Role of Dietary Copper in Enhancing Resistance to *Escherichia coli* Mastitis¹

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ABSTRACT

The role of dietary copper in enhancing resistance to *Escherichia coli* mastitis was investigated in first-lactation heifers. Twenty-three primigravid Holstein heifers were maintained on a basal (6.5 ppm copper; -Cu) diet or a diet supplemented (20 ppm) with copper sulfate (+Cu) beginning 60 d prepartum through 42 d of lactation. Liver biopsies and blood samples were taken for liver and blood minerals and plasma ceruloplasmin. Milk samples were taken weekly postpartum for bacteriology. The overall mean liver Cu concentration was about threefold higher, and the overall mean plasma Cu concentration was greater in the +Cu group than the -Cu group. At 34 d of lactation, one pathogen-free quarter per animal was infused with 22 cfu of *Escherichia coli* strain 727. Plasma Cu was greater at -24, 0, 18, 24, 36, 96, 192, and 240 h relative to infusion for +Cu animals. Plasma Zn concentration was higher at 24 h for the +Cu group. Milk bacterial count (log₁₀ cfu/ml) was lower at 12, 18, and 48 h for the +Cu group. Somatic cell count (log₁₀/ml) was lower at 18 h in +Cu animals. Clinical score at 24 h was lower for +Cu cows, while at 144 h, clinical score was lower for -Cu cows. Rectal temperature was lower at 18 h for the +Cu group. Plasma ceruloplasmin and Fe, dry matter intake and milk production did not differ. Copper supplementation reduced the clinical response during experimental *E. coli* mastitis, but duration was unchanged.

(**Key words:** mastitis, copper, *Escherichia coli*)

Abbreviation key: Cp = ceruloplasmin, HBSS = Hank's balanced salt solution, CNS = coagulase-negative staphylococcus

INTRODUCTION

Most of the efforts toward mastitis prevention have focused on management practices. Nutritional relation-

ships to host defense mechanisms have led to the idea of increasing the resistance of dairy cattle to mastitis through nutrition. The effect of dietary vitamin E and Se on mastitis resistance has been thoroughly documented (Erskine, 1993; Smith et al., 1997, 1991, 1984). Another supplemental nutrient showing beneficial effects on the immune system is Cu. The role of Cu has been documented in many species, including mice (Jones and Suttle, 1983; Jones, 1984; Prohaska and Lukasewycz, 1990), rats (Newberne et al., 1968; Johnson, 1986), sheep (Wooliams et al., 1986; Jones and Suttle, 1986), beef cattle (Gengelbach et al., 1997), and dairy cattle (Stabel et al., 1993; Harmon et al., 1994a; Harmon, 1998).

Mastitis is a costly disease to the dairy industry. Clinical mastitis due to environmental pathogens is an ongoing problem for even well-managed dairy herds that regularly maintain low herd SCC. However, the overall prevalence of environmental pathogen infections in a herd at any given time is generally low (Harmon, 1994). The udder is constantly exposed to environmental pathogens at times other than milking. These sources of pathogens are sometimes overlooked when attempting to decrease mastitis. One study has found that 38% of lactations in well-managed herds had cases of clinical mastitis. Environmental pathogens were isolated in 82% of these cases at a cost of \$107 per case (Hoblet et al., 1991). The nonlactating period, or dry period, is a part of the lactation cycle when cattle may be commonly housed in poorer environmental conditions than lactating cattle, resulting in the possibility of greater exposure to environmental pathogens. Antibiotic therapy for lactating cows is usually not efficacious or has a minor role in the treatment of coliform mastitis such as that caused by *Escherichia coli* (Crist et al., 1992). Furthermore, increased antibiotic use is associated with public health concerns regarding contamination of milk and meat for human consumption.

Recent studies by Harmon (1998) and Harmon et al. (1994a) have demonstrated the role of Cu on both incidence of IMI and response to infections during experimentally induced *E. coli* endotoxin mastitis in dairy

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Table 1. Calculated nutrient composition of prepartum and postpartum diets (DM basis).¹

Nutrient	Prepartum diet	Postpartum diet
	(% of diet)	
CP	14.21	17.33
ADF	29.50	23.30
NDF	42.01	33.76
Ca	0.97	0.80
P	0.44	0.48
Mg	0.20	0.23
K	1.92	1.61
Na	0.08	0.21
S	0.15	0.19
	(mg/kg in diet)	
Fe	396.98	324.79
Zn	34.12	50.78
Cu ²	6.71	6.14
Mn	62.60	59.89
Mo	1.44	2.10
Se ³	0.11	0.41 ⁴
NE _L , Mcal/kg ⁵	1.47	1.69

¹Nutrient composition obtained through wet chemistry of forages and concentrates.

²Does not include supplemental copper sulfate.

³Supplemental selenium.

⁴Diet provided 5.46 mg supplemental Se daily.

⁵Calculated according to NRC (1989).

cattle. The objective of the present study was to evaluate the effects of Cu status on the response of dairy cattle to live *E. coli* intramammary challenge.

MATERIALS AND METHODS

Experimental Design

Twenty-eight pregnant Holstein heifers were assigned to treatments in a completely randomized block design with eight blocks of two to four animals paired by expected calving date. At 60 d before anticipated calving, heifers were assigned to dietary treatments. Treatment groups were: 1) basal diet (6.5 ppm Cu) plus 20 ppm Cu as supplemental Cu sulfate in a ground corn carrier fed as a top-dress (+Cu group) or 2) basal diet with no supplemental Cu (–Cu group). The prepartum diet consisted of ad libitum corn silage and alfalfa silage (equal proportion, DM basis), 1.4 kg of concentrate, and 1.4 kg of alfalfa hay. The postpartum diet was fed as a TMR and consisted of corn silage (24% of diet DM), alfalfa silage (22% of diet DM), concentrate (37% of diet DM), whole cottonseed (9% of diet DM), and alfalfa hay (8% of diet DM). The prepartum diets met NRC (1989) requirements for all nutrients except Cu, Zn, and Se (Table 1). Prepartum supplemental Se was determined to be 0.11 ppm based on total DMI. The total Zn concentration of the prepartum diets was 34 ppm. The postpartum diets met NRC (1989) requirements for all nutrients except Cu (Table 1). Dietary Fe from naturally

occurring Fe in the feed ingredients was 400 ppm for the prepartum diet and 325 ppm for the postpartum diet. Experimental heifers were housed in a tie-stall barn and allowed access to a dirt lot about 4 h/d. Liver samples were used to determine copper status at 60 and 21 d prepartum, within 3 d postpartum, and at 42 d postpartum. Jugular blood samples were collected by venipuncture in lithium heparinized vacutainers (Becton Dickinson, Franklin Lakes, NJ) at the same sample times as liver, with an additional sample 21 d postpartum. Plasma ceruloplasmin (**Cp**) activity, plasma Cu concentration, and whole blood Se were determined.

Intramammary Bacterial Challenge

Escherichia coli strain 727, originally isolated from a naturally occurring IMI, was used as the intramammary challenge pathogen. Challenge inoculum was prepared as described by Hogan et al. (1995) with modification. The final inoculum dilution was in Hank's balanced salt solution (**HBSS**; Biowhittaker, Walkersville, MD) and not PBS. One quarter of each heifer was challenged by intramammary infusion of *E. coli* strain 727 5 h after the morning milking. Quarters chosen for challenge were bacteriologically negative and had a mean SCC of 30,000 at time of challenge. Mean SCC did not differ between groups ($P = 0.51$). The geometric mean of the colony-forming units for challenge inoculum was 21.3 (2.2 to 35) suspended in HBSS. Size of challenge inoculum between groups did not differ ($P = 0.98$). Heifers within the same block were challenged on the same day. The geometric mean DIM at challenge was 34 d (18 to 65 d).

Milk and Blood Samples during Challenge

Duplicate quarter foremilk samples were aseptically collected within 3 d of parturition, on lactation d 7, 14, 21, and 42 for microbiological analysis and SCC determination to verify mammary gland health. Milk microbiological status was determined using procedures recommended by the National Mastitis Council (Harmon et al., 1990). Culture results that did not match from duplicate samples were not included in the dataset. Somatic cell count was determined by commercial laboratory (Mid-South DHIA, Springfield, MO) using an electronic counter. Quarter foremilk samples also were collected 24 h before challenge, immediately before challenge (0 h), and 6, 12, 18, 24, 36, 48, 72, 96, 144, 192, and 240 h postchallenge. Plasma was collected as described earlier at the same times as quarter foremilk samples for Cu, Fe, Zn, and Cp analysis.

Colony-forming units of *E. coli* per milliliter and SCC were determined in quarter foremilk samples during

the postchallenge period. Colony-forming units were determined by appropriate 10-fold dilutions in PBS and surface plated in duplicate on McConkey agar plates. A 0.1-ml aliquot of undiluted milk was plated on the surface of a blood agar plate and in duplicate on the surface of McConkey agar plates. The SCC was determined as described earlier. Samples were diluted 1:10 in PBS from the 12-h sample through the 144-h sample to obtain accurate SCC for samples which exceeded the upper limit (9.7 million/ml) of the electronic cell counting equipment. Raw data were expressed as $\log_{10}$ cfu/ml and $\log_{10}$ SCC/ml of milk.

Clinical Signs

The clinical status of all quarters was recorded on a five-point scale at the time quarter foremilk samples were obtained following the scale as outlined by Hogan et al. (1995). The scale was: 1 = normal milk and normal quarter, 2 = normal quarter but questionable milk, 3 = normal quarter but abnormal milk, 4 = swollen quarter and abnormal milk, and 5 = swollen quarter, abnormal milk, and systemic signs of infection. Clots, discoloration, or a serous appearance were signs of abnormal milk. Rectal temperatures were measured immediately prior to challenge, and at each time quarter samples were collected postchallenge.

Milk Production and DMI

Milk production was measured from weigh jars and recorded following each milking. Milking times were 0430 and 1530 h. Daily feed intake was recorded for all heifers. Daily milk production and DMI were expressed as percentages of means for 7 d before challenge following the formula used by Hogan et al. (1995). The formula used was $[(b/a \times 100)]$, where a = mean value for the 7 d prior to challenge; and b = daily value postchallenge].

Analytical Procedures

Liver samples were obtained via biopsy according to the procedure of Erwin et al. (1956), rinsed in saline, and stored at -20°C until analysis by atomic absorption spectrophotometry (Instrumentation Laboratory, S11, Thermo Jarrell Ash Corp., Franklin, MA). The analysis process followed a modified wet-ashing procedure (Du et al., 1996). Samples were dried for 24 h at 100°C and weighed. Concentrated nitric acid (12 ml) was added, and samples were digested on an 80°C steam plate for 24 to 36 h. Samples were then dried on a flame plate, and 10 ml of 30% hydrogen peroxide was added. Digestion was continued for another 24 h followed by drying

on the steam plate. The ash was reconstituted in 10 ml of 1 N hydrochloric acid, and samples were analyzed using atomic absorption spectrophotometry. Any necessary dilutions were made with 1 N HCL. Plasma Cu was also determined using atomic absorption spectrophotometry. Samples were diluted (1:2, vol/vol) using distilled, deionized nanopure water. Plasma Cp activity was measured as oxidase activity using the procedure of Schosinsky et al. (1974), which uses *o*-dianisidine dihydrochloride as substrate. The assay was adapted to a microplate format that allows the assay of samples in quadruplicate. Whole blood was used for fluorometric determination of selenium concentration (AOAC, 1995).

All forages and concentrates were sampled biweekly or when a source changed. Dry matter was determined on the forages after drying samples at 100°C for 24 h. The samples were compiled trimonthly, subsampled, and sent to a commercial laboratory (Northeast DHIA Forage Analysis Laboratory, Ithaca, NY) for mineral and routine nutrient analysis by wet chemistry.

Statistical Analysis

Treatment differences for bacterial counts, SCC, rectal temperatures, clinical scores, blood and liver mineral concentrations, Cp activity, and DMI and milk production (as percentages of prechallenge means) were tested using the PROC MIXED procedure and least-square means of SAS (1999). Models included fixed effects of treatment, block, time of sampling, and interaction of main effects. Animal within treatment was included in the model for random subject effects. Infection prevalence (percentages) data were tested using chi-squared analyses. Significance was determined at $P < 0.05$, and trends were noted at $P < 0.10$. $\log_{10}$ transformations were done on bacterial counts and SCC raw data to normalize data before analysis.

RESULTS

Of the 28 Holstein heifers in the study, five were removed from the trial. Two were removed due to displaced abomasum shortly after calving, two were not challenged and removed due to high ambient temperatures early in the summer, and one heifer was challenged but was determined to be infected with coagulase-negative staphylococci (CNS) on the day of *E. coli* infusion. Data were analyzed for 11 heifers in the +Cu treatment and 12 heifers in the -Cu treatment.

General Parameters

Copper and Se status. Liver Cu (Table 2) was not different ($P = 0.76$) between treatment groups prior to

Table 2. Least-square means for liver and plasma Cu concentration, plasma ceruloplasmin activity, and whole blood Se concentration in first lactation heifers with (n = 11) or without (n = 12) supplemental (20 ppm) dietary copper.

Parameter		Day from calving					SEM
		-60	-21	0	21	42	
Liver Cu, ($\mu\text{g/g}$)	+Cu	28.4	134.2 ^a	162.7 ^a	NM ¹	256.2 ^a	16.3
	-Cu	34.8	45.2 ^b	33.4 ^b	NM ¹	45.4 ^b	10.3
Plasma Cu, ($\mu\text{g/ml}$)	+Cu	0.57	0.58	0.79 ^a	0.67	0.65 ^a	0.03
	-Cu	0.53	0.53	0.62 ^b	0.63	0.57 ^b	0.03
Ceruloplasmin, IU/L	+Cu	84.5	96.0	125.2	111.6	114.1 ^c	10.8
	-Cu	101.2	99.3	113.7	113.2	151.5 ^d	10.0
Whole blood Se, ($\mu\text{g/ml}$)	+Cu	0.085	0.079	0.080	NM ¹	0.083	0.007
	-Cu	0.080	0.079	0.079	NM ¹	0.090	0.005

^{a,b}Subcolumn means within parameter and column with different superscripts differ ($P < 0.001$).

^{c,d}Subcolumn means within parameter and column with different superscripts differ ($P < 0.05$).

¹Not measured.

treatments 60 d before parturition. However, at 21 d prepartum, at calving, and at 42 d postpartum, liver Cu concentrations were significantly higher ($P = 0.001$) in the +Cu group than in the -Cu group.

Similar to liver Cu, plasma Cu (Table 2) was not different ($P = 0.32$) between treatment groups on d -60. In contrast to liver Cu values, no difference ($P = 0.26$) in plasma Cu was observed on d -21. The +Cu group had higher ($P = 0.001$) plasma Cu levels than -Cu animals at parturition and on d 42 of lactation ($P = 0.05$). At 21 d postpartum, plasma Cu concentration was not different ($P = 0.29$) between treatment groups.

Heifers in the +Cu group had lower ($P = 0.017$) Cp activity (Table 2) than the -Cu group on d 42. Cp activity was not affected by treatment for any other sample time.

No differences ($P = 0.35$) among treatments were noted for whole blood Se concentration (Table 2). Overall Se concentrations were $0.082 \mu\text{g/ml}$ for both treatments.

DMI, BW, and milk production. No differences ($P = 0.94$, $P = 0.65$, and $P = 0.66$, respectively) were found between treatments for DMI, BW, or milk production during any of the sample times. DMI remained fairly constant for both treatment groups from 8 wk prepartum until calving (9 kg/d). DMI slightly decreased for both treatment groups from 1 wk prepartum (9.0 kg/d) until the week of calving (7.9 kg/d). As would be expected, DMI increased dramatically from calving until the end of the experiment (14.8 kg/d). Body weight increased gradually for both treatment groups from 8 wk before calving until calving (584 kg). Body weight decreased until about wk 4 and 5 postpartum (540 kg) and started to increase thereafter until the end of the experiment (548 kg). Milk production for both treatment groups increased from 20.5 kg/d in wk 1 to 29.2 kg/d in wk 7 of lactation.

Infection status. Copper-supplemented cows tended to have a lower proportion of quarters ($P < 0.06$) confirmed uninfected than -Cu animals at calving (Table 3). The +Cu group also had lower proportions of quarters confirmed negative at d 14 ($P < 0.05$) and -24 h prechallenge ($P < 0.09$), while CNS infection prevalence was higher for the +Cu group at d 14 ($P < 0.03$), -24 h prechallenge ($P < 0.03$), and +240 h postchallenge ($P < 0.06$).

Experimental *E. coli* Challenge

Bacteria count. Dramatic changes in *E. coli* numbers were observed during infection, exceeding 10^4 cfu/ml in the -Cu treatment (Figure 1). The +Cu group had lower mean numbers of *E. coli* in the milk at 12 ($P = 0.02$) and 18 h ($P = 0.05$) than the -Cu group (3.17 vs. $4.44 \log_{10}$ cfu/ml; 2.51 vs. $3.57 \log_{10}$ cfu/ml, respectively). At 48 h, +Cu animals had a numerically lower bacteria count ($P = 0.068$) than -Cu animals (1.77 and $2.71 \log_{10}$ cfu/ml, respectively). Neither treatment group had any infections in the challenged mammary quarters at 0 h as determined by bacteriological culture. The 6-h sample was the first sample from which *E. coli* was isolated. Bacterial counts increased sharply from 6 to 12 h with mean peak numbers of *E. coli* being reached at 12 h. *Escherichia coli* numbers decreased slightly at 18 through 36 h. After 36 h, *E. coli* counts in milk from both treatment groups gradually decreased until 144 h. No *E. coli* were isolated from -Cu cows after 144 h, and *E. coli* were only isolated from two +Cu cows after 144 h. These two +Cu cows eliminated the *E. coli* by 240 h. One +Cu cow did not become infected with *E. coli* after challenge, though the cow had an intramammary response with SCC increasing 10-fold. At 96 h, 4 of 11 +Cu cows had cleared the *E. coli* while 8 of 12 -Cu cows cleared the *E. coli* by this time.

Table 3. Quarter infection status (% quarters) at calving (d 1 to 3), d 7, 14, and 21 of lactation, 24 h prechallenge, and 240 h postchallenge of first lactation heifers with (n = 11) or without (n = 12) supplemental (20 ppm) dietary copper.

Bacteriological status	Sample					
	Calving	d 7	d 14	d 21	-24 h	+ 240 h
+Cu group						
Negative	50.0 ^a	84.1	70.5 ^c	84.1	81.8 ^a	84.1
CNS ¹	20.5	6.8	15.9 ^c	6.8	15.9 ^c	13.6 ^a
Major Pathogens ²	6.8	2.3	2.3	0.0 ^a	2.3	0.0
Other	4.5	0.0	0.0	0.0	0.0	2.3
Mixed Infection	0.0 ^a	0.0	2.3	0.0	0.0	0.0
-Cu group						
Negative	66.7 ^b	89.6	85.4 ^d	89.6	91.7 ^b	89.6
CNS ¹	14.6	2.1	4.2 ^d	4.2	4.2 ^d	4.2 ^b
Major Pathogens ²	6.3	2.1	2.1	4.2 ^b	2.1	2.1
Other	2.1	0.0	0.0	0.0	0.0	0.0
Mixed Infection	4.2 ^b	2.1	4.2	0.0	0.0	0.0

^{a,b}Subcolumn means within parameter and column with different superscripts differ ($P < 0.10$).

^{c,d}Subcolumn means within parameter and column with different superscripts differ ($P < 0.05$).

¹CNS = coagulase-negative staphylococci.

²Major pathogens = *Staphylococcus aureus*, *Streptococcus spp.*, and coliforms.

Somatic cell count. Beginning at 12 h, SCC (Figure 2) sharply increased for both groups until 18 h when SCC for +Cu animals were significantly lower ($P = 0.001$) compared with -Cu animals (6.52 and 7.65 $\log_{10}$ SCC/ml, respectively). The SCC of +Cu animals gradually reached a peak at 36 h. The -Cu animals reached peak SCC at 18 h, declined at 24 h, and then increased at 36 h when +Cu animals tended to be lower ($P = 0.064$) than -Cu animals (7.04 and 7.48 $\log_{10}$ SCC/ml). The peak geometric mean SCC was about fourfold higher in -Cu cows than in +Cu cows ($44.67 \times 10^6/\text{ml}$ and $10.96 \times 10^6/\text{ml}$). After 36 h, the SCC gradually decreased in

both treatments until 240 h. The +Cu animals had numerically lower SCC than -Cu animals throughout this period.

Clinical score. The mean clinical score of the +Cu group was significantly lower ($P = 0.03$) than that of the -Cu group at 24 h (3.2 and 4.1, respectively), and surprisingly, the score of the +Cu group was significantly higher ($P = 0.04$) than the -Cu group (2.0 vs. 1.3, respectively) at 144 h (Figure 3). A clinical score of 1.0 was recorded 24 h before and immediately before challenge, corresponding to both SCC and bacteria count data. The score increased slightly from 0 to 12 h.

Figure 1. Least-square mean ($\pm$ SEM) *Escherichia coli* numbers ($\log_{10}$ cfu/ml) in milk following intramammary challenge with *Escherichia coli* strain 727 at 0 h in first lactation heifers with (♦; n = 11) or without (○; n = 12) supplemental (20 ppm) dietary copper. Means within sample period differ (** $P = 0.02$, * $P = 0.05$, + $P = 0.068$).

Figure 2. Least-square mean ($\pm$ SEM) SCC ($\log_{10}$) following intramammary challenge with *Escherichia coli* strain 727 at 0 h in first lactation heifers with (♦; n = 11) or without (○; n = 12) supplemental (20 ppm) dietary copper. Means within sample period differ (* $P = 0.001$, + $P = 0.064$).

Figure 3. Least-square mean ($\pm$ SEM) clinical score following intramammary challenge with *Escherichia coli* strain 727 at 0 h in first lactation heifers with ($\blacklozenge$; $n = 11$) or without ($\circ$; $n = 12$) supplemental (20 ppm) dietary copper. Means within sample period differ (** $P = 0.03$, * $P = 0.04$).

Clinical score increased sharply at 18 h in +Cu animals, reaching their peak at 36 h; the -Cu animals reached their peak at 24 h and remained elevated at 36 h. From 36 to 96 h, clinical score gradually decreased in both treatment groups. The +Cu cows tended to have numerically lower clinical scores from 18 to 96 h postinoculation. A sharp decrease occurred by 144 h with a return to the preinfusion score by 192 h postchallenge.

Rectal temperature. Rectal temperatures (Figure 4) remained constant from 0 to 6 h for both treatments, slightly increased by 12 h, and then sharply increased

Figure 4. Least-square mean ($\pm$ SEM) rectal temperature ($^{\circ}\text{C}$) following intramammary challenge with *Escherichia coli* strain 727 at 0 h in first lactation heifers with ($\blacklozenge$; $n = 11$) or without ($\circ$; $n = 12$) supplemental (20 ppm) dietary copper. Means within sample period differ (* $P = 0.03$).

Figure 5. Least-square mean ($\pm$ SEM) plasma Cu concentration ($\mu\text{g/ml}$) following intramammary challenge with *Escherichia coli* strain 727 at 0 h in first lactation heifers with ($\blacklozenge$; $n = 11$) or without ($\circ$; $n = 12$) supplemental (20 ppm) dietary copper. Means differ ($P < 0.05$) for all times except 6, 12, 48, 72, and 144 h.

to its peak at 18 h. The +Cu animals had significantly lower ($P = 0.001$) rectal temperatures than -Cu animals at 18 h (40.0 vs. 40.8 $^{\circ}\text{C}$, respectively). Temperatures sharply declined at 24 h followed by a return to normal by 48 h and throughout the remainder of data collection.

Plasma copper. Generally, the +Cu group had a higher plasma Cu concentration than the -Cu group (Figure 5). Plasma Cu levels were higher ($P < 0.05$) in +Cu animals than -Cu animals at all times except 6, 12, 48, 72, and 144 h postinfusion. At 36 to 48 h, plasma Cu began to increase for both treatments. By 48 h postchallenge, plasma Cu for both treatment groups was greater ($P = 0.007$) than the 24-h and earlier samples. Plasma Cu levels continued to increase reaching a peak (0.68 $\mu\text{g/ml}$) at 96 h for the +Cu group and 72 h for the -Cu group (0.63 $\mu\text{g/ml}$). Copper levels at 240 h had decreased to levels closer to those found at -24 h for both treatments.

Plasma iron and zinc. Following infusion, hyperferremia and hyperzincemia were observed in both treatment groups (data not shown). Though no differences were noted between treatments ($P = 0.57$), plasma Fe concentration decreased sharply for both groups from 12 to 36 h postinfusion (1.3 to 0.60 $\mu\text{g/ml}$) and returned to the preinfusion concentration (1.40 $\mu\text{g/ml}$) by 72 h postinfusion. Similar to plasma Fe, plasma Zn sharply decreased at 12 h postchallenge until 24 h when both treatments reached their lowest plasma Zn concentration and then returned to prechallenge concentration (0.84 $\mu\text{g/ml}$) by 72 h. At 24 h, +Cu animals had a higher ($P = 0.001$) plasma Zn concentration compared to with -Cu animals (0.40 and 0.16 $\mu\text{g/ml}$, respectively). No other differences between treatments were found.

Figure 6. Least-square mean ($\pm$ SEM) plasma ceruloplasmin activity (IU/L) following intramammary challenge with *Escherichia coli* strain 727 at 0 h in first lactation heifers with ($\blacklozenge$; $n = 11$) or without ($\circ$; $n = 12$) supplemental (20 ppm) dietary copper. Dispersion bars represent standard errors for each treatment at each sample time.

Plasma Cp. Although +Cu animals had numerically lower Cp levels than -Cu animals, no significant differences ($P = 0.99$) existed between treatments at any sample time (Figure 6). Plasma Cp activity in both groups decreased from 6 to 18 h, then gradually increased and reached peak levels at 192 h.

DMI and milk production. DMI declined by 12 to 17% on the first day after inoculation. However, there were no differences ($P = 0.12$) between treatments at any of the sample times. Dry matter intake recovered by d 4 in both groups. Although DMI appeared to be quite variable, both groups averaged at or above the prechallenge mean beyond d 4.

On d 2 postchallenge, milk production was numerically higher for +Cu animals (84.7 vs. 73.8%). However, there were no differences ($P = 0.13$) between treatments at any of the sample times. Production of both groups gradually returned to d 1 levels by d 4. Milk production remained relatively constant from d 4 until d 15.

DISCUSSION

General Parameters

Dietary supplementation of Cu sulfate at 20 ppm for 60 d provided a sufficient Cu source to achieve adequate liver Cu concentration according to Puls (1994) and Mills (1987). Liver Cu in the +Cu group and -Cu group was 162 and 33 ppm at calving and 256 and 45 ppm at 42 d postpartum, respectively. However, the Cu level necessary for optimal host defense or antioxidant activity is not clear (Puls, 1994; Wikse et al., 1992).

The NRC (1989) dietary recommendation of Cu for dairy cattle was 10 ppm. The NRC (2001) dietary recom-

mendation remained 10 ppm for heifers up to d 270 of gestation when the recommendation increased to 16 ppm. In this study and others (Harmon et al., 1994a; Harmon, 1998), the -Cu group received 6 to 7 ppm of Cu in the basal diet. This Cu level was found naturally in the feedstuffs that were in the ration and was not from supplemental Cu. The unsupplemented heifers received about 60 to 70% of the NRC-suggested dietary Cu, yet encountered a moderate to severe liver Cu deficiency simply by lack of supplementation from about 5 mo of age through gestation and parturition. Paynter (1987) and Puls (1994) also demonstrated a Cu deficiency from the lack of Cu supplementation during gestation and lactation.

Plasma Cu concentration may not be a reliable indicator of liver Cu status within the range found in this study. Changes in Cu concentration in the liver did not parallel changes seen in plasma Cu between treatments. Perhaps plasma Cu is a reliable indicator of liver Cu status when the animal is clinically Cu-deficient (Stabel et al., 1993; Gengelbach and Spears, 1998). However, in the present trial, the cows were not clinically deficient ($< 30 \mu\text{g/g DM}$) but were marginally Cu deficient.

Supplemental dietary Se in the heifer diet prepartum was determined to be 0.11 ppm. This is below the NRC- (1989) recommended level of 0.3 ppm. Supplemental Se in the postpartum diet was calculated to be 5.5 mg/d. Erskine (1993) reported that the critical level of whole blood Se is $0.08 \mu\text{g/ml}$. The 60- and 42-d postpartum samples for both treatment groups were slightly above $0.08 \mu\text{g/ml}$ (range of 0.080 to $0.090 \mu\text{g/ml}$). The 21-d prepartum and calving samples for both treatment groups were approximately $0.08 \mu\text{g/ml}$ (range of 0.079 to $0.080 \mu\text{g/ml}$). Although it appears that supplemental Se was deficient in the prepartum heifer diet, the whole blood Se concentration over the entire trial was borderline deficient on all of the samples, based on Erskine's (1993) critical value. Both treatment groups were equal for whole blood Se, so differences in Se status should not have influenced results of the study.

Differences between treatment groups did not exist with respect to DMI, BW, and DMI as a percentage of BW from 8 wk prepartum until 7 wk postpartum. Also, there were no differences in milk production from calving until 7 wk postpartum. Growth rates also have been unaffected by copper deficiency in lambs (Suttle et al., 1970) and rats (Johnson, 1986; Koller et al., 1987). However, Prohaska et al. (1983) found a 25% reduction in the growth rate of second-generation, Cu-deficient mice averaging 6 wk of age. Xin et al. (1991) showed that animals with borderline deficient liver concentrations of Cu had normal growth.

Results of infection status at calving were different from results seen by others. Harmon et al. (1994a) found Cu-supplemented cows to have more uninfected quarters, fewer infections by major pathogens than in +Cu cows, and no differences for CNS infections at calving. In the present trial, the +Cu cows had fewer ($P < 0.06$) quarters negative (50%) than -Cu cows (66.7%). However, infections by major pathogens or CNS at calving were not different between treatments. Harmon (1998) found higher percentage quarters infected with major pathogens in Cu-supplemented cows. It appears that infection status at calving may vary between trials, with Cu source or amount of Cu supplemented.

***Escherichia coli* Challenge**

Responses of heifers to the *E. coli* challenge appeared to be influenced by supplemental Cu at 20 ppm. The NRC- (1989) recommended dietary level of Cu at 10 ppm seems adequate for growth and milk production but may be insufficient for optimal immune function and response.

Each cow challenged with *E. coli* strain 727, except one cow in the +Cu group, developed an IMI and clinical mastitis. Only one cow, which was in the -Cu group, had systemic signs of clinical mastitis that included depression, evident by droopy ears. All quarters used for challenge were bacteriologically negative with SCC that averaged 27,000 cells/ml.

The differences between treatments with respect to bacterial counts, SCC, clinical score, and rectal temperature appeared to be related to Cu status and the role of Cu in immune function. Macrophages and neutrophils are important for nonspecific cellular immune functions, protecting the mammary gland against infection through the phagocytosis and killing of invading microorganisms (Craven and Williams, 1985). Other studies in ewes (Jones and Suttle, 1981), dairy steers (Boyne and Arthur, 1981; Xin et al., 1991), and dairy heifers (Harmon et al., 1994a; Torre et al., 1996) have shown the role of Cu in immune response.

In the current trial, +Cu animals had lower bacterial counts, lower SCC, lower clinical scores, and lower peak rectal temperature than responses in -Cu animals. The decreased clinical severity could be due to increased capability of neutrophils in supplemented animals to kill the invading *E. coli*. Copper supplementation appeared to decrease the severity of an *E. coli* infection, while the duration of the infection was not changed. The ability of +Cu cows to limit *E. coli* growth in the mammary gland may have contributed to the lower magnitude of temperature response.

Plasma Cu concentration was significantly higher than that in -Cu animals at many sample times for the

+Cu group. Increased plasma Cu levels during infection and inflammation have also been noted in rats (Conforti et al., 1982; Oliva et al., 1987). Plasma Cp activity was numerically lower for the length of the challenge for the +Cu group compared with the -Cu group. This is contrasted by other studies in rats (Conforti et al., 1982), Holstein steers (Stabel et al., 1993), and Holstein heifers (Harmon, 1998), where Cp activity was greater in the Cu-supplemented animals. The data in the present trial may suggest, as indicated earlier, that Cu may be transported by a method other than Cp in Cu-adequate, lactating Holstein heifers during response to coliform mastitis. An alternate possibility might be stimulation of Cp activity in marginal deficiency to mobilize liver stores of Cu. In Cu-adequate animals, lower Cp activity may be adequate to transport Cu to other sites.

No differences between treatment groups for DMI were observed during the period following the challenge similar to results others have reported (Stabel et al., 1993; Harmon et al., 1994a). However, the +Cu group tended to have a lower DMI than the -Cu group from d 8 postchallenge until d 15 postchallenge. As expected, the greatest decrease in percentage of DMI was on the first day postchallenge. Interestingly, DMI nearly approached the prechallenge average by d 2 postchallenge.

Copper status had no effect on milk production changes during the period following the challenge. The greatest depression of milk production was about 19% for +Cu cows and 25% for -Cu cows ($P = 0.18$) on the d 2 postchallenge. Milk production approached the prechallenge average by d 4 postchallenge. Harmon et al. (1994a) also found no effects of Cu status on milk production following challenge in dairy heifers.

CONCLUSIONS

Liver mineral analysis may be a better indicator of an animal's overall mineral status than plasma analysis for Cu during marginal deficiency. Ceruloplasmin activity also may not be a reliable indicator of an animal's Cu status within the range of liver Cu concentration found in this study. No differences were noted with DMI, BW, and milk production. Surprisingly, Cu-supplemented heifers had a higher percentage of quarters infected at calving than unsupplemented heifers.

Copper-supplemented animals had lower bacterial counts, lower SCC, lower clinical udder scores, and lower peak rectal temperatures than responses in control animals after intramammary challenge with *E. coli* strain 727. The decreased clinical severity could be due to increased capability of neutrophils in supplemented animals to kill the invading *E. coli*. Copper supplementen-

tation appeared to decrease the severity of an *E. coli* infection, while the duration of the infection was unchanged. Copper-supplemented animals were also able to maintain higher plasma Cu concentrations over the duration of the challenge. Supplementation did not affect changes in DMI or milk production following challenge. Copper supplementation reduced the peak clinical response during experimental *E. coli* mastitis but had no effect on duration.

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REFERENCES

- Association of Official Analytical Chemists. 1995. Official Methods of Analysis. 16th ed. AOAC, Arlington, VA.
- Boyne, R. and J. R. Arthur. 1981. Effects of selenium and copper deficiency on neutrophil function in cattle. *J. Comp. Path.* 91:271-276.
- Conforti, A., L. Franco, R. Milanino, and G. Velo. 1982. Copper and ceruloplasmin (Cp) concentrations during the acute inflammatory process in the rat. *Agents Actions* 12:303-307.
- Craven, N., and M. R. Williams. 1985. Defenses of the bovine mammary gland against infection and prospects for their enhancement. *Vet. Immunol. Immunopathol.* 10:71-127.
- Crist, W. L., R. J. Harmon, J. O'Leary, A. J. McAllister, G. Heersche, Jr., D. Amaral-Phillips, and L. W. Turner. 1992. Mastitis and its control. ASC-140. University of Kentucky - Cooperative Extension Service.
- Du, Z., R. W. Hemken, and R. J. Harmon. 1996. Copper metabolism of Holstein and Jersey cows and heifers fed diets high in cupric sulfate or copper proteinate. *J. Dairy Sci.* 79:1873-1880.
- Erskine, R. J. 1993. Nutrition and mastitis. Pages 551-561 in *The Veterinary Clinics of North America: Food Animal Practice*. K. L. Andersen, ed. W. B. Saunders, Philadelphia, PA.
- Erwin, E. S., I. A. Dyer, T. O. Meyer, and K. W. Scott. 1956. Uses of aspiration biopsy technique. *J. Anim. Sci.* 15:428-434.
- Gengelbach, G. P., and J. W. Spears. 1998. Effects of dietary copper and molybdenum on copper status, cytokine production, and humoral immune response of calves. *J. Dairy Sci.* 81:3286-3292.
- Gengelbach, G. P., J. D. Ward, J. W. Spears, and T. T. Brown, Jr. 1997. Effects of copper deficiency and copper deficiency coupled with high dietary iron or molybdenum on phagocytic cell function and response of calves to a respiratory disease challenge. *J. Anim. Sci.* 75:1112-1118.
- Harmon, R. J. 1998. Trace minerals and dairy cattle: Importance for udder health. Pages 485-495 in *Biotechnology in the Feed Industry*. T. P. Lyons and K. A. Jacques, ed. Nottingham Univ. Press, Nottingham, UK.
- Harmon, R. J. 1994. Physiology of mastitis and factors affecting somatic cell counts. *J. Dairy Sci.* 77:2103-2112.
- Harmon, R. J., T. W. Clark, D. S. Trammell, B. A. Smith, P. M. Torre, and R. W. Hemken. 1994a. Influence of copper status in heifers on response to intramammary challenge with *Escherichia coli* endotoxin. *J. Dairy Sci.* 77(Suppl. 1):198.
- Harmon, R. J., R. J. Eberhart, D. E. Jasper, B. E. Langlois, and R. A. Wilson. 1990. Microbiological procedures for the diagnosis of bovine udder infection. Natl. Mastitis Council, Arlington, VA.
- Hoblet, K. H., G. D. Schnitky, D. Arbaugh, J. S. Hogan, K. L. Smith, P. S. Schoenberger, D. A. Todhunter, W. D. Hueston, D. E. Pritchard, G. L. Bowman, L. E. Heider, B. L. Brockett, and H. R. Conrad. 1991. Costs associated with selected preventive practices and with episodes of clinical mastitis in nine herds with low somatic cell counts. *J. Am. Vet. Med. Assoc.* 199:190-196.
- Hogan, J. S., W. P. Weiss, K. L. Smith, D. A. Todhunter, and P. S. Schoenberger. 1995. Effects of an *Escherichia coli* J5 vaccine on mild clinical coliform mastitis. *J. Dairy Sci.* 78:285-290.
- Johnson, M. A. 1986. Interaction of dietary carbohydrate, ascorbic acid and copper with the development of copper deficiency in rats. *J. Nutr.* 116:802-815.
- Jones, D. G. 1984. Effects of dietary copper depletion on acute and delayed inflammatory responses in mice. *Res. Vet. Sci.* 37:205-210.
- Jones, D. G., and N. F. Suttle. 1981. Some effects of copper deficiency on leucocyte function in sheep and cattle. *Res. Vet. Sci.* 31:151-154.
- Jones, D. G., and N. F. Suttle. 1983. The effect of copper deficiency on the resistance of mice to infection with *Pasturella haemolytica*. *J. Comp. Pathol.* 93:143-149.
- Jones, D. G., and N. F. Suttle. 1986. Copper and disease resistance in sheep: a rare natural confirmation of interaction between a specific nutrient and infection. *Proc. Nutr. Soc.* 45:317-325.
- Koller, L. D., S. A. Mulhern, N. C. Frankel, M. G. Steven, and J. R. Williams. 1987. Immune dysfunction in rats fed a diet deficient in copper. *Am. J. Clin. Nutr.* 45:997-1006.
- Mills, C. F. 1987. Biochemical and physiological indicators of mineral status in animals: copper, cobalt, and zinc. *J. Anim. Sci.* 65:1702-1711.
- National Research Council. 1989. Nutrient Requirements of Dairy Cattle. 6th revised ed. Natl. Acad. Sci., Washington, DC.
- National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7th revised ed. Natl. Acad. Sci., Washington, DC.
- Newberne, P. M., C. E. Hunt, and V. R. Young. 1968. The role of diet and the reticuloendothelial system in the response of rats to *Salmonella typhimurium* infection. *Br. J. Exp. Pathol.* 49:448-457.
- Oliva, J., M. Castell, J. Queralt, and C. Castellet. 1987. Effect of chronic inflammation on copper and zinc metabolism. *Rev. Esp. Fisiol.* 43:25-31.
- Paynter, D. I. 1987. The diagnosis of copper insufficiency. Pages 101-119 in *Copper in Animals and Man*, Vol 1. J. M. Howell and J. M. Gawthorne, ed. CRC Press, Inc., Boca Raton, FL.
- Prohaska, J. R., and O. A. Lukasewycz. 1990. Effects of copper deficiency on the immune system. Page 123 in *Antioxidant Nutrients and Immune Functions*. A. Bendich, M. Phillips, and R. P. Teng-erdy, ed. Plenum Press, New York.
- Prohaska, J. R., S. Downing, and O. A. Lukasewycz. 1983. Chronic dietary copper deficiency alters biochemical and morphological properties of mouse lymphoid tissues. *J. Nutr.* 113:1583-1590.
- Puls, R. 1994. Mineral levels in animal health. *Diagnostic Data*. 2nd ed. Sherpa International, Clearbrook, British Columbia, Canada.
- SAS. 1999. SAS User's Guide: Statistics, Version 8 Edition. SAS Inst., Inc., Cary, NC.
- Schosinsky, K. H., H. P. Lehman, and M. F. Beeler. 1974. Measurement of ceruloplasmin from its oxidase activity in serum by use of o-dianisidine dihydrochloride. *Clin. Chem.* 20:1556-1563.
- Smith, K. L., J. H. Harrison, D. D. Hancock, D. A. Todhunter, and H. R. Conrad. 1984. Effect of vitamin E and selenium supplementation on incidence of clinical mastitis and duration of clinical symptoms. *J. Dairy Sci.* 67:1293-1300.
- Smith, K. L., J. S. Hogan, and W. P. Weiss. 1991. Dietary selenium and vitamin E influence the resistance of cows to mastitis. *Proc. Ohio Dairy Nutrition Conf.*
- Smith, K. L., J. S. Hogan, and W. P. Weiss. 1997. Dietary vitamin E and selenium affect mastitis and milk quality. *J. Dairy Sci.* 75:1659-1665.

- Stabel, J. R., J. W. Spears, and T. T. Brown, Jr. 1993. Effect of copper deficiency on tissue, blood characteristics, and immune function of calves challenged with infectious bovine rhinotracheitis virus and *Pasturella hemolytica*. *J. Anim. Sci.* 71:1247–1255.
- Suttle, N., A. Field, and R. Barlow. 1970. Experimental copper deficiency in sheep. *J. Comp. Pathol.* 80:151–162.
- Torre, P. M., R. J. Harmon, R. W. Hemken, T. W. Clark, D. S. Trammell, and B. A. Smith. 1996. Mild dietary copper insufficiency depresses blood neutrophil function in dairy cattle. *J. Nutr. Immunol.* 4:3–24.
- Wikse, S. E., D. Herd, R. Field, and P. Holland. 1992. Diagnosis of copper deficiency in cattle. *J. Am. Vet. Med. Assoc.* 200:1625–1629.
- Woolliams, C., N. F. Suttle, J. A. Woolliams, D. J. Jones, and G. Wiener. 1986. Studies on lambs genetically selected for low and high copper status. 1. Differences in mortality. *Anim. Prod.* 43:293–301.
- Xin, Z., R. W. Hemken, D. F. Waterman, and R. J. Harmon. 1991. Effects of copper status on neutrophil function, superoxide dismutase, and copper distribution in steers. *J. Dairy Sci.* 74:3078–3085.