J. Dairy Sci. 91:1985–1995
doi:10.3168/jds.2007-0779

Effect of Trace Mineral Source on Lactation Performance, Claw Integrity, and Fertility of Dairy Cattle

J. L. Siciliano-Jones,* M. T. Socha,†[1] D. J. Tomlinson,† and J. M. DeFrain†
*FARME Institute, Homer, NY 13077
†Zinpro Corporation, Eden Prairie, MN 55344

ABSTRACT

Two hundred fifty multiparous and primiparous cows were assigned to a study at approximately 70 d prepartum to determine the effect of trace mineral source on lactation performance, claw integrity, and fertility. Cows received treatments from 3 wk prepartum through wk 35 postpartum. Treatments consisted of 1) all supplemental Zn, Mn, Cu, and Co provided in sulfate form (Sulfate) and 2) 360 mg of Zn, 200 mg of Mn, 125 mg of Cu, and 12 mg of Co supplied daily by Sulfate minerals replaced with similar amounts of minerals supplied by Availa-4 (CTM). Individuals involved with daily animal care or data recording, or both, were blinded to treatment assignments. Cows from all treatments were housed in common pens, and treatments were dispensed to cows via a computerized feeder. All claws of cows were examined before treatment administration and at 16 and 36 wk postpartum by personnel trained in identifying claw lesions. Cows fed the CTM diet tended to produce more milk and energy-corrected milk than cows fed the Sulfate diet. Cows fed the CTM diet also produced more milk protein and solids (fat + protein) than cows fed the Sulfate diet. Replacing Sulfate minerals with those supplied by CTM decreased incidence of sole ulcers at wk 36 postpartum and tended to decrease incidence of interdigital dermatitis at wk 16 and 36 postpartum. Severity of heel erosion tended to be less for cows fed CTM than cows receiving the Sulfate diet. Despite first service conception rates tending to be greater for cows fed the Sulfate diet, there was no effect of treatment on rate of conception. A greater percentage of cows fed the Sulfate diet tended to be culled from the herd before wk 36 postpartum than cows fed the CTM diet. Replacing Sulfate minerals with CTM resulted in improved lactation performance and claw integrity.

Key words: trace mineral, dairy cattle, claw lesion

INTRODUCTION

Trace minerals such as Zn, Mn, Cu, and Co have important roles in protein synthesis, vitamin metabolism, formation of connective tissue, and immune function (Miller et al., 1988; Cousins, 1996). The supply of these trace minerals affects several aspects of cattle performance and health, such as claw integrity, fertility, lactation, and immune function (Miller et al., 1988; Smart and Cymbaluk, 1997; NRC, 2001).

Amino acid complexes of trace minerals are more bioavailable (Wedekind et al., 1992; Paripatananont and Lovell, 1995) and are better retained by the body (Nockels et al., 1993) than inorganic sources of trace minerals. Clinical responses to improved bioavailability and retention of Zn, Mn, and Cu AA complexes have been demonstrated in numerous studies. For instance, in 12 studies, feeding cattle specific AA complexes of Zn reduced SCC (Kellogg et al., 2004), increased milk production (Kellogg et al., 2004), and improved claw integrity (Moore et al., 1989). Further improvements in milk production or reproduction, or both, have been demonstrated when Co glucoheptonate and AA complexes of Zn, Mn, and Cu (**OTM**) were added to diets of dairy cattle (Campbell et al., 1999; Kellogg et al., 2003; Griffiths et al., 2007) and when OTM replaced inorganic forms of these trace minerals (Uchida et al., 2001; Ballantine et al., 2002; Kellogg et al., 2003; Ferguson et al., 2004a; Kincaid and Socha, 2004; Kinal et al., 2005; Nocek et al., 2006).

The effect of OTM on claw integrity has been examined to a lesser extent. Although research has shown that adding OTM to diets of dairy cattle improves claw integrity (Nocek et al., 2000; Drendel et al., 2005), improvements in claw integrity when OTM replaced sulfate trace minerals have not been as consistent, with one study showing improvement (Ballantine et al., 2002) and another study showing no improvements (Nocek et al., 2006). Thus, the first objective of this study was to further investigate the effect of replacing sulfate trace minerals with OTM on claw integrity of dairy cattle. The second objective of this study was to determine the effect of replacing sulfate trace minerals with

Received October 15, 2007.
Accepted January 22, 2008.
[1]Corresponding author: msocha@zinpro.com

Table 1. Ingredient composition of pelleted concentrate fed to cows at the rate of 1.5 kg/d

Ingredient, % of concentrate	Sulfate	CTM[1]
Dried distillers grains	30.5	31.6
Corn grain, finely ground	30.6	31.6
Soybean meal, dehulled	30.6	31.6
Molasses, liquid	4.6	4.7
Trace mineral premix	3.7	0.5

[1]Daily, 360 mg of Zn, 200 mg of Mn, 125 mg of Cu, and 12 mg of Co were supplied by Availa-4 (Zinpro, Eden Prairie, MN).

OTM on lactation performance and fertility of dairy cattle.

MATERIALS AND METHODS

The experimental protocol was approved by the Institutional Committee for Animal Care of FARME Institute (Homer, NY). Two hundred fifty multiparous and primiparous cows were assigned to the study at approximately 70 d before parturition. Cows were blocked by expected calving date, lactation number, and 305-d mature equivalent milk yield (if lactating) and began receiving treatments approximately 3 wk before expected calving. Treatments consisted of 1) all supplemental Zn, Mn, Cu, and Co provided in sulfate form (Sulfate) and 2) diet similar to Sulfate with the exception that 360 mg of Zn, 200 mg of Mn, 125 mg of Cu, and 12 mg of Co supplied by sulfate minerals were replaced with a similar amount of trace minerals supplied by Availa-4 (Zinpro Corporation, Eden Prairie, MN; CTM). Availa-4 supplied approximately 21, 14, 37, and 100% of the supplemental Zn, Mn, Cu, and Co, respectively, whereas sulfate minerals supplied the rest of the supplemental Zn, Mn, and Cu in the CTM treatment. Cows remained on the study through wk 35 postpartum. The study was conducted blindly in that individuals involved with daily animal care or data recording, or both, were not aware of treatment assignments.

Primiparous and multiparous cows were commingled in the prefresh and postfresh pens. At 30 to 40 DIM, primiparous cows were separated from multiparous cows and housed in separate pens. All treatment groups were housed in common pens, thus eliminating any potential pen effect. Pens contained free stalls that were covered with a rubber-filled mattress. Approximately 2 cm of sawdust was applied to the top of the mattresses.

Treatments were blended into a pelleted concentrate at a commercial feed mill (Round House Mill, Cortland, NY; Table 1). The pelleted concentrate was formulated such that 1.5 kg of concentrate provided 360 mg of Zn, 200 mg of Mn, 125 mg of Cu, and 12 mg of Co from either Sulfate or CTM. The Availa-4 added to the CTM pelleted concentrate contained a tracer (Microtracer, MicroTracers Inc., San Francisco, CA), whereas the sulfate source added to the Sulfate pelleted concentrate did not contain a tracer. Samples of each concentrate batch were collected and analyzed for tracer by MicroTracers Inc. to ensure that the correct trace mineral source was added to the correct pelleted concentrate.

Approximately 1.5 kg of pelleted concentrate containing the treatments was delivered to all cows via computerized feeders (DeLaval feeding station standard, DeLaval, Tumba, Sweden). The computerized feeder identified cows, via a neck transponder, as they entered the feeding station and metered out the allotted amount of pelleted concentrate for the corresponding treatment. The computerized feeder tracked the amount of concentrate consumed by cows, and any cows not consuming the daily allotment of the pelleted concentrate were removed from the study. The pelleted concentrate was metered out at a rate such that if a cow was removed from the feeder by another cow before completion of its meal, only a small residual of the pelleted concentrate remained. Gates were added to the feeding station to minimize the number of times cows were dislodged from the feeder. Due to cows from the different treatments being housed in the same pen, feeders were monitored to ensure that little or no residual feed remained after cows left the feeder. Feeders were calibrated after delivery of each batch of pelleted concentrate.

Other than the pelleted concentrate delivered to cows via computerized feeders, all other dietary components were identical between treatments with cows receiving a common TMR. The basal diet was formulated to meet the nutrient requirements of cows (i.e., prefresh, postfresh, primiparous, multiparous), based upon DM intakes, weekly forage analyses, and consumption of the pelleted concentrate (Tables 2 and 3).

Feed intakes were monitored daily. Forages were sampled weekly, composited monthly by forage source, and analyzed for nutrient content (Cumberland Valley Analytical Services, Hagerstown, MD). Near-infrared was used to determine major nutrients, and wet chemistry was used to determine mineral content. Mineral content of forage samples, with the exception of sulfur and chloride, were determined using AOAC method 985.01 (AOAC, 2000), modified to ash 0.5 g of sample for 2 h at 535°C. The ashed sample was then digested in open crucibles for 20 min with 15% nitric acid. Samples were then diluted to 50 mL and analyzed by inductively coupled plasma mass spectrometry (Perkin Elmer 3300 XL ICP or 5300DV ICP, Perkin Elmer, Shelton, CT). Sulfur content of samples was determined using a Leco S-144DR Sulfur Combustion Analyzer with the use of tungsten oxide as a combustion aid (Leco, St. Joseph, MI). For chloride analysis, the sample was extracted

Table 2. Ingredient composition of diets

Ingredient, % of DM	Diet[1]		
	Prefresh	Postfresh	Lactation
Corn silage	57.08	34.25	32.29
Alfalfa silage	0.00	18.90	18.98
Straw	15.73	2.03	1.77
Corn grain, ground	0.00	16.77	18.47
Soybean meal, dehulled	12.95	6.61	7.25
Cottonseed, whole, fuzzy	3.29	7.79	7.66
Soybean hulls	6.72	0.00	0.00
Corn gluten feed	0.00	5.95	5.17
ProvAAI[2]	0.00	2.10	1.80
Beet pulp	0.00	0.00	1.85
Bypass fat[3]	0.00	1.17	0.92
Bypass choline[4]	0.91	0.00	0.00
Calcium carbonate	0.87	1.20	1.05
Sodium bicarbonate	0.00	0.79	0.69
Magnesium sulfate	0.40	0.12	0.10
Magnesium oxide	0.27	0.16	0.14
Liquid molasses	0.50	0.59	0.52
Urea	0.00	0.50	0.43
Salt	0.10	0.36	0.31
Yeast culture[5]	0.40	0.24	0.21
Vitamins and minerals[6]	0.76	0.46	0.42

[1]Sulfate and CTM diets were comprised of similar ingredients and differed only in trace mineral source. Prefresh diets were fed from 3 wk prepartum through parturition, postfresh diets were fed from parturition through 30 to 40 d postpartum, and lactation diets were fed from 30 to 40 d postpartum through wk 35 postpartum.

[2]Venture Milling, Fulton, NY.

[3]Megalac, Church and Dwight Animal Nutrition, Princeton, NJ.

[4]Reashure, Balchem Corporation, New Hampton, NY.

[5]Diamond V XP Yeast Culture, Diamond V Mills Inc., Cedar Rapids, IA.

[6]Prefresh diet contained Rumensin (Elanco Animal Health, Greenfield, IN), and postfresh and lactation diets contained Omnigen AF (Prince AgriProducts Inc., Quincy, IL).

Table 3. Chemical composition of diets

Chemical component, DM basis	Diet[1]		
	Prefresh	Postfresh	Lactation
CP, %	15.0	18.0	17.8
RUP, % CP	34.3	36.9	37.0
ADF, %	26.4	19.5	19.1
Forage NDF, %	35.1	22.9	22.3
Ether extract, %	2.9	5.2	5.1
Starch, %	17.6	24.1	25.1
Sugar, %	—	3.3	2.7
NFC, %	32.7	38.1	39.2
Ca, %	0.77	1.08	0.96
P, %	0.31	0.34	0.34
Mg, %	0.41	0.34	0.32
K, %	1.20	1.22	1.20
S, %	0.24	0.23	0.23
Na, %	0.08	0.43	0.37
Cl, %	0.42	0.41	0.38
Fe, mg/kg	232	233	211
Mn, mg/kg	61	96	86
Zn, mg/kg	105	112	102
Cu, mg/kg	24.0	23.4	21.4
Co, mg/kg	1.1	0.5	0.5
Se, mg/kg	0.64	0.36	0.31
I, mg/kg	2.0	2.8	2.4
Vitamin A, kIU/kg	24.1	11.9	10.3
Vitamin D, kIU/kg	1.8	2.2	1.9
Vitamin E, IU/kg	71	40	33

[1]Sulfate and CTM diets were similar in nutrient content and differed only in trace mineral source. Prefresh diets were fed from 3 wk prepartum through parturition, postfresh diets were fed from parturition through 30 to 40 d postpartum, and lactation diets were fed from 30 to 40 d postpartum through wk 35 postpartum.

with 1% nitric acid, and chloride content was determined using a Corning 925 Chloride Analyzer (Corning Inc., Corning, NY).

Cows were milked thrice daily, and milk yield was recorded at each milking. Milk was sampled twice monthly, and fat, protein, SCC, and MUN were determined (DairyOne, Ithaca, NY) according to approved procedures of AOAC (2000). Recombinant bovine somatotropin (Posilac, Protiva Inc., St. Louis, MO) was administered to cows beginning at d 63 postpartum with reinjection occurring every 14 d thereafter.

Reproduction

At 24 to 30 d postpartum, all cows were given a 5-cc intramuscular injection of $PGF_{2\alpha}$ (Lutalyse, Pfizer, New York, NY). Fourteen days later, all cows were given a second $PGF_{2\alpha}$ injection. Cow activity was measured electronically and used for estrus detection (ALPRO activity meter, DeLaval). Cows were deemed in estrus if activity increased at least 50% in a 1-h time period. Cows deemed in estrus after 45 DIM were artificially inseminated following the a.m.–p.m. rule.

Any cow that did not elicit estrus by 74 d postpartum entered a timed breeding program. A 2-cc intramuscular injection of GnRH (Cystorelin, Merial, Duluth, GA) was given at 74 d postpartum. Seven days later, cows received a 5-cc intramuscular injection of $PGF_{2\alpha}$. Forty-eight hours later, cows received a second 2-cc intramuscular injection of GnRH. Cows were artificially inseminated 12 h after the last GnRH injection.

Activity of all cows was monitored. Any cows deemed in estrus before being examined for pregnancy were artificially inseminated following the a.m.–p.m. insemination rule. Cows were checked for pregnancy 34 to 40 d postbreeding via rectal palpation. Cows that were not pregnant and were not observed in estrus reentered the timed breeding program outlined above.

Liver Biopsies

Twenty cows per treatment were selected for collection of liver biopsies before initiation of treatments and again at approximately 14 wk after calving. Liver biopsies were taken between the 10th and 11th right inter-

costals space using previously defined procedures (Arthington et al., 1995). Samples were placed in Whirl-Pak bags (Nasco, Fort Atkinson, WI) and stored at –20°C until all biopsies were collected. Samples were shipped, frozen, to the Michigan State Diagnostic Laboratory (Lansing) for determination of mineral concentration by flame atomic absorption spectroscopy.

Claw Evaluations

Claws of all cows were examined at dry-off, before treatment administration, and at 16 and 36 wk postpartum by personnel trained in identifying claw lesions. Claws were examined by placing cows in a trimming chute equipped with a hydraulic tilt table. All claws were directly examined for lesions after being functionally trimmed by trained personnel. Lesions were noted in all 8 claws were and scored for severity according to the following protocol: 1) dorsal wall ridge, 0 = none, 1 = ridges and grooves, and 2 = dorsal wall curls up; 2) heel erosion, 0 = smooth heels, 1 = corrugated or skin flaps, 2 = deep cracks >6 mm, and 3 = exposed corium in crack; 3) dorsal wall fissure, 0 = none, 1 = transverse cracks, thimbling, and 2 = vertical cracks; 4) double sole, 0 = none, 1 = separation of <1/3 sole, and 2 = separation of >1/3 sole; 5) white line separation, 0 = no defects, minor manure staining, 1 = red discoloration after trimming, 2 = white line abnormally wide, pancake foot, 3 = crevice still packed with manure after trimming, and 4 = wall separated and broken away from white line; 6) white line abscess, 0 = none, 1 = small abscess, <1/2 cc of pus, 2 = larger abscess undermining wall and some sole, and 3 = abscess breaking out at heel or coronary band; 7) sole hemorrhage, 0 = none, 1 = faint speckles or pink tinge or yellow tinge, 2 = red area larger than 21 mm, and 3 = more than 1/2 of sole colored; 8) sole ulcer, 0 = none, 1 = soft discolored sole at ulcer site, 2 = exposed corium at ulcer site, and 3 = complicated sole ulcer; 9) digital dermatitis, 0 = none and 1 = present; 10) interdigital dermatitis, 0 = none, smooth interdigital skin, 1 = roughened or cratered interdigital skin, and 2 = interdigital fibroma, quittor, corn, and 11) foot rot, 0 = none and 1 = present. For cows exhibiting a noted claw disorder, a claw lesion index was calculated by taking the number of claws affected and multiplying it by the average severity score of the lesions.

Statistical Analysis

Removal of cows from the study occurred only if cows did not adapt to the computer feeders or if health problems (contagious or recurrent mastitis) or injury necessitated pen changes. Data from cows removed from the study before the claw evaluation at 16 wk postpartum (22 cows fed the CTM diet and 14 cows fed the Sulfate diet) were not included in the data analysis. The UNIVARIATE procedure of SAS 9.1 (SAS Institute, Cary, NC) was used to determine outlier cows. An observation that was greater than 2.5 standard deviations from the mean for 3.5% FCM and ECM was considered an outlier. Two cows receiving the Sulfate diet were considered outliers, and their data were excluded from the analyses. Data for milk yield and composition were analyzed using the MIXED procedure of SAS 9.1 according to the following model

$$Y_{ij} = \mu + C_i + c_{ij} + W_k + CW_{ik} + P_l + E_{ijkl}$$

where Y_{ij} = dependent variable; μ = overall mean; C_i = fixed effect of the ith treatment, i = 1, 2; c_{ij} = random effect of the jth cow within the ith treatment, j = 1,…250; W_k = fixed effect of week of lactation, k = 1,…,35; CW_{ik} = fixed effect of the interaction between the ith treatment and the kth week; P_l = t fixed effect of parity, l = 1, 2; and E_{ijkl} = random residual ~N (0, σ^2_e).

Data for claw lesions and lesion severity were analyzed using the MIXED procedure of SAS 8.2 (SAS Institute) according to the following model

$$Y_{ij} = \mu + \beta X_{ij} + C_j + c_{ij} + W_k + CW_{jk} + P_l + E_{ijkl}$$

where Y_{ij} = dependent variable; μ = overall mean; β = the regression (covariate) coefficient; X_{ij} = covariate measurement for the ith cow on the jth treatment; C_j = fixed effect of the jth treatment, j = 1, 2; c_{ij} = random effect of the ith cow within the jth treatment, j = 1,…250; W_k = fixed effect of week of lactation, k = 16, 35; CW_{jk} = fixed effect of the interaction between the jth treatment and the kth week; P_l = fixed effect of parity, l = 1, 2; and E_{ijkl} = random residual ~N (0, σ^2_e).

In the models used to analyze the lactation and claw lesion data, the random effect of cows within treatment subclasses was used as the error term for the effect of trace mineral source. The residual errors, which are errors within cows across time and represent errors from repeated measurements in the experimental units (cows), were modeled using a first-order autoregressive covariance structure. Degrees of freedom were calculated using the Kenward-Roger option of the MIXED procedure of SAS. Least squares means were determined for the main effects of trace mineral source and trace mineral source × week interactions.

Data for reproduction were analyzed using the MIXED procedure of SAS according to the following model

$$Y_{ij} = \mu + C_i + c_{ij} + P_k + E_{ijk}$$

where Y_{ij} = dependent variable; μ = overall mean; C_i = fixed effect of the ith treatment, i = 1, 2; c_{ij} = random effect of the jth cow within the ith treatment, j = 1,…250; P_k = fixed effect of parity, k = 1, 2; and E_{ijk} = random residual ~N (0, σ_e^2).

Data for liver trace mineral concentrations were analyzed using the MIXED procedure of SAS according to the following model

$$Y_{ij} = \mu + \beta X_i + C_j + c_{ij} + P_k + E_{ijk}$$

where Y_{ij} = dependent variable; μ = overall mean; β = regression (covariate) coefficient; X_i = covariate measurement; C_j = fixed effect of the jth treatment, j = 1, 2; c_{ij} = random effect of the ith cow within the jth treatment, j = 1,…250; P_k = fixed effect of parity, k = 1, 2; and E_{ijk} = random residual ~N (0, σ_e^2).

In the models used to analyze the reproduction and liver data, the random effect of cows within treatment subclasses was used as the error term for the effect of trace mineral source. Degrees of freedom were calculated using the Kenward-Roger option of the MIXED procedure of SAS.

The LIFETEST procedure of SAS was used to perform the survival-curve analyses for days from calving to culling and days from calving to pregnancy. Culled cows were treated as censored on the day the cow left the herd; nonpregnant cows were censored on the last day of the 35-wk lactation period. Significant treatment effects were noted at $P \leq 0.05$, and trends for treatment effects were noted at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Lactation Parameters

Cows fed CTM tended to produce more milk ($P = 0.07$) and ECM ($P = 0.06$) than cows fed the Sulfate diet (Table 4). Yields of milk and ECM increased 2.9 and 3.2%, respectively, when Sulfate minerals were replaced with minerals supplied by CTM. Cows fed CTM also had increased yields of ($P \leq 0.05$) milk protein and solids (fat + protein) as compared with cows fed the Sulfate diet. Content of fat, protein, solids, and somatic cells in milk were not affected ($P > 0.10$) by treatment.

Ballantine et al. (2002) reported similar increases in lactation performance when portions of the Zn, Mn, Cu, and Co from sulfate sources were replaced with OTM. Kincaid and Socha (2004) observed increased lactation performance during peak lactation but not during the early or midlactation period in response to replacing a portion of the inorganic Zn, Mn, Cu, and Co with OTM. In contrast, Uchida et al. (2001) and Ferguson et al. (2004a) did not observe an increase in lactation performance when a portion of the inorganic sources of Zn, Mn, Cu, and Co were replaced with OTM.

Level of trace mineral fortification in diets fed in the forementioned studies was not indicative of potential lactation performance in response to replacing inorganic trace minerals with OTM. According to NRC (2001), a second-lactation, 635-kg cow (38 mo of age, mature BW 680 kg, 14 DIM, producing 36.3 kg of milk containing 3.74% fat and 3.08% true protein and consuming 16.6 kg of DM) has Zn, Mn, Cu, and Co requirements of 70, 19, 15, and 0.11 mg/kg of DM, respectively, when using absorption coefficients assigned to feedstuffs. In general (on a mg/kg of DM basis), trace mineral requirements are highest in early lactation and lowest in midlactation. Lactating diets in the Ballantine et al. (2002) study were fortified well in excess of NRC (2001) requirements, containing 155 mg/kg of Zn, 119 mg/kg of Mn, 23 mg/kg of Cu, and 1.5 mg/kg of Co. Similarly, lactating diets fed in the Uchida et al. (2001) study were fortified well in excess of NRC (2001) requirements, containing, on average, 134 mg/kg of Zn, 77 mg/kg of Mn, 26 mg/kg of Cu, and 1.2 mg/kg of Co. In contrast, only dietary Mn, Cu, and Co concentrations in the Kincaid and Socha (2004) and Ferguson et al. (2004a) studies were fortified well in excess of NRC (2001) trace mineral requirements. Lactating diets in the Kincaid and Socha (2004) study contained 75 mg/kg of Zn, 67 mg/kg of Mn, 20 mg/kg of Cu, and 1.7 mg/kg of Co, whereas lactating diets in the Ferguson et al. (2004a) study contained, on average, 80 mg/kg of Zn, 74 mg/kg of Mn, 23 mg/kg of Cu, and 1.4 mg/kg of Co.

Although improvements in yield of milk and milk components have been observed previously, in general, milk composition does not appear to be affected by level or source of trace mineral supplementation (Uchida et al., 2001; Ballantine et al., 2002; Kellogg et al., 2003). However, Ferguson et al. (2004a) and Kincaid and Socha (2004) reported greater milk protein content for cows supplemented with OTM.

Claw Parameters

White line separation and sole hemorrhages were the most prominent claw lesions, with more than 85% of cows being afflicted with these lesions at 16 wk postpartum (Table 5). Incidence rates of sole hemorrhages were similar to previous observations (Bergsten, 1994; Nocek et al., 2000), but incidences of white line separation were considerably greater (Nocek et al., 2000; Ballantine et al., 2002). Sole hemorrhages and white line separation are clinical manifestations of disturbances in the microcirculation of the corium of the claw with subsequent degeneration at the dermal-epidermal junction (Lischer and Ossent, 2002) and can be attributed to

Table 4. Effect of trace mineral source on lactation performance[1]

Measure	Treatment[2]		TRT,[3] P =	Time × TRT, P =
	Sulfates	CTM		
Milk, kg/d	36.7 ± 0.4	37.8 ± 0.5	0.07	0.82
ECM,[4] kg/d	36.6 ± 0.4	37.8 ± 0.5	0.06	0.81
3.5% FCM, kg/d	37.0 ± 0.5	38.1 ± 0.5	0.11	0.87
Fat, kg/d	1.31 ± 0.02	1.34 ± 0.02	0.21	0.88
True protein, kg/d	1.06 ± 0.01	1.11 ± 0.01	0.01	0.81
Solids,[5] kg/d	2.36 ± 0.03	2.45 ± 0.03	0.05	0.76
Milk composition				
Fat, %	3.59 ± 0.04	3.58 ± 0.04	0.88	0.80
True protein, %	2.91 ± 0.03	2.96 ± 0.03	0.18	0.19
Solids, %	6.50 ± 0.06	6.55 ± 0.06	0.59	0.33
SCC, 1,000/mL	210 ± 31	258 ± 31	0.28	0.71
MUN, mg/dL	15.6 ± 0.9	14.9 ± 1.0	0.58	0.71

[1]Least squares means (±SEM) are presented.

[2]In addition to trace minerals supplied by the basal diet, treatments supplied daily per cow, from 3 wk before calving through wk 35 postcalving, included 360 mg of Zn, 200 mg of Mn, 125 mg of Cu, and 12 mg of Co from either sulfate sources (Sulfates) or Availa-4 (CTM).

[3]Treatment.

[4]3.5% fat and 3.0% true protein.

[5]Fat plus protein.

subclinical acidosis and less-than-desired cow comfort. Factors contributing to poor cow comfort include excess time away from the pen, flooring that is rough and abrasive, and free stalls that are too narrow, too short, or do not provide adequate cushioning (Cook and Nordlund, 2007).

Replacing Sulfate minerals with those supplied by CTM decreased ($P \leq 0.05$) incidence of sole ulcers at wk 36 postpartum. In addition, cows fed CTM tended to have less ($P = 0.09$) incidence of interdigital dermatitis than cows fed the Sulfate diet at wk 16 and 36 postpartum (Table 5). Severity of heel erosion tended to be less ($P = 0.07$) for cows fed CTM than cows fed Sulfate minerals (Table 6).

Although treatment effects were only observed for incidence of sole ulcers and interdigital dermatitis, numerically, incidence of white line separation, sole hemorrhages, heel erosion, double sole, and white line ab-

Table 5. Effect of trace mineral source on incidence of claw lesions[1]

Lesion, % incidence	Treatment[2]		TRT,[3] P =	Time × TRT, P =
	Sulfates	CTM		
Dorsal wall ridge	0.85 ± 0.71	1.03 ± 0.71	0.86	0.34
Heel erosion	18.54 ± 2.96	17.44 ± 3.00	0.80	0.99
Dorsal wall fissure	0.00 ± 0.00	0.54 ± 0.36	0.29	0.29
Double sole	13.36 ± 2.43	12.56 ± 2.44	0.82	0.61
White line separation	77.24 ± 2.97	71.71 ± 2.98	0.19	0.87
White line abscess	4.54 ± 1.50	4.00 ± 1.51	0.80	0.63
Sole hemorrhage	84.46 ± 2.86	81.78 ± 2.89	0.51	0.46
Sole ulcer	10.74 ± 2.14	6.64 ± 2.17	0.18	0.11
16 wk postpartum	5.09 ± 2.54	4.76 ± 2.67	0.93	
36 wk postpartum	16.40 ± 2.86	8.51 ± 2.78	0.05	
Digital dermatitis	7.88 ± 1.84	8.55 ± 1.86	0.80	0.37
Interdigital dermatitis	29.93 ± 3.38	21.71 ± 3.43	0.09	0.85

[1]Claws were examined before trial initiation and at 16 and 36 wk postpartum. Data collected before trial initiation were used as a covatiate in analyses of the claw lesion data. Least squares means (± SEM) pooled across wk 16 and 36 are presented. Individual least squares means for week are presented if time × treatment effect is significant at $P \leq 0.15$.

[2]In addition to trace minerals supplied by the basal diet, treatments supplied daily per cow, from 3 wk before calving through wk 35 postcalving, included 360 mg of Zn, 200 mg of Mn, 125 mg of Cu, and 12 mg of Co from either sulfate sources (Sulfates) or Availa-4 (CTM).

[3]Treatment.

Table 6. Effect of trace mineral source on claw lesion severity index[1]

Lesion	Treatment[2]		TRT,[3] P =	Time × TRT, P =
	Sulfates	CTM		
Dorsal wall ridge	1.30 ± 1.30	1.50 ± 1.50	0.94	0.55
Heel erosion	3.83 ± 0.26	3.08 ± 0.30	0.07	0.79
Double side	1.69 ± 0.48	2.09 ± 0.48	0.56	0.32
White line separation	8.19 ± 0.41	8.35 ± 0.43	0.78	0.65
White line abscess	2.47 ± 0.80	3.13 ± 0.67	0.54	0.32
Sole hemorrhage	5.45 ± 0.20	5.35 ± 0.21	0.73	0.45
Sole ulcer	2.23 ± 0.31	2.05 ± 0.36	0.71	0.67
Digital dermatitis	1.27 ± 0.21	1.21 ± 0.17	0.85	0.35
Interdigital dermatitis	2.69 ± 0.18	2.63 ± 0.21	0.83	0.98

[1]Claws were examined before trial initiation and at 16 and 36 wk postpartum. Data collected before trial initiation were used as a covariate in analysis of claw lesion data. Claw lesion index was calculated only for cows with a claw lesion by multiplying numbers of zones affected by lesion by average severity score. Least squares means (±SEM) pooled across wk 16 and 36 are presented.

[2]In addition to trace minerals supplied by the basal diet, treatments supplied daily per cow from 3 wk before calving through wk 35 postcalving included 360 mg of Zn, 200 mg of Mn, 125 mg of Cu, and 12 mg of Co from either sulfate sources (Sulfates) or Availa-4 (CTM).

[3]Treatment.

scess were less for cows fed the CTM diet. The inability to detect more treatment differences on incidence of claw lesions can be attributed to the dichotomous nature of the data, requiring a large number of cows per treatment.

Decreased incidence of claw lesions with increased mineral availability is a reflection of the role of trace minerals in maintaining claw integrity. The biological basis for Zn in maintaining claw integrity is related to the Zn-dependent enzymes, RNA nucleotide transferases, RNA polymerase, alkaline phosphatase, carbonic anhydrase, and carboxypeptidase, which are integral in differentiation of keratinocytes (Cousins, 1996; NRC, 2001). The Zn-finger proteins (Cousins, 1996) have an integral role in the formation of keratin filaments in the keratinocytes. In addition, Zn affects Ca metabolism through the regulation of calmodulin and inositol phosphate (NRC, 2001). Calcium is needed for activation of epidermal transglutaminase, which is active in cross-linkage of the cell envelope keratin fibers and is involved in the initiation and regulation of the terminal differentiation of the epidermal cells (Tomlinson et al., 2004). Zinc supplementation has been shown to decrease incidence of claw lesions in dairy cattle (Demertizis, 1973; Moore et al., 1989).

Copper's role in the production of a healthy claw horn is related to the Cu enzyme, thiol oxidase, which strengthens claw horn through the formation of disulfide bonds between Cys residues of adjoining keratin filaments (O'Dell, 1990). The connective tissue that suspends the distal phalanx within the claw capsule is strengthened by the Cu-dependent enzyme lysyl oxidase, which forms the cross-linkages between collagen fibers (Smart and Cymbaluk, 1997). Overloading the suspensory connective tissue of the distal phalanx compresses the corium, resulting in the development of claw lesions such as sole hemorrhages, sole ulcers, and white line separation.

Although Co and Mn appear to have a lesser role in maintaining claw integrity, vitamin B_{12} deficiency has been shown to increase the risk of lameness (Smart and Cymbaluk, 1997). The Mn-dependent enzymes, galactotransferase and glycosyl transferase, are required for the formation of proteoglycans (Miller et al., 1988), which are components of synovial fluid, cartilage, and loose connective tissues (Murray et al., 1993). The fibrocartilaginous insertions in the claw wall and sole and insertion areas of the ligaments and tendons are currently being investigated to determine if failure of these components is a contributing factor to breakdown of the suspensory apparatus of the distal phalanx (Westerfield et al., 2004).

Improvements in claw integrity observed in this study have been observed previously. Ballantine et al. (2002) reported that replacing sulfate minerals with OTM, beginning 21 d prepartum, decreased incidence and severity of white line disease at both 75 and 250 d postpartum, severity of sole ulcers at 250 d postpartum, and severity of heel erosion at 75 d postpartum. Ferguson et al. (2004b) observed a reduction in sole lesions including sole ulcers at 30 d postpartum when OTM replaced inorganic minerals, beginning 60 d prepartum. In contrast, Nocek et al. (2006) only observed a reduction in severity of white line lesions when sulfate minerals were replaced with OTM. Limited effects of trace mineral source on claw integrity may be attributed to a lower incidence of claw lesions in the Nocek et al. (2006) study as compared with incidence of claw lesions

Table 7. Effect of trace mineral source on fertility parameter[1]

Measure	Treatment[2]		
	Sulfates	CTM	P =
Days to first service	72 ± 1	71 ± 1	0.92
First service conception rate, %	40.5 ± 4.6	28.6 ± 4.8	0.07
Days open[3]	115 ± 6	124 ± 6	0.27
Services/conception[3]	1.6 ± 0.1	1.7 ± 0.1	0.49

[1]Least squares means (±SEM) are presented.

[2]In addition to trace minerals supplied by the basal diet, treatments supplied daily per cow, from 3 wk before calving through wk 35 postcalving, included 360 mg of Zn, 200 mg of Mn, 125 mg of Cu, and 12 mg of Co from either sulfate sources (Sulfates) or Availa-4 (CTM).

[3]Includes only data from cows that conceived.

observed in this study as well as in the Nocek et al. (2000) and Ballantine et al. (2002) studies.

Fertility Parameters

Despite first service conception tending to be greater (P = 0.07) for cows fed the Sulfate diet, there was no effect of treatment ($P > 0.10$) on days to first service, services per conception, and days open (Table 7). There was no effect of treatment ($P > 0.10$) on days to conception (Figure 1), but there was a trend (P = 0.10) for a greater percentage of cows fed the Sulfate diet to be culled from the herd (Figure 2).

Similar to the results observed in this study, Toni et al. (2007) observed minimal effects of replacing inorganic minerals with OTM on fertility, but culling from the herd was decreased when OTM was fed. Failure of OTM to elicit improvements in fertility in these studies contradicts previous research in which cows fed OTM, either in addition to inorganic minerals or in substitution for inorganic minerals, had fewer days to first estrus (Campbell et al. 1999; Nocek et al., 2006), fewer services per conception (Uchida et al., 2001), fewer days to conception (Uchida et al., 2001; Ballantine et al., 2002; Kellogg et al., 2003), and increased percentage of cows pregnant (Ballantine et al., 2002; Ferguson et al., 2004a; Nocek et al., 2006).

Variability in response does not appear to be dependent on whether OTM was added to the diet (Kellogg et al., 2003; Nocek et al., 2006) or substituted for inorganic minerals (Uchida et al., 2001; Ballantine et al., 2002; Ferguson et al., 2004b). In addition, response does not appear to be dependent on whether cows were fed diets fortified with moderate (Ferguson et al., 2004b) or high levels of supplemental minerals (Uchida et al., 2001; Ballantine et al., 2002) as noted earlier.

However, improvements in fertility in response to increased availability of Zn, Mn, and Cu do have a

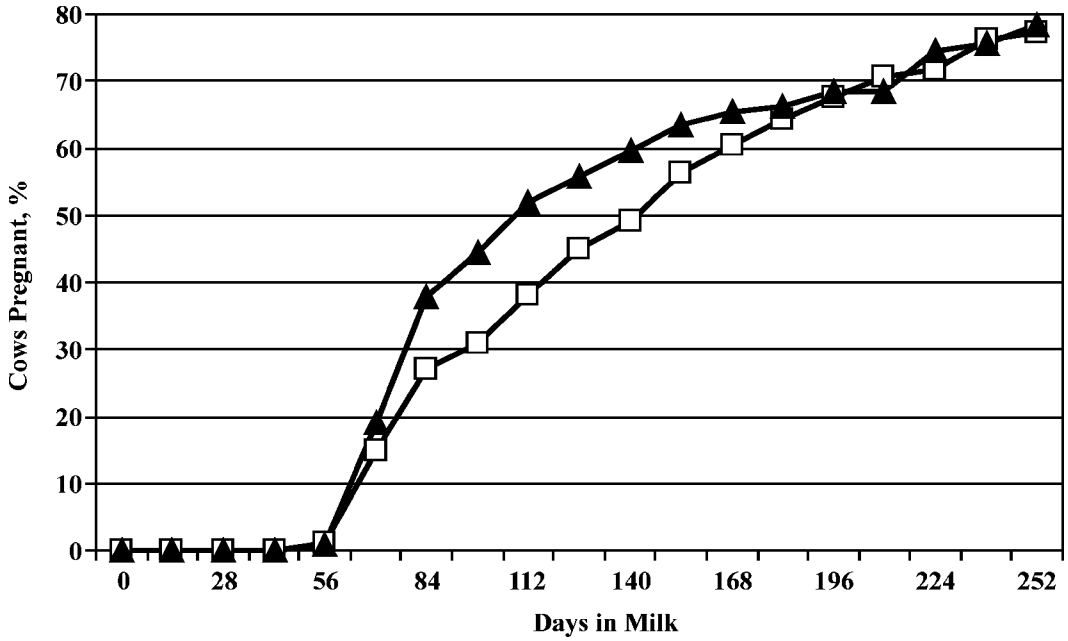

Figure 1. Effect of trace mineral source on days from calving to conception. In addition to trace minerals supplied by the basal diet, treatments supplied daily per cow, from 3 wk before calving through wk 35 postcalving, included 360 mg of Zn, 200 mg of Mn, 125 mg of Cu, and 12 mg of Co from either sulfate sources (Sulfates, ▲) or Availa-4 (CTM, □; log-rank test, χ^2 = 0.68, Sulfates SEM = 7.0, CTM SEM = 7.1, P = 0.41).

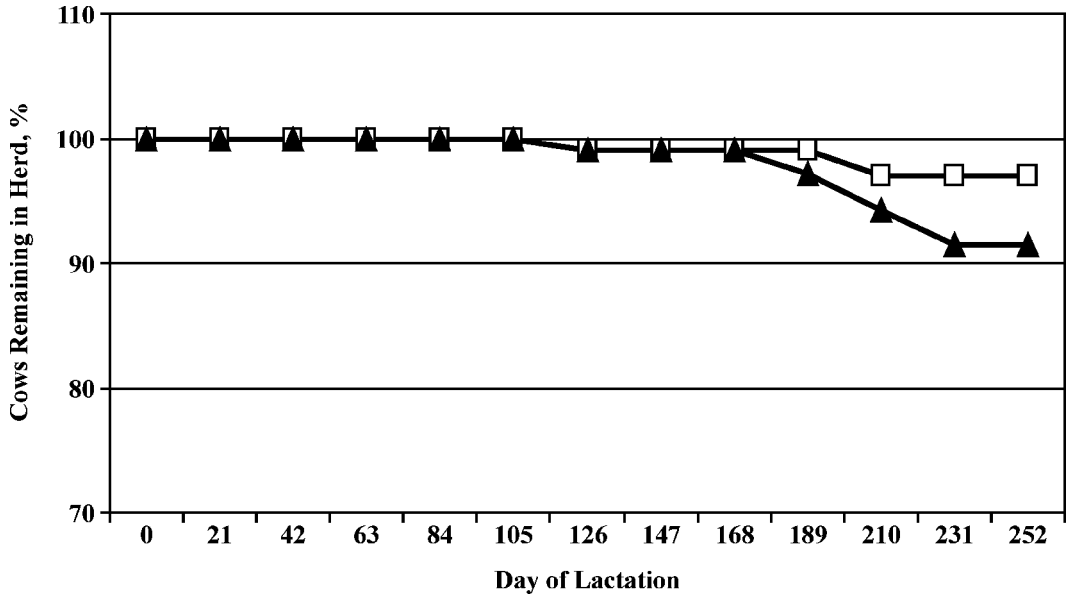

Figure 2. Effect of trace mineral source on cow removal from the herd. In addition to trace minerals supplied by the basal diet, treatments supplied daily per cow, from 3 wk before calving through wk 35 postcalving, included 360 mg of Zn, 200 mg of Mn, 125 mg of Cu, and 12 mg of Co from either sulfate sources (Sulfates, ▲) or Availa-4 (CTM, □; log-rank test, $\chi^2 = 2.79$, Sulfates SEM = 1.2, CTM SEM = 1.0, $P = 0.10$).

biological basis. Manganese is necessary for cholesterol synthesis, which, in turn, is required for synthesis of the steroids, estrogen, progesterone, and testosterone (Keen and Zidenberg-Cherr, 1990). In addition, the corpus luteum has a high Mn content and may be affected by level of Mn supplementation (Brown and Casillas, 1986). Symptoms of a Cu deficiency include early embryonic death, resorption of the embryo, increased retained placentas, and necrosis of the placenta (Miller et al., 1988; Puls, 1994). Weak and silent heats have been reported. Kappel et al. (1984) reported that dairy cows with greater serum Cu levels had significantly less days to first service, fewer services per conception, and fewer days open. Inadequate Zn levels have been associated with decreased fertility, abnormal estrus, abortion, and altered myometrial contractibility with prolonged labor (Duffy et al., 1977; Maas, 1987). Campbell and Miller (1998) found that feeding dry cows an additional 800 mg of Zn for the last 6 wk of gestation decreased days to first estrus and days to service in the subsequent lactation despite consuming a basal diet containing 102 mg/kg of Zn.

Liver Parameters

There was no effect of treatment ($P > 0.10$) on trace mineral content of the liver (Table 8). Average liver concentrations indicate that all groups of cows had adequate Cu, Mn, Zn, and Fe status, and Mo concentrations in the liver were normal (Puls, 1994). Despite cows having adequate Zn, Mn, and Cu status, as indicated by trace mineral content of liver, lactation performance and claw integrity of cows were affected by trace mineral source. These results suggest that either Zn, Mn, and Cu content of liver is a poor indicator of trace mineral status or that trace mineral content of liver is not an accurate predictor of whether cows will respond to different sources of trace minerals. Potential explanations include trace minerals in the liver being in a form that is of limited availability for metabolism or stores

Table 8. Effect of trace mineral source on liver mineral concentration, 14 wk postpartum[1]

Mineral, mg/kg of DM	Treatment[2]		
	Sulfates	CTM	P =
Cu	465 ± 31	492 ± 25	0.49
Fe	248 ± 18	227 ± 14	0.35
Mn	10.3 ± 0.4	10.2 ± 0.4	0.92
Mo	3.4 ± 0.1	3.4 ± 0.1	0.98
Zn	96.9 ± 5.5	105.0 ± 4.4	0.25

[1]Liver biopsies were collected before trial initiation and at 14 wk postpartum. Data collected before trial initiation were used as a covariate. Least squares means (±SEM) are presented.

[2]In addition to trace minerals supplied by the basal diet, treatments supplied daily per cow, from 3 wk before calving through wk 35 postcalving, included 360 mg of Zn, 200 mg of Mn, 125 mg of Cu, and 12 mg of Co from either sulfate sources (Sulfates) or Availa-4 (CTM).

of Zn, Mn, and Cu being mobilized at an insufficient rate to compensate for periods when animals are consuming inadequate amounts of these trace minerals to meet metabolic requirements. Similar results have been observed in other studies (Ballantine et al., 2002; Ferguson et al., 2004a,b; Nocek et al., 2006), in which cows had adequate Zn, Mn, Cu, and Co status, and despite minimal or no effect of treatment on liver trace mineral content, lactation performance, fertility, or claw integrity were improved when OTM were included in the diet.

CONCLUSIONS

Replacing Sulfate minerals with CTM resulted in improved claw integrity and lactation performance. Cows fed Sulfate minerals had greater first service conception rates yet were more likely to be culled from the herd. Although differences in performance were noted when cows were fed diets with different sources of trace minerals, mineral content of liver was not affected, indicating that trace mineral content of liver is not an accurate predictor of whether cows will respond to different sources of trace minerals.

REFERENCES

AOAC. 2000. Official Methods of Analysis. 17th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

Arthington, J. D., L. R. Corah, F. Blecha, and D. A. Hill. 1995. Effect of copper depletion and repletion on lymphocyte blastogenesis and neutrophil bactericidal function in beef heifers. J. Anim. Sci. 73:2079–2085.

Ballantine, H. T., M. T. Socha, D. J. Tomlinson, A. B. Johnson, A. S. Fielding, J. K. Shearer, and S. R. van Amstel. 2002. Effect of feeding complexed zinc, manganese, copper and cobalt to late gestation and lactating dairy cows on claw integrity, reproduction and lactation performance. Prof. Anim. Sci. 18:211–218.

Bergsten, C. 1994. Hemorrhages of the sole horn of dairy cows as a retrospective indicator of laminitis: Epidemiological study. Acta Vet. Scand. 35:55–67.

Brown, M. A., and E. R. Casillas. 1986. Manganese and manganese-ATP interactions with bovine sperm adenylate cyclase. Arch. Biochem. Biophys. 244:719–726.

Campbell, M. H., and J. K. Miller. 1998. Effect of supplemental dietary vitamin E and zinc on reproductive performance of dairy cows and heifers fed excess iron. J. Dairy Sci. 81:2693–2699.

Campbell, M. H., J. K. Miller, and F. N. Schrick. 1999. Effect of additional cobalt, copper, manganese, and zinc on reproduction and milk yield of lactating dairy cows receiving bovine somatotropin. J. Dairy Sci. 82:1019–1025.

Cook, N. B., and K. V. Nordlund. 2007. The influence of the environment on dairy cow behavior, claw health and herd lameness dynamics. Vet. J. doi:10.1016.j.tvjl.2007.09.016.

Cousins, R. J. 1996. Zinc. Pages 293–306 in Present Knowledge in Nutrition. 7th ed. E. E. Ziegler and L. J. Filer Jr., ed. ILSI Press, Washington, DC.

Demertizis, P. N. 1973. Oral zinc therapy in the control of infectious pododermatitis in young bulls. Vet. Rec. 93:219–222.

Drendel, T. R., P. C. Hoffman, N. R. St. Pierre, M. T. Socha, D. J. Tomlinson, and T. L. Ward. 2005. Effects of feeding zinc, manganese, and copper amino acid complexes and cobalt glucoheptonate to dairy replacement heifers on claw disorders. Prof. Anim. Sci. 21:217–224.

Duffy, J. H., J. B. Bingley, and L. Y. Cove. 1977. The plasma zinc concentration of nonpregnant, pregnant and parturient Hereford cattle. Aust. Vet. J. 53:519–522.

Ferguson, J. D., D. Tomlinson, and M. Socha. 2004a. Effects of inorganic and organic (4-PlexR) trace mineral supplementation on milk production and reproduction. J. Dairy Sci. 87(Suppl. 1):117. (Abstr.)

Ferguson, J. D., D. Tomlinson, and M. Socha. 2004b. Effects of inorganic and organic (4-PlexR) trace mineral supplementation on claw lesions. J. Dairy Sci. 87(Suppl. 1):117. (Abstr.)

Griffiths, L. M., S. H. Loeffler, M. T. Socha, D. J. Tomlinson, and A. B. Johnson. 2007. Effects of supplementing complexed zinc, manganese, copper and cobalt on lactation and reproductive performance of intensively grazed lactating dairy cattle on the South Island of New Zealand. Anim. Feed Sci. Technol. 137:69–83.

Kappel, L. C., R. H. Ingraham, E. B. Morgan, and D. K. Babcock. 1984. Plasma copper concentration and packed cell volume and their relationships to fertility and milk production in Holstein cows. Am. J. Vet. Res. 45:346–350.

Keen, C. L., and S. Zidenberg-Cherr. 1990. Manganese. Pages 279–286 in Present Knowledge in Nutrition. M. L. Brown, ed. Int. Life Sci. Inst., Nutr. Found., Washington, DC.

Kellogg, D. W., M. T. Socha, D. J. Tomlinson, and A. B. Johnson. 2003. Effect of feeding a combination of cobalt glucoheptonate and metal specific amino acid complexes of zinc, manganese and copper on lactation and reproductive performance of dairy cows: Eight-trial summary. Prof. Anim. Sci. 19:1–9.

Kellogg, D. W., D. J. Tomlinson, M. T. Socha, and A. B. Johnson. 2004. Effect of feeding zinc methionine complex on milk production and somatic cell count of dairy cattle: Twelve-trial summary. Prof. Anim. Sci. 20:295–301.

Kinal, S., A. Korniewicz, D. Jamroz, R. Zieminski, and M. Slupcznska. 2005. Dietary effects of zinc, copper and manganese chelates and sulphates on dairy cows. J. Food. Agric. Environ. 3:168–172.

Kincaid, R. L., and M. T. Socha. 2004. Inorganic versus complexed trace mineral supplements on performance of dairy cows. Prof. Anim. Sci. 20:66–73.

Lischer, C. J., and P. Ossent. 2002. Pathogenesis of sole lesions attributed to laminitis in cattle. Pages 82–89 in Proc. 12th Int. Symp. Lameness Rumin., Orlando, FL. Univ. Florida, Gainesville.

Maas, J. 1987. Relationship between nutrition and reproduction in beef cattle. Vet. Clin. N. Am. Food Anim. Pract. 3:633–646.

Miller, J. K., N. Ramsey, and F. C. Madsen. 1988. The trace elements. Pages 342–400 in The Ruminant Animal. D. C. Church, ed. Prentice Hall, Englewood Cliffs, NJ.

Moore, C. L., P. M. Walker, M. A. Jones, and J. M. Webb. 1989. Zinc methionine supplementation for dairy cattle. Trans. Ill. Acad. Sci. 82:99–108.

Murray, R. K., D. K. Granner, P. A. Mayes, and V. W. Rodwell. 1993. Harper's Biochemistry. Appleton and Lange Publ., Norwalk, CT.

Nocek, J. E., A. B. Johnson, and M. T. Socha. 2000. Digital characteristics in commercial dairy herds fed metal-specific amino acid complexes. J. Dairy Sci. 83:1553–1572.

Nocek, J. E., M. T. Socha, and D. J. Tomlinson. 2006. The effect of trace mineral fortification level and source on performance of dairy cattle. J. Dairy Sci. 89:2679–2693.

Nockels, C. F., J. DeBonis, and J. Torrent. 1993. Stress induction affects copper and zinc balance in calves fed organic and inorganic copper and zinc sources. J. Anim. Sci. 71:2539–2545.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.

O'Dell, B. L. 1990. Copper. Pages 261–267 in Present Knowledge in Nutrition. 6th ed. M. L. Brown, ed. ILSI Press, Washington, DC.

Paripatananont, T., and R. T. Lovell. 1995. Chelated zinc reduces the dietary zinc requirement of channel catfish, *Ictalurus punctatus*. Aquaculture 133:73–82.

Puls, R. 1994. Mineral Levels in Animal Health—Diagnostic Data. Sherpa Int., Clearbrook, British Columbia, Canada.

Smart, M., and N. F. Cymbaluk. 1997. Role of nutritional supplements in bovine lameness: Review of nutritional toxicities. Pages 145–161 in Lameness in Cattle. 3rd ed. P. R. Greenough and A. D. Weaver, ed. WB Sanders Co., Philadelphia, PA.

Tomlinson, D. J., C. H. Mülling, and T. M. Fakler. 2004. Formation of keratins in the bovine claw: Roles of hormones, minerals, and vitamins in functional claw integrity. J. Dairy Sci. 87:797–809.
Toni, F., L. Grigoletto, C. J. Rapp, M. T. Socha, and D. J. Tomlinson. 2007. Effect of replacing dietary inorganic forms of zinc, manganese, and copper with complexed sources on lactation and reproductive performance of dairy cows. Prof. Anim. Sci. 23:409–416.
Uchida, K. C., P. Mandebvu, C. S. Ballard, C. J. Sniffen, and M. P. Carter. 2001. Effect of feeding Availa-4 on performance of early lactation high producing dairy cows. Anim. Feed Sci. Technol. 93:193–203.
Wedekind, K. J., A. E. Hortin, and D. E. Baker. 1992. Methodology for assessing zinc bioavailability: Efficacy estimates for zinc-methionine, zinc sulfate and zinc oxide. J. Anim. Sci. 70:178–187.
Westerfield, I., R. M. Hirschfield, and K. D. Budras. 2004. The weight-bearing apparatus of the bovine claw. Pages 66–67 in Proc. 13th Int. Symp. and 5th Conf. Lameness Rumin., Maribor, Slovenia.