Effect of copper, zinc, and manganese supplementation and source on reproduction, mineral status, and performance in grazing beef cattle over a two-year period[1,2,3]

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ABSTRACT: Crossbred, multiparous beef cows (n = 178 in Year 1; n = 148 in Year 2) were used to evaluate the effects of Cu, Zn, and Mn supplementation and source on reproduction, mineral status, and performance in grazing cattle in eastern Colorado over a 2-yr period. Cows were stratified by expected calving date, age, BW, BCS, and liver mineral status and assigned to the following treatments: 1) control (no supplemental Cu, Zn, or Mn); 2) organic (ORG; 50% organic and 50% inorganic Cu, Zn, and Mn); and 3) inorganic (ING; 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$). Free-choice mineral feeders were used to provide current NRC-recommended concentrations of Cu, Zn, and Mn from 82 d (Year 1) and 81 d (Year 2) before the average calving date of the herd through 110 d (Year 1) and 135 d (Year 2) after calving. At the end of Year 1, supplemented cows had greater liver Cu ($P < 0.01$), Zn ($P < 0.05$), and Mn ($P < 0.01$) concentrations compared with controls, whereas liver Cu concentration was greater ($P < 0.01$) in ORG vs. ING cows. At the end of Year 2, supplemented cows had greater ($P < 0.01$) liver Cu concentrations relative to controls, whereas control cows had greater ($P < 0.02$) liver Mn concentration than did supplemented cows. In Year 1, pregnancy rate to AI in control cows did not differ ($P = 0.47$) from supplemented cows, but there was a trend ($P < 0.08$) for pregnancy rate to be higher for ORG than ING cows. In Year 2, supplemented cows had a higher ($P < 0.02$) pregnancy rate to AI than controls. In both years, when cows were inseminated after an observed estrus, supplemented cows had a higher ($P < 0.04$) pregnancy rate than did controls. Also, for both years, overall 60-d pregnancy rate tended ($P = 0.10$) to be higher for supplemented cows than for controls. In Year 1, kilograms of calf weaned per cow exposed was greater ($P < 0.02$) in controls than in supplemented cows, and kilograms of calf weaned per cow exposed was greater ($P < 0.01$) in ING than ORG treatments. However, in Year 2, kilograms of calf weaned per cow exposed was greater ($P < 0.02$) in controls than in supplemented cows, and tended ($P = 0.09$) to be greater in ORG than ING treatments. Results indicate that supplementation and source of trace minerals affected mineral status and kilograms of calf weaned per cow exposed in grazing beef cows. Supplementation also improved pregnancy rate to AI compared with cows not supplemented with Cu, Zn, or Mn for more than 1 yr. Furthermore, mineral source may influence pregnancy rate to AI.

Key Words: Beef Cattle, Performance, Reproduction, Trace Minerals

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Introduction

Responses in reproduction and performance to Cu, Zn, and Mn supplementation in ruminants have been variable (Underwood and Suttle, 1999). Olson et al. (1999) reported no difference in reproductive performance in cows supplemented with organic vs. inorganic forms of trace minerals. However, supplemented cows had lower pregnancy rates compared with controls, most likely because supplementation levels were two times NRC (1996) recommendations. In contrast, Stan-

[1]Use of trade names in this publication does not imply endorsement by Colorado State Univ. or criticism of similar products not mentioned.

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ton et al. (2000) reported that cows receiving organic trace minerals exhibited higher pregnancy rates to AI than those receiving inorganic trace minerals. Improved reproductive performance has been reported in dairy cows receiving organic mineral supplements (Manspeaker et al., 1987), which was attributed to improved repair of damaged uterine tissue following calving.

The increase in performance associated with organic trace minerals may be due to increased bioavailability that has been reported in several species. Du et al. (1996) and Kegley and Spears (1994) suggested that organic Cu sources are equally, if not more, bioavailable than $CuSO_4$ in rats and growing calves, respectively. Conversely, Cao et al. (2000) compared eight organic Zn products in chicks and lambs and found that only one was more bioavailable than $ZnSO_4$.

There has been tremendous variability in the concentration and source of trace mineral supplementation used in different studies, making interpretation of data and ability to reach a concise conclusion challenging. We hypothesized that not supplementing trace minerals would be detrimental to cow reproductive performance and that source of trace mineral supplementation may have different effects on reproductive performance in cows and on calf performance. The objective of this study was to determine the effect of the supplementation and source of Cu, Zn, and Mn at levels recommended by NRC (1996) on reproductive performance, mineral status, and performance in grazing beef cattle.

Materials and Methods

Experimental Design

Before starting this study, all animal use, handling, and sampling techniques described herein were approved by the Colorado State University Animal Care and Use Committee.

Twelve days before the start of this experiment (January 17, 2001), 178 crossbred (Red Angus-based), multiparous beef cows at the Eastern Colorado Research Center (Akron, CO) were stratified based on age, expected calving date, BW, BCS, and liver mineral status (Table 1) and randomly assigned to one of nine replicates. The replicates were then assigned to one of three treatments (n = 19 to 20 cows per replicate), resulting in three replicates per treatment in each year. Treatments were as follows: 1) control (no supplemental Cu, Zn, or Mn; n = 59); 2) organic (**ORG**; 50% organic and 50% inorganic Cu, Zn, and Mn; n = 60); and 3) inorganic (**ING**; 100% inorganic Cu, Zn, and Mn; n = 59).

All procedures described below were repeated over two consecutive years, except where noted. Cows remained in the same treatment for both years. Inorganic trace minerals were supplemented as $CuSO_4$, $ZnSO_4$, and $MnSO_4$, whereas organic trace minerals were provided from a commercially available mineral proteinate source (Bioplexes, Alltech Inc., Nicholasville, KY). Salt was added to the supplements to limit consumption of Cu, Zn, and Mn to approximately NRC (1996) recommended levels. Vitamins A, D, and E were added to meet NRC (1996) requirements and mixed thoroughly by hand at the time of mineral delivery to trace mineral feeders. Ingredient composition and laboratory analysis of the basal supplement and trace mineral treatments are shown in Table 2. Basal forage and water trace mineral concentrations were determined using samples collected from pasture, stored hay, and water sources. Trace mineral concentrations were as follows: pasture = 13.1 ppm Cu, 16.1 ppm Zn, and 36.6 ppm Mn; stored hay = 19.6 ppm Cu, 32.1 ppm Zn, and 52.2 ppm Mn; and water <0.01 ppm Cu, <0.01 ppm Zn, and 0.08 ppm Mn.

After replicates and treatments were assigned, animals were housed by replicate in nine separate pastures. Cows were maintained on native pastures that consisted primarily of blue grama (*Bouteloua gracilis*), prairie sandreed (*Calamovilfa longifolia*), and needle-and-thread grass (*Stipa comata*). Early in the first year of the study (December through March), supplemental millet hay and range cubes were provided to compensate for poor winter forage quality. Estimated intakes of millet hay and range cubes were similar across treatments. Forage and range cube supplementation were discontinued as range quality increased during early spring.

Mineral treatments were provided at a single location in each pasture in free-choice mineral feeders beginning 82 (d –82; Year 1) and 81 d (d –81; Year 2) before the average expected calving date (d 0) of the cowherd. Mineral treatments remained available for ad libitum consumption until 110 (d +110; Year 1) and 135 d (d +135; Year 2) after the average calving date of the cowherd. Mineral treatments were also made available exclusively to the calves via creep feeders in each pasture when calves within the pasture averaged 90 (Year 1) and 99 d (Year 2) of age. All calves received the same respective mineral treatment as their dam. In Year 1, on d +110 after the average calving date, treatments were discontinued and all cows within a treatment were combined and given access to the control (basal) mineral supplement, which did not contain any supplemental Cu, Zn, or Mn (Table 2) for a period of 160 d. Calves continued to have access to their respective mineral treatments until weaning at an age of 185 and 164 d in Years 1 and 2, respectively. Beginning 81 d before the average expected calving date of the cowherd in Year 2, cows were sorted into the same respective treatment groups as in Year 1, assigned to new replicates, and maintained on treatments until d +135 of Year 2. At the beginning of Year 2, there were fewer cows in the experiment (n = 148) than at the beginning of Year 1 (n = 178) because cows that were not pregnant after the final pregnancy rate was determined in Year 1 were removed from the experiment.

Replicates were rotated among pastures approximately every 28 d in order to minimize pasture effects. In conjunction with pasture rotations, mineral weigh-

Table 1. Least squares means for initial body weight, body condition score, age, and liver mineral status of multiparous cows

Item	Treatments			SEM	Contrast ($P <$)	
	Control	ORG[a]	ING[b]		Control vs. Supplement	ORG vs. ING
No. of cows	59	60	59	—	—	—
Age, yr	5.83	6.17	5.71	0.30	0.77	0.28
BW,kg	619.4	616.1	615.3	6.8	0.67	0.94
BCS[c]	5.91	5.97	5.97	0.06	0.51	0.99
Initial liver mineral status						
Cu, mg/kg of DM	55.1	42.8	66.5	13.4	0.97	0.19
Zn, mg/kg of DM	99.7	98.2	99.2	4.6	0.88	0.90
Mn, mg/kg of DM	7.2	6.7	7.2	0.3	0.66	0.31

[a]ORG = 50% organic and 50% inorganic Cu, Zn, and Mn.
[b]ING = 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$.
[c]BCS: 1 = emaciated; 9 = obese (Richards et al., 1986).

backs were performed at each rotation in order to calculate mineral disappearance. Replicates were pooled but remained within treatments for approximately 30 d around the time of calving and 60 d during the time of breeding to allow for more intensive management of the cattle. Mineral treatments continued to be available at these times, although mineral disappearance within replicates could not be monitored.

Mineral Status. Mineral status of the cows was measured using two methods. The first method involved the collection of a liver biopsy sample from every cow before the start of the experiment and then from a subgroup of animals (five per replicate) at the end of the supplementation period in Year 1 and at the beginning and end of the supplementation period in Year 2. This was accomplished by using the true-cut technique described by Pearson and Craig (1980), as modified by Engle and Spears (2000b). Following collection, samples were immediately rinsed with 0.01 *M* PBS, placed in an acid-washed polypropylene tube, capped, and placed on ice for 5 h before storing at –20°C. Liver samples were analyzed for trace mineral concentrations as described by Engle et al. (1997).

The second method used to determine mineral status involved the collection of whole blood via jugular venipuncture in heparinized, trace-mineral-free Vacutainer tubes (Becton Dickinson Co., Franklin Lakes, NJ) at the beginning, midpoint, and end of mineral supplementation from a subgroup of animals (five per replicate), the same animals from whom liver biopsies were obtained. Once collected, samples were placed on ice for 5 h before being centrifuged at 2,000 × *g* for 15 min. Plasma was then transferred to acid-washed storage vials and stored at –20°C.

Plasma Cu and Zn concentrations were measured after the samples were thawed at room temperature for 3 to 4 h. For Cu analysis, 1 mL of a 10% (wt/vol) trichloroacetic acid solution was added to 1 mL of plasma or standard and then vortexed vigorously for 20 s. To aid in precipitation, the sample was placed in a –20°C freezer for 30 min, and then centrifuged at 1,200 × *g* for 10 min at room temperature. The supernatant was removed and diluted in deionized water to concentrations that fit within a linear range of a standard curve generated by linear regression of known concentrations. Plasma Cu concentrations were read at 324.7 nm using a flame atomic absorption spectrophotometer (model 1275, Varian, Walnut Creek, CA). Plasma Zn concentrations were analyzed in a similar manner as described above. Five hundred microliters of plasma was diluted to a 1:5 ratio (plasma:deionized water). This dilution was then read at 213.9 nm using the same atomic absorption spectrophotometer previously mentioned. Only samples from Year 1 were analyzed for Cu and Zn concentrations.

Cow Performance. To determine the effects of trace mineral supplementation and source on reproductive performance, every cow was inseminated once following a modified Select-Synch (Geary et al., 2000) estrus synchronization protocol. Beginning on d +55 (Year 1) and

Table 2. Ingredient composition and laboratory analysis of the three trace mineral treatments

Item	Trace mineral treatments		
	Control	ORG[a]	ING[b]
Ca_2PO_4, %	52.0	52.0	52.0
NaCl, %	21.5	21.5	21.5
Dried distillers grain, %	15.6	15.6	15.6
MgO (52%), %	4.9	4.9	4.9
Soybean oil, %	4.0	4.0	4.0
Se (0.16%), %	1.9	1.9	1.9
Anise-fenugreek dry, %	0.11	0.11	0.11
EDDI (79.6%), %[c]	0.009	0.009	0.009
Chemical analyses			
Ca, %	10.7	10.7	10.7
P, %	11.4	11.4	11.4
Cu, mg/kg of DM	6.2	1,038.2	1,087.2
Zn, mg/kg of DM	17.1	3,173.1	3,241.0
Mn, mg/kg of DM	15.2	2,921.3	2,895.3

[a]ORG = 50% organic and 50% inorganic Cu, Zn, and Mn.
[b]ING = 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$.
[c]EDDI = ethylenediamine dihydroiodide.

d +69 (Year 2) after the average calving date of the cowherd, all cows were injected with 100 μg of GnRH (Fertagyl, Intervet, Millsboro, DE; i.m.), and then injected with 25 mg of $PGF_{2\alpha}$ 7 d later (Lutalyse, Pharmacia & Upjohn, Kalamazoo, MI; i.m.). Calves were removed for 72 h from all cows at the time of $PGF_{2\alpha}$ administration in an effort to increase estrous response. For management reasons, all cows were pooled and treatments were withheld during the 72-h synchronized AI breeding period. Cattle were observed for signs of behavioral estrus from 12 h before $PGF_{2\alpha}$ injection through 72 h after $PGF_{2\alpha}$ injection. Detection of estrus was accomplished by visual observation for a minimum of 1 h at dawn, noon, and dusk. Animals observed in behavioral estrus were inseminated approximately 12 h after first detected in estrus. At 72 h after $PGF_{2\alpha}$, all females that had not been observed in estrus were given a second injection of GnRH (100 μg; i.m.) and mass inseminated. Calves were returned to their dams following AI, and all cattle were sorted back into treatments and returned to appropriate pastures after mass insemination.

To minimize variation in breeding measurements, an effort was made to decrease confounding variables. Two AI technicians performed the inseminations, equally represented within each replicate, and one technician thawed semen. Semen from one purebred Charolais sire that had been acquired from a single collection was used to avoid a sire effect. To allow for the accurate differentiation between pregnancy to AI vs. pregnancy to natural service, cows were not exposed to bulls until 14 d after mass insemination. Six Red Angus-based composite bulls that had previously passed a breeding soundness evaluation were exposed to the cows for 46 d (two bulls per treatment).

In Year 2 only, immediately before the initiation of the synchronization protocol, two blood samples were collected via jugular venipuncture at 10-d intervals and progesterone concentrations were determined to identify cows that had cycled since parturition. Cows were identified as cycling if at least one blood sample had a serum progesterone concentration greater than 1.0 ng/mL, indicating the presence of a functional corpus luteum. Serum was harvested and stored at –20°C until samples were evaluated in duplicate for progesterone concentration by solid-phase RIA (Coat-a-Count kit; Diagnostic Products Corp., Los Angeles, CA), as described by Bellows et al. (1991). The intra- and interassay CV for the two assays were 5.9 and 2.2%, respectively. The sensitivity of the assay was 0.04 ng/mL of serum.

To determine pregnancy rate to AI, cattle were examined via rectal ultrasonography (Aloka 500V equipped with 5.0-MHz linear array transducer, Corometrics Medical Systems, Wallingford, CT) by a state-licensed veterinarian 40 d after mass mating. Cows with fetuses that were approximately 40 d old were classified as pregnant to AI, whereas all other cattle were classified as not pregnant to AI. Final pregnancy rates following the 60-d breeding season were determined via palpation per rectum with the aid of ultrasonography approximately 40 d after bull removal.

Cow performance was also monitored by collection of BW and BCS data at approximately 56-d intervals throughout the duration of the supplementation period in each year. The BCS measurements collected were on a scale of 1 to 9 (1 = emaciated, 9 = obese; Richards et al., 1986) and were assigned by the same technician throughout the study.

Calf Performance. Calf performance was measured in both years based on kilograms of calf weaned per cow exposed. Weaning weights were collected on each calf before shipment to the feedlot. At the end of Years 1 and 2, calves averaged 185 and 164 d of age, respectively.

Statistical Analysis

Cow performance, mineral status, and calf performance data (including BCS; BW; liver Cu, Zn, and Mn concentrations; plasma Cu and Zn concentrations; and weaning weight) were assessed using a restricted maximum likelihood-based, mixed-effects model, repeat-measures analysis (PROC MIXED; SAS Inst., Inc., Cary, NC). Initial cow performance and mineral status models contained fixed effects of treatment, time, and treatment × time interaction. Initial calf performance models included fixed effects of treatment, year, age of dam, age of calf, sex of calf, and all relevant two- and three-way interactions. A spatial power covariance structure was used in the analysis and the containment approximation was used to calculate denominator degrees of freedom. Pasture was used as the experimental unit. Reproductive response data (including estrous cyclicity, estrous response, pregnancy to a synchronized AI, and final pregnancy throughout the 60-d breeding season) were analyzed using logistic regression (PROC GENMOD of SAS). Initial models for reproductive response contained fixed effects of treatment, postpartum interval, BCS, BW, year, and AI technician, in addition to all relevant two- and three-way interactions. When an interaction was not significant, it was removed from the model. If the interaction of year × treatment was not significant, data were pooled across years; otherwise, data were reported for each year separately. Differences among means were determined using single-df contrasts; comparisons made were as follows: 1) control vs. supplemented and 2) ORG vs. ING.

Results and Discussion

Mineral Consumption

Average mineral disappearance for the cows during Year 1 was 0.12 ± 0.06, 0.11 ± 0.07, and 0.12 ± 0.07 $kg \cdot cow^{-1} \cdot d^{-1}$ for the ORG, ING, and control treatments, respectively. Average mineral disappearance during Year 2 was 0.13 ± 0.06, 0.12 ± 0.06, and 0.13 ± 0.06 $kg \cdot cow^{-1} \cdot d^{-1}$ for the ORG, ING, and control treatments, respectively. In both years, the amount of daily mineral

Table 3. Least squares means for liver mineral concentrations of cows supplemented with different trace mineral treatments[a]

Item	Treatments			SEM	Contrasts ($P <$)	
	Control	ORG[b]	ING[c]		Control vs. Supplement	ORG vs. ING
Cu, mg/kg of DM						
d –82 yr 1[d]	55.1	42.8	66.5	13.4	0.97	0.19
d +110 yr 1	50.3	150.6	97.3	12.9	0.01	0.01
d –81 yr 2	62.8	105.4	107.8	11.2	0.01	0.88
d +135 yr 2	43.7	156.1	141.8	10.9	0.01	0.39
Zn, mg/kg of DM						
d –82 yr 1[d]	99.7	98.2	99.2	4.6	0.88	0.90
d +110 yr 1	89.1	106.3	105.3	4.4	0.05	0.87
d –81 yr 2	90.6	87.3	130.5	15.6	0.32	0.07
d +135 yr 2	91.5	85.0	93.9	3.2	0.57	0.07
Mn, mg/kg of DM						
d –82 yr 1[d]	7.2	6.7	7.2	0.3	0.66	0.31
d +110 yr 1	8.0	8.9	9.2	0.3	0.01	0.52
d –81 yr 2	6.7	6.6	6.4	0.3	0.46	0.60
d +135 yr 2	9.5	8.9	8.3	0.3	0.02	0.25

[a]Day is relative to average calving date (d 0) of the cow herd.
[b]ORG = 50% organic and 50% inorganic Cu, Zn, and Mn.
[c]ING = 100% inorganic $CuSO_4$, $ZnSO_4$. And $MnSO_4$.
[d]Initial (d –82) liver values indicate concentrations measured before supplementation, which began on January 17, 2001. Day is relative to average calving date (d 0) of the cow herd.

disappearance indicated that the ORG and ING supplemented cattle consumed enough Cu, Zn, and Mn to meet their NRC-recommended requirements of 10 mg of Cu/kg of DM, 30 mg of Zn/kg of DM, and 40 mg of Mn/kg of DM (NRC, 1996; estimated DMI = 10.9 kg). Because forage basal trace minerals were at low concentrations relative to NRC (1996) recommendations, no effects on performance were attributed to excess supplementation of Cu, Zn, or Mn.

Liver Mineral Status

Liver mineral status for cows in this experiment was affected ($P < 0.05$) by trace mineral supplementation and source (Table 3). A year × treatment interaction was detected ($P = 0.03$) for liver Cu concentration. At the end of both years, liver Cu concentration was greater ($P < 0.01$) in supplemented vs. control cows; however, liver Cu concentration in control cows decreased in Year 2. In addition, at the end of Year 1, ORG cows had greater ($P < 0.01$) liver Cu concentration than did ING cows, whereas at the end of Year 2, liver Cu concentration did not differ ($P > 0.38$) between ORG and ING cows. Liver Cu results are consistent with those reported by other researchers (Du et al., 1996; Olson et al., 1999), wherein liver Cu concentrations increased with mineral supplementation when compared with controls. Although an increase in liver Cu concentration is often observed in conjunction with trace mineral supplementation, and in this case with source in Year 1, it is not clear as to the positive or negative effects of liver Cu retention on reproduction and/or calf performance.

Relative to liver Mn concentration, a year × treatment interaction was present ($P = 0.01$). Liver Mn concentration was greater ($P < 0.01$) in supplemented vs. control cows at the end of Year 1; however, at the end of Year 2, control cows had greater ($P < 0.02$) liver Mn concentration than did supplemented cows. Liver Mn concentration did not differ ($P > 0.51$ and $P > 0.24$, Year 1 and Year 2, respectively) between ORG and ING treatments. The data are difficult to interpret as to why liver Mn concentrations were greater in unsupplemented control cows relative to supplemented cows in Year 2. An animal's true Mn status may not be correctly evaluated when using a liver biopsy sample to determine Mn concentration. In ruminants, it has been reported that liver Mn concentration does not respond substantially to Mn supplementation, even at extreme dietary concentrations (Underwood and Suttle, 1999), as evidenced by only a fourfold increase in liver Mn when dietary Mn increased 130- to 140-fold (Ivan and Hidiroglou, 1980; Watson et al., 1973). Furthermore, the elevated liver Mn concentration observed in the control cows in Year 2 may not be biologically significant.

There was no year × treatment interaction ($P = 0.11$) for liver Zn concentration. Liver Zn concentration was greater ($P < 0.05$) in supplemented than in control cows at the end of Year 1; however, liver Zn concentration did not differ ($P > 0.56$) between supplemented and control cows at the end of Year 2. Liver Zn concentration did not differ ($P > 0.86$) between ORG and ING treatments at the end of Year 1, but ING cows tended ($P < 0.07$) to have greater liver Zn concentrations than ORG cows at the end of Year 2.

Plasma Mineral Status

Plasma trace mineral concentrations were impacted by trace mineral supplementation, but not by source.

Table 4. Reproductive performance of cows supplemented with different trace mineral treatments[a]

Item	Treatments			Contrasts ($P <$)	
	Control	ORG[b]	ING[c]	Control vs. Supplement	ORG vs. ING
Estrous cyclicity[d]	26% (11/43)[i]	28% (13/46)	23% (11/47)	0.95	0.61
Estrus observed within 72 h of $PGF_{2\alpha}$ administration	83% (80/96)	77% (78/101)	77% (79/103)	0.19	0.93
Pregnancy rate to AI (yr 1)[e]	65% (34/52)	67% (36/54)	52% (29/56)	0.47	0.08
Pregnancy rate to AI (yr 2)[e]	34% (15/44)	57% (26/46)	58% (25/43)	0.02	0.80
Pregnancy rate to AI if observed in estrus[f]	58% (46/80)	77% (59/77)	65% (49/75)	0.04	0.13
Pregnancy rate to AI if mass inseminated[g]	19% (3/16)	13% (3/23)	21% (5/24)	0.51	0.66
Overall pregnancy rate after 60-d season[h]	89% (85/96)	93% (94/101)	95% 98/103)	0.10	0.54

[a]Values reported are percentages, with ratios in parentheses.
[b]ORG = 50% organic and 50% inorganic Cu, Zn, and Mn.
[c]ING = 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$.
[d]Percentage of estrous cyclicity at the beginning of the breeding season was evaluated in Year 2 only via the collection of two blood samples at 10-d intervals. Cows were identified as "cycling" if at least one blood sample had a serum progesterone concentration greater than 1.0 ng/mL, indicating the presence of a functional corpus luteum.
[e]There was a year × treatment interaction ($P < 0.02$) for pregnancy to AI, so data could not be pooled across years. For each year, the value includes all cows in the study because all cows were inseminated once (either based on an observation of estrus within 72 h after $PGF_{2\alpha}$ administration or via mass-insemination at 72 h after $PGF_{2\alpha}$ administration).
[f]Values reported include only cows observed in estrus within 72 h of $PGF_{2\alpha}$ administration and inseminated based solely on this observation of behavioral estrus.
[g]Values reported include only cows never observed in estrus and subsequently mass-inseminated at 72 h after $PGF_{2\alpha}$.
[h]Overall 60-d pregnancy rate includes data from both Years 1 and 2.
[i]Number of animals observed/number of animals evaluated.

Plasma Cu concentrations at the end of the supplementation period in Year 1 tended ($P = 0.08$) to be greater in supplemented vs. control cows, and plasma Zn concentrations were greater ($P < 0.01$) in the supplemented cows vs. controls (data not shown).

Cow Performance

Mean BW and BCS did not differ among treatments throughout the 2-yr experiment (data not shown). However, there was a main effect of time ($P < 0.05$). Both BW and BCS declined following calving but returned to precalving levels by mid-summer. Similarly, previous experiments have reported that neither trace mineral supplementation nor source affected cow BW or BCS (Olson et al., 1999; Stanton et al., 2000; Muehlenbein et al., 2001).

Reproductive performance data throughout the experiment are reported in Table 4. There were no differences between control and supplemented cows, or between ORG and ING supplemented cows for the rate of estrous cyclicity at the start of the breeding season (Year 2 only) or percentage of cows exhibiting behavioral estrus in response to the synchronization protocol in either year. A year × treatment interaction ($P < 0.02$) for pregnancy rate to a synchronized AI was present, and therefore data were reported individually for each year. In Year 1, pregnancy rate to AI did not differ between control and supplemented cows; however, ORG-supplemented cows tended ($P < 0.08$) to have a higher pregnancy rate to AI than did ING-supplemented cows. Unlike Year 1, control cows had a lower ($P < 0.02$) pregnancy rate to AI than did supplemented cows in Year 2. Additionally, ORG- and ING-supplemented cows did not differ in pregnancy rate to AI in Year 2. To further evaluate the pregnancy response to AI data, time of AI was classified as being administered either 12 h after an observed estrus (**EAI**) or via mass insemination at 72 h after $PGF_{2\alpha}$ injection. Within the EAI group, supplemented cows had a higher ($P < 0.04$) pregnancy rate to AI than did control cows, and ORG-supplemented cows tended ($P = 0.13$) to have a higher pregnancy rate compared with ING-supplemented cows. Conversely, no differences were observed within the mass-insemination group; however, a much smaller number of cows were mass inseminated due to the high estrous response (approximately 80%) that occurred in both years.

The lack of a difference in pregnancy rate to AI between control and supplemented cows in Year 1, and the presence of a difference in Year 2, suggests that supplemented cows in Year 1 may not have benefited from ORG or ING supplementation. However, the lower pregnancy rate to AI that was present in the control cows in Year 2 seems to have been caused by the removal of supplemental Cu, Zn, and Mn for over 1 yr.

The tendencies for ORG-supplemented cows to have a higher pregnancy rate to AI compared with ING-supplemented cows in Year 1 and when AI was based on an observed estrus in Year 2, are supported by results from Stanton et al. (2000), where a higher pregnancy rate to AI was observed in cows receiving organic Cu, Zn, and Mn vs. the inorganic forms. However, it should be mentioned that many of the cows used by Stanton et al. (2000) had deficient liver Cu concentrations (defined as less than 20 to 30 ppm Cu; Mills, 1987) compared with the cows used in this study. Improved early pregnancy rates (pregnancies within the first 30 d of the breeding season) have also been reported by Muehlenbein et al. (2001) when Cu was supplied in an organic form compared with controls. Further research is needed to determine the likely physiological mechanisms that seem to enable trace minerals to affect pregnancy rates early in the breeding season.

Pregnancy rates collected at the end of the 60-d breeding season included pregnancies to both the synchronized AI and natural service. Supplemented cows tended ($P = 0.10$) to have a higher pregnancy rate compared with control cows; however, there was no difference between ORG- and ING-supplemented cows. To our knowledge, this is the first study to report that trace mineral supplementation may affect season-long reproductive performance in beef cattle. Trace mineral supplementation affected pregnancy rates to AI (as reported above) and possibly also natural service pregnancy rates during the 46-d period of bull exposure. Earlier studies have reported no differences at the end of a 60-d breeding season due to trace mineral supplementation (Arthington et al., 1995; Muehlenbein et al., 2001). However, Olson et al. (1999) reported a depressed 60-d pregnancy rate in cows that received supplemental Cu, Zn, and Mn in organic or inorganic forms at twice the NRC (1996) recommended levels compared with controls. The beneficial reproductive performance observed in the current study, when supplementation was provided at NRC (1996) recommended levels for 2 yr, suggests that trace minerals impact reproductive performance positively when supplementation follows NRC recommendations.

Calf Performance

From d +90 until weaning in Year 1, mineral disappearance from calf creep feeders was 0.010 ± 0.003, 0.014 ± 0.004, and 0.011 ± 0.004 kg·calf^{-1}·d^{-1} for ORG, ING, and control treatments, respectively. Conversely, from d +99 until weaning in Year 2, mineral disappearance from creep feeders of calves was 0.048 ± 0.012, 0.042 ± 0.12, and 0.061 ± 0.018 kg·calf^{-1}·d^{-1} for ORG, ING, and control treatments, respectively. In Year 2, mineral disappearance from creep feeders was greater ($P < 0.01$) than in Year 1. It is not clear why calves consumed more supplement in the second year of this experiment.

There was a year × treatment interaction ($P < 0.05$) for calf performance, as measured by kilograms of calf weaned per cow exposed, and therefore data were reported separately for each year (Table 5). In both years, trace mineral supplementation and source affected kilograms of calf weaned per cow exposed. In Year 1, there were more ($P < 0.02$) kilograms of calf weaned per cow exposed in the control treatment than in supplemented cows, and more ($P < 0.01$) kilograms of calf weaned per cow exposed in the ING treatment than in ORG. Year 2 results for the effect of supplementation were similar to Year 1 because controls had more ($P < 0.02$) kilograms of calf weaned per cow exposed vs. supplemented; however, unlike Year 1, the ORG treatment tended ($P = 0.09$) to have more kilograms of calf weaned per cow exposed than ING.

It is not clear why calf performance was higher in the control treatment vs. the supplemented treatment in both years, nor is it clear why calf performance was affected by trace mineral source differently in Year 1 than Year 2. Conflicting results have been reported in the literature as to the effects of trace minerals on calf performance in both preweaning (grazing) and postweaning (feedlot) situations. Preweaning calf ADG improved when Zn was supplemented from the time of bull removal until weaning (Mayland et al., 1980). Alternatively, calf gain was not affected by Cu, Co, Mn, and Zn supplementation compared with unsupplemented controls (Olson et al., 1999) or when calves were supplemented with organic Cu, inorganic Cu, or an organic Cu/Zn combination (Muehlenbein et al., 2001). In a study that evaluated the effect of Cu bolus administration before weaning, weaning weights were heavier in bull calves and tended to be heavier in heifer calves that received supplemental Cu compared with unsupplemented controls (Arthington et al., 1995). Studies have also reported that trace mineral source can impact calf performance. Stanton et al. (2000) reported increased preweaning ADG in calves that had access to a high level of supplemental Cu, Zn, and Mn in an organic form compared with calves receiving high or low levels of inorganic Cu, Zn, and Mn.

The effect of Cu on postweaning feedlot calf performance in the growing and finishing phases has been evaluated in several experiments. However, much like preweaning performance, postweaning performance has been shown to be positively affected, negatively affected, and also not affected by trace mineral supplementation. It has been reported that Cu supplementation does not impact the performance of growing or finishing steers (Engle and Spears, 2000a, 2001). However other experiments have shown both positive and negative effects of Cu on feedlot performance during the finishing phase. Copper has been shown to decrease feed intake, feed efficiency, and ADG when supplemented at 20 or 40 mg of Cu/kg of DM (Engle and Spears, 2000b). Conversely, Cu supplementation at 10 or 40 mg of Cu/kg of DM improved ADG and daily feed intake (Engle et al., 2000).

Table 5. Performance of calves from multiparous cows supplemented with different trace mineral treatments for Years 1 and 2

Item	Treatments			SEM	Contrasts ($P <$)	
	Control	ORG[a]	ING[b]		Control vs. Supplement	ORG vs. ING
Year 1						
Actual weaning weight, kg[cd]	212.7	200.0	209.3	3.0	0.04	0.01
205-d adjusted weaning weight, kg[ce]	266.5	247.7	262.9	4.2	0.04	0.01
Exposed cows that weaned a calf, %[f]	94.9%	93.4%	95.3%	0.7	0.49	0.29
Kilograms calf weaned per cow exposed[ch]	200.3	184.7	198.8	2.9	0.02	0.01
Year 2						
Actual weaning weight, kg[cd]	197.1	184.3	178.6	9.3	0.01	0.19
205-d adjusted weaning weight, kg[ce]	239.2	224.8	217.6	4.6	0.01	0.28
Exposed cows that weaned a calf, %[g]	78.0%	81.0%	79.0%	3.8	0.34	0.92
Ki;ograms calf weaned per cow exposed[ch]	151.1	148.4	139.2	2.8	0.02	0.09

[a]ORG = 50% organic and 50% inorganic Cu, Zn, and Mn.
[b]ING = 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$.
[c]Least squares means.
[d]Actual (unadjusted) weaning weight taken at weaning on October 2, 2001 (Year 1) and September 6, 2002 (Year 2), with age of dam, sex of calf, and age of calf included in the model statement.
[e]Weaning weight adjusted to 205 d of age using Beef Improvement Federation adjustments for age of dam and sex of calf.
[f]Percentage of cows pregnant at the start of the experiment that weaned a calf on October 2, 2001.
[g]Percentage of cows pregnant at Year 1 final pregnancy check and weaned a calf on September 6, 2002.
[h]Based on the actual (unadjusted) weaning weight taken on October 2, 2001 (Year 1) and September 6, 2002 (Year 2) and the "exposed cows that weaned a calf" percent.

It seems that Cu, Zn, and Mn supplementation and source affect calf performance; however, based on the highly variable results of other studies, it is not known why this might be occurring. Further research into the effect of trace mineral supplementation and source on calf performance is needed.

Implications

Both supplementation (at concentrations recommended by the NRC) and source of copper, zinc, and manganese affected the concentration of these minerals in the liver and plasma of multiparous beef cows, although the effect of these differing body mineral concentrations on reproduction or calf performance is unclear. After 1 yr without supplemental copper, zinc, and manganese, pregnancy rate to a synchronized artificial insemination may be decreased, and season-long reproductive performance also may be affected. Additionally, trace mineral source may affect pregnancy rate to a synchronized artificial insemination, particularly if the insemination was administered based on an observed estrus. Finally, under certain conditions, kilograms of calf weaned per cow exposed may be affected by both trace mineral supplementation and source, although the effects are not well understood and deserve further attention in future research.

Literature Cited

Arthington, J. D., R. L. Larson, and L. R. Corah. 1995. The effects of slow-release copper boluses on cow reproductive performance and calf growth. Prof. Anim. Sci. 11:219–222.

Bellows, R. A., R. B. Staigmiller, J. M. Wilson, D. A. Phelps, and A. Darling. 1991. Use of bovine FSH for superovulation and embryo production in beef heifers. Theriogenology 35:1069–1082.

Cao, J., P. R. Henry, R. Guo, R. A. Holwerda, J. P. Toth, R. C. Littell, R. D. Miles, and C. B. Ammerman. 2000. Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. J. Anim. Sci. 78:2039–2054.

Du, Z., R. W. Hemken, J. A. Jackson, and D. S. Trammell. 1996. Utilization of copper in copper proteinate, copper lysine, and cupric sulfate using the rat as an experimental model. J. Anim. Sci. 74:1657–1663.

Engle, T. E., C. F. Nockels, C. V. Kimberling, D. L. Weaber, and A. B. Johnson. 1997. Zinc repletion with organic or inorganic forms of zinc and protein turnover in marginally zinc-deficient calves. J. Anim. Sci. 75:3074–3081.

Engle, T. E., and J. W. Spears. 2000a. Dietary copper effects on lipid metabolism, performance, and ruminal fermentation in finishing steers. J. Anim. Sci. 78:2452–2458.

Engle, T. E., and J. W. Spears. 2000b. Effects of dietary copper concentration and source on performance and copper status of growing and finishing steers. J. Anim. Sci. 78:2446–2451.

Engle, T. E., and J. W. Spears. 2001. Performance, carcass characteristics, and lipid metabolism in growing and finishing Simmental steers fed varying concentrations of copper. J. Anim. Sci. 79:2920–2925.

Engle, T. E., J. W. Spears, L. Xi, and F. W. Edens. 2000. Dietary copper effects on lipid metabolism and circulating catecholamine concentrations in finishing steers. J. Anim. Sci. 78:2737–2744.

Geary, T. W., E. R. Downing, J. E. Bruemmer, and J. C. Whittier. 2000. Ovarian and estrous response of suckled beef cows to the select synch estrous synchronization protocol. Prof. Anim. Sci. 16:1–5.

Ivan, M., and M. Hidiroglou. 1980. Effect of dietary Manganese on growth and Manganese metabolism in sheep. J. Dairy Sci. 63:385–390.

Kegley, E. B., and J. W. Spears. 1994. Bioavailability of feed-grade copper sources (oxide, sulfate, or lysine) in growing cattle. J. Anim. Sci. 72:2728–2734.

Manspeaker, J. E., M. G. Robl, G. H. Edwards, and L. W. Douglass. 1987. Chelated minerals: Their role in bovine fertility. Vet. Med. 82:951–956.

Mayland, H. F., R. C. Rosenau, and A. R. Florence. 1980. Grazing cow and calf responses to zinc supplementation. J. Anim. Sci. 51:966–974.

Mills, C. F. 1987. Biochemical and biophysiological indicators of mineral status in animals: Copper, cobalt, and zinc. J. Anim. Sci. 65:1702–1711.

Muehlenbein, E. L., D. R. Brink, G. H. Deutscher, M. P. Carlson, and A. B. Johnson. 2001. Effect of inorganic and organic copper supplemented to first-calf cows on cow reproduction and calf health and performance. J. Anim. Sci. 79:1650–1659.

NRC. 1996. Nutrient Requirements of Domestic Animals. Nutrient Requirements of Cattle. 7th ed. Natl. Acad. Press, Washington, DC.

Olson, P. A., D. R. Brink, D. T. Hickok, M. P. Carlson, N. R. Schneider, G. H. Deutscher, D. C. Adams, D. J. Colburn, and A. B. Johnson. 1999. Effects of supplementation of organic and inorganic combinations of copper, cobalt, manganese, and zinc above nutrient requirement levels on postpartum two-year old cows. J. Anim. Sci. 77:522–532.

Pearson, E. G., and A. M. Craig. 1980. Diagnosis of liver-disease in equine and food animals. Mod. Vet. Pract. 61:233–237.

Richards, M. W., J. C. Splitzer, and M. B. Warner. 1986. Effect of varying levels of postpartum nutrition and body condition at calving on subsequent reproductive performance in beef cattle. J. Anim. Sci. 62:300–306.

Stanton, T. L., J. C. Whittier, T. W. Geary, C. V. Kimberling, and A. B. Johnson. 2000. Effects of trace mineral supplementation on cow-calf performance, reproduction, and immune function. Prof. Anim. Sci. 16:121–127.

Underwood, E. J., and N. F. Suttle. 1999. The Mineral Nutrition of Livestock. 3rd ed. CABI Publ. Wallingford, Oxon, U.K.

Watson, L. T., C. B. Ammerman, J. P. Feaster, and C. E. Roessler. 1973. Influence of manganese intake on metabolism of manganese and other minerals in sheep. J. Anim. Sci. 36:131–136.