Brief Research Update: Copper supplementation and its role in decreasing the severity of mastitis.

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Using nutrition to increase a cow's defense against infection has been a recent area of research. Vitamins and minerals, such as vitamin E, selenium, copper, and zinc, when properly supplemented, can enhance a cow's immunity against diseases, such as mastitis, by increasing resistance to infections and by decreasing severity of infections when they do occur. This article will discuss two recently completed trials at the University of Kentucky that looked at the effects of both level and source of supplemental dietary copper on mastitis in dairy heifers.

Experiment 1

Animals and Experimental Treatments

Twenty-three pregnant Holstein heifers were used for experiment 1. Heifers were paired by expected calving date and assigned to dietary treatments 60 days before expected calving. The treatments were: 1) basal diet which had 6.5 ppm of copper (-Cu) and 2) basal diet plus 20 ppm of additional copper sulfate that was top-dressed on the feed (+Cu). The precalving diet consisted of 3 pounds of both concentrate and alfalfa hay per day with ad libitum corn silage and alfalfa silage. The silages were mixed in equal proportion on a dry matter basis. The postcalving diet consisted of a TMR of 24% corn silage, 22% alfalfa silage, 37% concentrate, 9% whole cottonseed, and 8% alfalfa hay on a dry matter basis. Both diets followed the NRC (1989) guidelines for dairy cattle, except selenium which was 0.11 mg/kg in the prepartum diet. However, whole blood selenium concentrations were adequate in all heifers over the duration of the trial.

Heifers were housed in a tie-stall barn to allow for determination of each animal's feed intake. Animals were allowed to exercise on a sand lot for about 4 hours per day. Liver and blood samples were used to determine copper status at 60 and 21 days precalving, within 3 days postcalving, and 42 days postcalving. An additional blood sample was taken 21 days postcalving. Milk samples were taken from each quarter within 3 days of calving, and then 7, 14, 21 and 42 days postcalving for microbiological analysis and somatic cell count (SCC) determination to verify udder health.

The main part of the experiment was a bacterial challenge in the mammary gland. At day 34 of lactation, 1 quarter of each heifer was infused with *E. coli* which was originally isolated from a naturally occurring case of mastitis. Quarters used for challenge were uninfected and also had a history of low SCC since calving. Milk samples, plasma samples, and rectal temperatures were taken 24 hours before challenge, immediately prior to challenge, and 6, 12, 18, 24 and 36 hours postchallenge, with additional samples 2, 3, 4, 6, 8, and 10 days postchallenge. Milk samples were used to determine the severity of infections by counting the amount of *E. coli* in milk and also for SCC. Plasma samples were used to determine copper status during the challenge. Rectal temperatures and clinical udder scores were used to determine the severity of infections.

General Parameters

Dietary supplementation of copper sulfate at 20 ppm for 40 days provided a sufficient copper source to achieve adequate liver copper concentrations (Table 1). The liver copper concentrations of -Cu cows was low enough that they were considered to be marginally deficient (Puls, 1994). However, the copper level necessary for optimal host defense or antioxidant activity is not clear (Wikse, 1992).

The normal range of copper in the blood of healthy animals is 0.5 to 1.5 µg/ml with an average of about 0.9 µg/ml according to the NRC (1989) dairy guidelines. Plasma copper levels found in this study were lower than average (about 0.6 µg/ml) but were still in the normal range. However, plasma copper concentration may not be a reliable indicator of liver copper status within the range found in this experiment. Changes in liver copper concentration did not parallel changes seen in plasma copper between treatments. Plasma copper concentrations should not be used to determine the copper status of cows. There were no differences between treatment groups with dry matter intake, body weight, and milk production.

The NRC (1989) dietary recommendation of copper for dairy cattle is 10 ppm. In this study, the -Cu group received 6-7 ppm of Cu in the basal diet. This copper was found naturally in the feedstuffs that were in the ration and was not from supplemental copper. The unsupplemented heifers received about 60-70% of the NRC suggested dietary copper yet encountered a moderate to severe liver copper deficiency simply by lack of supplementation from about 5 months of age through gestation and parturition.

Experimental *E. coli* Challenge

Responses of heifers to the *E. coli* challenge will be discussed next as this is where the beneficial effects of supplemental copper at 20 ppm are evident. The NRC (1989) recommended dietary level of copper at 10 ppm seems adequate for growth and milk production but insufficient for optimal immune function and response.

Heifers supplemented with copper had lower peak bacterial counts (1,500 vs 28,000 colonies per ml of milk), lower peak SCC (11 vs 44 million), lower peak rectal temperatures (40.0 vs 40.8°C) and lower clinical udder scores than unsupplemented heifers. At 12 hours, +Cu animals had 1,500 colonies of *E. coli* per ml of milk while -Cu animals had 27,500 colonies of *E. coli* per ml of milk. At 18 hours, +Cu animals had 325 colonies of *E. coli* per ml of milk while -Cu animals had 3,700 colonies of *E. coli* per ml of milk. Two days postchallenge, +Cu cows had 60 colonies of *E. coli* per ml of milk and -Cu cows had 500 colonies of *E. coli* per ml of milk. The peak SCC was about 4-fold lower in +Cu cows compared to -Cu cows. Cows supplemented with Cu had body temperatures 0.8°C lower than unsupplemented cows showing that they were better able to limit bacteria growth and limit the severity of the infections. Dry matter intake and milk production were not different between treatments. The greatest decrease in percentage of dry matter intake was on the first day postchallenge while the greatest decrease in milk production was on the second day postchallenge.

Conclusions

Liver copper analysis is a better indicator of an animal's overall copper status than plasma analysis. No differences were noted with dry matter intake, body weight and milk production. Copper supplemented heifers had lower bacterial counts, lower SCC, and lower peak rectal temperatures than responses in control animals after intramammary challenge with *E. coli* 727. The decreased clinical severity of infection could be due to increased capability of copper 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.

Experiment 2

Animals and Experimental Treatments

Twenty-eight pregnant Holstein heifers were used for experiment 2. Heifers were grouped by expected calving date and assigned to dietary treatments 60 days before expected calving. The treatments were: 1) basal diet which had 7.1 ppm of copper (CON), 2) basal diet plus 10 ppm of additional copper sulfate that was top-dressed on the feed (CUS), 3) basal diet plus 10 ppm of additional copper proteinate (Bioplex; Alltech, Inc.). The precalving diet consisted of 4 pounds of concentrate per day with ad libitum corn silage and alfalfa silage. A transition diet was fed from 21 days before expected calving until calving containing 8 pounds of concentrate and ad libitum corn silage and alfalfa silage. The silages were mixed in equal proportion on a dry matter basis. The postcalving diet consisted of a TMR of 25% corn silage, 25% alfalfa silage, and 50% concentrate on a dry matter basis. All diets followed the NRC (1989) guidelines for dairy cattle, except for vitamin E which was supplemented at 1000 IU/d precalving and 535 IU/d postcalving. Heifers were housed and sampled following the similar procedures as listed in experiment one.

General Parameters

Animals supplemented with copper from either CUS or CUP for 40 days were no longer marginally copper deficient based on liver Cu concentrations (Table 2) and liver copper concentrations in animals from either copper source were not different from each other. Control heifers were marginally copper deficient over the length of the experiment. Overall copper concentrations in plasma tended to be higher in copper supplemented heifers with a marked difference at calving (Table 2) between copper supplemented heifers and control heifers. Plasma copper was in the normal range for cattle (NRC, 1989). As concluded in experiment 1, plasma copper concentration may not be a reliable indicator of liver copper status within the range found in this experiment. Changes in liver copper concentration did not parallel changes seen in plasma copper between treatments. There were no differences between treatment groups with dry matter intake, body weight, and milk production.

Experimental *E. coli* Challenge

The bacterial challenge with *E. coli* was on day 32 of lactation. Copper supplementation at 10 ppm from either CUS or CUP did not result in remarkable differences in responses compared to controls. There were no differences in peak responses among treatments in parameters measured following intramammary challenge though there were some variable benefits from copper supplementation during experimentally induced *E. coli* mastitis. Heifers supplemented with CUP had fewer colonies of *E. coli* in milk at 24 and 72 hours than CUS and CON heifers. At 24 hours, CUP heifers had 80 colonies of *E. coli* per ml of milk while the other heifers had 1270 cfu/ml and at 72 hours CUP heifers had 7 cfu/ml while the other heifers had 72 cfu/ml. At 48 hours, CUP heifers had fewer cfu/ml of *E. coli* than CON heifers (74 vs 830) and at 96 hours CUP heifers had fewer cfu/ml of *E.coli* than CUS heifers (4 vs 112). Clinical udder score was lower at 12 hours for CUS and CUP heifers than CON heifers (1 vs 2). Rectal temperature was lower at 24 hours for CON and CUS heifers than CUP heifers (38.78 vs 39.25°C). White blood cell count was higher at 36 hours for CUP heifers compared to the other groups (15.5 million vs 11.2 million cells/ml). Plasma iron concentration was higher at 24 hours in both CUP and CUS heifers compared to CON heifers. Milk production following challenge tended to be greater for CUP heifers than CUS heifers. Somatic cell count, dry matter intake, plasma copper and zinc, and plasma ceruloplasmin did not differ among treatments.

Conclusions

Similar to results reported from experiment one, liver copper analysis is a better indicator of an animal's overall copper status than plasma analysis. Though plasma copper tended to be higher in copper supplemented groups, plasma copper did not accurately parallel changes seen in liver copper except for the calving sample. No differences were noted with dry matter intake, body weight and milk production. Copper supplementation did not lower peak responses after intramammary challenge with *E. coli* 727 though there were variable benefits for copper supplementation from CUP compared to CUS and CON.

Overall Conclusions

Liver copper analysis is a better indicator of an animal's overall copper status than plasma analysis. Heifers supplemented with 20 ppm of copper as copper sulfate had lower bacterial counts, lower SCC, and lower peak rectal temperatures than responses in unsupplemented control animals. Copper supplementation with copper sulfate appeared to decrease the severity of an *E. coli* infection while the duration of the infection was not changed. Heifers supplemented with 10 ppm of copper as either copper sulfate or copper proteinate did not have different peak responses than control heifers but did have variable benefits from supplemental copper. Control animals in both studies were marginally copper deficient though the control diets contained about 6.8 ppm of copper. From the conditions encountered in these studies we suggest that supplementation of dairy cattle diets with copper between 10 and 20 ppm may be beneficial to udder health.

References

National Research Council. 1989. Nutrient Requirements of Dairy Cattle. 6th revised ed. Natl. Acad. Sci., Washington, D.C.

Puls, R. 1994. Mineral levels in animal health. Diagnostic Data. 2nd ed. Sherpa International, Clearbrook, British Columbia, Canada.

Wikse, S. E. 1992. Texas A&M Veterinary Beef Cattle Short Course.

Table 1. Least square means for liver and plasma copper in heifers with (n=11) or without (n=12) supplemental (20 ppm) dietary copper sulfate.

	Liver Cu[1] (µg/g dry weight)		Plasma Cu[2] (µg/ml)	
Day	+Cu group	-Cu group	+Cu group	-Cu group
-60	28.4	34.8	0.57	0.53
-21	134.2[a]	45.2	0.58	0.53
Calving	162.7[a]	33.4	0.79[b]	0.62
21	not measured	not measured	0.67	0.63
42	256.2[a]	45.4	0.65[c]	0.57

[1] Trt and Trt × Time, $P < 0.0001$

[2] Trt ($P < 0.04$), Trt × Time ($P < 0.01$)

[a] +Cu vs -Cu, $P = 0.0001$

[b] +Cu vs -Cu, $P = 0.0003$

[c] +Cu vs -Cu, $P = 0.05$

Table 2. Least square means for liver and plasma copper in heifers with 10 ppm supplemental copper as copper sulfate (CUS; n=10) or copper proteinate (CUP; n=9) and unsupplemented control heifers (CON; n=9).

	Liver Cu[1] (µg/g dry weight)			Plasma Cu[2] (µg/ml)		
Day	CUS	CUP	CON	CUS	CUP	CON
-60	63.73	43.84	73.14	0.71	0.73	0.72
-21	182.46[a]	139.90[a]	73.79[b]	0.67	0.67	0.62
Calving	197.98[a]	212.31[a]	64.77[b]	0.97[c]	0.96[c]	0.80[d]
21	181.14[a]	215.06[a]	60.6[b]	0.85	0.88	0.78
49	247.42[a]	252.62[a]	51.41[b]	0.75	0.73	0.66

[1] Trt and Trt × Time ($P < 0.001$)

[2] Trt ($P < 0.10$)

[a,b] Means within row with different superscripts differ ($P < 0.001$)

[c,d] Means within row with different superscripts differ ($P < 0.01$)