The Effect of Trace Mineral Fortification Level and Source on Performance of Dairy Cattle

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ABSTRACT

Five hundred seventy-three cows, balanced by parity and 305-d mature equivalent at dry off, were assigned to 1 of 4 treatments: 1) 75% complexed trace minerals (CTM; 75C): Zn, Mn, Cu, and Co supplied at 75% of NRC (2001) guidelines by Zn-, Mn-, and Cu-specific AA complexes, and cobalt glucoheptonate; 2) 100% inorganic (100I): Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by sulfate sources; 3) 100% complexed (100C): Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by CTM; and 4) complexed/inorganic (C/I): Zn and Cu supplied at 100% of NRC (2001) requirements using a combination of CTM and sulfates and Co and Mn supplied with sources at 9.1 and 3.3 times NRC (2001) requirements using a combination of CTM and sulfates. All percentages of Zn, Cu, Mn, and Co relative to NRC (2001) reflect supplemental contributions and do not include basal diet contributions. Experimental periods were dry period 1, full lactation 1, dry period 2, and 200 d into the subsequent lactation. Reproductive, health, and production information was collected during both lactations. Claw evaluations were conducted at trial start, 150 d into lactation 1, at the end of lactation 1, and 150 d into lactation 2. During lactation 1, C/I cows produced more milk, fat-corrected milk, energy-corrected milk, and fat than 100I cows. During lactation 2, yields of milk, fat-corrected milk, energy-corrected milk, fat, and protein were higher for 100C and C/I cows than for 75C or 100I cows. Fat percentage was highest for 100C cows with no treatment effect on protein content. During lactations 1 and 2, C/I cows had fewer days to first estrus than cows receiving the other treatments. During lactation 2, C/I cows had fewer services per conception and days open. There were no significant effects of treatment on health. White line separation incidence was lower for 100I cows than 75C cows, whereas heel erosion was higher for the 100I cows than for the C/I cows. Fortification of trace

elements with inorganic and complexed sources at or above NRC requirements improved reproductive and productive performance. In addition, cows can be supplemented with CTM at 75% of NRC requirements with no reduction in performance compared with supplementing at 100% of NRC requirements using only sulfate sources of Zn, Mn, Cu, and Co.

Key words: complexed trace minerals, reproduction, production, claw lesion

INTRODUCTION

Zinc, manganese, copper, and cobalt are required for the functionality of numerous structural proteins, enzymes, and cellular proteins. Numerous studies have confirmed that feeding amino acid complexes of Zn, Mn, and Cu, and cobalt glucoheptonate improves performance of dairy cattle. The benefits include preventing mastitis, improving fertility, and reducing the incidence of foot lesions (Nocek et al., 2000; Uchida et al., 2001; Ballantine et al., 2002; Kellogg et al., 2003, 2004). Improvements in animal performance in response to complexed trace minerals (CTM) supplementation appear to be related to increased availability of trace minerals for metabolism (Wedekind et al., 1992; Paripatananont and Lovell, 1994). Because bioavailability of these trace elements is increased, the amount of trace elements fed to cattle may be reduced while maintaining animal performance and potentially reducing excretion of trace minerals into the environment. However, research trials utilizing a substantial number of dairy cows to ascertain statistical differences in performance over more than one lactation have not been conducted to determine if supplementing diets with lower levels of trace mineral from CTM results in similar responses as supplementing diets with higher levels of trace minerals utilizing inorganic sources.

Recent research suggests that cows may benefit from diets supplemented above NRC (2001) requirements for Co and Mn. Kincaid et al. (2003) observed that supplementing lactating dairy diets with Co above NRC (2001) requirements resulted in improved lactational performance of multiparous, but not primiparous, cows. Likewise, in a summary of 8 balance studies, Weiss and

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Socha (2005) found that providing adult dairy cattle with amounts of Mn sufficient to meet NRC (2001) requirements resulted in cattle being in negative Mn balance. It was determined that adult dairy cattle must consume 580 mg of Mn/d to meet their maintenance requirement for Mn.

Therefore, the objectives of this study were to determine effects on dairy cattle lactation performance, fertility, and claw integrity of 1) supplementing Zn, Mn, Cu, and Co at 75% of NRC (2001) requirements using CTM; 2) supplementing Zn, Mn, Cu, and Co at 100% NRC (2001) requirements using either sulfate sources or CTM; and 3) supplementing at 100% of NRC (2001) Zn and Cu requirements and above NRC (2001) requirements for Mn and Co using a combination of CTM and sulfate sources.

MATERIALS AND METHODS

The study was conducted at Spruce Haven Farm and Research Center in Auburn, NY. Lactating cows were housed in a freestall barn that contained 8 individual pens, 4 on each side of a 6-row barn, that housed 75 cows per pen yielding 150 cows per treatment at trial initiation. This yielded 2 replications per treatment (pen = replication). Dry cows were housed in 4 separate group pens. Four treatments were utilized in this study: 1) 75% complexed (**75C**); Zn, Mn, Cu, and Co supplied at 75% of NRC (2001) requirements by CTM: zinc methionine (Zinpro Corp., Eden Prairie, MN), copper lysine (CuPlex, Zinpro), manganese methionine (ManPro, Zinpro), and cobalt glucoheptonate (CoPro, Zinpro); 2) 100% inorganic (**100I**); Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by zinc sulfate, manganese sulfate, copper sulfate, and cobalt sulfate; 3) 100% complexed (**100C**); Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements using CTM; and 4) complexed/inorganic (**C/I**); Zn and Cu supplied at 100% of NRC (2001) requirements using a combination of CTM and sulfates, and Co and Mn supplied at 9.1 and 3.3 times NRC (2001) requirements using a combination of CTM and sulfates. For the C/I treatment, a complexed product containing the same individual products as the 75C and 100C treatments (4-Plex, Zinpro), was added at the recommended level of 14 g/d in addition to the sulfate minerals.

Cows were balanced to 1 of 8 treatment group pens based on either previous lactation 305-d mature equivalent milk yield (multiparous) or sire PTA (primiparous) and parity. It is realized that animal assignment based on previous lactation production and expected genetic potential has limitations and does not guarantee that actual milk production will be uniform among treat-

ment groups. Treatment groups were randomized to pens on each side of the barn. Each pen contained an exit alley so that cows from one pen would not interfere with an adjacent pen when moved for milking. Beds were sand-based and water and feed space accessibility was equal in all pens. Cows started the experimental period at dry-off (approximately 55 d before expected calving). Cows remained on treatment during the dry period, the next entire lactation, a second dry period, and a subsequent lactation period through 200 DIM.

Basal diets for the prepartum program were fed as a single dry-cow group and were formulated to meet or exceed requirements (NRC, 2001) of a 635-kg cow, 250 d pregnant (Table 1). Because high haylage diets were fed to maintain body condition, calcium and potassium concentrations were higher than stated requirements. The lactation diet consisted of a 1-group TMR that was fed ad libitum once daily. The nutrient specifications were balanced to achieve 39 kg of milk (Table 2). Both dry and lactating diets were formulated assuming that feedstuffs contributed no Zn, Mn, Cu, and Co: only the treatment mixes contributed Zn, Cu, Mn, and Co. The treatment trace mineral premix was fed at 0.23 kg/cow per d and included designated mineral for specific treatment plus distillers grain as a carrier. Cows in both the dry and lactating group pens were housed initially with nontrial animals until enough cows were assigned in any given treatment pen to accommodate the group size. Trial animals were identified with specific treatment color-coded ear tags.

Parameter Measurements

Milk and Composition. Cows were milked twice daily and milk yields recorded at each milking and production summarized every 2 wk (biweekly). During lactation 1, milk was sampled every 4 wk for determination of milk composition. In lactation 2, milk was sampled every 2 wk for determination of milk composition. In lactations 1 and 2, milk samples were collected from both an a.m. and a p.m. milking, composited, and sent to Dairy One (Ithaca, NY) for analyses of fat, protein, somatic cells, lactose, and MUN content according to approved procedures of AOAC (1990).

DMI Estimate. Daily group intakes were recorded throughout the trial period for each treatment group pen (replication). The number of cows represented in a given group at any one time changed weekly and did not necessarily represent entirely cows on trial. However, the daily animal count per group represented cows actually consuming the experimental diet. Therefore, total feed offered divided by total cows in the group on a given day was then calculated. Mean DMI for dry period 1, lactation 1, dry period 2, and lactation 2 were

Table 1. Ingredient composition of experimental diets fed to dry and lactating cows^{1,2}

Ingredient	Dry cows				Lactating cows			
	75C	100I	100C	C/I	75C	100I	100C	C/I
	(% of DM basis)							
Haylage	53.7				6.8			
Corn silage	28.3				44.7			
Corn meal (fine ground)	9.1				16.8			
Carbohydrate-sugar mix	—				3.4			
Cottonseed with lint	—				3.5			
Corn distillers	1.02				0.8			
Corn gluten meal	0.41				1.0			
Fish meal	—				0.39			
Blood meal	0.83				0.59			
Calcium carbonate	0.32				1.39			
SoyPlus ³	1.02				2.9			
Soybean meal	2.26				9.76			
Soybean roasted	—				3.63			
Molasses	0.3				—			
Dairy premix	0.28				—			
Potassium carbonate	—				0.24			
Dynamate ⁴	0.02				0.07			
Magnesium sulfate	0.57				0.34			
Salt	0.02				0.67			
Megalac ⁵	—				0.77			
Amax yeast ⁶	—				0.23			
Sodium bicarbonate	—				0.7			
Calcium sulfate	0.1				0.2			
Novasil ⁷	—				0.45			
Monocal Phos 21	0.78				0.26			
Urea	0.21				0.14			
ADE Premix	—				0.12			
Vit E 20000	0.09				0.07			
Selenium 270	—				0.05			
Zinc sulfate	—	0.007	—	0.0131	—	0.021	—	0.017
Manganese sulfate	—	0.0062	—	0.017	—	0.0065	—	0.018
Copper sulfate	—	0.0053	—	0.0021	—	0.0063	—	0.002
Cobalt sulfate	—	0.00003	—	0.000015	—	0.00004	—	0
Zinpro 100 ⁸	0.019	—	0.025	0.0281	0.057	—	0.077	0.014
ManPro 160 ⁸	0.0088	—	0.011	0.01	0.0094	—	0.0125	0.0049
CuPlex100 ⁸	0.01	—	0.013	0.01	0.011	—	0.016	0.01
CoPro 25 ⁸	0.00037	—	0.00041	0.004	0.0004	—	0.0004	0.004

¹Mean $\pm$ SD of 32 chemical analyses; haylage: Zn = 28.3 ± 4.7 ppm, Cu = 11.7 ± 1.8 ppm, Mn = 33.2 ± 8.6 ppm; corn silage: Zn = 20.3 ± 7.7 ppm, Cu = 6.3 ± 0.8 ppm, Mn = 23.5 ± 3.9 ppm.

²Experimental supplements: 75C = 75% complexed: Zn, Mn, Cu, and Co supplied at 75% of NRC (2001) requirements by Zn-, Mn-, and Cu-specific AA complexes, and cobalt glucoheptonate; 100I = 100% inorganic: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by sulfate sources; 100C = 100% complexed: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by CTM; and C/I = complexed/inorganic: Zn and Cu supplied at 100% of NRC (2001) requirements using a combination of CTM and sulfates and Co and Mn supplied with sources at 9.1 and 3.3 times NRC (2001) requirements using a combination of CTM and sulfates.

³West Central Cooperative, Ralston, IA.

⁴Mosaic, Minneapolis, MN.

⁵Arm and Hammer Nutrition Group, Princeton, NJ.

⁶Varied Industries Corporation, Mason City, IA.

⁷Engelhard Corporation, Iselin, NJ.

⁸Zinpro Corporation, Eden Prairie, MN.

estimated when the maximum number of animals from a given stage of lactation was in a specific group. Samples of the forages and TMR were taken on a monthly basis and analyzed for trace mineral content. Body weight was measured and condition scored on all animals every 4 wk.

Reproduction. At 54 d postpartum, cows entered a timed breeding program. A 2-mL i.m. injection of GnRH (Cystorelin, Merial, Duluth, GA) was given at 54 d postpartum. Seven days later, cows received a 5-mL i.m. injection of prostaglandin. Forty-eight hours later, cows received a second 2-mL i.m. injection of GnRH. Cows

Table 2. Calculated chemical composition of experimental diets¹ fed to dry and lactating cows

Nutrients	Dry cows					Lactating cows				
	Basal	75C	100I	100C	C/I	Basal	75C	100I	100C	C/I
DM, %	34.6					48.1				
CP, %	15.9					18.0				
RUP, % CP	28.2					40.7				
RDP, % CP	71.8					59.3				
Soluble protein, % CP	50.2					31.8				
NE _L (Mcal/kg)	1.56					1.76				
NDF, %	43.3					30.7				
Total fat, %	3.4					5.01				
Calcium	1.52					1.07				
Phosphorus	0.46					0.42				
Magnesium, %	0.62					0.41				
Potassium, %	1.68					1.36				
Sulfur, %	0.21					0.23				
Sodium, %	0.03					0.49				
Chloride, %	0.03					0.45				
Zinc, ² ppm	26.9	19	25.3	25.2	75.2 (28)	28.6	58	78.4	77	75 (14)
Copper, ² ppm	9.8	10.0	13.1	13.2	15.0 (10)	8.0	12	16.2	16	15 (5)
Manganese, ² ppm	28.0	14.0	18.1	18.2	65.2 (16)	25.9	15	21	20	68 (8)
Selenium, ppm	0.31					0.31				
Cobalt, ² ppm	0.02	0.097	0.12	0.12	1.03 (1.03)	0.02	0.09	0.11	0.11	1.0 (1.0)
Iodine, ppm	91					0.9				
Vitamin A, kIU/kg	20.21					11.77				
Vitamin D, kIU/kg	6.74					2.62				
Vitamin E, IU/kg	114.45					37.05				

¹Experimental supplements: 75C = 75% complexed: Zn, Mn, Cu, and Co supplied at 75% of NRC (2001) requirements by Zn-, Mn-, and Cu-specific AA complexes, and cobalt glucoheptonate; 100I = 100% inorganic: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by sulfate sources; 100C = 100% complexed: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by CTM; and C/I = complexed/inorganic: Zn and Cu supplied at 100% of NRC (2001) requirements using a combination of CTM and sulfates and Co and Mn supplied with sources at 9.1 and 3.3 times NRC (2001) requirements using a combination of CTM and sulfates. For treatments, supplemental Zn, Cu, Mn and Co was in addition to basal diet.

²Number in parentheses represents ppm from complexed trace minerals (CTM).

were artificially inseminated 12 h after the last GnRH injection.

Cows were checked for pregnancy 34 to 40 d post-breeding via rectal palpation. Cows were visually observed for estrus twice daily, once during the a.m. period and once during the p.m. period. Any cows observed in estrus before being checked for pregnancy were artificially inseminated following the a.m./p.m. insemination rule. Cows that were not pregnant and were not observed in estrus during the a.m. and p.m. estrus detection periods reentered the timed breeding program outlined above.

Health. All health events were recorded. Placentas present 24 h after calving were considered retained. Metritis was diagnosed by either a purulent vaginal discharge or rectal palpitation. Ketosis was detected by either a high milk or urine ketone content and therapy included various combinations of intravenous injection of glucose, glucocorticoids, and oral administrative of propylene glycol. Displaced abomasums were detected by percussion with a stethoscope on either the left or right side of the cow.

Claw Parameter Measurements. Claws were evaluated by one evaluator at 4 time points during the

experiment: before initiation of treatments, at 150 d into lactation 1, at the start of the second dry period, and 200 d into lactation 2. All digits were examined at time of trimming and digit abnormalities were recorded and scored for both presence and severity according to the following scoring protocol: Sole hemorrhage (**SH**): 0 = no discoloration, 1 = <50% of the digit covered with a pinkish tinge, 2 = >50% of the digit area covered with a pinkish tinge, 3 = <50% of the digit covered with a deep red hemorrhage, and 4 = >50% of the digit covered with a deep red hemorrhage; heel erosion (**HE**): 0 = smooth heel, 1 = slight skin interruption, 2 = pronounced ridges, and 3 = broken skin; white line separation (**WLS**): 0 = no separation, 1 = <1/3 of the white line affected, 2 = >1/3 but <2/3 of the white line affected, and 3 = >2/3 of the white line affected on each digit; sole ulceration (**SU**): 1 = <25% of the digit with exposed corium, 2 = >25% but <50% of the digit with exposed corium, and 3 = >50% of the digit with exposed corium; and digital dermatitis (**DD**): 0 = no lesion, 1 = lesion < 2 cm, 2 = lesion > 2 cm in diameter.

Liver Biopsies. Liver biopsies were collected from the same 30 cows/treatment (15 per pen) at 3 times during the trial, including trial initiation, 150 d into

Table 3. The effect of complexed trace mineral (CTM) supplementation on production performance of lactating cows

Item	Treatments ¹				P-value		
	75C	100I	100C	C/I	SEM	Trt	Rep(Trt)
Lactation 1, ² n	101	109	102	107			
DMI dry group, kg/d	12.4	11.3	12.6	11.5			
DMI lactating group, kg/d	22.2	22.0	22.6	22.4			
Milk, kg/d	36.3 ^b	35.0 ^c	36.6 ^b	37.6 ^a	0.2	0.01	NS
3.5% FCM, kg/d	38.9 ^{bc}	37.9 ^c	39.4 ^{ab}	40.0 ^a	0.3	0.01	NS
ECM, ³ kg/d	38.3 ^b	37.3 ^c	38.7 ^{ab}	39.4 ^a	0.3	0.01	NS
Fat, %	3.95 ^{ab}	4.02 ^a	4.0 ^a	3.91 ^b	0.02	0.01	NS
Protein, %	3.06	3.07	3.07	3.04	0.01	NS	NS
SNF, %	6.61	6.67	6.67	6.59	0.03	NS	NS
Fat, kg/d	1.42 ^{bc}	1.40 ^c	1.46 ^{ab}	1.47 ^a	0.01	0.01	NS
Protein, kg/d	1.11	1.07	1.12	1.14	0.01	NS	NS
SNF, kg/d	2.42 ^b	2.33 ^c	2.44 ^{ab}	2.48 ^a	0.02	0.01	NS
Lactose, %	4.75	4.76	4.75	4.71	0.01	NS	NS
MUN	11.2	11.3	11.3	11.1	0.06	NS	NS
SCC, ×1,000	255	241	258	214	19	NS	NS
Lactation 2, ⁴ n	95	106	98	102			
DMI dry group, kg/d	14.5	14.7	15.6	14.2			
DMI lactating group, kg/d	25.7	24.4	25.4	25.6			
Milk, kg/d	41.5 ^b	41.5 ^b	43.8 ^a	43.5 ^a	0.2	0.01	NS
3.5% FCM, kg/d	44.2 ^b	44.7 ^b	47.5 ^a	46.7 ^a	0.3	0.01	NS
ECM, kg/d	43.3 ^b	43.6 ^b	46.3 ^a	45.6 ^a	0.3	0.01	NS
Fat, %	3.93 ^b	3.99 ^{ab}	4.03 ^a	3.98 ^{ab}	0.02	0.02	NS
Protein, %	2.95	2.93	2.94	2.92	0.01	NS	NS
SNF, %	6.88	6.92	6.97	6.90	0.03	NS	NS
Fat, kg	1.62 ^b	1.65 ^b	1.76 ^a	1.72 ^a	0.01	0.01	NS
Protein, kg/d	1.22 ^b	1.21 ^b	1.27 ^a	1.26 ^a	0.01	0.01	NS
SNF, kg/d	2.86 ^b	2.87 ^b	3.05 ^a	3.00 ^a	0.02	0.01	NS
Lactose, %	4.67	4.68	4.71	4.73	0.01	NS	NS
MUN	10.7 ^b	10.8 ^b	10.6 ^b	11.3 ^a	0.08	0.01	NS
SCC, ×1000	447 ^a	437 ^a	234 ^b	243 ^b	30	0.01	NS
BCS	3.35	3.36	3.37	3.31	0.01	NS	NS
BW, kg	700	701	697	691	4	NS	NS

^{a-c}Means within the same row with different superscripts differ ($P < 0.05$) by Tukey-Kramer test.

¹Experimental supplements: 75C = 75% complexed: Zn, Mn, Cu, and Co supplied at 75% of NRC (2001) requirements by Zn-, Mn-, and Cu-specific AA complexes, and cobalt glucoheptonate; 100I = 100% inorganic: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by sulfate sources; 100C = 100% complexed: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by CTM; and C/I = complexed/inorganic: Zn and Cu supplied at 100% of NRC (2001) requirements using a combination of CTM and sulfates and Co and Mn supplied with sources at 9.1 and 3.3 times NRC (2001) requirements using a combination of CTM and sulfates. For the 75C and 100C treatments, Zn, Mn, Cu and Co supplied by Zinpro, ManPro, CuPlex, and CoPro at 75 and 100% of NRC (2001) requirements; for the 100I treatment, Zn, Mn, Cu and Co supplied by ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at 100% of NRC (2001) requirements; For the C/I treatment, Zn, Mn, Cu and Co supplied by 4-Plex and ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at Zinpro Corporation's (Eden Prairie, MN) recommendations.

²Only cows that started a second lactation.

³ECM, kg = (kg of milk × 0.327) + (kg of milk fat × 12.95) + (kg of milk protein × 7.2).

⁴Cows that had at least 4 mo in lactation 2.

lactation 1, and 150 d into lactation 2. Approximately 50 mg of wet liver tissue was collected using a trocar and cannula. Liver tissue was stored in liquid nitrogen until all samples were collected. Liver samples were assayed for mineral content by flame atomic absorption spectroscopy (Michigan State University Diagnostic Laboratory, Lansing, MI).

Statistical Analyses

Production data from lactation 1 were only included in data analyses if cows completed lactation 1 and en-

tered lactation 2 of the study. Lactation data from lactation 2 were only included in data analyses if cows completed at least 4 mo in lactation 2. Statistical analyses of data for repeated measures such as milk, milk composition, liver mineral content, and claw measurements were conducted using the Multivariate model with repeated measures of JMP software (SAS Institute, Inc., Cary, NC). Effect of treatment on lactation performance was analyzed within a specific lactation. Effects of treatment on claw and liver indices were analyzed throughout the entire experimental period. Pen was used as

Figure 1. The effect of complexed trace mineral supplementation on biweekly milk yield profiles for lactation 2. Experimental supplements: 75C = 75% complexed: Zn, Mn, Cu, and Co supplied at 75% of NRC (2001) requirements by Zn-, Mn-, and Cu-specific AA complexes, and cobalt glucoheptonate; 100I = 100% inorganic: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by sulfate sources; 100C = 100% complexed: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by CTM; and C/I = complexed/inorganic: Zn and Cu supplied at 100% of NRC (2001) requirements using a combination of CTM and sulfates and Co and Mn supplied with sources at 9.1 and 3.3 times NRC (2001) requirements using a combination of CTM and sulfates. For the 75C and 100C treatments, Zn, Mn, Cu and Co supplied by Zinpro, ManPro, CuPlex, and CoPro at 75 and 100% of NRC (2001) requirements; for the 100I treatment, Zn, Mn, Cu and Co supplied by ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at 100% of NRC (2001) requirements; For the C/I treatment, Zn, Mn, Cu and Co supplied by 4-Plex and ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at Zinpro Corporation's (Eden Prairie, MN) recommendations.

the experimental unit. The model used to analyze the data was

$$Y_{ijk} = \mu + \text{trt}_i + \text{pen}_j(\text{trt}_i) + \text{period}_k + \text{trt}_i \times \text{period}_k + E_{ijk},$$

where Y_{ijk} = dependent variable; μ = overall mean; trt_i = fixed effect of the i th treatment, $i = 1, 2, 3, 4$; $\text{pen}_j(\text{trt}_i)$ = random effect of the j th pen within the i th treatment, $j = 1, \dots, 8$ (error term); period_k = fixed effect of sample collection/measurement period, $k = 1, \dots, 4$ for claw measurements, 1, ..., 3 for liver measurements, etc.; $\text{trt}_i \times \text{period}_k$ = fixed effect of the interaction between the i th treatment and the k th period; and E_{ijk} = random residual $\sim N(0, \sigma_e^2)$.

For nonrepeated measures within a lactation; that is, health and reproduction, pen was used as the experimental unit and the model was as follows:

$$Y_{ij} = \mu + \text{trt}_i + \text{pen}_j + \text{trt}_i \times \text{pen}_j + E_{ij}$$

where Y_{ij} = dependent variable; μ = overall mean; trt_i = fixed effect of the i th treatment, $i = 1, 2, 3, 4$; pen_j = random effect of the j th pen, $j = 1, \dots, 8$ (error term); $\text{trt}_i \times \text{pen}_j$ = fixed effect of the interaction between the i th treatment and the j th pen; and E_{ij} = random residual $\sim N(0, \sigma_e^2)$.

For both repeated and nonrepeated measures, the Tukey-Kramer test was used to separate means if a significant treatment effect ($P \leq 0.05$) was observed. Survival analysis was conducted on "time to event" reproductive parameters: days to first estrus, days to first service, and days open using univariate survival analysis of JMP. The χ^2 log-rank and Wilcoxon test were used to test treatment effects.

RESULTS AND DISCUSSION

Production Performance

Data listed in Table 3 are organized into 2 categories. Lactation 1 contains cows that had finished one lacta-

Table 4. The effect of complexed trace mineral (CTM) supplementation on reproductive performance of dairy cows

Item	Treatments ¹				SEM	P-value: Survival analysis (χ^2)			
	75C	100I	100C	C/I		Trt	Rep	Log-rank	Wilcoxon
Lactation 1, ² n	94	106	96	101					
First estrus, d	56 ^a	54 ^a	54 ^a	47 ^b	1.7	—	—	0.01	0.01
First service, d	66	66	65	65	1	—	—	NS	NS
Services/conception ³	2.3	2.4	2.2	1.9	0.2	NS	NS	—	—
Days open	120	118	115	104	6.4	—	—	NS	NS
Calving interval, mo ³	13.2	13.1	13	12.6	0.2	NS	NS	—	—
Lactation 2, ⁴ n	79	88	78	92					
First estrus, d	57 ^a	56 ^a	56 ^a	50 ^b	1.5	—	—	0.001	0.001
First service, d	65	64	65	64	0.6	—	—	NS	NS
Services/conception ³	2.6	2.5	2.7	2.2	0.2	NS	NS	—	—
Days open	129	132	135	116	6.1	—	—	0.02	0.04
First service conception, % ³	31.7 ^{ab}	29.6 ^b	33.5 ^{ab}	36.9 ^a	1	0.05	NS	—	—
Pregnant by 150 d, % ³	60.7 ^b	61.4 ^b	61 ^b	70.6 ^a	1.9	NS	NS	—	—
Pregnant by 200 d, % ³	73.4	73	70.4	73.9	2.8	NS	NS	—	—

^{a,b}Means within the same row with different superscripts differ ($P < 0.05$) by Tukey-Kramer test.

¹Experimental supplements: 75C = 75% complexed: Zn, Mn, Cu, and Co supplied at 75% of NRC (2001) requirements by Zn-, Mn-, and Cu-specific AA complexes, and cobalt glucoheptonate; 100I = 100% inorganic: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by sulfate sources; 100C = 100% complexed: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by CTM; and C/I = complexed/inorganic: Zn and Cu supplied at 100% of NRC (2001) requirements using a combination of CTM and sulfates and Co and Mn supplied with sources at 9.1 and 3.3 times NRC (2001) requirements using a combination of CTM and sulfates. For the 75C and 100C treatments, Zn, Mn, Cu and Co supplied by Zinpro, ManPro, CuPlex, and CoPro at 75 and 100% of NRC (2001) requirements; for the 100I treatment, Zn, Mn, Cu and Co supplied by ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at 100% of NRC (2001) requirements; For the C/I treatment, Zn, Mn, Cu and Co supplied by 4-Plex and ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at Zinpro Corporation's (Eden Prairie, MN) recommendations.

²Includes only those cows that completed lactation 1 and began lactation 2.

³Does not qualify for survival analysis because it is not a "time to event" parameter.

⁴Includes only those cows that completed lactation 2 to 200 d and were not designated "do not breed cows."

tion and includes primiparous cows. Lactation 2 included those animals that received treatments for at least 4 mo into lactation 2. All cows in lactation 2 were multiparous.

Lactation 1. Due to cows being group-fed, DM intakes were not statistically analyzed (Table 3). Cows receiving the C/I treatment produced 6.9, 5.4, and 5.5% more ($P \leq 0.05$) milk, 3.5% FCM, and ECM than 100I cows and 3.3, 2.9, and 2.9% more ($P \leq 0.05$) milk, 3.5% FCM, and ECM than 75C cows. Cows receiving the C/I treatment produced 2.8% more milk ($P \leq 0.05$) than 100C cows. Cows receiving the 75C and 100C treatments produced 3.6 and 4.2% more milk and 2.7 and 3.9% more ECM, respectively, than 100I cows ($P \leq 0.05$). In addition, 100C cows produced 3.9% more 3.5% FCM ($P \leq 0.05$) than 100I cows (Table 3).

There was no effect of treatment ($P > 0.05$) on protein, lactose, MUN, and SCC of milk or yield of milk protein (Table 3). Cows fed the 100I and 100C treatments produced milk with a higher fat content ($P \leq 0.05$) than that of C/I cows. However, 100I cows produced 3.7 and 4.6% less fat ($P \leq 0.05$), respectively, than 100C and C/I cows.

Lactation 2. Cows receiving C/I and 100C treatments produced more milk, ECM, and 3.5% FCM than 75C and 100I cows ($P \leq 0.05$). The differences in milk production among treatments were maintained throughout the entire experimental period of lactation 2 (Figure 1). Fat-corrected milk and ECM responses to treatment were similar to the milk production responses. Although not statistically significant ($P > 0.05$), cows consuming 100C produced 0.85 kg/d more ECM than those receiving C/I.

Cows supplemented with 100C and C/I produced more milk fat and protein ($P \leq 0.05$) than did 75C and 100I cows. There was no effect of treatment ($P > 0.05$) on protein and lactose contents of milk (Table 3). Cows fed the 100C treatment produced milk with a higher fat content ($P \leq 0.05$) than 75C cows. Treatment affected milk urea nitrogen, with C/I cows having higher MUN levels ($P \leq 0.05$) than cows receiving the other treatments. Overall, MUN levels were low in all groups, with none of the treatment groups averaging more than 11.3 mg/dL.

During lactation 1 there was no treatment effect on SCC; however, during lactation 2, SCC was lower ($P \leq$

Figure 2. The effect of complexed trace mineral supplementation on survival analyses of days to first estrus for lactation 1. Experimental supplements: 75C = 75% complexed: Zn, Mn, Cu, and Co supplied at 75% of NRC (2001) requirements by Zn-, Mn-, and Cu-specific AA complexes, and cobalt glucoheptonate; 100I = 100% inorganic: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by sulfate sources; 100C = 100% complexed: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by CTM; and C/I = complexed/inorganic: Zn and Cu supplied at 100% of NRC (2001) requirements using a combination of CTM and sulfates and Co and Mn supplied with sources at 9.1 and 3.3 times NRC (2001) requirements using a combination of CTM and sulfates. For the 75C and 100C treatments, Zn, Mn, Cu and Co supplied by Zinpro, ManPro, CuPlex, and CoPro at 75 and 100% of NRC (2001) requirements; for the 100I treatment, Zn, Mn, Cu and Co supplied by ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at 100% of NRC (2001) requirements; For the C/I treatment, Zn, Mn, Cu and Co supplied by 4-Plex and ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at Zinpro Corporation's (Eden Prairie, MN) recommendations.

0.05) for 100C and C/I cows than for 75C and 100I cows (Table 3). Somatic cell counts of 100C cows were 46.5 and 47.7% lower than those of 100I and 75C cows, respectively, and SCC of C/I cows were 44.4 and 45.6% lower than those of 100I and 75C cows. Cows on 75C and 100I treatments had unusually high SCC relative to cows on other treatments and nontrial herd mates. There was no effect of treatment ($P > 0.05$) on BCS or BW.

Production levels were higher in lactation 2 than in lactation 1 primarily because more first-calf heifers were present in lactation 1 and they normally produce less milk than in subsequent lactations. In lactation 2, there was a clear delineation between 75C and 100I vs. 100C and C/I groups. Increase in lactation performance with the 100C treatment vs. 75C treatment can be attributed to increased supply of Zn, Mn, Cu, and Co, whereas increased lactation performance with the 100C treatment vs. the 100I treatment can be attributed to increased bioavailability of Zn, Mn, Cu, and Co. Similarly, Ballantine et al. (2002) reported increased lacta-

tion performance when a portion of the Zn, Mn, Cu, and Co from sulfate sources was replaced with CTM. 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 CTM. 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 was replaced with CTM. Level of trace mineral fortification of diets was not indicative of whether lactation performance will improve in response to replacing inorganic trace minerals with CTM. Diets in the Ballantine et al. (2002) and Uchida et al. (2001) studies were fortified well in excess of NRC (2001) Zn, Mn, Cu, and Co requirements, whereas only dietary Co and Mn concentrations in the Kincaid and Socha (2004) and Ferguson et al. (2004a) studies were fortified well in excess of NRC (2001) requirements.

In general, milk composition does not appear to be affected by level or source of trace mineral supplementa-

Figure 3. The effect of complexed trace mineral supplementation on survival analyses of days to first estrus for lactation 2. Experimental supplements: 75C = 75% complexed: Zn, Mn, Cu, and Co supplied at 75% of NRC (2001) requirements by Zn-, Mn-, and Cu-specific AA complexes, and cobalt glucoheptonate; 100I = 100% inorganic: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by sulfate sources; 100C = 100% complexed: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by CTM; and C/I = complexed/inorganic: Zn and Cu supplied at 100% of NRC (2001) requirements using a combination of CTM and sulfates and Co and Mn supplied with sources at 9.1 and 3.3 times NRC (2001) requirements using a combination of CTM and sulfates. For the 75C and 100C treatments, Zn, Mn, Cu and Co supplied by Zinpro, ManPro, CuPlex, and CoPro at 75 and 100% of NRC (2001) requirements; for the 100I treatment, Zn, Mn, Cu and Co supplied by ZnSO_4 , MnSO_4 , CuSO_4 and CoSO_4 at 100% of NRC (2001) requirements; For the C/I treatment, Zn, Mn, Cu and Co supplied by 4-Plex and ZnSO_4 , MnSO_4 , CuSO_4 and CoSO_4 at Zinpro Corporation's (Eden Prairie, MN) recommendations.

tation (Uchida et al., 2001; Ballantine et al., 2002; Kellogg et al., 2003); however, Ferguson et al. (2004a) and Kincaid and Socha (2004) reported higher milk protein content for cows supplemented with CTM.

Reproductive Performance

Reproductive performance was divided into 2 categories: cows that completed lactation 1 and those that entered lactation 2 with reproductive data through 200 DIM. The effect of replication was not significant ($P > 0.05$).

Lactation 1. There was no effect of treatment ($P > 0.05$) on days to first service, services per conception, days open, or calving interval (Table 4). There was a numeric tendency ($P > 0.05$) for services per conception, days open, and the calving interval to be lowest for C/I cows. Cows receiving the C/I treatment had fewer days to first estrus ($P \leq 0.05$) than cows receiving 1 of the other 3 treatments. In addition, survival analysis of days to first estrus indicated that more C/I cows

cycled sooner after calving than cows fed the other treatments (Figure 2, log-rank test, $P \leq 0.05$).

Lactation 2. There was no effect of treatment ($P > 0.05$) on days to first service, services per conception, days open, and percentage of cows pregnant at 200 d postpartum (Table 4). More C/I cows were pregnant ($P \leq 0.05$) at 150 d postpartum than cows receiving the other treatments. Cows receiving the C/I treatment cycled sooner after calving ($P \leq 0.05$) than cows fed the other treatments (Table 4). Cows fed the C/I treatment also had higher first-service conception rates ($P \leq 0.05$) than 100I cows. Survival analysis of the days to first estrus data indicated that C/I cows cycled for the first time postcalving at a faster rate than cows fed the other treatments (Figure 3, $P \leq 0.05$). Furthermore, C/I cows conceived at a faster rate than cows fed the other treatments (Figure 3, $P \leq 0.05$); this was reflected in more C/I cows being pregnant ($P \leq 0.05$) at 150 d postpartum than cows fed the other treatments.

For both lactation periods, days to first estrus was lower for cows receiving the C/I regimen compared with

Table 5. Effect of trace mineral complexed supplementation on health parameters in dairy cattle

Item	Treatments ¹				SEM	P
	75C	100I	100C	C/I		
Lactation 1, ² n	143	152	151	147		
	(% of cows)					
Retained placenta	9.9	8	15.9	9.5	2.9	NS
Metritis	18.9	15.9	21.9	21.8	3.8	NS
Displaced abomasum	6.3	8.6	8.7	8.2	1.5	NS
Ketosis	7.7	9.8	8.7	10.2	3.6	NS
Mastitis	15.5	16.5	23.8	21.1	4	NS
Lactation 2, ³ n	87	104	90	99		
	(% of cows)					
Retained placenta	10.2	10.7	15.3	10.1	2.3	NS
Metritis	9.2	14.4	16.3	15.6	4.6	NS
Displaced abomasum	8	8.9	12.2	9.2	1.4	NS
Ketosis	17.4	6.7	17.5	13.2	2.7	NS
Mastitis	20.9	23	26.3	15.2	5	NS

¹Experimental supplements: 75C = 75% complexed: Zn, Mn, Cu, and Co supplied at 75% of NRC (2001) requirements by Zn-, Mn-, and Cu-specific AA complexes, and cobalt glucoheptonate; 100I = 100% inorganic: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by sulfate sources; 100C = 100% complexed: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by CTM; and C/I = complexed/inorganic: Zn and Cu supplied at 100% of NRC (2001) requirements using a combination of CTM and sulfates and Co and Mn supplied with sources at 9.1 and 3.3 times NRC (2001) requirements using a combination of CTM and sulfates. For the 75C and 100C treatments, Zn, Mn, Cu and Co supplied by Zinpro, ManPro, CuPlex, and CoPro at 75 and 100% of NRC (2001) requirements; for the 100I treatment, Zn, Mn, Cu and Co supplied by ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at 100% of NRC (2001) requirements; For the C/I treatment, Zn, Mn, Cu and Co supplied by 4-Plex and ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at Zinpro Corporation's (Eden Prairie, MN) recommendations.

²Includes only those cows that completed lactation 1 and began lactation 2.

³Includes only those cows that completed lactation 2 to 200 d and were not designated "do not breed cows."

others. Although not significant ($P > 0.05$), days open followed the same pattern during lactation 2, which agrees with others (Campbell and Miller, 1998; Uchida et al., 2001; Ballantine et al., 2002). Kellogg et al. (2003) also demonstrated in the 8-cow summary that cows fed complexed zinc, manganese, copper, and cobalt had fewer days open and fewer days to first service.

In both lactations 1 and 2, treatments containing 100% of NRC levels of trace minerals from either CTM or sulfates performed similarly to cows supplemented at 75% of NRC (2001) requirements using CTM. Reproductive responses were most favorable when cows were supplemented with Zn and Cu at NRC (2001) recommendations using a combination of CTM and sulfates and Co and Mn at 9.1 and 3.3 times NRC (2001) requirements. Improved fertility may be reflective of Mn supply in particular, because Mn 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 manganese supplementation (Brown and Casillas, 1986).

Health

Health data are reported as 2 categories: cows that completed at least 50 d in lactation 1 or 2. During lactation 1, there were no significant ($P > 0.05$) effects of treatment on health parameters (Table 5). Replacing inorganic Zn, Mn, Cu, and Co with CTM has resulted in only numeric effects on incidence of health disorders in other studies (Ballantine et al., 2002; Ferguson et al., 2004a).

Claw Characteristics

Solar hemorrhaging had the highest incidence (16.1 to 19.7%) of all claw characteristics measured and was not affected by treatment ($P > 0.05$). The incidences of WLS and HE were affected by treatment ($P \leq 0.05$, Table 6). The incidence of WLS was generally <2% and was lowest for cows receiving 100I compared with cows receiving 75C, whereas 100C and C/I were not different from either. Heel erosion was lowest for those supplemented with C/I compared with 100I, whereas 75I and 100C were not different from either. The incidence of sole ulceration and DD was <1% for all treatment regi-

Table 6. Effect of complexed trace mineral (CTM) supplementation on the incidence and severity of foot characteristics of cows with 2 lactations

Item	Treatments ¹				SEM	Probability of significance			
	75C	100I	100C	C/I		Trt	R(Trt)	Period	Period × Trt
Cows/treatment, n	95	99	99	100					
Incidence (% of cows)									
Solar hemorrhage	19.7	19.2	18.0	16.1	1.5	NS	NS	0.01	NS
White line separation	2.1 ^a	1.0 ^b	1.4 ^{ab}	1.1 ^{ab}	0.3	0.05	NS	0.05	NS
Heel erosion	5.4 ^{ab}	8.2 ^a	6.0 ^{ab}	4.9 ^b	0.8	0.05	NS	0.05	NS
Sole ulcer	0.7	0.4	0.6	0.7	0.1	NS	NS	0.01	NS
PDD ²	0.3	0.3	0.6	0.3	0.1	NS	NS	NS	NS
Severity									
Solar hemorrhage	1.4	1.4	1.3	1.3	0.03	NS	NS	0.01	0.05
White line separation	1.3 ^{ab}	1.5 ^a	1.2 ^b	1.2 ^b	0.07	0.05	NS	NS	NS
Heel erosion	1.8	1.9	1.8	1.7	0.30	NS	NS	NS	NS
Sole ulcer	1.5	1.8	1.5	1.8	0.10	NS	NS	0.05	NS
PDD ²	1.1	1.2	1.2	1.1	0.10	NS	NS	NS	NS

^{a,b}Means within the same row with different superscripts differ ($P < 0.05$) by Tukey-Kramer test.

¹Experimental supplements: 75C = 75% complexed: Zn, Mn, Cu, and Co supplied at 75% of NRC (2001) requirements by Zn-, Mn-, and Cu-specific AA complexes, and cobalt glucoheptonate; 100I = 100% inorganic: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by sulfate sources; 100C = 100% complexed: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by CTM; and C/I = complexed/inorganic: Zn and Cu supplied at 100% of NRC (2001) requirements using a combination of CTM and sulfates and Co and Mn supplied with sources at 9.1 and 3.3 times NRC (2001) requirements using a combination of CTM and sulfates. For the 75C and 100C treatments, Zn, Mn, Cu and Co supplied by Zinpro, ManPro, CuPlex, and CoPro at 75 and 100% of NRC (2001) requirements; for the 100I treatment, Zn, Mn, Cu and Co supplied by ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at 100% of NRC (2001) requirements; For the C/I treatment, Zn, Mn, Cu and Co supplied by 4-Plex and ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at Zinpro Corporation's (Eden Prairie, MN) recommendations.

²PDD = Papillomatosis digital dermatitis.

mens. The only severity parameters affected by treatment regimen was WLS ($P \leq 0.05$), which was lowest for cows receiving the 100C and C/I treatments compared with those receiving 100I.

For all treatments, the incidence of solar hemorrhaging (Table 7) tended to elevate after the first period (dry, pretreatment), then decline with subsequent periods. The only significant period effect ($P \leq 0.05$) was for 75C, which had a higher incidence in the second period (150 DIM, lactation 1) compared with period 1. During period 3 (start of second dry period), animals treated with 100C had the highest and C/I the lowest incidence of SH. Solar hemorrhaging is a clinical manifestation of subclinical laminitis, which is thought to be a hemodynamic process that is aggravated by parturition and subsequent lactations (Boosman et al., 1989). The highest proportions of first-lactation cows were on trial during period 1. In addition, these claw evaluations occurred in the dry period. Researchers (Nocek, 1997; Donovan et al., 2004) have found that dry cows generally have a lower incidence of claw disorders than lactating cows. Others (Bergsten, 1994; Bradley et al., 1989) have shown SH to be as high as 80% in first-lactation cows.

Severity of SH increased from period 1 to 3, and then declined for the fourth period. Period differences were significant ($P \leq 0.05$) for all treatments. The only treat-

ment effect occurred in period 3, in which cows supplemented with 100I had a higher severity score than C/I, with other treatments not being different from either. The most likely explanation for this occurrence is that all cows were younger and dry during period 1. Ballantyne et al. (2002) reported an incidence of SH of 0 for control and 4% for cows receiving CTM. Nocek et al. (2000) reported an incidence of SH of up to 80%, with occurrence being highest in the hind feet.

The overall incidence of WLS in the present study was <3% (Table 8). Period within treatment incidence of WLS was higher ($P \leq 0.05$) in period 4 than in period 1 for cows consuming C/I. Severity scores for WLS were not influenced by period. Incidence of white line disease was 14.6 and 9.5% for control and CTM-supplemented cows, respectively, at 75 DIM and 8.8 vs. 4.9% at 250 DIM (Ballantyne et al., 2002). Nocek et al. (2000) showed that the incidence of WLS was reduced from 33.6 to 15.4% with supplementation of CTM. These researchers also showed WLS incidence to increase from 6 to 18% from lactation 1 to 3.

Heel erosion (Table 9) was influenced ($P \leq 0.05$) by treatment during period 4 with animals supplemented with C/I demonstrating the lowest incidence and animals supplemented, with 100I demonstrating the highest incidence. Cows supplemented with 100I exhibited the highest ($P \leq 0.05$) incidence during periods 3 and

Table 7. Effect of complexed trace mineral (CTM) supplementation on the incidence and severity of sole hemorrhage by period for cows with 2 lactations

Variable	Treatments ¹			
	75C	100I	100C	C/I
n	99	99	99	100
Incidence (% of cows)				
Period ²				
1	12.1 ^b	13.6	14.1	15.4
2	28.4 ^a	23.5	20.6	19.3
3	18.5 ^{ab,AB}	20.9 ^{AB}	22.0 ^A	13.9 ^B
4	19.7 ^{ab}	18.6	15.2	16.0
SEM	0.30	2.9	2.4	2.7
Probability of difference	0.01	NS	NS	NS
Severity				
Period				
1	1.16 ^b	1.16 ^b	1.18 ^c	1.19 ^b
2	1.40 ^a	1.31 ^{ab}	1.26 ^{bc}	1.25 ^{ab}
3	1.48 ^{a,AB}	1.65 ^{a,A}	1.55 ^{a,AB}	1.34 ^{ab,B}
4	1.42 ^a	1.37 ^{ab}	1.38 ^{ab}	1.38 ^a
SEM	0.05	0.06	0.05	0.06
Probability of difference	0.01	0.01	0.01	0.01

^{a-c}Period means within the same column with different superscripts differ ($P < 0.05$) by Tukey-Kramer test.

^{AB}Treatment means within the same row with different superscripts differ ($P < 0.05$) by Tukey-Kramer test.

¹Experimental supplements: 75C = 75% complexed: Zn, Mn, Cu, and Co supplied at 75% of NRC (2001) requirements by Zn-, Mn-, and Cu-specific AA complexes, and cobalt glucoheptonate; 100I = 100% inorganic: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by sulfate sources; 100C = 100% complexed: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by CTM; and C/I = complexed/inorganic: Zn and Cu supplied at 100% of NRC (2001) requirements using a combination of CTM and sulfates and Co and Mn supplied with sources at 9.1 and 3.3 times NRC (2001) requirements using a combination of CTM and sulfates. For the 75C and 100C treatments, Zn, Mn, Cu and Co supplied by Zinpro, ManPro, CuPlex, and CoPro at 75 and 100% of NRC (2001) requirements; for the 100I treatment, Zn, Mn, Cu and Co supplied by ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at 100% of NRC (2001) requirements; For the C/I treatment, Zn, Mn, Cu and Co supplied by 4-Plex and ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at Zinpro Corporation's (Eden Prairie, MN) recommendations.

²Periods: 1 = start of trial (40 to 60 d before expected calving date); 2 = 150 DIM: Lactation 1; 3 = start of second dry period; 4 = 200 DIM: Lactation 2.

4 and the lowest incidence during period 1. During period 2, cows supplemented with 100I and 100C had higher severity scores than cows supplemented with 75C and C/I. During period 3, cows supplemented with 100I had higher severity than those supplemented with 100C. For period 4, cows supplemented with 100I had a higher severity score than cows supplemented with C/I. Within treatment, cows supplemented with 100C had the lowest severity score during period 3 and highest severity score during period 2. For C/I cows, HE severity was less in the period 4 than in period 1 or 2 (1.43 vs. 1.98 and 1.71). Ferguson et al. (2004b) indicated that the heel erosions were the most frequent

Table 8. Effect of complexed trace mineral (CTM) supplementation on the incidence and severity of white line separation by period for cows with 2 lactations

Variable	Treatments ¹			
	75C	100I	100C	C/I
n	99	99	99	100
Incidence (% of cows)				
Period ²				
1	1.3	0.7	1.2	0.4 ^b
2	2.5	0.8	1.6	1.3 ^{ab}
3	1.8	1.2	1.0	0.9 ^{ab}
4	2.6	1.4	1.6	2.0 ^a
SEM	0.65	0.45	0.5	0.43
Probability of difference	NS	NS	NS	0.05
Severity				
Period				
1	1.53	1.5	1.07	1.0
2	1.33	1.47	1.33	1.3
3	1.21	1.30	1.0	1.3
4	1.25	1.67	1.45	1.40
SEM	0.13	0.17	0.12	0.14
Probability of difference	NS	NS	NS	NS

^{a,b}Period means within the same column with different superscripts differ ($P < 0.05$) by Tukey-Kramer test.

¹Experimental supplements: 75C = 75% complexed: Zn, Mn, Cu, and Co supplied at 75% of NRC (2001) requirements by Zn-, Mn-, and Cu-specific AA complexes, and cobalt glucoheptonate; 100I = 100% inorganic: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by sulfate sources; 100C = 100% complexed: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by CTM; and C/I = complexed/inorganic: Zn and Cu supplied at 100% of NRC (2001) requirements using a combination of CTM and sulfates and Co and Mn supplied with sources at 9.1 and 3.3 times NRC (2001) requirements using a combination of CTM and sulfates. For the 75C and 100C treatments, Zn, Mn, Cu and Co supplied by Zinpro, ManPro, CuPlex, and CoPro at 75 and 100% of NRC (2001) requirements; for the 100I treatment, Zn, Mn, Cu and Co supplied by ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at 100% of NRC (2001) requirements; For the C/I treatment, Zn, Mn, Cu and Co supplied by 4-Plex and ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at Zinpro Corporation's (Eden Prairie, MN) recommendations.

²Periods: 1 = start of trial (40 to 60 d before expected calving date); 2 = 150 DIM: Lactation 1; 3 = start of second dry period; 4 = 200 DIM: Lactation 2.

lesion observed in all treatments and periods, with no effect of CTM supplementation on incidence. Ballantine et al. (2002) reported an incidence of HE of 15.8 vs. 12.2 for inorganic and organic trace mineral supplementation, respectively, at 75 DIM and 0 vs. 0 at 250 DIM. Livesey et al. (1998) found that diet had little effect on the incidence of HE; however, its prevalence increased in early lactation. They concluded that metabolic stress in late pregnancy or early lactation contributed to HE development. Heel erosion has been characterized as an infectious disease that occurs independently of other claw diseases. The incidence of SU (Table 10) was generally less than 1.0%. A period effect was exhibited for cows consuming C/I—a higher incidence of ulcers was observed in period 4 compared with period 1. Cows supplemented with C/I had the highest ($P < 0.01$) sever-

Table 9. Effect of complexed trace mineral (CTM) supplementation on the incidence and severity of heel erosion by period for cows with 2 lactations

Variable	Treatments ¹			
	75C	100I	100C	C/I
n	95	99	99	100
Incidence (% of cows)				
Period ²				
1	4.4	4.3 ^b	2.9	3.9
2	6.2	7.7 ^{ab}	5.9	5.8
3	4.7	9.9 ^a	7.4	4.8
4	6.2 ^{AB}	10.9 ^{a,A}	7.7 ^{AB}	5.4 ^B
SEM	1.4	2.0	1.9	1.4
Probability of difference	NS	0.05	NS	NS
Severity				
Period				
1	1.78	1.95	1.87 ^{ab}	1.98 ^a
2	1.72 ^B	1.97 ^A	1.95 ^{a,A}	1.71 ^{b,B}
3	1.84 ^{AB}	1.91 ^A	1.73 ^{b,B}	1.86 ^{ab,AB}
4	1.76 ^{AB}	1.96 ^A	1.79 ^{ab,AB}	1.43 ^{c,B}
SEM	0.05	0.28	0.04	0.07
Probability of difference	NS	NS	0.05	0.05

^{a-c}Period means within the same column with different superscripts differ ($P < 0.05$) by Tukey-Kramer test.

^{AB}Treatment means within the same row with different superscripts differ ($P < 0.05$) by Tukey-Kramer test.

¹Experimental supplements: 75C = 75% complexed: Zn, Mn, Cu, and Co supplied at 75% of NRC (2001) requirements by Zn-, Mn-, and Cu-specific AA complexes, and cobalt glucoheptonate; 100I = 100% inorganic: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by sulfate sources; 100C = 100% complexed: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by CTM; and C/I = complexed/inorganic: Zn and Cu supplied at 100% of NRC (2001) requirements using a combination of CTM and sulfates and Co and Mn supplied with sources at 9.1 and 3.3 times NRC (2001) requirements using a combination of CTM and sulfates. For the 75C and 100C treatments, Zn, Mn, Cu and Co supplied by Zinpro, ManPro, CuPlex, and CoPro at 75 and 100% of NRC (2001) requirements; for the 100I treatment, Zn, Mn, Cu and Co supplied by ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at 100% of NRC (2001) requirements; For the C/I treatment, Zn, Mn, Cu and Co supplied by 4-Plex and ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at Zinpro Corporation's (Eden Prairie, MN) recommendations.

²Periods: 1 = start of trial (40 to 60 d before expected calving date); 2 = 150 DIM: Lactation 1; 3 = start of second dry period; 4 = 200 DIM: Lactation 2.

ity during periods 2 and 4 compared with periods 1 and 3. The incidence and severity of DD was <1% for any given period and was not affected ($P > 0.05$) by treatment or period within treatment.

Liver Mineral Concentrations

Liver Zn concentration was highest ($P < 0.05$) for cows supplemented with 100C compared with those supplemented with 100I and C/I, with 75C not different from other treatments (Table 11). There were no period within treatment effects for Zn. Liver Cu concentration was higher for 100C and C/I ($P < 0.05$) groups compared with 75C and 100I groups. Hepatic Cu concentration was affected ($P < 0.01$) by period for each treatment

Table 10. Effect of complexed trace mineral (CTM) supplementation on the incidence and severity of sole ulcers by period for cows with 2 lactations

Variable	Treatments ¹			
	75C	100I	100C	C/I
n	95	99	99	100
Incidence (% of cows)				
Period ²				
1	0.3	0.24	0.14	0.2 ^b
2	1.1	0.65	0.90	1.1 ^{ab}
3	0.4	0.24	0.63	0.54 ^{ab}
4	0.9	0.49	0.88	1.2 ^a
SEM	0.29	0.22	0.37	0.25
Probability of difference	NS	NS	NS	0.05
Severity				
Period				
1	1.6	1.3	1.0	1.0 ^b
2	1.3 ^b	1.7 ^{ab}	1.5 ^{ab}	1.9 ^a
3	1.7	2.0	1.67	1.5 ^b
4	1.45	2.0	1.67	2.0 ^a
SEM	0.22	0.38	0.24	0.13
Probability of difference	NS	NS	NS	0.01

^{a,b}Period means within the same column with different superscripts differ ($P < 0.05$) by Tukey-Kramer test.

¹Experimental supplements: 75C = 75% complexed: Zn, Mn, Cu, and Co supplied at 75% of NRC (2001) requirements by Zn-, Mn-, and Cu-specific AA complexes, and cobalt glucoheptonate; 100I = 100% inorganic: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by sulfate sources; 100C = 100% complexed: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by CTM; and C/I = complexed/inorganic: Zn and Cu supplied at 100% of NRC (2001) requirements using a combination of CTM and sulfates and Co and Mn supplied with sources at 9.1 and 3.3 times NRC (2001) requirements using a combination of CTM and sulfates. For the 75C and 100C treatments, Zn, Mn, Cu and Co supplied by Zinpro, ManPro, CuPlex, and CoPro at 75 and 100% of NRC (2001) requirements; for the 100I treatment, Zn, Mn, Cu and Co supplied by ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at 100% of NRC (2001) requirements; For the C/I treatment, Zn, Mn, Cu and Co supplied by 4-Plex and ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at Zinpro Corporation's (Eden Prairie, MN) recommendations.

²Periods: 1 = start of trial (40 to 60 d before expected calving date); 2 = 150 DIM: Lactation 1; 3 = start of second dry period; 4 = 200 DIM: Lactation 2.

whereby it was lowest at trial initiation and increased linearly from 150 DIM to the end of the study. There was no treatment effect ($P > 0.05$) on liver Mn concentration, but there was a period effect ($P < 0.01$). Manganese was low at trial initiation, increased to a threshold at 150 DIM, and then stabilized.

Wright and Spears (2001) evaluated Zn source and level on Zn metabolism in bull calves. Calves supplemented with zinc sulfate at 20 mg/kg of DM had a higher liver concentration of Zn than calves supplemented with zinc proteinate at the same level of inclusion. However, when calves were switched to a higher level of zinc (500 mg of Zn/kg of DM), liver Zn concentration was higher for calves fed the zinc proteinate than zinc sulfate. Sneed et al. (2001) dosed supplemental Zn, Cu, Mn, and Co at up to 4 times the level of the same

Table 11. The effect of complexed trace mineral (CTM) supplementation on liver mineral concentrations at different times

Item	Treatments ¹				SEM	P-value		
	75C	100I	100C	C/I		Treatment	Period	Trt × Period
Zinc (ppm dry wt)								
Treatment	105.9 ^{ab}	103 ^b	111.7 ^a	104.1 ^b	1.9	0.01	NS	NS
Period								
Trial initiation	104.9	106.1	113.6	108.6				
150 DIM	104.6	103.2	111.7	100.9				
End of trial	108.6	99.3	110.8	103.3				
SEM	3.2	2.7	3.9	3.5				
P	NS	NS	NS	NS				
Copper (ppm dry wt)								
Treatment	531 ^b	521 ^b	575 ^a	570 ^a	16	0.05	0.01	NS
Period								
Trial initiation	419.2	381.8	439.2	438.6				
150 DIM	532.8	542.5	602.7	614.4				
End of trial	642.5	639.6	681	657.38				
SEM	27.4	25.7	25.8	30.7				
P	0.01	0.01	0.01	0.01				
Manganese (ppm dry wt)								
Treatment	9.3	9.4	9.6	9.2	0.2	NS	0.01	NS
Period								
Trial initiation	8.3	8.2	8.1	8.2				
150 DIM	9.6	9.9	10.2	9.8				
End of trial	9.8	10	10.6	9.5				
SEM	0.3	0.24	0.39	0.23				
P	0.05	0.01	0.01	0.01				

^{a,b}Means within the same row with different superscripts differ ($P < 0.05$) by Tukey-Kramer test.

¹Experimental supplements: 75C = 75% complexed: Zn, Mn, Cu, and Co supplied at 75% of NRC (2001) requirements by Zn-, Mn-, and Cu-specific AA complexes, and cobalt glucoheptonate; 100I = 100% inorganic: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by sulfate sources; 100C = 100% complexed: Zn, Mn, Cu, and Co supplied at 100% of NRC (2001) requirements by CTM; and C/I = complexed/inorganic: Zn and Cu supplied at 100% of NRC (2001) requirements using a combination of CTM and sulfates and Co and Mn supplied with sources at 9.1 and 3.3 times NRC (2001) requirements using a combination of CTM and sulfates. For the 75C and 100C treatments, Zn, Mn, Cu and Co supplied by Zinpro, ManPro, CuPlex, and CoPro at 75 and 100% of NRC (2001) requirements; for the 100I treatment, Zn, Mn, Cu and Co supplied by ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at 100% of NRC (2001) requirements; For the C/I treatment, Zn, Mn, Cu and Co supplied by 4-Plex and ZnSO₄, MnSO₄, CuSO₄ and CoSO₄ at Zinpro Corporation's (Eden Prairie, MN) recommendations.

product used in the present study and show no significant dose effect on liver mineral concentration at -21, 7, 50, and 120 DIM. In the present study, liver Cu increased with time and was higher for the 100C and C/I treatments. This suggests that liver Cu concentrations respond to Cu source.

Trace mineral content of liver indicated that all trace mineral supplementation regimens provided sufficient amounts of Zn, Mn, and Cu over 2 lactations and dry periods to maintain adequate trace mineral status (Puls, 1994). Despite cows having adequate Zn, Mn, and Cu, as indicated by trace mineral content of liver, performance of cows was affected by level and source of trace minerals. 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 and levels of trace minerals. Potential explanations include trace minerals in liver being in a form that is of limited availability for metabolism or stores of Zn, Mn, and Cu are 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 (Sneed et al., 2001; Ballantine et al., 2002; Ferguson et al., 2004a,b) in which cows had adequate Zn, Mn, Cu, and Co status and despite a lack of a treatment effect on liver trace mineral content, lactation performance, fertility, and claw integrity were improved when CTM was included in the diet.

CONCLUSIONS

The results of this study suggest that cows could be supplemented with 75% of NRC requirements for Zn, Cu, Mn, and Co using CTM and achieve similar reproductive, health, and productive performance as cows receiving 100% of NRC (2001) requirements for these minerals in an inorganic form. Cows supplemented with 100% of NRC (2001) requirements from CTM demonstrated similar reproductive and health performance as cows supplemented with the same elements in the inorganic form; however, milk and component yields

were higher and severity of white line separation was lower for the former. Improved fertility for C/I cows vs. cows receiving the other treatments maybe attributed to increased supply of Mn, Co, or both and indicates that current NRC (2001) requirements may not provide adequate amounts of Mn and Co for maximizing fertility of dairy cattle.

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