Effect of dietary copper source on response to coliform mastitis in dairy cows

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ABSTRACT

The effect of organic or inorganic dietary Cu on *Escherichia coli* mastitis was investigated in first-lactation heifers. Twenty-eight primigravid Holstein heifers were assigned to 3 treatments in a completely randomized block design with 10 blocks of 3 animals grouped by expected calving date. Treatments were as follows: basal diet [7.1 mg Cu/kg of dry matter (DM); CON] and diets supplemented with Cu (10 mg/kg of DM) as Cu sulfate (CUS) or as Cu proteinate (CUP). Treatments were fed individually from 60 d prepartum through 49 d of lactation. All heifers were marginally deficient at the onset of the experiment (liver Cu of 60 mg/kg) and did not differ between groups. Mean liver Cu concentrations were about 3-fold greater in CUS and CUP compared with CON at d 0, 21, and 42 of lactation. At d 34 postpartum, one pathogen-free quarter per cow was infused with *Escherichia coli* strain 727. Copper supplementation did not lower peak responses to challenge; however, CUP tended to offer some benefits: milk bacterial count with CUP was lower compared with CON at 24, 48, and 72 h and lower than CUS at 24 and 96 h, and postchallenge milk production tended to be greater for CUP. Clinical udder score was lower at 12 h for CUP and CUS compared with CON, and at 144 h CUP had lower clinical scores compared with CUS or CON. Somatic cell count, dry matter intake, plasma Cu, and plasma ceruloplasmin did not differ between treatments. Compared with the control diet or Cu sulfate supplement, supplementation with Cu proteinate tended to improve the clinical status of cows after live *E. coli* intramammary challenge.

Key words: dairy cow, mastitis, copper, coliform

INTRODUCTION

Sound mastitis control is critical in producing high quality milk and preserving dairy cattle health. Nutritional interventions that address essential micronutrients, such as vitamin E, selenium, zinc, and copper,

have been shown effective in improving udder health and reducing SCC in milk (Smith et al., 1984, 1997; Erskine, 1993; Harmon and Torre, 1997). Copper sulfate supplementation of dairy cattle has been shown to reduce the incidence and severity of IMI in response to experimentally induced mastitis using endotoxin (Harmon et al., 1994) or live *Escherichia coli* (Scaletti et al., 2003) in primiparous Holstein heifers.

Supplementation of animal feed with micronutrients has traditionally relied on inorganic sources. However, in recent years research in ruminants indicates that organic forms of some micronutrients may be more biologically available. Such benefits have been demonstrated in dairy cattle for both selenium (Malbe et al., 1995; Knowles et al., 1999; Ortman and Pehrson, 1999; Malbe et al., 2003; Ceballos-Marquez et al., 2010) and zinc (Wright and Spears, 2001; Cope et al., 2009). A study by Harmon (1998) found that supplementation of heifers with organic, proteinated Cu (Bioplex, Alltech Inc., Nicholasville, KY), compared with Cu sulfate, resulted in a lower percentage of quarters infected with major pathogens. Moreover, unlike Cu sulfate, the Bioplex treatment was linked to increased plasma Cu levels without marked stimulation of ceruloplasmin (Cp), suggesting that the mechanism of Cu absorption and (or) transport differs between organic and inorganic forms (Harmon, 1998).

The objective of this study was to compare the effects of organic and inorganic sources of dietary Cu on the response of primiparous heifers to live *E. coli* intramammary challenge.

MATERIALS AND METHODS

This experiment was approved in advance by the Institutional Animal Care and Use Committee of the University of Kentucky and conducted in accordance with the principles and guidelines on animal care and use of the Federation of Animal Science Societies (1999).

Experimental Design

Forty-two pregnant Holstein heifers were assigned to treatments in a completely randomized block design, with animals blocked in groups of 3 by expected calv-

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ing date. Of the 42 Holstein heifers in the study, 14 were removed from the study. Seven were removed after the prepartum concentrate was determined to have supplemental Cu (about 100 mg/kg of DM) due to an error with the commercial feed mill that supplied the concentrate. Three were removed because of retained placentas (1 CUS and 1 CUP) and uterine infections (1 CUP) postpartum. One heifer was removed for each of the following reasons: displaced abomasum after parturition (CUS), leg injury (CUP), and death (CON). The final heifer that was removed was normal (CUS), but all other heifers in her block were removed. Data were analyzed for 9 CON, 10 CUS, and 9 CUP heifers. The trial began in August 2000 and was completed in July 2002. Heifers were not fed supplemental Cu from about 5 mo of age until study enrollment, which created marginal liver Cu deficiency (60 mg/kg of DM; Puls, 1994). At 60 d before anticipated calving, heifers were assigned to dietary treatments. Treatment groups were (1) basal diet (7.1 mg/kg of DM of Cu, CON; n = 9), (2) basal diet plus 10 mg/kg of DM of Cu as Cu sulfate in a ground corn carrier individually fed as a top-dress (CUS; n = 10), and (3) basal diet plus 10 mg/kg of DM of Cu as Cu proteinate (Bioplex Cu; Alltech Inc.) in a ground corn carrier individually fed as a top-dress (CUP; n = 9). A prepartum diet was fed ad libitum from 60 to 21 d before anticipated parturition and consisted of corn silage and alfalfa silage (equal proportion, DM basis) and 1.8 kg of concentrate per day mixed with forages (Table 1). A prepartum transition diet was fed ad libitum from 21 d before parturition until parturition and consisted of corn silage and alfalfa silage (equal proportion, DM basis) and 3.6 kg of concentrate per day mixed with forages (Table 1). The postpartum diet (Table 1) was fed as a TMR and consisted of corn silage (25% of diet DM), alfalfa silage (25% of diet DM), and concentrate (50% of diet DM). The prepartum diet met NRC (1989) requirements for all nutrients except Cu, Zn, Se, and Na (Table 1). Selenium was supplemented at 0.23 mg/kg of DM (NRC, 1989 requirement 0.3 mg/kg of DM) to provide 2.7 mg of Se per day. Dietary Na was 0.09% of diet DM, total dietary Zn was 28.5 mg/kg of DM, and total dietary Cu was 7.7 mg/kg of DM (NRC, 1989 requirement 0.10%, 40 mg/kg of DM, and 10 mg/kg of DM, respectively). The prepartum transition diet met NRC (1989) requirements for all nutrients except Cu and Na (Table 1). Dietary Na was 0.09% of DM and dietary Cu was 6.7 mg/kg of DM (NRC, 1989 requirement 0.10% and 10 mg/kg of DM, respectively). Vitamin E was supplemented at 1,004 IU/d and supplemental Se was 3.6 mg/d. The postpartum diet met NRC (1989) requirements for all nutrients except Cu and ADF (Table 1). Acid detergent fiber was 18.7% of DM and dietary Cu was 6.8 mg/kg

of DM (NRC, 1989 requirement >19% and 10 mg/kg of DM, respectively). Vitamin E was supplemented at 535 IU/d and supplemental Se was 5.7 mg/d in the postpartum diet. Dietary Fe was about 460 mg/kg of DM for all diets. Experimental heifers were housed in a tie-stall barn bedded with sawdust and allowed access to a dirt lot about 4 h/d. Dry matter intake was measured daily and BW obtained weekly. Heifers were moved to a box stall bedded with straw about 3 d before parturition and returned to the tie-stall barn about 3 d after parturition. After parturition, animals were milked at 0430 and 1530 h daily. All forages and concentrates were sampled every other week and stored at -20°C . The samples were compiled every 3 mo, sub-sampled, and sent to a commercial laboratory (Northeast DHIA Forage Analysis Laboratory, Ithaca, NY) for mineral and nutrient analysis by wet chemistry.

Intramammary Bacterial Challenge

Escherichia coli strain 727, originally isolated from a naturally occurring IMI, was used as the challenge pathogen. Challenge inoculum was prepared as described by Scaletti et al. (2003). One quarter of each heifer was challenged by intramammary infusion of the challenge strain 5 h after the morning milking and feeding. Quarters chosen for challenge were bacteriologically negative and had a geometric mean SCC of 55,000 at the time of challenge. The geometric mean of the colony-forming units for inoculum was 29 (20 to 40) suspended in Hanks' balanced salt solution. Heifers within the same block were challenged on the same day. The arithmetic mean DIM at challenge was 32 d (25 to 50 d).

Sample Collection and Analysis

Liver Biopsies. At 60 and 21 d prepartum, within 3 d postpartum, and at 21 and 49 d postpartum, liver samples (average weight 0.16 g of dry weight) were obtained via biopsy (Erwin et al., 1956) to determine Cu status. After collection, samples were analyzed as previously reported (Scaletti et al., 2003). Samples were rinsed with saline and stored at -20°C until analysis by atomic absorption spectrophotometry (Solaar, Thermo Jarrell Ash Corp., Franklin, MA) using the wet-ashing procedure previously reported (Scaletti et al., 2003). Dried samples were digested in 12 mL of concentrated nitric acid and then digested again in 10 mL of 30% hydrogen peroxide. Samples were dried and reconstituted in 10 mL of 1 N hydrochloric acid and analyzed for Cu. Results were reported on a dry weight basis.

Milk and Blood Samples. Duplicate quarter foremilk samples were aseptically collected within 3 d of

Table 1. Calculated nutrient composition of prepartum, prepartum transition, and postpartum diets from assayed values of feeds (DM basis)¹

Item	Prepartum	Prepartum transition	Postpartum
CP, %	15.92	15.31	17.75
ADF, %	26.96	23.36	18.72
NDF, %	39.33	34.25	28.65
Ca, %	1.05	1.17	1.40
P, %	0.46	0.50	0.64
Mg, %	0.20	0.20	0.26
K, %	1.79	1.55	1.49
Na, %	0.09	0.09	0.55
S, %	0.18	0.18	0.27
Fe, mg/kg	467.0	461.2	443.5
Zn, mg/kg	28.5	39.6	46.8
Cu, ² mg/kg	7.7	6.7	6.8
Mn, mg/kg	72.6	78.8	118.4
Mo, mg/kg	1.0	1.0	1.0
Se, ³ mg/kg	0.23	0.37	0.34
Vitamin A, ⁴ IU/d	8,092	32,000	80,000
Vitamin D, ⁴ IU/d	3,197	6,400	16,000
Vitamin E, ⁴ IU/d	125	1,004	535
NE _L , Mcal/kg	1.53	1.61	1.71

¹Nutrient composition obtained through wet chemistry of forages and concentrates (Northeast DHIA Forage Analysis Laboratory, Ithaca, NY).

²Does not include supplemental Cu.

³Calculated supplemental Se provided 2.7, 3.6, and 5.7 mg/d, respectively.

⁴Calculated supplemental vitamin level.

parturition, and on lactation d 7, 14, 21, and 49 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 from duplicate samples that did not match were not included in the data set. Somatic cell count was determined by commercial laboratory (Mid-South DHIA, Springfield, MO) using an electronic counter. Quarter foremilk samples (for SCC and bacteriology) were also 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. Jugular blood samples were also collected at these sample times by venipuncture in Vacutainers (Becton, Dickinson and Co., Franklin Lakes, NJ) containing lithium heparin (for plasma mineral analysis and Cp activity) and Vacutainers containing potassium EDTA (for hematocrit, white blood cell count, and erythrocyte sedimentation rate). Whole blood was centrifuged for 20 min at 1,000 × g, and plasma was frozen at −20°C in aliquots for later use. Thawed plasma was diluted 1:4 (vol/vol) in distilled, deionized nanopure water and analyzed using atomic absorption spectrophotometry to determine Cu, Fe, and Zn concentrations. Plasma Cp activity was measured as oxidase activity using the procedure of Schosinsky et al. (1974) which uses *o*-dianisidine dihydrochloride as substrate. The procedure was adapted to a microplate format that allowed the assay of samples

in quadruplicate. Whole blood was used to determine white blood cell count (**WBC**) by an electronic cell counter (Coulter Corporation, Miami, FL); erythrocyte sedimentation rate was determined following the procedure of Sox and Liang (1986); and hematocrit was determined using a microhematocrit procedure. Colony-forming units of *E. coli* per milliliter and SCC were determined in quarter foremilk samples postchallenge. 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. Milk samples from 12 to 144 h were diluted 1:10 in PBS to obtain accurate SCC for samples that exceeded the upper limit (9.7 million cells/mL) of the electronic cell counting equipment. Raw data were expressed as log₁₀ cfu/mL and log₁₀ SCC/mL of milk.

Clinical Signs

The clinical status of all quarters was recorded on a 5-point scale at the time quarter foremilk samples were obtained following the scale outlined by Hogan et al. (1995). The scale was as follows: 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 before challenge and each time quarter samples were collected postchallenge.

Milk Production and DMI During Challenge

Milk production was recorded from weigh jars after 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 the 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 before challenge, and b = daily value postchallenge.

Statistical Analysis

Treatment differences for bacterial counts, SCC, rectal temperatures, clinical scores, blood and liver mineral concentrations, Cp activity, white blood cell count, and DMI and milk production (as percentages of prechallenge means) were tested using the PROC MIXED procedure and protected least significant difference (SAS Institute, 1985). 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, SCC, and white blood cell counts to normalize data before analysis.

RESULTS AND DISCUSSION

Liver Cu

Dietary supplementation with Cu at 10 mg/kg of DM from CUS or CUP for 40 d provided sufficient Cu sources to achieve adequate liver Cu concentration according to levels defined by Puls (1994) and Mills (1987). Liver Cu did not differ ($P = 0.58$) between groups before dietary treatments 60 d prepartum. At d 21 prepartum and at d 0, 21 and 49 postpartum liver Cu was greater [treatment (**Trt**) $\times$ time, $P < 0.0001$; time, $P < 0.0001$; Trt, $P < 0.0003$] in Cu-supplemented groups compared with unsupplemented control heifers. Overall liver Cu concentrations were 64.7, 174.5, and 172.8 mg/kg of DM for CON, CUS, and CUP heifers, respectively. Differences between CUS and CUP treatments were not significant ($P = 0.93$). Liver Cu 10 d before intramammary challenge was 60.6 mg/kg of DM

for CON heifers and 181.1 and 215.1 mg/kg of DM for CUS and CUP heifers, respectively.

Experimental *E. coli* Challenge

Responses of heifers to *E. coli* challenge were moderately influenced by supplemental Cu at 10 mg/kg of DM. Copper supplementation did not lower peak responses after intramammary challenge although variable benefits were observed for Cu supplementation. Supplementation with CUP had greater effects for some parameters compared with CON and CUS. Heifers supplemented with CUP were better able to limit *E. coli* growth in the mammary gland, which appeared to translate to improved clinical udder score and tendency for improved recovery to normal milk yield. Each quarter challenged developed an IMI and clinical mastitis. All quarters used for challenge were bacteriologically negative at the time of challenge with SCC that averaged 55,000 cells/mL. A review of the literature revealed limited studies investigating effects of organic and inorganic Cu supplementation on mastitis (Harmon, 1998) and no other studies investigating effects of organic Cu supplementation on experimentally induced mastitis compared with inorganic sources. Studies in ewes (Jones and Suttle, 1981), dairy steers (Boyne and Arthur, 1981; Xin et al., 1991), and dairy heifers (Harmon et al., 1994; Torre et al., 1996; Scaletti et al., 2003) have shown the role of Cu in immune response. 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).

Bacterial Count

Escherichia coli numbers increased dramatically in milk from the challenged quarters after infusion, approaching or exceeding 10^4 cfu/mL in all treatments (Figure 1; Trt $\times$ time, $P < 0.05$; time, $P < 0.0001$; Trt, $P < 0.11$). All challenged quarters were bacteriologically negative by culture of milk at 0 h. *Escherichia coli* was first isolated from the 6 h sample from all treatment groups. Bacterial counts increased sharply from 6 to 12 h, with peak numbers of *E. coli* being reached at 12 h. Peak numbers did not differ ($P = 0.55$) and were 3.92, 4.43, and 4.24 $\log_{10}$ cfu/mL for CON, CUS, and CUP heifer groups, respectively. Heifers supplemented with CUP had lower ($P = 0.02$) bacterial counts at 24 h compared with CUS and CON heifers. At 48 h, CUP heifers tended to have ($P = 0.10$) less *E. coli* in milk than CON heifers. Heifers supplemented with CUP continued to have less ($P = 0.05$) bacteria in

milk at 72 h compared with CUS and CON heifers. At 96 h, CUP heifers tended to have lower ($P = 0.07$) bacterial counts compared with CUS heifers. No other differences between treatments were observed, although bacterial numbers in CUP animals tended to remain numerically lower than those of the other 2 treatment groups throughout the remainder of the trial. Supplementation with CUP provided some benefit for the elimination of the infection, or in limiting bacterial growth, or both. Other factors not measured may have improved bacterial elimination and subsequently improved milk production recovery. Some of these factors could include modifying the environment of the gland affecting neutrophil function or affecting lactoferrin, which is an Fe-binding antibacterial protein (Anderson et al., 1985).

SCC

The SCC of milk from the infused mammary quarter was constant from 0 to 6 h for all treatment groups (Figure 2). No differences were observed for any of the samples (Trt $\times$ time, $P < 0.76$; time, $P < 0.0001$; Trt, $P < 0.74$). Beginning at 12 h, SCC sharply increased by 20-fold for all treatment groups and at 18 h SCC increased more than 30-fold for all treatment groups. Peak SCC was reached at 36 h and was 7.84, 7.48, and 7.60 $\log_{10}$ cells/mL for CON, CUS, and CUP heifers, respectively. After 36 h, SCC gradually decreased in all 3 treatment groups until 240 h.

Figure 1. Least squares means *Escherichia coli* numbers ($\log_{10}$ cfu/mL) in milk after intramammary challenge with *E. coli* strain 727 at 0 h in first-lactation heifers with 10 mg/kg of DM supplemental dietary copper as Cu sulfate ($\blacktriangle$; $n = 10$) or Cu proteinate ($\blacksquare$; $n = 9$) and unsupplemented control heifers ($\circ$; $n = 9$). Pooled SEM = 0.33. Treatment $\times$ time, $P < 0.05$. ^a $P = 0.02$, CUP versus CUS and CON; ^b $P = 0.10$, CUP versus CON; ^c $P = 0.05$, CUP versus CUS and CON; ^d $P = 0.07$, CUP versus CUS.

Figure 2. Least squares means SCC ($\log_{10}$ cells/mL) in milk after intramammary challenge with *Escherichia coli* strain 727 at 0 h in first-lactation heifers with 10 mg/kg of DM supplemental dietary copper as Cu sulfate ($\blacktriangle$; $n = 10$) or Cu proteinate ($\blacksquare$; $n = 9$) and unsupplemented control heifers ($\circ$; $n = 9$). Pooled SEM = 0.20. Treatment $\times$ time, $P < 0.74$.

Clinical Score

The mean clinical udder score (Figure 3; Trt $\times$ time, $P < 0.03$; time, $P < 0.0001$; Trt, $P < 0.14$) of the infused quarter was lower ($P = 0.03$) at 12 h in CUP and CUS heifers compared with CON heifers (1.0, 1.2, and 2.0 respectively). At 96 h, CUS animals tended to have lower scores ($P = 0.08$) compared with CON (2.3 vs. 3.1, respectively); at 144 h CUP heifers (1.3) had lower ($P = 0.01$) clinical scores compared with CUS or CON heifers, and CUS heifers (1.9) had lower scores compared with CON heifers (2.6). A clinical score of 1.0 was observed 24 h before and immediately before *E. coli* infusion into one mammary quarter. All groups reached peak clinical score, which did not differ ($P < 0.85$), at 24 h and then gradually declined for the remaining samples. Clinical udder scores showed that CON animals displayed a clinical response more quickly and maintained an elevated score for a longer period, suggesting that CUS and CUP supplementation modified the clinical response.

Rectal Temperature

Fever is one of the more obvious signs of the systemic effects of inflammation (Kumar et al., 1997). Peak rectal temperatures were similar between treatments and were about 2°C greater than prechallenge temperatures. Rectal temperatures (Figure 4; Trt $\times$ time, $P < 0.47$; time, $P < 0.0001$; Trt, $P < 0.65$) remained constant from 0 to 12 h for all treatments and then sharply increased to peak values at 18 h. Temperatures returned to normal by 24 h in CUS and CON heifers (38.8°C each) and were numerically lower than tem-

Figure 3. Least squares means clinical udder score after intramammary challenge with *Escherichia coli* strain 727 at 0 h in first-lactation heifers with 10 mg/kg DM supplemental dietary copper as Cu sulfate ($\blacktriangle$; $n = 10$) or Cu proteinate ($\blacksquare$; $n = 9$) and unsupplemented control heifers ($\circ$; $n = 9$). Pooled SEM = 0.3. Treatment $\times$ time, $P < 0.03$. ^a $P = 0.02$, CUP and CUS versus CON; ^b $P < 0.08$, CUS versus CON; ^c $P < 0.01$ CUP versus CUS versus CON.

peratures of CUP heifers (39.3°C). Temperatures of heifers supplemented with CUP returned to normal by 36 h and temperatures from all groups were normal for the remaining sample times.

WBC and Hematocrit

White blood cell count (Figure 5; Trt $\times$ time, $P < 0.92$; time, $P < 0.0001$; Trt, $P < 0.24$) remained constant for all treatment groups from 0 to 12 h and then dramatically declined and reached its lowest level at 18 h. The WBC was similar between treatments at 12 h (7.03, 7.03, and 7.06 $\log_{10}$ cells/mL for

CON, CUS, and CUP groups, respectively) and then declined by about 4.5 million cells/mL (6.83, 6.77, and 6.82 $\log_{10}$ cells/mL for CON, CUS, and CUP groups, respectively). Depressed WBC continued through 24 h and then increased sharply for all treatment groups by 36 h. At 36 h, WBC returned to preinfusion levels for all groups, with CUP heifers having numerically greater WBC than CUS and CON heifers. Decreased WBC count was also observed by Kremer et al. (1993) after infusion of *E. coli* into one quarter of the mammary gland, although dietary Cu concentrations were not stated. Migration of WBC from the blood into the udder is thought to be of major importance in host defense against *E. coli* IMI (Craven and Williams, 1985; Kremer et al., 1993). Leukocytosis is a common feature of inflammatory reactions especially those induced by bacterial infection and results from release of cells from bone marrow (Kumar et al., 1997). Hematocrit did not differ (Trt $\times$ time, $P < 0.99$; time, $P < 0.004$; Trt, $P < 0.74$) between treatments (ranging from 36.1 to 42.7%) and declined over time after intramammary challenge.

Plasma Cu

Plasma Cu was similar between treatments (Trt $\times$ time, $P < 0.18$; time, $P < 0.0001$; Trt, $P < 0.61$) for all times except 24 h when CUS heifers had numerically greater plasma Cu concentration compared with CON and CUP heifers (0.87 vs. 0.72 and 0.76 $\mu\text{g/mL}$ for CUS, CON, and CUP heifers, respectively). Least squares means concentrations were constant between treatments (0.83 $\mu\text{g/mL}$) over the sample period. Our previous work (Scaletti et al., 2003) showed significantly greater plasma Cu concentrations compared with con-

Figure 4. Least squares means rectal temperature (°C) after intramammary challenge with *Escherichia coli* strain 727 at 0 h in first-lactation heifers with 10 mg/kg of DM supplemental dietary copper as Cu sulfate ($\blacktriangle$; $n = 10$) or Cu proteinate ($\blacksquare$; $n = 9$) and unsupplemented control heifers ($\circ$; $n = 9$). Pooled SEM = 0.1. Treatment $\times$ time, $P < 0.47$.

Figure 5. Least squares means white blood cell (WBC) count ($\log_{10}$ cells/mL) after intramammary challenge with *Escherichia coli* strain 727 at 0 h in first-lactation heifers with 10 mg/kg of DM supplemental dietary copper as Cu sulfate ($\blacktriangle$; $n = 10$) or Cu proteinate ($\blacksquare$; $n = 9$) and unsupplemented control heifers ($\circ$; $n = 9$). Pooled SEM = 0.05. Treatment $\times$ time, $P < 0.92$.

trols when heifers were supplemented with Cu sulfate at 20 mg/kg of DM. Increased plasma Cu levels during infection and inflammation have also been noted in rats (Conforti et al., 1982; Oliva et al., 1987).

Plasma Cp

Ceruloplasmin activity after intramammary challenge did not differ between treatments (Trt $\times$ time, $P < 0.74$; time, $P < 0.0001$; Trt, $P < 0.33$). Overall Cp activity was 61.2, 68.8, and 64.7 IU/L for CON, CUS, and CUP heifers, respectively. Copper-supplemented heifers had numerically greater Cp activities than CON heifers for several samples. Activity increased from -24 h to 0 h and then decreased after challenge with *E. coli* for all groups until 18 h. Other studies in rats (Conforti et al., 1982) and Holstein steers (Stabel et al., 1993) have shown increased Cp activity during inflammation. Our previous research (Scaletti et al., 2003) when supplementing with Cu at 20 mg/kg of DM as Cu sulfate to Holstein heifers found numerically lower plasma Cp activity after intramammary challenge. Harmon (1998) reported increased Cp activity in heifers supplemented (10 mg of Cu/kg of DM) with Cu sulfate compared with heifers unsupplemented or supplemented with organic Cu. It appears that effects of supplemental Cu may vary with amount and form provided.

DMI and Milk Production

No differences between treatment groups (Trt $\times$ time, $P < 0.80$; time, $P < 0.0001$; Trt, $P < 0.60$) for percentage DMI (expressed as percentages of means for 7 d before challenge) were observed during the period after the challenge, similar to results reported by others in dairy heifers and dairy steers (Stabel et al., 1993). As expected, the greatest decrease in percentage of DMI was on the first day postchallenge. Dry matter intake declined by 20 to 32% on the first day after intramammary challenge. Anorexia is one of the more obvious signs of systemic effects of inflammation (Kumar et al., 1997). By d 2, DMI started to increase and was 9 to 15% lower than the prechallenge mean. However, treatments did not differ (Trt $\times$ time, $P < 0.60$) at any of the sample times. Dry matter intake recovered to prechallenge intake by d 7 in CON and CUP heifers and by d 9 in CUS heifers.

Milk production (Figure 6; Trt $\times$ time, $P < 0.05$; time, $P < 0.0001$; Trt, $P < 0.16$) declined 1 d after challenge for CON and CUS heifers, whereas CUP heifers maintained milk production levels after intramammary challenge (94.4 and 95.8 vs. 100.7%, for CON, CUS, and CUP, respectively), although this difference in production was not significant ($P = 0.58$). Milk pro-

duction continued to decrease until d 2 when all groups reached their lowest milk production (71.0, 73.6, and 80.9% for CON, CUS, and CUP, respectively). By d 3, milk production tended to be greater ($P = 0.06$) for CUP heifers compared with CON and CUS heifers (101.3 vs. 90.0 and 86.7%, respectively). Differences in milk production were also observed on d 4 when CUP and CON heifers had greater ($P = 0.05$) milk yield than CUS heifers (103.5 and 99.1 vs. 89.0%, respectively). Heifers in the CUP group tended to have greater milk production ($P = 0.09$) than CUS heifers on d 5. Production was similar between treatments from d 6 until d 10 when CUP heifers tended to have greater ($P = 0.06$) milk yield than CON and CUS heifers (112.4 vs. 99.3 and 99.7%, respectively). Milk yield was similar between treatments for the remaining samples after the intramammary challenge. Differences in milk production were observed although DMI did not differ.

Comparison with a Previous Challenge Study

Few challenge studies in lactating dairy cattle have evaluated the effects of organic and inorganic forms of supplemental Cu on mastitis. Results after intramammary challenge with *E. coli* in the present study differ somewhat from results of our previous study (Scaletti et al., 2003), when Holstein heifers were supplemented at a greater rate (20 mg of Cu/kg of DM) with Cu sulfate. Inorganic Cu supplementation at 20 mg/kg of

Figure 6. Least squares means daily milk yield after intramammary challenge with *Escherichia coli* strain 727 at 0 h in first-lactation heifers with 10 mg/kg of DM supplemental dietary copper as Cu sulfate (▲; $n = 10$; 31.4 kg average 7-d milk yield) or Cu proteinate (■; $n = 9$; 30.8 kg average 7-d milk yield) and unsupplemented control heifers (○; $n = 9$; 32.0 kg average 7-d milk yield). Data are expressed as percentages of means for 7 d before challenge following the formula used by Hogan et al. (1995): $[(b/a) \times 100]$, where a = mean value for 7 d before challenge, and b = daily value postchallenge. Pooled SEM = 4.3. Treatment $\times$ time, $P < 0.05$. ^a $P < 0.06$ CUP versus CON and CUS; ^b $P < 0.05$ CUP versus CUS and CUS versus CON; ^c $P < 0.09$ CUP versus CUS.

DM resulted in lower peak bacterial counts, lower SCC, lower clinical udder scores, and lower peak rectal temperatures compared with responses in unsupplemented control animals. Liver Cu values were similar among Cu-supplemented heifers in both trials regardless of Cu source or level. Another drawback in making comparisons between the present study and our previous work (Scaletti et al., 2003) is the difference in Cu status of the control animals in each study. Control heifers in the present study had liver Cu concentrations averaging 65 mg/kg of DM, whereas control heifers in the previous study averaged 40 mg/kg of DM over the 110-d supplementation period. The concentration at which liver Cu is considered clinically deficient varies between reports in the literature. Mills (1987) reported that when liver Cu concentrations fell below 30 mg/kg of DM, normal plasma Cu could not be maintained and Cp activity (Cu-dependent) may be lost. Tiffany et al. (2002) stated that liver Cu concentrations under 25 mg/kg of DM indicated severe deficiency, which is in agreement with Underwood and Suttle (2001). Puls (1994) reported liver Cu concentrations of 2.0 to 35 mg/kg of DM as deficient and those from 17.5 to 87.5 mg/kg of DM as marginal based on diagnostic data. According to Tiffany et al. (2002), Cu liver concentrations below 75 mg/kg of DM may indicate onset of deficiency. However, the Cu concentration necessary for optimal host defense is not clear (Wikse et al., 1992; Puls, 1994).

CONCLUSIONS

Both Cu sulfate and Cu proteinate supplemented at 10 mg of Cu/kg of DM were equally able to return marginally Cu-deficient Holstein heifers to Cu-adequate status after 40 d of supplementation. Responses of heifers to *E. coli* challenge were moderately influenced by supplemental Cu at 10 mg/kg of DM. Copper supplementation did not lower peak responses after intramammary challenge although benefits were observed for Cu supplementation. Supplementation with CUP was associated with improvements in milk yield, clinical udder score, and *E. coli* numbers compared with CON and CUS treatments. This study is the first to investigate effects of organic Cu supplementation on experimentally induced mastitis in dairy cattle compared with an inorganic source. Continued investigation is warranted concerning the appropriate level of Cu supplementation.

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