An Abstract of the Thesis of

Calvin L. Nunn for the degree of Master of Science in Animal Science presented on April 16, 1996. Title: Effect of Vitamin E on Scours in Neonatal Beef Calves.

Abstract approved: Redacted for Privacy

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Objectives of this study were to test the effects of supplementing vitamin E to cows and calves fed diets deficient in vitamin E on calves' immunocompetence; morbidity of calves, specifically scours; and subsequent productive and reproductive performance of the cows and calves. This study was conducted during the winter and calving periods of 1994 and 1995 at the Eastern Oregon Agricultural Research Center (EOARC) Union, Oregon. Station cattle are Hereford x Simmental with 66 and 35 primiparous and 144 and 158 multiparous cows utilized in 1994 and 1995 respectively. Cattle were stratified by age with stratum assigned randomly to treatment. Cows in 1995 remained on the same treatment which they were assigned in 1994. Treatments consisted of pellets fed at the rate of .45 kg per day. Vitamin E treated pellets contained 3,310.3 IU per kg (1,500 IU d^{-1}). Controls received no additional vitamin E in their pellets. Calves were assigned to the treatment regimen corresponding to their dams. Control calves received no vitamin E supplementation. Vitamin E treated calves received intramuscular injections of vitamin E at birth (3,000 IU), and 900 IU at 2 weeks and 900 IU at 4 weeks of age. Calves were observed daily for incidence of scours. Severity of scours was scaled from 0 (no scours) to 4 (severe dehydration and requiring I.V. electrolytes). Ten percent of the cow herd was bled prior to initialization of treatment 30 days prepartum (-

30 days) and again 28 days post-partum. Calves were bled at birth and at weekly intervals for 4 weeks. Cow plasma was analyzed for vitamin E and Se. Plasma from calves was analyzed for vitamin E, Se, and Cu in both 1994 and 1995. Calve's plasma from 1994 were analyzed for plasma zinc, immunoglobulin gamma, and serum soluble interleukin-2 receptor. Calves in 1995 were additionally analyzed for polymorpho nuclear cells and challenged with keyhole limpet hemocyanin.

A treatment by sample time interaction was observed ($P < .01$) in 1994 cow plasma vitamin E. Post partum plasma vitamin E concentrations were elevated in vitamin E treated cows, as compared to pre treatment levels ($P < .01$). Post-partum plasma vitamin E concentration in 1995 increased ($P < .01$) for cows fed vitamin E pellets compared to control pellets. Plasma vitamin E levels were decreased ($P < .05$) in control cows in 1995 at 28 days post partum compared to pre partum samples. Vitamin E treated cows had similar plasma vitamin E at both -30 days and 28 days post-partum. Plasma Se was increased from -30 to 28 days post-partum with vitamin E having no effect ($P > .1$) in either 1994 or 1995.

There was a tendency ($P < .1$) for calves from multiparous dams to have increased scours incidence in both 1994 and 1995. Treatments had no affect ($P > .1$) on scours incidence . Vitamin E treatment in 1994 increased ($P < .01$) plasma vitamin E concentrations of calves when averaged over time (age of calf) and scours. Vitamin E injections increased ($P < .01$) plasma vitamin E in 1994 calves from one week through four weeks of age. Scours tended to decrease plasma vitamin E concentrations in vitamin E treated calves at four weeks of age ($P < .1$) in 1994. Vitamin E treatment increased 1995 plasma vitamin E concentrations in weeks one through three ($P < .05$) compared to

controls. Plasma Se levels decreased ($P < .01$) in healthy calves from birth to four weeks of age in 1994 and 1995. No affect ($P > .1$) of vitamin E treatment was observed on plasma Se concentration. Plasma Cu concentrations of calves increased with age ($P < .01$) in both 1994 and 1995. Vitamin E treatment increased ($P < .05$) plasma Cu concentration independent of calf age and scours.

Birth weight, average daily gain (ADG), adjusted ADG, weaning weight, 245 day weaning weight, and calving interval were unaffected ($P > .1$) by vitamin E treatment in 1994. Birth weight (1995 data) was increased by vitamin E treatment ($P < .01$) in both primiparous and multiparous cows. Treatment did not affect ($P > .1$) ADG, adjusted ADG, weaning weight or 245 day weaning weight. Vitamin E treatment had no effect ($P > .1$) on conception rate or weaning percentages for either herd of cattle in either year.

Vitamin E treatment was successful in raising plasma vitamin E concentrations of both the cows and their calves. Vitamin E did not influence scours incidence in this study; however, plasma vitamin E was affected by scours. Plasma Cu concentrations were influenced by vitamin E treatment. In the second year of the study vitamin E treated cows produced larger offspring. Further research is warranted to determine the role of vitamin E in these interactions.

Effect of Vitamin E on Scours in Neonatal Beef Calves

by

Calvin L. Nunn

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented April 16, 1996
Commencment June 1996

Master of Science thesis of Calvin L. Nunn presented on April 16, 1996

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Acknowledgments

I would like to thank all the people who have helped me with my work on this project. I do not think there was a door in Animal Sciences which I did not go knock on and have opened, to be given assistance. I especially would like to thank my mentors and committee members Drs. Harley Turner, Phillip Whanger, and Robert Van Saun. I visited their offices with a large number of questions and usually left with those answered and a few new questions. Financial support for this study was provided by Hoffman-LaRoche Nutley, New Jersey. Injectable vitamin E for this study was provided by Schering-Plough Animal Corp. Kenilworth, New Jersey.

I learned several techniques from a large number of people. Drs. Hiedel and Bayne helped me to work out my neutrophile procedures. Statistical analysis was assisted along by Dr. Thomas, Dr. DelCurto, and some thought provoking questions from Dr. Carroll. Judy Butler and Dawn Whanger helped me to learn about mineral analysis and ordering of supplies. Robert Lowry, Gene Johnson, and Bobby Loper helped me to struggle with vitamin E analysis using the ominous HPLC. Rocky Baker taught me how to run the ELISA for my KLH challenged calves. Lynette, Laura, Jackie, Terri, Noreen, and Sheila were there to help answer computer questions and help order supplies. Mike Beilstein was always around to lend a second perspective to any problem, no matter what it was.

I received a lot of help from the people of the EOARC at Union including Teena Tibbs, Kenny Fite, Wade Titus, Ronnie Slater, John Jaeger, the mechanic Larry, Allison

Early, and the only other graduate student there at that time, Amaya Lowery. These people taught me about cows, people, and how to drink coffee.

One of the biggest things for getting through graduate school is peer support and advise. Fellow graduate students include Qu Ping Gu, Yu Sun, Jan Yeh, Tim Hazzard, Shelby Filley, Tina Clark, Dale Evers, Matt Cannon, Rick Christenson, Loni Quinlan, Jennifer Duncan, as well as many others. Other people who were around to keep me on track include my parents. Also my brothers and their girl friends were usually around. Beth Fox came around and helped me to get through the last two or three terms, which were the worst.

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Effect of Vitamin E on Scours in Neonatal Beef Calves

Introduction

In Oregon it is estimated that scours cost cattlemen an average of 10 million dollars annually, and in peak scour years such as 1989 this figure can be five to six times higher (Dr. Donald Hansen, personal communication, OSU School of Veterinary Medicine). Studies have been underway to investigate factors influencing the incidence and severity of scours. Cell mediated and humoral immune responses of calves are enhanced by supplemental vitamin E (Hidiroglou et al., 1992a; Gogu and Blumberg, 1993). Van Ryssen et al. (1994) found plasma vitamin E to be lowest during the winter for steers at Eastern Oregon Agricultural Research Center (EOARC) at Union, Oregon. Vitamin E and Se have been shown to be important in disease resistance (Droke and Loerch, 1990).

Since calving season is during the late winter the neonatal calves are at risk of scours, this is the time vitamin E has been reported to be lowest. A study was initiated and plasma vitamin E concentrations were found to be lower in calves with scours than those without scours (Table 1, Appendix 1). Objectives of the current study were 1) test the effects of providing vitamin E to cows and calves fed diets deficient in vitamin E, 2) perform tests on the immunocompetence of calves, 3) investigate the effect of vitamin E on morbidity of calves, specifically scours, and 4) evaluate subsequent productive and reproductive performance of the cows and calves.

Literature Review

Vitamin E

Vitamin E is available in several forms and is a general name for a group of alcohols and esters referred to as tocopherols and tocotrienols, respectively. According to Coehlo (1991a) in a recent literature review vitamin E is known to have several functions in the body including tissue antioxidant, coenzyme Q synthesis, vitamin C synthesis, involvement in sulfur amino acid and selenium (Se) metabolism, involvment in cell respiration, and boosts the immune system. Vitamin E has also been shown to reduce signs of zinc (Zn) deficiency and protect against silver, mercury, and lead poisoning. Furthermore this vitamin interacts with the clotting functions of vitamin K by inhibiting peroxidation of arachidonic acid. Consequences of lipid peroxidation include loss of membrane structure, inhibited enzyme activity, accumulation of reaction products, and altered membrane permeability.

According to Church and Pond (3rd ed., 1988) vitamin E deficiency has been implicated with several diseases, namely reproductive failure, cell lysis, and hemolysis in rats and chickens. Encephalomalacia in chickens is only responsive to vitamin E, but exudative diathesis is responsive to both vitamin E and Se. Nutritional muscular dystrophy (NMD) is commonly associated with vitamin E and Se deficiencies in calves, lambs, pigs, and poultry. Nutritional muscular dystrophy is often characterized by muscular degeneration with white streaks in the muscles, commonly called white muscle disease. Clinical signs of NMD often include muscle weakness and stiff muscles. Pigs

are affected in the heart, often called mulberry heart disease, with necrosis of the liver (hepatosis dietetica). When both organs are affected death usually occurs.

Combs (1991) has reviewed vitamin E absorption, transportation, and storage. Vitamin E absorption, transportation and storage involves moving a hydrophobic molecule through hydrophilic environments. During ingestion vitamin E becomes associated with fat droplets in the diet; therefore, absorption depends upon fat digestive ability of the individual. Lipase enzymes and bile salts in the small intestine play key roles in absorption of vitamin E and fat. Vitamin E is then transported in lipoproteins within the plasma while a portion binds, with specificity, to the erythrocytes.

Assessment of Vitamin E in Tissue

There is debate about the appropriate method for assessing total body status of the fat soluble vitamin E. The liver acts as a storage organ for vitamin E in many species, but vitamin E is transported in the blood. Hidiroglou and Charmley (1990) found the liver to be the major regulatory organ for temporary storage of vitamin E. Of tissues tested Njeru et al. (1994a) showed that injections and dietary supplementation of vitamin E resulted in the liver and pancreas having the highest levels. They suggested that liver tocopherol concentrations may depend on the form of vitamin E as well as the route of administration. These researchers also found serum concentrations of α-tocopherol did not reflect large tissue concentrations. The α-tocopherol content of serum, platelet and muscle were found to increase as daily dietary supplementation of DL-α-tocopheryl acetate increased (Njeru et al. 1994b). A low correlation was found between either the cholesterol fraction, triglycerides, or the sum of the two lipid fractions with serum or

platelet tocopherol. The researchers also found muscle tocopherol content to have a low correlation with serum or platelet tocopherol. Njeru et al. (1994b) concluded that serum tocopherol concentration were a reliable indicator of vitamin E status while expressing serum tocopherol relative to blood lipids did not improve the relation between intake and serum tocopherol levels. Njeru et al. (1994c) found the relationship between serum α-tocopherol and vitamin E intake was not improved by expressing serum tocopherol relative to blood lipids. Njeru et al. (1995) found that serum and liver tocopherol levels in yearling beef heifers could be used to predict total body vitamin E stores. This study showed no effect of vitamin E on blood lipid fractions. The ratio of serum tocopherol to the sum of cholesterol and triglycerides followed serum α-tocopherol trends with respect to treatment. Weiss et al. (1992) found positive correlations between serum tocopherol and cholesterol in dairy cows.

Researchers have studied plasma and serum levels of vitamin E in relation to assessment of total body vitamin E stores (Nockels, 1991; Roquet et al, 1992; and Hidiroglou et al., 1994a; Njeru et al., 1994c). Nockels (1991) suggests that serum may not be the most accurate predictor of total body vitamin E status; however, red blood cells or vitamin E content of neutrophils may be a better indicator. Sheep given dietary vitamin E supplementation had platelet tocopherol concentrations which were more sensitive to vitamin E intake than either muscle or serum (Njeru et al. 1994c). Differences due to different forms of vitamin E were found to be more evident in plasma α-tocopherol levels than in red blood cell levels (Roquet et al. 1992). Hidiroglou et al.

(1994a); however, considers plasma α-tocopherol concentrations a direct reflection of dietary vitamin E levels.

Vitamin E Routes of Administration

Injections and dietary supplementation are the most common routes of administration to increase plasma vitamin E of animals. DL-α-tocopheryl acetate is the common form of vitamin E used in the livestock feed industry (Lynch, 1991b). Pure D isomers of vitamin E are expensive to acquire in comparison to the racemic DL mixture. Alcohol molecules are less stable than acetate molecules because the former are potent antioxidants (Lynch, 1991b). Green plants and whole cereal grains produce the largest quantities of vitamin E (Lynch, 1991a). According to Coelho (1991) α-tocopherol is more preferable for increasing total body vitamin E stores than γ-tocopherol. The latter is unable to bind membrane receptors as well as α forms, leading to more rapid elimination of γ-tocopherol mixtures from the blood. A standard was established that 1 mg of racemic dl-α-tocopherol acetate equals 1 International unit (IU) of activity. National Research Council estimates vitamin E requirements are 15-60 IU per kg of dietary dry matter for young animals (NRC 1984). Normal diets for adult animals provide adequate amounts of vitamin E.

Different forms of injectable vitamin E have been used to increase serum and plasma tocopherol levels in cattle (Reddy et al. 1985, Hogan et al. 1992, Hidiroglou et al. 1992a, and Charmley and Nicholson 1993) and sheep (Njeru et al. 1992). Reddy et al. (1985) found that calves given a weekly 1,400 IU injection of dl-α-tocopherol had serum tocopherol concentrations which were higher than serum levels of calves given dietary

boluses of 1,400 or 2,800 mg dl-α-tocopherol acetate at weekly intervals. According to Hidiroglou et al. (1994a) maximum plasma concentrations following intramuscular injections occurs within 24 hours. They consider plasma levels to be returned to normal within ten days of injection. Hogan et al. (1992) injected cows subcutaneously with 3,000 IU of vitamin E at 10 and 5 days prepartum. This study found increased plasma α-tocopherol concentrations 5 days after the first injection, at calving, and 1 week after calving compared to controls. Increased concentrations of vitamin E due to treatment were not observed at two and four weeks post partum. Hidiroglou et al. (1992a) injected newborn Hereford cross calves with 900, 1,800, or 2,700 IU injections of D-α-tocopherol at birth and then once every three weeks. This study found injected calves to have increased plasma concentrations of vitamin E compared to non injected control calves. Dairy cows were injected with 1,500 and 3,000 IU of α-tocopherol and peak plasma tocopherol levels occurred in one day, while peak milk values occurred three days post injection (Charmley and Nicholson 1993). Sheep injected with DL-α-tocopherol reached peak serum tocopherol concentrations within 8-12 hours, and then returned to pretreatment values within 56 to 74 hours when given 125 and 1,000 IU injections respectively (Njeru et al. 1992). Hidiroglou et al. (1994a) found vitamin E concentration in cow plasma unaffected by injections with values of 2.90 μg/ml and 3.07 μg/ml for control and injected (3,000 IU α-tocopherol) animals, respectively. Njeru et al. (1994a) suggests care in choosing oil carriers for α-tocopherol injections, a carrier which is to thick may not allow for adequate absorption of vitamin E.

Injections can have a side affect which is undesirable to beef producers, swelling at the injection site. Reddy et al. (1986) found vitamin E injections to cause a slight inflammation at the injection site in Holstein calves; however, swelling went down with age. Njeru et al. (1992) also reported inflammation at the injection site.

Dietary supplementation has been used to increase plasma and red blood cell tocopherol concentrations in cattle (Hidiroglou et al. 1989, Hidiroglou et al. 1992c, Roquet et al. 1992), sheep (Hidiroglou et al. 1994b; Njeru et al., 1994a), and swine (Chung et al. 1992; Mahan, 1994). Hidiroglou et al. (1989) conducted a trial with four beef heifers, given single oral doses of DL-α-tocopherol and DL-α-tocopheryl acetate, and found the area under the concentrations curves to be greater for DL-α-tocopherol. Hidiroglou et al. (1992c) tested several forms of vitamin E in cattle and found a mixture of D-α-tocopheryl acetate plus D-α-tocopheryl polyethylene glycol 1,000 succinate to have the greatest response of the seven forms tested. All treated groups did exhibit increased serum α-tocopherol above unsupplemented controls. Roquet et al. (1992) found all calves receiving vitamin E supplementation had increases in plasma and red blood cell α-tocopherol, compared to controls. Calves were fed daily the equivalent of 228 mg of α-tocopherol in one of four mixtures. 1) D-α-tocopheryl acetate, 2) DL-α-tocopheryl acetate, 3) D-α-tocopheryl polyethylene glycol 1,000 succinate, and 4) experimental blend made up of the first and third. Hidiroglou et al. (1989) administered single oral doses of DL-α-tocopherol and DL-α-tocopheryl acetate to sheep. The researchers reported time to maximum concentration of plasma α-tocopherol was shorter for the DL-α-tocopherol group compared to the DL-α-tocopheryl acetate treated group.

Brzezinska-Slebodzinska et al. (1994) treated dairy cows daily with capsules of 1,000 IU vitamin E, starting six weeks prepartum. This study found vitamin E supplementation to increase serum α-tocopherol about 75% above non supplemented cows, during the first two weeks of supplementation. After 2 weeks of supplementation all cows experienced declining tocopherol values. Stowe et al (1988) found 500 IU of vitamin E per day sufficient to increase serum concentrations in Holstein cows within one month. Eicher et al. (1994b) supplemented Holstein calves with vitamin E in the milk replacer and found increases in plasma concentrations reflective of level of supplementation. Milking Holstein cows fed vitamin E supplement for 30 days, starting 21 days post partum, had increased plasma α-tocopherol 51 days post partum (Hogan et al. 1990). Hidiroglou et al. (1994b) studied bioavailability of D-α-tocopherol and D-α-tocopheryl succinate with the use of tritiated tocopherols administered directly to the rumen of cannulated wethers. Bioavailability of tocopherol was proven greater compared to the ester form, by an increased tissue concentration of radioactivity. Njeru et al. (1994a) found sheep given dietary supplementation of vitamin E reached a maximum serum vitamin E concentration at 7 days. Chung et al. (1992) found that weanling swine absorbed and retained dietary D-α-tocopherol more effectively than DL-α-tocopheryl acetate. Sows experienced increases in serum and colostral α-tocopherol concentrations when fed a vitamin E Se fortified diet (Mutetikka and Mahan 1993). Mahan (1994) was able to increase sow serum and colostral tocopherol content with increased dietary vitamin E.

Dietary supplementation of vitamin E has some drawbacks, not all of the vitamin E reaches the intestine, and that which does is not always absorbed. A larger amount

must be used to obtain the same increase of serum vitamin E as found with injections. Preintestinal disappearance of vitamin E has been studied and found to be higher in animals fed high concentrate diets. Alderson et al. (1971) found the disappearance of vitamin E was 8.4% for animals fed a diet of 20% corn but as high as 42.8% disappearance in those fed a diet of 80% corn. The investigators found the loss of vitamin E to be less when alfalfa was used to replace corn. The researchers also investigated the absorption of vitamin E in ligated intestinal loops of sheep. After administering a 3.56 g α-tocopherol gelatin capsule there was a rise of serum tocopherol in non ligated ewes; however, the ligated ewes did not have an increase in serum vitamin E (Alderson et al. 1971). Roquet et al. (1992); however, found steers given vitamin E directly into the rumen had greater increases in plasma tocopherol compared to duodenally dosed steers. McDiarmid et al. (1994) developed a procedure for analysis of vitamin E in bovine ruminal fluid. These researchers found no evidence of ester hydrolysis or degradation of α-tocopherol acetate after a 24 hour incubation in bovine ruminal fluid. Leedle et al. (1993) examined microbial degradation of DL-α-tocopheryl acetate with undiluted ruminal contents from a steer fed a corn based high concentrate diet. Tocopheryl acetate was not found destroyed by ruminal microorganisms in the 24 hours monitored. Composition and type of diet influence the absorption of vitamin E (Hidiroglou et al. 1992b). After dietary supplementation with various forms of vitamin E Hidiroglou et al. (1992c) found concentrations of serum tocopherol to peak between 15 and 21 days, before reaching a plateau.

Vitamin E in Forage

Variations in natural vitamin E content of forages can be caused by many factors (Shingoethe et al., 1982; Smith et al., 1988; Hidiroglou et al. 1992b; Hidiroglou et al., 1994a). Vitamin E content was shown to decline in stored forages, and be highest in green forages (Smith et al. 1984). Smith et al. (1988) showed vitamin E content of forages declined both with growing season and storage. Vitamin E concentration in grass silage was higher than concentration in grass hay; however, fresh grass had increased concentrations above grass silage (Shingoethe et al. 1982, Hidiroglou et al. 1994a). Loss of vitamin E can be affected by management of hay and silage, drying of hay in the field can account for a 60% loss of vitamin E activity within four days (Hidiroglou et al. 1992b). Hidiroglou et al. (1994a) were able to show that cows receiving silage had significantly increased concentrations of plasma α-tocopherol above cows receiving timothy hay. Cattle have been reported to have lower vitamin E levels in the winter than during the fall (Shingoethe et al. 1982).

Vitamin E at Calving

Plasma vitamin E of cows has been shown to decline around calving time (Smith et al. 1988, Weiss et al. 1990, Hogan et al. 1992, Hogan et al. 1993, Brzezinska-Slebodzinska et al. 1994). The exhibited decline in plasma vitamin E of cows at parturition has been attributed to a decreased transport capacity and decreased consumption of vitamin E (Hogan et al. 1993). Holstein cows supplemented with 1,040 IU/day of vitamin E during the dry period had increased vitamin E 10 days prior to calving compared to unsupplemented cows (Hogan et al. 1992). Vitamin E supplemented

cows exhibited no difference in plasma tocopherol levels above controls five days pre partum, at parturition, or during early lactation.

Declining plasma vitamin E in the dam leads to a lower concentration of vitamin E in colostrum. Vitamin E is transported into the mammary gland of the cow for colostrum and milk; colostrum is the richer source of vitamin E in cows (Van Saun et al. 1989b, Weiss et al. 1990, Hidiroglou et al. 1992b). There is little or no placental transfer of vitamin E in cows (Van Saun et al. 1989b, Hidiroglou et al. 1994a) or sheep (Njeru 1994b). Neonatal calves must receive most of their vitamin E via colostral transfer (Van Saun et al. 1989b, Hidiroglou et al. 1994a). Weiss et al. (1990) found that dry period supplementation of dairy cows increased α-tocopherol concentrations of colostrum. A linear effect of supplementation on vitamin E content was found in colostrum of sheep (Njeru et al. 1994b). Babinszky et al. (1991) concluded an increased concentration of serum vitamin E in week old pigs was from high levels of vitamin E in the sow's diet. One reason for high colostral concentrations of vitamin E may be interactions with the immune system (Hidiroglou et al. 1992a). Interactions of vitamin E and the immune system are discussed later in this review.

Vitamin E in the Newborn

Newborn calves have low blood tocopherol levels at birth prior to suckling colostrum (Hidiroglou et al. 1994a). Blood concentrations of vitamin E in newborn calves without vitamin E treatment were 0.14 μg/ml with an increase to 1.04 μg/ml at 10 days, which dropped to 0.80 μg/ml by thirty days of age (Hidiroglou et al. 1994a). Serum vitamin E levels in newborn calves, prior to nursing were not affected by dietary

supplementation (Stowe et al. 1988). Cipriano et al. (1982) found that supplementing newborn dairy calves with 1 g DL-α-tocopheryl acetate daily increased plasma tocopherol concentrations.

Effect of Vitamin E on Livestock

Vitamin E supplementation has been shown to affect cows (Reddy et al. 1987a, Charmley et al. 1993, Charmely and Nicholson 1993, and Hogan et al. 1993) but others found no affect upon cows (Stowe et al. 1988 and Weiss et al. 1990). Swine (Mahan 1994) have been shown to be affected by dietary vitamin E. However, other researchers have shown swine (Chung et al. 1992) and sheep (Njeru et al. 1992) to not respond to vitamin E supplementation. Nockels (1991) maintains that vitamin E improves weight gain and feed efficiency by depression of cortisol, a stress responsive hormone. This is supported by data showing stressed feedlot cattle which received 1,600 IU vitamin E in the diet, gained 22.2% faster than controls. Reddy et al. (1987a) found vitamin E supplementation of neonatal Holstein heifer calves at 125 and 250 IU per day improved weight gain when compared to zero supplementation. Calves receiving 500 IU per day had intermediate weight gains. Feed intakes for all treatments were similar. Serum α-tocopherol increased with supplementation, 0.89, 1.67, 2.69, and 2.89 μg/ml for diets with 0, 125, 250, and 500 IU per day supplementation, respectively. Pehrson et al. (1991) found that on average calves given dietary vitamin E supplementation gained more weight than non supplemented controls. Dairy cows have been shown to exhibit decreased incidence of mastitis, intra mammary infection, and milk somatic cell count when receiving 1,000 IU /day vitamin E (Hogan et al. 1993). Smith et al. (1984)

supplemented multiparous cows with vitamin E and found the vitamin E supplemented group exhibited a 37% reduction in clinical mastitis and a 44% reduction in duration of clinical symptoms of mastitis compared to controls. Supplementation of dairy cows with vitamin E has been shown to increase milk production (Charmley et al. 1993). Charmely and Nicholson (1993) found mid lactation dairy cows to have increased milk fat concentration as the level of α-tocopherol injected increased. The same study found intake and milk yield similar between treatments; however, there was a numerical decline in body weight gain as treatment level of tocopherol increased. Weiss et al. (1990) found no effect of vitamin E supplementation on milk production or feed intake of Holstein cows. Reddy et al. (1985) found no statistical differences in feed intake or weight gain; although, vitamin E supplemented calves tended to exhibit an increase in weight gain and feed intake. Dietary vitamin E supplementation did not affect post weaning measures of swine production including weight gain, feed intakes, or gain to feed ratios (Chung et al. 1992). Njeru et al. (1992) found no effect of vitamin E injections on body weight gain of crossbred wether sheep.

Brzezinska-Slebodzinska et al. (1994) found that dairy cows which retained fetal membranes at parturition had a lower antioxidant status as defined by glutathione peroxidase and fast acting antioxidant levels in plasma. This study also found cows supplemented with vitamin E were likely to have an antioxidant status above the mean. Harrison et al. (1984) found a decrease in days to first estrus for cows supplemented with vitamin E. Supplementation with vitamin E for 6 months prior to breeding of heifers increased the pregnancy rate above controls (reviewed by Hidiroglou et al. 1992b). The

same review also reports that age to first heat, breeding and calving were unaffected by vitamin E supplementation. Stowe et al. (1988) found no effect of vitamin E supplementation on reproductive performance in Holstein Freisian cows. Vitamin E has been reported to decrease the anestrus period in gilts (Grandhi et al. 1993); however, supplemental vitamin E had no effect on ovulation rate, number of live embryos or fetal survival. Mahan (1994) reports an increased number of pigs born as dietary vitamin E increased; however, sow weights, litter birth and weaning weights were unaffected.

Selenium

Since selenium (Se) is an essential element for animals it is appropriate to discuss the most common ways animals acquire the element. Ruminants consume plants which accumulate Se; however, it can be present in several forms and at different concentrations. Whanger (1989) discussed Se in plants and provided details on different forms. Selenium uptake by plants is affected by soil type, soil pH, plant species, type of fertilizer, climate, and chemicals previously applied to the land. There are three classes of plants which accumulate Se at different levels; primary indicators, secondary indicators, and non accumulators. Primary indicators accumulate Se in tissue as nonprotein seleno-amino acids. Secondary indicators do not accumulate Se at the concentration of primary indicators and non accumulators generally contain less than 25 ppm Se. Selenium can be present in soil as selenide (-2), selenite (+4), selenate (+6), elemental Se (0) and possibly organic; however, selenate is the predominate form of this

element. Selenate is the most readily absorbed form of Se in plants. Selenium is known to be lost from the soil by leaching, therefore areas of heavy rainfall have decreased Se levels.

Selenium absorption and pools in Livestock

Whanger (1989) discussed the absorption of Se in ruminants. Sheep given oral selenite absorbed only 25% but swine given oral selenite absorbed 77% of the dose. Selenite is primarily absorbed in the small intestine and cecum of sheep and swine. Differences in retention are attributed to ruminal microbial populations of sheep. Ruminal microbial populations can change inorganic Se to organic forms and incorporate them into their microbial proteins. The microbial population reduces dietary Se to forms which are unabsorbed by ruminants. Inorganic Se compounds may be changed to organic Se in animal tissues, but the reverse is not true. Selenium excretion by monogastric animals occurs primarily as trimethyl selenide via the urine. In contrast, ruminants excrete most dietary Se in feces as unavailable forms due to microbial reduction; however, Se given by injections is excreted in the urine. Selenium is available to the animal through dietary supplementation and injections. Selenite is absorbed from the gastrointestinal tract; however very little Se is found in the body as free selenite. Free Se is toxic to the body as are free forms of most metals. Selenium supplements are traditionally in the form of selenite, but some researchers believe this may not be the best form. Ruminants absorb selenomethionine better than selenite.

Selenium's primary mode of action is as glutathione peroxidase (GSH-Px) which reduces peroxides to alcohols. NRC (1984) recommends .05-.10 mg Se per kg diet, on a

dry matter basis, for cattle; however, this is variable and depends on the vitamin E status of the animal. Mertz (1986) reports 100 ppb as an adequate dietary Se intake for sheep and cattle, while 60 ppb is a minimum value for prevention of NMD. Van Saun (1989) reports serum Se concentration of 40-70 ng/ml to be marginal for cattle.

Yeh et al. (1995) discussed the presence of Se in mammalian selenoenzymes and selenoproteins. The selenoenzymes are the Se-dependent glutathione peroxidase family (GSH-Px), and types I, II, and III iodothyronine deiodinases. Selenoproteins P and W are the most studied selenoproteins without a known function. The majority of plasma Se is bound to selenoprotein P (Read et al. 1990), but selenoprotein W is found predominantly in muscle, brain, testes and spleen (Yeh et al. 1995). Yeh et al. (1995) notes that selenoprotein W is higher in sheep heart and muscle than other tissues; however it is low in the rat heart. They indicated no cardiac damage to rats in Se deficiency but Schubert et al. (1961) found cardiac damage in Se deficient sheep.

Affects of Se supplementation on plasma and blood concentrations

Selenium supplementation has been shown to increase plasma Se in cattle (Stowe et al., 1988; Hogan et al., 1990; Weiss et al., 1990; Nicholson et al., 1993; Hidiroglou et al., 1994a). Supplementation with Se rapidly increased plasma Se concentrations; however, whole blood Se and GSH Px levels are slower to respond (Weiss et al. 1990). Yearling beef heifers fed Se fertilized forage had increased blood Se and GSH-Px compared to control animals; however, neither group was different from those fed inorganic Se as Se enriched yeast (Nicholson et al. 1993). Daily dietary supplementation of 2 mg sodium selenite in Holstein dairy cows increased serum Se concentrations within

one month (Stowe et al. 1988). Hogan et al. (1990) found increases in whole blood and plasma Se concentrations of multiparous Holstein cows within 30 days after giving sodium selenite injections and dietary sodium selenite supplementation. A single 30 mg injection to beef cows 21 days prepartum increased plasma Se concentrations above controls (Hidiroglou et al. 1994a).

Selenium in the Neonate

Selenium concentrations in the blood and serum of the neonatal calf is dependent upon the Se level of the dam (Kincaid and Hodgson 1989). Selenium is transferred across placental barriers with limited mammary gland transfer of the mineral (Weiss et al. 1984, Van Saun et al. 1989a, Hidiroglou et al. 1994a). Weiss et al. (1984) found whole blood Se levels of calves to be high at birth from cows which received prepartum Se supplementation. Cows supplemented with dietary Se at 2 mg per day had increased Se in colostrum and serum of their presuckle calves (Stowe et al. 1988). Selenium concentration of milk 7 day post-partum and serum of week old calves was unaffected by Se supplementation of the dam. Beef cows injected with Se 21 days prior to expected parturition had increased Se concentrations of placental tissues and plasma Se of presuckle calves compared to controls (Hidiroglou et al. 1994a).

Selenium's affects on Livestock

Cattle have been shown to respond to Se in several ways, which is dependent upon Se status of the animal. Selenium responsive disorders of cattle include white muscle disease, nutritional muscle degeneration, certain forms of neonatal weaknesses,

infertility, abortions and stillbirths, retained placentas, immune responsive disorders, loss of body condition, and diarrhea (Ammerman and Miller 1975, Church and Pond 1988, 3rd ed., Van Saun 1989). Stowe et al. (1988) reported that cows supplemented with Se daily tended to have a lower incidence of retained placenta and metritis. Incidence of metritis and cystic ovaries was reduced in dairy cows by prepartum Se injections (Harrison et al. 1984). Cows which retained fetal membranes were found to have lowered levels of GSH-Px (Brzezinska-Slebodzinska 1994).

Weight gain of cattle has been shown to be increased by Se supplementation (Ammerman et al. 1980, Weiss et al. 1984, and Swecker et al. 1991); however, there are reports showing no affect of Se upon weight gain of cattle (Weiss et al. 1983, Reffett et al. 1988, and Nicholson et al. 1993). Se deficient rats have been reported to have increased growth rates when given dietary Se supplementation (Whanger and Weswig, 1975). Weiss et al. (1984) reports improved weight gains for calves given milk based diets with Se supplementation. Ammerman et al. (1980) found that weaning weight of calves tended to increase with Se supplementation of cows, but no influence was found on live weight of dams or birth weight of calves. Swecker et al. (1991) found that calves whose dams received mineral salt supplement with 120 ppm Se had an increased ADG for the first sixty days after birth. Appendix 2 contains unpublished data showing steers receiving Se supplementation to exhibit an increased feed efficiency. However, Nicholson et al. (1993) found no effect of Se supplementation on weight gain, feed intake or feed conversion in yearling beef cattle, but these cattle exhibited no clinical signs of disease. Reffett et al. (1988) challenged Se deficient and Se supplemented neonatal

Holstein calves with infectious bovine rhino tracheitis virus (IBRV) and found no effect of Se status upon feed intake or final weight. Kincaid and Hodgson (1989) found Se supplementation to have no affect on average daily gain in young calves. Weiss et al. (1983) found no affect on rate of gain or total gain from Se/vitamin E injections. Spears et al. (1986) suggests that selenium deficiency can decrease calf gains, without showing any clinical signs of deficiency. Weiss et al. (1990) found no effect of Se supplementation on feed intake or milk production in Holstein cows.

Vitamin E and Selenium Interaction

Supplementation with either vitamin E or Se has been reported to affect plasma levels of the opposite element (Whanger and Weswig; 1975; Stowe et al., 1988; Van Ryssen et al., 1994), while others have found no effect (Weiss et al., 1990; Brezezinska-Slebodzinska et al., 1994). Selenium and vitamin E act together as antioxidants (Van Saun et al. 1989b, Hogan et al. 1993). Vitamin E prevents peroxidation within the lipid membrane. Selenium works within the cytoplasm of the cell in GSH-Px (Hogan et al. 1993). These two antioxidants act synergistically to improve animal health and performance. In a review, Turner and Finch (1991) indicate that few researchers working with Se neither report vitamin E status or take efforts to control vitamin E status of animals, despite evidence connecting the two nutrients in biological systems. Stowe et al. (1988) found serum Se concentration of cows supplemented with vitamin E and Se to be lower than cows given Se supplementation alone, between two and six months post partum, in lactation 1. Cows treated with vitamin E and Se in lactation 2 of a 2 year study had serum Se higher than the Se supplemented group. Vitamin E supplementation

increased serum vitamin E in the presence and absence of Se supplementation. A reduction in plasma tocopherol levels occurred with dietary increases in Se too Se deficient rats which were adequate in vitamin E (Whanger and Weswig 1975). Vitamin E deficient rats have been reported to have 20% less plasma Se than rats adequate in vitamin E given the same amount of Se (Turner and Finch 1991). Van Ryssen et al. (1994) found plasma Se to be negatively correlated with plasma vitamin E in beef steers. Weiss et al. (1990) fed dairy cows supplemental vitamin E and/or Se, they found blood Se values unaffected by vitamin E supplementation. These researchers also found no affect of Se supplementation on plasma α-tocopherol concentrations. Dietary Se supplementation did not affect serum α-tocopherol concentrations (Brzezinska-Slebodzinska et al. 1994). Van Saun et al. (1989b) studied Se and vitamin E interactions in slaughtered dairy cows and their fetuses. No interactions of maternal vitamin E and Se status were reported.

Vitamin E and Se given in conjunction have been shown to either enhance (Smith et al. 1984, Hogan et al. 1993) or have no affect on performance of cows (Harrison et al. 1984, Stowe et al. 1988, and Weiss et al. 1990). No sign of a vitamin ExSe interaction was detected with incidence of mastitis in dairy cows (Smith et al. 1984). This study did find that duration of clinical symptoms was greatest in control animals with the shortest duration of clinical symptoms in cows supplemented with both vitamin E and Se. Hogan et al. (1993) reports that cows supplemented with vitamin E and Se had a shorter duration of clinical signs of mastitis than cows supplemented with either nutrient separately. Harrison et al. (1984) found retained placentas to be unaffected by oral vitamin E or Se

injections when given separately; however, when both given to the same cow there was a reduced incidence of retained placenta from 17.5% to 0%. Stowe et al. (1988) found vitamin E and Se supplementation to have no effect on reproductive performance of Holstein cows; however, they found that cows receiving vitamin E plus Se supplementation tended to have fewer services per conception than other cows. Weiss et al. (1990) found no effect of vitamin E or Se on milk production or feed intake. Nicholson et al. (1993) supplemented dairy cows with vitamin E and Se and found no affect on feed intake. This study showed milk yield tended to be larger for cows receiving one of the two nutrients compared to other cows.

Miscellaneous Interactions

Vitamin E x Vitamins

Vitamin E interacts with other fat soluble vitamins. Studies have investigated interactions of vitamin E with various forms of vitamin A. β-carotene is converted to vitamin A, which exists as retinol, retinoic acid and retinal (Chew 1993). Various forms of vitamin A are important in vision, growth, reproduction, and general health (Chew 1993).

Anderson et al. (1995) fed growing finishing swine high levels of vitamin A while varying the level of vitamin E. These researchers found no consistent evidence to indicate that 2,000 or 20,000 IU retinyl acetate per kg diet interfered with swine performance or serum and tissue α-tocopherol concentrations. Eicher et al. (1994b) found plasma vitamin E concentration of Holstein calves to be unaffected by vitamin A

supplementation. Chew et al. (1993) administered β-carotene to 3-4 month old Angus bull calves and found no influence on α-tocopherol concentration of plasma. Harrison et al. (1984) found vitamin E supplementation in multiparous dairy cows tended to decrease plasma vitamin A concentration. Daily dietary vitamin E supplementation did not affect plasma β-carotene concentrations in non-lactating cows (Weiss et al. 1994).

Effect of vitamin C concentration on vitamin E concentrations has been investigated, but no effect was found on plasma vitamin E concentrations in neonatal calves (Eicher-Pruiett 1992).

Vitamin E x Minerals

Certain minerals complement the actions of the antioxidant vitamins, among these are selenium (Se), copper (Cu), and zinc (Zn). Plasma Cu is known to increase after calves have experienced a microbial challenge (Mertz 1986 and Stabel et al. 1993). Copper is involved in antioxidant activities through the enzyme superoxide dismutase. Dove and Ewan (1991) supplemented pigs with Cu and α-tocopheryl acetate and found serum tocopherols reduced by addition of Cu and increased by addition of α-tocopheryl acetate. Copper supplementation had no effect on tocopherol concentrations of tissues measured; however, α-tocopheryl acetate supplementation did increase tissue tocopherol concentrations. Xin et al. (1991) found no effect of Cu status upon GSH-Px in Holstein steers. Koenig et al. (1991) found no affect of Cu on Se absorption in dairy cows. Dill et al. (1991) found immune response of calves improved with Se injections; however, the response was not as great when Se and Cu were injected together. Dove and Ewan (1990) supplemented pigs with iron (Fe), Zn and Cu and they found no effect of Fe or Zn

on serum tocopherols; however, Cu decreased serum tocopherol levels. They found high levels of Cu, Zn, and Fe each destroyed tocopherols present in the feed, during storage. Several minerals have been shown to affect the vitamin E-Se interaction. When subtoxic levels are used, several elements lead to vitamin E and/or Se deficiencies. Silver, zinc, cadmium, tellurium, cobalt, copper, mercury, tin, lead, arsenic, iron, and sulfur have all been shown to produce deficiencies of vitamin E and/or Se in ducks (Van Vleet 1982); however, the concentration required to produce the deficiency is well above nutritional requirements. The vitamin E and/or Se deficiency produced was overcome by additional supplementation with vitamin E and Se.

Immunology

Since the immune system is an integral part of the animal's body, a brief description of sources of immune system cells would be appropriate. Tizzard (1992) describes the source of primary lymphoid cells in the young fetus as being the liver and yolk sac. The source of the primary cells in the older fetus and the adult animal is the bone marrow. Primary lymphoid cells (stem cells) migrate to different areas in the body where maturation continues. Stem cells which migrate to the thymus to mature and differentiate are known as T cells. The T cells are responsible for cell mediated immunity and are the predominant circulating lymphocytes. A second class of lymphocytes are B cells. Avian B cells differentiate in the Bursa of Fabricius, while the bone marrow is used in primates and rodents. Ruminants are not the same because their B cells differentiate in areas located near the small intestine known as Peyer's Patches. The B cells are responsible for differentiating to produce antibodies. Life span of B cells is shorter than

T cells. A third population of cells active in the immune system is the natural killer (NK) cells.

Cell Mediated Background

Tizzard (1992) briefly describes a portion of the immune response known as cell mediated response. These responses are directed against cells which are non-self (foreign to the body). Foreign cells are found by the immune surveillance mechanism, which is beyond the scope of this paper. Cell mediated responses are best demonstrated by tissue grafts from a donor animal given to a host animal. A graft will survive for a short period after the first attempt to graft. The second attempt to graft tissue leads to rapid rejection of the graft, provided the original donor and host were used. Tizzard (1992) notes cell mediated immunity can be transferred by taking lymphocytes from the spleen and lymph nodes of the sensitized animal and transferring them to another animal. The immune system is prevented from attacking self tissue due to tolerance (Tizzard 1992). Tolerance allows the immune system to differentiate between self antigens and non-self antigens, which prevents random indiscriminate immune responses.

Humoral Background

According to Tizzard (1992) the humoral immune system is that portion of the immune system which is active in the serum of the animal. Antibodies are proteins produced by B cells as a result of B cell antigen interactions. These proteins are found in the serum of animals exposed to an antigen, provided the animal has produced an immune response to the antigen. An antigen is a foreign substance which stimulates an

animal to produce antibodies. Antibodies react to specific regions of the antigen. The antibody is able to bind and neutralize the antigen which is often toxic to the animal. A good antigen has certain characteristic features which stimulate the immune system. The antigen must be large enough to stimulate the immune system, the larger the better. Antigens need to be complex, bacterial lipopolysaccharide and proteinaceous compounds are potent antigens. The immune system needs to be able to break down antigens; however, the antigen needs to be stable enough to allow processing. Antibodies are produced against foreign antigens, the more different from self tissue the better.

Antibody Production

The immune system may be stimulated to produce antibodies through either natural exposure or vaccination. When vaccinating an animal the immune system is stimulated with injections of vaccines, which are made less virulent than a natural infection. After exposure to an antigen, for the first time, there is a short lag period of approximately one week before antibodies are present in serum. The quantity of antibodies present after the first exposure to an antigen is small and transient, ten to fourteen days. The second exposure to the same antigen has a shorter lag period and an increased concentration of antibodies are produced. This secondary response is specific, it can only be initiated by the same antigen or one which is identical to the original antigen. The amnestic response (i.e., memory response) produces the more effective secondary immune response because the immune system remembers the antigen and has previously produced this specific antibody.

Tizzard (1992) refers to immunoglobulins (Ig) as proteins with antibody activity. There are five major classes of Igs; mu (M), gamma (G), alpha (A), epsilon (E), and delta (D). This discussion will focus on IgM and IgG, as cattle have these two immunoglobulins present in the highest concentration. IgG is made of two heavy chains and two light chains, held together by disulfide bonds, in a 'Y' shape with an antigen binding site on the upper end of each branch, giving two antigen binding sites. This Ig is the smallest of the Igs and is also of the highest concentration in mammalian blood of all immunoglobulins. The small size of IgG allows the molecule to easily be transported out of blood vessels and into other systems within the body, which explains its prevalence in colostrum and milk from cows. Production of IgG is only after the animal has been exposed to a specific antigen. IgG is produced from the amnestic response and not during the primary immune response. IgM is the isotype of second highest concentration in cattle. The IgM molecule is made up of five monomers, which are shaped in a similar fashion to IgG monomers using a different heavy chain than IgG. The five monomers give IgM ten antigen binding sites; however, they are not all bound at one time due to structural hindrances. IgM is involved in the primary immune response because it binds more general antigens than IgG. The large size of IgM does not allow easy transport out of the blood stream; therefore, it is not found in large quantities in the milk of cows.

Measures of the Immune System

A simple intradermal skin test measures cell mediated immunity according to Tizzard (1992). These test are designed to measure T lymphocyte proliferation or

lymphokine production. Tests of cell mediated immunity are not readily available or easily performed, except with intradermal inoculations such as tuberculosis.

Tizzard (1992) defined serology as the science of detecting specific antibodies in body fluids. Antiglobulins are immunoglobulins from one species injected into a second species. An example would be bovine IgG injected into a rabbit, the rabbit produces antibodies to bovine IgG, the rabbit antibovine IgG antibodies are harvested. This molecule will bind to bovine IgG molecules in the serum of the rabbit or in culture. The rabbit antibody is often tagged with enzymes for identification and used as a reagent in laboratory analysis. Primary binding tests measure direct binding of antigen and antibody, one reactant is normally labeled. A competitive radioimmunoassay has reagents labeled with radioactive compounds. An enzyme linked immunosorbent assay (ELISA) measures antibody concentration using enzyme tags on one reagent in the test, while other tests use fluorescent tags. These tests vary in incubation and wash steps, with variations for all tests. The antigen and antibody must be soaked together and any unbound antigen or antibody washed off, the tagged reagent must also be incorporated. Secondary binding tests use a two stage process. The first step is an interaction of antigen and antibody which causes some measurable event to occur. Several different forms of this test are used with cell lysis, precipitation of molecules, clumping of cells and activation of the complement pathway as commonly used measures of antigen and antibody interaction. The third level of antibody tests include in vivo testing procedures.

Poly Morphic Nuclear Cells

Myeloid cells originate in the bone marrow and are rapidly acting cells incapable of long term effort (Tizzard 1992). Neutrophils (Poly Morphic Nuclear Cells (PMNs)) account for about 20-30% of blood leucocytes in cattle and sheep (Tizzard, 1992). Neutrophils migrate to the blood where they live about 12 hours until they move to the tissues, where they live for days. Toxic oxygen radicals produced by PMNs are designed to kill invading bacteria; however, the PMNs may spill enzymes and radicals. The leakage of oxygen radicals causes the surrounding tissue to be oxidized and further cell damage occurs to the host. Damage to the plasma membrane by radicals decreases the ability of these cells to function (Mottola 1980, Eicher et al. 1994a). Lipid peroxidation, caused by free oxygen radicals, decreases membrane fluidity; therefore, affecting cellular interactions of the immune system (Reffett et al. 1988, Bendich 1993). Antioxidants aid the immune system by stabilizing free radicals; however, the level needed to improve the immune responses may be greater than current dietary recommendations (Bendich 1993).

Increases in oxygen metabolism due to the respiratory burst of neutrophils, requires more antioxidants to control increased levels of toxic oxygen radicals (Hogan et al. 1993). Phagocytic ability of neutrophils is unaffected by different levels of selenium and vitamin E (Boyne and Arthur, 1978; Aziz et al., 1984; Hogan et al. 1992). Microbicidal ability, i.e. ability to kill microorganisms, of the PMN is affected with different levels of vitamin E and selenium (Boyne and Arther, 1978; Aziz et al., 1984; Hogan et al. 1992). Aziz et al. (1984) showed that a diet deficient in Se affects the function of PMNs in goats. Boyne and Arthur (1978) showed the ability of the

neutrophile to kill ingested yeast was three times higher in the Se supplemented group compared to the Se depleted group. There was no difference in the ability of PMNs to ingest yeast across treatments. Ability of neutrophils to phagocytize bacteria was shown independent of dietary supplementation with vitamin E and Se; however, supplemental vitamin E did influence intracellular kill by bovine neutrophils (Hogan et al. 1992). A suppression of PMN function with a deficiency of vitamin E and Se was seen in the sow (Wuryastuti et al. 1993). Multiparous Holstein cows were used to determine the effect of dietary supplementation of vitamin E and Se on in vitro phagocytosis and intracellular kill of bacteria (Hogan et al. 1990). These researchers found an increased intracellular kill rate of *Staphylococcus aureus* and *Escherichia coli* from neutrophils of vitamin E supplemented cows. Selenium supplementation increased kill rates of *Staphylococcus aureus* above cows not supplemented with Se. Gyang et al. (1984) found no difference in the ability of PMNs to phagocytize bacteria (*Staphylococcus aureus*); however, Se and vitamin E injected cows had neutrophils which were better able to kill ingested bacteria than those of Se deficient cattle.

Research has shown PMNs from Holstein calves supplemented with vitamins E and A to have increased bactericidal activity, compared to those given vitamins A or E separately (Eicher et al. 1994b). Daniel et al. (1991) studied phagocytosis of blood and milk phagocytic cells in Holstein cows supplemented with β-carotene, retinol, and retinoic acid. These researchers found no influence of β-carotene on phagocytosis by blood or milk phagocytes. The same animals experienced increased intracellular kill by phagocytic cells, compared to other treatments. Retinoic acid decreased phagocytic

rates and intracellular kill rates, while retinol had no affect on either measure. Eicher et al. (1994a) studied blood neutrophils and pulmonary alveolar macrophages after isolation from calves at 3 and 6 weeks of age. Cells were incubated with vitamins added to the incubation medium at the rate of 100 μg/dl vitamin A, 1000 μg/dl vitamin E, vitamin A plus E, or .25 μg/dl β-carotene plus vitamin E. Chemotactic indexes and random migration of vitamin E supplemented neutrophils were higher than control cells or vitamin A supplemented cells. Decreased kill rates of bacteria were found in neutrophils treated with vitamin E at 3 weeks; however, vitamin E and vitamin A plus E treatment also decreased kill rates at 6 weeks. Eicher-Pruiett et al. (1992) studied supplementation of dairy calves with vitamins E and C, starting at 3 days of age, and thier effect on neutrophils and lymphocytes. The researchers found vitamin C supplemented calves to have a lower neutrophile mediated phagocytosis compared to controls. The group with both vitamins E and C had near or slightly higher measures than controls. Eicher-Pruiett et al. (1992) suggest that vitamin E negated adverse affects of vitamin C on neutrophile function. Subcutaneous doses of ascorbic acid resulted in enhanced neutrophil oxidative metabolism in cattle (Roth and Kaeberle 1985).

Immunoglobulins are Affected by Vitamin E and Se

Immunoglobulins have been shown to be affected by vitamin E (Tengerdy et al. 1983; Reddy et al. 1987b; Nemec et al. 1990; Hidiroglou et al. 1992a) as well as Se (Kiremidjiian-Shumacher and Stotzky 1987; Reffett et al. 1988; Turner and Finch 1991; Nicholson et al. 1993). Nemec et al. (1990) showed the natural antibody to *Salmonella typhimurium*, IgM, to be increased with vitamin E supplementation of beef heifers.

Neonatal Hereford crossbred calves challenged with Keyhole Limpet Hemocyanin (KLH) tended to have increased concentrations of IgG class 1 and 2 with increasing levels of supplemental vitamin E (Hidiroglou et al. 1992a). These investigators also found calves given 2,700 IU injections of vitamin E every three weeks had higher IgM values than controls. Supplemented calves were suggested to have an increased ability to synthesize immunoglobulins because IgM is rapidly metabolized and not provided in large quantities in colostrum. Vitamin E supplemented calves have shown an increased IgG response to vaccination for antibovine herpesvirus type one (Reddy et al. 1987b). Tengerdy et al. (1983) found an increased humoral antibody production in sheep vaccinated with *Clostridium perfringens* when fed vitamin E at 300 mg/kg diet. The same researchers found a larger increase when a vitamin E adjuvant was used in the vaccine. Antibodies were measured by ELISA. Reddy et al. (1987b) found calves fed vitamin E daily tended to have higher IgG titre to *Antibovine Herpesvirus Type 1* (anti BHV-1) in response to an injection at 7 weeks of age. Following a second injection at 21 weeks of age calves given 125 IU per day had higher titres than controls. Other researchers found IgG1 and 2 to be unaffected by vitamin E treatments of 0, 1,400, 2,800 mg of dl-α-tocopheryl acetate given orally, or 1,400 mg dl-α-tocopherol injection given at weekly intervals (Reddy et al. 1986). The same researchers tested calf serum in tissue culture for the ability to inhibit replication of bovine infectious rhinotracheitis virus (IBRV). At 12 weeks of age calves given 2,800 mg orally and calves given 1,400 mg injections of vitamin E exhibited an inhibition of viral replication as compared to controls.

Selenium deficiency reduces resistance to infections and inhibits antibody production (Kiremidjian-Schumacher and Stotzky 1987). Concentrations of IgG and IgM can be most effectively boosted by doses of Se considerably in excess of levels required for normal growth; however, that is dependent on species, age, and sex (Turner and Finch 1991). Selenium supplemented Holstein calves challenged with IBRV had increased serum IgM, compared to controls (Reffett et al. 1988). These researchers also found serum IgG concentrations of steers to be unaffected by Se status. Nicholson et al. (1993) supplemented yearling beef cattle with Se, differences in antibody titre were small but tended to be higher in Se supplemented cattle.

Many scientific trials are able to show increases in basic immune parameters with laboratory tests; however, they are unable to show a difference in morbidity and mortality rates of cattle. Droke and Loerch (1989) monitored health of feedlot steers injected with vitamin E and/or Se. Performance and average days sick were unaffected by a single injection, but serum IgG increased linearly with treatment. *Pasteurella haemolytica* antibody response was enhanced with the combination injection of vitamin E and Se. Supplementation with vitamin E and Se often increases immunoglobulin concentrations; however, not all studies find the same results. Differences arise due to different conditions, types of tests performed, types of supplements used, and individual animal differences. Nemec et al. (1990) administered Se and vitamin E boluses to 7 month old Charolais x Simmental heifers. They found no treatment differences in anti-*Brucella abortus* immunoglobulin classes G1, G2, and M; following vaccination with *Brucella abortus* strain 19 vaccine.

Neonatal Calf

Tizzard (1992) discussed immunity of the fetus and newborn. The fetus in utero is capable of mounting an immune response against viruses; however, at birth there is depression of the immune system, due to elevated steroid levels associated with parturition. Newborn calves have a naive immune system, unprimed, which makes them susceptible to invading microorganisms. Newborn calves acquire immunoglobulins from the dam through colostrum. Proteolytic activity of the digestive system is low to prevent immunoglobulin destruction. Quigley et al. (1995) hypothesized from their research that trypsin inhibitor may serve to protect immunoglobulins in the colostrum from digestive enzymes of the calf. These researchers found trypsin inhibitor in colostrum to be closely related to the IgG concentration of colostrum. Immunoglobulins are absorbed intact in the ileum of the small intestine through the process of pinocytosis. Pinocytosis is non-selective in ruminants any immunoglobulin present is absorbed; however, IgG is the most common. Absorptive ability declines after birth and absorption is nearly non-existent by the time the neonate is 24 hours old. Work with newborn calves is difficult to prove significant differences, because there are many factors contributing to large variations in plasma immunoglobulin levels. Colostral quality from the dam is a factor contributing to large variations in calf immunoglobulin levels. Researchers have tried to circumvent this by feeding colostrum from the same source with a known concentration of antibodies. Cipriano et al. (1982) fed colostrum to Holstein calves, giving six of the twelve vitamin E (1mg daily), and variations were still too high to allow for significant differences. Besser et al. (1985) found a negative correlation between efficiency of absorption and quantity of

immunoglobulins fed. These researchers suggest a transport maximum for immunoglobulin absorption in the lower gut of the neonatal calf. Variation in serum immunoglobulins is also attributed to the calves ability to absorb immunoglobulin, age of calf at first feeding, amount of colostrum fed, immunoglobulin concentration in colostrum, and age of cow (Cipriano et al. 1982).

Scours

Field cases of neonatal diarrhea are often due to infection with more than one agent (Haggard 1985). Symptoms of scours include diarrhea, lethargy, loss of appetite, dehydration, and death. Scours incidence is known to be influenced by weather, viruses, bacteria, and plane of nutrition. Several other factors are known to contribute to introduction of scours to a herd including crowded calving pens, age of dam, and low non-specific herd immunity. A study on risk factors associated with neonatal diarrhea was conducted on 5 herds in North Dakota by King et al. (1994). The researchers found herd, year of study, age of dam (primiparous vs multiparous), time of calving, and calf sex to be risk factors with associations to neonatal diarrhea. Quigley et al. (1994) found scours to be less severe for calves housed in individual hutches. These researchers found *Cryptosporidium* infections to occur primarily during the first 2 weeks of life and as a result body weight gain was reduced. Of the microorganisms tested for in feces of scoured calves *Cryptosporidium* was most closely related to scours; however, rotavirus reduced body weight gain and increased severity of scours. *Escherichia coli* bacterial infections occur wherever calves are, however, a contributing factor leading to an outbreak of scours is lowered colostrum consumption (Haggard, 1985). Huang et al.

(1992) analyzed fecal specimens from 78 calves involved in diarrhea outbreaks in Australia in 1988. Samples were analyzed for Rotaviruses and 38 were found positive by enzyme-linked immunosorbent assay. Twenty samples contained double-stranded RNAs detected by polyacrylamide gel electrophoresis. Torres-Medina et al. (1985) reviewed rotaviruse and coronaviruse as causative agents of neonatal diarrhea in cattle. These viruses are the most common causes, with detection involving collection of fresh fecal samples at the onset of diarrhea. These reviewers site bacterial infection and dehydration as secondary complications extending the presence of clinical symptoms. Data on a survey of 1,667 beef herds in Alberta and Saskatchewan found scours to account for 36% of all death losses on average between birth and 30 days in the herds surveyed (Acres and Radostits, Department of Veterinary Internal Medicine, Western College of Veterinary Medicine in Saskatoon Saskatchewan, Canada, published a VIDO fact sheet on calf scours). Of calves born, approximately 27% and 14% born to primiparous and multiparous cows, respectively, scoured prior to one month of age. Two year old cows have calves which are more susceptible to weather conditions than calves born to older cows (Azzam et al. 1993). These researchers also documented temperature and precipitation on day of calving as affecting calf survival rates. The negative effect of precipitation on calf survival increased with decreasing temperature (Azzam et al. 1993). Jenny et al. (1978) found dry matter concentrations of the milk had more influence on scours than did fluid intake level in Holsteins calves. Calves receiving 20% dry matter at 10% body weight all scoured. The researchers also found dry matter concentration and water intake to affect incidence and duration of scours.

Acres and Radostits list five principles for prevention of scours. 1) Remove the infectious agent from the calf's environment. 2) Move the calves to a new environment, free of infectious agents. 3) Increase nonspecific resistance of the calf. 4) Increase specific immunity of the calf, for the infectious agent. 5) Reduce stress to the calves, which will allow them to fight infections more effectively. Vitamin E's immunostimulatory effects may aid in decreasing losses due to morbidity and mortality caused by exposure to pathogens (Hidiroglou et al. 1992a).

Materials and Methods

Cattle and Facilities

The facilities and cattle of EOARC at Union, Oregon, were used for this study. HerefordxSimmental cattle were utilized and consisted of 66 primiparous and 144 multiparous cows for 1994. The 1995 scours study utilized the same herd of cattle including 35 primiparous and 158 multiparous cows. Wind breaks were provided to cows in adequate quantities. Cows had ad libitum access to water. Cows were maintained in pastures containing dormant grasses. Grass hay containing $\leq$ .05 ppm Se was provided daily, for ad libitum consumption.

Treatment

Thirty days prior to parturition cows were stratified by age with stratum randomly assigned to one of two treatments. Treatments consisted of pellets (2.20 mg Se/ kg pellet) which were mill run based with alfalfa and ground corn added for flavor. Pellet components are detailed in Table 1. Pellets were fed at the rate of .45 kg per head per day for both treatments. Vitamin E pellets contained an additional 3310 IU/kg of vitamin E above control pellets which provided 1,500 IU/day above control cows, in the form of dl-α-tocopheryl acetate. Treatments for calves consisted of no supplementation for controls, with vitamin E treated calves receiving intramuscular injections of vitamin E (d-α-tocopherol) at birth (3,000 IU), 2 weeks of age (900 IU), and 4 weeks of age (900 IU). Calves were placed on the treatment regimen corresponding to their dams.

Nutrient	Units	Control	Vitamin E
Calcium	%	3.1984	3.1985
Total Phosphorus	%	3.9979	3.9985
Sodium	%	0.0778	0.0770
Potassium	%	0.9523	0.9429
Manganese	mg/kg	877.2383	876.7158
Zinc	mg/kg	876.4061	875.9028
Iron	mg/kg	416.2776	415.8566
Copper	mg/kg	129.8334	129.7697
Cobalt	mg/kg	2.0392	2.0388
Iodine	mg/kg	7.9194	7.9191
Vitamin A	KIU/kg	111.87	111.87
Vitamin D-3	KIUC/k	11.00	11.00
Vitamin E	IU/kg	18.00	3310.9
Niacin	mg/kg	66.4169	65.9340
Magnesium	%	0.4287	0.4259
Sulfur	%	0.2343	0.2329
Selenium	mg/kg	2.1998	2.1988
Chloride	%	0.1107	0.1093

[a]Formulation of the pellets as determined by Land O'Lakes/Cenex. Vitamin E was provided as D-L-α-tocopheryl acetate.

Samples and Data Collection

Calves were observed daily for scours incidence. Severity of scours was scaled from 0 (no scours) to 4 (severe dehydration and requiring I.V. electrolytes). Ten percent of the cow herd was bled at initialization of treatment (-30 days) and the same cows again 28 days post-partum. Calves were bled at birth and at weekly intervals for 4 weeks.

Venous blood was collected into 10 ml Na-EDTA Vacutainers (Becton Dickinson Vacutainer Systems Rutherford, NJ), and centrifuged to obtain plasma. Plasma was frozen at -20 °C until analyzed. Plasma vitamin E was determined by the method of Chow and Omaye (1983) with modifications. Briefly, vitamin E was extracted in heptane/ethyl acetate (70/30 v/v) and concentrated in methanol. Vitamin E concentration was determined by high performance liquid chromatography (HPLC). Vitamin E concentrations were expressed in μg/ml with a zero value equivalent to less than 0.15 μg/ml. Plasma Cu concentrations were determined via Perkin-Elmer Atomic Absorption/Flame Emission Spectrometer (Perkin-Elmer Corp. Norwalk, CT.; Analytical Methods for Atomic Absorption Spectrophotometry, 1976). Plasma Se was determined with a Perkin-Elmer 3030 instrument (Perkin-Elmer Corp., Norwalk, CT) equipped with an electrode-less discharge lamp and automatic Zeeman-effect background correction (Techniques in Graphite Furnace Atomic Absorption Spectrophotometry, 1985).

Samples from healthy calves were pooled for plasma Se analysis in 1994. Immunoglobulin gamma (IgG) and immunoglobulin mu (IgM) determinations were performed using a commercial radial immunodiffusion kit (VMRD, Inc., Pullman, WA). Serum soluble interleukin-2 receptor (sIL-2R) was determined with the use of radioactive (iodinated with ^{125}I) rabbit IL-2 in a competitive radio immunoassay as described by Khosraviani (1994).

Polymorphonuclear cells (PMN) were isolated for determination of bactericidal activity as modified from Carlson and Kaneko (1973, Appendix 3). Bactericidal ability of PMNs was determined on one week old calves. Six calves per week were chosen for

PMN analysis. Animals were chosen using random numbers during weeks with more than six new calves. Assay, as modified from Metcalf et al. (1986), employed Staphlycoccus auereus, as described in detail in Appendix 3. Anti-Keyhole Limpet Hemocyanin (KLH) IgG concentrations were determined via ELISA, on 22 calves from each treatment within the main cow herd. Calves challenged with KLH were selected from the main cow herd during a period of high birth rate to decrease variation due to age. Serum was collected before calves were injected at one week of age. Calves were injected again at four weeks of age with final serum collected at five weeks of age. The ELISA procedure, as detailed in Appendix 4, used the peroxidase enzyme, as modified from Dill (1991).

Ten bales were used for vitamin E analysis of the forage in each year. Core samples were taken of hay which was made available for the cows. Samples were taken starting July 1993 and at 6 time points through the calving season the first year of the study. During year 2 of the study, samples were started in July of 1994 and taken once a month until the hay was fed in March 1995. Core samples were frozen until shipment to Hoffman-LaRoche laboratories Paramus, New Jersey, for analysis.

Statistical Analysis

Statistical analysis were performed using Statistical Analysis Systems (SAS ver. 6.08) (SAS, 1989). The general linear models (GLM) procedure was used in all analysis with repeated measures used in analysis over time (age of calf). Analysis was performed as a completely randomized design. Factors considered were vitamin E treatment ,

scours, and parity. Non-parametric analysis procedure of SAS, Wilcoxon T test, was used due to non uniform data distribution of PMNs and plasma vitamin E data from 1995.

Statistical Model

$$Y_{iklm}=\mu+\tau_i+\epsilon_{k(I)}+\omega_l+\omega\tau_{il}+\lambda_{k(I)l}$$

Y_{iklm} =dependent variable

μ =overall mean

τ_i =treatment effect where I=vitamin E level

$\epsilon_{k(I)}$ =difference between animals receiving the same treatment where k(ij)=individual animal within treatment group

ω_l =effect of week where l=sample week

$\omega\tau_{il}$ =effect of treatment by week interaction

$\lambda_{k(I)l}$ =difference between animals within weeks and treatment

Results and Discussion

Table 2 displays plasma vitamin E concentrations of cows pre treatment and 28 days post-partum. Plasma vitamin E was confounded in 1994, pretreatment values for vitamin E treated cows were lower than controls ($P < .01$). Cows were assigned to treatment without regard to plasma vitamin E levels. A likely explanation is that by chance the 10% were not representative of the population. It is highly unlikely when randomly assigning 144 cows to treatment that one group would be greatly different than the other. Post partum plasma vitamin E concentrations were increased with vitamin E treatment. Differences within treatment were analyzed and vitamin E treated cows increased .24 μg/ml while control animals decreased .22 μg/ml ($P < .01$). Plasma vitamin E (1995 data) was not different ($P > .1$) between the two groups at initiation of treatment, 30 days pre-partum. Vitamin E concentrations in 1995 control cows were affected by sampling time ($P < .05$), with plasma vitamin E at 28 days post-partum being lower than pretreatment values. Plasma vitamin E levels were lower ($P < .05$) in control cows at 28 days post partum. Vitamin E treated cows had similar plasma vitamin E concentrations at both -30 days and 28 days post-partum. Post-partum vitamin E levels in the vitamin E fed groups were elevated compared to the control groups in both years. This data indicates that vitamin E pellets were effective in elevating plasma concentrations of vitamin E above non supplemented cows. This is in agreement with other researchers who found increased plasma vitamin E concentration with dietary supplementation of vitamin E (Stowe et al. 1988, Hogan et al. 1990, Hidiroglou et al. 1992b, Brzezinska-Slebodzinska et al. 1994).

Table 2: Plasma Vitamin E and Se Concentrations of Cows Orally Supplemented with 1,500 IU Vitamin E per Day[a]

Plasma Component	Year	----Control----		----Vitamin E----	
		Pre-Partum	Post-Partum	Pre-Partum	Post-Partum
Vitamin E	1994	.884 ± .04[b]	.666 ± .04[c]	.693 ± .04[c]	.928 ± .04[b]
Vitamin E	1995	.937 ± .054[b]	.707 ± .054[c]	.990 ± .052[b]	.948 ± .056[b]
Se	1994[y]	24.3 ± 4.5[b]	36.1 ± 4.5[bc]	24.3 ± 4.5[b]	40.3 ± 4.5[c]
Se	1995	12.8 ± 5.1[d]	33.2 ± 5.1[e]	16.1 ± 4.9[d]	39.3 ± 5.3[e]

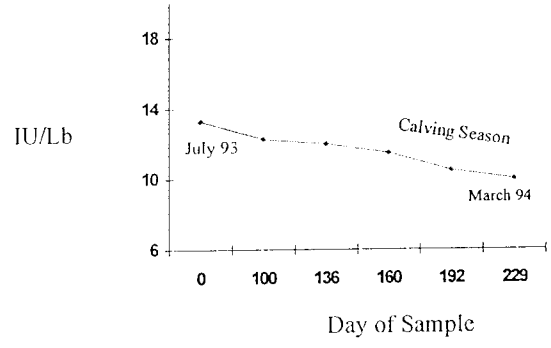
[a] Values are least square means ± standard errors of the mean, vitamin E is displayed in μg/ml and Se in ppb.
[bc] Rows not connected by superscripts different at $P < .01$ or $P < .05$ ([de]).
[y]Pre-Partum Se was analyzed with samples pooled across treatment to obtain a background Se level in 1994.

Cows were fed a diet of stored forage containing 11.5 IU in January 1994 decreasing to 10.0 IU/vitamin E per pound of hay in March 1994 (Figure 1). A decline in forage vitamin E content from harvest in Summer 1993 to Spring 1994 is shown in Figure 1. Forage vitamin E levels for the 1995 calving season have not been completed. The vitamin E content of forage has been shown to decline with storage (Smith et al., 1988). There was very little dormant grass available to the cows at the time of calving. Vitamin E was only available to the cows through the pellets and hay.

Selenium content of plasma for cows was determined (Table 2). The 1994 Se samples were unaffected ($P > .1$) by treatment. Selenium concentrations from 1995 were also unaffected ($P > .1$) by vitamin E treatment. Data is in agreement with Weiss et al. (1990) who found blood Se values to be unaffected by vitamin E supplementation. Stowe et al. (1988); however, found serum Se concentrations of cows to be lower when the cows

were supplemented with vitamin E and Se than Se supplementation alone. Plasma Se levels in cows increased ($P < .01$) between initiation of treatments and post partum samples. Selenium values in pre-treatment cows were at deficient levels. Post partum levels were approaching 40 ppm both years, Van Saun (1989) considers 40-70 ppm marginal. The mineral mix in the pellets included Se for both treatment groups, and this appeared sufficient to increase plasma Se in the study cows. The Se levels were controlled and monitored as vitamin E and Se have been shown to interact (Whanger and Weswig; 1975; Stowe et al., 1988; Van Ryssen et al., 1994).

Figure 1: Vitamin E Content of Hay



Severity of scours was rated on a scale of zero to four, with zero given to animals with no visible signs of scours, and four being very severe. Observed incidence of scours is displayed in Table 3 for 1994 and 1995. Effects of age of dam (heifer versus cow) and vitamin E versus control were statistically analyzed. There was a tendency ($P < .1$) for age of dam to affect scours where 16% of calves scoured from the cow herd but only 7%

of calves from heifers experienced scours in 1994. Vitamin E treatment had no affect on scours incidence (P > .1).

Table 3: Scours Incidence in Beef Calves Injected with Vitamin E

Age[a]	Year	Control	Vitamin E	Overall
Heifers	1994	3/27 (10%)	1/24 (4 %)	4/55 (7%)
Cows	1994	12/51 (19%)	10/58 (15%)	22/131 (16%)
Overall	1994	15/78 (16%)	11/82 (12%)	26/186 (14%)
Heifers	1995	1/13 (8%)	3/18 (17%)	4/31 (13%)
Cows	1995	20/72 (28%)	22/71(31%)	42/143 (29%)
Overall	1995	21/85 (25%)	25/89 (28%)	46/174 (26%)

Injections were given at birth (3,000 IU), 2 weeks (900 IU) and 4 weeks (900 IU). Values within parenthesis are percentages calculated from the actual incidence of scours which are represented in fractions.
[a] Age of dam effect (P = .1) with Fisher's exact 2 tail test.

The 1995 scours incidence was unaffected (P > .1) by vitamin E treatment. Age of dam tended (P < .1) to affect scours incidence, where calves from heifers had fewer cases of scours (13%) than calves from cows (29%). Scours incidence was lower for calves from first calf heifers in both years, when compared to calves from cows, with no treatment affect either year. Colostral quality can affect the health of calves; however, colostrum was not analyzed in this study. One inducer of scours can be over consumption of milk. Cows are known to have increased quantities of milk compared to first calf heifers, which could be one explanation for the increased incidence of scours in calves from cows. The more likely explanation is heifers calved a month ahead of the main cow herd and had more space per animal than the mature cows. Scours has been shown to be influenced by crowded calving grounds (King et al., 1994 and Quigley et al.,

1994). Cow's calves were not excessively crowded but the increased number of calves increases stress and gives infectious agents more opportunity to survive and pass between calves. Calves from heifers were in a cleaner environment and not exposed to as many infectious agents as calves from cows. The smaller number of heifers to calve, space considerations, and consistent weather although colder in late January may have contributed to the differences. The cold weather also kept the pasture frozen and clean. When the older cows calved the weather was usually either wet snow or rain, leading to wet ground and variable weather switching from cold to warm can lead to increased health problems. These observations may partially explain why more of the cow's calves tended to become clinical, as apposed to King et al. (1994), who found age of dam to affect scours incidence in calves, with heifers calves being 3.9 times more likely to contract diarrhea.

Statistical comparisons were not made across year but there was 14% of calves scoured in 1994, while 26% of the 1995 calves scoured. Weather has been shown to affect incidence of scours in beef calves (Azzam et al., 1993). Weather in Northeastern Oregon is variable during February, March and April. One year the temperature will range from the single digits to the thirties during the day. The next year the temperature may not drop below thirty-two and it will rain during these months. These variations in weather from year to year make it difficult to produce consistent levels of scours incidence. Variable weather from day to day will produce an enviroment condusive to

increased scours. Weather data collected at the Union Station during the winters of the scours study has been provided in Appendix 5 for the readers personal edification.

Plasma vitamin E concentrations of calves are shown in Table 4. Vitamin E treatment in 1994 increased ($P < .01$) plasma vitamin E concentrations when averaged over time (age of calf) and scours. Due to a lack of normal distribution, 1995 data was analyzed with pair-wise comparisons using non parametric methods. This analysis did not allow for comparisons over time. Vitamin E treatment increased ($P < .05$) plasma vitamin E concentrations in weeks one through three compared to controls.

Table 4: Plasma Vitamin E Concentrations of Calves Injected with Vitamin E[a]

		----Control----		----Vitamin E----	
Age[b]	Year	Non Scour	Scours	Non Scour	Scour
Birth	1994	.16 ± .03	.19 ± .03	.18 ± .03	.23 ± .03
Week 1	1994	.34 ± .13[c]	.29 ± .13[c]	.92 ± .14[d]	1.15 ± .10[d]
Week 2	1994	.34 ± .10[c]	.27 ± .10[c]	.87 ± .11[d]	.82 ± .11[d]
Week 3	1994	.22 ± .10[c]	.22 ± .10[c]	.98 ± .10[d]	.94 ± .10[d]
Week 4	1994	.15 ± .04[c]	.46 ± .04[c]	.65 ± .04[d]	.51 ± .04[e]
Birth	1995	.43 ± .14[c]	.29 ± .06[c]	.42 ± .04[d]	.46 ± .06[cd]
Week 1	1995	.28 ± .03[c]	.38 ± .08[c]	.93 ± .11[d]	.83 ± .08[d]
Week 2	1995	.30 ± .05[c]	.40 ± .09[c]	1.10 ± .24[d]	.95 ± .13[d]
Week 3	1995	.32 ± .05[c]	.35 ± .08[c]	.90 ± .07[d]	.73 ± .10[d]
Week 4	1995	.62 ± .18[c]	.20 ± .05[d]	.64 ± .09[c]	.53 ± .07[c]

[a] Values are least square means ± the standard error of the mean displayed in µg/ml. Injections were given at birth (3,000 IU), 2 weeks (900 IU) and 4 weeks (900 IU).
[b] Age of calf at which sample was obtained.
[cde] Values within rows not connected by superscripts are different at $P < .05$.

These data indicate our injection regimen was successful in increasing plasma vitamin E concentrations in calves during both years, which agrees with the results of

Hidiroglou et al. (1992a). A treatment by time interaction ($P < .01$) was present (1994 data) where plasma vitamin E concentration increased ($P < .01$) between birth and one week of age in both control and vitamin E injected calves. Vitamin E concentration decreased ($P < .01$) in calves from week 3 to week 4. Plasma vitamin E concentration in 1995 calves was also observed to decline in 3 and 4 week old scoured calves and vitamin E injected calves, while it increased in non-scoured controls. Hidiroglou et al (1994a) suggested that plasma levels from intramuscular injections reach maximum levels within 24 hours, and returned to normal levels within 10 days. The rapid loss of injected vitamin E viewed by Hidiroglou et al. (1994a) would explain decreased plasma values in week 4 both years. Hidiroglou et al. (1994a) found blood vitamin E concentrations of vitamin E injected newborn calves to increase in the first ten days and then decline until thirty days of age. Samples taken at birth were obtained within 24 hours of birth. Calves which had not obtained colostrum prior to sample collection did not receive colostral vitamin E to boost their plasma vitamin E levels. Vitamin E increases due to colostrum content did appear at 1 week of age. Concentrations were not statistically analyzed between 1994 and 1995 but concentrations were increased at birth in 1995 compared to samples at birth in 1994.

One purpose of the study was to establish a relationship between scours incidence and plasma vitamin E concentrations. Scours agents are combated by the immune system of the calf and vitamin E has been shown to increase non specific immunity of calves (Reddy et al., 1986, Reddy et al., 1987b, Nemec et al., 1990, Hidiroglou et al., 1992a,). Scours tended to decrease ($P < .1$) plasma vitamin E

concentrations in vitamin E treated calves at four weeks of age in 1994 (Table 4). Treated calves (Table 4) had lower plasma concentrations of vitamin E when scoured, compared to non scoured vitamin E injected calves. Control calves of the same age did not show this affect. Four week old 1995 calves treated with vitamin E had lower plasma vitamin E concentrations when affected by scours than non scoured calves of the same age and treatment. These scoured calves had numerically lower plasma vitamin E concentrations at weeks 1, 2, and 3. These observations agree with previous years work, in Appendix 1, in which calves with scours had lower plasma vitamin E than non scoured calves. Plasma vitamin E in control calves with scours fluctuated across the board. Some weeks were lower than others, while values in 1995 were increased above those of 1994.

Table 5 is provided as observational data from 1994 because there was not enough animals in each group to provide proper statistical values. Plasma vitamin E concentrations are grouped by treatment, scours, and age of calf at which scours occurred. Table 5 lists the week of scours and the plasma vitamin E concentrations of the calves in both treatments, with and with out scours, in an effort to establish a relationship between scours and plasma vitamin E concentrations. Data from the previous two years work on this herd (Appendix 1) has shown that calves with scours have lower plasma vitamin E concentrations than calves without scours. Plasma vitamin E concentrations in 1994 and 1995 are consistently lower than the pilot study and the 1993 scours study. The 1994 and 1995 calves were provided with increased levels of vitamin E than 1993 calves. A possible explanation is this may simply be year to year variation. Cows in 1993 may

have been exsposed to an earlier spring, with warmer weather in March, leading to earlier development of fresh green grass, making more vitamin E available for the cows.

Table 5: Mean Plasma Vitamin E Levels (µg/ml) ±Standard Error 1994

Tmnt	Status	Week of Scours	n	Birth	Week 1	Week 2	Week 3	Week 4
Control	Non		8	.13 ± .04	.37 ± .06	.30 ± .06	.17 ± .02	.15 ± .02
Control	Scour	1	8	.20 ± .06	.32 ± .09	.22 ± .03	.24 ± .05	.14 ± .01
Vitamin	Non		1	.17	.52	.48	.64	1.29
Vitamin	Scour	1	1	.15	2.04	.64	.66	.61
Control	Non		1	.24	.47	.45	.58	.18
Control	Scour	2	1	.27	.41	.28	.44	.19
Vitamin	Non		2	.12 ± .05	.72 ± .1	.56 ± .06	.63 ± .06	.83 ± .37
Vitamin	Scour	2	2	.13 ± .02	.53 ± .10	.48 ± .23	.73 ± .11	.55 ± .16
Control	Non		1	.25	.16	.29	.16	.12
Control	Scour	3	1	.56	.16	.11	.13	.08
Vitamin	Non		3	.31 ± .15	.66 ± .07	.77 ± .13	.73 ± .10	.67 ± .10
Vitamin	Scour	3	3	.17 ± .02	.72 ± .06	.92 ± .18	.64 ± .23	.50 ± .09
Control	Non		3	.19 ± .08	.28 ± .09	.18 ± .02	.25 ± .05	.11 ± .02
Control	Scour	4	3	.15 ± .05	.19 ± .02	.19 ± .03	.13 ± .01	.13 ± .03
Vitamin	Non		8	.19 ± .05	.90 ± .21	.93 ± .33	1.00 ± .26	.58 ± .06
Vitamin	Scour	4	8	.23 ± .05	1.32 ± .49	.77 ± .09	.96 ± .14	.47 ± .06
Control	Non		7	.17 ± .05	.32 ± .11	.43 ± .16	.20 ± .04	.16 ± .02
Control	Scour	Post	7	.14 ± .04	.29 ± .05	.39 ± .20	.23 ± .05	.19 ± .05
Vitamin	Non		4	.08 ± .03	1.35 ± .27	1.09 ± .47	1.41 ± .52	.54 ± .11
Vitamin	Scour	Post	4	.33 ± .11	1.24 ± .44	1.05 ± .29	1.31 ± .39	.56 ± .09

Vitamin E concentrations from non-scoured calves in 1994 are shown in Table 6. Plasma samples from non-scoured calves were pooled. Plasma vitamin E concentration tended to be increased ($P < .1$) at birth in calves from heifers. As noted in Table 4 a treatment by age interaction was also present. Calves from the vitamin E treated heifers

consistently had increased plasma vitamin E above calves from vitamin E treated cows, with a difference ($P < .05$) at two of the five sample times. Plasma vitamin E concentrations of control calves fluctuated with no consistent pattern between calves from cows and heifers.

Table 6: Vitamin E Concentration of Calves Injected with Vitamin E*

	----Control----		----Vitamin E----	
Age[a]	Heifer	Cow	Heifer	Cow
Birth	.27 ± .05[ef]	.17 ± .04[e]	.54 ± .22[f]	.16 ± .01[e]
Week 1	.31 ± .06[e]	.30 ± .08[e]	1.32 ± .07[f]	1.10 ± .12[f]
Week 2	.23 ± .04[e]	.41 ± .10[e]	1.32 ± .11[f]	.86 ± .11[h]
Week 3	.35 ± .11[e]	.32 ± .07[e]	1.41 ± .11[f]	1.19 ± .17[f]
Week 4	.24 ± .05[e]	.48 ± .28[e]	1.27 ± .12[f]	1.07 ± .24[f]

* Values are least square mean (μg/ml) ± standard error of the mean. Injections were given at birth (3,000 IU), 2 weeks (900 IU) and 4 weeks (900 IU).

[a] Age of calf at which plasma sample was taken.

[efh] Values within the same row not connected by superscripts differ by $P \leq .05$.

Plasma Se levels of calves were analyzed because vitamin E and Se have been shown to interact (Whanger and Weswig, 1975; Stowe et al., 1988; Van Saun et al., 1989a; Van Ryssen et al., 1994). Healthy calves from 1994 were pooled for plasma Se concentrations at the various ages and are shown in Table 7. Plasma Se levels decreased ($P < .01$) in healthy calves from birth to four weeks of age. No affect ($P > .1$) of vitamin E treatment was observed on plasma Se concentration.

Plasma Se concentrations in 1995 were not affected by treatment ($P > .1$, Table 8). Selenium concentrations have been shown unaffected by vitamin E injections (Weiss et al., 1990; Brzezinska-Slebodzinska et al., 1994). Selenium concentrations were

marginal at birth and decreased from birth to four weeks of age ($P < .01$). Other researchers have found plasma Se concentrations to be higher at birth, with declines as long as samples were collected (Weiss et al., 1984; Kincaid and Hodgson, 1989). One explanation of declining Se values in calves seen in our study and other researchers is that little mammary gland transfer of the mineral occures as seen by Van Saun et al. (1989).

Table 7: 1994 Selenium Levels from Pools of Healthy Calves Injected with Vitamin E[a]

Age[b]	Control	Vitamin E	SE[c]
Birth	46.4	51.5	2.90
Week 1	37.9	36.2	3.32
Week 2	34.6	35.6	2.17
Week 3	33.3	33.4	1.82
Week 4	29.0	32.4	2.66

[a] Injections were given at birth (3,000 IU), 2 weeks (900 IU) and 4 weeks (900 IU).
[b] Age of calf at which sample was taken. Plasma Se (ppb) concentrations of pooled samples decreased as age of calf increased ($P < .01$).
[c] Standard error of the least square mean for the treatment effect on Se concentration.

Table 8: Plasma Selenium Concentrations of Scoured Calves Injected with Vitamin E in 1995[a]

	----Control----		----Vitamin E----	
Sample Time[b]	Non-Scours	Scours	Non-Scours	Scours
Birth	31.1 ± 2.9	33.4 ± 3.3	30.5 ± 2.9	33.1 ± 3.2
Week 1	18.3 ± 2.4	22.2 ± 2.8	20.2 ± 2.4	21.3 ± 2.6
Week 2	21.7 ± 2.3	26.0 ± 2.6	22.8 ± 2.3	20.9 ± 2.5
Week 3	21.3 ± 2.4	21.4 ± 2.7	21.3 ± 2.4	21.9 ± 2.6
Week 4	16.8 ± 2.4	22.9 ± 2.7	19.2 ± 2.4	18.8 ± 2.6

[a] Values are LS Means ± standard error of the mean, displayed in parts per billion (ppb). Injections were given at birth (3,000 IU), 2 weeks (900 IU) and 4 weeks (900 IU).
[b] Age of calf at which sample was collected. Plasma Se concentrations declined ($P < .01$) from birth to four weeks of age.

Plasma Se concentrations of 1995 were not affected (P > .1) by scours which supports earlier work shown in Appendix 1. These data indicates no effect of scours incidence upon plasma Se concentrations.

Table 9 shows the effects of vitamin E treatment and scours incidence on plasma Cu concentration of calves from birth to four weeks of age. Plasma Cu concentrations increased (P < .01) with age in the 1994 study. In 1995 plasma Cu levels increased (P < .01) as calves aged with increases between birth and week 1 (P < .01) and between weeks one and two (P < .01).

Table 9: Copper Levels in Scoured Calves Injected with Vitamin E[a]

		----Control----		----Vitamin E----	
Sample Time[b]	Year	Non-Scours	Scours	Non-Scours	Scours
Birth	1994*	.328 ± .021[c]	.397 ± .028[cd]	.359 ± .022[cd]	.409 ± .033[d]
Week 1**	1994*	.960 ± .061[c]	.920 ± .084[c]	1.18 ± .065[d]	1.40 ± .096[d]
Week 2**	1994*	1.08 ± .080[c]	.990 ± .109[c]	1.39 ± .085[d]	1.58 ± .126[d]
Week 3	1994*	1.20 ± .061[cd]	1.03 ± .083[c]	1.22 ± .064[cd]	1.32 ± .096[d]
Week 4	1994*	1.26 ± .064	1.24 ± .088	1.29 ± .068	1.24 ± .101
Birth	1995	.68 ± .05	.66 ± .05	.60 ± .05	.69 ± .05
Week 1**	1995	1.07 ± .06[c]	1.25 ± .06[d]	1.29 ± .06[d]	1.29 ± .07[d]
Week 2	1995	1.32 ± .07	1.38 ± .07	1.32 ± .06	1.38 ± .07
Week 3**	1995	1.08 ± .07[c]	1.21 ± .07[cd]	1.31 ± .06[dc]	1.47 ± .07[e]
Week 4**	1995	.92 ± .06[c]	1.06 ± .06[cd]	1.09 ± .05[de]	1.22 ± .06[e]

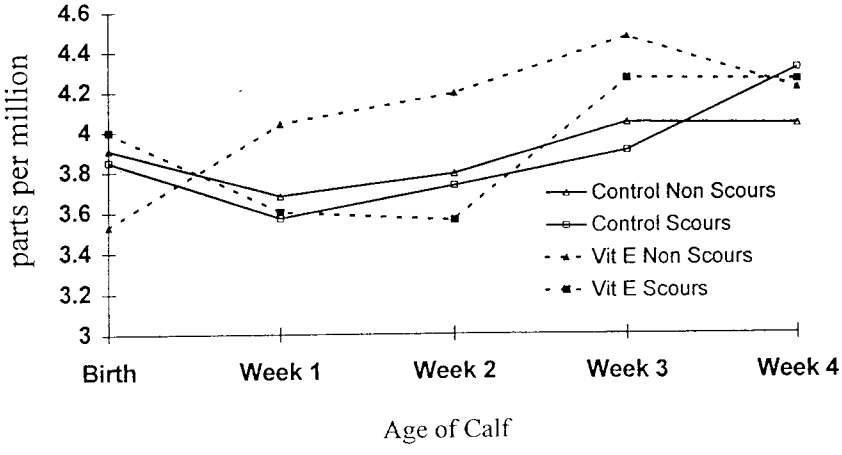
[a] Values are least square means ± the standard error of the mean in ppm. Injections were given at birth (3,000 IU), 2 weeks (900 IU) and 4 weeks (900 IU).
[b] Age of calf at which sample was taken.
[cd] Values within rows which are not connected by superscripts are different (P < .05).
*Copper values increased over time (P < .01)
**Treatment affect significant at P < .05.

Calves from 1995 experienced declining ($P < .01$) Cu concentrations between weeks 3 and 4. Copper concentrations increased between birth and 4 weeks of age in both 1994 and 1995, with the largest increase between birth and one week of age. These increases are consistent with previous year's data (1993, Appendix 1). In 1994, the vitamin E treatment increased ($P < .05$) plasma Cu concentration independent of calf age and scours. Vitamin E treated calves had increased ($P < .05$) plasma Cu concentrations at one and two weeks of age, compared to controls. A treatment by sample time interaction was present ($P < .01$) for plasma Cu in 1995. Vitamin E treatment increased plasma Cu at weeks 1 ($P < .05$), 3 ($P < .01$) and 4 ($P < .01$). Vitamin E treatment increased plasma copper concentrations ($P < .01$), without regards to incidence of scours. Copper concentrations have not been shown to be influenced by vitamin E supplementation of this herd; however, past studies have not included Cu supplementation to the dams. The pellets provided Cu to the cows in this study, as the area has been shown to be marginal in Cu availability. However, Dove and Ewan (1990) found the addition of 250 ppm Cu to the diet decreased serum tocopherol in 5 week old swine during an 8 week trial, compared to pigs fed 5 ppm Cu. Their previous study with Fe, Zn, and Cu found no affect of the minerals on plasma tocopherol concentrations. Dove and Ewan (1991) found the addition of 250 ppm Cu to the diet decreased serum α-tocopherol in pigs with significant differences at some time points, weeks 1-4, 6 and 7; however, it was numerically lower the remainder of the trial. The researchers offered no explanation in the later paper, while Dove and Ewan (1990) suggested the lowered tocopherols was a result of lowered tocopherol in the diet of pigs supplemented with 250 ppm Cu.

Scours increased ($P < .05$) 1995 plasma Cu values at weeks 3 and 4. Scours resulted in increased ($P < .01$) Cu concentrations in calves when averaged over treatment. Plasma Cu has been shown to increase in calves with viral and bacterial challenges (Stabel et al., 1993). Plasma Cu concentration was initially analyzed to see what affect scours incidence has upon Cu levels. Previous years work on this herd, displayed in Appendix 1, has consistently shown calves with scours to have increased plasma Cu levels. Copper concentrations in 1994 and 1995 were increased above those of 1993, likely due to Cu supplementation of the dam in the pellets.

Figure 2 displays plasma Zn concentrations of calves at various ages as affected by scours and vitamin E treatment in 1994. Plasma Zn concentrations increased in calves from birth to four weeks of age ($P < .01$). There were no other consistent significant influences upon plasma Zn levels. Zinc concentrations were not affected by scours incidence ($P > .1$) or vitamin E treatment ($P > .1$). Plasma Zn was analyzed in 1994 to

Figure 2: Plasma Zinc Concentrations 1994 Calves



confirm previous years research which indicates no influence of scours on plasma Zn concentrations as shown in Appendix 1, Table 1.

Figure 3 displays the effect of vitamin E and scours on plasma IgG concentrations in calves between birth and four weeks of age. IgG concentrations declined ($P < .01$) in calves from birth to four weeks of age. Total IgG concentration in the plasma was measured, including quantity acquired from colostrum through pinocytosis and the quantity produced by the calf. IgG has one of the highest turnover rates of the immunoglobulins, so a rapid decline after birth is expected. Neonatal animals have a naive immune system for weeks to months after birth. Vitamin E treatment tended ($P < .1$) to affect IgG concentration in calves differently as the calves aged, compared to controls. Concentrations of IgG in the two treatments did not decline in a like fashion. Scours had no affect ($P > .1$) on IgG levels, but the scoured calves had numerically lower concentrations of IgG in both treatments by four weeks of age.

Figure 3: Plasma IgG Concentrations of Calves

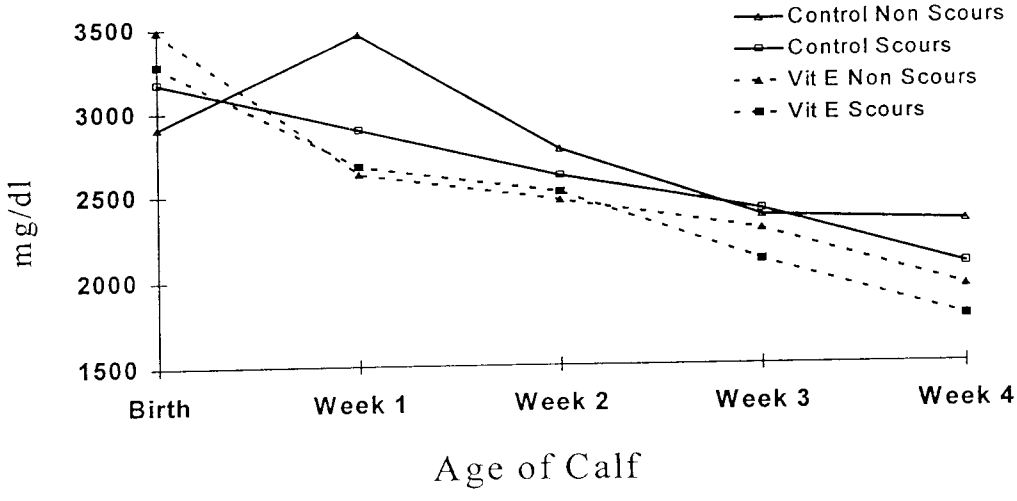


Table 10 displays values from different immune system assays including serum soluble IL-2 receptor, KLH titre, and PMN bactericidal assay. No treatment effect ($P > .1$) was found for any of the variables measured with these assays. Plasma samples from 1994 calves were analyzed in a sIL-2R assay, no treatment effect was found, also there was no affect of scours on the nonspecific binding of the interleukin receptor. Titre to keyhole limpet hemocyanin (KLH) was assayed in 1995, to determine response of neonatal calves to a challenge. No treatment effect was found on the IgG titre against KLH as measured by ELISA. The response of calves to KLH appeared to be responsive to vitamin E supplementation; however, the variation was to high to allow for any significant differences. Transformations were performed on this data in an attempt to eliminate variation, with no success. Bactericidal activity of PMNs in week old calves, from 1995, was not affected by vitamin E injections. Neutrophils from calves injected with vitamin E appeared to kill fewer bacteria, initially and then caught up with PMNs from control animals. This observation was seen while performing laboratory analysis, and can also be seen in Table 10 at the 20 and 40 minute time points. Modifications to the laboratory analysis of PMNs may in the future find a response of PMNs, from neonatal calves, to vitamin E injections. These tests of the cell mediated (PMN and sIL-2R) and humoral (IgG and KLH) immune system test different portions of the immune system. Neonates have a suppressed immune system at birth due to high levels of corticosteroid released during parturition (Tizzard, 1992). The immune system of the neonate is naive, because they have not been exposed to antigens and have not developed memory cells. Calves were not sampled beyond four weeks of age, except KLH

challenged calves which were five weeks old. Hidiroglou et al. (1992a) found calves given injections of vitamin E (2,700 IU) every three weeks had IgM concentrations increased above the control calves. Reddy et al. (1987b) found 24 week old calves to have increased IgG titre when fed vitamin E at 125 IU/day with inoculations of intranasal Antibovine herpesvirus type 1 at 7 and 21 weeks. This may suggest that either the calves were too young or vitamin E injections need to be larger or more frequent to obtain a positive response. Improvements in immune responses in the literature are usually found in older animals. The observations of immune responses and the supporting literature suggest that the calves which we worked with may be to young to expect significant differences.

Table 10: Results of Immune System Function Tests of Beef Calves Supplemented with Vitamin E[a]

Measure	Year	Control	Vitamin E	P value
IL-2 Receptor (specific binding)	1994	3.14 ± .47	2.33 ± .50	P>.1
KLH Titre	1995	11,618 ± 8,736	25,624 ± 9,162	P >.1
Neutrophile assay (% survival of bacteria) (Wilcoxon 2-sample test used to approximate P)				
20 minutes	1995	70.9 ± 5.2	64.4 ± 4.5	P>.1
40 minutes	1995	.18 ± .07	5.12 ± 2.8	P>.1
60 minutes	1995	.05 ± .01	.03 ± .01	P>.1

[a] Values are least square means ± the standard error of the mean. Injections were given at birth (3,000 IU), 2 weeks (900 IU) and 4 weeks (900 IU).

Table 11 displays production data which was analyzed separately for heifers and cows in 1994. Birth weight, average daily gain (ADG), adjusted ADG, weaning weight, 245 day weaning weight, and calving interval were unaffected (P > .1) by vitamin E

treatment in 1994. Production data from 1995 was analyzed across age (cows vs. heifers) and for treatment affects. Birth weight was increased by vitamin E treatment ($P < .01$) for both heifers and cows.

Table 11: Production Data of Calves Injected with Vitamin E from Cows fed 1,500 IU Vitamin E per Day

		----Heifers----		----Mature Cows----	
Variable	Year	Control	Vitamin E	Control	Vitamin E
Birth Weight[b]	1994[a]	36.4 ± .86	36.3 ± .95	39.9 ± .64	40.1 ± .59
ADG[c]	1994[a]	.68 ± .03	.68 ± .03	.86 ± .02	.87 ± .02
Adjusted ADG[c]	1994[a]	.71 ± .03	.72 ± .03	.90 ± .02	.91 ± .02
Wean Weight[b]	1994[a]	205 ± 8.2	209 ± 9.1	235 ± 5.0	238 ± 4.5
245 Day Weight[b]	1994[a]	210 ± 7.7	214 ± 8.6	259 ± 4.5	264 ± 4.4
Calving Interval	1994[a]	395 ± 3.9	390 ± 4.0	363 ± 2.1	366 ± 2.0
Birth Weight[bd]	1995	35.4 ± 1.4[z]	39 ± 1.2[x]	40.9 ± .6[yx]	42.3 ± .5[y]
ADG[ce]	1995	.77 ± .05	.80 ± .05	.85 ± .03	.88 ± .03
Adjusted ADG[ce]	1995	.80 ± .05	.82 ± .05	.87 ± .03	.89 ± .03
Wean Weight[be]	1995	248 ± 13	249 ± 12	229 ± 8	238 ± 7
245 Day Weight[be]	1995	199 ± 10	206 ± 10	220 ± 6	225 ± 5
Calving Interval	1995				

[a] Heifers and cows were analyzed as separate groups of cattle. Calves injections were given at birth (3,000 IU), 2 weeks (900 IU) and 4 weeks (900 IU).
[b] Weight in Kg.
[c] Kg per day gain.
[d] Birth weight increased by vitamin E treatment ($P = .01$) and increased in cows compared to heifers.
[e] Values are confounded by different summer pastures for the cows ($P < .01$).

Adjusted ADG, ADG, weaning weight and 245 day weaning weight were confounded by 1995 summer location ($P < .01$). Cattle were in 5 different pastures with

no respect to treatment. Different pastures had differing quality forages, affecting weight gains of calves. Treatment did not affect ADG, adjusted ADG, weaning weight or 245 day weaning weight ($P > .1$). Average daily gains were adjusted for sex in both years with heifers adjusted up to the steers, based on actual weight gains. Weaning weights were adjusted to a uniform age at weaning for comparisons. Calves in the 1995 weaned at an average of 245 days, so these calves were adjusted to 245 days.

Table 12 displays conception and weaning rates which were analyzed separately for heifers and cows. Vitamin E treatment in 1994 had no effect ($P > .1$) on conception rate or weaning percentages for cows in either herd of cattle. Conception and weaning rates in 1995 were similar to those of 1994. Conception rate in 1995 was unaffected ($P > .1$) by vitamin E treatment in heifers. Cows tended to have a decreased ($P < .1$) conception rate in the vitamin E treated group. This effect carried over to the herd averages ($P < .1$). Other researchers have found production and reproduction parameters to be positively affected (Reddy et al., 1987a; Nockels, 1991;Pehrson et al., 1991; Hidiroglou et al., 1992b) while others have not found any effect (Stowe et al., 1988) of vitamin E.

Table 12: Conception and Weaning Rates of Cows fed 1,500 IU Vitamin E d^{-1}

Age of Dam	Year of Study	------- Conception Rate ------			------- Weaning Rate ------		
		Control	Vitamin E	Overall	Control	Vitamin E	Overall
Heifers	1994	19/30 (63%)	17/25 (68%)	36/55 (65%)	30/33 (91%)	24/29 (83%)	54/62 (87%)
Cows	1994	58/70 (83%)	62/74 (84%)	120/144 (83%)	67/70 (96%)	73/74 (99%)	140/144 (97%)
Overall	1994	77/100 (77%)	79/99 (79%)	156/199 (78%)	97/103 (94%)	97/103 (94%)	194/206 (94%)
Heifers	1995	10/14 (71%)	13/18 (72%)	23/32 (72%)	14/16 (88%)	18/18 (100%)	32/34 (94%)
Cows	1995[a]	63/74 (85%)	53/73 (73%)	116/147 (79%)	70/78 (90%)	73/79 (91%)	143/157 (91%)
Overall	1995[a]	73/88 (83%)	66/91 (73%)	139/179 (78%)	84/94 (89%)	91/97 (94%)	175/191 (92%)

Values in parenthesis are percentages of cows conceived (calves weaned) with the actual numbers listed as the fraction in the same box.

[a] 1995 conception rate was lower in the vitamin E treated cows ($P < .1$) and overall ($P < .1$) with heifers and cows combined.

Conclusions and Implications

Vitamin E treatment was successful in increasing plasma vitamin E, in both cows and calves. Tests performed on the immunocompetence of the calves found no effect of vitamin E. Incidence and severity of scours in calves was not affected by vitamin E treatment. Neonatal calves are still developing at birth, including the immune system. The immune system is often suppressed in new born calves due to hormones involved in parturition. These factors lead to difficulty in finding differences with immune parameters of neonatal calves. Vitamin E treatment was not effective in stimulating any differences in the immune parameters measured. Vitamin E treatment was found to have an effect on birth weights in the second year of the study to slightly repress the pregnancy rate in vitamin E treated cows. All production and reproduction parameters were unaffected by treatment in the first year of the study. Production data in the second year was confounded by location of the cows on summer range, so that any treatment effect could not be analyzed properly.

Further studies should be conducted on scours at a more intense level by injecting calves with an infectious agent, while monitoring vitamin E supplementation and tissue and plasma concentrations. Several factors affect the health of calves and we can only control a small portion of the entire system.

References

Alderson, N.E., G.E. Mitchell, Jr., C.O. Little, R.E. Warner, and R.E. Tucker. 1971. Preintestinal Disappearance of Vitamin E in Ruminants. J. Nutrition. 101:655.

Ammerman, C. B. and S. M. Miller. 1975. Selenium in Ruminant Nutrition: A Review. J. Dairy Sci. 58:1561.

Ammerman, C. B., H. L. Chapman, G. W. Bouwman, J. P. Fontenot, C. P. Bagely, and A. L. Moxon. 1980. Effect of Supplemental Selenium for Beef Cows on the Performance and Tissue Selenium Concentrations of Cows and Suckling Calves. J. Anim. Sci. 51:1381.

Analytical Methods for Atomic Absorption Spectrophotometry. 1976. Perkin Elmer Corp. Norwalk, CT.

Anderson, L.E. Sr., R.O. Myer, J.H. Brendemuhl, and L.R. McDowell. 1995. The Effect of Excessive Dietary Vitamin A on Performance and Vitamin E Status in Swine Fed Diets Varying in Dietary Vitamin E. J. Anim. Sci. 73:1093.

Aziz, E.S., P. H. Klesius, J. C. Frandsen. 1984. Effects of Selenium on Polymorphonuclear Leukocyte Function in Goats. Am. J. Vet. Res. 45:1715.

Azzam, S.M., J.E. Kinder, M.K. Nielsen, L.A. Werth, K.E. Gregory, L.V. Cundiff, and R.M. Koch. 1993. Environmental Effects on Neonatal Mortality of Beef Calves. J. Anim. Sci. 71:282.

Babinszky, L., D. J. Langhout, M. W. A. Verstegen, L. A. den Hartog, P. Joling, M. Nieuwland. 1991. Effect of Vitamin E and Fat Source in Sows' Diets on Immune Response of Suckling and Weaned Piglets. J. Anim. Sci. 69:1833.

Bendich, A. 1993. Physiological Role of Antioxidants in the Immune System. J. Dairy Sci. 76:2789.

Besser, T.E., A.E. Garmedia, T.C. McGuire, and C.C. Gay. 1985. Effect of Colostral Immunoglobulin M Concentrations on Immunoglobulin Absorption in Calves. J. Dairy Sci. 68:2033.

Boyne, R. and J. R. Arthur. 1978. Alterations of Neutrophile Function in Selenium-Deficient Cattle. J. Comp. Path. 89:151.

Brzezinska-Slebodzinska, E., J.K. Miller, J.D. Quigley, III, and J.R. Morre, and F.C. Madsen. 1994. Antioxidant Status of Dairy Cows Supplemented Prepartum with Vitamin E and Selenium. J. Dairy Sci. 77:3087.

Carlson, G. P. and J. J. Kaneko. 1973. Isolation of Leukocytes from Bovine Peripheral Blood. Proc. Soc. Exp. Biol. Med. 142:853.

Charmley, E. and J. W. G. Nicholson. 1993. Injectable α-tocopherol for Control of Oxidized Flavour in Milk from Dairy Cows. Can. J. Anim. Sci. 73:381.

Charmley, E., J. W. G. Nicholson, and J. A. Zee. 1993. Effect of Supplemental Vitamin E and Selenium in the Diet on Vitamin E and Selenium Levels and Control of Oxidized Flavor in Milk from Holstein Cows. Can. J. Anim. Sci. 73:453.

Chew, B.P. 1993. Effects of Supplemental β-Carotene and Vitamin A on Reproduction in Swine. J. Anim. Sci. 71:247.

Chew, B. P., T. S. Wong, and J. J. Michal. 1993. Uptake of Orally Administered β-Carotene by Blood Plasma, Leukocytes. and Lipoproteins in Calves. J. Anim. Sci. 71:730.

Chow, F. I. and S. T. Omaye. 1983. The Use of Antioxidants in the Analysis of Vitamins A and E in Mammalian Plasma by High Performance Liquid Chromatography. Lipids. 18:837.

Chung, Y. K., D. C. Mahan, and A. J. Lepine. 1992. Efficacy of Dietary D-α-Tocopherol and DL-α-Tocopheryl Acetate for Weanling Pigs. J. Anim. Sci. 70:2485.

Church, D.C. and W.G. Pond. 1988. Basic Animal Nutrition and Feeding (3rd ed.). John Wiley and Sons Inc., New York, NY.

Cipriano, J.E., J.L. Morrill, and N.V. Anderson. 1982. Effect of Dietary Vitamin E on Immune Responses of Calves. J. Dairy Sci. 65:2357.

Coelho, M.B. 1991. Bioavailability Methodology. In: M.B. Coelho (Ed.) Vitamin E in Animal Nutrition and Management. BASF Reference Manuel 1991. p. 7.

Combs, G.F. 1991. Mechanism of Absorption, Transport and Tissue Uptake of Vitamin E. In: M.B. Coelho (Ed.) Vitamin E in Animal Nutrition and Management. BASF Reference Manuel 1991. p. 19.

Daniel, L. R., B. P. Chew, T. S. Tanaka, L. W. Tjoelker. 1991. β-Carotene and Vitamin A Effects on Bovine Phagocyte Function In Vitro During the Peripartum Period. J. Dairy Sci. 74:124.

Dill, T.O. 1991. Effect of Zinc, Copper and Selenium Supplementation on the Humoral Immune Response of Weaned Beef Steers. PhD Dissertation. Oregon State University.

Dill, T.O., H.A. Turner, D.W. Weber, D.E. Mattson, R.J. Baker and P.D. Whanger. 1991. Humoral Immune System Response to Copper and Selenium Supplementation in Weaned Beef Steers. Proc. Western Section, Amer. Soc. Anim. Sci. 42:8.

Dove, C. R. and R. C. Ewan. 1990. Effect of Excess Dietary Copper, Iron or Zinc on the Tocopherol and Selenium Status of Growing Pigs. J. Anim. Sci. 68:2407.

Dove, C. R. and R. C. Ewan. 1991. Effect of Vitamin E and Copper on the Vitamin E Status and Performance of Growing Pigs. J. Anim. Sci. 69:2516.

Droke, E.A. and S.C. Loerch. 1989. Effects of Parenteral Selenium and Vitamin E on Performance, Health and Humoral Immune Response of Steers New to the Feedlot Environment. J. Anim. Sci. 67:1350.

Eicher, S. D., J. L. Morrill, F. Blecha. 1994a. Vitamin Concentration and Function of Leukocytes from Dairy Calves Supplemented with Vitamin A, Vitamin E, and β-Carotene In Vitro. J. Dairy Sci. 77:560.

Eicher, S. D., J. L. Morrill, F. Blecha, C. G. Chitko-McKown, N. V. Anderson, and J. J. Higgins. 1994b. Leukocyte Functions of Young Dairy Calves Fed Milk Replacers Supplemented with Vitamins A and E. J. Dairy Sci. 77:1399.

Eicher-Pruiett, S. D., J. L. Morrill, F. Blecha, J. J. Higgins, N. V. Anderson, and P. G. Reddy. 1992. Neutrophil and Lymphocyte Response to Supplementation with Vitamins C and E in Young Calves. J. Dairy Sci. 75:1635.

Grandhi, R. R., M. W. Smith, M. Frigg, and P. A. Thacker. 1993. Effect of Supplemental Vitamin E During Prepubertal Development and Early Gestation on Reproductive Performance and Nutrient Metabolism in Gilts. Can. J. Anim. Sci. 73:593.

Gogu, S. R. and J. B. Blumberg. 1993. Vitamin E Increases Interleukin-2 Dependent Cellular Growth and Glycoprotein Glycosylation in Murine Cytotoxic T-Cell Line. Biochem. Biophys. Res. Commun. 193:872.

Gyang, E.O., J. B. Stevens, W. G. Olson, S. D. Tsitsamis, and E. A. Usenik. 1984. Effects of Selenium-Vitamin E Injection on Bovine Polymorphonucleated Leukocytes Phagocytosis and Killing of *Staphylococcus aureus*. Am. J. Vet. Res. 45:175.

Haggard, K.L. 1985. Bovine Enteric Colibacillosis. Veterinary Clinics of North America: Food Animal Practice. 1:495.

Harrison, J. H., D. D. Hancock, and H. R. Conrad. 1984. Vitamin E and Selenium for Reproduction of the Dairy Cow. J. Dairy Sci. 67:123.

Hidiroglou, N., L. R. Mcdowell and O. Balbuena. 1989. Plasma Tocopherol in Sheep and Cattle After Ingesting Free or Acetylated Tocopherol. J. Dairy Sci. 72:1793.

Hidiroglou, M. and E. Charmley. 1990. Vitamin E Concentration in Blood Plasma of Sheep and in Sheep Tissues after a Single Intraruminal or Intraperitoneal Administration of DL-α-Tocopheryl Acetate. Res. Vet. Sci. 48:158.

Hidiroglou, M., T. R. Batra, L. F. Laflamme, and F. Markham. 1992a. Possible Roles of Vitamin E in Immune Response of Calves. Internat. J. Vit. Nutr. Res. 62:308.

Hidiroglou, N., N. Cave, A. S. Atwal, E. R. Farnworth, and L. R. McDowell. 1992b. Comparative Vitamin E Requirements and Metabolism in Livestock. Ann. Rech. Vet. 23:337

Hidiroglou, N., L. R. McDowell, A. M. Papas, M. Antapli, and N. S. Wilkinson. 1992c. Bioavailability of Vitamin E Compounds in Lambs. J. Anim. Sci. 70:2556.

Hidiroglou, M., T. R. Batra, G. L. Roy. 1994a. Changes in Plasma α-Tocopherol and Selenium of Gestating Cows Fed Hay or Silage. J. Dairy Sci. 77:190.

Hidiroglou, M., M. Ivan and P.L. Toutain. 1994b. Metabolism of Tritiated D-α-Tocopherol and D-α-Tocopheryl Succinate in Intraruminally Dosed Sheep. J. Anim. Sci. 72:2124.

Hogan, J. S., K. L. Smith, W. P. Weiss, D. A. Todhunter, W. L. Schockey. 1990. Relationships Among Vitamin E, Selenium, and Bovine Blood Neutrophils. J. Dairy Sci. 73:2372

Hogan, J.S., W. P. Weiss, D. A. Todhunter, K. L. Smith, P. S. Schoenberger. 1992. Bovine Neutrophil Responses to Parenteral Vitamin E. J. Dairy Sci. 75:399.

Hogan, J.S., W.P. Weiss, and K.L. Smith. 1993. Role of Vitamin E and Selenium in Host Defense Against Mastitis. J. Dairy Sci. 76:2795.

Huang, J., H.S. Nagesha, D.R. Snodgrass, and I.H. Holmes. 1992. Molecular and Serological Analyses of Two Bovine Rotaviruses (B-11 and B-60) Causing Calf Scours in Australia. J. Clinical Microbiology. 30:85.

Jenny, B.F., S.E. Mills, W.E. Johnston and G.D. O'Dell. 1978. Effect of fluid Intake and Dry Matter Concentration on Scours and Water Intake in Calves Fed Once Daily. J. Dairy Sci. 61:765.

Khosraviani, M. 1994. Interaction Between the Immune and Endocrine Systems in Ruminant Animals. Phd Dissertation. Oregon State University

Kincaid, R. L. and A. S. Hodgson. 1989. Relationship of Selenium Concentrations in Blood of Calves to Blood Selenium of the Dam and Supplemental Selenium. J. Dairy Sci. 72:259.

King, M.E., J.C. Clement, M.D. Salmanand K.G. Odde. 1994. Risk Factors Associated with Neonatal Diarrhea in Five North Dakota Cow-Calf Operations. J. Anim. Sci. 72:(Supp 2)99. (Abst.)

Kiremidjian-Schumacher, L. and G. Stotzky. 1987. Selenium and Immune Responses. Environmental Research 42:277.

Koenig, K.M., W. T. Buckley, and J. A. Shelford. 1991. True Absorption of Selenium in Dairy Cows: Stable Isotope Tracer Methodology and Effect of Dietary Copper. Can. J. Anim. Sci. 71:175.

Leedle, R. A., J. A. Z. Leedle, and M. D. Butine. 1993. Vitamin E is not Degraded by Ruminal Microorganisms: Assessment with Ruminal Contents from a Steer Fed a High-Concentrate Diet. J. Anim. Sci. 71:3442.

Lynch, G.L. 1991a. Natural Occurrence and Content of Vitamin E in Feedstuffs. In: M.B. Coelho (Ed.) Vitamin E in Animal Nutrition and Management. BASF Reference Manuel 1991. p. 43.

Lynch, G.L. 1991b. Vitamin E Structure and Bioavailability. In: M.B. Coelho (Ed.) Vitamin E in Animal Nutrition and Management. BASF Reference Manuel 1991. p. 1.

Mahan, D.C. 1994. Effects of Dietary Vitamin E on Sow Reproductive Performance over a Five-Parity Period. J. Anim. Sci. 72:2870.

McDiarmid, R.E., W. Majak and K.J. Cheng. 1994. Procedure for Analysis of α-Tocopherol Acetate in Bovine Ruminal Fluid. Can. J. Anim. Sci. 74:391.

Mertz, W. 1986. Trace Elements in Human and Animal Nutrition Volume 2 (5th ed.). Harcourt Brace Jovanovich, Orlando, Fl.

Metcalf, J. A., J. I. Gallin, W. M. Nauseef, and R. K. Root. 1986. Laboratory Manual of Neutrophil Function. p. 134.

Mottola, C., R. Gennaro, A. Marzallo, and D. Romeo. 1980. Isolation and Partial Characterization of the Plasma Membrane of Purified Bovine Neutrophils. Eur. J. Biochem. 111:341.

Mutetikka, D.B. and D.C. Mahan. 1993. Effect of Pasture, Confinement, and Diet Fortification with Vitamin E and Selenium on Reproducing Gilts and their Progeny. J. Anim. Sci. 71:3211.

Nemec, M., M. Hidiroglou, K. Nielsen, J. Proulx. 1990. Effect of Vitamin E and Selenium Supplementation on some Immune Parameters Following Vaccination Against Brucellosis in Cattle. J. Anim. Sci. 68:4303.

Nicholson, J. W. G., R. S. Bush, and J. G. Allen. 1993. Antibody Responses of Growing Beef Cattle fed Silage Diets with and without Selenium Supplementation. Can. J. Anim. Sci. 73:355.

Njeru, C. A., L. R. McDowell, N. S. Wilkinson, S. B. Linda, S. N. Williams, and E. L. Lentz. 1992. Serum α-Tocopherol Concentration in Sheep After Intramuscular Injection of DL-α-Tocopherol. J. Anim. Sci. 70:2562.

Njeru, C. A. , L. R. McDowell, N. S. Wilkinson, S. B. Linda, L. X. Rojas, and S. N. Williams. 1994a. Serum and Tissue Tocopherol in Sheep after Intramuscular Injection and(or) Dietary Vitamin E Supplementation. J. Anim. Sci. 72:739.

Njeru, C. A., L. R. McDowell, N. S. Wilkinson, S. B. Linda, and S. N. Williams. 1994b. Pre- and Postpartum Supplemental DL-α-Tocopheryl Acetate Effects on Placental and Mammary Vitamin E Transfer in Sheep. J. Anim. Sci. 72:1636.

Njeru, C.A., L.R. McDowell, N.S. Wilkinson, and S.N. Williams. 1994c. Assessment of Vitamin E Nutritional Status in Sheep. J. Anim. Sci. 72:3207.

Njeru, C.A., L.R. McDowell, R.M. Shireman, N.S. Wilkinson, L.X. Rojas, and S.N. Williams. 1995. Assessment of Vitamin E Nutritional Status in Yearling Beef Heifers. J. Anim. Sci. 73:1440.

Nockels, C.F. 1991. Vitamin E Requirement of Stressed Cattle. In: M.B. Coelho (Ed.) Vitamin E in Animal Nutrition and Management. BASF Reference Manuel 1991. p. 43.

NRC. 1984. Nutrient Requirements of Beef Cattle (6th Ed.). National Academy Press, Washington, DC.

Pehrson, B., J. Hakkarainen, M. Tornquist, K. Edfors, and C. Fossum. 1991. Effect of Vitamin E Supplementation on Weight Gain, Immune Competence, and Disease Incidence in Barley-Fed Beef Cattle. J. Dairy Sci. 74:1054.

Quigley, J.D. III, K.R. Martin, D.A. Bemins, L.N.D. Potgieter, C.R. Reinemeyer, B.W. Rohrbach, H.H. Dowlen and K.C. Lamar. 1994. Effects of Housing and Colostrum Feeding on Prevalence of Selected Organisms and Incidence of Diarrhea in Jersey Calves. J. Anim. Sci. 72:(Supp 1)297. (Abst).

Quigley, J.D. III, K.R. Martin, and H.H. Dowlen. 1995. Concentrations of Trypsin Inhibitor and Immunoglobulins in Colostrum of Jersey Cows. J. Dairy Sci. 78:1573.

Reddy, P.G., J.L. Morrill, R.A. Frey, M.B. Morrill, H.C. Minocha, S.J. Galitzer, and A.D. Dayton. 1985. Effects of Supplemental Vitamin E on the Performance and Metabolic Profiles of Dairy Calves. J. Dairy Sci. 68:2259.

Reddy, P.G., J. L. Morrill, H. C. Minocha, M. B. Morrill, A. D. Dayton, and R. A. Frey. 1986. Effect of Supplemental Vitamin E on the Immune System of Calves. J. Dairy Sci. 69:164.

Reddy, P.G., J.L. Morrill, and R.A. Frey. 1987a. Vitamin E Requirements of Dairy Calves. J. Dairy Sci. 70:123.

Reddy, P.G., J. L. Morrill, H. C. Minocha, and J. S. Stevenson. 1987b. Vitamin E is Immunostimulatory in Calves. J. Dairy Sci. 70:993.

Reffett, J.K., J. W. Spears, and T. T. Brown Jr. 1988. Effect of Dietary Selenium on the Primary and Secondary Immune Response in Calves Challenged with Infectious Bovine Rhinotracheitis Virus. J. Nutr. 118:229.

Roquet, J., C. F. Nockels, and A. M. Papas. 1992. Cattle Blood Plasma and Red Blood Cell α-Tocopherol Levels in Response to Different Chemical Forms and Routes of Administration of Vitamin E. J. Anim. Sci. 70:2542.

Roth, J. A. and M. L. Kaeberle. 1985. In Vivo Effect of Ascorbic Acid on Neutrophil Function in Healthy and Dexamethasone-Treated Cattle. Am. J. Vet. Res. 46:2434.

Salih, Y., L.R. McDowell, J.F. Hentges, R.M. Mason, Jr., and C.J. Wilcox. 1987. Mineral Content of Milk, Colostrum, and Serum as Affected by Physiological State and Mineral Supplementation. J. Dairy Sci. 70:608.

SAS. 1989. SAS® Language: Reference, Version 6.08. SAS Inst. Inc., Cary, NC.

Schubert, J.R., O.H. Muth, J.E. Oldfield, and L.F. Remmert. 1961. Experimental Results with Selenium in White Muscle Disease of Lambs. Federation Proc. 20:689.

Shingoethe, D.J., C.A. Kirkbride, I.S. Palmer, M.J. Owens, and W. L. Tucker. 1982. Response of Cows Consuming Adequate Selenium to Vitamin E and Selenium Supplementation. J. Dairy Sci. 65:2338

Smith, K.L., J.H. Harrison, D.D. Hancock, D.A. Todhunter, and H.R. Conrad. 1984. Effect of Vitamin E and Selenium Supplementation on Incidence of Clinical Mastitis and Duration of Clinical Symptoms. J. Dairy Sci. 67:1293.

Smith, L.K., J. S. Hogan, H. R. Conrad. 1988. Selenium in Dairy Cattle: Its Role in Disease Resistance. Veterinary Medicine. 83(1):72.

Spears, J.W., R.W. Harvey, and E.C. Segerson. 1986. Effects of Marginal Selenium Deficiency and Winter Protein Supplementation on Growth, Reproduction and Selenium Status of Beef Cattle. J. Anim. Sci. 63:586.

Stabel, J. R., J. W. Spears, and T. T. Brown, Jr. 1993. Effect of Copper Deficiency on Tissue, Blood Characteristics, and Immune Function of Calves Challenged with Infectious Bovine Rhinotracheitis Virus and *Pasteurella hemolytica.* J. Anim. Sci. 71:1247.

Stowe, H. D., J. W. Thomas, T. Johnson, J. V. Marteniuk, D. A. Morrow, and D. E. Ullrey. 1988. Responses of Dairy Cattle to Long-Term and Short-Term Supplementation with Oral Selenium and Vitamin E. J. Dairy Sci. 71:1830.

Swecker Jr., W.S., D.E. Eversole, C.D. Thatcher, and D.J. Blodgett. 1991. Selenium Supplementation of Gestating Beef Cows on Selenium-Deficient Pastures. Agri-Practice. 12:25.

Techniques in Graphite Furnace Atomic Absorption Spectrophotometry. 1985. Perkin Elmer Corp. Ridgefield, CT.

Tengerdy, R.P., D. L. Meyer, L. H. Lauerman, D. C. Lueker, and C. F. Nockels. 1983. Vitamin E-Enhanced Humoral Antibody Response to Clostridium perfringens Type D in Sheep. Br. Vet. J. 139:147.

Tizzard, I. 1992. Veterinary Immunology An Introduction (4th ed.). WB Saunders Company, Philadelphia, PE.

Torres-Medina, A., D.H. Schlafer, and C.A. Mebus. 1985. Rotaviral and Coranaviral Diarrhea. Veterinary Clinics of North America: Food Animal Practice. 1:495.

Turner, R. J. and J. M. Finch. 1991. Selenium and the Immune Response. Proc. Nutr. Soc. 50:275.

van Ryssen, J. B. J., P. D. Whanger, H. A. Turner, and I. J. Tinsley. 1994. Mineral and Vitamin Interactions of Steers in a Mediterranean Climate. Livest. Prod. Sci. 38:107.

Van Saun, R.J. 1989. A Rational Approach to Selenium Supplementation in Cattle. Proceedings 1989 Cornell Nutrition Conference for Feed Manufactures. pg. 113.

Van Saun, R. J. , T. H. Herdt, and H. D. Stowe. 1989a. Maternal and Fetal Selenium Concentrations and their Interrelationships in Dairy Cattle. J. Nutr. 119:1128.

Van Saun, R. J. , T. H. Herdt, and H. D. Stowe. 1989b. Maternal and Fetal Vitamin E Concentrations and Selenium-Vitamin E Interrelationships in Dairy Cattle. J. Nutr. 119:1156.

Van Vleet, J.F. 1982. Amounts of Eight Combined Elements Required to Induce Selenium-Vitamin E Deficiency in Ducklings and Protection by Supplements of Selenium and Vitamin E. A. J. Vet. Res. 43:1049.

Weiss, W.P., V.F. Colenbrander, M.D. Cunningham, and C.J. Callahan. 1983. Selenium/Vitamin E: Role in Disease Prevention and Weight Gain of Neonatal Calves. J.Dairy Sci. 66:1101

Weiss, W. P., V. F. Colenbrander, and M. D. Cunningham. 1984. Maternal Transfer and Retention of Supplemental Selenium in Neonatal Calves. J. Dairy Sci. 67:416.

Weiss, W.P., D. A. Todhunter, J. S. Hogan, K. L. Smith. 1990. Effect of Duration of Supplementation of Selenium and Vitamin E on Periparturient Cows. J. Dairy Sci. 73:3187.

Weiss, W.P., J.S. Hogan, K.L. Smith, D.A. Todhunter, and S.N. Williams. 1992. Effect of Supplementation on Periparturient Cows with Vitamin E on Distribution of α-Tocopherol in Blood. J. Dairy Sci. 75:3479

Weiss, W. P., J. S. Hogan, K. L. Smith, and S. N. Williams. 1994. Effect of Dietary Fat and Vitamin E on α-Tocopherol and β-Carotene in Blood of Peripartum Cows. J. Dairy Sci. 77:1422.

Whanger, P.D. and P.H. Weswig. 1975. Effects of Selenium, Chromium and Antioxidants on Growth, Eye Cataracts, Plasma Cholesterol and Blood Glucose in Selenium Deficient, Vitamin E Supplemented Rats. Nutrition Reports Inter. 12:345.

Whanger, P.D. 1989. Selenocompounds in Plants and their Effects on Animals: In P.R. Cheeke (Ed.) Toxicants of Plant Origin Volume III Proteins and Amino Acids. p. 141. CRC Press, Boca Raton, Fl.

Wuryastuti, H., H.D. Stowe, R.W. Bull, and E.R. Miller. 1993. Effects of Vitamin E and Selenium on Immune Responses of Peripheral Blood, Colostrum, and Milk Leukocytes of Sows. J. Anim. Sci. 71:2464

Xin, Z., D.F. Waterman, R.W. Hemken, and R.J. Harmon. 1991. Effects of Copper Status on Neutrophil Function, Superoxide Dismutase, and Copper Distribution in Steers. J. Dairy Sci. 74:3078.

Yeh, J-Y., M.A. Beilstein, J.S. Andrews, and P.D. Whanger. 1995. Tissue Distribution and Influence of Selenium Status on Levels of Selenoprotein W. Faseb J. 9:392.

APPENDICES

Appendix 1:

Effect of Vitamin E and Selenium on Neonatal Beef Calves[1]

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[1] To be submitted in an appropriate journal.

Abstract

The objectives of this study were to determine the effect of vitamin E, selenium (Se) or a combination on scours incidence, immunoglobulin (Ig) concentration of neonatal beef calves and certain production and reproduction parameters. Hereford x Angus (n=160) and Hereford x Simmental (n=209) crossbred cows were randomly assigned to vitamin E and Se treatments in a 2 x 2 factorial design. Se treatments consisted of either a 120 day prolonged release bolus (3 mg/d) 30 d prior to expected first parturition or no supplementation. Vitamin E treatments consisted of either a single 10,000 IU intramuscular injection 30 d prior to initiation of expected parturition on a herd basis or no supplementation. All cows received an identical diet consisting of alfalfa-grass hay (.025 ppm Se) and free choice mineral salt devoid of Se or vitamin E. Calves were assigned to the same treatment groups as their dams. Selenium injections (.06 mg/kg of birth weight) were given at birth and 2 wks of age. Vitamin E injections were given at birth (1,500 IU), wk 2 (750 IU) and wk 4 (750 IU) of age. Plasma was obtained from calves at birth (within 24 hrs) and weekly for the first 4 wk. Plasma was analyzed for vitamin E, Se, copper (Cu) and immunoglobulin gamma (IgG) and immunoglobulin mu (IgM) concentrations. Vitamin E supplementation increased ($P < .01$) plasma levels of vitamin E at each week. Calves experiencing scours had decreased ($P < .05$) plasma vitamin E levels as compared to healthy calves within all treatment groups. Plasma Se concentrations were increased ($P < .01$) by Se treatment with them peaking at wk 2 and declining in wks 3 and 4 ($P < .01$). Vitamin E treatment decreased ($P < .01$) plasma Se concentrations. Plasma Cu concentrations increased ($P < .01$) over time across all

treatments. The largest increase in Cu occurred between birth and wk 1 ($P < .01$). Calves with scours had increased ($P < .01$) plasma Cu concentrations over time compared to unaffected calves. IgM concentrations were not affected ($P > .1$) by either treatment or scours. IgG concentrations were decreased ($P < .05$) in calves with scours. IgG concentrations decreased over time ($P < .01$). Se injections slowed the rate of decrease ($P < .01$) in scouring animals, with vitamin E having no effect. The combination of vitamin E and Se was the most effective for slowing the rate of decrease of IgG ($P < .01$). In non scouring animals the only effect ($P < .05$) of treatment was on wk 3 in Se alone which had higher IgG levels than controls. Vitamin E, Se or combination treatment had no influence on scours incidence or severity. Reproductive and production parameters evaluated were not consistently affected by treatment within any of the mature cow groups. Treatment tended ($P < .1$) to affect weaning weights and adjusted average daily gain (ADG) in the heifer group.

Vitamin E and Se treatments were effective in increasing plasma levels of these elements. Calves which experienced scours were found to have a decreased concentration of plasma vitamin E compared to non scoured calves.

Introduction

In Oregon it is estimated that scours cost cattlemen an average of 10 million dollars annually, and in peak scour years such as 1989 this figure can be five to six times higher (Dr. Donald Hansen, personal communication, OSU School of Veterinary Medicine). Thus studies have been underway to investigate the factors influencing the incidence and severity of scours. Selenium and vitamin E have been shown to be important in disease resistance (Droke and Loerch 1990). Cell mediated and humoral immune responses of calves are enhanced by supplemental vitamin E (Hidiroglou et al. 1992; Gogu et al. 1993). Objectives of this study were to determine the effect of vitamin E, Se, or the combination treatment on scours incidence and plasma concentration of vitamin E, Se, Cu, IgG, and IgM in neonatal beef calves, and evaluate production and reproduction parameters in beef cattle.

Materials and Methods

The facilities of Eastern Oregon Agricultural Research Center (EOARC) at Union, Oregon, were used for this study, with cows from both Union and Burns, Oregon Stations. HerefordxSimmental cattle were utilized at Union and consisted of 49 primiparous and 160 multiparous cows. Cattle from Burns were HerefordxAngus multiparous cows (160). Thirty days prior to parturition cows were assigned to one of four treatments, in a 2x2 factorial arrangement, using a randomized block design with calving date as the blocking factor. Treatments consisted of a controlled release Se bolus at 3 mg/d and an intramuscular injection of 10,000 IU of α-tocopherol (+vit +Se), vitamin E injection (+vit), Se bolus alone (+Se), and no additional supplementation (control). Treatments for calves consisted of intramuscular injections of Se by birth weight (.06 mg/kg birth wieght) at birth and two weeks of age and vitamin E at birth (1,500 IU), two and four weeks of age (750 IU) (+vit +Se), vitamin E injections (+vit), Se injections (+Se), and no injections (control). Calves continued on the treatment regimen corresponding to their dams.

Calves were bled at birth and at weekly intervals for 4 weeks. Blood was collected into 10 ml Na-EDTA tubes, and centrifuged to obtain plasma. Plasma was frozen at -20 °C until analysis. Plasma vitamin E was extracted in heptane and concentrated in methanol and concentration was determined by HPLC as modified from Chow and Omaye (1983). Copper plasma levels were determined via Perkin-Elmer Atomic Absorption/Flame Emission Spectrometer (Perkin-Elmer Corp. Norwalk, CT.) Healthy calves were pooled for plasma Se analysis. Plasma Se concentrations were

determined with a Perkin-Elmer 3030 instrument (Perkin-Elmer Corp., Norwalk, CT) equipped with an electrode-less discharge lamp and automatic Zeeman-effect background correction. IgG and IgM determinations were performed using a commercial radial immunodiffusion kit (VMRD, Inc., Pullman, WA). *Phaseolus vulgaris* phytohemagglutinin (PHA-P) lectin was used to challenge calves for determination of cell mediated immunity. Briefly, PHA-P powder was mixed with saline and 2 week old calves injected intradermally. Calves were clipped then shaved with a razor and cleaned with alcohol on the neck at two sites approximately 10 cm apart, control and treated sites. Double skin fold thickness was measured with constant tension calipers (Ralmike's Tool-A-Rama, South Plainfield, NJ). Measures of change in double skin-fold thickness at both sites were used in data analysis. Severity of scours was scaled from 0 (no scours) to 4 (severe dehydration and requiring I.V. electrolytes).

Statistical analysis were performed using Statistical Analysis Systems (SAS ver. 6.08, SAS 1989). The GLM procedure was used in all analysis with repeated measures used in analysis over time (age of calf). Analyses were performed as a completely randomized design, as the effect of the blocking factor (calving date) was inefficient for plasma values. The three factors considered were vitamin E, Se, and scours. Production and reproduction parameters were analyzed considering vitamin E, Se, and the blocking factor.

Statistical Model

$$Y_{ijklm}=\mu+\alpha_i+\beta_j+\alpha\beta_{ij}+\varepsilon_{k(ij)}+\omega_l+\alpha\omega_{il}+\beta\omega_{jl}+\alpha\beta\omega_{ijl}+\lambda_{k(ij)l}$$

Y_{ijklm} =dependent variable

μ =overall mean

α_i =treatment effect due to Se where I=Se level

β_j =treatment effect due to vitamin E where j=level of E

$\alpha\beta_{ij}$ =interaction effect of a specific combination of Se and E

$\epsilon_{k(ij)}$ =difference between animals receiving the same treatment where k(ij)=individual animal within treatment group

ω_l =effect of week where l=sample week

$\alpha\omega_{il}$ =Se treatment interaction with week

$\beta\omega_{jl}$ =interaction of vitamin E with week

$\alpha\beta\omega_{ijl}$ =interaction of specific combination of Se and E with week

$\lambda_{(ij)l}$ =difference between animals on the same treatment and sample week

Results

Previous years work at the Union Experiment Station has shown cattle to have lower plasma vitamin E levels during the winter feeding months (Van Ryssen et al. 1994). This observation led to the pilot study displayed in Table 1, which shows plasma values for calves with and without scours. The pilot study with the experimental herd at EOARC showed plasma vitamin E to be decreased ($P < .01$) and Cu increased ($P < .05$) in calves with scours as shown in Table 1. Plasma levels of Zn, Se, and Vitamin A were unaffected by scours ($P > .1$). Plasma Cu concentration of cattle is known to increase during viral and bacterial challenges (Stabel et al. 1993). The current study was designed to study interactions between scours and plasma vitamin E concentration in calves, during the 1993 winter calving period.

Table 1. Plasma Values in Calves with and without Scours

Mineral or Vitamin	-----Calf Scours----	
	Without	With
Zinc	2.49 ± .22	2.61 ± .22
Copper	.76 ± .13	.89 ± .22*
Selenium	.039 ± .014	.041 ± .018
Vitamin A	3.96 ± .77	3.64 ± 1.14
Vitamin E	2.54 ± 1.16	1.52 ± .54**

*Significantly different (P<.05)
**Significantly different (P<.01)

Plasma vitamin E concentrations of calves (Table 2) were increased ($P < .01$) by intramuscular injections of vitamin E. Calves affected with scours had lower ($P < .05$) plasma vitamin E than healthy calves within the same treatment. This is consistent with

preliminary work displayed in Table 1. Calves which scoured had numerically lower plasma levels of vitamin E at birth (Table 2) than did calves of the same age and treatment which did not scour.

Table 2: Mean (±SEM) Plasma Vitamin E Concentration (μg/ml) within Treatment Group and Disease Status

Disease Status	Control	+Se	+Vit E	+Se +Vit E
		Birth[a]		
Scours	0.33 ± 0.20	0.52 ± 0.18	0.54 ± 0.21	0.90 ± 0.18
Non-Scours	0.91 ±0.20	0.84 ± 0.24	0.69 ± 0.21	0.88 ± 0.22
		Week 1[a]		
Scours	0.52 ± 0.41[b]	0.76 ± 0.37[b]	2.25 ± 0.43[c]	1.67 ± 0.36[bc]
Non-Scours	0.83 ± 0.41[b]	1.10 ± 0.48[be]	1.46 ± 0.43[bc]	2.56 ± 0.48[cd]
		Week 2[a]		
Scours	0.89 ± 0.37	0.57 ± 0.33	1.23 ± 0.38	0.70 ± 0.32
Non-Scours	0.67 ± 0.37[b]	0.87 ± 0.43[bc]	1.92 ± 0.38[c]	1.66 ± 0.39[bc]
		Week 3[a]		
Scours	0.50 ± 0.38[b]	0.67 ± 0.34[bd]	1.78 ± 0.39[ce]	1.02 ± 0.33[bc(*)]
Non-Scours	0.44 ± 0.38[b]	0.79 ± 0.44[b]	2.15 ± 0.39[c]	2.39 ± 0.40[c(*)]
		Week 4[a]		
Scours	0.36 ± 0.16[b]	0.28 ± 0.15[b]	1.43 ± 0.17[c]	1.07 ± 0.14[c(*)]
Non-Scours	0.69 ± 0.16[b]	0.60 ± 0.19[b]	1.85 ± 0.17[c]	1.84 ± 0.17[c(*)]

[a] Age of calf at which plasma sample was obtained.
[bcd] Means within row with different superscripts differ by $P < .05$.
[ef] Means within row with different superscripts differ by $P < .07$.
[(*)] Means within columns different between scours and non-scours by $P < .05$.

Calves experienced scours at one week of age or later. Morbidity and mortality of calves was found to be unaffected by the treatment regimen; however, few researchers

find differences in whole animal measures but differences are usually found in laboratory analysis.

Scours incidence for 1993 calves are displayed in Table 3. No effect of either vitamin E or Se treatment ($P > .1$) was seen on scours incidence. There was an effect of calving group on scours incidence ($P < .01$), as determined with Chi square test, where the two Burns groups had increased incidence of scours above any Union herds.

Table 3: Scour Incidence Data (1993)

Calving Group[a]	Control	+Se	+Vit	+Vit +Se	Group
Heifers	3/8 (38%)	2/12 (17%)	1/12 (8%)	3/9 (33%)	9/41 (22%)
Burns Early	5/14 (36%)	5/14 (36%)	2/13 (15%)	6/14 (43%)	18/55 (33%)
Burns Late	9/26 (35%)	8/21 (38%)	12/20 (60%)	9/24 (38%)	38/91 (42%)
Union Early	3/23 (13%)	0/26 (0%)	4/21 (16%)	3/22 (14%)	10/96 (10%)
Union Late	3/13 (23%)	3/13 (23%)	6/14 (43%)	3/14 (21%)	15/54 (28%)
Overall	23/84 (27%)	18/86 (21%)	25/84 (30%)	24/83 (29%)	90/337 (27%)

[a] Calving group affect ($P < .01$) with Chi-Square test.

Plasma Se values of calves are shown in Table 4, displayed by treatment and age of calf. Treatment of calves with vitamin E decreased ($P < .01$) plasma Se levels across all treatments. This is in agreement with Stowe et al. (1988) who found vitamin E and Se supplemented cows to have a lower serum Se concentration than cows supplemented with Se alone. However, Weiss et al. (1990) found blood Se values of dairy cows to be unaffected by supplemental vitamin E. Plasma Se was increased ($P < .01$) by treatment with intramuscular injections of Se. Plasma Se values peaked at wk 2 and declined in

weeks 3 and 4, ($P < .01$). Calves were injected with Se at birth and 2 weeks of age, this was anticipated to maintain increased plasma Se levels; however, levels declined in weeks 3 and 4. Van Saun (1989) considers a serum Se concentration of < 40 ppb as deficient. This would indicate that the only group that had even marginal Se levels was the group receiving only Se injections.

Table 4: Effect of Vitamin E on Mean (±SEM ng/ml) Plasma Se Concentrations in Healthy Calves

Week[a]	Control	+Se	+Vit E	+Se +Vit E
Birth	23.2 ± 4.6^{b}	40.0 ± 4.6^{c}	16.8 ± 4.6^{b}	26.9 ± 4.6^{bc}
Week 1	22.6 ± 4.5^{b}	50.9 ± 4.5^{c}	18.2 ± 4.5^{b}	41.2 ± 4.5^{c}
Week 2	23.7 ± 3.8^{b}	54.9 ± 3.8^{c}	16.9 ± 3.8^{b}	33.1 ± 3.8^{b}
Week 3	21.7 ± 4.6^{bc}	49.3 ± 4.6^{d}	10.8 ± 4.6^{b}	31.8 ± 4.6^{c}
Week 4	21.4 ± 4.9^{b}	40.8 ± 4.9^{c}	13.4 ± 4.9^{b}	27.8 ± 4.9^{bc}

[a] Age of calf at which plasma sample was obtained.
[bcd] Means within row with different superscripts differ by $P<.05$.

Plasma Cu concentrations (Table 5) were not dependent upon treatment of calves with either vitamin E, Se or the combination of the two; however, plasma Cu increased ($P < .01$) with age. The largest increase of plasma Cu occurred between birth and week 1 ($P < .01$). Scours, over the sample dates, increased ($P < .01$) plasma Cu concentrations when compared to non scoured calves, in agreement with Table 1.

Total IgG concentration of calf plasma was measured and is displayed in Table 6. IgG molecules are provided to the calf from the cow through colostrum, in order to boost the calve's immune system. IgG concentrations decreased over time ($P < .01$), which is

representative of metabolism of colostral antibodies. IgG concentrations were decreased ($P < .05$) in calves with scours indicative of a loss of IgG due to combating of infection.

Table 5: Influence of Scours Incidence and Se and Vitamin E Supplementation on Mean (±SEM) Plasma Cu Levels (μg/ml)

Disease Status	Control	+Se	+Vit E	+Se +Vit E
		Birth[a]		
Scours	.408 ± .054	.360 ± .049	.397 ± .070	.404 ± .069
Non-Scours	.508 ± .083	.414 ± .058	.421 ± .099	.371 ± .067
		Week 1[a]		
Scours	.882 ± .054	.798 ± .049	.947 ± .070	.894 ± .067
Non-Scours	.689 ± .082	.830 ± .058	.917 ± .099	.894 ± .067
		Week 2[a]		
Scours	.922 ± .039[bc]	.867 ± .036[b]	1.187 ± .051[d]	1.049 ± .048[cd]
Non-Scours	.802 ± .060[b]	.849 ± .042[b]	1.082 ± .072[c]	.995 ± .048[c]
		Week 3[a]		
Scours	.892 ± .095	1.060 ± .087	1.100 ± .123	1.061 ± .118
Non-Scours	.735 ± .145	.976 ± .103	.685 ± .174	.837 ± .118
		Week 4[a]		
Scours	1.106 ± .076[b]	.802 ± .069[c]	.877 ± .098[bc]	.953 ± .094[bc]
Non-Scours	.969 ± .116[b]	.853 ± .082[c]	.932 ± .139[bc]	.747 ± .094[bc]

[a] Age of calf at which plasma sample was obtained.
[bcd] Means within row with differing superscripts different by $P<.05$.

Se injections slowed the rate of decrease ($P < .01$) of IgG in scouring animals, with vitamin E having no effect (Table 6). The combination of vitamin E and Se was the most effective for slowing the decrease of IgG ($P < .01$). The decline in IgG concentration was slowed by Se injections and the combination treatment, the mechanism of which is unknown. Nicholson et al. (1993) has shown Se supplemented cattle tend to have a

higher antibody titer than non supplemented animals. Slowing the loss of immunoglobulins leaves more IgG for combating infection, allowing for a healthier calf. In non scouring animals the only significant effect of treatment was on week 3 in Se alone which had higher IgG levels than controls ($P < .05$).

Table 6: Mean (±SEM) Plasma IgG Concentration (mg/dl) in Calves With and Without Scours by Treatment Group

Disease	Control	+Se	+Vit E	+Se +Vit E
		Birth[a]		
Scours	3629 ± 659^{bd}	2163 ± 435^{e}	2048 ± 579^{e}	2839 ± 478^{de}
Non-Scours	3756 ± 510	2557 ± 477	3289 ± 510	2659 ± 581
		Week 1[a]		
Scours	$1993 \pm 282^{bd(*)}$	2320 ± 186^{bc}	$2071 \pm 248^{bd(*)}$	2642 ± 205^{ce}
Non-Scours	$2748 \pm 219^{de(*)}$	2641 ± 204^{d}	$3217 \pm 219^{e(*)}$	2670 ± 249^{de}
		Week 2[a]		
Scours	$1648 \pm 268^{b(**)}$	$1959 \pm 177^{b(**)}$	$1868 \pm 236^{b(**)}$	$2606 \pm 195^{c(**)}$
Non-Scours	$2246 \pm 208^{(**)}$	$2450 \pm 194^{(**)}$	$2478 \pm 208^{(**)}$	$1964 \pm 236^{(**)}$
		Week 3[a]		
Scours	$1415 \pm 234^{d(*)}$	1780 ± 155^{de}	1818 ± 206^{de}	1950 ± 170^{e}
Non-Scours	$2134 \pm 181^{(*)}$	1785 ± 170	2087 ± 181	2072 ± 206
		Week 4[a]		
Scours	$1266 \pm 165^{b(**)}$	$1495 \pm 109^{bd(*)}$	$1457 \pm 145^{bd(*)}$	1804 ± 120^{e}
Non-Scours	$1651 \pm 128^{d(**)}$	$1973 \pm 119^{e(*)}$	$1992 \pm 128^{e(*)}$	1710 ± 145^{de}

[a] Age of calf at which plasma sample was taken.
[bc] Means within row with differing superscript differ by $P < .05$.
[de] Means within row with differing superscript differ by $P < .1$.
[(*)] Means within columns different between scours and non-scours by $P < .05$ or [(**)] $P < .1$.

IgM concentrations were found to be unaffected ($P > .1$) by scours or either of the vitamin E and Se treatments. IgM concentrations in cow colostrum are low, leaving low

levels in calf plasma. Low levels and high variability of IgM did not allow any statistical differences due to either treatment or scours ($P > .1$).

Skin fold thickness was analyzed using calf (treatment) as the error term, to account for between calf variation (Table 7). The differences between control and challenge site at the initial and final time points were analyzed. Treatment affected ($P < .01$) the skin fold thickness measurement. The combination treatment had an increased thickness above the controls and these two treatments had increased thickness above the groups receiving either vitamin E or Se. Time of measurement ($P < .01$) affected the measurement, with initial measurement difference being smaller than the final difference. The initial measurement is expected to be the same as the same amount of solution was injected under the skin in each site.

Table 7: Double Skin Fold Thickness after Calves were Challenged with *Phaseolus vulgaris*

Treatment	Before	After[a]	Difference between control and challenge sites
Control	0.3 ± 0.1	2.5 ± 0.2^{bc}	1.4 ± 0.1^{de}
+ Vit E	0.2 ± 0.1	2.2 ± 0.2^{c}	1.2 ± 0.1^{ef}
+ Se	0.2 ± 0.1	2.1 ± 0.2^{c}	1.1 ± 0.1^{f}
+ Vit E + Se	0.4 ± 0.1	2.7 ± 0.2^{b}	1.6 ± 0.1^{d}

[a] Treatment affect ($P < .1$) on the differences between control and challenge sites with the two time points analyzed separately.
^{bc} Values within a column not connected by superscripts differ at $P < .1$.
^{def} Values within column not connected by superscripts differ at $P \leq .05$.

Between calf variation (error term) ($P < .01$) had a significant affect on skin thickness measurements. When differences between injection sites on the calf were analyzed, within treatment, the combination group had the largest difference. The control treated calves had a larger difference than the groups receiving either vitamin E or Se treatment alone.

Production and reproduction data from these cows is displayed in Table 8. The cows were assigned to treatments within a blocking factor (calving group), and production and reproductive data were analyzed within calving group. Average daily gain of calves from heifers tended to be affected by treatment, with a vitamin E x Se interaction ($P < .1$). Selenium treatment increased ADG ($P < .01$) in the Burns Early group, while Se decreased ($P < .05$) this variable in the Burns Late group. The ADG of calves from the mature Union cows were not affected by any treatment ($P > .1$). Weaning weights of calves are adjusted to 205 days for comparison. Weaning weights of calves from heifers tended to be affected with a vitamin E x Se treatment interaction ($P < .1$). Heifers treated with either vitamin E or Se weaned heavier calves than those treated with the combination or no supplement. Weaning weights of calves from Burns cows were affected by Se treatment ($P < .05$); however, early calving cows weaned heavier when supplemented with Se while late calving cows weaned lighter calves. Weaning weights of calves from the mature Union cows were unaffected by treatment ($P > .1$). Vitamin E treatment increased ($P < .05$) the calving interval for cows in the Burns Early calving group, while no other groups were affected by any treatment ($P > .1$). Conception rate of

cows (Table 9) was unaffected (P > .1) by treatment. Treatment also had no affect (P > .1) on weaning rates of calves.

Table 8: Production Data for Cows Injected with Vitamin E and Se 30 Days Pre-Partum

		Zero Se		Se	
Calving Group		Zero Vitamin E	Vitamin E	Zero Vitamin E	Vitamin E
Heifers[A]	adg[x]	.50 ± .052[a]	.63 ± .040[b]	.63 ± .052[b]	.57 ± .061[ab]
Burns Early[B]	adg[x]	.67 ± .025	.65 ± .026	.76 ± .026	.71 ± .025
Burns Late[C]	adg[x]	.75 ± .020	.76 ± .022	.70 ± .022	.71 ± .021
Union Early	adg[x]	.85 ± .028	.85 ± .027	.89 ± .027	.86 ± .029
Union Late	adg[x]	.91 ± .038	.84 ± .038	.90 ± .038	.86 ± .036
Heifers[A]	WW[y]	151 ± 13.1[a]	183 ± 10.0[b]	182 ± 13.09[b]	167 ± 15.50[ab]
Burns Early[C]	WW[y]	187 ± 6.45	183 ± 6.68	205 ± 6.68	199 ± 6.45
Burns Late[C]	WW[y]	201 ± 5.73	187 ± 6.09	177 ± 6.23	178 ± 5.82
Union Early	WW[y]	241 ± 6.95	242 ± 6.77	252 ± 6.82	243 ± 7.27
Union Late	WW[y]	256 ± 9.50	240 ± 9.50	256 ± 9.50	244 ± 9.14
Heifers	CI[z]	405 ± 10.9	396 ± 7.1	393 ± 6.6	381 ± 7.7
Burns Early[D]	CI[z]	367 ± 7.4	388 ± 7.8	393 ± 7.1	395 ± 7.1
Burns Late	CI[z]	377 ± 5.4	365 ± 5.9	364 ± 5.8	366 ± 5.6
Union Early	CI[z]	368 ± 3.5	371 ± 3.3	376 ± 3.4	371 ± 4.0
Union Late	CI[z]	357 ± 3.2	353 ± 4.2	357 ± 5.5	358 ± 4.7

Production variables measured for cows in 1993 scour study were [x]adjusted average Daily gain (kg/day) [y]205 day weaning weight (kg) and [z] calving interval in days.
[ab] Means within rows not connected by superscripts differ by P < .1.
[A] Interaction of vitamin E and Se at P < .1.
[B] Se treatment effect at P < .01, or [C] at P < .05.
[D] Vitamin E effect at P < .05.

Treated heifers had numerically shorter calving intervals compared to controls, with the shortest calving interval in the vitamin E and Se treated group. Vitamin E and Se injection tended to interact to affect weaning weight and ADG of calves from these

cows. Treatment of calves with either vitamin E or Se improved weight gain above controls, while calves receiving both vitamin E and Se had an intermediate performance. Mature cows from Union were completely unaffected by treatment. Cows from the Early calving Burns group had calves which weaned heavier and gained faster with Se treatment. Late calving cows from Burns had calves which gained slower and weaned lighter when they received Se supplementation.

Investigations have been conducted as to why the two Burns groups performed differently when given Se supplementation under the same conditions. Cattle from Burns are accustomed to a High Desert type of climate, and were transported to Union for this winter due to a hay shortage in Burns. Northeastern Oregon is a forest type environment. The different types of climates may explain the performance differences of the Burns cattle.

Table 9: Percent of Cows Pregnant (preg), or Weaned a Calf (wean) which were given Vitamin E and/or Se Injections 30 Pre-Partum

Calving Group		Control	+Se	+Vit	+Se +Vit	Overall
Heifers	preg	3/7 (43%)	9/11 (82%)	9/12 (75%)	6/6 (100%)	27/36 (75%)
Burns Early	preg	11/14 (79%)	11/14 (79%)	13/13 (100%)	12/14 (86%)	47/55 (85%)
Burns Late	preg	24/27 (89%)	20/26 (77%)	22/25 (88%)	23/26 (88%)	89/104 (86%)
Union Early	preg	19/23 (83%)	21/25 (84%)	22/24 (92%)	16/21 (75%)	78/93 (84%)
Union Late	preg	11/13 (85%)	11/13 (85%)	7/13 (54%)	10/14 (71%)	39/53 (74%)
Overall	preg	68/84 (81%)	72/89 (81%)	73/87 (84%)	67/81 (83%)	280/341 (82%)
Heifers	wean	7/7 (100%)	7/11 (64%)	12/12 (100%)	5/6 (83%)	31/36 (86%)
Burns Early	wean	14/14 (100%)	13/14 (93%)	13/13 (100%)	14/14 (100%)	54/55 (98%)
Burns Late	wean	25/27 (93%)	22/26 (85%)	22/25 (88%)	24/26 (92%)	93/104 (89%)
Union Early	wean	23/23 (100%)	24/25 (96%)	24/24 (100%)	21/21 (100%)	92/93 (99%)
Union Late	wean	13/13 (100%)	13/13 (100%)	13/13 (100%)	14/14 (100%)	53/53 (100%)
Overall	wean	82/84 (98%)	79/89 (89%)	84/87 (97%)	78/81 (96%)	323/351 (95%)

Conclusions

Calves that experience scours have lower vitamin E plasma levels, but more research is needed to determine if decreases in plasma vitamin E are a cause or an effect of scours. Vitamin E injections decreased the calving interval for first calf heifers, which allows an earlier breed back after calving and increased weaning weights of their calves. Morbidity and mortality differences may need more severe weather conditions or a higher level of challenge from disease organisms before differences are apparent. Further work is being conducted on this relationship between scours and vitamin E.

References

Chow, F. I. and S. T. Omaye. 1983. The use of Antioxidants in the Analysis of Vitamins A and E in Mammalian Plasma by High Performance Liquid Chromatography. Lipids. 18:837.

Droke, E.A. and S.C. Loerch. 1989. Effects of Parenteral Selenium and Vitamin E on Performance, Health and Humoral Immune Response of Steers new to the Feedlot Environment. J. Anim. Sci. 67:1350.

Gogu, S. R. and J. B. Blumberg. 1993. Vitamin E Increases Interleukin-2 Dependent Cellular Growth and Glycoprotein Glycosylation in Murine Cytotoxic T-Cell Line. Biochem. Biophys. Res. Commun. 193:872.

Hidiroglou, M., T. R. Batra, L. F. Laflamme, and F. Markham. 1992. Possible Roles of Vitamin E in Immune Response of Calves. Internat. J. Vit. Nutr. Res. 62:308.

Nicholson, J. W. G., R. S. Bush, and J. G. Allen. 1993. Antibody Responses of Growing Beef Cattle Fed Silage Diets with and without Selenium Supplementation. Can. J. Anim. Sci. 73:355.

Stabel, J. R., J. W. Spears, and T. T. Brown, Jr. 1993. Effect of Copper Deficiency on Tissue, Blood Characteristics, and Immune Function of Calves Challenged with Infectious Bovine Rhinotracheitis Virus and *Pasteurella hemolytica*. J. Anim. Sci. 71:1247.

Stowe, H. D., J. W. Thomas, T. Johnson, J. V. Marteniuk, D. A. Morrow, and D. E. Ullrey. 1988. Responses of Dairy Cattle to Long-Term and Short-Term Supplementation with Oral Selenium and Vitamin E. J. Dairy Sci. 71:1830.

Weiss, W.P., D. A. Todhunter, J. S. Hogan, and K. L. Smith. 1990. Effect of Duration of Supplementation of Selenium and Vitamin E on Periparturient Cows. J. Dairy Sci. 73:3187.

van Ryssen, J. B. J., P. D. Whanger, H. A. Turner, and I. J. Tinsley. 1994. Mineral and Vitamin Interactions of Steers in a Mediterranean Climate. Livest. Prod. Sci. 38:107.

Appendix 2:

Effect of Selenium Boluses on Weight Gain and Feed Efficiency of Wintering Beef Steers[1]

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Abstract

The objective of this study was to test the effect of selenium (Se) boluses on weight gain and feed efficiency of yearling beef steers wintered on Se deficient hay. This study was conducted over two winter feeding periods. Year one consisted of 87 Hereford x Simmental yearling steers. Steers were bled and weighed on days 0, 56, and 105 of the study. In year two, 85 Hereford x Simmental yearling steers were bled and weighed on days 0, 49, and 98 of the study. Each weight was taken after overnight depravation of food and water. Whole blood was collected on weigh dates in EDTA tubes and analyzed for Se. Whole blood Se determination was performed by fluorometry following nitric and perchloric acid digestion and dissolution of whole blood sample. Animals were stratified by initial weight and randomly assigned to treatments. Treatments were control (no bolus) or Se treatment (Dura Se-120 releasing 3 mg d^{-1} for 120 days). Steers were allowed ad libitum access to water and grass hay (≤ .023 ppm Se). Intake was determined on 20 animals each year by estimation of fecal output, with the use of chromium (Cr) marker boluses (Captec Chrome Captec PTY. Ltd. Australia). Steers were paired based on their interim weight, utilizing 10 from each treatment group. Chromium boluses were administered on interim weigh dates. Fecal samples were obtained on 7 different days for each of the 20 steers, between 8 and 18 days after administration of Cr boluses. Fecal samples were composited for each animal to achieve a representative sample. Hay samples were taken by core sampling bales fed to Cr bolused steers. In vitro digestibility was determined and along with fecal output

estimates used to calculate feed intake. Statistical analysis were performed using Statistical Analysis Systems (SAS ver 6.08).

Steers in year one lost weight with no treatment effect ($P > .1$), however Se treated animals tended ($P > .1$) to have lower feed intake (6.92 vs. 7.31 kg/day). Whole blood Se in year one was increased ($P < .01$) by Se boluses when averaged over all time points. Selenium treatment in year two increased feed efficiency ($P < .1$) from 25.6 to 41.3 (g gain d^{-1})/(kg intake d^{-1}), respectively, for control and Se bolused animals. Weight gain was increased ($P = .05$) by Se treatment in year two. Selenium boluses increased ($P < .01$) whole blood levels from the initial 93.5 ± 4.0 to 114.5 ± 4.0 and 184.0 ± 4.0 ppb for the interim and final bleedings, respectively. Control animal Se levels decreased ($P < .01$) from 91.4 ± 4.0 initially to 73.8 ± 4.1 and 51.6 ± 4.0 ppb on the interim and final bleedings, respectively. Selenium blood levels were not different between treatments at the initial bleeding, but were higher ($P < .01$) for the Se bolused group throughout the remainder of the trial. In summary Se boluses when utilized with Se deficient hay for wintering yearling steers improved feed efficiency. Feed intake was less for Se supplemented steers when gain was not affected by treatment, intake was the same between treatment groups when Se supplements improved weight gains. This data would suggest that Se supplementation is beneficial even in situations where a gain response is not realized.

Introduction

Selenium (Se) has been shown to affect cattle in many ways. Se affects reproduction (Van Saun et al. 1989), scours, and IgM concentrations (Weiss et al. 1983). The effect of Se on weight gain and feed efficiency is not well understood. Weiss et al. (1983) found no affect on rate of gain or total gain from Se/vitamin E injections. Swecker et al. (1991) found that calves whose dams received mineral salt supplement with 120 ppm Se had an increased average daily gain for the first sixty days after birth. Spears et al. (1986) suggests that a Se deficiency can decrease calf gains, without showing any clinical signs of deficiency.

This study was conducted at the Eastern Oregon Agricultural Research Center of Oregon State University (EOARC), in Northeastern Oregon. Much of the feed produced on the Union Station is Se deficient (< .05 ppm). Station cattle traditionally respond to supplemental Se in terms of additional weight gains or improved reproductive performance. Past studies have not measured feed efficiency. The objective of this study was to test the effect of Se boluses on weight gain and feed efficiency of yearling beef steers wintered on Se deficient hay.

Materials and Methods

In year 1, 87 Hereford X Simmental yearling steers from EOARC at Union were utilized to test the effect of supplemental Se on weight gain and feed efficiency, with a Se deficient diet. Year two consisted of 85 Hereford x Simmental yearling steers. Steers were stratified by weaning weight and randomly assigned to treatments of Se or control. Selenium treated steers received controlled release Se boluses releasing 3 mg per day (Dura Se-120 Shering -Plough Animal Corp. Kenilworth, New Jersey), on day 0 of the study. Steers ran together at Union with adequate shelter, free access water, and free access to Se deficient hay ($\leq$ .023 ppm). Hay, in 500 kg round bales, was fed in four round bale feeders. Year one steers (1994) were started on Se deficient hay at inception of study. Year two steers (1995) received Se deficient hay four weeks prior to initial weigh date.

Steers were weighed three times throughout the study representing beginning, interim, and final weights. Year one weights were collected on days 0, 56, and 105. Year two weights were collected on days 0, 49, and 98. Animals were gathered the previous afternoon and shrunk overnight, without access to feed or water. Blood was collected from each animal on weigh dates in 10 ml purple topped EDTA Vacutainer tubes for determination of Se content of whole blood. Whole blood Se determination was performed by fluorometry following nitric and perchloric acid digestion and dissolution of whole blood sample.

Intake and feed efficiency were determined on 20 steers with the use of chromium marker boluses (Captec Chrome Captec PTY. Ltd. Australia). Steers were paired based

on their interim weight, 10 control and 10 Se treated. Chromium boluses were administered on interim weigh dates. Steers receiving Cr boluses ran in a pen separate from the remaining steers, until the end of fecal collection. Seven fecal samples were collected between days 8 and 18 after administering chromium boluses, and then composites were made to achieve a representative sample for each animal. Twelve core samples were taken from six round bales which were fed to steers with chromium boluses to analyze in vitro digestibility. In Vitro digestibility and fecal output estimates were used to estimate feed intake.

Statistical analysis was performed on weights and whole blood Se using repeated measures of proc GLM of SAS (ver 6.08). Statistical analysis of forage intakes was performed using proc Ttest of SAS (ver 6.08). Feed efficiency was determined by ratio of the means (Casella and Berger 1990) dividing Kg of feed intake per day into g of gain per day.

Results and Discussion:

Steers receiving Se in year one consumed less hay (P > .1), as shown in Table 1. Intake of steers receiving the Se boluses was 0.39 kg/day less on a dry matter basis than the controls. Weight gains over the length of the trial were similar between control and Se supplemented groups with both groups losing. Losses were over 4 kg for both control and Se supplemented groups. Weight loss was due to poor quality forage with no concentrate supplements. Both protein and energy were deficient. Properly fed this class of animal should gain between .34 and .68 kg per day to realize an economic return on a winter feeding program. Actual target levels would depend on future management of these steers.

Table 1: Se Feed Efficiency Study Value means ± standard error

	Year	Control	Se treated
Total Gain[a]	1994	-4.19 ± 1.5	-4.31 ± 1.6
	1995[e]	20.7 ± 2.0	26.5 ± 2.1
Whole Blood Se Conc.[b]	1994	192 ± 9.7	213 ± 10.4
	1995	51.6 ± 4.0	184.0 ± 4.0
Forage Intake % body weight[c]	1994	2.80 ± .10	2.66 ± .14
	1995	2.69 ± .06	2.72 ± .08
Actual Forage[d] Intake	1994	7.31 ± .31	6.92 ± .27
	1995	7.47 ± .20	7.58 ± .18

[a] Total gain of steers over trial period (kg).
[b] Whole blood Se values (ppb), final bleeding.
[c] Intake on a dry matter basis as a percentage body weight.
[d] Kg intake per day, dry matter basis.
[e] Se treatment increased gain (P = .05) compared to controls in 1995.

Reported adequate blood Se levels are approximately 100 ppb (Mertz 1986). Whole blood Se values of 130 to 212 ppb for steers in year one did not indicate a deficiency for either group. Selenium boluses did not increase blood Se levels ($P > .1$) in year 1. Possibly due to a saturation of tissue levels in both treatment groups. Lack of response to supplementation would indicate the steers were not Se deficient. Weight gains were also not affected. However feed intake was less for steers receiving the Se bolus.

Figure 1 demonstrates whole blood Se increased ($P < .01$) in year one when averaged over all time points with no differences between treatments. Whole blood Se increased ($P < .05$) between initial and interim samples and middle and final ($P < .01$) sample times when averaged over treatment. In year two, Se boluses were effective in increasing whole blood Se ($P < .01$) throughout the trial, whereas controls experienced declining values ($P < .01$).

Steers in year two gained more ($P = .05$) when treated with Se boluses than controls by 5.8 kg. Intake (Kg\day) was similar between treatments for control and Se treated steers. Feed efficiency was increased ($P < .1$) in Se bolused steers to 41.3 from 25.6 (g gain d^{-1})/(kg intake d^{-1}) for controls.

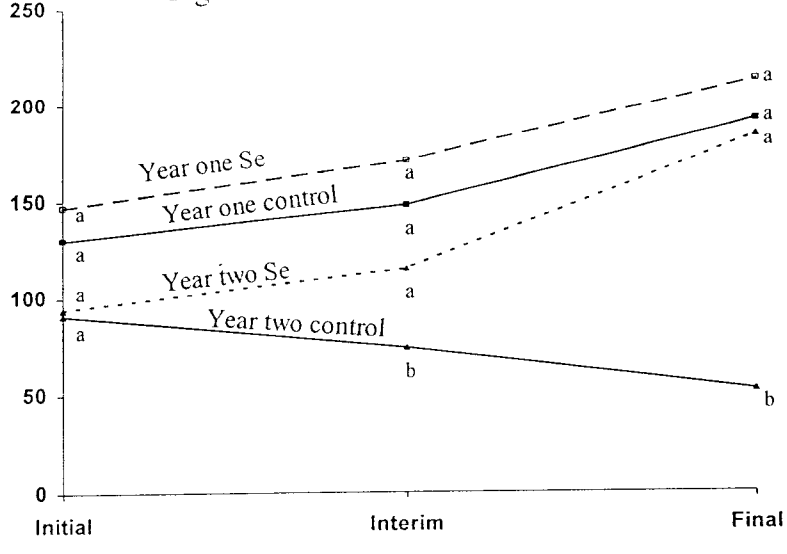
Initial blood Se levels were lower in year two at 91.4 ± 4.0 for control and 93.5 ± 4.0 ppb in Se bolused ($P > .1$). Control animals experienced decreased whole blood Se levels at interim (73.8 ± 4.1) and final (51.6 ± 4.0) bleedings compared to initial sampling ($P < .01$). Selenium boluses increased ($P < .01$) blood Se levels from initial to interim (114.5 ± 4.0 ppb) and final (184.0 ± 4.0 ppb) bleedings.

The effect of Se on weight gain and feed consumption was tested in Alturas, California a few years back. These studies found similar gains in controls and Se injected groups; however, there was a decreased hay consumption of 0.72 kg (Pierce 1976)[a] one year and 0.28 kg (Pierce 1977)[b] a second year, in the Se injected group. It is interesting to note that despite the poor feeding regime and adequacy of Se blood levels throughout year one, our results were similar to those of the California studies in terms of feed efficiency.

[a]Peirce, Cecil. 1976. Effect of Selenium on Average Daily Gain and Feed Consumption of Steer and Heifer Calves. Extension Handout. Univ. of California.

[b]Peirce, Cecil. 1977. Effect of Selenium on Average Daily Gain and Feed Consumption of Heifer Calves. Extension Handout. Univ. of California.

Figure 1: Whole Blood Se Concentrations



Conclusions:

Supplemental Se for steers wintered on Se deficient hay improved feed efficiency. In year one, feed intake was less for those receiving Se with gains being equal. Se supplemented steers consumed 0.39 kg/day less, dry matter basis, with 90 percent dry matter hay that is 0.43 kg/day per steer. A 150 day winter feeding period with 100 steers would save approximately 6,500 kg hay for the winter. Steers in year two gained more weight when treated with Se with intake equal to that of the controls, improving feed efficiency. This data would suggest that Se supplementation improves feed efficiency with or without a corresponding positive gain response.

References Cited

Casella, G. and R.L. Berger. 1990. Statistical Inference. p. 330. Brooks/Cole Publishing Company. Belmont, California.

Mertz, W. 1986. Trace Elements in Human and Animal Nutrition (5th edition). Academic Press Inc. Orlando Florida.

SAS. 1989. SAS® Language: Reference, Version 6.08. SAS Inst. Inc., Cary, NC.

Spears, J.W., R.W. Harvey, and E.C. Segerson. 1986. Effects of Marginal Selenium Deficiency and Winter Protein Supplementation on Growth, Reproduction and Selenium Status of Beef Cattle. J. Anim. Sci. 63:586.

Swecker Jr., W.S., D.E. Eversole, C.D. Thatcher, and D.J. Blodgett. 1991. Selenium Supplementation of Gestating Beef Cows on Selenium-Deficient Pastures. Agri-Practice. 12:25.

Van Saun, R. J. , T. H. Herdt, and H. D. Stowe. 1989. Maternal and Fetal Selenium Concentrations and Their Interrelationships in Dairy Cattle. J. Nutr. 119:1128.

Weiss, W.P., V.F. Colenbrander, M.D. Cunningham, and C.J. Callahan. 1983. Selenium/Vitamin E: Role in Disease Prevention and Weight Gain of Neonatal Calves. J. Dairy Sci. 66:1101

Appendix 3:

Neutrophile Procedures for Scours Study

Collection of plasma will be as follows:

1. Collect blood in 10 ml EDTA Vacutainer (Becton Dickinson Vacutainer systems, Rutherford, NJ).
2. Tip blood to mix with anticoagulant, keep from freezing to prevent hemolysis.
3. Centrifuge blood samples for 25-30 minutes at about 3,000 rpm.
4. Remove plasma and place in numbered scintillation vials.
    a. number the plasma samples oldest to youngest
    b. use a different colored pen for every 50 samples
    c. record the number on the corresponding data sheet, for the appropriate calf
5. Box in numerical order, freeze, mark as plasma samples and await shipment to OSU for analysis.

Isolation of PMNs from Whole Calf Blood

1. Collect 50 mls calf blood into one 60 cc syringe containing 3 mls of anticoagulant, invert syringe to mix anticoagulant and blood.
2. Pour into 50 ml tube and spin at 2,800 rpm for 20 min.
3. Suction off serum, buffy coat and some Red Blood Cells (RBCs), leaving approximately 15-20 mls of RBCs.
4. Pour RBCs into flask, rinse out tubes with Hanks Balanced Salt Solution (HBSS).
5. Lyse RBCs by adding 50 mls 0.001 M phosphate buffer.

6. Stop lysis at end of 50 seconds by adding 25 mls of 0.001 M phosphate buffer plus 2.7% NaCl.
7. Pour into 50 ml tubes and spin at 1,500 RPM for 10 minutes.
8. Pour off supernatant and resuspend pellet in 10 mls HBSS.
9. Spin at 1,500 rpm for 10 minutes.
10. Pour off supernatant and resuspend pellet in HBSS with 1.0% ovalbumin.
11. Spin at 1,500 rpm for 10 minutes.
12. Pour off supernatant and resuspend pellet in 2.0 mls HBSS with 1.0% ovalbumin.
13. Stain with trypan blue for live dead, do differential stain for purity.
14. Count PMNs with hemocytometer and adjust to $5x10^6$ in HBSS with 1.0% ovalbumin.

Serum Collection for the Neutrophile Experiment

1. Collect blood in tubes with no anticoagulant (red topped vacutainers).
2. Allow blood to sit at room temperature for one hour.
3. Aseptically remove the plug and ring the clot.
   a. flame a pipet
   b. release the clot from the top of the tube
4. Recap the tube and let sit overnight in the refrigerator, or allow to sit two to three hours at room temperature.
5. Centrifuge and collect the serum from the top of the clot.

6. Pool the serum and mix thoroughly, then put 2.0 ml aliquots in cryovials and freeze.
7. Store the vials in the liquid nitrogen tank until needed for experiment.

Preparation of Bacteria for Neutrophile Experiment

1. Inoculate 10 ml of Tryptic Soy Broth (TSB) from stock bacteria culture, *Staphylococcus aureus*, allow to grow overnight.
2. Inoculate 10 ml of TSB with 0.15 ml of culture grown overnight.
3. Place on shaker until broth reaches 50% transmittance at 650 nm, as determined by previously performed series of dilutions.
4. Centrifuge at 1,000 g for 10 minutes and wash the pellet twice in HBSS.
5. Resuspend pellet in HBSS, adjusting to $2.5x10^8$ cells/ml.

Assay Control for Test of Microbicidal Activity of Neutrophils

1. Run one set of controls for each batch. Controls do not have any neutrophils in the test tubes.
2. Duplicates are run of every experimental tube and control.
3. Pre-label experimental tubes, water tubes, and petri dishes (label bottom). The label should include the #, time of incubation, and dilution.
4. Each control tube will contain 0.1 ml serum (pooled) and 0.8 ml HBSS.
5. Add 0.1 ml bacteria to control tubes first and mix.
    a. Take 0.01 ml from control tube, place in water tube containing 10 ml distilled water and vortex.
    b. Plate 10 minutes later using 0.01 ml for 10^{-5} and 0.1 ml for 10^{-4} dilution.

c. Immediately pour agar and bacteria, swirl to mix.

6. Incubate the cell free controls for 60 min on the shaker.

a. At 20 min add 0.01 ml lysostaphin, after the experimental tubes have been sampled at 20 minutes.

b. Sample the controls at 60 min as at the initial sampling, in step 5.

7. Allow agar to harden and incubate petri dishes upside down overnight at 37°C.

8. Count the colony forming units and record.

Assay of Microbicidal Activity of Neutrophils

1. Include a set of controls each time the experiment is conducted, all tubes are duplicated.

2. Pre-label the water tubes, petri dishes, and the experimental tubes. Petri dishes should have sample time, dilution, and calf number.

3. Contents of experimental tubes: 0.5 ml of neutrophils, 0.1 ml pooled serum, 0.3 ml HBSS; warm for five minutes

4. Add 0.1 ml bacteria to each experimental tube, cap and place on shaker.

5. Sample at 20, 40, and 60 minutes adding 0.01 ml lysostaphin to all tubes after 20 minute sample period.

6. Sampling procedure

a. 20 and 40 minute samples for experimental tubes

1. 0.01 ml is removed from the experimental tube and added to 10 ml distilled sterile water.

2. Vortex water tubes and let them sit for ten minutes to lyse the neutrophils
3. Re-vortex mixture and plate 0.01 ml for the 10^{-5}, 0.1 ml for 10^{-4}, and 1.0 ml for 10^{-3} dilutions.
4. Pour agar and swirl with bacteria to mix.

b. 60 minute sampling for the experimental tubes is the same as 20 and 40 minute sampling except

1. 0.1 ml of bacteria is extracted from the experimental tube.
2. 0.01 ml of water bacteria mixture is plated for 10^{-4}, 0.1 ml for 10^{-3} and 1.0 ml for 10^{-2} dilutions.

7. Allow agar to harden and incubate upside down overnight at 37°C.
8. Count colony forming units and record.

Appendix 4:

Elisa Procedure for KLH

Coating wells with antigen:

1. Dilute antigen to 5 µg/ml in PBS coating buffer.
2. Add 0.1 ml of diluted antigen to each well of a 96 well microtiter plate. (Linbro/Titertek EIA Plate, ICN catalog # 76-381-04).
3 Seal wells with parafilm and store at 4°C until needed. (At least overnight but okay to store for 10-14 days).

ELISA Procedure:

In this analysis 12 known positive serums will be serially diluted in a separate microtitre plate and then transfered to an EIA plate, which is coated with Rabbit anti-bovine IgG. Known negatives were tested and it was determined to use a 1:100 dilution for known negatives to calculate a corrected absorbance. Titrecal (software program) used the corrected absorbance to calculate the titre from the ELISA procedure.

1. Dilute serums to be tested 1:25 (25 µl serum + 600 µl diluent) in a test tube.
2. Add 150 µl diluent to a clean microtitre plate as in the following diagram:

	1	2	3	4	5	6	7	8	9	10	11	12
A	empty row.....											
B	150	150	150	150	150	150	150	150	150	150	150	150
C	150	150	150	150	150	150	150	150	150	150	150	150

through row H.

3. Transfer 150 µl of the 1:25 dilution of each serum into row A of the dilution plate, so 12 separate diluted serums are in row A. Using a multichannel pipet mix and

transfer 50 µl of row A to row B and so forth through row H. The result is a 1:4 dilution of each serum down the plate from row A to row H.

Dilutions

row A: 1:25

row B: 1:100

row C: 1:400

row D: 1:1600

row E: 1:6400

row F: 1:256000

row G: 1:1024000

row H: 1:4096000

4. Remove an antigen coated microtitre plate from the freezer and wash 3 times with the plate washer, filled with wash solution. Allow one 10 minute soak before the last rinse. Following the last rinse, vaccum the plate dry and tap any residual wash solution out on a stack of paper towels. Proceed to step 5 immediately.
5. Transfer 100 µl of each well from the diluting plate to the corresponding well antigen coated plate. This is done with a 12 channel multipipetor starting in row H, the most dilute, and ending with row B, leaving row A empty.
6. Cover the plate with parafilm and incubate 30 minutes on the rotator in a 37°C incubator.

7. Remove the plate and wash twice, leaving the plate to soak 5 minutes after the 2nd wash. Rinse plate once more and tap residual wash solution out on paper towels.

8. Dilute peroxidase labeled rabbit anti bovine IgG (H+L) F(ab)2 according to previously performed titration. Dilute with ELISA diluent, fresh for each plate. This antibody was diluted 1:5000, 4 µl into 20 ml. Add 100 µl of conjugate to each well of the plate.

9. Cover the plate with parafilm and incubate 30 minutes on the rotator in a 37°C incubator.

10. Wash the plate, again, as in step 7. Prepare the substrate solution.

11. Immediately after the final wash tap the plate dry and add 100 µl of substrate solution to each well, start the watch at the filling of the last well.

12. Allow color to develop, on the rotator in a 37°C incubator. Monitor the color of the positive control until it reaches the predetermined reading, then read the entire plate. Read the plate at 410 nm on the EIA plate reader.

TEN Buffer (Stock Solution)

Tris base (0.05M)	6.05 g	12.1 g
EDTA disodium salt dihydrate (0.001M)	0.37 g	0.74 g
NaCl (0.15M)	8.76 g	17.52 g
distilled H_2O	1000 ml	2000 ml

adjust to pH 7.5 with concentrated HCl (~3.5 ml/1000 ml TEN)

ELISA Diluent (for serum and conjugate dilutions):

TEN Buffer	100 ml
Tween 20	0.2 ml
Mix well	

ELISA Wash Solution: (TEN + 0.05% tween 20)

TEN Buffer	900 ml	1000 ml	2000 ml
Tween 20	0.45 ml	0.50 ml	1.0 ml
Mix well			

Citrate Buffer (0.01M) (mix fresh each day):

Citric Acid monohydrate	0.21 g
Distilled H_2O	100 ml
Adjust pH to 4.0 with NaOH	

Substrate Solution

Solution A: dilute 150 µl of 40 mM stock ABTS in 20 ml Citrate buffer

Solution B: dilute 0.1 ml stock H_2O_2 (30%) in 0.9 ml citrate buffer

Solution A is light sensitive

Just before use: Mix 50 µl solution B in 20 ml solution A

Appendix 5:

Weather Data from Calving Seasons of 1993, 1994, and 1995

Date	Temperatures (degrees F) 24 Hr			Precipitation (inches) 24 Hr. Amounts		At obser.
Jan. 1993	Maximum	Minimum	Observation	Rain, melted snow, etc	Snow, ice, hail, etc	Snow on ground
1	35	28	30	.09	1.4	2
2	30	13	16	.33	3.7	6
3	29	15	19	0	0	5
4	25	17	24	0	0	5
5	28	21	26	0	0	5
6	38	11	23	0	0	4
7	24	9	10	T	T	4
8	24	9	22	.01	.4	4
9	24	12	14	.20	3.5	7
10	19	0	9	.02	.5	8
11	21	7	10	.07	1.5	8
12	20	2	10	.01	.2	8
13	27	10	19	T	T	8
14	33	19	33	.05	1	8
15	38	27	31	.02	0	6
16	34	25	32	0	0	6
17	33	27	29	.03	.2	6
18	36	27	27	0	0	6
19	30	26	30	.01	T	6
20	41	29	40	.01	T	5
21	45	32	33	.04	0	4
22	40	31	31	.18	.1	3
23	34	18	19	0	0	3
24	29	18	29	.04	.5	3
25	34	25	27	.02	.5	3
26	38	25	26	0	0	3
27	33	22	25	0	0	3
28	31	24	27	0	0	3
29	31	24	28	.02	.2	3
30	38	20	26	0	0	3
31	29	22	22	0	0	3

Date	Temperatures (degrees F) 24 Hr			Precipitation (inches) 24 Hr. Amounts		At obser.
Feb. 1993	Maximum	Minimum	Observation	Rain, melted snow, etc	Snow, ice, hail, etc	Snow on ground
1	28	16	18	0	0	3
2	28	8	12	0	0	3
3	26	12	24	0	0	2
4	34	23	30	0	0	2
5	36	28	33	0	0	2
6	39	32	36	0	0	2
7	39	34	38	0	0	1
8	41	31	36	0	0	1
9	44	35	40	.02	0	T
10	44	36	35	.05	0	T
11	46	34	38	.02	0	T
12	40	32	34	.07	0	T
13	39	33	36	0	0	T
14	41	29	30	0	0	T
15	43	22	22	.16	1.6	2
16	24	9	12	0	0	1
17	24	4	7	0	0	1
18	26	6	26	.02	.2	1
19	40	25	39	.03	T	1
20	47	27	29	T	T	T
21	42	26	32	0	0	T
22	36	22	26	.05	.7	1
23	35	26	30	.02	.5	1
24	32	20	22	.17	.6	1
25	27	7	15	0	0	1
26	30	7	14	0	0	T
27	28	11	16	0	0	T
28	30	15	19	0	0	T
29						
30						
31						

Date	Temperatures (degrees F) 24 Hr			Precipitation (inches) 24 Hr. Amounts		At obser.
March 1993	Maximum	Minimum	Observation	Rain, melted snow, etc	Snow, ice, hail, etc	Snow on ground
1	30	15	19	0	0	T
2	31	19	28	.01	.1	T
3	34	27	30	.02	.5	1
4	37	27	28	T	T	T
5	36	27	36	.15	.2	T
6	45	34	35	0	0	T
7	42	34	37	0	0	T
8	42	35	38	0	0	T
9	47	27	33	T	0	0
10	45	33	37	T	0	0
11	48	25	31	0	0	0
12	49	24	34	0	0	0
13	41	30	34	.01	T	T
14	45	33	38	0	0	0
15	45	37	45	.08	0	0
16	54	34	37	.19	0	0
17	44	36	42	.03	0	0
18	50	41	43	.10	0	0
19	57	39	44	.28	0	0
20	57	39	45	.04	0	0
21	54	31	36	.01	0	0
22	58	36	51	0	0	0
23	62	51	54	T	0	0
24	58	37	38	.46	0	0
25	43	32	37	.02	0	0
26	49	37	44	.01	0	0
27	57	36	41	0	0	0
28	59	29	41	0	0	0
29	58	27	40	0	0	0
30	46	26	37	.05	0	0
31	55	27	39	0	0	0

Date	Temperatures (degrees F) 24 Hr Values			Precipitation (inches) 24 Hr. Amounts		At obser.
April 1993	Maximum	Minimum	Observation	Rain, melted snow, etc	Snow, ice, hail, etc	Snow on ground
1	63	39	46	.14	0	0
2	51	39	42	.08	0	0
3	58	41	49	.01	0	0
4	56	36	39	.28	0	0
5	43	30	47	.14	0	0
6	48	26	33	T	0	0
7	54	33	46	.04	0	0
8	62	45	50	0	0	0
9	62	35	41	.04	0	0
10	52	34	37	.31	0	0
11	52	33	37	.24	0	0
12	46	35	37	.14	0	0
13	52	29	41	0	0	0
14	59	34	40	.13	0	0
15	56	39	46	T	0	0
16	48	28	33	.05	0	0
17	60	33	48	.01	0	0
18	55	39	43	.08	0	0
19	52	31	46	.02	0	0
20	58	29	38	0	0	0
21	61	37	50	0	0	0
22	62	40	46	0	0	0
23	54	34	46	.04	0	0
24	59	40	52	.13	0	0
25	56	41	48	.02	0	0
26	65	36	44	.23	0	0
27	53	36	43	.02	0	0
28	57	30	41	0	0	0
29	60	40	50	.05	0	0
30	57	42	46	.43	0	0
31						

Date	Temperatures (degrees F) 24 Hr			Precipitation (inches) 24 Hr. Amounts		At obser.
Jan. 1994	Maximum	Minimum	Observation	Rain, melted	Snow, ice,	Snow on
				snow, etc	hail, etc	ground
1	42	31	35	.25	0	0
2	47	34	39	.01	0	0
3	42	36	38	.22	0	0
4	44	37	44	.04	0	0
5	49	34	35	.14	0	0
6	39	32	33	.10	.5	T
7	37	29	32	0	0	0
8	38	32	35	.01	T	T
9	37	33	35	.04	.2	T
10	38	30	32	0	0	0
11	38	32	35	.04	0	0
12	44	34	35	.01	0	0
13	39	35	39	.15	0	0
14	45	37	40	0	0	0
15	45	35	35	0	0	0
16	48	26	27	0	0	0
17	46	27	32	0	0	0
18	45	31	33	0	0	0
19	44	30	30	0	0	0
20	46	25	29	0	0	0
21	40	28	39	0	0	0
22	45	36	40	0	0	0
23	50	37	39	0	0	0
24	47	37	38	T	0	0
25	50	32	34	.01	0	0
26	44	30	32	0	0	0
27	44	29	35	0	0	0
28	43	25	28	0	0	0
29	44	30	20	0	0	0
30	45	19	23	.02	T	T
31	42	13	14	0	0	0

Date	Temperatures (degrees F) 24 Hr			Precipitation (inches) 24 Hr. Amounts		At obser.
Feb. 1994	Maximum	Minimum	Observation	Rain, melted snow, etc	Snow, ice, hail, etc	Snow on ground
1	39	14	23	0	0	0
2	40	21	23	0	0	0
3	41	23	25	0	0	0
4	41	13	15	0	0	0
5	44	13	23	0	0	0
6	44	23	24	0	0	0
7	44	24	31	.05	1.3	1
8	31	2	3	.08	.7	2
9	26	2	26	.01	.3	2
10	30	23	25	.04	.1	2
11	33	24	30	T	T	1
12	39	23	29	0	0	0
13	38	29	33	0	0	0
14	44	28	33	.01	.1	T
15	49	33	40	0	0	T
16	48	38	43	0	0	T
17	53	42	48	0	0	0
18	49	29	30	.03	0	0
19	43	27	32	.01	0	0
20	45	29	34	0	0	0
21	44	31	37	0	0	0
22	44	25	31	.07	.8	1
23	39	30	33	.07	1.8	2
24	42	29	29	1.00	4.5	5
25	33	25	27	.24	3.0	6
26	40	27	33	.02	0	3
27	43	33	34	.44	0	1
28	47	34	41	.02	0	T
29						
30						
31						

Date	Temperatures (degrees F) 24 Hr			Precipitation (inches) 24 Hr. Amounts		At obser.
March 1994	Maximum	Minimum	Observation	Rain, melted snow, etc	Snow, ice, hail, etc	Snow on ground
1	48	40	43	0	0	0
2	61	42	51	0	0	0
3	62	39	48	.01	0	0
4	65	40	42	.08	0	0
5	58	27	30	0	0	0
6	47	26	28	0	0	0
7	49	21	23	0	0	0
8	48	21	25	0	0	0
9	52	21	26	0	0	0
10	60	25	42	.02	0	0
11	51	35	41	.03	0	0
12	53	24	31	0	0	0
13	63	29	42	0	0	0
14	64	30	40	0	0	0
15	70	40	66	0	0	0
16	75	35	36	0	0	0
17	52	31	42	0	0	0
18	55	42	53	T	0	0
19	59	21	36	.01	T	T
20	44	24	36	.01	0	0
21	52	31	36	.02	0	0
22	48	19	26	0	0	0
23	41	20	24	T	0	0
24	47	18	20	0	0	0
25	54	20	22	0	0	0
26	55	22	36	0	0	0
27	62	25	34	0	0	0
28	66	28	29	0	0	0
29	72	27	55	0	0	0
30	68	42	49	0	0	0
31	67	44	45	.09	0	0

Date	Temperatures (degrees F) 24 Hr			Precipitation (inches) 24 Hr. Amounts		At obser.
April 1994	Maximum	Minimum	Observation	Rain, melted snow, etc	Snow, ice, hail, etc	Snow on ground
1	53	35	52	.10	0	0
2	65	34	35	0	0	0
3	66	34	39	.28	0	0
4	50	32	37	T	0	0
5	49	30	41	0	0	0
6	57	39	44	.14	0	0
7	50	33	35	.32	T	T
8	48	34	45	.02	0	0
9	57	36	39	.62	0	0
10	51	38	44	.01	0	0
11	56	29	39	0	0	0
12	66	39	53	0	0	0
13	56	33	40	0	0	0
14	55	35	37	T	0	0
15	52	32	46	0	0	0
16	71	40	57	0	0	0
17	78	43	57	0	0	0
18	79	47	52	0	0	0
19	83	49	56	0	0	0
20	70	44	45	0	0	0
21	78	45	52	0	0	0
22	62	45	48	0	0	0
23	62	37	45	.14	0	0
24	64	45	45	.16	0	0
25	53	40	42	.50	0	0
26	55	40	45	.02	0	0
27	50	42	45	.03	0	0
28	58	32	42	0	0	0
29	61	28	35	0	0	0
30	65	34	48	.16	0	0
31						

Date	Temperatures (degrees F) 24 Hr			Precipitation (inches) 24 Hr. Amounts		At obser.
Jan. 1995	Maximum	Minimum	Observation	Rain, melted snow, etc	Snow, ice, hail, etc	Snow on ground
1	30	9	20	0	0	0
2	32	7	9	0	0	0
3	29	8	17	0	0	0
4	31	16	24	0	0	0
5	36	18	20	0	0	0
6	30	19	28	T	T	T
7	34	28	34	.06	.5	1
8	43	33	41	.08	0	0
9	49	29	47	.08	0	0
10	51	43	48	.08	0	0
11	52	38	39	.05	0	0
12	49	38	41	T	0	0
13	43	37	40	.13	0	0
14	50	38	48	.46	0	0
15	53	28	29	0	0	0
16	35	29	34	.11	.10	T
17	40	26	31	0	0	0
18	39	28	35	.02	.3	T
19	39	31	34	.02	T	0
20	40	33	34	0	0	0
21	43	18	22	0	0	0
22	29	17	21	0	0	0
23	39	19	32	0	0	0
24	43	31	37	0	0	0
25	45	33	40	T	0	0
26	45	34	35	.08	0	0
27	47	32	34	.08	0	0
28	46	28	31	0	0	0
29	42	29	41	0	0	0
30	46	37	40	.01	0	0
31	48	37	46	.56	0	0

Date	Temperatures (degrees F) 24 Hr			Precipitation (inches) 24 Hr. Amounts		At obser.
Feb. 1995	Maximum	Minimum	Observation	Rain, melted snow, etc	Snow, ice, hail, etc	Snow on ground
1	57	44	51	0	0	0
2	55	35	39	.13	0	0
3	54	36	44	0	0	0
4	54	42	43	0	0	0
5	55	40	48	0	0	0
6	58	33	37	0	0	0
7	56	32	37	0	0	0
8	38	35	38	.03	0	0
9	48	26	27	0	0	0
10	52	24	26	0	0	0
11	47	25	33	.05	1.4	1
12	34	25	25	.09	1.0	2
13	32	14	15	.14	1.6	3
14	23	3	10	T	.1	3
15	27	9	27	0	0	2
16	32	25	28	.03	.8	2
17	40	27	39	T	0	1
18	44	36	41	.19	0	0
19	51	40	49	.02	0	0
20	58	47	49	0	0	0
21	65	37	39	0	0	0
22	62	29	32	0	0	0
23	61	28	29	0	0	0
24	66	28	50	0	0	0
25	62	31	46	0	0	0
26	59	41	51	0	0	0
27	59	25	29	0	0	0
28	45	15	17	0	0	0
29						
30						
31						

Date	Temperatures (degrees F) 24 Hr			Precipitation (inches) 24 Hr. Amounts		At obser.
March 1995	Maximum	Minimum	Observation	Rain, melted snow, etc	Snow, ice, hail, etc	Snow on ground
1	45	17	22	0	0	0
2	44	18	20	0	0	0
3	50	19	38	0	0	0
4	43	35	37	.11	0	0
5	47	29	34	.05	.3	T
6	40	28	32	T	T	T
7	42	23	29	0	0	0
8	51	28	44	0	0	0
9	52	38	49	.12	0	0
10	58	45	45	.10	0	0
11	57	43	47	.04	0	0
12	62	38	46	.03	0	0
13	52	34	41	.10	0	0
14	58	38	50	0	0	0
15	55	32	34	.75	2.0	2
16	51	29	32	.03	0	0
17	51	26	29	T	0	0
18	58	27	47	T	0	0
19	54	35	38	.32	0	0
20	53	37	47	T	0	0
21	55	35	37	.06	0	0
22	52	28	40	T	T	T
23	46	35	39	.21	0	0
24	47	31	33	.03	.40	T
25	44	27	30	0	0	0
26	46	24	45	0	0	0
27	50	23	29	0	0	0
28	54	28	32	0	0	0
29	53	23	28	0	0	0
30	58	25	32	0	0	0
31	62	26	44	0	0	0

Date	Temperatures (degrees F) 24 Hr			Precipitation (inches) 24 Hr. Amounts		At obser.
April 1995	Maximum	Minimum	Observation	Rain, melted snow, etc	Snow, ice, hail, etc	Snow on ground
1	67	40	40	.07	0	0
2	52	30	33	T	0	0
3	58	27	28	0	0	0
4	68	27	40	0	0	0
5	64	36	40	.01	0	0
6	58	39	40	.15	0	0
7	60	39	49	.11	0	0
8	61	35	35	.43	0	0
9	49	33	36	.05	0	0
10	50	27	32	.01	0	0
11	56	32	44	.01	0	0
12	55	41	45	.01	0	0
13	59	39	40	.20	0	0
14	44	30	32	.12	0	0
15	46	24	35	.01	0	0
16	49	26	34	0	0	0
17	56	27	30	0	0	0
18	50	29	38	0	0	0
19	50	34	35	0	0	0
20	48	34	37	.03	0	0
21	48	36	39	.06	0	0
22	55	29	35	0	0	0
23	62	28	45	T	0	0
24	66	34	39	0	0	0
25	70	33	46	0	0	0
26	60	33	37	0	0	0
27	67	36	44	0	0	0
28	66	41	43	.84	0	0
29	55	35	42	.17	0	0
30	49	39	47	.85	0	0
31						