

## **ANIMAL RESPONSE TO GRAZING ON RECLAIMED MINE TAILINGS**

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### **ABSTRACT**

In 1994 and 1995, 32 cow/calf pairs were grazed on molybdenum (Mo) enriched herbage (21-65 mg kg<sup>-1</sup> DM) for a 12 week period at a reclaimed mine site located at the Highland Valley Copper Mine near Logan Lake, British Columbia. The scientific literature indicates that feedstuffs with high levels of Mo (>10 ppm) may induce a copper (Cu) deficiency in ruminants (referred to as molybdenosis), which results in poor animal health and productivity and may result in death of affected animals. This study was undertaken to evaluate the efficacy of grazing cattle on Mo enriched forage on reclaimed mine tailings, with or without an All-Trace copper-enriched bolus supplement, and its effects on Cu and Mo levels in milk, blood and liver tissue. Weight gains and health were normal for all the animals and no visual signs of a Mo induced Cu deficiency were observed. Serum Cu concentrations remained in the normal range of 0.7 to 1.5 ug ml<sup>-1</sup> and did not differ (P<0.05) for the supplemented and control groups for cows or calves in either year. Liver tissue Cu concentration increased in the Cu supplemented cows and calves for the first sampling period after treatment but was in the normal range throughout the remainder of the season for both treatment groups. Copper supplementation did not affect the concentrations of Mo in the serum, liver tissue, or milk; however, Mo did accumulate linearly in these components throughout the grazing period. Our results to date suggest that prolonged periods of Mo-enriched herbage consumption will result in increased concentrations of Mo in the serum and liver tissue of cows and calves but this may not result in molybdenosis when the Cu requirements of the animals are met. Supplementing animals with Cu-enriched boli enhanced liver Cu storage, while the effects on serum and milk Cu were minimal.

### **INTRODUCTION**

Trace mineral complications in ruminants consuming feedstuffs with high concentrations of molybdenum (Mo) are well documented (Dick et al. 1975; Mason 1978; Ademosum et al. 1982). Not only can Mo cause toxicities, but it can also induce copper (Cu) deficiencies, resulting in adverse effects on animal health and productivity (Cunningham et al. 1952; Hornick et al. 1976; Hidiroglou 1981). Cu intake, Cu availability, sulfur intake, iron intake and the physical form of the feed are all factors related to a Mo induced Cu deficiency (Ward 1989).

It has been documented that the form of Mo ingested by ruminants may affect what levels can be tolerated without illiciting a response. For example, Huber et al. (1971) fed dairy cattle levels of up to 100 ppm Mo in the form of an inorganic Mo salt without causing any adverse effects. However, Smith et al. (1975) found that beef calves consuming fresh forage that contained 2-6 ppm Mo were exhibiting signs of a Cu deficiency, also termed molybdenosis, and Leech and Thornton (1987) suggested that critical concentrations in pasture

herbage are reached at 3 ppm, especially in the presence of antagonists such as zinc, iron and cadmium. In a literature review paper Ward (1989) concluded that Mo levels below 100 ppm can be added to dry feed without evidence of ill effects but that animals grazing fresh forage containing Mo are much more sensitive.

It has also been established that the Cu:Mo ratio is critical in maintaining Cu availability (Fletcher and Brink 1969) and if it falls below 2:1, Cu deficiency symptoms may arise (Miltimore and Mason 1971). Normal blood serum and liver copper concentrations were recorded at 0.7-1.5 ug ml<sup>-1</sup> and 90-400 mg kg<sup>-1</sup> dry matter basis (DMB), respectively, while 0.5 jug ml<sup>-1</sup> for serum and 25 mg kg<sup>-1</sup> DMB for liver were indicative of a deficiency (Baldwin et al. 1981; Meldrum and Trout 1985; Puis 1988 in Corah and Ives 1991; Gengelbach et al. 1994).

Sulfur compounds also play a major role in Mo induced Cu deficiency, particularly in ruminants (Mason 1978). In the rumen, thiomolybdates ( $\text{MoO}_n\text{S}_{(4-n)}^2$ ; where n is 0 to 3) are formed by interaction of Mo compounds with sulfide (Dick et al. 1975). High sulfur concentrations in the diet lead to increased formation of thiomolybdates, which bind with Cu, thereby making it unabsorbable (Suttle 1980; Mason 1978; Dick et al. 1975).

Treatment of a Mo induced Cu deficiency may involve removal of the animals from the feed source or supplementation with Cu (Ward 1989). Different forms of Cu supplementation have been tested and proven to be effective. These range from soil and foliar application, Cu salt injection, Cu Calcium EDTA, Cu methionine, Cu glycinate and Cu complexed with sulphate, oxide, chelate, proteinate or diamine peptide (Smart et al. 1992).

The objective of this research was to study the effect of grazing cattle on Mo-rich reclaimed mine-tailings on the Mo and Cu concentrations in serum, milk and liver tissue and to evaluate the efficacy of All-trace Cu-enriched boli in providing Cu for these animals.

## **MATERIALS AND METHODS**

### Study Area

The experiment was conducted from July 7 to September 20 in 1994 and from June 30 to September 19 in 1995. The site was on 55 ha of reclaimed mine tailings at Highland Valley Copper Mines near Logan Lake, British Columbia and was stocked continuously at 0.85 AU ha<sup>-1</sup>. Pasture vegetation consisted of crested wheatgrass (*Agropyron cristatum*), pubescent wheatgrass (*Agropyron trichophorum*), smooth brome grass

(*Bromus inermis* Leyss.), orchardgrass (*Dactylis glomerata*), red fescue (*Festuca rubra*), alfalfa (*Medicago sativa*), sainfoin (*Onobrychis viciaefolia*), timothy (*Phleum pratense*), Canada bluegrass (*Poa compressa*) and Kentucky bluegrass (*Poa pratensis*). The site was irrigated during the months of August and September in 1994 and continuously in 1995. Reclamation on the site began in 1988 and soils in the area were derived from mining excavations and are therefore not classified. Precipitation during the 1994 growing season was 20.0 mm, which was below average, while in 1995 precipitation increased to 36.0 mm.

#### Animals and Data Collection

Thirty-two Hereford and Hereford-Angus cow/calf pairs and eight mature Jersey steers fitted with ruminal cannulae were used in the experiment. Before arrival on pasture 16 cows and their calves were supplemented (SUP) with 'All-Trace' trace element boli (Agrimin Ltd.), while the remaining animals served as the control group (CON). Animals were offered free-choice cobalt-iodized salt, but no mineral was provided. Blood and milk were collected from all animals every three weeks and animal weights were recorded. Every six weeks liver tissue samples were collected from one half of each treatment and control cows and calves (n=32) using the tru-cut biopsy technique (Davies and Jebbeth 1981) with a liver biopsy instrument for large animals (Buckley et al. 1986). Serum, milk and liver tissue samples were digested in nitric acid and hydrogen peroxide and metal scans on serum and milk were done using a Thermo Jarrell Ash ICAP61 simultaneous inductively coupled argon plasma atomic emission spectrophotometer (ICP). Liver tissue was analyzed with the same apparatus, but an ultrasonic nebulizer was used. All animals used in the experiment were cared for according to recommended codes of practice (Canadian Council on Animal Care 1980). Forage was hand-plucked to represent the animals' diets (Edlefsen et al. 1960) concurrent with serum sampling dates. Samples were dried at 60°C for 24 h and ground to pass a 1 mm steel sieve using a Wiley Mill and analyzed for Cu, Mo, sulfur and crude protein.

#### Statistical

Differences within season in Cu and Mo of serum, milk and liver tissues of supplemented and control groups were determined using the GLM procedure (SAS 1990). Repeated measures analysis was used to test for effects of season and its interaction with supplemented and control groups using the GLM procedure (SAS 1990). Further comparisons of multiple means were done using the LSD test in the GLM package (SAS 1990).

## **RESULTS**

### Study Area

Cu and Mo in the forage averaged 19.4 and 44 ppm (DMB) in 1994 and 13.1 and 37.3 ppm in 1995 respectively. Crude protein (CP) had a mean of 12.1 % in 1994 and 11.3 % in 1995 (Table 2). The average daily gain ( $\text{kg d}^{-1}$ ) of calves did not differ ( $P>0.05$ ) between the SUP and CON groups in either years (Table 1) and similarly, dams maintained good condition through both seasons which was reflected by increases in their weights.

### Serum

Serum Cu content remained normal ( $0.7\text{-}1.5 \text{ u.g ml}^{-1}$ ) for SUP and CON groups (Tables 3; 4) in 1994 and 1995. Throughout each grazing season, no differences ( $P>0.05$ ) between SUP and CON groups were recorded. A linear increase ( $P<0.05$ ) in serum Mo was observed for cows and calves in both years. However, despite elevated levels of serum Mo, serum Cu was not depressed.

### Liver

In 1994, the liver Cu of the SUP cows was greater ( $P<0.05$ ) than that of the CON cows in weeks 1, 6 and for the mean of the periods sampled (Table 3). The results were similar for the calves except that no differences were found in week 6 (Table 4). In 1995, for weeks 6, 12 and the mean of the periods sampled, Cu in liver tissue of SUP cows was greater ( $P<0.05$ ) than in CON cows with the calves showing the same results except for week 12 (Table 3; 4). Mo content of liver tissue in SUP and CON groups did not differ ( $P>0.05$ ) for cows and calves and throughout the 1994 or 1995 grazing season (Tables 3; 4). However, similar to serum Mo, the Mo content in liver tissue in all animals increased linearly ( $P<0.05$ ) from week 0 to week 12.

### Milk

Cu and Mo content in milk of SUP and CON groups did not differ in either year (Table 5), however, Mo increased linearly ( $P<0.05$ ) as the seasons progressed.

## **DISCUSSION**

Performance of cattle did not differ for either treatment group and weight gains were similar to cattle grazing within these regions (Quintan 1987). Although the concentration of Cu in herbage consumed by cattle was well above the requirement and Mo was more than two times as high as Cu and within toxic ranges (NRC

1984), serum and liver Cu content was within normal reported ranges (Baldwin et al. 1981; Meldrum and Trout 1985; Puis 1988 in Corah and Ives 1991; Gengelbach et al. 1994) and did not differ for cows and calves in SUP and CON groups. Therefore, consumption of forage containing increased concentrations of Mo did not appear to affect serum or liver Cu. However, an increase in serum, liver and milk Mo was recorded as the grazing season progressed. These results indicate that the high levels of Mo present in the forage were accumulating in the animals but without affecting the availability of Cu.

In 1994 the animals were dosed with Cu boli 7 days before the first samples were collected. Therefore, as the animals were administered the Cu supplement before grazing commenced, the increase in liver Cu in the SUP versus CON cows at week 1 could only be explained by an immediate transfer of Cu from the bolus to the liver. In 1995 cattle received the bolus when the first samples were collected (week 1), thus no differences were found between the SUP and CON groups at this time. However, liver tissue Cu was higher in both the SUP cows and calves at various sampling periods throughout the grazing season. These results indicate that in both the cows and calves the Cu boli appeared to increase Cu storage in the liver. However, in all cases liver tissue Cu remained in the normal range of 90 - 400 mg kg<sup>-1</sup> (Baldwin et al. 1981; Meldrum and Trout 1985; Puis 1988 in Corah and Ives 1991; Gengelbach et al. 1994), indicating that the high levels of Mo present in the forage were not rendering the Cu unavailable for absorption.

## **CONCLUSION**

Increases in Mo over time were documented in the serum and liver of all animals. Despite continuous consumption of Mo-rich forage, Cu in both serum and liver remained within normal ranges. The bolus was effective in providing Cu to liver tissues of supplemented cows and calves, although this was often only numerical. Animal health was not adversely affected and data from liver tissues and blood serum support this finding. It is possible that other factors such as the form of the Mo in the forage, the high concentration of Cu in the diet and the interaction of those minerals with sulfur may have prevented a Mo induced Cu deficiency from occurring.

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**Table 1. Performance of cows and calves grazing reclaimed mine tailings at Logan Lake.**

	Control		Copper supplement		SEM	
	1994	1995	1994	1995	1994	1995
Calves						
ADG (kg d <sup>-1</sup> )	1.13	1.18	1.14	1.13	0.05	0.07
Cows						
ADG (kg d <sup>-1</sup> )	0.50	0.36	0.54	0.43	0.04	0.06

ADG - average daily gain (kg d<sup>-1</sup>); SEM - standard error of the mean

**Table 2. Copper (ppm), molybdenum (ppm), sulfur (%) and crude protein (%) of forage.**

	Copper		Molybdenum		Sulfur		Crude Protein	
	1994	1995	1994	1995	1994	1995	1994	1995
mean	19.4	13.1	44.0	37.3	0.26	0.17	12.1	11.3
SEM	2.1	2.6	2.6	2.8	0.05	0.05	1.5	1.2

**Table 3. Cu and Mo content in serum (µg ml<sup>-1</sup>) and liver tissue (ppm DM) of supplemented and control cows.**

Test period	Treatment	Serum Cu		Serum Mo		Liver Cu		Liver Mo	
		1994* <sup>1</sup>	1995	1994* <sup>2</sup>	1995* <sup>2</sup>	1994	1995* <sup>1</sup>	1994	1995* <sup>2</sup>
Week 1	Control	0.82	0.80	0.15	0.06	118.9a	161.5	4.0	4.8
	Supplement	0.76	0.80	0.15	0.05	219.4b	151.7	4.2	4.4
	SEM	0.02	0.04	0.00	0.004	24.2	22.0	0.18	0.18
Week 3	Control	0.92	1.01	1.20	1.09				
	Supplement	0.87	0.95	1.64	1.09				
	SEM	0.03	0.03	0.11	0.04				
Week 6	Control	0.77	1.02	4.73	1.36	146.8a	145.6a	11.3	6.5
	Supplement	0.75	1.02	4.36	1.11	372.8b	356.8b	12.9	6.3
	SEM	0.02	0.03	0.29	0.07	36.2	33.9	0.89	0.19
Week 9	Control	0.82	0.93	3.32	1.92				
	Supplement	0.76	0.88	3.45	1.43				
	SEM	0.03	0.03	0.18	0.15				
Week 12	Control	1.08	1.03	8.48	4.73	217.8	323.7a	13.3	13.3
	Supplement	1.09	1.02	8.26	5.33	247.9	535.9b	11.1	13.8
	SEM	0.04	0.03	0.16	0.23	27.0	46.0	0.71	0.57
Mean	Control	0.88	0.94	3.73	1.13	161.2a	210.2a	9.5	8.2
	Supplement	0.86	0.91	3.57	0.95	280.0b	348.1b	9.4	8.1
	Control SEM	0.03	0.02	0.23	0.08	28.5	39.0	0.45	0.39
	Suppl. SEM	0.03	0.03	0.24	0.07	24.1	33.8	0.87	0.27
	mean SEM	0.02	0.02	0.16	0.06	23.7	30.6	0.47	0.23

\*<sup>1</sup> Quadratic component (P<0.05); \*<sup>2</sup> Linear component (P<0.05); <sup>a,b</sup> difference between treatments within sampling periods significant (P<0.05).



**Table 4. Cu and Mo content in serum ( $\mu\text{g ml}^{-1}$ ) and liver tissue (ppm DM) of supplemented and control calves.**

Test period	Treatment	Serum Cu		Serum Mo		Liver Cu		Liver Mo	
		1994	1995	1994* <sup>2</sup>	1995* <sup>2</sup>	1994	1995* <sup>1</sup>	1994	1995* <sup>2</sup>
Week 1	Control	0.73	0.86	0.15	0.04	132.8a	92.3	2.5	2.9
	Supplement	1.30	0.88	0.15	0.05	213.1b	91.6	2.4	3.1
	SEM	0.30	0.06	0.00	0.004	0.7	7.01	0.19	0.20
Week 3	Control	0.72	0.78	1.14	0.63				
	Supplement	0.73	0.76	1.01	0.67				
	SEM	0.02	0.03	0.07	0.05				
Week 6	Control	0.63	0.78	1.66	0.60	90.3	65.9a	6.0	4.3
	Supplement	0.63	0.79	1.36	0.67	127.3	205.3b	5.8	4.4
	SEM	0.02	0.03	0.16	0.04	11.8	24.92	0.40	0.20
Week 9	Control	0.72	0.86	1.15	0.71				
	Supplement	0.91	0.76	1.29	0.79				
	SEM	0.02	0.03	0.83	0.06				
Week 12	Control	0.91	0.98	2.19	1.24	106.5	191.2	7.1	6.4
	Supplement	0.91	0.91	2.15	1.62	121.6	245.1	6.6	6.5
	SEM	0.03	0.04	0.20	0.13	12.4	23.1	0.50	0.35
	Supplement	0.87	0.80	1.12	0.55	154.0b	180.7b	4.9	4.7
	Control SEM	0.02	0.03	0.13	0.04	12.8	20.3	0.42	0.17
	Suppl. SEM	0.13	0.04	0.09	0.05	19.0	18.3	0.26	0.27
mean SEM	0.08	0.02	0.09	0.03	12.4	15.6	0.24	0.15	

\*<sup>2</sup> Linear component ( $P < 0.05$ ); <sup>a,b</sup> difference between treatments within sampling periods significant ( $P < 0.07$ ).

**Table 5. Cu and Mo content ( $\mu\text{g ml}^{-1}$ ) in milk of supplemented and control cows.**

Test period	Treatment	Cu		Mo	
		1994	1995	1994* <sup>1</sup>	1995* <sup>1</sup>
Week 1	Control	0.05	0.07	0.15	0.03
	Supplement	0.05	0.06	0.15	0.03
Week 3	Control	0.06	0.04	0.50	0.17
	Supplement	0.05	0.06	0.40	0.18
Week 6	Control	0.05	0.04	1.30	0.27a
	Supplement	0.06	0.05	1.26	0.20b
Week 9	Control	0.05	0.04	0.98	0.62a
	Supplement	0.06	0.05	1.03	0.41b
Week 12	Control	0.05	0.03	2.32	1.18
	Supplement	0.05	0.03	2.14	1.21
Mean	Control	0.05	0.05	1.05	0.45
	Supplement	0.06	0.05	1.00	0.41

\*<sup>1</sup> Linear component ( $P < 0.05$ ).