5 ^b	1.37
5	18.1 ^{ab}	20.0 ^a	12.6 ^b	13.8 ^b	2.41
6	30.3 ^{ab}	32.7 ^a	23.0 ^b	24.5 ^b	3.31
7	44.0	45.7	34.9	37.7	4.44
8	59.0	62.1	49.8	53.7	5.08
9	76.0	80.3	67.4	71.3	5.77
10	93.7	99.4	85.3	90.8	6.98
11	113.6	119.6	105.7	112.8	8.19
12	137.0	142.7	128.7	138.1	9.12

^{a,b} means in the same row with differing superscripts differ (P < .10).

¹M=milk, PS=prestarter, S=starter.

significant differences in feed intake, cumulative or weekly, between treatments from weeks 7 through 12.

The cumulative average daily gain (ADG), Table 5, shows significant differences at wk 2, 3, 4, 6, and 7. Calves with lasalocid in M,PS,S had equal to or greater cumulative ADG than any of the other three treatments for all but wk 2 and 12. The body weights shown in Table 2 reflect a similar pattern for the four treatments. The greater gains shown during the first 4 wk by calves with lasalocid in M,PS,S and then the calves with lasalocid in S may be attributed to earlier weaning attained by these groups. The larger gains at weeks 4-6 for calves fed lasalocid in M,PS,S may be due to levels of lasalocid intake near 1.5 mg/kg of body wt. The equal gains in wk 7-12 reflect similar levels of lasalocid intake, near 1.0 mg/kg, for all treatment groups from wk 7 on. This is discrepant with results of Anderson et al. (1988) which showed gain differences between treatments during wk 7-12. During the first 6 wk of that study, lasalocid intake was < 1 mg/kg of body wt/day and reached 1.5 mg/kg/d during the last 6 wk when the greatest weight gains were observed. Therefore, in both studies greater weight gain was seen when lasalocid consumption was > 1.0 mg/kg/d, regardless of age.

TABLE 5. Cumulative daily gain (kg/d).

Age WK	Lasalocid Administration				Pooled SE
	None	M, PS, S ¹	PS, S	S	
1	.30	.41	.25	.38	.070
2	.13 ^b	.24 ^a	.16 ^{ab}	.24 ^a	.040
3	.17 ^b	.27 ^a	.22 ^{ab}	.27 ^a	.039
4	.23 ^{ab}	.30 ^a	.22 ^b	.26 ^{ab}	.035
5	.32	.39	.29	.33	.047
6	.41 ^{ab}	.50 ^a	.36 ^b	.43 ^{ab}	.044
7	.49 ^{ab}	.52 ^a	.41 ^b	.49 ^{ab}	.052
8	.53	.57	.49	.56	.038
9	.55	.60	.54	.58	.037
10	.57	.65	.55	.61	.039
11	.63	.67	.60	.67	.042
12	.67	.71	.66	.73	.044

a,b means in the same row without a common superscript differ (P < .10).

¹M=milk, PS=prestarter, S=starter.

Average daily gain and lasalocid intake for all lasalocid-fed calves are shown in Figures 1, 2, and 3. Calves with lasalocid delivered in M,PS,S gained more than calves on the other two lasalocid administration treatments from weeks 2 through 6. The decrease between weeks 6-7 is present in all three lasalocid treatments. Lasalocid administration in PS,S appeared to lack the beneficial effect that delivery of lasalocid in all three feeds had on gain. However, the gain of calves with lasalocid in S only increased as the lasalocid intake increased with starter consumption. The ADG of all three lasalocid treatments reflect the lasalocid intake, increased lasalocid intake followed by increased gain and lasalocid decreases followed by stable or decreased gain.

Fecal scores (Figure 4) showed significant differences at two different weeks. Week 4 fecal scores for the control group were significantly greater ($P < .10$) than those of calves fed lasalocid in M,PS,S. The fecal score of calves receiving lasalocid in S only was different ($P < .10$) than for the other three treatments at week 7. At week 2, a typical increase in fecal scores was observed in all treatments. We observed no incidence of coccidiosis during this study. This is consistent with Anderson et al.(1988), who reported no incidence of coccidiosis in conjunction with lasalocid consumption. However, in contrast to our data, Anderson et

Figure 1. Daily gain and lasalocid intake of calves fed
lasalocid in milk, prestarter, and starter.
M=milk, PS=prestarter, S=starter.

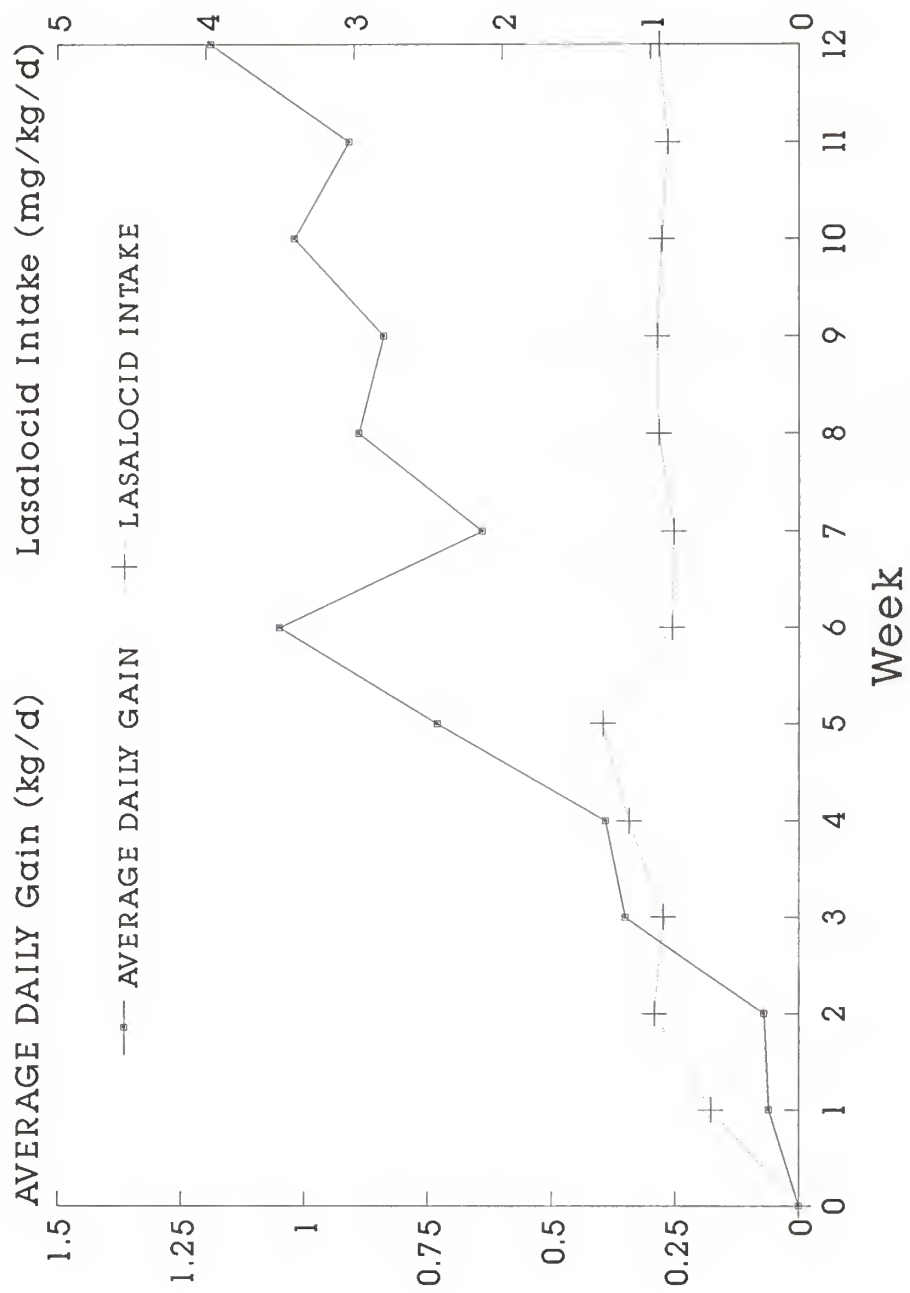


Figure 2. Daily gain and lasalocid intake of calves fed
lasalocid in prestarter and starter.

¹PS=prestarter, S=starter.

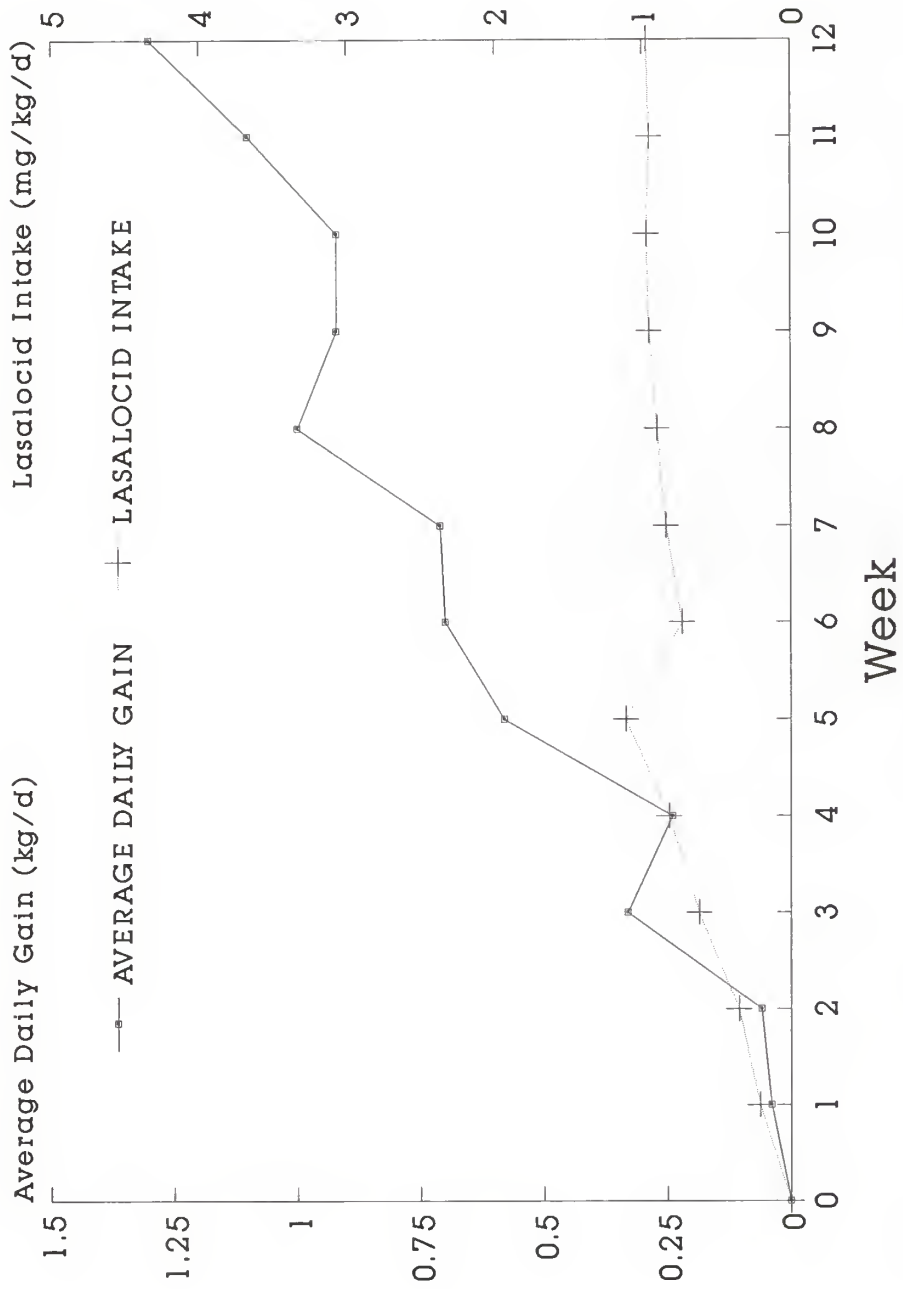


Figure 3. Daily gain and lasalocid intake of calves fed
lasalocid in starter.

¹S=starter.

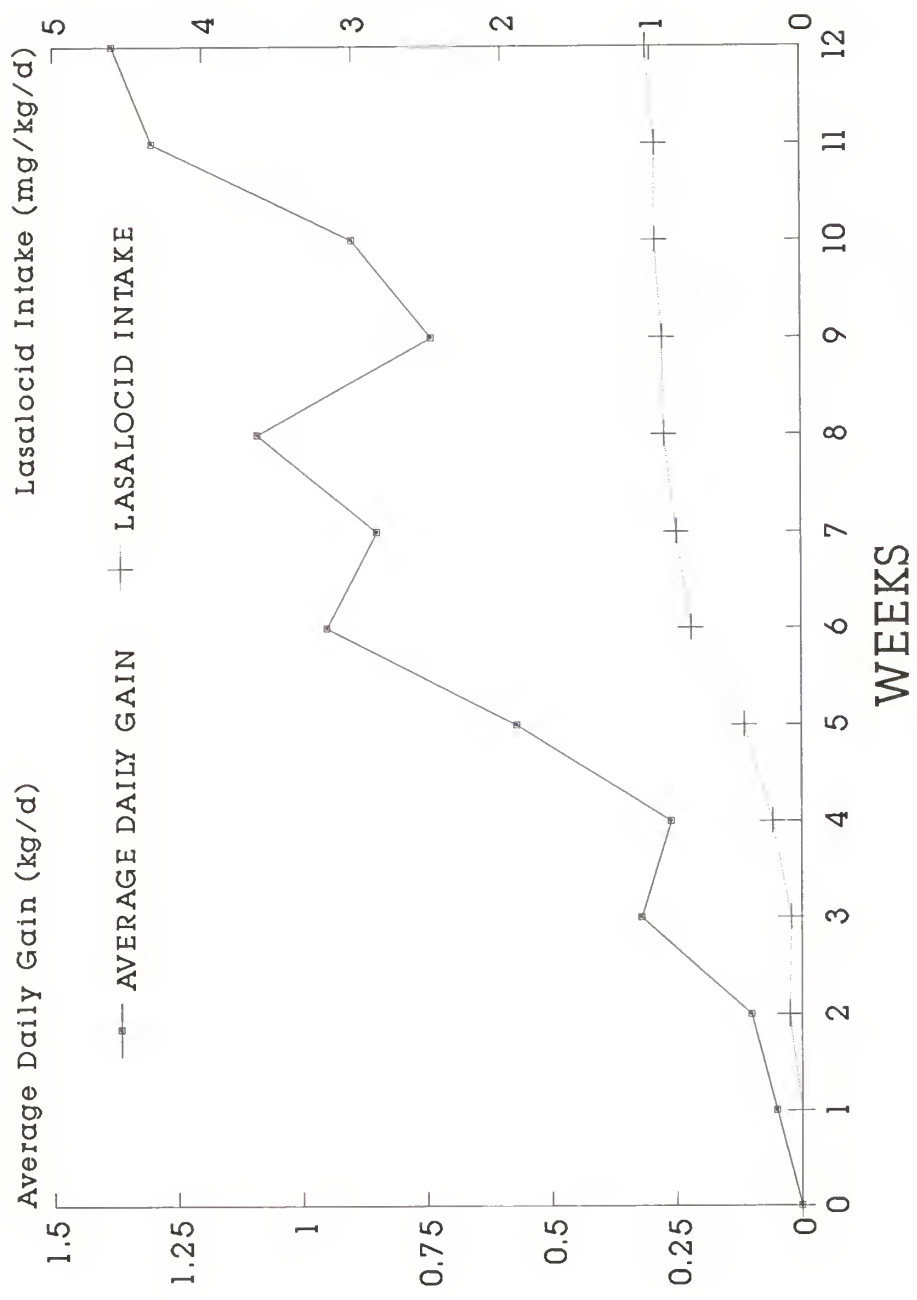
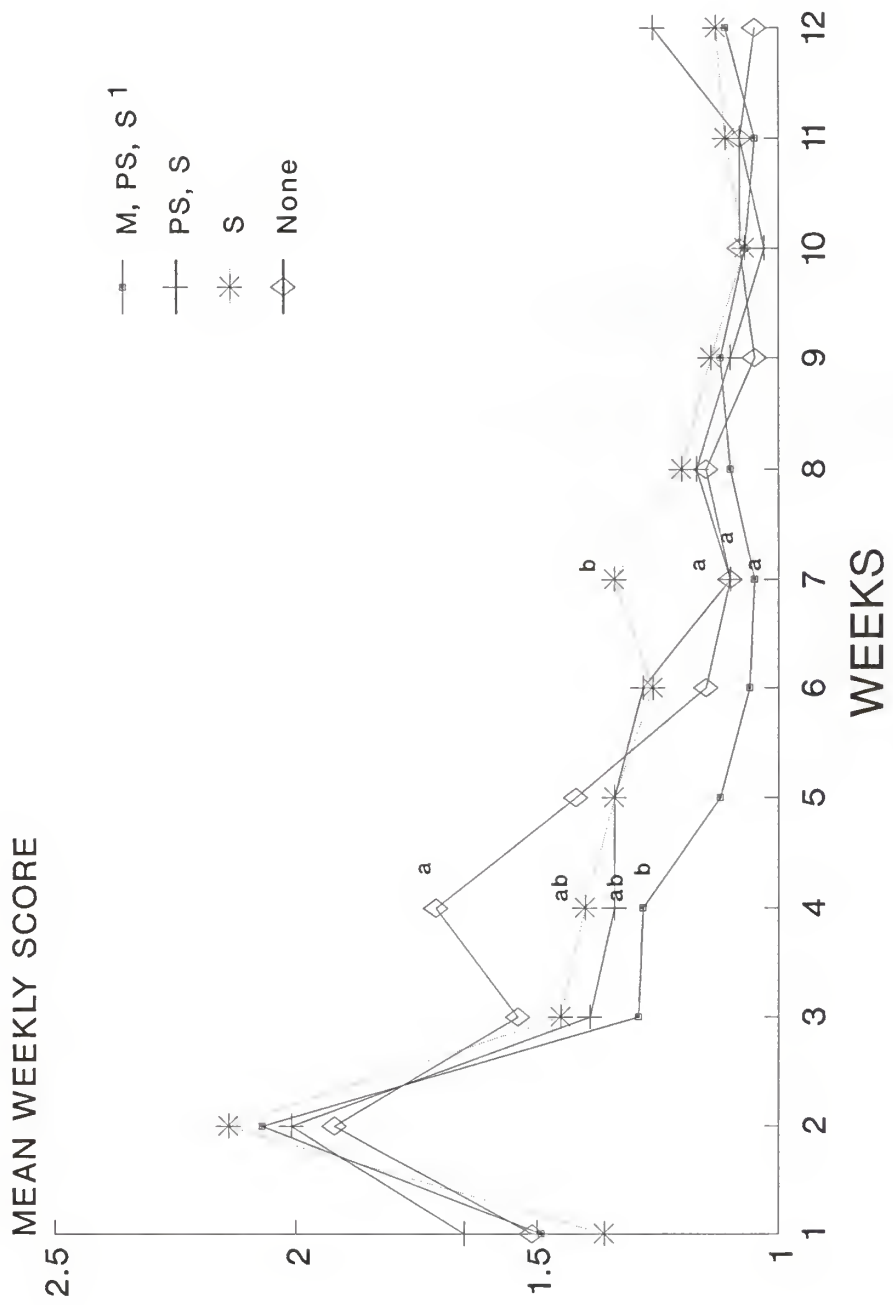


Figure 4. Mean weekly fecal scores for calves receiving lasalocid in M,PS,S; PS,S; S; or none. Differing superscripts within the same week denote means with statistically significant differences ($P < .10$).

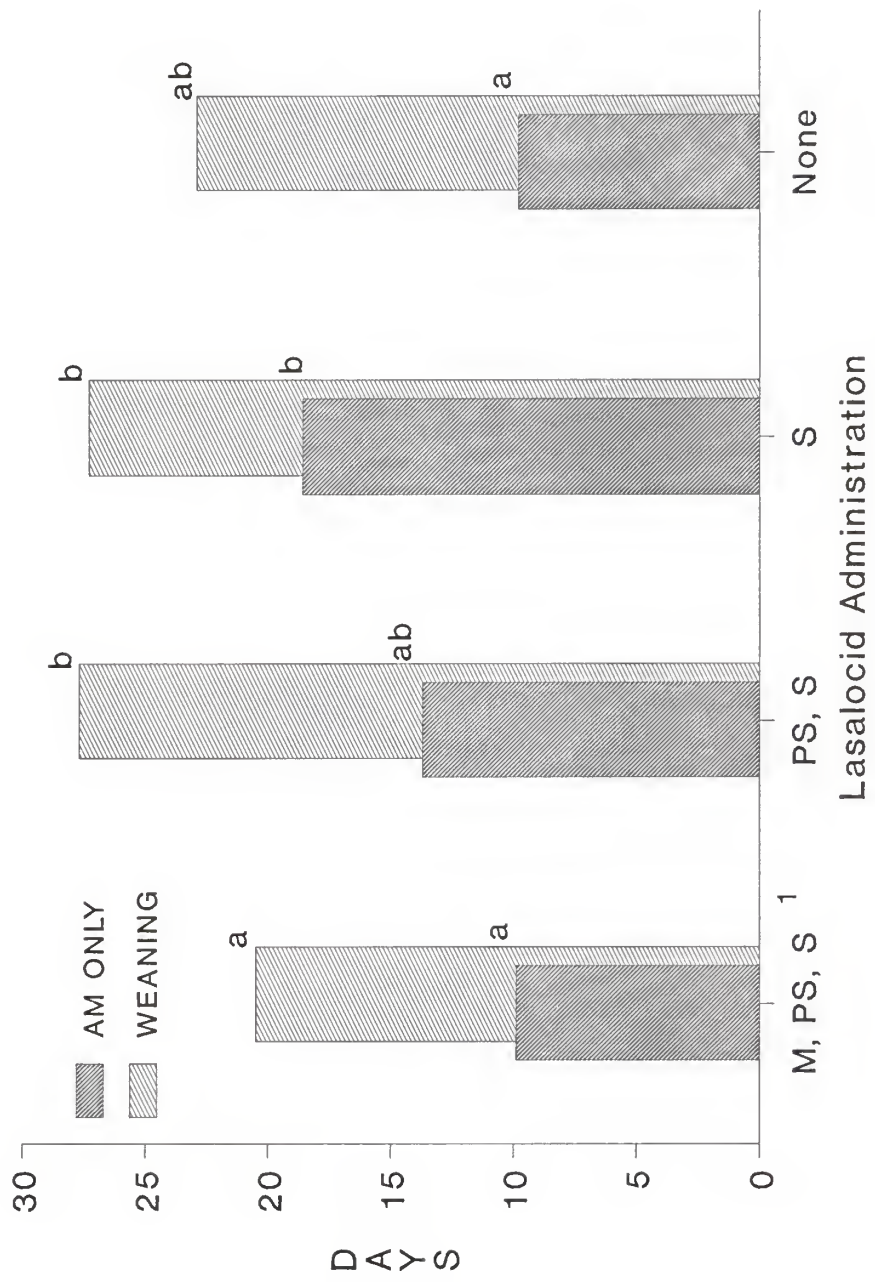
¹M=milk, PS=prestarter, S=starter.



al. (1988) did not observe differences in fecal scores.

Days required to reach the criterion for A.M. only feeding (Figure 5) show significant differences between the control group, as well as the group receiving lasalocid in M,PS,S, and those with lasalocid delivery in S only. Significant differences between calves with lasalocid in M,PS,S and the other two lasalocid fed groups were seen for days to weaning ($P < .10$). The standard deviation for calves with lasalocid delivered in M,PS,S was least for days to A.M. only and significantly less for days to weaning than the other treatments. Standard deviations for days to A.M. only were; 8.0, 5.8, 11.0, and 10.8, and for days to weaning were; 5.9, 2.8, 8.2, and 6.5 for lasalocid delivery in none; M,PS,S; PS,S; and S respectively. This study demonstrated a greater consistency for calves fed lasalocid in milk, prestarter and starter to achieve earlier 1 X daily feeding and weaning, probably due to earlier rumen development. Anderson et al. (1988) investigated the value of lasalocid for stimulating rumen development, thereby increasing dry feed consumption and utilization and allowing earlier weaning. Early weaning stimulated earlier dry feed consumption which led to early development of rumen microflora (Anderson et al., 1987 a,b). The rumen microbes of early weaned calves contained high numbers of methanogens, leading to increased methanogenesis,

Figure 5. Days required to reach criterion for 1 time daily feeding and weaning for calves fed lasalocid in M,PS,S;PS,S; S; or none. Differing superscripts within the same week denote means with statistically significant differences ($P < .10$).



possibly reducing efficiency of weight gain (Thornton and Owens, 1981). The data in Figure 5 suggests that, not only did it assist calves that are weaned early, but when delivered in M,PS, and S, lasalocid encouraged earlier weaning.

Results of blood metabolite analyses are shown in Table 6, 7 and 8. Differences among treatment groups were observed for blood glucose and urea nitrogen concentrations at week 4; chloride, urea nitrogen, and creatinine concentrations at week 8; and chloride, urea nitrogen, creatinine, and potassium concentrations at week 12. The concentration of blood glucose of calves fed lasalocid in M,PS,S at week 4 was lower than the other groups' and urea nitrogen concentrations were higher.

These differences can be attributed to weaning and the onset of rumen development, however the values were within the normal range for bovine species. Blood urea nitrogen increases as rumen fermentation increases. This is consistent with the higher $\text{NH}_3\text{-N}$ values found in rumen fluid by Anderson (1987b) for early weaned calves and for lasalocid fed calves (Anderson et al., 1988). Lasalocid consumption in older animals caused an increase in $\text{NH}_3\text{-N}$ concentrations. Studies of older cattle have shown decreased blood glucose concentration with lasalocid consumption (Scheling, 1984, Spears and Harvery, 1982) and studies with young animals show a decrease in blood glucose concentration up to 8 weeks of age

TABLE 6. Blood metabolite analysis, week 4, of calves fed no lasalocid, lasalocid in M,PS,S, lasalocid in PS,S, or lasalocid in S.

Metabolite	Lasalocid Administration			
	None	M,PS,S ¹	PS,S	S
Creatinine (mg/dL)	.98	1.05	1.07	1.04
Potassium (mmol/L)	5.3	5.4	5.3	5.4
Chloride (mmol/L)	103.	104.	102.	103.
Glucose (mg/dL)	84. ^{ab}	74. ^a	82. ^{ab}	88. ^b
BUN ² (mg/dL)	13.1 ^{ab}	16.0 ^a	12.7 ^b	12.3 ^b
Sodium (mmol/L)	140.	141.	140.	140.
CO ₂ (mmol/L)	25.2	24.7	24.6	25.2
ALP ³ (U/L)	103.	116.	117.	138.
Total Protein (g/dL)	5.5	6.0	5.5	5.8
Albumin (g/dL)	3.1	3.1	3.1	3.2
Globulin (g/dL)	2.4	2.9	2.4	2.6
A:G ⁴	1.3	1.1	1.8	1.2
Calcium (mg/dL)	10.5	10.5	10.4	10.6
Phosphorus (mg/dL)	7.8	7.7	7.7	7.9

^{abc} means in the same row with differing superscripts differ (P < .10)

¹M=milk, PS=prestarter, S=starter.

²BUN=blood urea nitrogen.

³ALP=alkaline phosphatase.

⁴A:G=albumin to globulin ratio.

(Otterby and Linn, 1981).

Urea nitrogen at week 8 was highest for the control group and lowest for calves with lasalocid administered in PS,S. These data are consistent with the feed consumption and weaning data, all of which reflect rumen development. The control group was the second group to attain weaning criteria and second to increase feed consumption. The serum chloride concentration of the control group at week 8 was high and out of the normal range for the adult bovine. Creatinine for calves with lasalocid in PS,S was below the normal range at week 8, however this is not unusual for calves in confinement.

At week 12 blood urea nitrogen concentration was high and chloride concentration was above the normal range for control calves. The group fed lasalocid in PS,S had significantly lower chloride concentration, but the concentration was within the normal range. Creatinine at week 12 was below the normal range for adult bovine for the PS,S lasalocid-fed group and the control group. Potassium at week 12 was lowest for calves receiving lasalocid in M,PS,S and highest for the control group, but all fell within the normal range. These potassium values appear to reflect the time the calves in the treatment groups had been consuming lasalocid.

TABLE 7. Blood metabolite analysis, week 8, of calves fed no lasalocid, lasalocid in M,PS,S, lasalocid in PS,S, or lasalocid in S.

Metabolite	Lasalocid Administration			
	None	M,PS,S ¹	PS,S	S
Creatinine (mg/dL)	.83b ^{ab}	.81 ^a	.77 ^a	.88 ^b
Potassium (mmol/L)	5.2	5.0	5.0	5.1
Chloride (mmol/L)	104. ^b	100. ^a	102. ^{ab}	102. ^{ab}
Glucose (mg/dL)	89.	93.	90.	90.
BUN ² (mg/dL)	9.9 ^b	9.5 ^{ab}	7.6 ^a	9.2 ^{ab}
Sodium (mmol/L)	142.	141.	142.	143.
CO ₂ (mmol/L)	25.1	25.7	26.1	24.8
ALP ³ (U/L)	363.	292.	272.	326.
Total Protein (g/dL)	5.6	5.8	6.0	5.8
Albumin (g/dL)	3.2	3.2	3.2	3.2
Globulin (g/dL)	2.3	2.7	2.7	2.6
A:G ⁴	1.4	1.2	1.2	1.3
Calcium (mg/dL)	11.1	11.2	11.3	11.2
Phosphorus (mg/dL)	8.0	8.7	8.6	8.6

^{abc} means in the same row with differing superscripts differ (P < .10).

¹M=milk, PS=prestarter, S=starter.

²BUN=blood urea nitrogen.

³ALP=alkaline phosphatase.

⁴A:G=albumin to globulin ratio.

TABLE 8. Blood metabolite analysis, week 12, of calves fed no lasalocid, lasalocid in M,PS,S, lasalocid in PS,S, or lasalocid in S.

Metabolite	Lasalocid Administration			
	None	M,PS,S ¹	PS,S	S
Creatinine (mg/dL)	.74 ^c	.84 ^a	.75 ^{bc}	.81 ^{ab}
Potassium (mmol/L)	4.99 ^c	4.72 ^a	4.74 ^{ab}	4.93 ^{bc}
Chloride (mmol/L)	103. ^b	102. ^{ab}	99. ^a	101. ^{ab}
Glucose (mg/dL)	106.	106.	102.	109.
BUN ² (mg/dL)	13.3 ^b	9.2 ^a	9.9 ^a	9.5 ^a
Sodium (mmol/L)	145.	145.	140.	142.
CO ₂ (mmol/L)	26.0	26.4	25.2	25.5
ALP ³ (U/L)	312.	362.	274.	412.
Total Protein (g/dL)	6.0	6.6	6.1	6.2
Albumin (g/dL)	3.5	3.6	3.4	3.6
Globulin (g/dL)	2.4	3.0	3.0	2.6
A:G ⁴	1.5	1.2	1.2	1.4
Calcium (mg/dL)	11.6	11.6	11.3	11.4
Phosphorus (mg/dL)	8.8	8.7	8.6	8.6

^{abc} means in the same row with differing superscripts differ (P < .10).

¹M=milk, PS=prestarter, S=starter.

²BUN=blood urea nitrogen.

³ALP=alkaline phosphatase.

⁴A:G=albumin to globulin ratio.

CONCLUSIONS

Lasalocid delivery in milk, prestarter and starter promoted early weaning with less variability. Although the control group had the same mean number of days to reach criterion for weaning, there was a greater variation in that group. Calves receiving lasalocid in all three feeds showed increased ADG during wk 4-6 and equal to or greater ADG during wk 1-11 than calves receiving no lasalocid, lasalocid in PS and S, or S only. The feed intake of the group with lasalocid in all three feeds was greater than the other treatment groups throughout the twelve weeks of the experiment.

Lasalocid in PS and S was less effective than either lasalocid in milk, prestarter, and starter, or in starter only. Delivery at approximately 1.5 mg/kg of body weight appeared to be most effective, based on these data and those of Anderson et al.(1988). These data suggest that for use with young calves, lasalocid should be delivered in all three feeds. Further investigations of the most effective dosage levels are needed.

EFFECT OF SUPPLEMENTAL VITAMINS C AND E
IN MILK REPLACER
ON GROWTH AND HEALTH IN BULL CALVES

INTRODUCTION

Ascorbic acid is produced by the liver of many animals, including cattle. Lundquist and Phillips (1942) believed that ascorbic acid synthesis begins in calves between the second and third week of life and Bouda et al. (1980) established that adult levels of vitamin C are not present in the calf until after 3 months of age.

Milk, which has with a relatively low ascorbic acid content (Wegger and Moustgaard, 1982) is often exposed to air and light before being consumed by the calf. Both are destructive agents of vitamin C and therefore the milk may not contain adequate vitamin C for the calf. Hypovitaminosis C is therefore a potential problem with the young milk fed calf. This would be especially true for dairy calves whose only source of vitamin C for the first few weeks of life is bucket or bottle fed milk or milk replacer.

Vitamin C deficiency has been linked to compromised immunocompetence and elevated ascorbic acid concentrations have been linked to increased immune response in catfish (Li and Lovel, 1985, Durve and Lovell, 1982) poultry (Moffat et al., 1972, Ghoshal and Bhattacharyga, 1985, Thaxton and Pardue, 1984), cattle (Roth and Kaeberle, 1985, Blair and Cummins, 1984) and swine (Kristensen et al., 1986).

Conversely, others have found that vitamin C supplementation had no beneficial effect on the immune parameters measured (Siegel and Morton, 1984, Bendich et al., 1984, and Cummins and Brunner, 1989).

The value of vitamin E to the immune system of the young calf has been established (Cipriano et al., 1982, Reddy et al., 1986; 1987). The value of vitamin C and E supplementation together has been supported for other species (Bendich et al., 1984, Anderson, 1981, Packer et al., 1979, and Niki et al., 1982). Vitamin E is primarily responsible for protection of the cell membrane. As vitamin E interacts with free radicals at the membrane, it is reduced. A high concentration of vitamin C at the cell membrane is thought to regenerate the reduced vitamin E created during the oxidation-reduction process, which increases the available concentration of vitamin E (Bendich, 1987). Therefore, vitamins C and E work cooperatively to protect the cell membranes against peroxidation.

The purpose of the present study was to determine the effects of supplemental vitamin C alone and vitamins C and E together on the function of neutrophils and lymphocytes of the young calf's immune system, as well as their effect on the growth and general health of the calf during the first 8 wk of life.

MATERIALS AND METHODS

Thirty Holstein bull calves were removed from their dams at 24 h following birth. They were given transition milk for two more d then assigned to a block by date of birth. Calves within each block were randomly assigned to one of three treatment groups. The treatments consisted of milk replacers containing 1) no supplemental vitamin C or E, 2) 10g vitamin C per kg and no supplemental vitamin E, 3) 10g vitamin C and 57 IU vitamin E per kg. The basic milk replacer was an all milk, milk replacer with the following analysis: 45% lactose, 20.1% protein, 20.3% fat, 6.7% ash, 4170 Kcal/kg energy, 2.3 IU/kg vitamin E, and 0 vitamin C¹. Calves were bottle fed to increase the probability of rumen bypass for the eight wk of the study. Milk replacer was reconstituted to 13.5 % dry matter and was fed in two equal daily feedings at 10% of body weight, adjusted weekly. Calves were housed in individual hutches with straw bedding. Water was available ad libitum.

Body weights were measured and milk replacer allocations were adjusted weekly. Twice daily fecal (Larson et al., 1977) and general appearance scores were recorded, and checks for eye or nasal discharge or signs of enteric or respiratory

¹Milk Specialties Company, Dundee, IL.60118.

illness were noted. Blood samples were collected in heparinized tubes on experimental d 1, 14, 28, and 56 for vitamin C and E determinations. Day 14 and 28 samples were used to assay lymphocyte proliferation and neutrophil function. A lymphocyte transformation assay (LTA), and antibody-dependent cellular-cytotoxicity (ADCC) and S. aureus phagocytosis assays were used to determine lymphocyte cellular function.

Lymphocyte and Neutrophil Isolation and Cryopreservation

All blood samples were kept on ice until reaching the lab. Whole blood samples were centrifuged at 700 x g for 10 minutes and the plasma was removed and frozen (-20° C) for later determination of vitamins C and E. Isolation and proliferation of mitogen-stimulated peripheral blood mononuclear cells (PBMC) were measured as previously described (Blecha and Baker, 1986). Briefly, 30 ml of heparinized blood were centrifuged at 600 x g for 15 min at 25°C. The buffy coat, diluted 1:2 with RPMI, was layered onto ficoll/sodium diatrizoate (Histopaque-1077)². The remaining red cell layer was saved in an ice bath for neutrophil isolation. After centrifugation at 400 x g for 40 min at 25°C, the mononuclear

²Sigma Chemical Co., St. Louis, MO 63178.

cell layer was collected. Cells were then washed three times in RPMI, counted and suspended at 2.5×10^6 cells/ml in RPMI supplemented with 20% FBS, MEM-nonessential amino acids, MEM essential amino acids, MEM vitamins, 1 mM sodium pyruvate, 2 mM L-glutamine, 100 U/ml penicillin, 100 μ g/ml streptomycin, fungizone, 24 mM NaHCO_3 , 10 mM HEPES (cDMEM)³ and 10% dimethylsulfoxide (DMSO) according to methods previously described (Kleinschuster et al., 1979, Kuil, 1984, Birkeland, 1976, and Takahashi et al., 1985). The cells were frozen immediately in a Que Cryostar at -135°C .

Neutrophil isolation and antibody-dependent cellular-cytotoxicity (ADCC) assay procedures were a modification of the methods of Rouse et al., (1976) and Roth and Kaeberle, (1981a,b). The red cell layer was lysed by suspension in 10 ml cold sterile deionized water for neutrophil separation. An equal volume of RPMI was added to restore isotonicity. The cells were centrifuged at 700 RPM at 4°C for 10 min. This lysis procedure was repeated two more times. Throughout the procedure all media and tubes were kept in an ice bath. The neutrophils were frozen as described for the lymphocytes.

Thawing procedures were modifications of those described by Kleinschuster et al. (1979). Briefly, cells, either

³GIBCO, Grand Island, NY 14072.

neutrophils or lymphocytes, were placed in a 37° C water bath just until the last crystal melted. The cells were added to 5 ml RPMI and centrifuged, lymphocytes at 1600 RPM and neutrophils at 700 RPM. Both cells were washed 2 more times with RPMI. Five samples each from lymphocytes at 2 and 4 wk and neutrophils at 2 and 4 wk were counted using Trypan Blue exclusion method for viability. All cells were counted on a Coulter Counter and lymphocytes adjusted for viability to 2×10^6 and neutrophils to 2.5×10^6 cells/ml.

Antisera Preparation

Two 3 mo old calves were hyperimmunized for three wk at 1 wk intervals with washed chicken red blood cells (CRBC) from 10 ml fresh chicken blood. Calf blood samples were collected for sera separation at 1 and 2 wk following the final immunization. Sera were heat inactivated (30 min in a 57° C water bath) and frozen at - 20° C. Sera were later diluted by a factor of 10 with RPMI and refrozen until thawed at 23° C just prior to use in the ADCC assay.

Target Cells

Approximately 1 ml of whole chicken blood was collected into a heparinized tube, then centrifuged at 1500 RPM for 15 min. The plasma and buffy coat were discarded.

The CRBC were washed with RPMI 3 times. Two tenths cc sodium-chromate was added to the CRBC and incubated for 3 h. The cells were then washed 3 time in RPMI and counted for use in the ADCC.

ADCC Procedures

The labeled CRBC were diluted to 100,000 cells/ml for a 25:1 effector to target ratio. Spontaneous release mixture, (.1 ml CRBC and .1 ml RPMI), maximum release mixture, (.1 ml CRBC and .1 ml Triton), and reaction mixtures, (.1 ml CRBC, 20 μ l CRBC antibody, and .1 ml neutrophils), were each added to three wells of a 96 well Costar microtiter plate. The cells were incubated at 37⁰ C for 18 h. The supernatant was harvested and the gamma emissions counted. Results were expressed as a percent of specific release with:

Percent specific release =

$$\frac{\text{mean CPM test release} - \text{mean CPM spontaneous release}}{\text{mean CPM maximum release} - \text{mean CPM spontaneous release}} \times 100$$

Lymphocyte Transformation Assay (LTA)

Proliferative responses to mitogens were evaluated by incubating 100 μ l of PBMC at 2.5×10^6 cells/ml with 100 μ l of

concanavalin A (20 $\mu\text{g}/\text{ml}$)⁴, phytohemagglutinin (10 $\mu\text{g}/\text{ml}$)⁵, and pokeweed mitogen (100 $\mu\text{l}/\text{ml}$)⁶ for 66 hours in a humidified 93% air-7% CO_2 atmosphere. Tritiated thymidine (20 μl at 0.075 $\mu\text{Ci}/\mu\text{l}$, sp act.=6.7 Ci/mM)⁷; was added for the last 18 hours of incubation. Cells were harvested onto filter paper for liquid scintillation spectrophotometry⁸ with an automated cell harvester⁹

Vitamin C Determination

A modification of the method of Albanese, et al. (1975) was used for vitamin C analysis. Modification was the use of 4 ml 30% Trichloroacetic acid (TCA) to extract ascorbic acid from 1 ml plasma sample, due to low concentrations of ascorbic acid in samples. Briefly, 1 ml plasma was added to 4 ml 30% TCA and centrifuged at 2500 RPM for 10 minutes. One ml of

⁴Pharmacia Inc., Piscataway, NJ.

⁵Burroughs Wellcome Co., Research Triangle Park, NC.

⁶GIBCO, Grand Island, NY.

⁷ICN Chemicals, Irvine, Ca.

⁸Packard Tri-carb, model 4450, Packard Instrument Co., Downers Grove, Il.

⁹PHD Cell Harvesting System, model 200A, Cambridge Technology Inc., Cambridge, MA.

tenths ml of 2-4 dinitrophenylhydrazine was added to each tube, the tube was stoppered, and heated for 20 min in a 90° C water bath. The tubes were cooled for 5 min in an ice bath. One and one half ml 65% H₂SO₄ was added to each tube. Absorbance was then determined on a spectrophotometer at 520 nm.

S. aureus Phagocytosis

Staph aureus phagocytosis was conducted as described by Roth and Kaeberle (1981a). Briefly, heat killed ³H-Glycine-labeled S. aureus cells were mixed with neutrophils and incubated for 10 min at 37°C. Lysostaphin was then added and the mixture incubated for 30 min more. The cells were washed twice in phosphate buffer saline and the radioactivity of the remaining pellet was determined in a gamma counter. Results were expressed as a percent of total S. aureus ingested with:

Percent ingestion =

$$\frac{(\text{CPM in reaction tube}) - (\text{CPM in background tube})}{(\text{CPM in standard tube}) - (\text{CPM in background tube})} \times 100$$

RESULTS AND DISCUSSION

Milk Replacer Vitamin Concentration Verification

To check stability of vitamin C in milk replacers, samples were collected for analysis from an unopened bag older than one month, an unopened bag less than two weeks old, and from a covered bucket in which the milk replacer was stored for daily use. Results are in Table 1. All control milk replacer samples had about 1 mg/kg of vitamin C concentration. The samples of milk replacers with vitamin C only (10 mg/kg) had about 7.0 mg/kg vitamin C concentration. The mix with both vitamins C and E taken directly from the bags had less oxidation of the vitamin C (7.6 and 9.0 mg/kg) while the mix from the bucket was similar to that of all the vitamin C only mixes.

Daily Health and Growth

Plasma vitamin C and E concentrations for wk 0, 2, 4, and 8 are in Figures 1 and 2. The plasma vitamin C concentration of the control group dropped gradually through week 4 and then returned to approximately the same concentration as all other treatments by week 8. The group receiving vitamin C in the milk replacer began with a greater concentration of plasma vitamin C and gradually fell at week

Table 1. Vitamin C content of milk replacers from old mix (older than 1 mo, from an unopened bag), new mix (less than 2 wk old, from an unopened bag), or mix stored in a daily use bucket.

Mix	Vitamin C (g/kg)
Old: Control	1.0
Vit. C	7.0
Vit. C & E	9.0
New: Control	1.0
Vit. C	6.9
Vit. C & E	7.6
Bucket: Control	1.1
Vit. C	7.2
Vit. C & E	7.1

Figure 1. Plasma vitamin C concentrations at 0, 2, 4, and 8 weeks for calves supplemented with 0 vitamin C and 0 vitamin E, 10 g/kg vitamin C and 0 vitamin E, or 10 g/kg vitamin C and 57 IU/kg vitamin E.

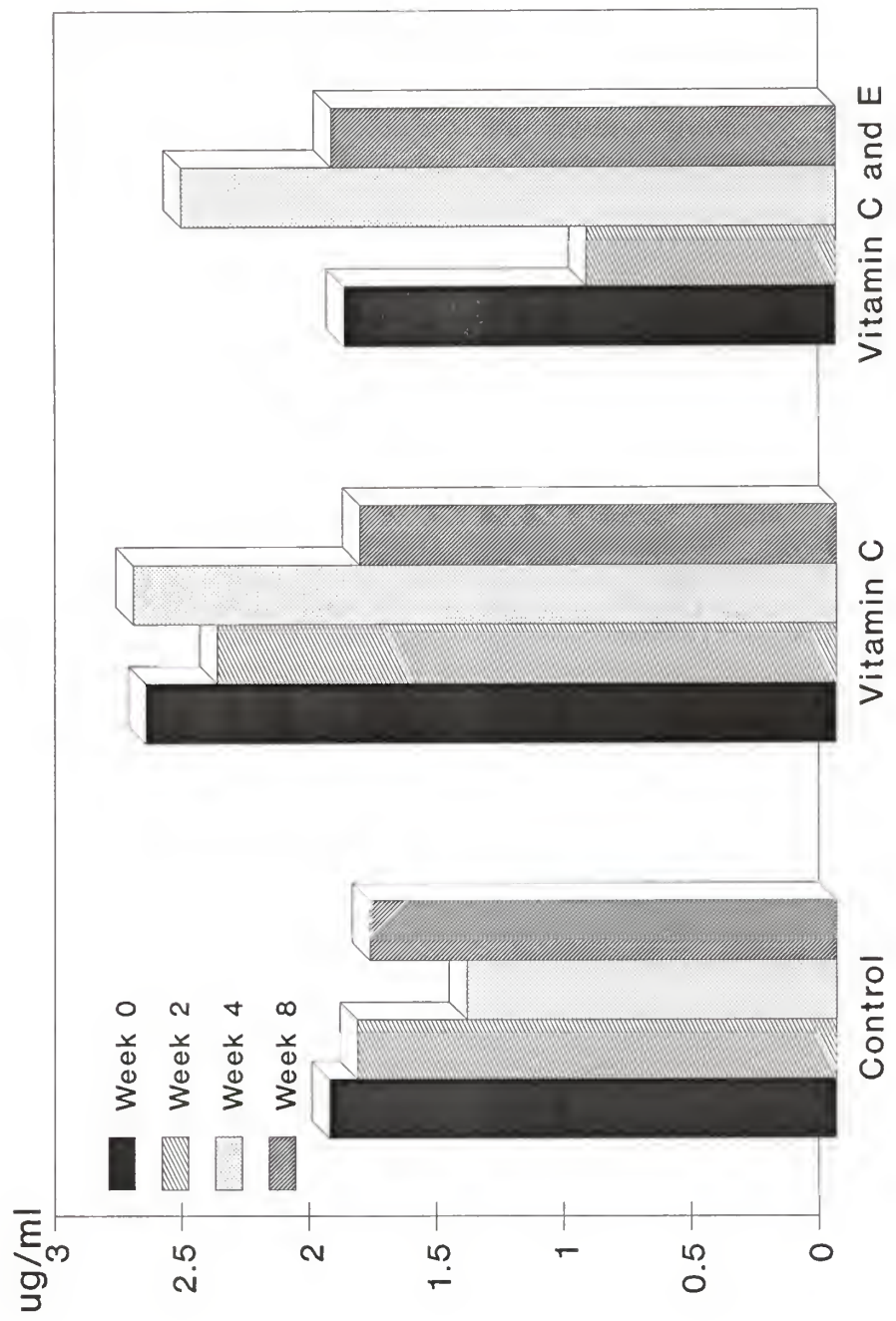
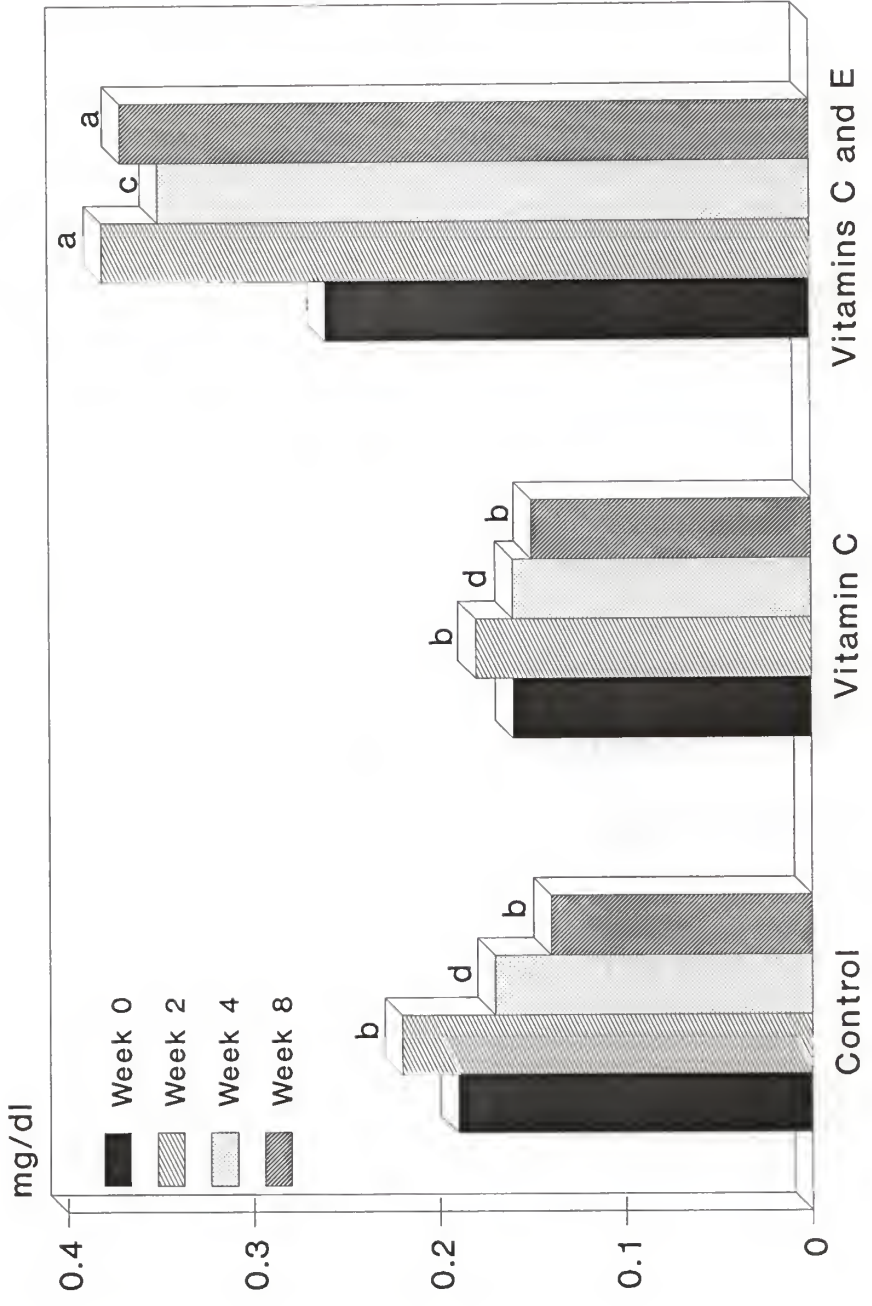


Figure 2. Plasma vitamin E concentrations at 0, 2, 4, and 8 weeks for calves supplemented with 0 vitamin C and 0 vitamin E, 10 g/kg vitamin C and 0 vitamin E, or 10 g/kg vitamin C and 57 IU/kg vitamin E. Differing superscripts within the same week denote means with statistically significant differences. ^{a,b} (P < .05. ^{c,d} (P < .10).



2 and wk 8, reaching the same concentrations as the other two groups at week 8. The group with vitamins C and E supplementation in the milk replacer showed a large decrease in plasma vitamin C concentration by week 2. This group showed a large rebound in plasma vitamin C concentration at week 4 which then returned to the same concentration as the other groups by week 8. The values reported here are low compared to previous reports of vitamin C concentration of young calves. However, in this study, all samples were taken 30 minutes prior to feeding, which is the lowest point of vitamin C concentration during the day. The plasma samples did not contain any antioxidant during freezing which may account for some of the reduced concentrations.

Plasma vitamin E concentrations reflected the supplementation of that vitamin. A similar pattern of vitamin E plasma concentration occurred for the control calves and the calves supplemented with only vitamin C. A slight increase at week 2 and a continued decrease throughout the experiment of plasma vitamin E concentration was seen in both groups. It appears vitamin C had no effect on the vitamin E plasma content at this low concentration. The vitamin C and E supplemented group, although beginning at a higher level, had significant increases in plasma vitamin E concentrations at wk 2, 4, and

8 above that of the control and of the vitamin C only groups.

Total gain, overall feed efficiency and, total ocular and nasal discharge data are presented in Table 2. The net gains of the calves on treatments were not significantly different from the net gain of the control calves. However, the smaller gains of the vitamin C supplemented calves were statistically different from the increased gains of the vitamin C and E supplemented group. The net feed efficiency was determined by calculating kg dry weight of milk replacer fed per kg gain. The net efficiency of the vitamin C and E supplemented group was significantly lower, indicating greater feed efficiency, than those of the vitamin C supplemented group or of the control group. The three groups performed similarly in daily gain through wk 5, except for a decrease of the vitamin C and E supplemented group at wk 3. Weekly average daily gain (Figure 3) showed significant differences at week 6 and 8 between the two treatment groups and a significant difference between the vitamin C and E supplemented calves and the control calves at week 7. The vitamin C and E group showed more variation between weeks throughout the eight weeks while the vitamin C group deviated from the control group only at week 6.

Feed efficiency data (Figure 4) show no differences between treatments until week 6 and 7. At week 6 vitamin C

Table 2. Overall gain and feed efficiency and total ocular and nasal discharge for calves supplemented with no vitamin C and no vitamin E, 10 g/kg vitamin C and no vitamin E, or 10 g/kg vitamin C and 57 IU/kg vitamin E.

	Control	Vit. C	Vit. C & E
Gain (kg)	28.1 ^{ab}	26.2 ^a	29.4 ^b
Feed Efficiency (kg dry feed/kg gain)	1.65 ^a	1.61 ^a	1.41 ^b
Discharge	17.0	10.5	9.0

a,b

Differing superscripts within the same variable differ (P < .10).

Figure 3. Average daily gain of calves supplemented with vitamin C and 0 vitamin E, 10 g/kg vitamin C and 0 vitamin E, or 10 g/kg vitamin C and 57 IU/kg vitamin E. Differing superscripts within the same week denote means with statistically significant differences ($P < .10$).

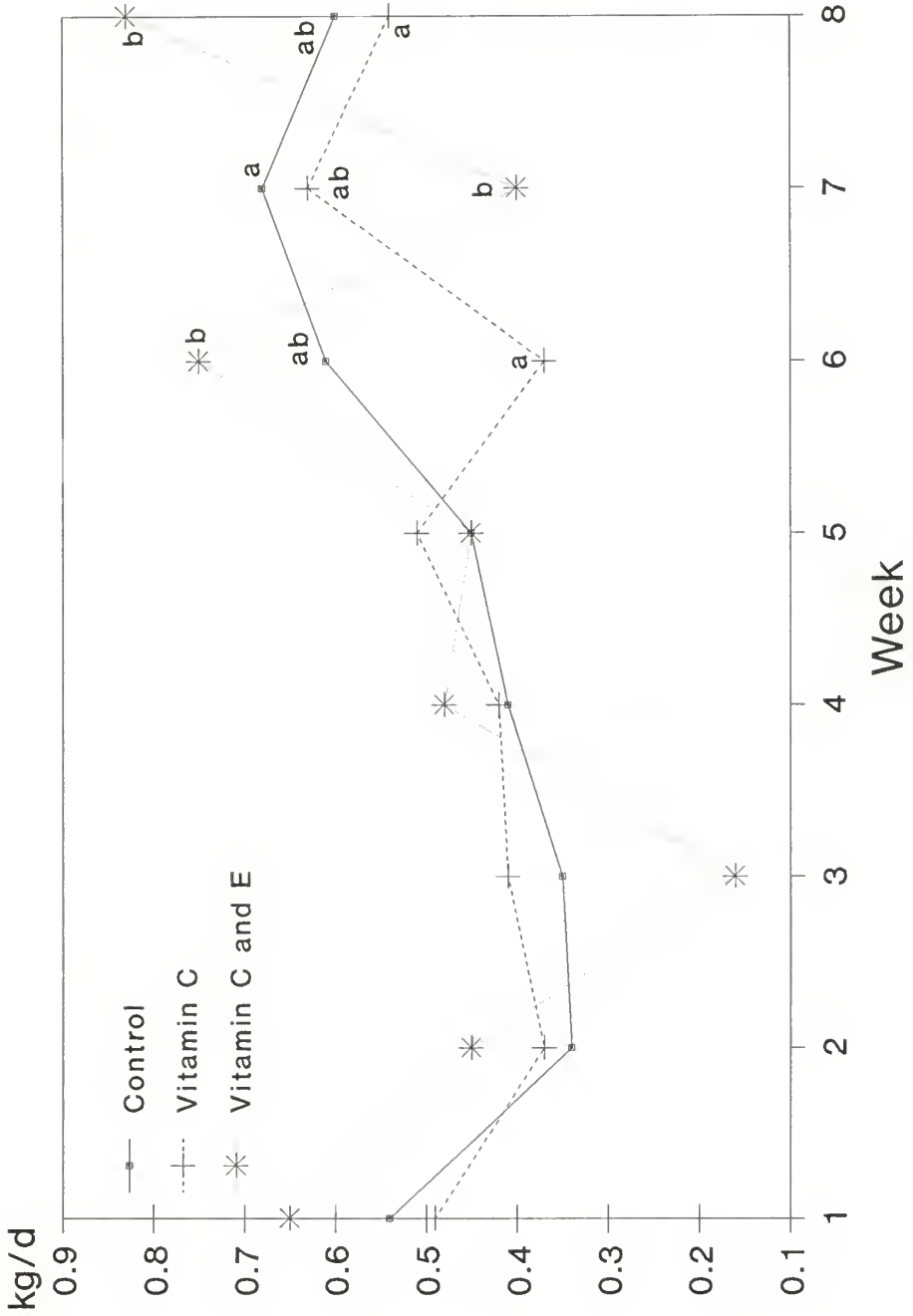
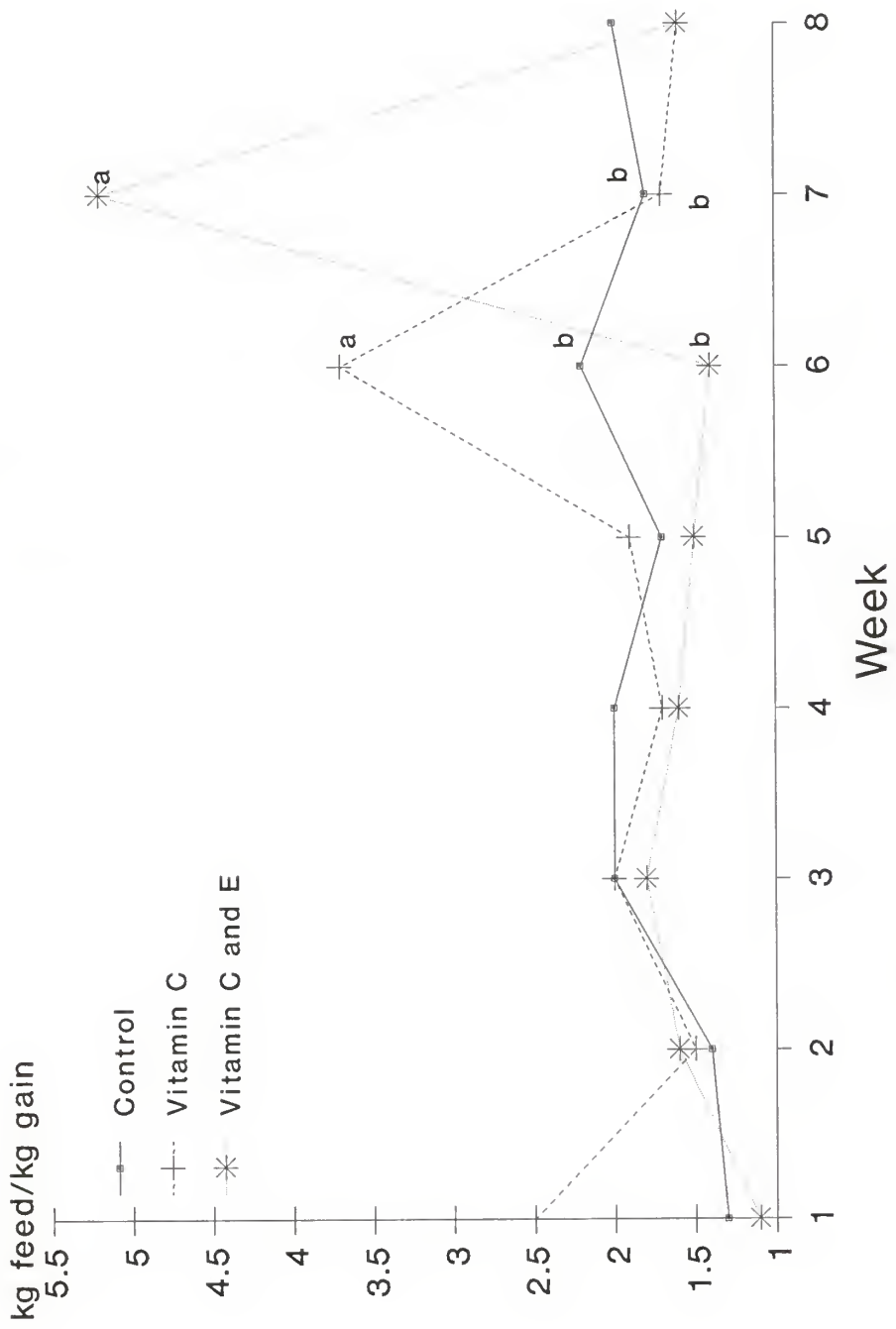


Figure 4. Feed efficiency of calves supplemented with 0 vitamin C and 0 vitamin E, 10 g/kg vitamin C and 0 vitamin E, or 10 g/kg vitamin C and 57 IU/kg vitamin E. Differing superscripts within the same week denote means with statistically significant differences ($P < .10$).



supplementation group was statistically different from the control and the vitamin C and E supplemented group. At week 7 the vitamin C and E group was significantly different from the control and vitamin C only groups. It was at week 7 that a decrease in ADG was seen for that group as well.

Figure 5 shows weekly mean fecal scores for all groups. The control group tended to have the lowest fecal scores throughout the 8 wk and the vitamin C supplemented group tended to have the highest scores. The control and vitamin C supplemented groups were statistically different ($p < .10$) at wk 2, 6, and 8, and at week 8 the vitamin C group was also significantly higher than the vitamin C and E supplemented group.

Mean eye and nose discharge observations tended to be higher for the control group for all 8 wk (Figure 6). At week 3 the vitamin C and E supplemented calves had significantly lower observations of discharges than the control group and the vitamin C only supplemented group was significantly different than the control calves at week 7.

Cryopreservation and Fresh Cell Comparisons

The reductions in function of cryopreserved cells from that of fresh cells are shown in Table 3. Both lymphocytes and neutrophils demonstrated acceptable percentages of

Figure 5. Fecal scores of calves supplemented with 0 vitamin C and 0 vitamin E, 10 g/kg vitamin C and 0 vitamin E, or 10 g/kg vitamin C and 57 IU/kg vitamin E. Differing superscripts within the same week denote means with statistically significant differences ($P < .10$).

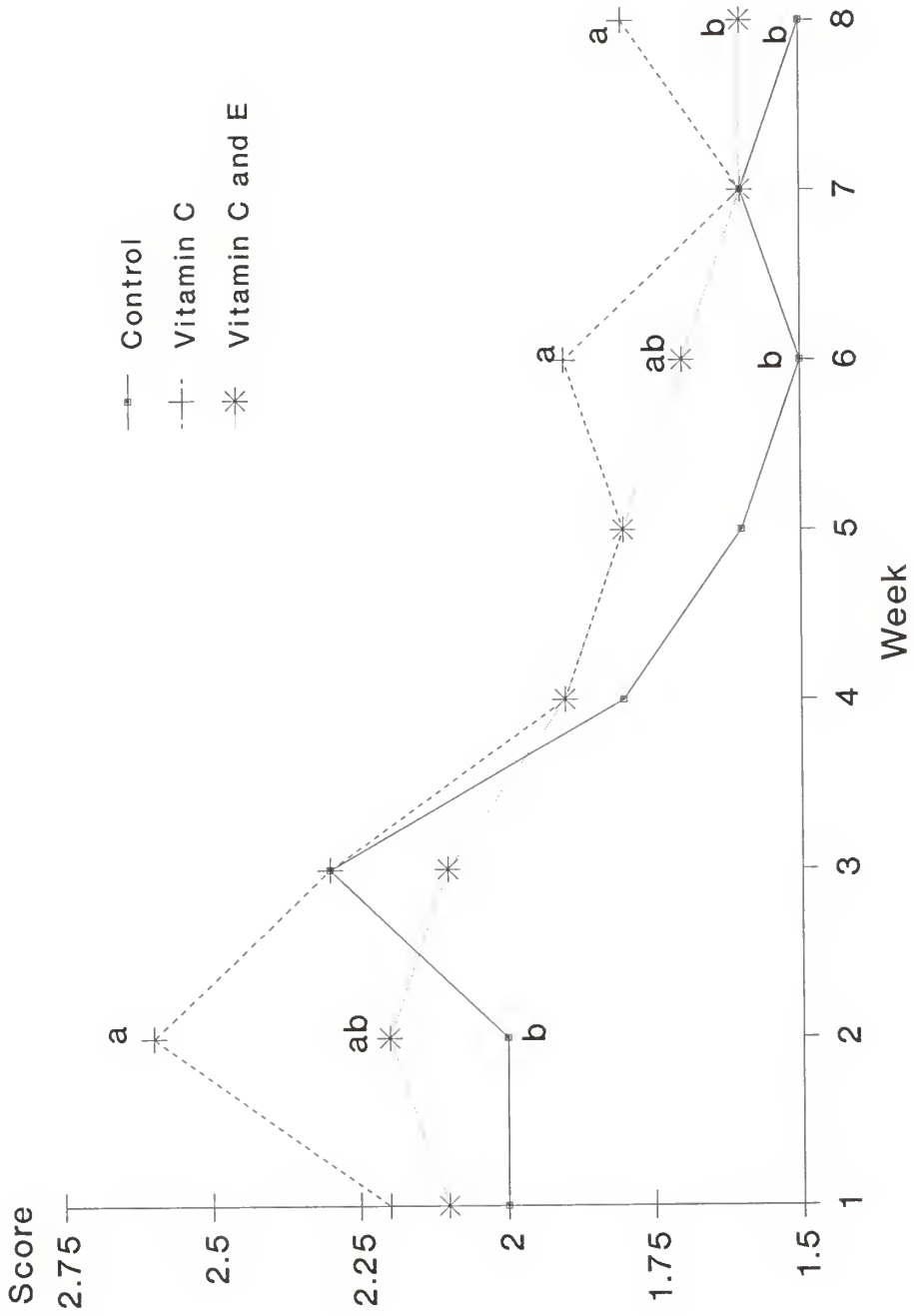


Figure 6. Ocular and nasal discharge of calves supplemented with 0 vitamin C and 0 vitamin E, 10 g/kg vitamin C and 0 vitamin E, or 10 g/kg vitamin C and 57 IU/kg vitamin E. Differing superscripts within the same week denote means with statistically significant differences ($P < .10$).

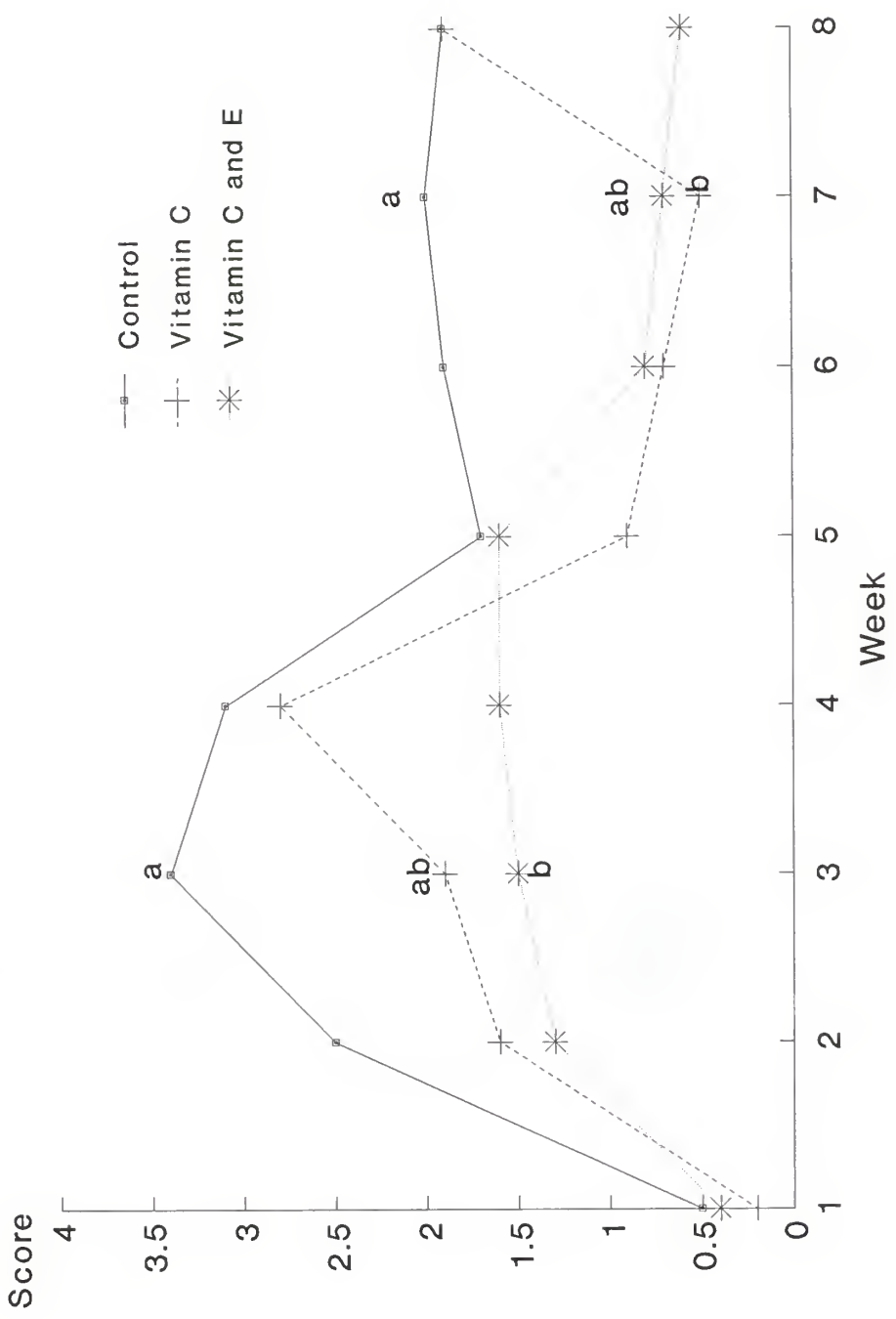


Table 3. Percent reduction in function and viability after cryopreservation for lymphocytes and neutrophils.

Trt.	Con A	PHA	PWM	% reduction in Proliferation (cpm x 1000)	% reduction in Neutrophil-mediated specific lysis
Control	55	70	60		42
Vit. C	52	70	28		44
Vit. C & E	47	57	62		29
Viability	92%				90%

viability, 90% and 92% for neutrophils and lymphocytes, respectively. The percent reduction of lymphocyte proliferation in response to Con-A mitogen from fresh to frozen cells was similar across treatments. The PHA mitogen stimulated lymphocytes and the neutrophils were similar in percent reduction of function for the control and vitamin C supplemented groups. There was less reduction of cellular function of cells from calves supplemented with vitamins C and E. This was true for both neutrophils and lymphocytes stimulated with PHA. The cryopreserved lymphocytes stimulated with PWM displayed a great variability from the response of fresh cells, some increasing and some decreasing from the fresh cell response, resulting in a reduction in function overall. It appeared from these data that neutrophils and lymphocytes stimulated with Con-A and PHA were predictably effected by cryopreservation. However, the data obtained with the use of cryopreserved cells stimulated with PWM are questionable due to the great variability in response of cells following cryopreservation as well as the mean differences between treatments for percent reduction in function.

Immune Function

Lymphocyte transformation assay results appear in Figures 7, 8, and 9. Due to the variability in the lymphocyte

Figure 7. Lymphocyte proliferation with concanavalin A mitogen for calves supplemented with 0 vitamin C and 0 vitamin E, 10 g/kg vitamin C and 0 vitamin E, or 10 g/kg vitamin C and 57 IU/kg vitamin E.

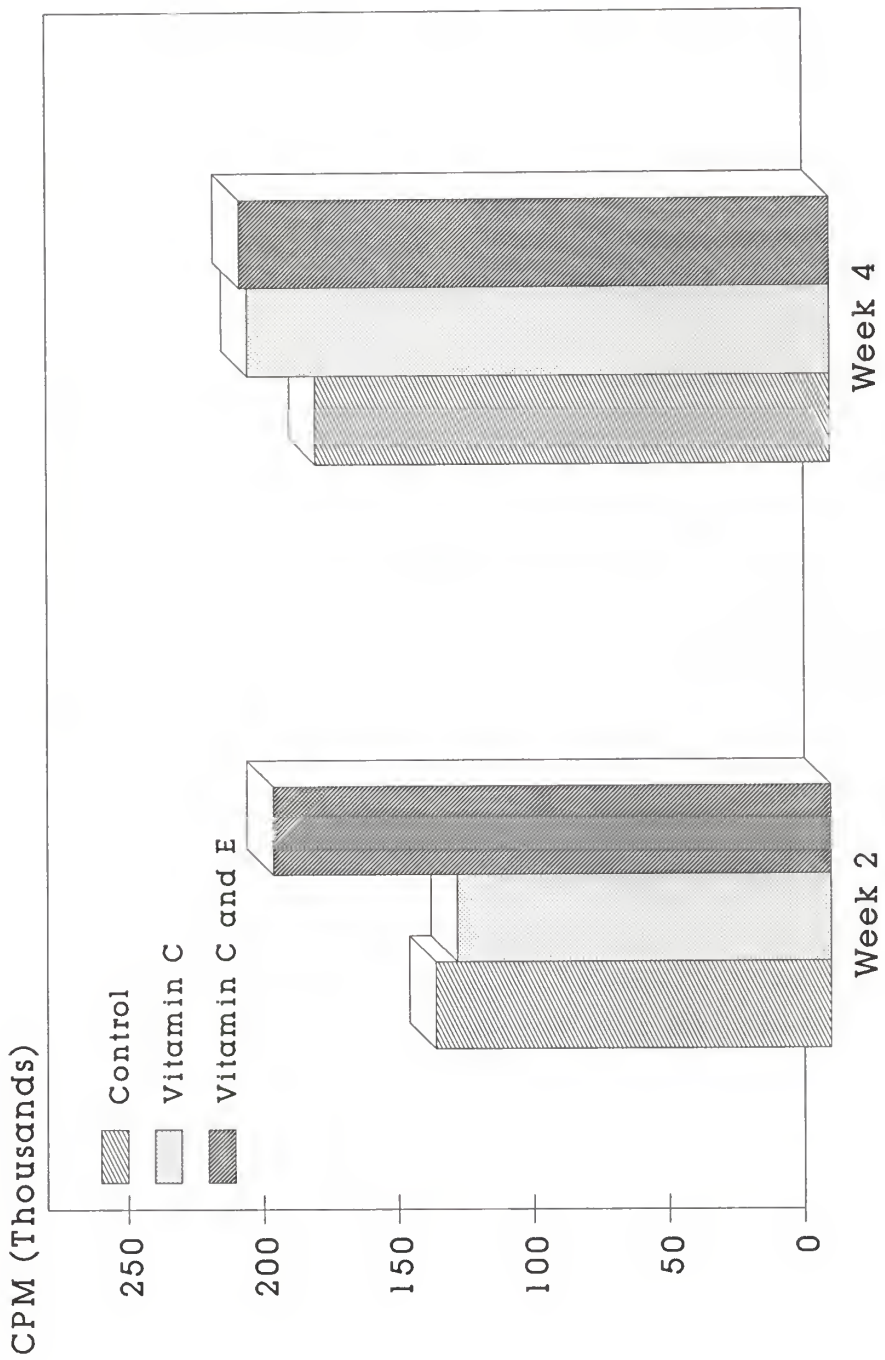


Figure 8. Lymphocyte proliferation with phytohemagglutinin for calves supplemented with 0 vitamin C and 0 vitamin E, 10 g/kg vitamin C and 0 vitamin E, or 10 g/kg vitamin C and 57 IU/kg vitamin E.

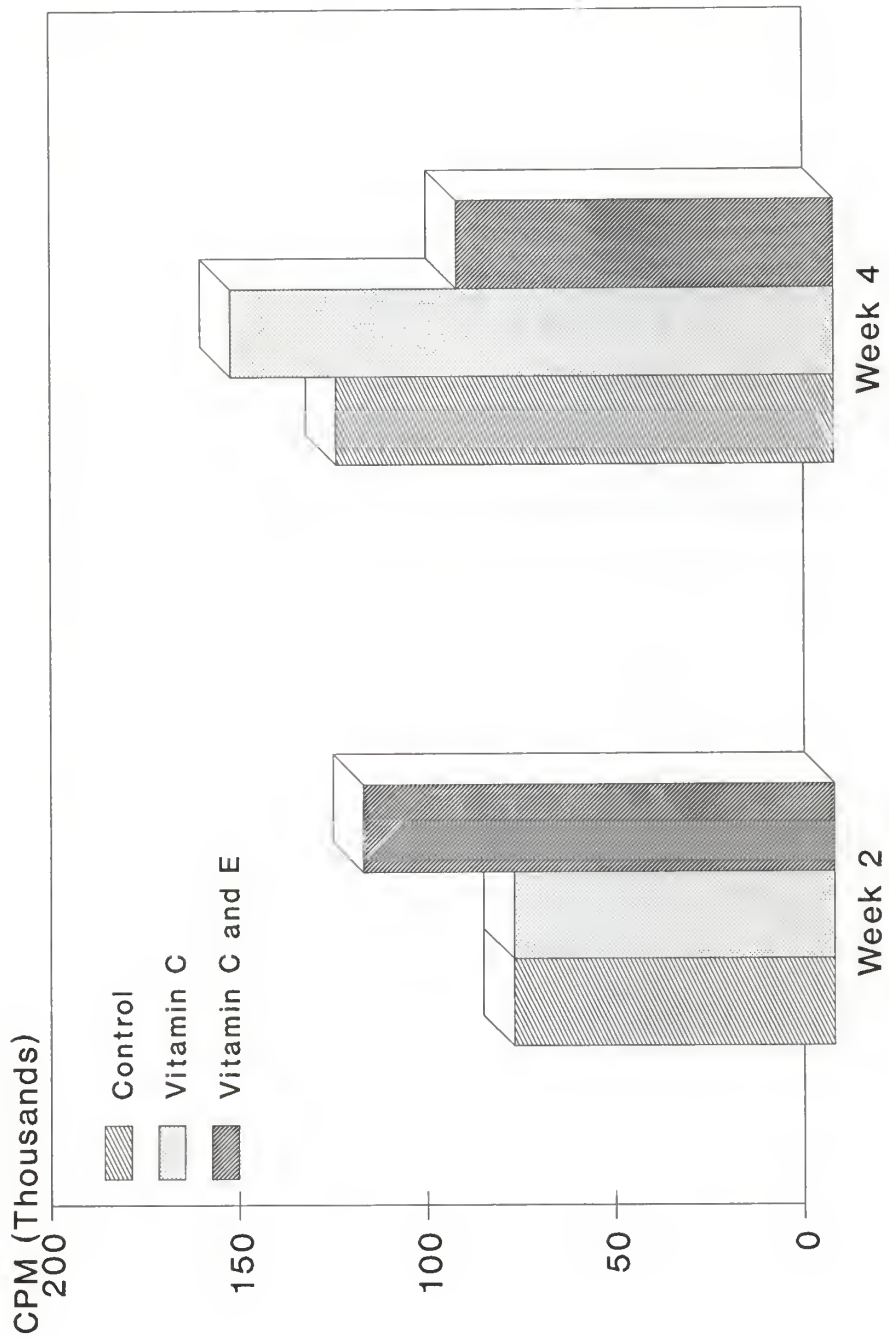
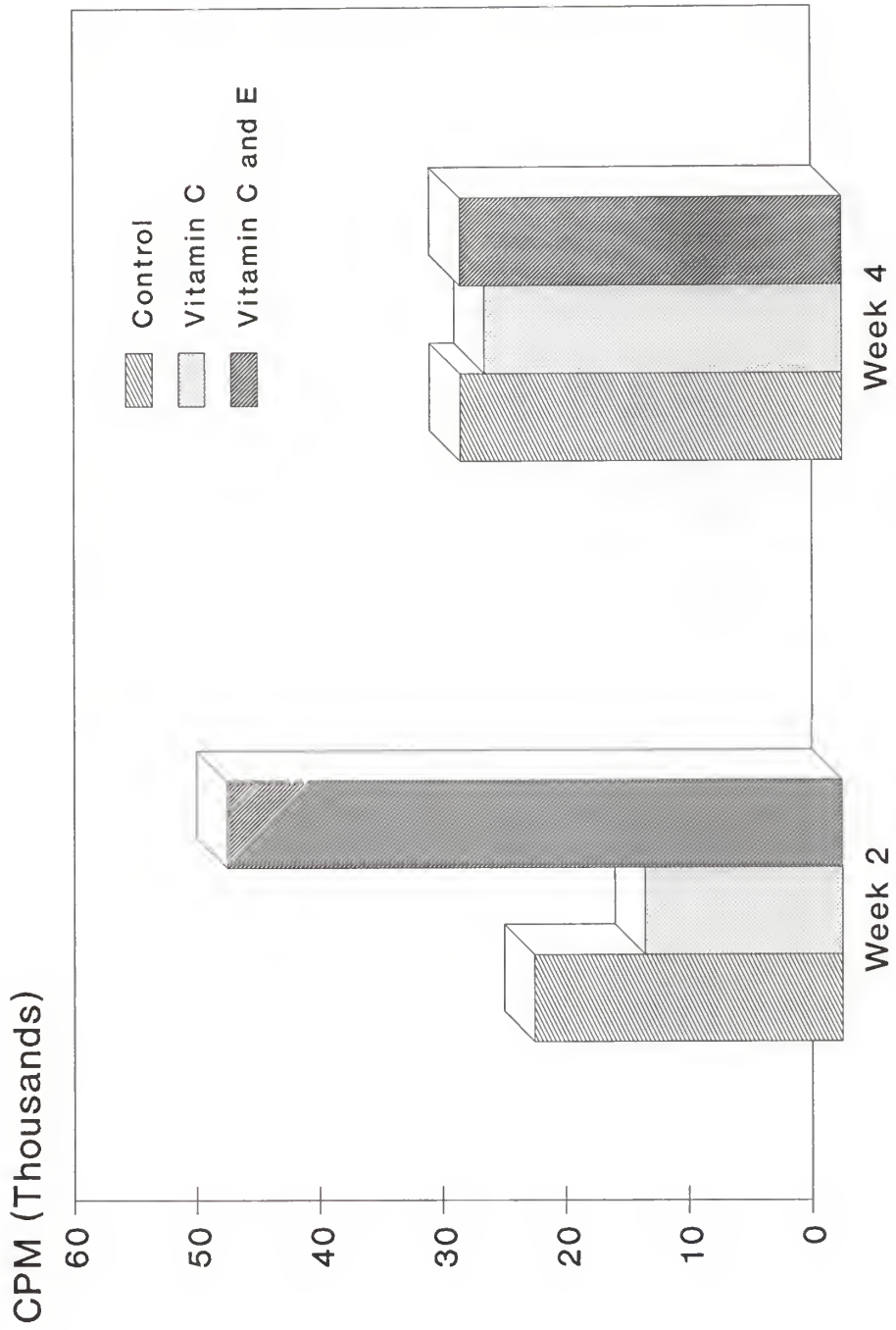


Figure 9. Lymphocyte proliferation with pokeweed mitogen for calves supplemented with 0 vitamin C and 0 vitamin E, 10 g/kg vitamin C and 0 vitamin E, or 10 g/kg vitamin C and 57 IU/kg vitamin E.



functions of the 2 and 4 week old calf no significant differences between the treatment groups can be noted in the Con-A and PHA mitogen induced proliferation means. Week 2 lymphocytes of vitamin C and E supplemented calves had greater proliferation. By week 4 the lymphocytes of vitamin C vitamin supplemented calves had equal to or greater proliferative responses than that of the C and E supplemented calves. The PWM responses had extreme variability within each group and did not appear to be a good measure for these calves.

The neutrophil mediated ADCC at week 2 (Figure 10) showed significant differences between the vitamin C supplemented group and the control at week 4, and between the two vitamin supplemented groups at week 2. The same cells used in S. aureus phagocytosis assays showed the vitamin C group significantly different from the control a week 2, but no significant differences were detected at week 4 with this measure (Figure 11). However the same trend as seen in the ADCC is evident at week 4.

CONCLUSION

The supplementation of vitamin C alone and vitamins C and E together did not have a beneficial effect on any of the immune responses measured here. However, the lower incidence of mucous discharge suggests a beneficial effect that was not

Figure 10. Neutrophil-mediated antibody-dependent cellular cytotoxicity % specific-lysis for calves supplemented with 0 vitamin C and 0 vitamin E, 10 g/kg vitamin C and 0 vitamin E, or 10 g/kg vitamin C and 57 IU/kg vitamin E. Differing superscripts within the same week denote means with statistically significant differences ($P < .10$).

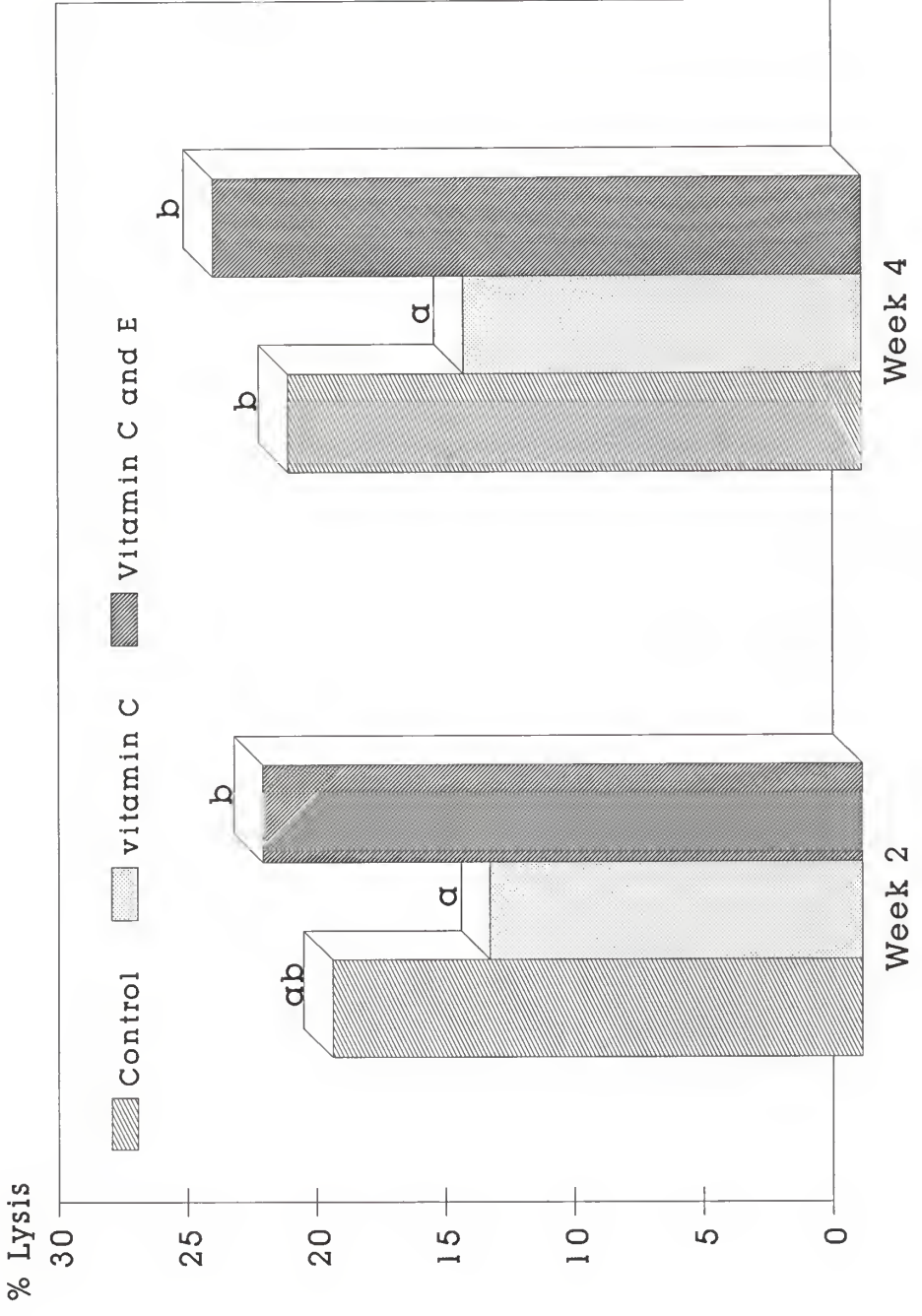
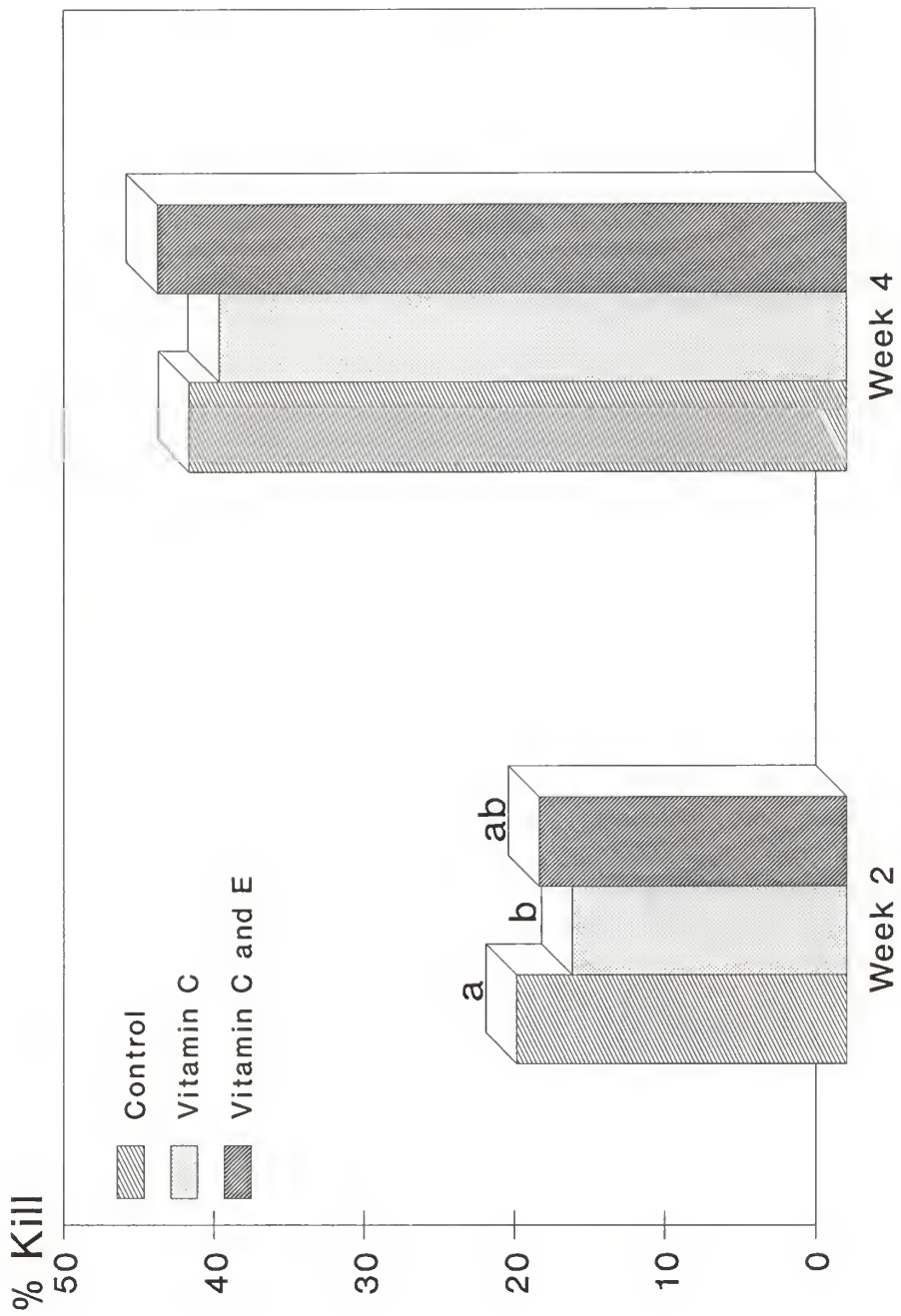


Figure 11. Neutrophil-mediated S. aureus phagocytosis % kill by neutrophils from calves supplemented with 0 vitamin C and 0 vitamin E, 10 g/kg vitamin C and 0 vitamin E, or 10 g/kg vitamin C and 57 IU/kg vitamin E. Differing superscripts within the same week denote means with statistically significant differences ($P < .05$).



reflected by the cellular functions we measured. The use of vitamins C and E together appeared to negate the adverse effects of vitamin C alone on neutrophil functions at both 2 and 4 wk and on lymphocyte proliferation at wk 2. The synergistic relationship of vitamin C and E and its effect on the immune cell's functions and other possible roles of vitamin C in the immune system merit further investigation.

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GROWTH AND HEALTH OF YOUNG CALVES WITH
VARIOUS ROUTES OF LASALOCID ADMINISTRATION or
ASCORBIC ACID OR ASCORBIC ACID AND VITAMIN E SUPPLEMENTATION

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Measurements of the growth and health of young dairy calves as influenced by lasalocid administration or vitamin C and vitamins C and E supplementation were determined. Forty Holstein bull calves were assigned to one of four treatment groups; no lasalocid, lasalocid in starter, lasalocid in prestarter and starter, or lasalocid in milk, prestarter and starter for a twelve week trial period. Calves were fed milk at 8% of birth weight daily divided into two equal feedings, until they consumed 227 g of prestarter. Then the afternoon milk feeding was discontinued and as much starter as the calf would consume was added to the prestarter. When total dry feed consumption was at 1.3% of birth weight the calf was weaned. At five weeks of age the prestarter was discontinued.

Daily gain was greatest during the first six weeks for the calves with lasalocid in milk, prestarter and starter. The calves receiving lasalocid in milk, prestarter and starter were weaned earlier with less variation. There were no differences in gain by week twelve between the treatments. Lasalocid in milk, prestarter and starter was the most effective delivery of lasalocid of the three routes of lasalocid administration given.

Thirty Holstein bull calves were bottle-fed one of three milk replacers at 10% of weekly adjusted body weight for eight weeks. Milk replacers were supplemented with

vitamins C and E at : (1) 0.0 and 0.0, (2) 10g and 0.0, or (3) 10g and 57 IU/kg, respectively. Lymphocytes and neutrophils isolated from blood samples collected on day 14 and 28 were cryopreserved and later analyzed for neutrophil-mediated antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis of S. aureus, and for concanavalin A (con A), phytohemagglutinin (PHA), and pokeweed mitogen (PWM) induced proliferation.

Cryopreservation of cells adversely affected immunological functions in all assays, however function was affected predictably in all but PWM stimulated cells of calves supplemented with vitamin C. The ocular and nasal discharge scores of calves supplemented with vitamin C and vitamins C and E were lower than those of control calves for all eight weeks. Neutrophils of calves supplemented with vitamin C had phagocytosis and lysis functions decreased from control calf neutrophil functions at weeks two and four. Neutrophil function of calves supplemented with vitamins C and E together was near the level of controls or slightly higher for weeks two and four, suggesting that the addition of vitamin E with vitamin C negated the adverse effects that vitamin C alone had on neutrophil mediated ADCC and phagocytosis. A similar trend, reduced gain in vitamin C supplemented calves and a slight increase in gain in vitamin C and E supplemented calves from controls was seen. Lymphocyte proliferation with all three

mitogens showed a trend for vitamin C and E supplemented calves to have a higher response at week two but no difference between treatments at week four.