THESIS

THE EFFECTS OF MARGINAL ZINC DEFICIENCY ON BIOCHEMICAL AND PHYSIOLOGICAL PARAMETERS IN BEEF HEIFER AND HOLSTEIN STEER CALVES

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY TERRY E. ENGLE ENTITLED THE EFFECTS OF MARGINAL ZINC DEFICIENCY ON BIOCHEMICAL AND PHYSIOLOGICAL PARAMETERS IN BEEF HEIFER AND HOLSTEIN STEER CALVES BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

THE EFFECTS OF MARGINAL ZINC DEFICIENCY ON BIOCHEMICAL AND PHYSIOLOGICAL PARAMETERS IN BEEF HEIFER AND HOLSTEIN STEER CALVES

Three experiments were conducted to determine if certain biochemical and physiological parameters were altered in a marginal zinc (Zn) deficiency. In experiment 1, ten weaned crossbred Hereford Angus heifer calves, weighing 163 ± 2 kg were utilized. Five calves were fed a Zn deficient (-Zn) brome/alfalfa hay diet containing 17 mg Zn/kg and five calves were fed a Zn adequate (+Zn) diet with 23 mg Zn/kg from $ZnSO_4$ supplemented to the -Zn diet (total diet, 40 mg Zn/kg), for 32 d. At 21 d the -Zn calves had a reduction ($P<.05$) in feed efficiency. By 25 d, plasma Zn and alkaline phosphatase concentrations were reduced ($P<.05$) in -Zn calves. Blood urea nitrogen, glucose, insulin, plasma Cu concentration, red blood cell (RBC), and liver Zn and Cu concentrations were not altered ($P>.05$) by the -Zn diet through 25 d. In response to a single i.m. injection of dexamethasone (20 mg) on 25 d, calves fed the two dietary Zn amounts showed no changes ($P>.05$) in plasma or RBC Zn and Cu concentrations, serum IGF-I, insulin, and glucose when measured at 6, 12, 24, 48,72

and 96 h post injection. In response to an intradermal injection of phytohemagglutinin (PHA) on 30 d, cell mediated immune (CMI) response was reduced ($P<.05$) in the -Zn calves. These observations indicated that during a marginal Zn deficiency in calves, there was a decrease in feed efficiency, plasma Zn, serum alkaline phosphatase, and CMI response.

In experiment 2, a study determined the affects of Zn repletion using different Zn chemical forms on reversing certain physiological parameters in marginally -Zn deficient calves. Forty calves were allocated by weight with ten calves to each of four treatment groups. One group served as controls and received 23 mg Zn/kg from Zn sulfate ($ZnSO_4$) supplemented to the -Zn diet containing 17 mg Zn/kg. The three other groups were fed the -Zn diet. After 21 d, when greater than 90% of the calves in each group fed the -Zn diet demonstrated at least a 50% decrease in feed efficiency, they were deemed -Zn. Liver biopsies, blood plasma and RBC were taken for Zn analysis. Cell mediated immune response to PHA and feed efficiency were reduced ($P<.05$) in the -Zn calves but plasma and liver Zn concentrations were unaffected. Zn repletion occurred after feeding iso-Zn amounts of 23 mg Zn/kg diet, from Zn lysine (ZnLys), Zn methionine (ZnMet) and $ZnSO_4$. After 12 d of Zn repletion, liver biopsies and final blood samples were taken. Feed efficiency of the calves fed ZnMet returned to that of controls in 3 d, whereas feed efficiency of calves fed ZnLys and $ZnSO_4$ returned to that of control values by 9 d.

Cell mediated immune response to PHA in the ZnMet-fed group was greater and more rapid than that of the controls followed by the ZnLys and $ZnSO_4$ fed groups. These observations indicated that during a marginal Zn deficiency in heifer calves, repletion of Zn was the most rapid from ZnMet.

In experiment 3, a study was designed to determine the composition of gain in marginally -Zn calves. Ten Holstein steer calves 160 ± 1.0 kg were utilized in this experiment. Five calves were fed a -Zn diet (17 mg Zn/kg), and five were fed a +Zn diet of 40 mg Zn/kg (23 mg Zn/kg from $ZnSO_4$). Two consecutive 24 h urine collections were obtained from both groups of calves when the -Zn calves showed a decrease ($P<.05$) in feed efficiency. A second urine collection was conducted when the -Zn calves were repleted with 23 mg Zn/kg from $ZnSO_4$, and their feed efficiencies returned to that of the controls. Urinary 3-methylhistidine (3MH) was analyzed to determine muscle protein degradation. Zinc deficient calves excreted less ($P<.05$) g protein/kg of body weight/day than the controls when feed efficiencies of the -Zn calves were less than ($P<.05$) controls. Upon refeeding of Zn to the -Zn group there was no change in protein excretion or change in body weight. Following a rapid increase ($P<.05$) in growth, the control group excreted less ($P<.05$) g protein/kg of body weight/day than the -Zn group. These results indicated that a marginal Zn deficiency did not decrease muscle mass or increase protein

turnover in Holstein steer calves but did alter the rate of protein turnover and urine excretion.

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Chapter 1

INTRODUCTION

Overt signs of severe Zn deficiency in cattle are easily identified by inflammation of the nose and mouth with submucous hemorrhages, rough hair coat, bowing of hind legs, and parakeratosis of the skin which is probably the most obvious clinical sign of severely Zn deficient cattle (Blackman et al., 1967; Miller, 1979). However, marginal Zn deficiency is probably more prevalent than cattle exhibiting severe Zn deficiency signs. Easily measured indicators of marginal Zn deficiency have yet to be determined. Marginal Zn deficiency has been shown to decrease growth, feed efficiency, and resistance to infection and decrease immune function (Miller, 1970; Spears,1991), however, visual assessment of these clinical signs is not possible. Furthermore, plasma Zn concentrations of either severe and marginally Zn deficient cattle have provided variable data, thus, making plasma Zn somewhat of an unreliable indicator for Zn status in cattle.

The benefits of mineral supplementation to animals have been known for a long time. Mineral supplements originate from different sources, with the most commonly used sources being inorganic in nature. Different forms of inorganic minerals may

be absorbed with varying efficiencies. Organic minerals are those that have an organic molecule (e.g., amino acid, protein) attached to the metal. A metal-amino acid complex is a product resulting from complexing a soluble metal salt with an amino acid (Hambidge, 1986). The ligand formed in this complex is biodegradable and, therefore, the element may be more bioavailable.

Bioavailablity of the element is of utmost importance to an animal. If the mineral is poorly absorbed and retained by the animal, it is excreted into the environment. In areas of intense livestock concentration, heavy metal contamination of soil and ground water may someday be regulated by the United States Government as it is in other countries. The allowable amounts of these elements may not necessarily be determined by animal need, but by waste content. Therefore, in order to minimize excretory losses of metals such as Zn, sources providing better utilization to the animal must be found. In order to determine differences in utilization, criteria of Zn adequacy need to be established using immunological, hormonal and biochemical indices. The purpose of this study was to determine the biochemical and physiological changes which occur in marginal Zn deficiency and the effects of feeding organic and inorganic Zn on biochemical and physiological parameters in Zn deficient calves.

Chapter 2

LITERATURE REVIEW

History

Todd et al.(1934) was the first to establish that Zn is needed for the normal growth of the rat. The element Zn became a more predominant concern nutritionally when it was found to be deficient in swine diets in 1955, poultry diets in 1958, cattle diets in 1960, and in humans in 1961 (McDowell, 1992).

Availability

The amount of a particular element available to an animal depends on both intrinsic and extrinsic factors (O'Dell, 1983) which are also referred to as endogenous and exogenous factors. The intrinsic factors are physiological in nature and are much harder to control. They include species and genotype (Kinkade et al.,1976), stage of production (Berg et al., 1979), physiological stress (Orr et al., 1990), nutritional status (Stuart et al., 1986), and intestinal well being (Bafundo et al., 1984). The extrinsic factors are those which are present in the diet and include actual concentration in the diet, chemical or physical form of the element, presence

of chelating agents, solubility of the source, presence of interacting nutrients, and protein concentration of the diet (Shafey et al., 1991). Overall, the extrinsic factors can be controlled more efficiently to improve availability.

Bioavailability

Bioavailability is the proportion of a nutrient in a feedstuff that can be absorbed and utilized by an animal (O'Dell, 1983). It is important to realize that the total concentration of a particular element in a feedstuff may not reflect what is absorbed by the animal. Furthermore, even if the element is absorbed, it may not be metabolized for body functions and may be excreted immediately. It is the combination of absorption and utilization of an element, that determines bioavailability (Fox et al., 1981). In trace elements, therefore, bioavailability refers to the proportion of a nutrient which can be utilized by the animal to fulfill the functions for which the element is needed (Miller et al., 1979).

Source

The primary Zn supplements utilized by the animal feed industry today, are $ZnSO_4$ (36% Zn) and ZnO (72% Zn). However, the bioavailability of both of these minerals is uncertain. Many contradictory results have been reported as to the different effects and bioavailabilities of these different

sources. Clydesdale. (1990) determined that mineral source plays an important role in the formation of unknown complexes inside the digestive tract which in turn limits their absorption and further metabolism.

In recent years, animal nutritionists have investigated the concept, of using as a portion of the supplemental trace mineral mix, an organically complexed form. Wapmir et al., (1985), as well as Spears et al., (1991) have demonstrated that essential trace minerals in chelated form can improve animal function and production in certain species. Very little is known, however, about the physiological characteristics and physiological modes of action of the chelated nutrients.

In cattle, Zn methionine (ZnMet) is not catabolized by ruminal microorganisms (Heinrichs and Conrad, 1983) and was found to be more bioavailable than ZnO (Chirase et al., 1991). In lambs, however, ZnO and ZnMet were absorbed similarly but were metabolized differently after absorption (Spears, 1989). ZnMet supplementation has been shown to increase antibody titer against BHV-1 in cattle when compared to ZnO supplementation (Spears, 1991). Zinc methionine supplementation when compared to ZnO supplemention has also been shown to increase gain in feedlot cattle, and reduce somatic cell counts and increase milk production in dairy cattle (Herrick, 1989). In chickens, significant differences in bioavailability among $ZnSO_4$, ZnO, and Zn Met have been shown. Zinc methionine had a bioavailability of 206% relative

to $ZnSO_4$ (100%), and ZnO (62%) (Wedekind and Baker, 1992). The results from research using organic vs. inorganic minerals have often given opposing results. However, it has been determined that organically chelated minerals are absorbed differently than inorganic minerals. With a different absorption pathway, the organic forms of Zn may prove to be more available when feedstuffs high in fiber and phytate are being consumed.

Metabolism

Absorption: The mechanisms by which Zn is absorbed are not fully understood. In cattle, Zn is absorbed by the lower gastrointestinal tract primarily in the small intestine (Miller and Cragle, 1965). It is important to note that Zn absorption can be hindered by other components of the diet. Excess amounts of copper (Cu), calcium (Ca), phosphorous (P) and cadmium (Cd) can impair Zn absorption by competing for the same absorption site or by binding Zn and making it unavailable (Miller et al., 1979; Roberts et al., 1973). Fiber and phytate decrease Zn absorption and increase its excretion by binding positively charged Zn ions to negatively charged fiber and phytate particles thus, decreasing its availability (Miller et al.,1979). Iron has been shown to decrease Zn absorption, but the interactions between the two minerals are still unclear (Solomons and Cousins, 1984). Low dietary protein intake has been shown to decrease Zn absorption and

increase Zn excretion (Gerger and Snedeker, 1980). Aging also decreases Zn absorption because of decreased absorption efficiency by the ageing intestine (Lockitch et al., 1983).

Two kinetic processes are involved in Zn absorption, passive diffusion, and a carrier-mediated process which represents paracellular and intracellular absorption pathways (Hoodley et al., 1987). Zn uptake by carrier-mediated high molecular weight proteins in the intestinal mucosa is an active process requiring ATP (Menard and Cousins., 1983). In contrast, the diffusion component of Zn absorption is unaffected by Zn deficiency, and absorption via this process is proportional to luminal Zn concentration.

Uptake of Zn by intestinal cells is bidirectional. Zn is taken up from the intestinal lumen during times of need and also from the blood supply during times of abundance. Carrier mediated Zn absorption may increase during periods of low Zn intake, which suggests that the carrier mediated response is activated during periods of Zn deficiency (Hoodley et al., 1987).

Within the mucosal cell, absorbed Zn is localized largely in the cytosol and associated with either high molecular weight proteins such as metalloproteins or metallothionein (Hambidge, 1986). Metallothionein synthesis is closely related to dietary Zn intake and Zn plasma level concentration. It appears that the Zn metallothionein complex present in the intestinal mucosal cells is present longer or at least as long

as the life span of the mucosal cell itself. Zn that is bound to metallothionein in the enterocyte is unable to be absorbed into the blood and is lost with the enterocyte. Therefore, metallothionein represents a mechanism to help maintain the amount of Zn entering and leaving the body (Wapmir, 1985)

The entry of Zn from the enterocyte into the plasma is slower than the uptake of Zn into the enterocyte, thus being the rate limiting step in Zn absorption (Miller, 1970). Zn transport across the basolateral membrane of the enterocyte appears to be regulated by an ATP driven mechanism (Vikbladh, 1950). A small portion is complexed with alph-2-macroglobulin (Parisi and Vallee, 1970), transferrin (Charlwood, 1979), the amino acid cystine and histidine and the majority attached to albumin (Morgan, 1981).

Transport: Plasma Zn represents less than 1% of the total body content but serves as a primary source of the element accessible to all cells (Vallee and Falchuck, 1993). Following absorption into the portal blood, approximately two thirds of plasma Zn is loosely bound to albumin, and most of the remainder is tightly bound to alpha-2-macroglobulin (Giroux et al, 1976). The albumin-bound Zn is primarily taken up by the liver via an energy dependent process (Failla and Cousins, 1978). Within the hepatocyte, Zn is distributed among several metabolic pools. Approximately half of the Zn is in the cytosol, with a small portion associated with metallothionein. Metallothionein acts as a major storage form

of Zn in the liver and is mobilized during metabolic need (Richards and Cousins, 1976).

Regulation of liver metallothionein is under control of both dietary Zn and hormonal signals. The primary inducer of metallothionein mRNA appears to be, glucocorticoids, glucagon, and epinephrine, as well as by a number of other factors including cAMP, interferon, and interleukin-1 (Cousins, 1985).

Approximately 30-40% of the Zn entering the hepatic blood supply is extracted by the liver, from which it is incorporated, at different rates, into venous extrahepatic tissues which have different rates of Zn turnover (Hambidge et al., 1986).

Storage: Animals have a limited capacity to store Zn in a form that can be mobilized rapidly to prevent Zn deficiency (Underwood, 1977). Metallothionein, as mentioned before acts as a major storage form of Zn in the liver and is mobilized during metabolic need. Muscle contains approximately 65% of the total body Zn. Primarily, Zn is associated with protein and skeletal tissues, with little found in the lipid portion. One biochemical characteristic of Zn is its ability to form complexes with side chains of proteins (Riordan, 1976). Zn in muscle is not released in direct response to low circulating levels of plasma Zn, although muscle catabolism can result in a significant release of Zn into the circulation during severe Zn deficiency. This should be considered when measuring plasma Zn to determine Zn status of an animal.

Functions

Enzymes: Zn is essential for the function of more than 200 enzymes (Vallee, 1983), and is part of the structure of many proteins. Zn containing enzymes are found in all of the major pathways involved in carbohydrate, lipid, protein, and nucleic acid metabolism (Kaneko, 1989). Also Zn has the ability to function as a structural component away from the active enzymatic site, as a proton donor, and as a bridging atom between the substrate and enzyme (Vallee, 1983). Animal Zn containing enzymes include carboxypeptidase, alkaline phosphatase, alcohol dehydrogenase, carbonic anhydrase, and superoxide dismutase. In severe deficiencies activities of alkaline phosphatase, liver, and pancreatic carboxypeptidase A may be depressed. Hambidge, (1986) reported that one of the earliest signs of Zn deficiency in calves was a decrease in serum alkaline phosphatase, then a loss of appetite, followed by poor growth and reduced feed efficiency.

Zn has also been shown to be important in the synthesis of proteins. Zn is involved in stabilizing the structures of RNA, DNA, and ribosomes (Prask and Plocke, 1971). Berg, (1986) reported that there are several binding domains in DNA (Zn-binding fingers), which suggests that gene expression may,in part, be regulated by Zn, therefore linking Zn to growth.

Immune system: Because of the diverse roles of Zn in nucleic acid and protein synthesis and in gene expression, a deficiency in the mineral can have detrimental effects on the

immune system. In both mice and monkeys, marginal Zn deficiency resulted in an impairment in immunoglobulin M production and a decreased sensitivity to a number of mitogens (Gershwin et al., 1987). Beach et al. (1982) observed that these immune defects persist well into adulthood despite the introduction of Zn replete diets at birth.

Research conducted by Droke and Spears (1993) demonstrated that in lambs fed a severely Zn deficient diet, there was a lower percentage of lymphocytes and a higher percentage of neutrophils in peripheral blood. It is still not clear why a severe Zn deficiency altered the percentage of neutrophils and lymphocytes in this study. Droke and Spears speculated that perhaps a higher percentage of neutrophils may indicate an increased incidence of infection, however, no clinical signs of infection were observed. In contrast, Reffett et al. (1986) supplemented stressed (weaned and shipped) calves, fed a diet containing 27 mg/kg Zn, of which 30 mg/kg Zn was supplemented from $ZnSO_4$, and found no difference in percentages of neutrophils and lymphocytes. However, monocytes were increased by Zn supplementation. Reffett et al., noted that low plasma Zn levels may not be a factor in this alteration. Fracker et al., (1989) demonstrated in young adult Zn deficient mice, reductions in the absolute number of spleenocytes, and greatly depressed responses to both T-cell dependent and T-cell independent antigens. In response to sheep red blood cells, the Zn deficient mice

produced 40% fewer IgM and IgG placque forming cells per spleen as did the Zn adequate mice. The T-cell to B-cell ratio was unaltered, however, the deficient mice had nearly double the proportion of B-cells bearing high amounts of surface IgM as did the control mice.

Further studies suggested that a greater proportion of immature B-cells were accumulating in spleens of Zn deficient mice (Fracker et al., 1989). With the insufficiency of the mineral, the cells were unable to differentiate and proliferate.

Growth and Protein Turnover: Several physiological and biochemical changes have been reported in Zn deficient animals (Hambidge, 1986), including a loss of appetite and poor feed efficiency. Growth retardation during Zn deficiency has been identified in cattle (Wheaton et al., 1986). Insulin like growth factor-I (IGF-I) serum levels parallel changes in growth hormone and are relatively constant throughout the day, making IGF-I a relatively easy and less expensive alternative to measuring growth hormone in cattle (Ellenberger et al., 1989). IGF-I has been shown to respond to Zn deficiency (Oner et al., 1984) and nutritional reductions (Bass et al., 1991, McGuire et al., 1992) and was used to address growth changes in cattle. Pancreatic and plasma concentrations of insulin were also markedly reduced by Zn deficiency (McDowell, 1992).

Many studies have consistently shown that Zn was required for normal growth and skeletal development in all species

(Hambidge, 1986; O'Dell and Reeves, 1985). In rats, markedly impaired growth caused by the consumption of a Zn deficient diet for four to five days was restored by Zn supplementation (Givgliano and Milward, 1984). Humans with Zn deficiency have shown improved growth after supplementation with Zn (Hambidge, 1986).

Because growth hormone plays a key role in regulation of postnatal growth (Etherton et al., 1993), a defect in the growth hormone signaling pathway in Zn deficiency accounts for the growth retardation observed (McNall et al., 1995). Growth hormone stimulates the synthesis and release of IGF-I which is a potent mitogen that enables growth hormone to act on specific target cells (Froesch et al., 1985). Serum levels of IGF-I have been found to be markedly reduced by Zn deficiency (Droke and Spears, 1993). However, growth hormone deficiency in itself does not account for this decrease because the release of growth hormone from somatotrophs is not changed by Zn deficiency (Focht et al ., 1991) thus, serum growth hormone levels are not reduced by Zn deficiency (Droup et al., 1991). Also Boltz et al., (1987) have shown that the ability of IGF-I to stimulate sulfate incorporation into cartilage is reduced during Zn deficiency.

Zn is required for normal DNA polymerase activity and is a component of many zinc-finger DNA binding proteins (Chesters, 1980). Recent studies have shown that the RNA abundance for several genes is reduced by Zn deficiency (Shay

and Cousins, 1993). McNall et al., (1995) determined that Zn deficiency markedly affects the expression of genes involved in the growth hormone intracellular signalling pathway. Lower levels of IGF-I growth hormone receptor and growth hormone binding protein mRNA are associated with Zn deficiency.

Zinc and muscle composition: Zinc concentration of muscles varies with their color and functional activity (Mertz, 1986). The highest Zn concentrations in muscle are found in muscles that are highly oxidative and have a large proportion of slow-twitch muscle fibers (Maltin, 1982). The increased Zn content of slow twitch muscle fibers is thought to be associated with higher levels of carbonic anhydrase present in these more oxidative tissues (Cassens, 1976).

During Zn deficiency, the zinc content of muscles is generally unaltered. However the size and number of the various muscle fibers may be reduced and relative distribution altered, with a reduction in the oxidative muscles and an increase in fast twitch glycolytic muscle types (Jackson et al., 1982).

Three-methylhistidine as an Indicator of Protein Turnover: A major component of growth in farm animals is the increase in muscle mass that contributes about 45% of the body weight in mature mammals, regardless of their size (Munro, 1969), as well as being an important tissue for protein synthesis, reserve, and turnover (Cheek, 1971). The net deposition of protein in mammals is determined by the balance

between the rates of synthesis and degradation. Millward et al., (1975) reported that there is a direct correlation between growth rate and muscle protein breakdown rate, and that rapid growth is always accompanied by high rates of protein turnover.

Quantitative procedures to study muscle protein metabolism under different physiological conditions have become necessary. Muscle protein is of major economic importance, is harvested from food animals, and is determined by the balance between muscle protein synthesis and degradation.

Excretion of 3-methylhistidine (3-MH) in urine has been widely used as a nondestructive technique for measuring in vivo muscle protein degradation (Young and Munro, 1978). The 3-MH originates from the degradation of actin and myosin heavy chains in which it is present after post-translational methylation of specific histidine residues (Young and Munro, 1978). During the catabolism of myofibular proteins 3-MH is released and is not reutilized for protein synthesis or metabolized oxidatively (Long et al., 1975) but is excreted in the urine (Young et al., 1972). Therefore, urinary excretion of 3-MH has been suggested as a valid in vivo indictor of muscle protein degradation (Ward and Buttery, 1978).

The determination of 24 hour urinary excretion of 3-MH to measure muscle protein degradation has been used in man (Tomas

et al., 1979), rats (Santidrian et al., 1981), and validated for cattle (Harris and Milnein, 1981).

Muscle Protein and 3-methylhisdine calculations: Total skeletal muscle mass of cattle, calculated as 33% of the total body weight, has been validated by Allen et al., (1968), and Brannang, (1971). Gopinath and Kitts,(1984) determined that the total protein content of wet skeletal muscle in cattle is 157 mg/g wet. The 3-MH content of skeletal muscle protein has been determined by Nishizawa et al., (1979) to be 3.5106 umol 3-MH/g of wet muscle protein. Gopinath and Kitts,(1984) also have verified that the calculated 3-MH pool of skeletal muscle, when determined by multiplying the estimated skeletal muscle mass by the protein content of skeletal muscle and the 3-MH content of skeletal muscle protein, is a valid index for determining the 3-MH pool of cattle.

Chapter 3

MATERIALS AND METHODS

Protocols for all 3 experiments described herein were approved by the Colorado State University Animal Care and Use Committee. All calves were transported to the Department of Animal Sciences feedlot at Fort Collins, Colorado.

Adaptation and Nutrient Repletion phase: In experiments 1, 2, and 3 calves were given ad libitum access to their respective diets (Table 1). The diets for experiments 1 and 2 consisted of brome grass hay and alfalfa hay (17 mg Zn/kg), and for experiment 3 the diet contained corn silage and sudan hay (17 mg Zn/kg). All control diets were fed with a corn based supplement containing 23 mg Zn/kg from $ZnSO_4$ and other nutrients (Table 1) to meet or exceed the suggested NRC (1984) requirements for beef cattle (total dietary Zn concentration, 40 mg Zn/kg). The supplement was topped dressed immediately after the diet was put in the bunk. Each day the cattle were monitored and were found to consume the supplement prior to the roughage portion of the diet. This enabled the calves to become repleted with nutrients if previously insufficient. Dietary CP, Ca, P, Cu, and Zn were analyzed in all diets by Weld Laboratories Inc., (Greeley, CO). Individual feed

consumption was determined daily and animals were weighed weekly.

Zinc Depletion phase: After the adaptation and nutrient repletion phase, the calves were weighed on two consecutive days and were assigned to a control or treatment group. On 0 d, the treatment group received the control diet in which the $ZnSO_4$ was omitted to give a total dietary Zn content of 17 mg Zn/kg, and the control group was fed the same diet as was fed in the adaptation and nutrient repletion phase (40 mg Zn/kg). The calves were weighed and bled, prior to feeding.

Immune challenge: To determine CMI response throughout all experiments, hair on the right side of the neck was clipped, and .75 ug of phytohemagglutinin (PHA; Sigma Chemical, St. Louis MO) in 0.1 ml of 0.9% saline solution in a phosphate buffer was injected intradermally at two separate sites (Fritz et al.1990) in the neck. Inflammatory response was measured in mm as a change from the heifers skin thickness prior to and after injection with PHA, at certain times using skin fold calipers (Slim Guide, Creative Health Products, Plymouth, Michigan) (Fritz et al., 1990).

Tissue sampling: Blood samples taken throughout all experiments were collected by venipuncture from the jugular vein using an 18 gauge needle inserted into vacutainer (Becton Dickinson Vacutainer Systems, Becton Dickinson and Company, Franklin Lakes, NJ 07417-1885) tubes. Samples analyzed for Zn and Cu were collected in trace mineral free vacutainer (Becton

Dickinson Vacutainer Systems, Becton Dickinson and Company, Franklin Lakes, NJ 07417-1885) tubes. Blood was immediately chilled in ice, returned to the laboratory, centrifuged at 1000 x g for 15 minutes and plasma or serum was harvested and frozen at -20 °C until analyzed. Red blood cells were washed three times with 0.9% saline solution and centrifuged at 1000 x g for 15 minutes. The packed cell volume of the RBC was determined after the final centrifugation, and the cells frozen at -20 °C.

Liver biopsy sites were clipped of hair, given three scrubs with Betadine (Purdue Fredrick, Norwalk, CT), and the area was locally anesthetized with lidocaine (5 ml/animal Vedco, St. Joseph, MO). A liver biopsy was obtained through an incision made between the 11th and 12th ribs on a line from the tubercoxae to the point of the shoulder. A core sample of liver weighing approximately 50 mg was taken by the true-cut technique (Pearson and Craig, 1980) using a modified Jan Shide bone marrow biopsy punch (.5 cm in diameter x 14 cm in length). Liver biopsies were immediately rinsed with deionized water and drained to remove contaminating blood. The biopsy was placed in a polyethylene tube, capped and frozen at - 20C.

Tissue Analyses: Liver samples were dried in a 60 C drying oven for 24 h to determine dry matter. The tissue samples were then wet-ashed in .5 ml of 7 N nitric acid in an acid washed centrifuge tube. The tubes were then placed in a

50 C water bath for 12 h, and analyzed directly using flame atomic absorption spectrometry. All Zn and Cu analyses were determined using flame atomic absorption spectrometry in the Diagnostic Laboratory (Dept. of Pathology, Colorado State Univ., Fort Collins; Varian Model 1275). Plasma was diluted 1:5 (vol/vol) with deionized water for Zn and 1:3 (vol/vol) for Cu. Red blood cells were diluted 1:10 (vol/vol) with deionized water for Zn and 1:3 (vol/vol) for Cu.

Experiment 1

Adaptation and Nutrient Repletion phase: Ten weaned crossbred Hereford Angus heifer calves weighing 163 ± 2 kg were placed in individual pens (1.8 m x 10 m) and for 28 d given ad libitum access to the control diet (Table 1). Throughout the trial, no supplement was recovered upon determination of daily feed intake. During the first 28 d the calves were adapted to the individual feeding pens, diet, and gentled to reduce the stress of handling and jugular bleeding while calves were haltered and tied. Stress has been shown to increase the release of glucocorticoids (Cousins, 1985), and cause a decrease in plasma Zn and increase hepatic Zn content (Weeks, 1989). On 25, 26, 27, 28 d of the repletion phase the calves were bled prior to feeding to provide baseline biochemical measurements.

Zinc Depletion phase: After the 28 d adaptation the calves were paired by feed intake per body weight$^{0.75}$. The

calves were weighed and bled, prior to feeding, on 3, 7, 10, 14, 17, 21, and 24 d. On 24 d, liver biopsies were taken. On day 25, each calf was bled and then given 20 mg i.m. (Booth and McDonald, 1982) of aqueous dexamethasone (Anthony Products Arcadia, CA), to examine the effects of a synthetic glucocorticoid (dexamethasone) on serum glucose (Heitzman and Baird, 1969), alkaline phosphatase (Miyahara et al., 1991), insulin (Kirchgessner et al., 1978) and IGF-I (Mile et al., 1993) in Zn adequate and marginally deficient calves. Jugular blood samples were taken at 6, 12, 24 48, 72, and 96 h post-dexamethasone injection.

Immune challenge: At 30 d of the Zn depletion phase, the immune system was challenged with an injection of PHA, and measurements were taken after 8, 24 and 48 hours.

Tissue sampling: Blood samples were collected into three tubes : one 10-ml heparinized tube, one 10-ml nonheparinized tube, and one 7-ml trace mineral-free tube containing heparin (Becton Dickinson Vacutainer Systems, Becton Dickinson and Company, Franklin Lakes, NJ 07417-1885).

Tissue Analyses: Liver, plasma, and RBC Zn and Cu concentrations were determined as previously described. Serum IGF-1 concentrations were determined by double-antibody radioimmunoassay (Holland et al., 1988). Insulin was quantified using an RIA kit (Diagnostic Products Corp., Los Angles, CA). Alkaline phosphatase activity, glucose, and blood urea nitrogen concentrations were determined using procedures

in Diagnostic Kits No. 245-20, 245-20, and 640-20, respectively, from Sigma Chemical (St. Louis MO). McNall et al. (1995) showed IGF-I serum concentrations to decrease in severely Zn rats. Dietary Zn deficiency has been shown, in cattle, to markedly reduce serum insulin concentrations, resulting in increased levels of plasma glucose (Kirchgessner et al.,1978). Hambidge, (1986) reported decreased alkaline phosphatase activity during Zn deficiency. Rabbani and Prasad, (1978) determined that blood urea nitrogen in Zn deficient rats increases during the first week of Zn deficiency and then begins to decrease over time.

Experiment 2

Adaptation and Nutrient Repletion phase: Forty weaned crossbred Hereford Angus heifer calves weighing 209 ± 14 kg were utilized. All calves upon arrival were placed in one pen (500 m^2) and for 28 days given ad libitum access to the control diet (Table 1). On 25, 26, 27, 28 d of the repletion phase the calves were bled prior to feeding to provide baseline biochemical measurements. On 27 and 28 d consecutive body weights were taken and on 28 d, liver biopsies were taken.

Zinc Depletion phase: After the 28 d adaptation and nutrient repletion phase, the calves were allocated by weight to each of four final treatment groups. One group received the control diet of 40 mg Zn/kg with 23 mg Zn/kg from $ZnSO_4$ while the other three groups to be depleted of Zn received the same

diet with no supplemental Zn (17 mg Zn/kg by analysis). Each group was fed in one of four ten head pens (400 m^2). Each pen was equipped with one pinpointer (Pinpointer 4000 USI Cooperation, Cookeville, TN); an electronic feeding device allowing measurement of individual feed intakes. The pinpointer feeders were housed under a roof and on concrete pads. The roughage portion of the diet was cubed (50 cm x 25 cm^2) at Morning Fresh Dairy Farms (Graves Dairy, Fort Collins, Co) cubing facility (model; John Deere 390 hay compressor). The pinpointers were filled every 2 d with approximately 125 kg of total mixed ration (TMR). Prior to filling the pinpointer tubs, the supplement was mixed with the cubed hay. A consistent mixture of hay and supplement was obtained, due to the cubes flaking apart upon mixing. The mean particle size of the TMR placed in the pinpointer tub ranged from 5 cm x 2 cm^2 to 40 cm x 10 cm^2. Bridging of the feed was prevented by placing a curved rod in the center of the pinpointer tub that extended to the feeding bunk. Upon consumption of the diet the animals were observed to move the rod with their muzzle thus preventing bridging of the feed, much like that of a pig feeder.

Body weights and blood samples were taken prior to feeding, on 3, 7, 10, 14, 17, and 21 d. When 90% or more of the calves in each of the 3 pens fed the -Zn diet demonstrated at least a 50% decrease in feed efficiency relative to controls, they were deemed Zn deficient. Deficiency of the

element occurred 21 days after removing the supplemental Zn from the diet.

Immune challenge: On day 22 of the Zn depletion phase, response to PHA at 8, 12, 24, and 48 hours were measured.

Zinc Repletion Phase. On 0 d, Zn repletion was begun by feeding 23 mg Zn/kg from one of the following sources; ZnMet, ZnLys, and $ZnSO_4$ sulfate to give a total dietary Zn content of 40 mg/kg. The same weighing and bleeding schedule was maintained as in the Zn depletion phase. On day 7 of the Zn repletion phase the second PHA test was conducted. After 12 days of Zn refeeding the third and final liver biopsies were taken, cattle were weighed on 13 and 14 d and the experiment terminated.

Tissue sampling: Blood samples were collected into two 7-ml trace mineral-free tube containing heparin (Becton Dickinson Vacutainer Systems, Becton Dickinson and Company, Franklin Lakes, NJ 07417-1885).

Tissue Analyses: Liver, plasma, and RBC Zn were determined as previously described.

Experiment 3

Adaptation and Nutrient Repletion phase: Ten Holstein steer calves weighing approximately 125 ± 1.6 kg were transported from Badger Creek Farm, Inc. (Fort Morgan, CO) to the Department of Animal Sciences feedlot. The calves were housed in individual pens (10 m x 1.5 m). The feed bunks were

under a roof and on concrete pads. The calves were fed for 14 d the control diet (Table 1). Throughout the trial, no supplement was recovered upon determination of daily feed intake. This two week period allowed the calves to become accustomed to individual pens, jugular bleeding, handling, tethering, haltering and urine harnesses. Stress has been shown to increase the release of glucocorticoids (Cousins, 1985), and cause a decrease in plasma Zn and increase hepatic Zn content (Weeks et al., 1989). At the end of the adaptation period, the calves were weighed and bled on 13 and 14 d and their feed efficiency determined. On 14 d liver biopsies were taken.

Zinc depletion phase: Calves were then paired by body weight$^{.75}$ (kg) divided by feed intake, into five pairs with one calf of each pair allocated to the control group and fed the same diet as fed in the adaptation period (40 mg Zn/kg). The other five calves from each pair constituted the experimental group which were fed the same diet as the controls, but with no supplemental Zn added to the diet (total diet, 17 mg Zn/kg), for 21 d until all five calves had a decrease ($P<.05$) in feed efficiency. These calves were then considered Zn deficient and blood plasma and liver biopsies were taken for Zn analysis. The calves were then tethered to their feed bunks for two days where they were fed their diets and given water. A urine collection bag was strapped to each animal and was connected to a vacuum pump via 9.5 mm. i.d. plastic tubing

(Morgan et al. 1993). During these two days, two consecutive 24-h urine collections were obtained. The urine was collected in a 20-L glass container to which 200 ml of 6 N HCl was added to prevent nitrogen loss. At the end of each 24-h collection period, total volume of excreted urine was measured, mixed and a 50 ml sample obtained and frozen at -20 C until analyzed (Morgan et al. 1993).

Zinc repletion phase: After two complete days of urine collection all calves were fed the control diet containing 40 mg Zn/kg. On 14 d, when the deficient group had their feed efficiency returned to that of the controls, a liver biopsy was taken, a two day collection of urine was taken and the experiment terminated.

Analyses: Urinary concentration of 3-methylhistidine was determined by the HPLC procedures (Warren Labs, Greeley Co.) as described by Wassner et al., (1980) and as modified by Wheeler and Koohamaraie (1992). Analyses of blood and liver samples for Zn were performed at the Soils, Water and Plant: Diagnostic Laboratory at Colorado State University.

Calculations: The calculations of muscle protein (MP) metabolism were preformed according to Gopinath and Kitts (1984) and McCarthy et al., (1983). The 3-MH pool in skeletal muscle was calculated by multiplying the estimated muscle mass (33% of body weight; Allen et al., 1968; Brannagan, 1971) by the protein content of skeletal muscle mass (157 mg/g fresh weight; Gopinath and Kitts, 1984) and the 3-MH content of

skeletal muscle protein (3.5106 umol 3-methylhistidine/g muscle protein; Nishizawa et al.,1979). The fractional degradation rate (FDR) of muscle protein was calculated by dividing the daily excretion of 3-MH in urine by the amount of 3-MH in the skeletal muscle pool and multiplying by 100.

Fractional accretion rate (FAR) was calculated as the rate of skeletal muscle protein gain divided by total skeletal muscle protein pool at the time urine samples were obtained. The calculation was: FAR = $([MP_1 - MP_0]/T \div MP_1] \times 100$, where MP_1 was the measurement of total muscle protein at the current urine collection period, MP_0 was the measure of muscle protein from the previous urine collection period, and T is the number of days between collection periods. The numerator of the FAR is equal to the absolute rate of muscle protein accretion (MPA, g/d).

The fractional synthesis rate (FSR) of the mixed muscle protein pool was calculated as the sum of FDR and FAR. Measurements of 3-MH excretion can be used to determine degradation of myofibrillar proteins, primarily actin (Young and Munro 1978). However, the differences in turnover rate between myofibrillar sarcoplasmic and stromal proteins were not sufficient to negate the use of 3-MH excretion to measure skeletal muscle protein breakdown (Bates and Millward, 1983). Thus, 3-MH excretion is useful as an estimate of muscle protein turnover. This calculation is a modification of the calculation used by Millward et al., (1975), who used the FSR

and FAR to calculate FBR. Myofibrillar protein degradation (MPD) was calculated by dividing the daily 3-MH excretion by the concentration of 3-MH in muscle. The rate of muscle protein synthesis (MPS) was calculated as the sum of MPD and MPA.

Statistical analysis

In experiments 1, 2, and 3, the model where appropriate contained treatment, calf within treatment, period, time, and the treatment by time interactions. In experiment 1, repeated measures ANOVA was preformed using General Linear Models Procedure of SAS (SAS,1989) on changes during the Zn depletion phase, in Zn and Cu concentrations in plasma, RBC and liver, in blood alkaline phosphatase, urea nitrogen, IGF-I, insulin, and glucose, and CMI response and feed efficiency. In Experiment 2, repeated measures ANOVA was preformed using General Linear Models Procedure of SAS (SAS,1989) on changes during the Zn depletion and repletion phase, in Zn concentrations in plasma, RBC and liver, and CMI response and feed efficiency. In experiment 3, repeated measures ANOVA was preformed using General Linear Models Procedure of SAS (SAS,1989) on changes during the Zn depletion and repletion phase, in Zn concentrations in plasma and liver, feed efficiency and urinary concentration of 3MH. Treatment least squares means were tested by a protected F test, with significance determined at ($P<.05$).

Chapter 4

RESULTS AND DISCUSSION

Experiment 1

At the end of the 28 d repletion period, there was no difference between calf pair body weights ($P>.05$, Table 2). Feed efficiency of the calves fed adequate dietary Zn was constant over the entire trial. However, the calves fed the -Zn diet were characterized by a steady reduction in feed efficiency, beginning approximately 17 d after feeding the -Zn diet, and reaching a 50% decrease ($P<.05$) in feed efficiency by 21 d (Figure 1). At this time, feed efficiency was reduced by 50% and remained constant through 24 d. Based on the 50% reduction in feed efficiency in the -Zn calves at 21 d, animals were deemed marginally Zn deficient at this time. The reduction in feed efficiency in the -Zn calves is similar to that reported by Essatara et al., (1986) in rats, and was the earliest and most reliable parameter of Zn deficiency in this study. Earlier studies by Mayland et al. (1980) noted that grazing cattle fed a Zn supplement gained 6% more ($P<.05$) weight and were more efficient than the control group which received no supplemental Zn.

Plasma alkaline phosphatase was reduced (P<.05) in the -Zn group on 25 d of the deficiency period (Figure 2). This reduction in plasma alkaline phosphatase from Zn deficiency supports the results of Hambidge, (1986). However, a decrease in plasma alkaline phosphatase activity was not observed until after the heifers were deemed -Zn at 21 d when a decrease in their feed efficiencies was observed (Figure 2).

After the dexamethasone injection, a gradual reduction in serum alkaline phosphatase occurred during the first 48 h (P<.05 vs. 0 time) in both +Zn and -Zn groups (Figure 3). Dexamethasone has been reported to increase osteoblast metallothionein production and alkaline phosphatase activity in rats (Miyahara et al., 1991) and liver metallothionein production in sheep (Peterson and Mercer 1988). An increase in cellular metallothionein enhances clearance of zinc from the blood. Zinc deficient calves would be expected to show a decrease in alkaline phosphatase as Zn was unavailable for alkaline phosphatase production. However, a reduction (P<.05) of serum alkaline phosphatase was observed in both +Zn and -Zn calves. Alkaline phosphatase reduction may have been due to a down regulation of osteoblast and liver metallothionein production as a result of dexamethasone administration. This decrease in serum alkaline phosphatase, to our knowledge, has never been reported in cattle. The decrease in serum alkaline phosphatase demonstrated that dexamethasone had a negative effect on alkaline phosphatase in +Zn and -Zn heifer calves.

Serum IGF-I levels in calves were not altered during a marginal Zn deficiency from 0 through 25 d (Figure 4). McNall et al. (1995) showed IGF-I serum concentrations to decrease in severely -Zn rats. Their study also indicated that Zn deficiency markedly decreased the expression of genes involved in the growth hormone intracellular signaling pathway. Because McNall et al. (1995) used rats which were severely Zn deficient and our results are from marginally deficient calves, the studies are not directly comparable. Marginally Zn deficiency in calves did not affect IGF-I levels.

Dexamethasone administration on 25 d, induced a rapid reduction of IGF-I levels during the first 6 h ($P<.05$ vs. 0 time) in both +Zn and -Zn groups (Figure 5). Six hours after dexamethasone treatment, serum concentrations of IGF-I in +Zn and -Zn calves were reduced by 35-40%. Insulin-like growth factor-I levels remained depressed for 1-2 days after dexamethasone treatment and slowly returned to pre-injection amounts at the end of the study period. This may suggest that dexamethasone had a negative effect on IGF-1 in heifer calves, in contrast to other species such as humans where dexamethasone treatment increased IGF-I and insulin concentrations in blood (Mile et al.,1993).

Blood insulin, glucose, and urea nitrogen concentrations were not affected by Zn status (Figure 4) through 25 d. Kirchgessner et al, (1978) determined, that in cattle, Zn was associated with insulin release from the pancreas and that

pancreatic Zn concentrations were markedly reduced during dietary zinc deficiencies, resulting in reduced concentrations of serum insulin and hyperglycemia. Rabbani and Prasad, (1978) determined that blood urea nitrogen in Zn deficient rats increased during the first week of Zn deficiency and then began to decrease over time. In this study no change occurred in blood insulin, glucose, and urea nitrogen concentrations which possibly reflects the marginal Zn deficiency induced in the calves. A more severe Zn deficiency may be needed to alter insulin, urea, and glucose levels.

Insulin, IGF-1 and glucose serum concentrations were not different between the +Zn and -Zn treatments post-dexamethasone injection (Figure 5). However, serum concentrations of glucose approximately 24 hours post-dexamethasone, increased rapidly ($P<.05$), whereas insulin levels decreased slightly ($P>.05$) then began to increase in response to increasing glucose concentrations. These results concur with those of Andersson and Olsson, (1984) who observed an increase in plasma glucose in dairy cattle after the administration of dexamethasone.

According to McDowell (1992), cattle develop characteristic severe skin lesions when their plasma Zn is less than .6 mg/kg and decreased growth was noted when plasma was between .6 and 1.0 mg/kg. Because the -Zn calves in the present experiment had a mean plasma Zn of .75±.11 mg/kg and a reduced feed efficiency, but displayed no lesions and no

decrease in feed intake, we concluded that these calves were marginally Zn deficient.

At the end of the depletion phase, plasma Zn but not Cu was decreased ($P<.05$) in the heifers fed the low Zn diet (Table 3). In contrast to our results, data obtained by Abdulla, (1983), showed that low dietary intakes of Zn increased Cu plasma levels in cattle.

Packed red blood cells from these marginally Zn deficient calves also had a slight reduction in Zn ($P<.07$) but not in Cu content (Table 3). Hepatic Zn and Cu concentrations were not affected by dietary Zn (Table 3).

After a single dexamethasone injection on 25 d of the depletion phase, plasma and RBC Zn and Cu, were not different among treatments at 6, 12, 24 48, 72, and 96 h post-dexamethasone injection (Table 4). Weeks et al, (1989) observed that cattle injected with dexamethasone had decreased serum Zn concentrations, and Cousins and Cooper, (1985) noted that glucocorticoids cause acute hypozincenima, increased hepatic metallothionein gene expression, and redistribution of hepatic Zn. In this study no change was observed in plasma and RBC Zn and Cu concentrations post-dexamethasone.

The marginally zinc-deficient calves had a lower CMI response ($P<.05$) at 8-h post-injection than the controls (Figure 6). Zinc is essential to the integrity of the immune system (McDowell 1992). Severe effects of Zn deficiency on immunocompetence are related to thymic hormone production and

activity; lymphocyte function, and antibody dependent cell-mediated cytotoxicity (Hambidge, 1986). An impairment in cell-mediated immunity has not been previously reported in calves marginally deficient in Zn, but is similar to the results reported by Droke and Spears, (1993) in severely Zn deficient lambs.

In summary, this study found that during a marginal Zn deficiency in beef heifer calves there was a decrease in feed efficiency, plasma alkaline phosphatase, CMI, and plasma Zn concentration.

Experiment 2

At the end of the 28 d repletion phase, there was no difference ($P>.05$) between calf pair body weights, feed intake, and Zn concentration of plasma and liver (Table 5). However, following the Zn depletion phase, there was a reduction ($P<.05$) in average daily gain (Table 5), feed efficiency (Table 5) and PHA skin swelling response (Figure 7). Based on the 50% reduction in feed efficiency in the -Zn calves by 21 d, animals were considered marginally Zn deficient at this time. The reduction in feed efficiency in the -Zn calves is similar to that reported by Essatara et al., (1986) in rats. During the Zn repletion phase, feed efficiency of the calves fed ZnMet returned to that of the controls by 3 d, whereas feed efficiency of calves fed ZnLys and $ZnSO_4$ returned to that of the control values by 9 d (Table 5). Skin

swelling response to PHA in the ZnMet fed group was greater and more rapid than that of the controls followed by ZnLys and ZnSO groups (Figure 8). There were no differences in plasma or liver Zn during the depletion or repletion phases. Spears et al., (1991) as well as Wapmir et al., (1985) have demonstrated that essential trace minerals in chelated form can improve animal function and production in certain species. In cattle, ZnMet is not catabolized by ruminal microorganisms (Heinrichs and Conrad, 1983) and the Zn was found to be more bioavailable than Zn in ZnO (Chirase et al., 1991). In lambs, however, Zn from ZnO and ZnMet were absorbed similarly but were metabolized differently after absorption (Spears, 1989). ZnMet supplementation has been shown to increase antibody titer against BHV-1 in cattle when compared to Zn in ZnO supplementation (Spears, 1991). Zinc Met supplementation when compared to ZnO supplemention has also been shown to increase gain in feedlot cattle, and reduce somatic cell counts and increase milk production in dairy cattle (Herrick, 1989). In chickens, significant differences in Zn bioavailability among $ZnSO_4$, ZnO, and Zn Met have been shown. Zinc Met had a bioavailability of 206% relative to $ZnSO_4$ (100%), and ZnO (62%) (Wedekind et al., 1992). The results from research using organic vs. inorganic minerals have been contradictory. However, it has been determined that organically chelated minerals are absorbed differently than inorganic minerals. With a different absorption pathway, the organic forms of Zn

may prove to be more available when feedstuffs high in fiber and phytate are being consumed (Miller et al., 1979). Very little is known, however, about the physiological characteristics and physiological modes of action of the chelated nutrients.

Experiment 3

The initial body weights, feed intake, average daily gain, feed efficiency, and plasma Zn were not different among the treatment groups at the end of the adaptation and nutrient repletion phase (Table 6). Body weight, average daily gain, and feed efficiency of the -Zn group were decreased ($P<.05$) and urine excretion was increased ($P<.001$) when compared to the +Zn group (10.1 ± 2.9 and 3.2 ± 1.1 L/d, respectively), at the end of the 21 d Zn depletion phase (Table 6). Muscle protein degradation at the end of the Zn depletion period was greater ($P<.05$) in the +Zn group than in the -Zn group (Table 7). Instead of observing a decrease in muscle mass due to an increase in protein degradation which was hypothesized, results showed that during a marginal Zn deficiency FDR, FAR, and FSR decreased ($P<.05$; Table 7). It is speculated that the marginally Zn deficient calves, which had a decrease in feed efficiency due to a decrease in gain, in conjunction with lower MPD, FAR, FDR and FSR, may have down regulated proteolytic enzymes involved in muscle protein degradation in order to maintain a balance of the mineral and protein.

Therefore, this reduction in growth during marginal Zn deficiency coupled with the same feed intake is a possible cause of the observed change in feed efficiency. However, the decrease in feed efficiency of the -Zn group is probably only in part due to the decreased rate of protein turnover. At the time of the decreased feed efficiency, the -Zn calves' urine excretion was greater ($P<.05$) than that of the controls. Research conducted by Song (1987) demonstrated that Zn appears to be beneficial for Na-K balance in rats fed a -Zn diet. Because this study did not utilize cattle, it is hypothesized that -Zn deficient cattle while showing a decreased feed efficiency, had a decreased rate of protein turnover coupled with a Na-K imbalance, as shown by their increased urine excretion.

Upon Zn repletion to the -Zn group, there was no change ($P<.05$) in body weight gain (Table 6), MPD and FDR but there was an increase ($P<.05$) in FAR and FSR (Table 7) and a decrease ($P<.05$) in urine excretion (5.1 ± 1.3 l/d) from the measurements obtained at the end of the Zn depletion phase. This suggests that as the -Zn group became repleted with Zn, any down regulation of protein metabolism as well as any Na-K imbalance may have been reduced and FSR and feed efficiency returned to that of the controls. Following a rapid increase in body weight (Table 6), the +Zn group had no change ($p>.05$) in urine excretion, but had a decrease ($P<.05$) in 3MH excretion, MPD, FDR, and FSR (Table 7). It is speculated that

this rapid growth observed in the +Zn calves was due to maintenance of the FAR (Table 7), in conjunction with the reduction of MPD and FDR.

Plasma Zn concentration showed no change between groups ($P>.05$) at any time (Table 6). During the depletion phase the liver Zn content of the -Zn group was greater ($P<.05$) than that of the +Zn group (Table 6) in contrast to research by Smart et. al.,(1981) who showed a decrease in liver Zn content during Zn deficiency.

Data from this research indicates that a marginal Zn deficiency did not yield a decrease in muscle mass or an increase in protein turnover in Holstein steer calves but did alter the rate of protein turnover.

Implications

Marginal Zn deficiency is probably far more prevalent than cattle exhibiting Zn deficiency signs. Plasma Zn and alkaline phosphatase were reduced in marginally Zn deficient calves. However, these differences were no longer evident after the administration of dexamethasone a synthetic glucocorticoid. This suggests that if dexamethasone acts physiologically like cortisol after stresses, such as transportation or restraint, cattle marginally deficient in Zn may have differences in plasma Zn and alkaline phosphatase concentrations masked compared to Zn adequate cattle. Furthermore, cell mediated immune response was reduced in

marginally Zn deficient calves allowing them to be more susceptible to disease. Repletion of Zn with ZnMet rather than ZnLYS or $ZnSO_4$ produced the most rapid improvement in feed efficiency, and cell mediated immune response indicating that Zn from different sources may be metabolized differently.

Table 1. Composition of control diet.

Experiment 1 and 2		Experiment 3	
Ingredient	% DM basis	Ingredient	% DM basis
Brome Grass Hay	54.0	Sudan Hay	44.0
Alfalfa	39.0	Corn silage	21.0
		Alfalfa	11.0
Supplement		Supplement	
Ground corn	5.0	Ground corn	21.0
Trace minerals[a,b]	1.0	Urea	1.4
Soybean meal	.33	Trace minerals[a,b]	1.0
Salt (iodized)	.25	Limestone Salt (iodized)	.56
Urea	.22	Salt (iodized)	.25
Vitamins and Rumensin[c]	.20	Vitamin and Rumensin[c]	.20
Analyzed composition, DM[d]		Analyzed composition, DM[d]	
Crude Protein, %	10.8	Crude Protein, %	10.8
Calcium, %	.40	Calcium, %	.40
Phosphorus, %	.24	Phosphorus, %	.24
Zinc, mg/kg	40.0	Zinc, mg/kg	40.0
Calculated Content		Calculated Content	
Dry Matter, %	89.0	Dry Matter, %	62.6
NEm, Mcal/kg	1.6	NEm, Mcal/kg	1.4

[a]Trace minerals added per kilogram of diet: $CuSO_4 \cdot 5H_2O$, 15.7 mg; MgO, 26.3 mg; Na_2SeO_3, .15 mg; $ZnSO_4$, 81.7 mg.

[b]$ZnSO_4$ was omitted from the -Zn diet during the depletion phase (total Zn for the -Zn calves, 17 mg/kg). For experiment 2 Zn was repleated with ZnMet, 120 mg; ZnLys, 120 mg; and $ZnSO_4$, 81.7 mg were added to the diet during the repletion phase. All diets during the repletion phase contained 40 mg/kg Zn.

[c]Vitamins provided per kilogram of diet: vitamin A, 3000 IU; rumensin, .5 mg; and vitamin E, 500 IU/hd/d.

[d]Analyzed composition of the total diet.

Table 2. Effects of adequate and deficient dietary zinc on performance of heifers fed a roughage based diet.

	Dietary Zinc		
Item	+Zn	-Zn	SEM
Repletion phase, 0-28d			
Initial wt, kg	164.0[a]	162.0[a]	2.0
Final wt, kg	178.0[a]	175.0[a]	1.2
ADG, kg	.50[a]	.46[a]	.04
Feed intake, kg/d	4.2[a]	3.9[a]	.07
Gain/feed	.12[a]	.12[a]	.01
Depletion phase, 0-25d			
Initial wt, kg	180.2[a]	179.0[a]	.26
Final wt, kg	194.0[a]	185.9[b]	.10
ADG, kg	.54[a]	.28[b]	.05
Feed intake, kg/d	4.5[a]	4.0[a]	.18
Gain/feed	.13[a]	.07[b]	.03

[a,b] Means in a row lacking a common superscript letter differ($P<.05$).

Table 3. Tissue zinc and copper content of heifers fed zinc adequate deficient diets for 25 days.

Item	+Zn	-Zn	SEM
Plasma Zn, mg/dl	1.05[a]	.75[b]	.11
Plasma Cu, mg/dl	.88[c]	.80[d]	.09
RBC Zn, mg/dl[e]	.07[a]	.05[a]	.25
RBC Cu ng/dl[f]	.64[a]	.61[a]	.30
Liver Zn, mg/kg DM	120.0[a]	113.0[a]	.26
Liver Cu mg/kg DM	240.0[a]	231.0[a]	.39

[a,b] Means in a row lacking a common superscript letter differ (P<.05).

[c,d] Means in a row lacking a common superscript letter differ (P<.10).

[e] mg/dl packed red blood cells.

[f] ng/dl packed red blood cells.

Table 4. The effects of dexamethasone administration on day 25 of the depletion phase on tissue zinc and copper content 6,12, 24, 48, 72, and 96 hours post-dexamethasone administration.

Item	Dietary Zinc +Zn	-Zn	SEM
6 hours, post dex.			
Plasma Zn, mg/dl	.85[a]	.75[a]	.10
Plasma Cu, mg/dl	.90[a]	.81[a]	.04
RBC Zn, mg/packed dl	.07[a]	.05[a]	.03
RBC Cu mg/packed dl	.0007[a]	.0071[a]	.0005
12 hours, post dex.			
Plasma Zn, mg/dl	.95[a]	.86[a]	.11
Plasma Cu, mg/dl	.88[a]	.80[a]	.04
RBC Zn, mg/packed dl	.10[a]	.08[a]	.03
RBC Cu mg/packed dl	.0008[a]	.0009[a]	.005
24 hours, post dex.			
Plasma Zn, mg/dl	.90[a]	.75[a]	.12
Plasma Cu, mg/dl	.90[a]	.81[a]	.04
RBC Zn, mg/packed dl	.08[a]	.05[a]	.03
RBC Cu mg/packed dl	.0007[a]	.0007[a]	.005
48 hours, post dex.			
Plasma Zn, mg/dl	.95[a]	.85[a]	.10
Plasma Cu, mg/dl	.88[a]	.83[a]	.04
RBC Zn, mg/packed dl	.05[a]	.08[a]	.09
RBC Cu mg/packed dl	.0007[a]	.0008[a]	.005
72 hours, post dex.			
Plasma Zn, mg/dl	1.10[a]	.95[a]	.10
Plasma Cu, mg/dl	.90[a]	.81[a]	.04
RBC Zn, mg/packed dl	.07[a]	.05[a]	.03
RBC Cu mg/packed dl	.0007[a]	.0007[a]	.005
96 hours, post dex.			
Plasma Zn, mg/dl	1.05[a]	.95[a]	.10
Plasma Cu, mg/dl	.89[a]	.84[a]	.04
RBC Zn, mg/packed dl	.06[a]	.05[a]	.03
RBC Cu mg/packed dl	.0008[a]	.0008[a]	.007

[a,b] Means in a row lacking a common superscript letter differ (P<.05).

Table 5. Effects of feeding heifer calves, Zn adequate diets for 28 days, Zn deficient diets for 21 days and repletion from Zn from Zn lysine, Zn methionine and $ZnSO_4$ for 14 days.

Treatment Group	Body Wt. (kg)	ADG (kg)	Feed Intake (kg DM)	Gain/Feed	Plasma Zn (mg/dl)	Liver Zn (mg/g DM)
Day 0-28, adaptation and nutrient repletion phase						
Source of added Zn[c]						
1, $ZnSO_4$[d]	204 ± 14[a]	.56 ± .03[a]	5.9 ± .60[a]	.10 ± .08[a]	.94 ± .21[a]	108 ± 10.0[a]
2, $ZnSO_4$	210 ± 12[a]	.56 ± .09[a]	5.6 ± .47[a]	.10 ± .03[a]	.89 ± .32[a]	112 ± 8.9[a]
3, $ZnSO_4$	210 ± 10[a]	.56 ± .03[a]	5.9 ± 1.0[a]	.10 ± .04[a]	.96 ± .10[a]	100 ± 7.9[a]
4, $ZnSO_4$	208 ± 10[a]	.45 ± .08[a]	6.0 ± .35[a]	.08 ± .04[a]	.85 ± .19[a]	110 ± 7.9[a]
Day 0-21, Zn Depletion phase						
Source of added Zn[e]						
1, $ZnSO_4$[d]	216 ± 10[a]	.57 ± .09[a]	6.1 ± .36[a]	.10 ± .04[a]	.97 ± .30[a]	106 ± .36[a]
2, 0[e]	216 ± 13[a]	.33 ± .10[b]	7.1 ± .18[a]	.05 ± .04[b]	.73 ± .36[a]	100 ± .30[a]
3, 0	217 ± 10[a]	.31 ± .05[b]	6.2 ± .46[a]	.05 ± .08[b]	.96 ± .12[a]	97 ± .30[a]
4, 0	214 ± 9[a]	.32 ± .02[b]	6.5 ± .33[a]	.06 ± .03[b]	.82 ± .10[a]	106 ± .12[a]
Day 0-14, Zn Repletion phase						
Source of added Zn						
1, $ZnSO_4$[d]	223 ± 9[a]	.50 ± .11[a]	6.1 ± .40[a]	.09 ± .10[a]	.95 ± .18[a]	109 ± 1.9[a]
2, Zn Lys[f]	221 ± 6[a]	.41 ± .11[a]	6.6 ± .40[a]	.07 ± .05[a]	.87 ± .12[a]	110 ± .90[a]
3, Zn Met[g]	222 ± 10[a]	.39 ± .10[a]	5.7 ± .50[a]	.07 ± .11[a]	1.07 ± .38[a]	101 ± .98[a]
4, $ZnSO_4$[h]	221 ± 4[a]	.54 ± .07[a]	6.6 ± .15[a]	.08 ± .10[a]	.90 ± .20[a]	111 ± .13[a]

[a,b] Means in a column followed by unlike letters are different (P<.05).

[c] All groups fed 23 mg Zn/kg DM from $ZnSO_4$ added to the basal diet containing 17 mg Zn/kg DM throughout the adaptation and nutrient repletion phase (Total diet, 40 mg Zn/kg DM).

[d] Control group fed 23 mg Zn/kg DM from $ZnSO_4$ added to the basal diet containing 17 mg Zn/kg DM throughout entire trial (Total diet, 40 mg Zn/kg DM).

[e] No Zn added to diets of groups 2,3, and 4 (Total diet, 17 mg Zn/kg DM).

[f] Group fed 23 mg Zn/kg DM from Zn lysine added to the basal diet containing 17 mg Zn/kg DM throughout Zn repletion phase (Total diet, 40 mg Zn/kg DM).

[g] Group fed 23 mg Zn/kg DM from Zn methionine added to the basal diet containing 17 mg Zn/kg DM throughout Zn repletion phase (Total diet, 40 mg Zn/kg DM).

[h] Group fed 23 mg Zn/kg DM from $ZnSO_4$ added to the basal diet containing 17 mg Zn/kg DM throughout Zn repletion phase (Total diet, 40 mg Zn/kg DM).

Table 6. Effects of feeding Holstein steer calves adequate diets for 14 days, Zn deficient diets for 21 days and repletion with $ZnSO_4$ for 14 days.

Treatment	Body Wt. (kg)	ADG (kg)	Feed Intake (kg DM)	Gain/Feed	Plasma Zn (mg/dl)	Liver Zn (mg/kg DM)
Day 0-14, adaptation and nutrient repletion phase						
Control.[d]	125 ±1.60[a]	.50 ±.09[a]	3.6 ±.25[a]	.13 ±.40[a]	.89 ±.10[a]	320 ±44[a]
-Zn[e]	125 ±1.60[a]	.50 ±.08[a]	3.7 ±.41[a]	.14 ±.30[a]	.92 ±.13[a]	343 ±32[a]
Day 0-21, Zinc depletion phase						
Control[d]	137 ±1.80[a]	.60 ±.40[a]	5.3 ±.20[a]	.11 ±.09[a]	.96 ±.02[a]	266 ±43[b]
-Zn[f]	130 ±2.00[b]	.28 ±.35[b]	5.5 ±.20[a]	.06 ±.05[b]	.94 ±.02[a]	395 ±37[a]
Day 0-14, Zn Repletion phase						
Control[d]	146 ±3.10[c]	.60 ±1.50[a]	5.6 ± 1.51[a]	.11 ±.20[a]	1.23 ±.18[a]	396 ±18[a]
-Zn[g]	136 ±2.50[ab]	.49 ±.40[a]	5.7 ±.40[a]	.09 ±.25[a]	1.11 ±.12[a]	356 ±26[a]

[a,b,c] Means in a column followed by unlike letters are different (P<.05).

[d] Control group fed 23mg Zn/kg DM from $ZnSO_4$ throughout entire trial. (Total diet, 40 mg Zn/kg DM).

[e] Experimental groups fed 23 mg Zn/kg DM from $ZnSO_4$ (Total diet, 40 mg Zn/kg DM).

[f] Experimental groups fed no supplemental Zn. (Total diet, 17 mg Zn/kg DM).

[g] Experimental groups fed 23 mg Zn/kg DM from $ZnSO_4$ (Total diet, 40 mg Zn/kg DM).

Table 7. The effects of marginal Zn deficiency on muscle protein turnover.

Treatment	Urine 3-methylhistidine, mmol/d	Total 3-methylhistidine pool, mmol*	Muscle Protein degradation, g/kg BW excreted/d	Fractional degradation rate, %/d	Fractional accretion rate, %/d*	Fractional synthesis rate, %/d[d]
Day 21 end of depletion						
Control[e]	1.5 ± .02[a]	25.1 ± 1.60[a]	3.1 ± .20[a]	5.9 ± .05[a]	.45 ± .10[a]	6.4 ± .70[a]
-Zn[f]	1.1 ± .02[a]	24.0 ± 1.70[a]	2.4 ± .10[b]	4.5 ± .02[b]	.18 ± .20[b]	4.6 ± .20[b]
Day 14 end of repletion						
Control[e]	.80 ± .01[b]	26.5 ± 1.60[a]	1.5 ±.5[b]	3.0 ± .40[c]	.39 ± .03[a]	3.4 ± .07[b]
-Zn[g]	1.2 ± .03[a]	25.0 ± 1.80[a]	2.4 ±.60[b]	4.8 ± .50[b]	.36 ± .04[a]	5.2 ± .15[a]

[a,b,c] Means in a column followed by unlike numbers are different (P<.05).
[d] The summation of fractional degradation rate and fractional accretion rate.
[e] Control group fed 23 mg Zn/kg DM from $ZnSO_4$ throughout trial(Total diet contained 40 mg Zn/kg DM).
[f] -Zn group fed no supplemental Zn, 0-21 d (Total diet contained 17 mg Zn/kg DM).
[g] -Zn group fed 23 mg Zn/kg DM from $ZnSO_4$ 0-14 (Total diet contained 40 mg Zn/kg DM).
* Calculated not determined.

Figure 1. Mean feed efficiencies (gain/feed) for each treatment during the zinc depletion phase. The +Zn calves received the control diet which contained 40 mg Zn/kg (23 mg Zn/kg from $ZnSO_4$). The -Zn calves received no supplemental Zn (total Zn, 17 mg Zn/kg) a,b ($P<.05$).

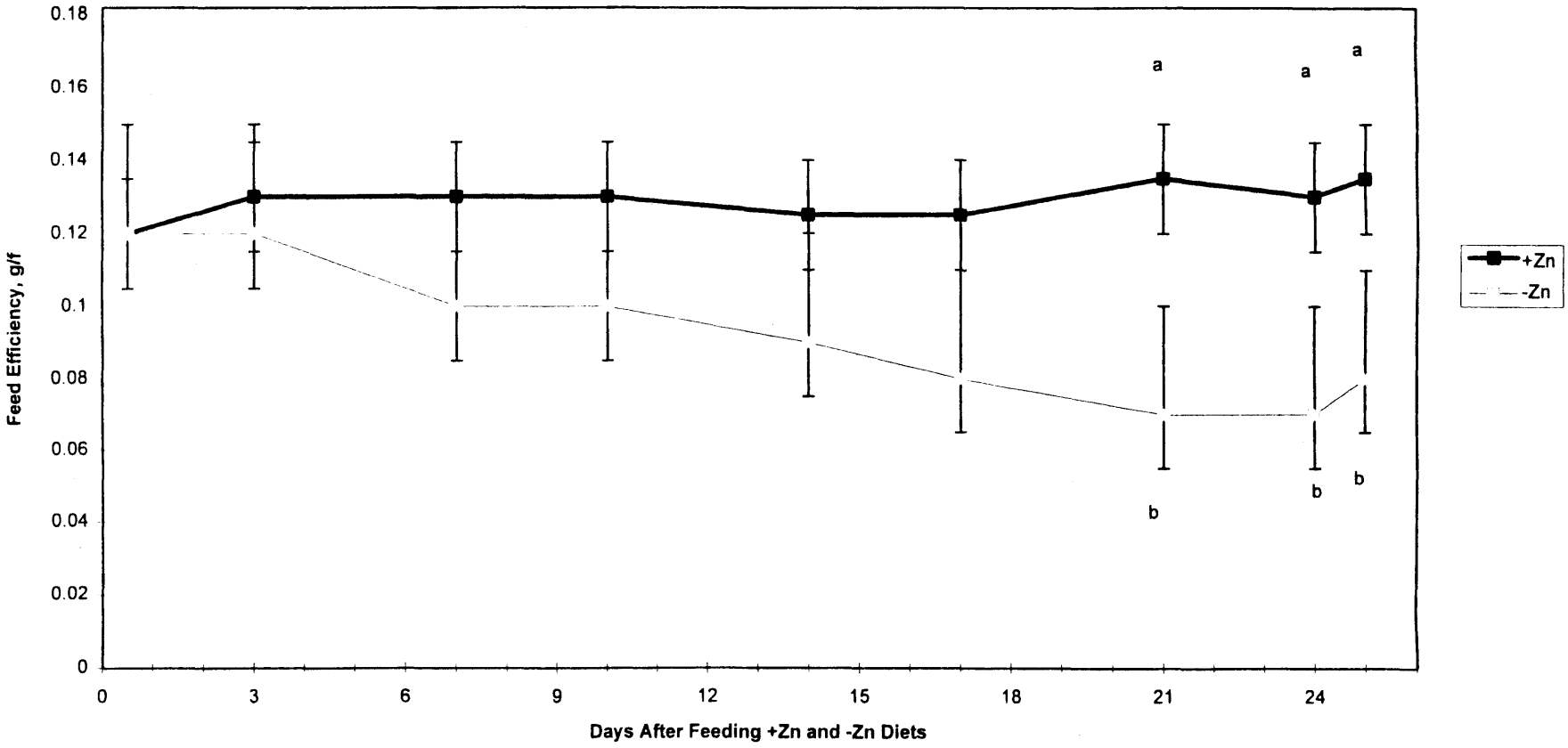

0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Feed Efficiency, g/f
0
3
6
9
12
15
18
21
24
Days After Feeding +Zn and -Zn Diets
a
a
a
b
b
b
+Zn
-Zn

Figure 2. Mean alkaline phosphatase activity in serum from heifers during the zinc depletion phase. The +Zn calves received the control diet which contained 40 mg Zn/kg (23 mg Zn/kg from $ZnSO_4$). The -Zn calves received no supplemental Zn (total Zn, 17 mg Zn/kg). a,b ($P<.05$).

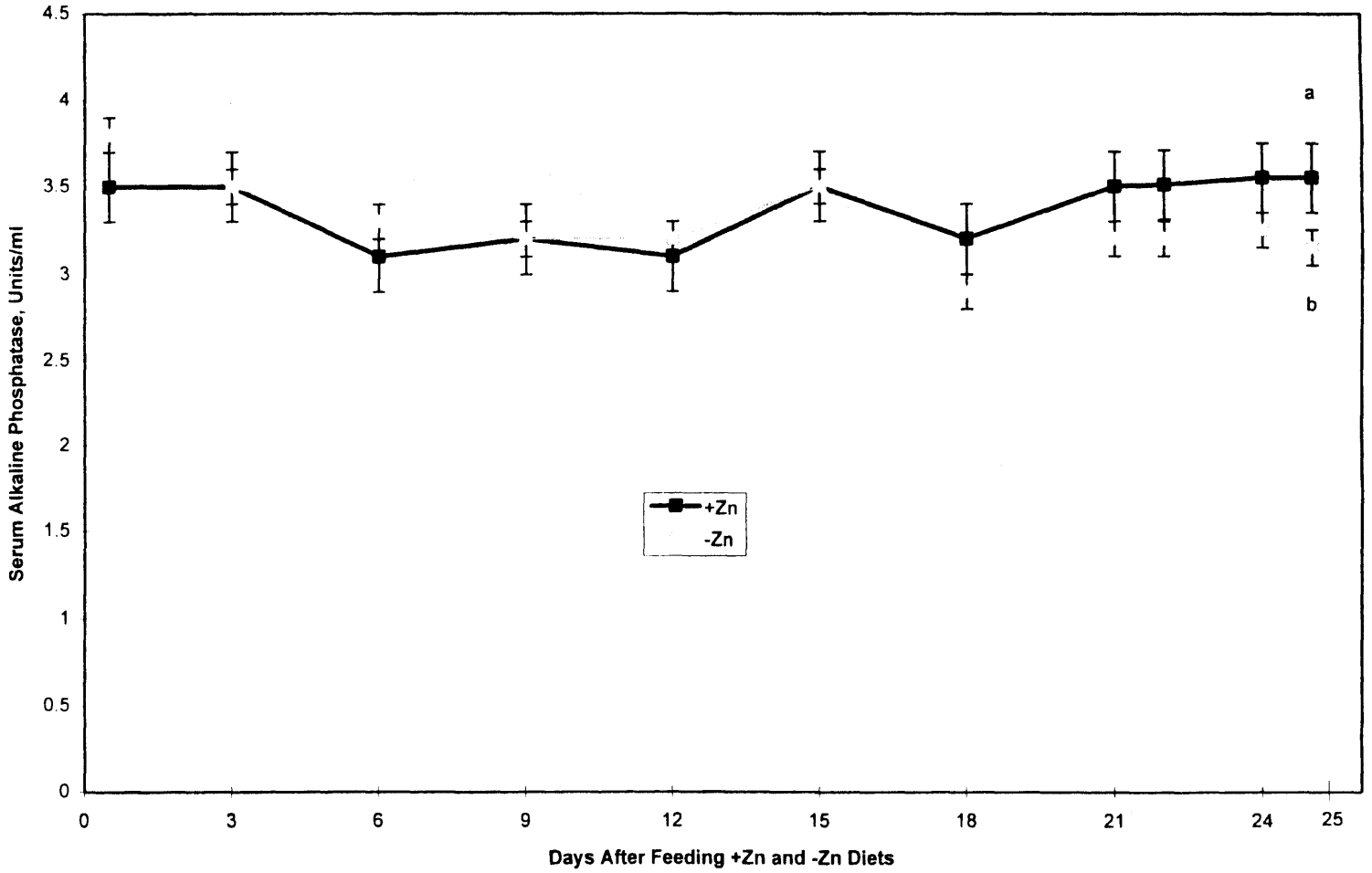

4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Serum Alkaline Phosphatase, Units/ml
a
b
+Zn
-Zn
0
3
6
9
12
15
18
21
24
25
Days After Feeding +Zn and -Zn Diets

Figure 3. Mean serum alkaline phosphatase concentrations at 6, 12, 24, 48, 72, and 96 h, after i.m. administration of 20 mg of aqueous dexamethasone, beginning on 25 d of the depletion phase. The +Zn calves received the control diet which contained 40 mg Zn/kg diet (23 mg Zn/kg from $ZnSO_4$). The -Zn calves received no supplemental Zn (total Zn, 17 mg Zn/kg). Treatment means did not differ ($P>.05$) at each time measured throughout 96 h. However, alkaline phosphatase means were different ($P<.05$) just prior to dexamethasone administration, and there was a decrease a,c; b,c ($P<.05$) over time, (0 vs. 48 h) for both treatments.

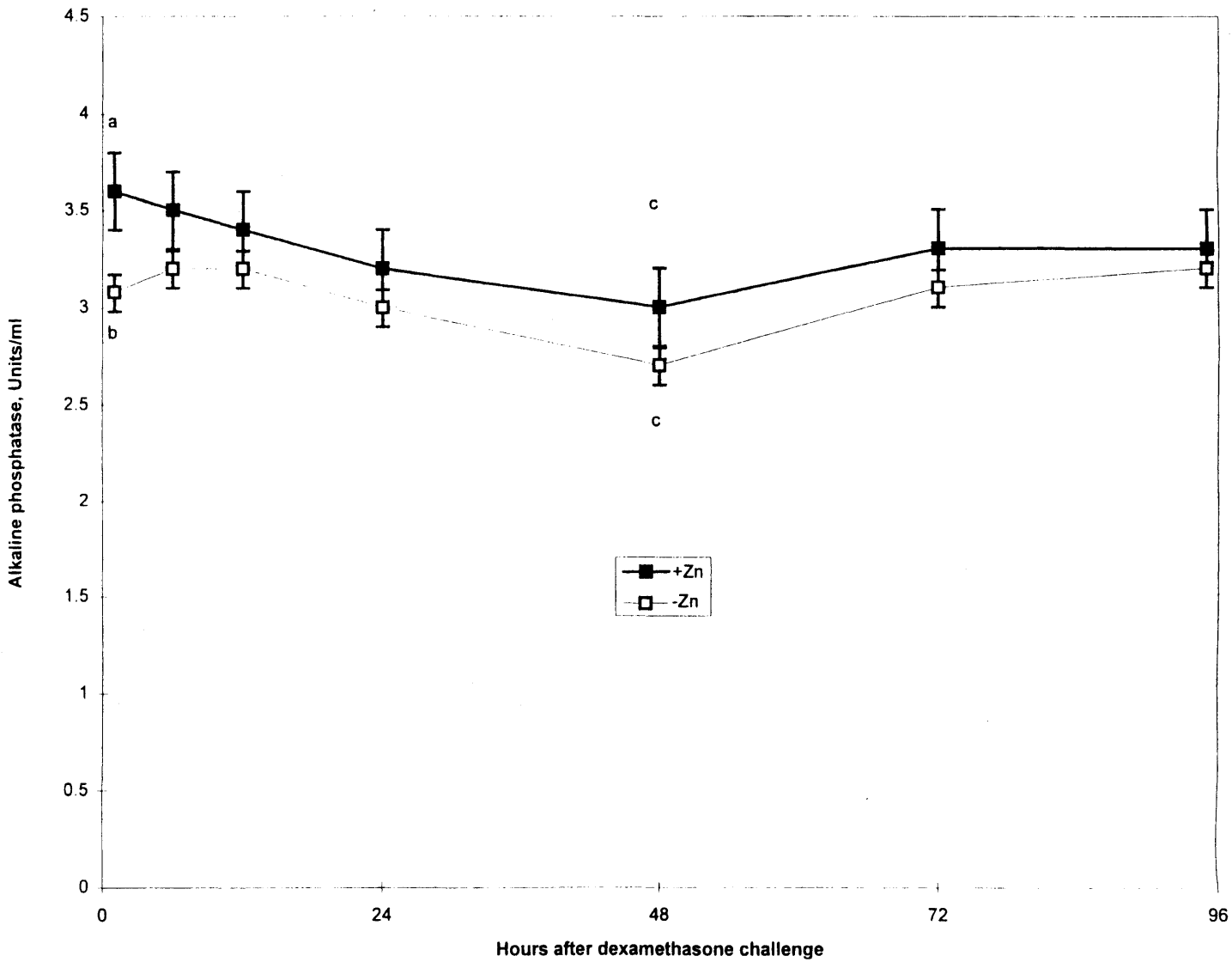

4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
a
b
c
c
+Zn
-Zn
0
24
48
72
96
Alkaline phosphatase, Units/ml
Hours after dexamethasone challenge

Figure 4. Mean serum glucose, IGF-I, urea nitrogen, and insulin concentrations from heifers fed during the zinc depletion phase. The +Zn calves received the control diet which contained 40 mg Zn/kg diet (23 mg Zn/kg from $ZnSO_4$). The -Zn calves received no supplemental Zn (total Zn, 17 mg Zn/kg). Treatment means did not differ ($P>.05$) at each measurement from 0-25 d for any of the parameters measured.

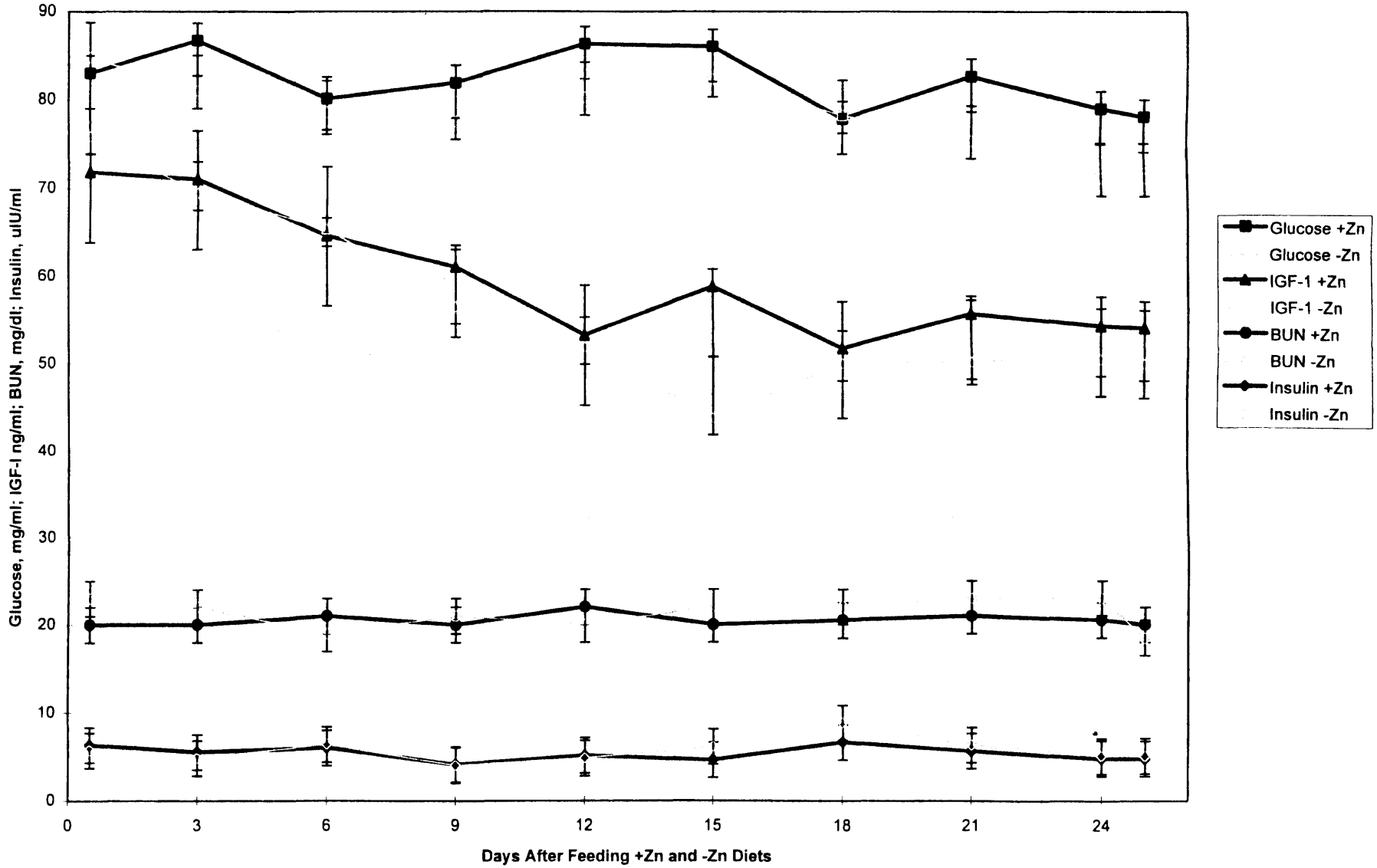

Glucose, mg/ml; IGF-I ng/ml; BUN, mg/dl; Insulin, ulU/ml
90
80
70
60
50
40
30
20
10
0
0
3
6
9
12
15
18
21
24
Days After Feeding +Zn and -Zn Diets
Glucose +Zn
Glucose -Zn
IGF-1 +Zn
IGF-1 -Zn
BUN +Zn
BUN -Zn
Insulin +Zn
Insulin -Zn

Figure 5. Mean serum IGF-I, glucose, and insulin concentrations at 6, 12, 24, 48, 72, and 96 h, after administration of i.m. 20 mg of aqueous dexamethasone, beginning on 25 d of the depletion phase. The +Zn calves received the control diet which contained 40 mg Zn/kg diet (23 mg Zn/kg from $ZnSO_4$). The -Zn calves received no supplemental Zn (total Zn, 17 mg Zn/kg). Treatment means did not differ ($P>.05$) at each time measured throughout 96 h. However, glucose concentrations were increased and IGF-I concentrations were decreased a,b ($P<.05$) over time, for both treatments.

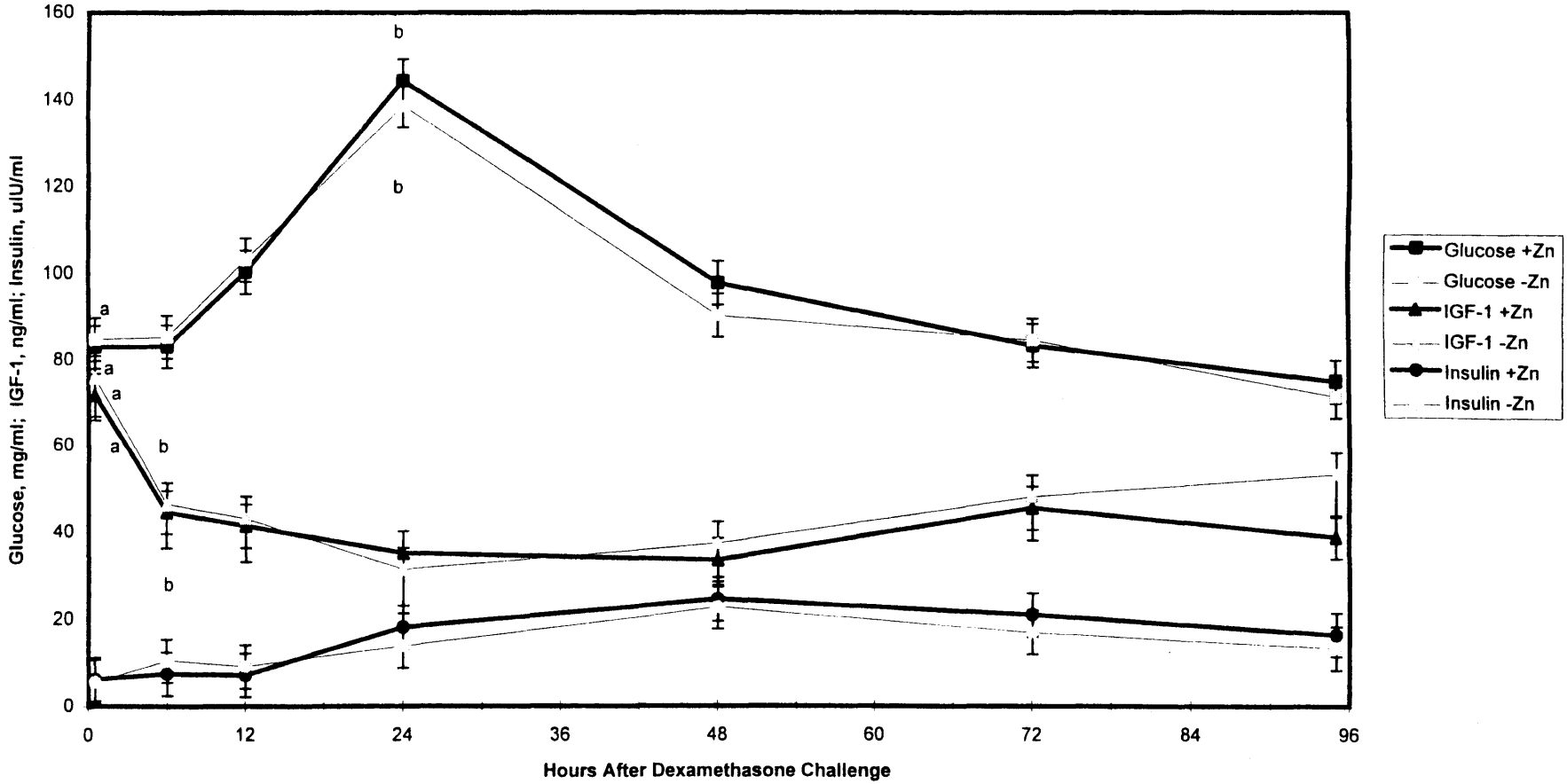

Glucose, mg/ml; IGF-1, ng/ml; Insulin, uIU/ml
Hours After Dexamethasone Challenge
Glucose +Zn
Glucose -Zn
IGF-1 +Zn
IGF-1 -Zn
Insulin +Zn
Insulin -Zn
0
20
40
60
80
100
120
140
160
0
12
24
36
48
60
72
84
96
a
b

Figure 6. Mean skin swelling response to PHA from heifers fed during the zinc depletion phase. The +Zn calves received the control diet which contained 40 mg Zn/kg (23 mg Zn/kg from $ZnSO_4$). The -Zn calves received no supplemental Zn (total Zn, 17 mg Zn/kg). Skin swelling response was measured as a change in mm from skin thickness prior to the subdermal injection of .75 ug of PHA in 0.1 ml of physiological saline at two separate sites in the neck. Measurements were taken at 8, 24, and 48 hours post-injection. The injection was given 30 d after initiation of the -Zn diet. At 8 h post injection, +Zn calves had greater swelling a,b ($P<.05$) than the -Zn group.

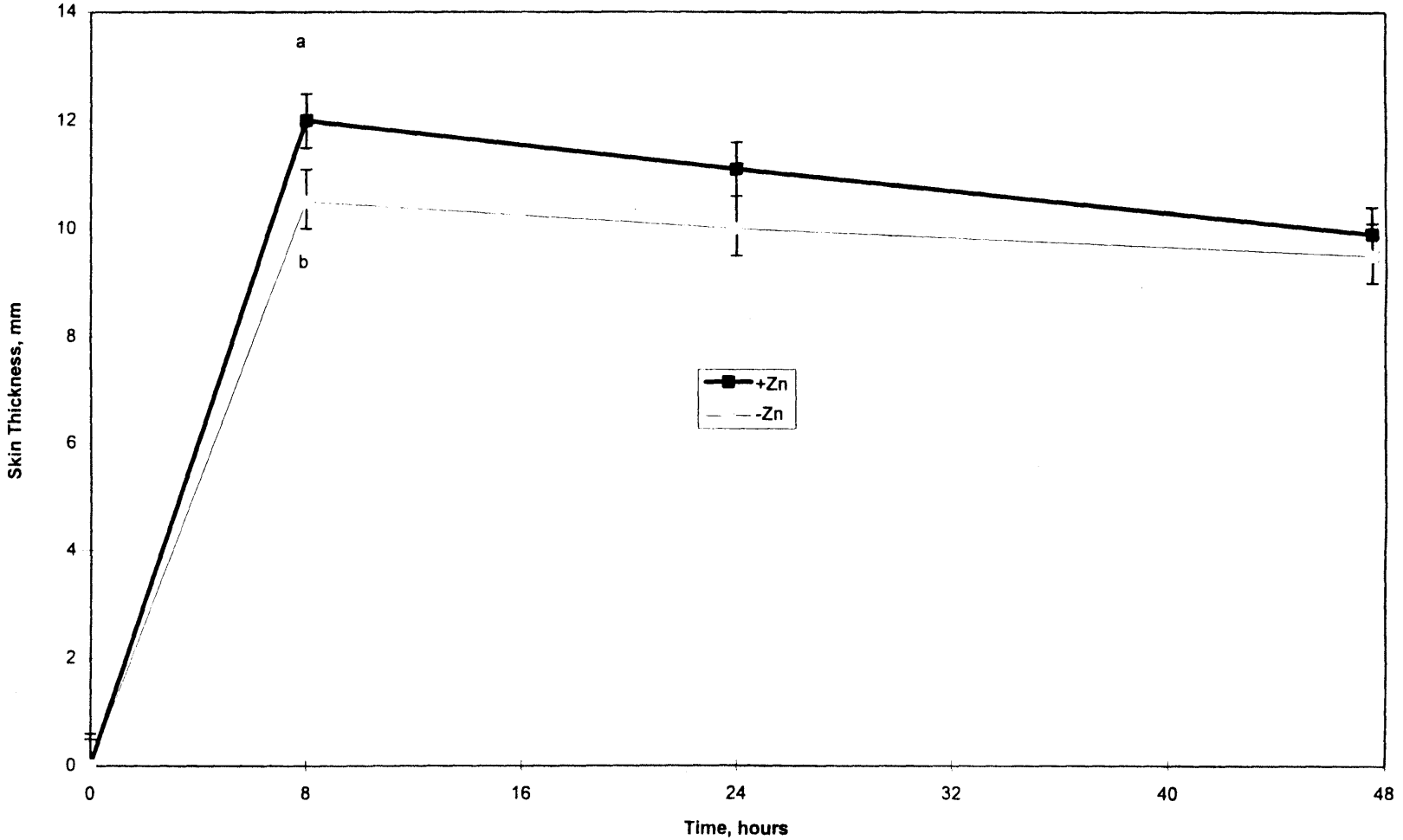

14
12
10
8
6
4
2
0
a
b
Skin Thickness, mm
+Zn
-Zn
0
8
16
24
32
40
48
Time, hours

Figure 7. Mean skin swelling response to PHA from calves fed during the zinc depletion phase. The +Zn calves received the control diet which contained 40 mg Zn/kg (23 mg Zn/kg from $ZnSO_4$). The -Zn calves received no supplemental Zn (total Zn, 17 mg Zn/kg). Skin swelling response was measured as a change in mm from skin thickness prior to the subdermal injection of .75 ug of PHA in 0.1 ml of physiological saline at two separate sites in the neck. Measurements were taken at 8, 12, 24, and 48 hours post-injection. The injection was given 21 d after initiation of the -Zn diet. At 8 and 12 h post injection, +Zn calves had greater swelling a,b ($P<.05$) than the -Zn groups.

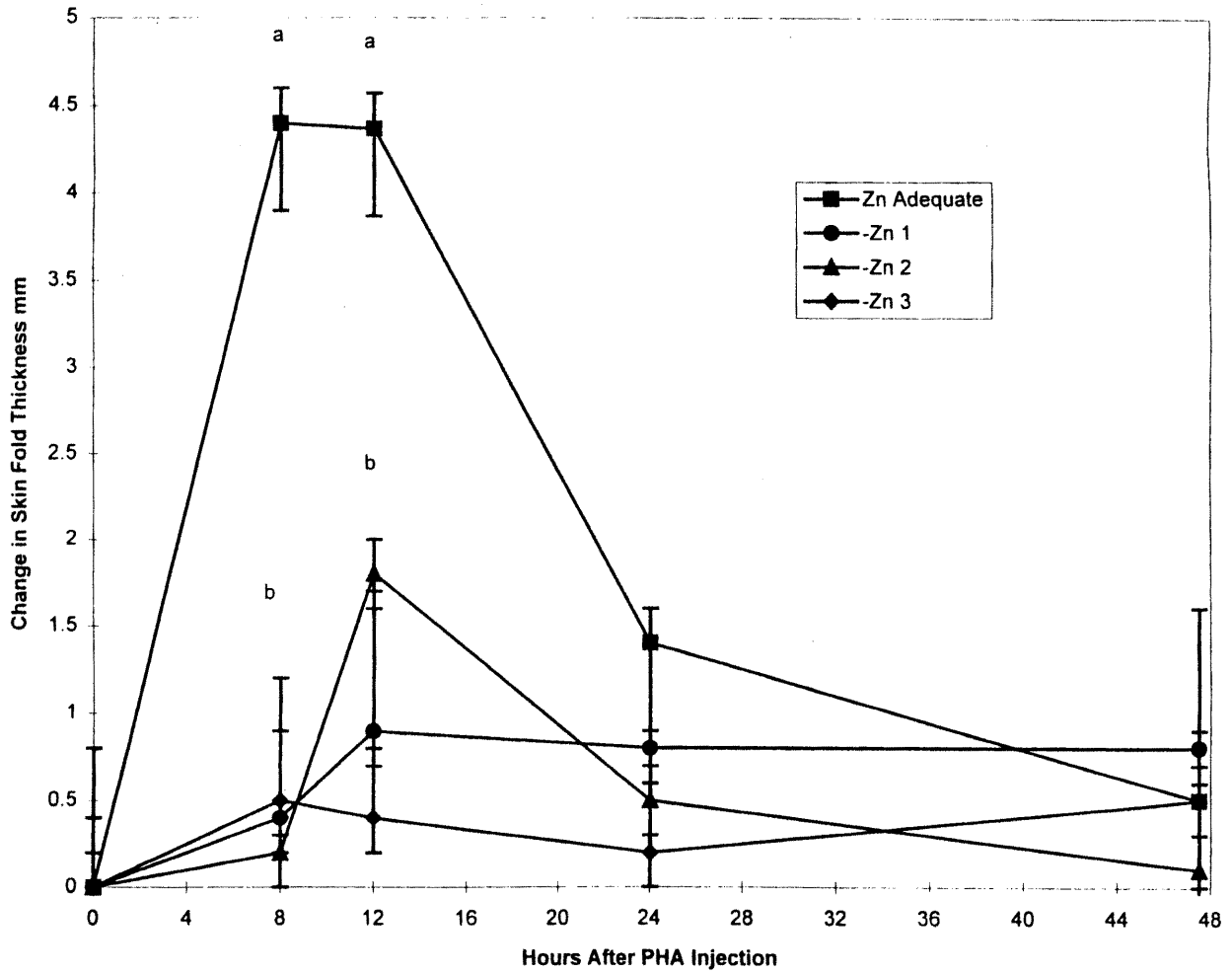
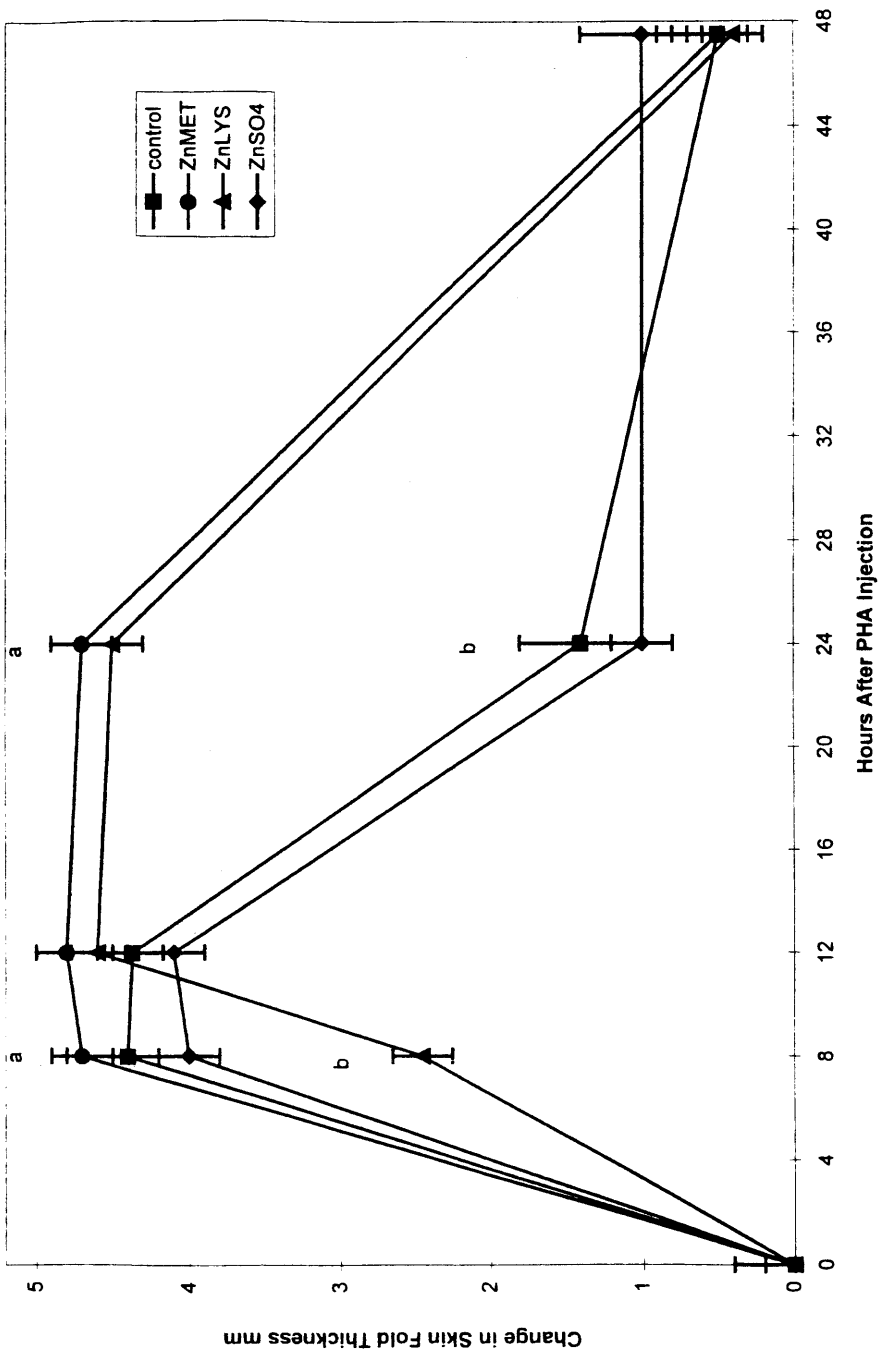

Change in Skin Fold Thickness mm
Hours After PHA Injection
Zn Adequate
-Zn 1
-Zn 2
-Zn 3
a
a
b
b
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
0
4
8
12
16
20
24
28
32
36
40
44
48

Figure 8. Mean skin swelling response to PHA from calves fed during the Zn depletion phase. The +Zn calves received the control diet which contained 40 mg Zn/kg (23 mg Zn/kg from $ZnSO_4$). The -Zn calves received 23 mg Zn/kg from ZnMet, ZnLys, or $ZnSO_4$ (total Zn, 40 mg Zn/kg). Skin swelling response was measured as a change in mm from skin thickness prior to the subdermal injection of .75 ug of PHA in 0.1 ml of physiological saline at two separate sites in the neck. Measurements were taken at 8, 12, 24, and 48 hours post-injection. The injection was given 14 d after initiation of the Zn replete diets. At 24 h post injection, ZnMet and ZnLys calves had a longer and greater swelling response a,b ($P<.05$) than the $ZnSO_4$ and control groups.


control
ZnMET
ZnLYS
ZnSO4
a
b
a
b
0
1
2
3
4
5
0
4
8
12
16
20
24
28
32
36
40
44
48
Change in Skin Fold Thickness mm
Hours After PHA Injection

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