Marginal Zinc Deficiency Affects Biochemical and Physiological Parameters in Beef Heifer Calves[1]

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ABSTRACT : A study determined whether certain biochemical and physiological variables were altered during marginal Zn deficiency. 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 diet DM, and five calves were fed a Zn-adequate ($+Zn$) diet with 23 mg Zn/kg diet DM from $ZnSO_4$ added to the $-Zn$ diet (total diet, 40 mg Zn/kg diet DM), 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 the $-Zn$ calves. Blood urea nitrogen, glucose, insulin, IGF-I, Cu plasma concent-ration and Zn and Cu concentrations of red blood cell (RBC) and liver 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 d 25, 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 after injection. In response to an intradermal injection of phytohemagglutinin on d 30, cell mediated immune (CMI) response was reduced ($p < .05$) in the $-$ Zn calves. These observations indicate that during a marginal Zn deficiency in calves, there was a decrease in feed efficiency, plasma Zn, serum alkaline phosphatase, and CMI response.

(Key Words: Zinc, Cattle, Feed Efficiency, Immune Response, Insulin-Like Growth Factor I, Dexamethasone)

INTRODUCTION

Several biochemical and physiological changes have been reported in Zn deficient ($-$ Zn) animals (Hambidge et al., 1986), including an early decrease in serum alkaline phosphatase, then a loss of appetite, followed by poor growth and reduced feed efficiency. After prolonged Zn deficiency, serum and red blood cell (RBC) Zn decreases. Reductions of hepatic Zn stores in ruminants, have also been reported after prolonged Zn deficiency (Kaneko, 1989). Interactions among Zn and Cu have been described in animal and human studies (Mills, 1985). Many aspects of immunity are impaired by Zn deficiency including lymphocyte blastogenesis in response to phytohemag-glutinin (PHA), a measure of cell mediated immunity (Droke and Spears, 1993). Pancreatic and plasma insulin concentrations in nonruminants are also reduced by Zn deficiency (McDowell, 1992).

Zinc deficiency has been shown to reduce growth, by reducing serum growth hormone (GH) concentrations in steers (Wheaton et al., 1986). In cattle, insulin-like growth factor-I (IGF-I) serum concentrations parallel changes in GH and are relatively constant (Ellenberger et al., 1989). Insulin-like growth factor serum concentrations have been shown to decrease during Zn deficiency (Oner et al., 1984). The purpose of this study was to determine biochemical and physiological changes during marginal Zn deficiency with the goal of identifying an early measurable marker of Zn deficiency.

MATERIALS AND METHODS

Adaptation and nutrient repletion phase

Ten weaned crossbred Hereford Angus heifer calves weighing 163 ± 2 kg were transported to the Department of Animal Sciences feedlot at Fort Collins. The calves were placed in individual pens (1.8 m × 10 m) and for 28

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d, given ad libitum access to the control diet of brome grass hay, alfalfa hay, and a corn based supplement that supplied 23 mg of supplemental Zn/kg diet DM 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 diet DM). This enabled the calves to become repleted with nutrients if previously insufficient. The supplement was top dressed immediately after the diet was put in the bunk. Each day the cattle were monitored and they were found to consume the supplement prior to the roughage portion of the diet. Throughout the trial, no supplement was recovered upon determination of daily feed intake. Dietary CP, Ca, P, Cu, and Zn were analyzed by Weld Laboratories (Greeley, CO). Crude protein content was determined using the Kjeldahl nitrogen determination method, Ca was determined using the dry ash method, and P was determined using the photometric method as described in the AOAC Official Methods of Analysis (Helrich, 1990; procedures 976.05, 927.02, and 965.17 respectively). Copper and Zn were analyzed using flame absorption spectrometry (Varian Model 1275). Plasma

Table 1. Composition of control diet

Ingredient	% DM basis
Brome Grass Hay	54.0
Alfalfa	39.0
Supplement	
Ground corn	5.0
Trace minerals[a,b]	1.0
Soybean meal	.33
Salt (iodized)	.25
Urea	.22
Vitamins and Rumensin[c]	.20
Analyzed Composition, (DM[d])	
Crude Protein (%)	10.8
Calcium (%)	.40
Phosphorus (%)	.24
Zinc (mg/kg)	40.0
Calculated Content	
Dry Matter (%)	89.0
NEm (Mcal/kg)	1.6

[a] Trace minerals added per kilogram of diet: $CuSO_4$ $5H_2O$, 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).
[c] Vitamins provided per kilogram of diet: vitamin A, 3,000 IU; rumensin, .5 mg; and vitamin E, 500 IU/ha/d.
[d] Analyzed composition of the total diet.

was diluted 1:3 (vol/vol) with deionized water for Cu and 1:5 (vol/vol) for Zn. During the first 28 d the calves were adapted to the individual feeding pens, diet, and gentled by handling, haltering and grooming several times a day, to reduce the stress of jugular bleeding while calves were haltered and tied. Individual feed consumption was determined daily and the calves were weighed weekly before feeding. On d 25, 26, 27, and 28 of the repletion phase, the calves were bled prior to feeding.

Protocol for this research was approved by the Colorado State University Animal Care and Use Committee.

Zinc depletion phase

After the 28 d adaptation and nutrient repletion phase, the calves were weighed prior to feeding on two consecutive days and were paired by feed intake per body weight[75]. On d 0, one calf from each pair was fed the control diet in which the $ZnSO_4$ was omitted to give a total dietary Zn content of 17 mg Zn/kg diet DM, and the other calves were fed the control diet. The calves were weighed and bled, prior to feeding, on 3, 7, 19, 14, 17, 21, and 24 d. On d 24, liver biopsies were taken. On d 25, each calf was bled and then given i. m. 20 mg (Booth and McDonald, 1982) of aqueous dexamethasone (Anthony Products Arcadia, CA), to determine the effect of a synthetic glucocorticoid (dexamethasone) on serum glucose, alkaline phosphatase, insulin, and IGF-I in Zn adequate and 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, hair on the right side of the neck was clipped, and .75 µg of phytohemagglutinin (PHA; Sigma Chemical, St. Louis, MO) in .1 ml of physiological saline was injected intradermally at two separate sites (Fritz et al., 1990). Inflammatory respones was measured in mm as a change from the heifers skin thickness prior to injection with PHA, and after 8, 24 and 48 h, using skin-fold calipers (Slim Guide, Creative Health Products, Plymouth, Michigan) (Fritz et al., 1990).

Tissue sampling

Blood samples were collected by jugular venipuncture using an 18 gauge needle. Samples were collected into three tubes; one 10-ml heparinized tube, one 10-ml non heparinized tube, and one 7-ml trace mineral-free tube containing heparin (Becton Dickinson Vacutainer Systems,

Becton Dickinson and Company, Franklin Lakes, NJ 07417-1885). Blood was immediately chilled in ice, returned to the laboratory, centrifuged at 1,000 × g for 15 min and plasma or serum harvested and frozen at −20°C until analyzed. Red blood cells were washed three times with .9% saline solution and centrifuged at 1,000 × g for 15 min. 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 Vadco, 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 × 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 −20°C.

Tissue analysis

Liver, plasma, and RBC Zn and Cu conctrations were determined using flame atomic absorption spectrometry (Varian Model 1,275) in the Diagnostic Laboratory (Dept. of Pathology, Colorado State Univ., Fort Collins). Liver samples were dried in a 60°C drying oven for 24 h to determine DM. The tissue samples were then wet-ashed in .5 ml of 7N nitric acid in acid washed centrifuge tubes. The tubes were then placed in a 50°C water bath for 12 h, and analyzed directly for Zn and Cu content. Plasma was diluted 1:5 (vol/vol) with deionized water for Zn and 1:3 (vol/vol) for Cu. RBC were diluted 1:10 (vol/vol) with deionized water for Zn and 1:3 (vol/vol) for Cu.

Serum IGF-I 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 Kiks No. 245-20, 245-20, and 640-20, respectively, from Sigma Chemical (St. Louis, MO).

Statistical analysis

Repeated measures ANOVA were performed 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. The model, where appropriate, contained treatment, calf within treatment, period, time, and the treatment by time interaction. Treatment least squares means were tested by a proteceed F test, with significance determined at (p < .05).

RESULTS AND DISCUSSION

At the end of 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 had a steady decline in feed efficiency, beginning approximately 17 d after feeding the − Zn diet, and reaching a minimum (p < .05) feed efficiency at 21 d (figure 1). At which time, feed efficiency was reduced by 50% and remained constant through d 24. Based on the 50% reduction in feed efficiency in the − Zn calves at d 21, 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 measure 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 than the control group which received no supplemental Zn.

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

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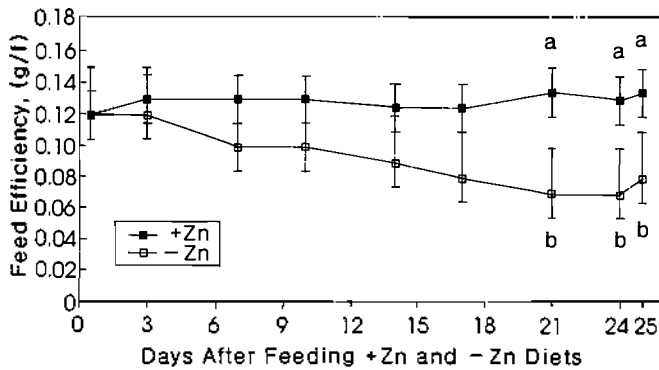
Figure 1. Mean feed efficiencies (gain/feed) for each treatment during the zinc depletion phase. The +Zn calves received the control diet which contained 40 mg Zn/kg (23 mg Zn/kg from $ZnSO_4$). The − Zn calves received no supplemental Zn (total Zn, 17 mg Zn/kg). a,b (p < .05).

Serum alkaline phosphatase was reduced (p < .05) in the − Zn group on d 25 of the deficiency period (figure 2a). This reduction in serum alkaline phosphatase from Zn deficiency supports previous studies (Hambidge et al., 1986). However, a decrease in serum alkaline phosphatase activity was not observed until after the heifers were deemed − Zn at d 21 when a decrease in their feed efficiencies was observed (figure 2a).
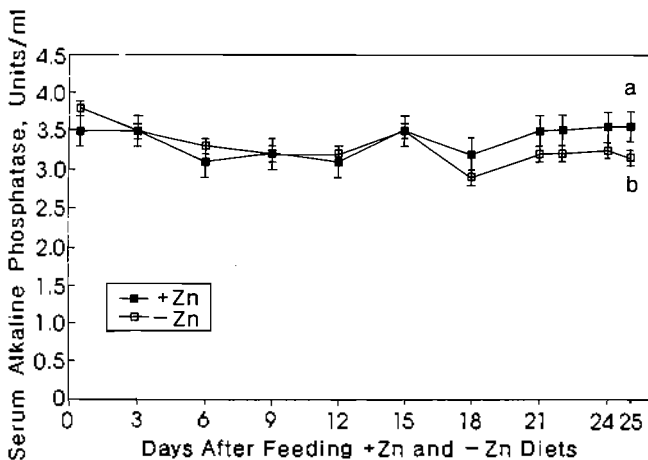
Figure 2a. Mean alkaline phosphatase activity in serum from heifers during the zinc depletion phase. The +Zn calves received the control diet which contained 40 mg Zn/kg (23 mg Zn/kg from $ZnSO_4$). The − Zn calves received no supplemental Zn (total Zn, 17 mg Zn/kg). a,b (p < .05).

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 2b).
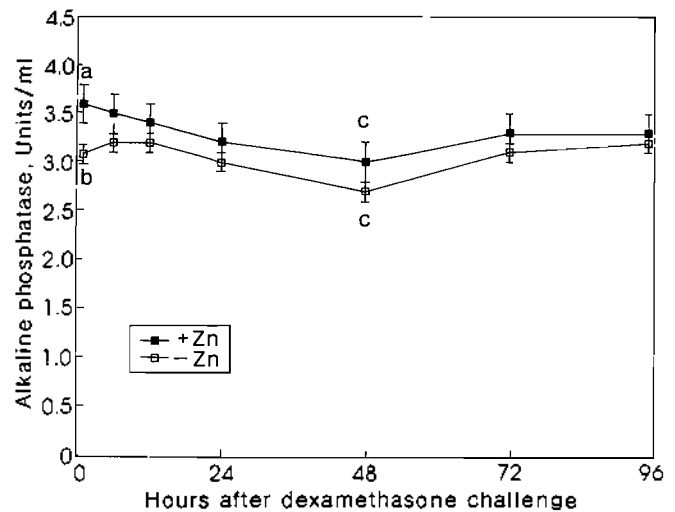
Figure 2b. Mean serum alkaline phosphatase concentrations at 6, 12, 24, 48, 72 and 96 h, after i. m. administration of 20 mg of aqueous dexamethasone, beginning on 25 d of the depletion phase. The +Zn calves received the control diet which contained 40 mg Zn/kg diet (23 mg Zn /kg from $ZnSO_4$). The − 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.

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). However, a reduction (p < .05) was observed in both +Zn and − Zn calves. This reduction may have been due to a down regulation of osteoblast and liver metallothionein production. A decrease in serum alkaline phosphatase due to the administration of dexamethasone, to our knowledge, has never been reported in cattle.

Serum IGF-I levels in calves were not altered during a marginal Zn deficiency through d 25 (figure 3a). McNall et al. (1995) showed IGF-I serum concentrations to decreased 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 marginal deficient ruminants, the studies are not directly comparable.

Dexamethasone administration on d 25, induced a rapid reduction of IGF-I levels during the first 6 h by 35 to 40% (p < .05 vs. 0 time) in both +Zn and − Zn groups (figure 3b). IGF-I levels remained depressed for 1

to 2 d 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-I in heifer calves, in contrast to other species such as humans where dexamethasone treatment has been shown to increase 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 3a) through d 25. However, 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 Zn 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 begin 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, nitrogen and glucose levels.
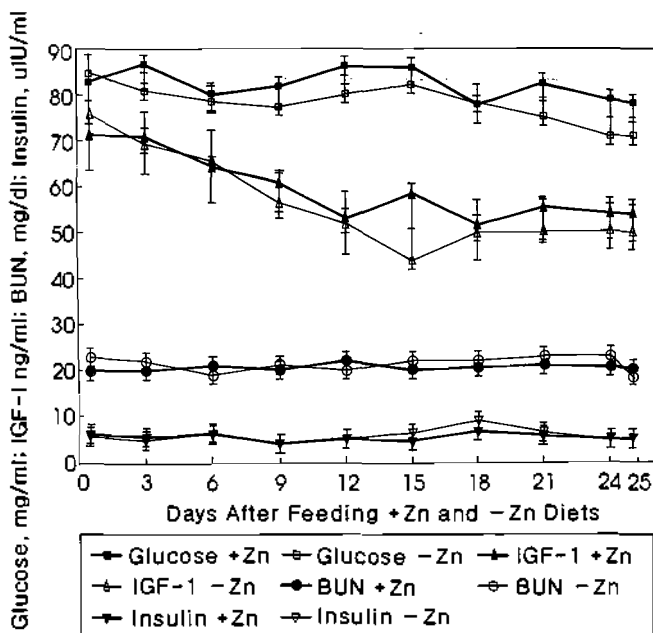
Figure 3a. Mean serum glucose, IGF-I, urea nitrogen, and insulin concentrations from heifers fed during the zinc depletion phase. The +Zn calves received the control diet which contained 40 mg Zn/kg diet (23 mg Zn/kg from ZnSO₄). The – Zn calves received no supplemental Zn (total Zn, 17 mg Zn/kg). Treatment means did not differ (p>.05) at each measurement from 0-25 d for any of the parameters measured.

Insulin, IGF-I and glucose serum concentrations were not different between the +Zn and – Zn treatments post-

dexamethasone injection (figure 3b). However, serum concentrations of glucose, approximately 24 h post-dexamethasone, increased rapidly (p < .05), whereas insulin levels 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. Furthermore, adrenal glucocorticoids are released in response to stress and act to mobilize energy stores, such as glycogen (Kaneko, 1989). As there were no differences (p > .05) between treatments in response to dexamethasone, a marginal Zn deficiency did not alter insulin, IGF-I and glucose serum concentrations.
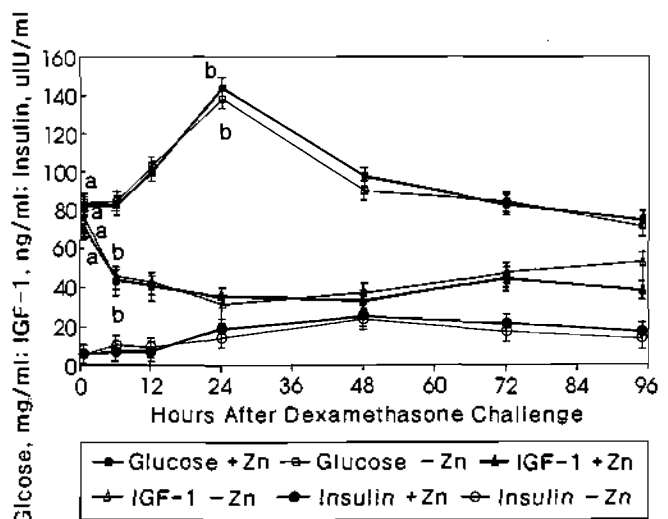
Figure 3b. Mean serum IGF-I, glucose, and insulin concentrations at 6, 12, 24, 48, 72, and 96 h, after administration of i. m. 20 mg of aqueous dexamethasone, beginning on 25 d of the depletion phase. The +Zn calves received the control diect which contained 40 mg Zn/kg diet (23 mg Zn/kg from ZnSO₄). 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.

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. Previous studies (Graham, 1991) have determined that the normal range of plasma Zn for cattle is .8 mg/l to 1.4 mg/l and that marginal levels of plasma Zn range between .4 and .8 mg/ l. Because the – Zn calves in the present experiment had a mean plasma Zn of .75 ± .11 mg/l and a reduced feed efficiency without a reduction in feed intake, we

concluded that these calves were marginally Zn deficient. 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).

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

Item	+Zn	− Zn	SEM
Plasma Zn (mg/l)	1.05[a]	.75[b]	.11
Plasma Cu (mg/l)	.88[c]	.80[c]	.09
RBC Zn (mg/l[e])	7.7[c]	4.2[d]	1.8
RBC Cu (mg/l[e])	.64[a]	.61[a]	.30
Liver Zn (mg/kg DM)	120.0[a]	113.0[a]	3.6
Liver Cu (mg/kg DM)	240.0[a]	231.0[a]	6.9

[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/l packed red blood cells.

After a single dexamethasone injection on d 25 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. Mean plasma Zn and Cu concentrations (mg/l) through 96 h for the +Zn calves were .99 ± .12 and .89 ± .10, and for the − Zn calves were .92 ± .10 and .87 ± .09, respectively. Mean RBC Zn and Cu concentrations (mg/packed l) through 96 h for the +Zn calves were 8.0 ± .3, .7 ± .05 and for the − Zn calves were 7.0 ± .3, .7 ± .4 respectively. Weeks et al. (1989) observed that cattle injected with dexamethasone had decreased serum Zn concentrations, and Cousins, (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, possibly reflecting the marginal Zn deficiency induced in the calves.

The marginally Zn deficient calves had a lower CMI response (p < .05) at 8 h post-injection than the controls (figure 4). 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, natural killer cell function, antibody dependent cell-mediated cytotoxicity, and neutrophil function (Hambidge et al., 1986). An impairment in cell-mediated immunity has not been previously reported in calves marginally deficient in Zn. The depressed cell-mediated immunity is similar to that

reported by Droke and Spears (1993) in severely Zn deficient lambs.

In summary, a marginal Zn deficiency in beef heifer calves resulted in a decreased feed efficiency, plasma alkaline phosphatase, CMI response, and plasma Zn concentration.
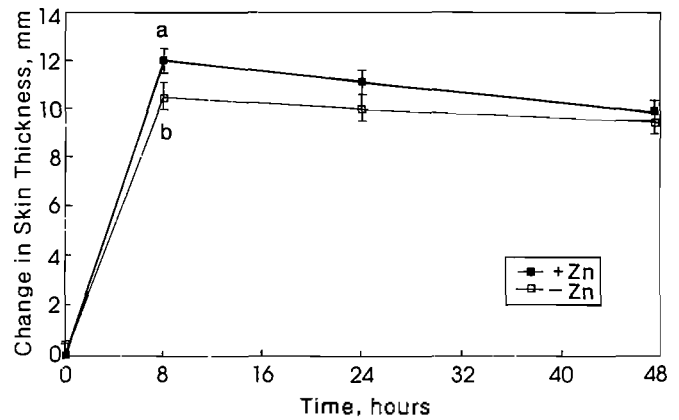
Figure 4. Mean skin swelling response to PHA from heifers fed during the zinc depletion phase. The +Zn calves received the control diet which contained 40 mg Zn/kg (23 mg Zn/kg from $ZnSO_4$). The − Zn calves received no supplemental Zn (total Zn, 17 mg Zn/kg). Skin swelling response was measured as a change in mm from skin thichkess 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.

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