To the Graduate Council:

I am submitting herewith a thesis written by Pamela F. Cunningham entitled "Effectiveness of Vitamin E or Zinc in Reducing Udder Edema in Heifers Fed Excess Iron." I have examined the final copy of this thesis for form and content and recommeded that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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Effectiveness of Vitamin E or Zinc in Reducing Udder Edema of Heifers Fed Excess Iron

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CHAPTER I

INTRODUCTION

The health of a dairy cow is of extreme economic importance to the dairy farmer especially at the time of parturition. Parturition and the period immediately before and after parturition are the most stressful times in the life of adult dairy cows. Most health problems will occur early in the cow's lactation cycle. It is an advantage to the farmer to have his or her cows in top physical condition as this time approaches. According to Shanks et al. (1981) mammary and reproductive costs were over 50% of the total health costs in the first 30 days of lactation. Because these are the two most important areas of production, they effect the income of the farmer the most. Reproductive and mammary gland diseases effect the quality and quantity of milk as well as the cost of the treatments (Shanks et al., 1981, and Vestwebber et al., 1984).

The health of the mammary gland is of extreme importance to the dairy farmer, because this is where the majority of his or her income is obtained. An animal with udder edema requires more labor to milk, is more prone to injury, and is under an extreme amount of stress (Dentine et al., 1983). Although the exact cause of udder edema is not known, there have been numerous proposal as to its origin (Vestwebber et al., 1984). Miller et al. (1993) reported a reduction in the number of animals with udder edema when an adequate amount of vitamin E and Se were received from the diet before parturition. This might suggest that oxidative stress could play a role in the incidence and severity of udder edema.

Oxidative stress is caused by a build up of oxygen free radicals in the cow's blood system. The reactive oxygen metabolites, which include superoxide and hydrogen peroxide, are produced as a result of normal metabolic functions. They are often involved in important

physiological roles such as phagocytic action and enzymatic control (Rice-Evans et al., 1993). When produced at normal metabolic rates these free radicals are not harmful, however, if they are converted to more reactive species by transition metals, such as Fe and Cu, they can cause great harm to the body (Miller et al., 1993; Halliwell, 1991; Beger et al., 1990; and Groot, 1994).

Iron is essential for life so the body protects itself by sequestering Fe in the proteins, transferrin and ferritin. However in cases where Fe is excessive in the diet or during infection, inflammation and environmental stress, Fe may become available. This occurs because Fe binding sites become saturated allowing Fe to become decompartmentalized or nonspecifically bound. Decompartmentalized Fe then becomes the initiator of free radical chain-reactions. Iron can be involved in several different types of oxidative reactions. Iron catalyses the reaction between superoxide and hydrogen peroxide to produce the hydroxyl radical (Groot, 1994). The hydroxyl radical which is the most reactive radical (Burton, 1994), causes specific damage to phospholipids, proteins, carbohydrates, and DNA strands (Miller et al., 1993). Iron may also be involved in lipid peroxidation to form alkoxyl and peroxyl radicals (Halliwell, 1991).

It is important to terminate the free radical reactions before it causes extensive damage to the body. Antioxidants can prevent free radical reactions by several different mechanisms. There are at least three lines of defense against free radical damage (Miller and Madsen, 1994). First macromolecules compartmentalize transition elements preventing their catalytic activity. Second, antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase remove superoxide and peroxides before they can approach catalytic transition metals. Finally, chemical antioxidants terminate peroxidative chains initiated by free-radicals which escaped the first two lines of defense. These exogenous antioxidants include vitamin E which protects

against lipid peroxidation (Bendich, 1993).

Unfortunately, it takes more than vitamin E to contain all free radical reactions. Vitamin E is ineffective against site specific radical damage. Site specific damage occurs when Fe is loosely bound to a protein and causes the formation of the hydroxyl radical at that site (Har-el and Chevion, 1991). Zinc, which only recently has been recognized as an antioxidant, can prevent site specific damage. Zinc prevents these reactions because of its similar chemical make up to Fe (Bettger et al., 1990). Zinc has the unique ability to displace Fe during redox reactions producing a non-reactive product (Har-el, 1991). Because so often dairy cattle diets contain high concentrations of Fe, it may be necessary to consider supplementation with Zn as a preventative measure for the damaging effect that decompartmentalized Fe may cause.

Parturition is an extremely stressful time for the animal, therefore it is possible that oxidative stress may increase the severity and the incidence of udder edema in dairy cows. Oxidative stress may cause udder edema by increasing membrane permeability or decreasing the production of reproductive hormones causing and increase in water retention (Vestwebber et al., 1984; Miller et al., 1993). This experiment was designed to test the effectiveness of vitamin E or Zn in reducing udder edema of heifers fed excess Fe.

CHAPTER II

LITERATURE REVIEW

OXIDATIVE STRESS

Oxidative stress is caused by the build up of oxygen free radical in the animals system. A free radical is a molecule that can exist with one or more unpaired electrons (Halliwell, 1991). Oxygen free radicals have been the focus of many studies in recent years. Reactive oxygen metabolites (ROM) are produced daily in metabolic functions. Free radicals are involved in phagocytic action, endothelial-derived relaxing factor, and controlled use in enzymatic reactions (Rice-Evans and Burdon, 1993). Free radicals are not harmful under normal metabolic rates, however, when they are produced too rapidly and can not be disposed of properly, they increase the oxidative stress of an animal (Miller et al., 1993). Free radicals may increases as a result of:

1. Raised oxygen concentration.
2. Adding certain toxins that increase intracellular formation of reactive oxygen species.
3. Activating a large number of phagocyte at a localized site. (Halliwell, 1991)

Because most free radicals are derivatives of molecular oxygen, they are named for their oxygen counterpart. (Table 1). Superoxide radical is the least reactive species where as, the hydroxyl radical is extremely reactive with any organic molecule in the area (Burton, 1994). Free radical propagation is initiated by metal ions such as Fe and Cu. These reactions occur by Fenton (eq. 1 and 2) and Haber-Weiss reactions (eq. 3)(Berger et al, 1990, Rice-Evans et al, 1993, Halliwell, 1991, Groot, 1994).

$Fe^{2+} + H_2O_2 + H^+ \text{----->} Fe^{3+} + OH^{\bullet} + H_2O$ {eq 1}

$Fe^{3+} + OH \text{ <----->} Fe^{2+} + OH^{\bullet}$ {eq 2}

Table 1 Reactive oxygen species

FORMULA	NAME
O_2	Superoxide anion (radical)
H_2O_2	Hydrogen peroxide
$\bullet OH$	Hyroxyl (radical)
$^1O_2$	Singlet molecular oxygen
RO	Alkoxyl (radical)
ROO	Peroxyl (radical)
ROOH	Organic hydroperoxide
HOCl	Hypochlorous acid
$Fe^{2+}O$	Ferryl ion
NO	Nitric oxide (radical)
NO_2	Nitrogen dioxide (radical)

(Groot, 1994)

$$O^{-}_2 + H_2O_2 + H^+ \text{-----> } O_2 + OH\bullet + H_2O \quad \{eq\ 3\}$$

Equation three is the Haber-Weiss reaction it proceeds extremely slow unless catalyzed by a metal ion (Groot 1994).

IRON

Iron (Fe) is the most abundant metal ion in the body. It is an important co-factor for many enzymatic reactions and is important for oxygen transport. Iron exists in the body bound to proteins. It is transported through the plasma by transferrin and stored intracellularly by ferritin. Excess Fe in the diet is more likely to saturate Fe binding sites and increase likelihood of Fe available to catalyze free radical reactions (Emery, 1991, Seis, 1991). Catalytic Fe can also come from: Fe released from ferritin by a compound capable of reducing Fe (III) in the iron core and Fe containing proteins attacked by the body's defense system (Ryan, 1992). The many oxidation states of Fe and the other transition metals allows their participation in single electron transfer reactions (Halliwell, 1993). This makes them excellent catalysts for free radical reactions.

Free radicals are involved in the damage of phospholipids, proteins, carbohydrates, and DNA molecules (Miller et al, 1993). Phospholipids are the most susceptible to attack because of their polyunsaturated fatty acid side chains (Rice-Evans and Burdon, 1993). The attack on lipid membranes by free radicals involves lipid peroxidation and the formation of peroxyl and alkoxyl radicals (eq. 4 and 5) (Halliwell, 1991).

$$LOOH + Fe^{2+} \text{-----> } LO\bullet + Fe^{3+} + OH^{-} \quad \{eq\ 4\}$$

$$LOOH + Fe^{3+} \text{-----> } LOO\bullet + Fe^{2+} + H^{+} \quad \{eq\ 5\}$$

According to Seis (1985), Fe is an excellent catalyst in the "peroxidation of unsaturated fatty acids of membrane phospholipids." Iron contributes to lipid peroxidation in two ways; catalyzing the formation of initiation species, and stimulating peroxidation by reacting with lipid

hydroperoxides and decomposing them to peroxyl radicals and alkoxyl radicals (Halliwell, 1992). Lipid peroxidation causes changes in membrane permeability, transmembrane ion gradients, lipid fluidity, and lipid-protein interactions (Rice-Evans and Burdon, 1993). Areas of the body like the brain and central nervous system are extremely susceptible to free radical attack because of their high concentration of polyunsaturated fatty acids, high concentration of oxygen and low molecular weight Fe complexes (Halliwell, 1992, Reiter, 1995).

DNA may also be affected by free radical damage through strand breakage, base damage, cross-linking, and chromosomal aberrations (Thompson, 1994, and Halliwell, 1993). Protein modification occurs at the amino acid side chain. These modifications cause alterations in enzymatic activity (Rice-Evans and Burdon, 1993).

Free radicals have been implicated in many diseases, however it is not certain whether they are the primary cause of the disease or a secondary condition (Halliwell, 1987, and Rice-Evans et al 1993). Diseases in which free radicals are produced are cancer, cardiovascular disease, arthritis, and atheroscelorsis. Free radicals have also been implicated in the aging process (Edgington, 1994).

Many studies have been done relating free radicals and iron-overload to disease. Wills (1972), showed an increase in lipid peroxidation in the liver of rats injected with iron dextran. Iron-overload has also shown to increase lipid peroxidation in the plasma of rats (Dabbagh, 1994). Using the intact heart as a model, Lesmefsky et al (1992), showed that Fe catalyzed reaction generated hydroxy radicals and increased the lipid peroxidation of the membrane. Higher levels of lipid peroxidation were found in babies that had rhesus hemolytic disease than normal babies (Berger et al., 1990).

Deficiencies in other transition metals can also cause an increase in free radical damage. Freedman et al (1992), found that hamsters that were deficient in magnesium (Mg) were more

susceptible to free radical damage, because of reduced antioxidant capacity. Rats deficient in Mg and given an excess amount of Fe produced more hydroxyl radicals (Rock et al., 1995). Excess Fe may not be the only problem. Leiter et al (1995), found that mice with Lupus Erythematosus and deficient in Fe suffered just as severely as those mice fed an excessive amount of Fe. How can oxidative stress and the production of free radicals be lessened?

ANTIOXIDANTS

Antioxidants can be defined as "any substance that when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate," (Halliwell and Gutteridge, 1989). Antioxidants have several modes of action. They scavenge ROM, inhibit their formation, bind transition metal ions, or any combination of these (Halliwell, 1991). The primary antioxidant defense are the proteins that compartmentalize Fe such as, transferrin, ferritin, and ceruloplasmin. The secondary defense is through enzymes (superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH)). The last line of defense are the chemical antioxidants, such as, vitamins and minerals (Vitamin E and C, Beta-carotene, and Zn) (Ryan, 1992). Antioxidants found in low concentrations have been associated with many diseases.

VITAMIN E

Vitamin E is the major lipid soluble antioxidant. The term vitamin E is a generic term that applies to all tocopherols. Alpha-tocopherol has the greatest biopotency of the eight tocopherols that occur naturally (Moore, 1992). Located in all cellular membranes vitamin E protects against lipid peroxidation and prevents the loss of membrane fluidity (Bendich, 1993). In the presence of peroxyl and alkoxyl radicals vitamin E combines with the radicals to convert into a new less active radical (eq. 6) (Halliwell, 1991).

Vitamin E + R• ----> Vitamin E• + RH {eq 6}

"The hydrogen atom of the OH group of the vitamin E is easily removed. Free radicals preferentially combine with it instead of the adjacent lipids" (Thompson, 1994). This breaks the oxidative chain because the vitamin E radical is not very reactive. In assays using the free radical generator 2,2'-azobis (2-amidinopropane) dihydrochloride, plasma from cows supplemented with vitamin E protected the fluorescing indicator phycoerythrin from free radicals more effectively than plasma from cows not given vitamin E (Brzezinska-Slebodzinska et al., 1994).

Vitamin E may be replenished in the body by Vitamin C and GSH. This restoration occurs on the surface of the membrane (Thompson, 1991). [fig 1].

What role does vitamin E play in the diseased animal? Bendich (1993), and Meydani (1995), both suggest that vitamin E has an immunostimulatory effect. However, most vitamin E research focuses on deficiencies and the control of lipid peroxidation. Moore (1992), found that in both rats and pigs feeding a supplement of vitamin E decreased lipid peroxidation and increased the plasma level of vitamin E. High concentration of vitamin E have been associated with reduced risk of heart disease (Thompson, 1994, Rice-Evans et al., 1993, and Burton, 1994). Burton (1994), also states that vitamin E decreased the incidence of cancer in mice, chemically induced with oesophageal cancer. Demling et al. (1995), showed that pretreatment with vitamin E improved the survival rate of rats after inflammatory disease. Heliovaara et al. (1994) showed that low concentrations of vitamin E were associated with an elevated risk of rheumatoid arthritis.

Not only has vitamin E been effective in reducing the incidence of diseases affecting humans but, it has also been shown to reduce the incidence of disease in cattle. Inadequate vitamin E has been associated with both reproductive and mammary gland diseases. According to Sies, (1985), vitamin E is involved in the protection of the amniotic membrane against

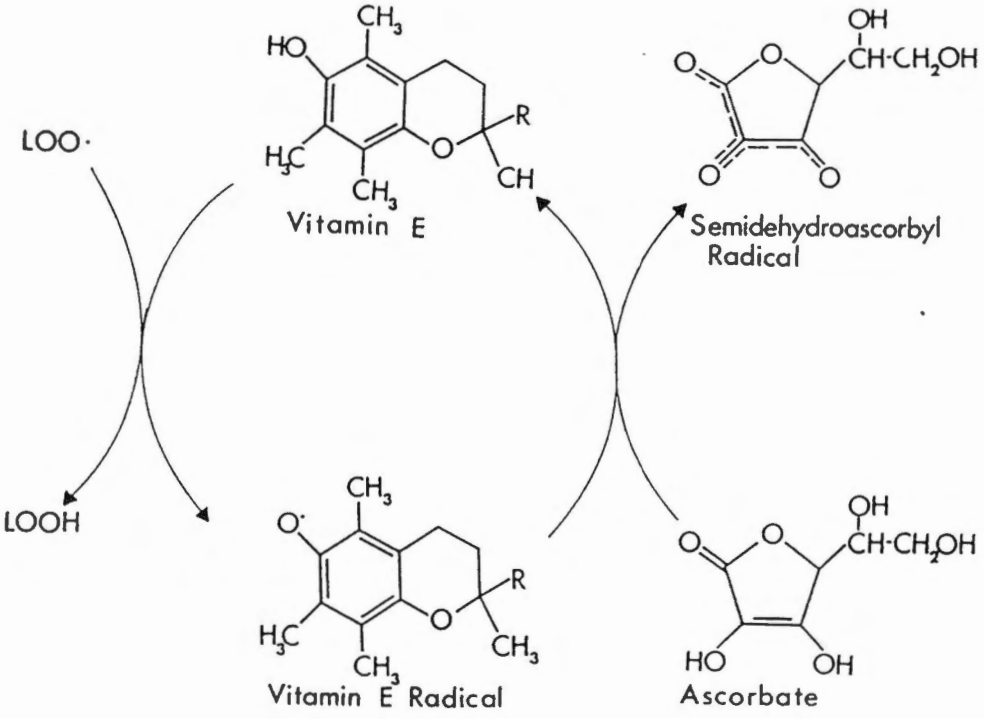

Figure 1. Mechaism of ascorbate-dependant regeneration of vitamin E from the vitamin E radical (Ryan et al., 1992).

peroxidation. Vitamin E has also been found to play a role in the defense against mastitis (Hogan et al., 1993). Supplementation of the diet with vitamin E allows the animal to maintain a higher level of plasma alpha-tocopherol (Goff and Stabel, 1990). Vitamin E decreases as a result of infection, inflammation, and trauma (Demling et al., 1995). Weiss et al., (1990), discovered that the plasma concentration of tocopherol dropped 50% the last 7 d prior to calving and remained at that level for 20-30 d postpartum. This is a very stressful and traumatic time for the cow. The animal is under a high level of oxidative stress at this time. Brzenzinska-Slebodzinska et al. (1994) showed that cows fed excess vitamin E prior to parturition showed a 75% increase in serum alpha-tocopherol during the first weeks of supplementation and although the concentration declined at the same rate as cows not supplemented, the supplemented cows did not fall below the initial concentration level. It must be noted that vitamin E alone can not take care of all free radical production.

ZINC

Zinc may also be consider an antioxidant. There are two theories of antioxidant protection for Zn. The first is the protection of sulfhydryl groups against oxidation. The second is the prevention of the hydroxyl and superoxide radical production by transition metals (Bray and Bettger, 1990). The mechanism for this reaction occurs because of the similarity between the charges of Zn and Fe. Zinc displaces Fe during redox reactions diverting the potential for the production of free radicals (Har-el, 1991; Bray, 1990). Because of Zn low reactivity, zinc does not continue the oxidative path of Fe. Zinc is also an important co-factor for the enzyme SOD and metallothionein (Grimble, 1994; Machlin et al., 1987).

Deficiencies of Zn in the diet may cause an increase in oxidative damage. "Mild Zn deficiencies can affect susceptibility to an injury thought to involve the actions of oxygen free radicals." (Parsons et al., 1994). Taylor et al. (1988) showed an increase in free radicals in the

lungs due to a deficiency in dietary Zn. Dietary deficiencies of Zn have been shown to cause an accumulation of Fe in several tissues in the body. Oteiza et al. (1995) found an increase in protein carbonyl groups, an indicator of protein oxidation, in Zn deficient groups of rats. They also found that after two weeks on a diet deficient in Zn, there was an accumulation of Fe in the testes of rats. Zinc has also been shown to improve glucose tolerance in diabetics and patients with inflammatory acne (Silvas, 1992, and Dreno, 1989). Powell et al. (1994) believed that Zn processes a cardiac cytoprotective qualities.

Zinc also has an indirect effect on the elimination of free radicals. Grimble (1994), says that Zn deficiency reduced liver and lung metallothoinein and can affect ceruloplasmin and metallothoinein synthesis in response to inflammatory stimulus. Zinc has also been shown to have a synergistic effect with vitamin E. Bunk et al. (1989) reported that deficiency of Zn resulted in a marked reduction in the plasma alpha-tocopherol. Zinc and vitamin E work together to stabilize the cellular membrane (Bunk et al., 1989). Diseases affecting the skin and joints caused by Zn deficiencies show remarkable recovery after administration of vitamin E (Bunk et al., 1989). Bettger et al. (1989), found that supplementation with vitamin E decreased the severity of skin and joint pathology in chicks fed Zn deficient diets. The synergistic effects may aid in preventing or lessening other health problems.

UDDER EDEMA

Udder edema is described as excessive accumulation of fluid in the interstitial space of the mammary gland occurring either just prior to parturition or immediately after parturition (Vestwebber et al., 1984). Heifers have been shown to be more severely effected by udder edema than cows (Dentine et al., 1983). Hayes and Albright, (1976) found that over 90% of heifers show some sign of udder edema upon calving. Cows and heifers with edematous udders require more labor and are more prone to injury.

There are many theories about what causes udder edema, however, the exact cause has not been identified. Hayes and Albright (1976), discovered that udder edema could be associated with breed, length of dry period and season of calving. Diet may also contribute to the severity of edema. Emery et al. (1969), found that heifers on high concentrate diets prior to parturition had more incidence of udder edema than heifers fed only roughage. Sodium chloride, and potassium chloride have also been associated with severe udder edema, when fed in excess prior to calving (Randall et al., 1974). According to Miller et al. (1993) udder edema could be linked to oxidative stress through steroidogensis. "Suppression of androgen and estrogens induce by oxidative stress accompanied by elevated corticosterone can impair reproduction but also increase sodium and water retention, thereby contributing to udder edema." Lipid peroxidation may cause udder edema. Changes in the membrane permeability caused by oxidation of phospholipids within the membrane results in the accumulation of fluid in the interstitial space (Vestwebber et al., 1984). There may be many other ways in which udder edema is linked to oxidative stress but because the actual cause of udder edema is unknown it is hard to speculate on what those ways might be.

Antioxidants, such as vitamin E and selenium, have been shown to be beneficial in lessening the severity of udder edema when fed as a supplements during the dry period. Mueller et al. (1989), found the cows fed vitamin E during the dry period had less severe udder edema. Miller et al. (1994), discovered that heifers with below average plasma fast-acting antioxidants were 4.7 times more likely to have udder edema than heifers with average or above average fast-acting antioxidants. Numerous studies have shown that supplementation of vitamin E prior to calving results in a decrease in the severity of udder edema. There seems to be a possible connection between udder edema and oxidative stress. The questions reasearchers must answer are, what causes udder edema and what can be done to prevent it.

CHAPTER III

MATERIALS AND METHODS

ANIMALS AND TREATMENTS

Eight Jersey and 48 Holstein heifers were assigned to seven blocks by breed. Within each block treatments of, vitamin E, zinc, iron, or all combination of these and a control were assigned at random in a 2 x 2 x 2 factorial arrangement. Treatments began 6 wks before parturition. Heifers were administered 1000 IU of vitamin E (d,l alpha-tocopheryl acetate), Zinc (.8 g half by Zn methionine and half by $ZnSO_4$), or an excess of iron (12 g as $FeSo_4$ $7H_2O$) or any and all combinations of these. With expected feed intakes, amounts of supplemented should average 100 IU of vitamin E, 80 mg of Zn, and 1200 mg of Fe per Kg of DM consumed. The heifers diet consisted of pasture and hay ad libitum as well as 4 to 5 kg of concentrate per cow per day.

PROCEDURES AND ANALYSIS OF SAMPLES

Blood samples were taken prior to the beginning of treatment and every two weeks up until parturition. Two samples of blood were taken and placed on ice in low light conditions to prevent loss of vitamin E due to UV electromagnetic radiation. Plasma and serum were removed from their respective tubes by centifugation and placed in storage tubes. The plasma was then halved so that it could be analyzed for the concentration of alpha-tocopherol and fast-acting antioxidants. Analysis for alpha-tocopherol was carried out utilizing a general lipid extraction procedure (Moore, 1992). The remaining plasma was analyzed for the activity of fast-acting antioxidants. This procedure involved the use of phycoerythrin fluorescence-based assay (Glazer, 1990). This assay depends on the detection of damage to the phycoerythrin by decreasing its fluorescence emission. The increase of ROM causes a decrease in the

fluorescencing ability of the phycoerythrin. This loss of fluorescencing emission is an index of free radical damage (Glazer, 1990).

UDDER MEASUREMENT

After parturition, udder measurements were taken on days 1, 2, 3, and 7. Measurements were taken by placing a blank piece of paper against wet teat ends before and after milking(Seykora and McDaniel, 1986). Area of the quadrilateral defined by the resulting four spots was determined by connecting two of the spots with a diagonal line from which perpendicular lines were drawn to each of the remaining two spots. The base and altitudes of the four right triangles were then measured in centimeters. Udder floor area was then calculated as one-half of the sum of the products of base x altitude of the four right triangles. The percent shrink was then determined by taking the ratio between the two measurements. The mean shrink for the population was then determined. Heifers that were below the average shrink for the population were deemed edematuous.

STATISTICAL ANALYSIS

The data was analyzed by General Linear Mixed Models (GLMM) procedure of the Statistical Analysis System (SAS, 1987). Least square means were used to find treatment interactions. Odds ratio was also used to compare the treatments effects on udder edema (Fletcher et al., 1988). Regression analysis was used to find the relationship between udder edema and plasma alpha-tocopherol.

CHAPTER IV

RESULTS AND DISCUSSION

An objective measurement was needed to determine the degree of edema associated with each treatment. A variation of Seykora and McDaniel's (1986) measurement of teat distances was used to obtain an objective measurement of decrease in udder floor area due to removal of milk. It was assumed that a rigid, edematous udder would be less likely to decrease in volume during milking than a flexible, nonedematous udder. Contrary to other studies which used subjective measurements to asses the degree of udder edema (Dentine and McDaniel, 1983; 1984; Vestwebber and Al-Ani, 1984; 1983), the determination of the % shrink in the area of the udder floor should decrease human error associated with a subjective estimate. However, this type of measurement was not without other sources of error. Because the measurements are taken before and after milking, there is the possibility that the first measurement could have been made before the heifer had time to let down her milk. This would underestimate the first measurement in relation to the second measurement and in turn would exagerate the appearence of edema. Another potential problem with this type of measurement is the conformation of the udder. In high producing animals the teats have a tendency to strut when the udder is full of milk, but after milking the teats will hang straight down. Measurement of this udder would overestimate udder shrink. This type of measurement is much more reliable that using a subjective estimate to determine udder edema.

Udder edema occurs at a crucial time in the lactation cycle of a cow. Over 90% of all first calf heifers show some sign of udder edema at calving (Hayes and Albright, 1976). This is an overwhelming percentage of animals considering the other diseases and health problems associated with udder edema. Heifers with edema are more prone to mastitis, teat injury, and trauma (Vestwebber and Al-Ani, 1983; Dentine and McDaniel, 1983). The dairy farmer will

suffer lost revenue from decrease in production as well as for treatment of the cow. In the most severe, case edema causes the breakdown of udder ligaments. This breakdown results in pendulous udders. Edema may also cause scar tissue to develop reducing milk production (Dentine and McDaniel, 1984). This type of damage a decreases the length of productivity and total yield of the animal.

Many causes of edema have been proposed over the years. Dentine and McDaniel (1983) as well as Vestwebber and Al-Ani (1984), suggest that udder edema is the result of diet, age, body condition, and length of dry period. However, udder edema may be initiated on the cellular level. Edema may result from an increase in the vascular permeability through damage to endothelial tissue (Vestwebber and Al-Ani, 1983). Endothelial damage may be the result of free radical damage to the lipid membrane. As a result of the increase in vascular permeability fluid can enter the interstitial space of the mammary gland (Vestwebber, 1984). In this situation antioxidants may be used to reduce udder edema in first calf heifers. Heifers supplemented with vitamin E maintained higher ($P<.01$) alpha-tocopherol levels in plasma on days 28 and 14 before calving (fig 2). Although the levels of plasma alpha-tocopherol in cows supplemented with vitamin E tended to decrease as parturition approached, the levels remained above pre-treatment levels. This is consistent with research done by Goff and Stabel (1990), Weiss et al. (1992), Miller et al. (1993), Brzezinska-Slebodzinska et al. (1994). The sharp decrease in plasma alpha-tocopherol in both heifers supplemented with vitamin E and those not supplemented with vitamin E is due partially to the partitioning of vitamin E into the production and secretion of colostrum (Goff and Stabel, 1990; Hidiroglou et al., 1993). Another reason for the decrease in plasma alpha-tocopherol may be the result of increased consumption of alpha-tocopherol in quenching free radical reactions caused by the increase in oxidative stress at the time of parturition (Miller et al., 1993). Weiss et al. (1992) suggested that at the time of

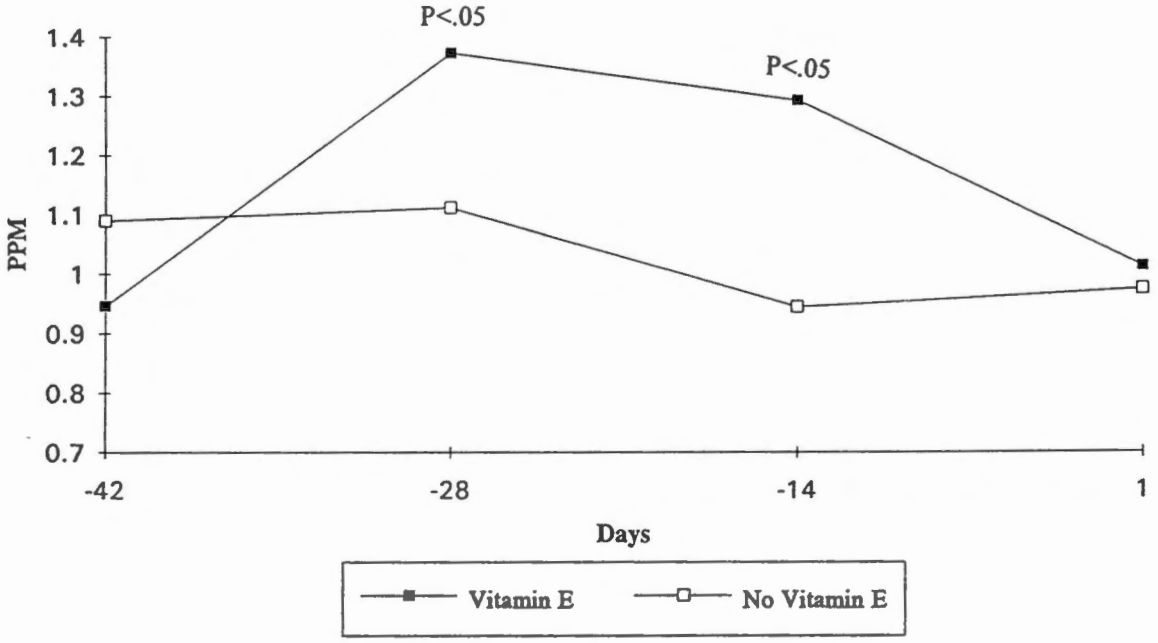

Figure 2. Cows supplemented with vitamin E daily had increased levels of plasma alpha-tocopherol.

at the time of parturition (Miller et al., 1993). Weiss et al. (1992) suggested that at the time of parturition there is a decrease in the transport capacity for vitamin E. During a normal oxidative challenge the cow may mobilize vitamin E to combat the excessive oxygen load. Therefore the cow is not able to mobilize vitamin E to handle the oxidative challenge produced by parturition thus causing the depletion of plasma alpha-tocopherol at the time of calving. Although feeding a higher concentration of vitamin E in the ration will not prevent the decrease in plasma alpha-tocopherol, it will help to maintain levels that should be suitable for minimizing oxidative stress (Halliwell, 1991; Benchich, 1993; Thompson, 1994;).

Vitamin E had no effect on udder edema when cows were fed excess Fe (fig. 3) (table 2). Iron has several paths of free radical initiation; it is possible that in the case of udder edema Fe causes the formation of an OH radical at a specific site (Ryan et al., 1992; Groot, 1994). If this is the case then vitamin E would not be able to prevent the oxidative damage because its path of action is as a chain breaking antioxidant (Bendich, 1993; Halliwell, 1991). In this case it may be necessary to use an antioxidant such as zinc, which can prevent site specific damage. Udder edema was significantly reduced ($P<.05$) on day 2 after parturition (fig. 4) and tended to continue to decrease (table 3) on days 3 and 7 for those cows supplemented with vitamin E and not given excess iron. These results are similar to others which found that supplementation on vitamin E reduced the severity of udder edema in first calf heifers (Miller and Madsen, 1994; Miller et al., 1993; Mueller et al., 1989). Vestwebber et al. (1984) suggested that udder edema may be the result of damage to the membrane permeability allowing fluid to flow into the interstitial space. If so free radicals may increase the severity of udder edema by damaging membrane permeability. Vitamin E because of its location in the cell membrane and its ability to prevent lipid peroxidation (Meydani, 1995; Halliwell, 1991; Rice-Evans and Burdon, 1993; Thompson, 1994) may successfully retard the damage caused by

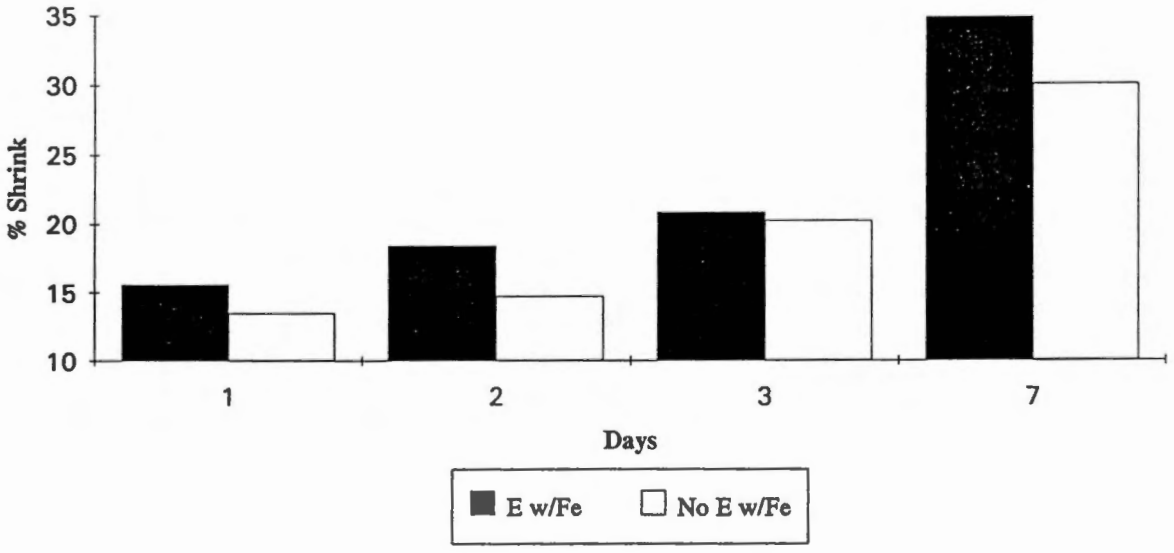

Figure 3. Vitamin E had no effect on udder edema in cows fed excess iron.

Table 2. Vitamin E had no effect on udder edema in cows fed excess iron

	-E	-E	+E	+E	ODDS RATIO	CHI^2	P VALUE
DAY	+EDEMA	-EDEMA	+EDEMA	-EDEMA			
1	5*	7	7	5	0.49	0.17	0.68
2	7	6	5	8	1.3	0	1
3	7	5	5	8	1.6	0.02	0.9
7	5	5	5	5	1.2	0.05	0.82

* Number of cows

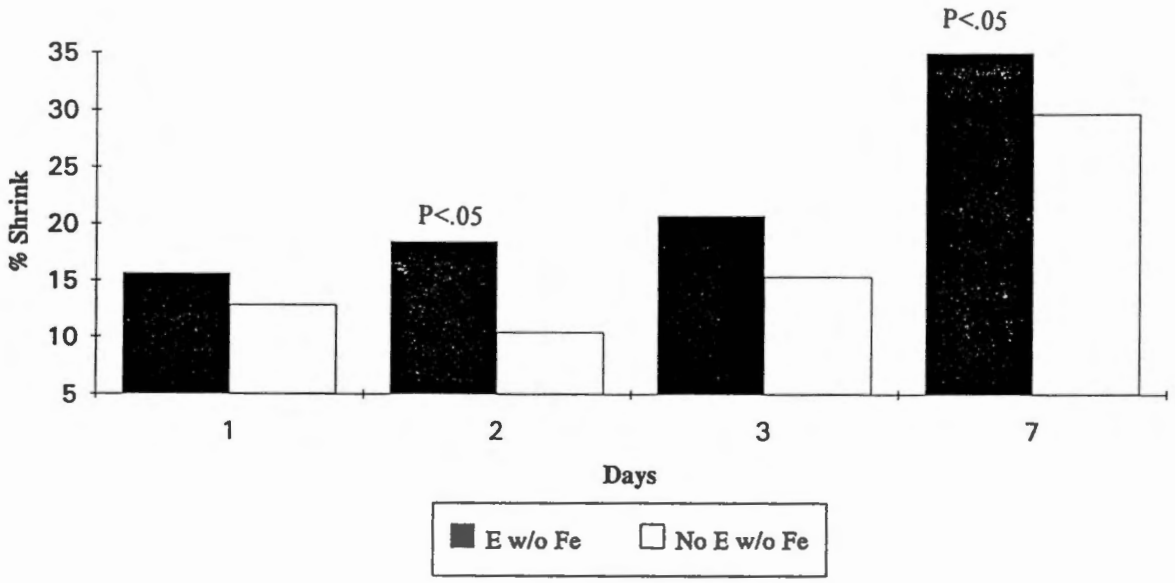

Figure 4. Vitamin E reduced the severity of udder edema in cows not fed excess iron two out of the 4 d measured.

Table 3. Vitamin E reduced udder edema in cows not fed excess iron

DAY	-E +EDEMA	-E -EDEMA	+E +EDEMA	+E -EDEMA	ODDS RATIO	CHI ^{2}	P VALUE	
1	7 *	7	6	7	1.2	0	1	
2	10	3	5	8	5.3	3.9	0.05	**
3	8	4	4	9	4.5	3.2	0.08	
7	6	6	1	9	9	4	0.05	**

* Number of cows

** $P < .05$

peroxidative chain reactions to the membrane and thus reduce the severity of udder edema.

Zinc supplementation had no effect on plasma alpha-tocopherol levels (fig. 5). Bunk et al. (1989) also observed no increase in the concentration of plasm alpha-tocopherol in Zn supplemented rats as compared to rats given zinc-adequate diets. Bettger et al. (1980) suggest that vitamin E and Zn work together to maintain cellular integrity. Therefore, one would expect Zn supplementation to alter plasma concentration of vitamin E. Because of its antioxidant status, zinc would help to control an oxidative challenge and therefore one would expect Zn to spare the level of vitamin E. Zinc, however, has a different mode of action than vitamin E. By replacing iron at sensitive sites, zinc prevents redox generation of radical species capable of initiating peroxidative chains (Har-el et al., 1991; and Bray et al., 1990) whereas vitamin E terminates chains after they have been started. Because of their different modes of action, zinc may not prevent the consumption of vitamin E by free radicals in chain reactions thus vitamin E would not be spared. Contrary to expectation, iron had no effect on plasma alpha-tocopherol levels (fig. 6). However, other research indicated that Fe decreases the level of plasma alpha-tocopherol (Moore, 1992). High dietary Fe could increase generation of OH radical which is capable of initiation peroxidative chain reactions. The resulting oxidative load would be expected to consume alpha-tocopherol in quenching reactions.

What causes the oxidative stress on the mammary gland? It has been speculated that the mammary gland may be in a similar situation to that of a heart during by-pass surgery. The heart first suffers from ischaemia, which only allows enough blood flow to keep the muscle alive (Guyton, 1981). Reestablishment of blood flow to the heart or reperfusion may promote free radical damage to the tissue by the generation of oxygen free radicals and lipid peroxidation (Meydani, 1995). In comparison the mammary gland has only a minimal amount of blood flow until the time of parturition. At or near parturition an extremely large amount

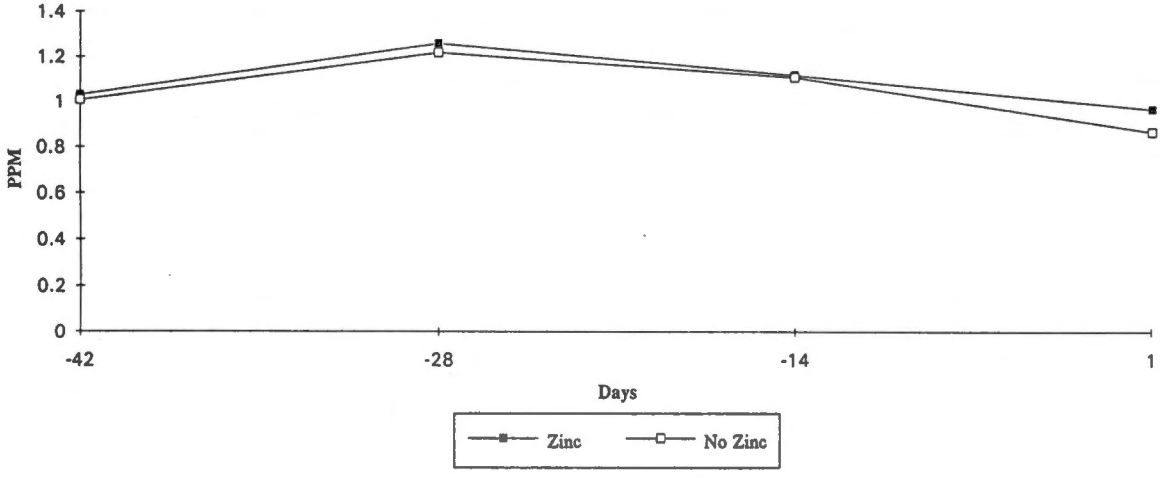

Figure 5. Zinc supplementation had no effect on the level of plasma alpha-tocopherol.

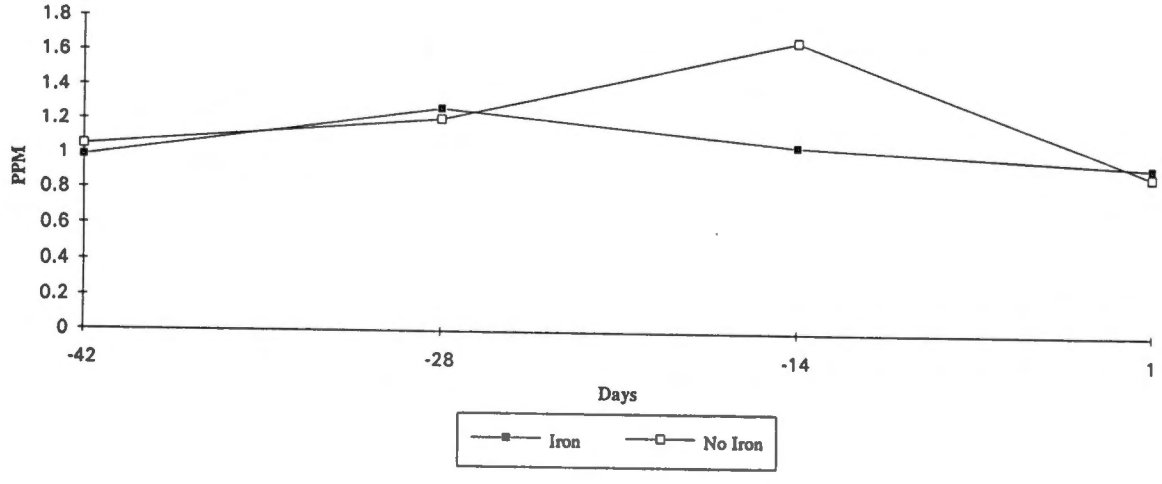

Figure 6. Iron supplementation had no effect on the level of plasma alpha-tocopherol.

of oxygenated blood begins flowing to the mammary gland (Vestwebber and Al-Ani, 1983). Studies have shown that in the case of ischaemia/reperfusion injuries patients pre-treated with vitamin E suffered less tissue damage as well as lipid peroxidation (Meydani, 1995). Vitamin E supplemented before parturition may have the same influence on the mammary gland.

Udder edema was also significantly reduced (P=.05) on day 3 when cows fed excess iron were supplemented with zinc (fig. 7) (table 4). The small number of heifers in the sample may have effected the results. However from this experiment it appears that, zinc has no effect on reducing the severity of udder edema. Because there were no trends on the other 3 days measured, the signifacance on day 3 must have been due to the number of animals measured on that day. If the number of heifers in the sample were increase, we would expect zinc to have a positive effect in reducing udder edema through the control of site specific damage. Site specific damage occurs when, iron non-specifically bound to a molecule, catalyses the formation of the hydroxy radical which reacts with the molecule right where the metal is located. Zinc possesses chemistry similar to Fe and may displace Fe from the binding site thus preventing the damage caused by free radicals (Har-el and Chevion, 1991; Bray and Bettger, 1990). This is a different mechanism from vitamin E which can only intercede after the free radical chain has been started. Although very little research has been done using Zn at a pharmacological level, research using zinc-deficient animals has shown an increase in free radical production both in tissues and isolated membranes (Bray and Bettger, 1990; Rodgers et al., 1987). Zinc deficiency has been shown to alter membranes resulting in an increase in the ability of free radicals to cause damage. In the case of udder edema, increased levels of Zn may be able to reduce the site specific damage caused by excessive iron in the diet. When excess Fe was not present in the diet zinc had no effect on udder edema (fig. 8) (table 5). Bray and Bettger (1990) note that under normal conditions zinc does not have antioxidant properties like those of vitamin E, but

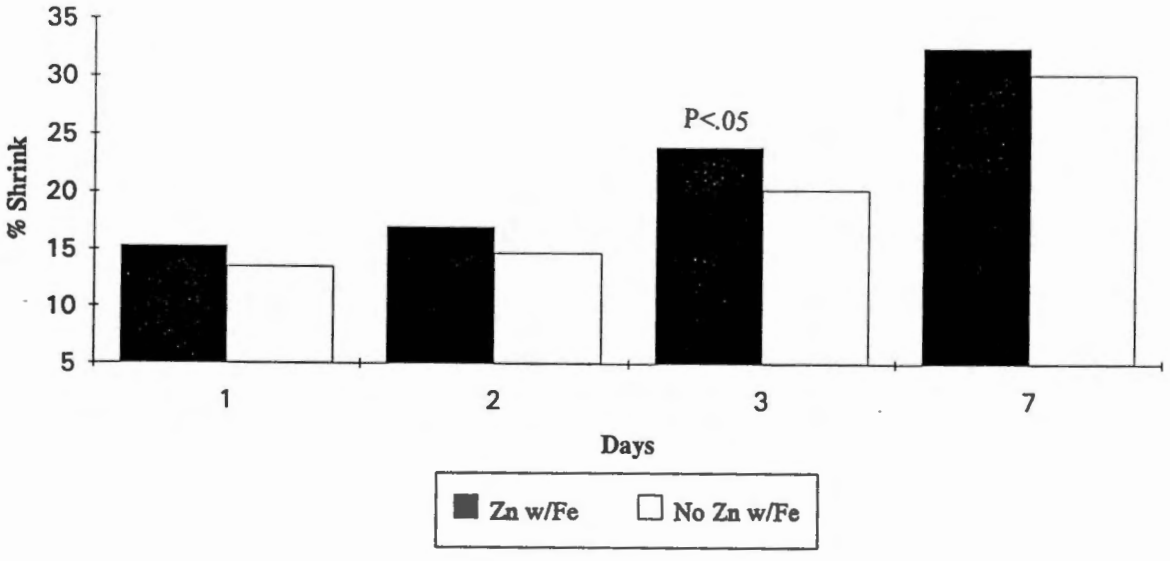

Figure 7. Zinc reduced the incidence of udder edema on one of four days measured in cows fed excess iron.

Table 4. Zinc reduced udder edema in cows fed excess iron only on 3 d

	-ZN	-Zn	+Zn	+Zn	ODDS RATIO	CHI	P VALUE
DAY	+EDEMA	-EDEMA	+EDEMA	-EDEMA			
1	5*	8	8	4	0.31	1.9	0.31
2	6	7	4	7	1.5	0.24	0.65
3	8	5	2	8	6.4	4	0.05
7	3	2	5	6	1.8	0.24	0.65

*Number of cows

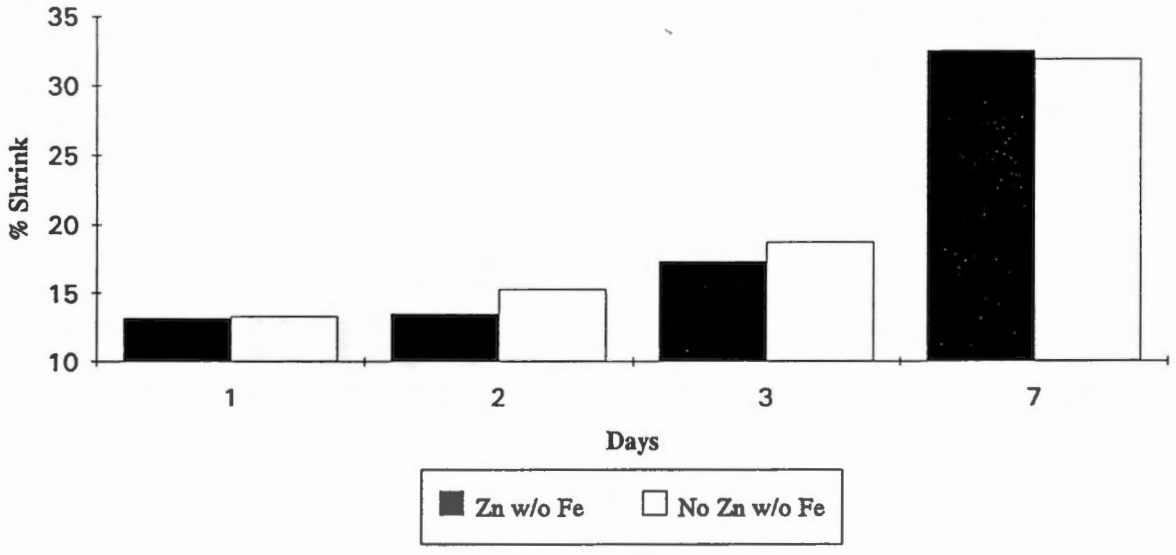

Figure 8. Zinc had no effect on udder edema in cows not fed excess iron.

Table 5. Zinc had no effect on udder edema in cows not fed excess iron

	-ZN	-Zn	+Zn	+Zn	ODDS RATIO	CHI^2	P VALUE
DAY	+EDEMA	-EDEMA	+EDEMA	-EDEMA			
1	6 *	8	7	6	0.64	0.03	0.85
2	8	5	8	5	1	0.16	0.69
3	5	7	7	6	0.61	0.04	0.84
7	5	8	5	4	0.5	0.13	0.72

* Number of cows

when challenged with a prooxidant such as iron, zinc exhibits antioxidant abilities. In areas where there is a high iron content in the soil it may be necessary to feed a higher level of zinc in the diet to protect against the oxidative effect of iron.

Plasma fast acting-antioxidants (FAA) were not increased or decreased as a result of the treatments. Research by Brzezinska-Slebodinska et al. (1992) showed that supplementation with vitamin E increased plasma total antioxidants by 40% in the sixth week of supplementation. Discrepancies between the two sets of data may be due to the random nature of free radical reactions. Free radicals are produced and propagated by random events and are subject to uncontrolled factors such as changes in feed, environment, stress etc. As F. C. Madsen (1995) pointed out, "biological phenomena are affected by many causal factors, uncontrollable in their variation and often unidentifiable." This often makes an animal response in different experiments as measured by an analytical procedure very difficult to reproduce. There was no relationship between fast acting-antioxidants and udder edema in this experiment. However, Miller and Madsen (1994) found that heifers with below average plasma FAA in late gestation were 4.7 times more likely to have udder edema than heifers with average or above FAA. The lack of treatment effects in the present experiment may have prevented a measurable relationship between FAA and udder edema.

CHAPTER V

CONCLUSION

This experiment was designed to determine the effects of vitamin E and Zn on udder edema in first calf heifers fed excess iron. Plasma alpha-tocopherol was maintained at significantly higher levels in heifers supplemented with vitamin E, which was consistent with other studies (Miller et al., 1993; Brzezinska-Slebodinska et al., 1994; Moore, 1992; Goff and Stabel, 1990; Weiss et al., 1992). The reduction of plasm alpha-tocopherol was partially due to the partitioning of vitamin E into the production and secretion of colostrum (Goff and Stabel, 1990; Hidiroglou et al., 1993). The quenching of free radical reactions may account for the rest of the decrease in plasma alpha-tocopherol. Cows not supplemented with vitamin E may not have enough plasma alpha-tocopherol to fight free radical production. Zinc had no significant effect on the level of plasma alpha-tocopherol. Zinc may work synergistically with vitamin E to maintain cellular integrity (Bettger et al., 1980). Zinc also has a different mechanism for preventing free radical reactions (Har-el et al., 1991; Bray et al., 1990) and thus may not have an effect on the level of vitamin E in plasma. Iron as well had no effect on the level of plasma alpha-tocopherol, unlike studies with pigs where supplemental Fe caused a significant decrease in plasma alpha-tocopherol (Moore, 1992). An excessive level of dietary Fe would be expected to generate free radical production of the hydroxyl radical, which in turn would intitiate peroxidative chain reactions. The resulting oxidative load would be expected to consume alph-tocopherol in quenching the reactions.

Treatments had no effect on the level of fast-acting antioxidants (FAA). This was inconsistent with data that showed cows supplemented with vitamin E had an increase in total plasma antioxidants by the sixth week of supplementation (Brzezinska-Slebodinska et al., 1994). The failure to find treatment effects on FAA may be due to the randomness of free radical

reactions. Biological sciences are often hard to replicate due to the many uncontrolable variations involved (Madsen, 1995). There was no relationship between udder edema and FAA, contrary to research done by Miller and Madsen (1994) where heifers with below average FAA were 4.7 times more likely to have udder edema than heifers with average or above FAA. Lack of treatment effects in this experiment may have prevented a measurable relationship between FAA and udder edema.

Udder edema was reduced when heifers were supplemented with vitamin E but not fed excess iron, which was consistent with other research (Miller and Madsen, 1994; Miller et al., 1993; Mueller et al., 1989). It is possible that under these circumstances vitamin E reduced udder edema by preventing the damaging effects of free radicals on membrane permeability. In the presence of excess Fe vitamin E was not able to reduce udder edema. One of the many ways in which iron initiates free radical reactions is through site specific damage (Har-el et al., 1991). Vitamin E is not able to prevent site specific damage because it is a chain breaking antioxidant (Bendich, 1993; Halliwell, 1991). Therefore vitamin E may not be able to reduce udder edema in the presence of high dietary iron. It may be necessary to find an antioxidant such as Zn that can prevent site specific damage. Udder edema was significantly reduced by Zn in the presence of excess iron. Feeding an increased dietary level of Zn may help prevent the site specific damage of excess Fe and thus, reduce udder edema in first calf heifers. However, Zn had no effect on udder edema when heifers were not fed excess iron. Zinc seems only to be an effective antioxidant in the presence of a prooxidant such as iron (Bray and Bettger, 1990).

Vitamin E reduced the severity of udder edema in first calf heifers. It may be cost effective to add vitamin E as a supplement to the diet of heifers during the last trimester of pregnancy. Zinc did not reduce the severity of udder edema in first calf heifers in this

experiment. However, sample size may have contributed to the lack of significant effects. Future experiments should increase the number of animals used in order to substantiate the results found in this research. At this time I would not suggest increasing zinc in the diet to reduce udder edema.

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