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# EFFECT OF MOLYBDENUM/SULFUR-INDUCED COPPER DEFICIENCY UPON ENZYME LEVELS AND REPRODUCTION IN HEIFERS

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## Summary

To evaluate the effects of molybdenum (Mo)- and sulfur (S)- induced copper (Cu) deficiency, 99 prepubertal heifers were allotted into two groups. Treated heifers (n = 72) were fed a grass hay naturally high in Mo (Mo = 8 ppm and Cu = 3.2 ppm). Sulfur was supplemented to achieve a dietary level of .3%. Control heifers (n = 27) received a grass hay with normal levels of Mo (Mo = .47 ppm and Cu = 1.5 ppm). Copper was supplemented to achieve a dietary level of 8 ppm. Diets were fed for a period of 163 d. Liver samples were collected and analyzed for total Cu. Copper-containing enzyme activity (ceruloplasmin and Cu,Zn-superoxide dismutase [Cu,Zn-SOD]), and progesterone were measured in blood serum. Heifers in the treated group had lower concentrations of liver Cu and decreased ceruloplasmin activity, with no differences noted in Cu,Zn-SOD activity or onset of puberty. No difference was detected in first-service conception rate following artificial insemination (AI). Overall AI pregnancy rate tended to be higher for control heifers.

(Key Words: Heifers, Copper, Ceruloplasmin, Cu,Zn-SOD, Reproduction.)

## Introduction

Recently in Kansas, copper (Cu) was determined to be deficient in several beef cow herds. In many instances, forage Cu was adequate, but the deficiency appeared to be due to unusually high levels of sulfur (S) or molybdenum (Mo) or both. In the rumen, S and Mo combine to form a thiomolybdate complex. Thiomolybdates have the ability to recombine with elemental Cu, forming an insoluble Cu-thiomolybdate complex. This complex renders Cu unavailable to the animal; therefore, causing a subsequent Cu deficiency.

The activity of two Cu-containing enzymes, ceruloplasmin and Cu,Zn-SOD, has been shown to be depressed in instances of Cu deficiency. Ceruloplasmin is the major carrier protein that delivers Cu to body cells. The enzyme Cu,Zn-SOD is the primary scavenger of toxic oxygen radicals that are produced during normal cellular respiration. By measuring the activity of these two enzymes, an estimate of the biological impact created by a Cu deficiency can be determined.

The experimental diet was formulated using native grass hay from a Kansas pasture with a history of high Mo content. Because no exogenous Mo was used, subsequent deficiency was thought to represent that occurring naturally in grazing beef herds under similar dietary conditions.

## Experimental Procedures

Ninety-nine prepubertal heifers were allotted into treated (n = 72) and control (n = 27) groups. Heifers assigned to the treated group received a grass hay naturally high in Mo (Mo = 8 ppm and Cu = 3.2 ppm). Sulfur was supplemented to achieve a dietary level of .3%. Control heifers received grass hay with normal molybdenum levels (Mo = .47 ppm and Cu = 1.5 ppm). Copper was supplemented to achieve a dietary level of 8 ppm. Jugular blood and liver samples were collected on d 0, 41, 79, 110, 130, and 163 of the experiment. Liver samples were collected using a liver biopsy technique. Blood samples

were collected from the jugular vein. Liver Cu was analyzed utilizing inductively coupled plasma spectroscopy (ICP). Ceruloplasmin activity was determined by the *p*-phenyl-enediamine oxidase method. The enzyme Cu,Zn-SOD was determined by measuring the initial rate of inhibition of pyrogallol autoxidation. The number of heifers attaining puberty by the start of the breeding season (April 27) was determined by analysis of serum progesterone using a conventional radioimmunoassay technique. Estrus was synchronized in all heifers by feeding MGA for 17 d, followed by a single injection of prostaglandin $F_2\alpha$. Heifers were inseminated artificially (AI) upon observation of standing estrus. The breeding season extended from April 27 until June 8.

## Results and Discussion

Even though heifers in the treated group had lower ($P < .03$) liver Cu concentrations at the beginning of the study they experienced dramatic reductions in liver Cu by d 163 (Table 1). Ceruloplasmin levels were depressed ($P < .01$) in the treated group on d 130 and 163. No differences were detected in Cu,Zn-SOD activity.

No differences were detected in the percentage of heifers reaching puberty at the start of the breeding season (66.6% for both control and treated groups) Overall AI pregnancy rate tended to be higher ($P = .34$) for control (70.4%) than treated heifers (58.3%).

Molybdenum- and S-induced Cu deficiency decreased liver copper and serum ceruloplasmin, but had no effect upon serum Cu,Zn-SOD activity, attainment of puberty, and pregnancy rate.

**Table 1. Copper and Enzyme Levels in Control (C) and Treated (T) Heifers**

| | | Day of experiment | | | | | |
|---|---|---|---|---|---|---|---|
| Trait | | 0 | 41 | 79 | 110 | 130 | 163 |
| Liver copper, ppm | C | 148 | 238 | 111 | 72 | 68 | 115 |
| | T | 93[b] | 147[b] | 68 | 37 | 21[b] | 24[b] |
| Ceruloplasmin, mg/100ml | C | 13.9 | 14.1 | 11.6 | 12.0 | 16.5 | 15.2 |
| | T | 14.4 | 15.6 | 11.6 | 11.1 | 12.8[b] | 11.2[b] |
| Cu,Zn-SOD, units[a] | C | .998 | --- | .785 | --- | --- | .937 |
| | T | .988 | --- | .800 | --- | --- | .880 |

[a]1 unit = 50% inhibition of initial rate.
[b]Different ($P < .05$) from controls within day.