Efficacy of Copper Supplementation in the Prevention of Molybdenosis in Cattle

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Abstract

Revegetation and sustainable cattle grazing are major objectives in the reclamation of mine tailings at the Highland Valley Copper mine in British Columbia, Canada. A total of 150 cows with their calves grazed forage extremely high in molybdenum (Mo) for 5–6 weeks in the summer and fall for 3 consecutive years (2002–2004). The average stocking rate was 0.89 ha per animal unit month. The animals' diet consisted primarily of alfalfa (*Medicago sativa* L.) and orchardgrass (*Dactylis glomerata* L.) containing 100–400 ppm Mo. Each year, the herd was divided into 2 groups of approximately 25 cow–calf pairs. One group was supplemented with 2.5% copper sulphate ($CuSO_4 \cdot 5H_2O$) in loose salt, and the other group was only given loose salt. Clinical signs of Mo toxicity, including lameness, diarrhea, and a faded hair coat, were significantly reduced in the supplement cows compared to control cows, which demonstrated the efficacy of the copper supplement treatment. Cattle also developed a tolerance to grazing high-Mo forage, as lameness and diarrhea were reduced in cows that had previous exposure to the site. However, lameness, the primary sign, and diarrhea were resolved in all cows by the end of each trial without treatment and hair coats returned to normal by the following spring. Only 4 calves showed signs of lameness or diarrhea in the 3-year study. Cattle grazing is a viable option for the end land use plan for Highland Valley Copper, provided a $CuSO_4 \cdot 5H_2O$ supplement is available to counteract the toxic effects of the high-Mo forage. As well, animals with previous exposure to the site should be utilized, as they appear to develop a tolerance to the Mo in the forage.

Resumen

La revegetación y el apacentamiento sostenible de ganado son los principales objetivos en la restauración de las pilas de colas de la mina Highland Valley Copper en British Columbia, Canadá. Un total de 150 vacas con sus becerros apacentaron forraje con contenidos extremadamente altos de molibdeno (Mo) durante cinco o seis semanas en el verano y otoño por tres años consecutivos (2002–2004). La carga animal promedio fue de 0.89 ha por unidad animal mes. La dieta de los animales consistió principalmente de "Alfalfa" (*Medicago sativa* L.) y "Orchardgrass" (*Dactylis glomerata* L.) conteniendo 100–400 ppm de Mo. Cada año, el hato se dividió en dos grupos de aproximadamente 25 pares de vaca-becerro, uno de los grupos fue suplementado con 2.5% de sulfato de cobre ($CuSO_4 \cdot 5H_2O$) mezclado con sal suelta mientras que al otro se le suministro solo sal suelta (control). Los signos clínicos de intoxicación por Mo, incluyendo la incapacidad para caminar por cojera, diarrea y decoloración del pelo, fueron reducidos significativamente en las vacas suplementadas en comparación con las vacas del grupo control, lo cual demostró la eficacia del cobre como tratamiento suplementario. El ganado también desarrolló una tolerancia a apacentar forraje con alto contenido de Mo ya que la cojera y la diarrea se redujeron en vacas que previamente habían sido expuestas al sitio. Sin embargo, la cojera, el principal signo, y la diarrea se solucionaron en todas las vacas al final de cada ensayo sin la aplicación de algún tratamiento y el pelaje retorno a su normalidad en la siguiente primavera. En los tres años de estudio solo cuatro becerros mostraron signos de cojera o diarrea. El apacentamiento del ganado es una opción viable como uso final del terreno de la mina de Highland Valley Copper proveyendo un suplemento de $CuSO_4 \cdot 5H_2O$ para contrarrestar los efectos tóxicos del alto contenido de Mo en el forraje. También, animales con exposición previa al sitio deben ser utilizados, ya que aparentemente ellos desarrollan una tolerancia al Mo en el forraje.

Key Words: cattle grazing, mine reclamation, molybdenum toxicity

INTRODUCTION

The Highland Valley Copper mine (HVC) in British Columbia, Canada, is one of the largest open pit mining operations in the world. It is restoring 6 000 ha of rangeland disturbed by over 20 years of mining. Of primary concern are the tailings ponds, which contain elevated levels of molybdenum (Mo), a residue from the copper (Cu) and Mo extraction process. With a milling recovery of 42–55% Mo, the mineral is a prominent residue in the tailings. Environmental reclamation of tailings areas consist primarily of establishing agronomic vegetation with the aid of fertilizers (C. E. Jones and Associates Ltd. 1999, 2000). A proposed end land use plan for these areas includes livestock grazing and hay production, thus restoring the terrain to sustainable agricultural use. Studies have shown that residual Mo in the tailings is imbibed by vegetation and can accumulate

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Table 1. Range of values for copper (Cu) and molybdenum (Mo) levels (ppm dry weight) in agronomic grasses, Kentucky bluegrass, white clover, and pinegrass at the Stevenson Allotment, for alfalfa and orchardgrass at Highmont (Highland Valley Copper mine) and for the Cu:Mo ratios.

Location	Year	Species	*n*	Cu	Mo	Cu:Mo
				--------------- (ppm)	-------------	
Stevenson Allotment						
	2002[1]	Agronomic grasses	13	5–16	6–23	0.3–1.4
		Kentucky bluegrass	3	8–9	10–11	0.7–0.9
		White clover	2	11–17	18–64	0.3–0.6
		Pinegrass	9	5–11	2–15	0.3–3.2
	2003[1]	Agronomic grasses	12	3–7	3–11	0.5–1.6
		Pinegrass	9	3–6	2–7	0.7–3.4
Highmont						
	2002[2]	Alfalfa	4	9–18	222–378	0.03–0.07
		Orchardgrass	4	9–11	181–232	0.04–0.05
	2003[2]	Alfalfa	6	7–12	132–304	0.03–0.05
		Orchardgrass	6	6–7	98–232	0.03–0.07
	2004[2]	Alfalfa	8	5–12	151–305	0.03–0.06
		Orchardgrass	8	3–9	128–253	0.02–0.04

[1]Samples were collected approximately every 4 weeks from June to August.
[2]Samples were collected before and after the grazing trial.

to extremely high levels depending on plant species and site (Gardner et al. 2003; Majak et al. 2004). Accordingly, grazing studies were initiated to determine the feasibility of utilizing the tailings areas at HVC for cattle production. We are unaware of any other long-term grazing studies utilizing high-Mo forage. Forages are also high in Mo on reclaimed lands at the URAD mine in Colorado (Trlica and Brown 2000) and at the Brenda Mines site in British Columbia (Taylor and McKee 2003), but long-term grazing trials have not been initiated.

The Highmont tailings area at HVC was chosen for cattle studies because it shows some of the highest levels of forage Mo (100–400 ppm) on the mine site. Animal health guidelines recommend a maximum level of 5 ppm Mo in feed for beef cattle (NRC 1996). Elevated levels of Mo in forage can induce molybdenosis, a form of Mo toxicity in ruminants. Molybdenosis is also known as secondary Cu deficiency because the ingestion of excess Mo results in the reduction of biologically available Cu. To determine the impact of the high-Mo forage on grazing cattle, a 3-year (1999–2001) clinical evaluation was initially conducted at Highmont. The study demonstrated that grazing cows were susceptible to some degree of molybdenosis (Majak et al. 2004). Clinical signs of molybdenosis are much varied (Ward 1978; Swan et al. 1998), but clinical evaluations at Highmont only revealed signs of lameness, manifested as a stiff shuffling gait, diarrhea, and hair-coat depigmentation (Majak et al. 2004). It should be noted that lameness and diarrhea were resolved in all animals by the end of each grazing season without treatment and hair coats returned to normal by the following spring.

To prevent the onset of clinical signs, some form of supplementation would be required if humane and profitable cattle grazing was to be included in an end land use plan for the Highmont site. A supplement such as Cu sulphate in salt has been recommended for forage rations or pastures with excessive amounts of Mo in plant tissue (Merck 1967; Radostits et al. 2000). Accordingly, a second 3-year study (2002–2004) was initiated to test the efficacy of a 2.5% $CuSO_4 \cdot 5H_2O$ supplement in salt to prevent the onset of clinical signs at Highmont and to further determine the status of Cu and Mo in liver and serum of grazing cattle. Should the supplement prove to be effective at Highmont, the treatment could be applied to other areas of HVC, which show lower levels of forage Mo (Gardner et al. 2003).

MATERIALS AND METHODS

Site Descriptions

Before the commencement of each trial, cattle were held at the Stevenson Allotment (lat 55°32′N, long 120°51′W, elevation 1 050 m) for 12 weeks. The site is near the mine and adjacent to Witches Brook, the holding area used for the clinical evaluation during 1999–2001 (Majak et al. 2004). Kentucky bluegrass (*Poa pratensis* L.), white clover (*Trifolium repens* L.), pinegrass (*Calamagrostis rubescens* Buckl.) and a mixture of agronomic grasses were the major forage components at Stevenson and the major source of drinking water was an alkali pond. Cattle were provided with cobalt iodized salt blocks during the pretrial grazing.

The cattle were trailed to Highmont at the end of August for a 6-week grazing period in 2003 and 5-week grazing periods in 2002 and 2004. The description of the experimental site at Highmont and the associated ground cover were reported earlier (Majak et al. 2004). The grazing period was reduced to increase the stocking rate and it was conducted later in the growing season to reduce the risk of bloat.

Forage and Water Samples

Aerial portions of plant samples were randomly clipped during the grazing periods at Stevenson and at Highmont. At least 20 individual plants of each species were pooled into composite samples (approximately 0.5 kg fresh weight per species per sampling date). Sampling intervals are indicated in Table 1. Agronomic grasses, bluegrass, clover, and pinegrass were collected at Stevenson in 2002, but bluegrass and clover were not collected in 2003 due to a drought. Alfalfa and orchardgrass were collected at Highmont in each year of the trial. The forages were obtained from areas occupied by cattle to approximate components of diet. Samples were transported on ice, frozen, and then freeze-dried. They were ground in a Wiley mill with the use of a 1-mm screen.

Water samples were obtained from the pond (pH 8.4–9.3) at Stevenson 4 times and the S-2 seepage pond (pH 7.6–8.5) at Highmont 7 times during the 3 years to determine the suitability of the water for livestock consumption. The Stevenson pond was sampled from the shoreline with the use of acid-washed sample bottles. At Highmont, water samples were obtained directly from the trough filled with S-2 seepage pond water.

Grazing Trials

The average stocking rate at Highmont was maintained at 0.89 ha per animal unit month. The herd consisted of Hereford or British crossbred cows (3–10 years of age) with nursing Charolais-sired calves. A total of 53, 46, and 51 cows were

Table 2. Guidelines for determining severity of clinical signs in cattle at Highmont (Highland Valley Copper mine).

Condition	Description	Scale
Lameness		
Normal		0
Mild	Stiff on rising, slow shuffling gait, warms out of it	1
Moderate	Reluctant to move, always trailing the herd, shuffling, tiptoe gait	2
Severe	Recumbent, cannot go to water, gaunt	3
Diarrhea		
Absent	Normal consistency for green alfalfa/grass diet	0
Moderate	Fluid feces, fecal staining on tail/perineum/hocks	1
Severe	Watery feces, explosive diarrhea	2
Hair coat		
Normal		0
Dull	Decrease in luster	1
Faded	Depigmentation	2
Hair loss[1]		3

[1]Other than mechanical.

Table 3. Average daily gains (ADG; kg head^{-1} day^{-1} ± SE) in cows and calves at Highmont (Highland Valley Copper mine).

		Cows		Calves	
Year	Treatment	n	ADG	n	ADG
2002	Control	26	0.76 ± 0.13	26	1.23 ± 0.04
	Supplement	26	1.66 ± 0.12	27	1.53 ± 0.04
2003	Control	23	0.90 ± 0.06	22	1.24 ± 0.04
	Supplement	23	1.04 ± 0.08	23	1.47 ± 0.03
2004	Control	26	1.13 ± 0.13	26	1.44 ± 0.05
	Supplement	25	0.78 ± 0.07	25	1.42 ± 0.05

grazed in 2002, 2003, and 2004, respectively. Animals were divided in 2 groups with similar numbers of previously exposed and naïve cattle in each group to determine carryover effects, if any. The control group had 11, 12, and 5 returning cows that had grazed the area the previous year, and 16, 11, and 21 naïve cows in 2002, 2003, and 2004, respectively. The supplement group had 10, 11, and 4 returning cows and 16, 12, and 21 naïve cows in 2002, 2003, and 2004, respectively. The 56-ha study area was divided with a permanent fence to accommodate the 2 groups of cow calf pairs in Pastures 1 and 2. Copper sulphate ($CuSO_4 \cdot 5H_2O$), 2.5% in loose salt as prescribed by the consulting veterinarian (Merck 1967; Radostits et al. 2000), was provided free choice to one group, whereas the other group, which served as a control, was given only loose salt. The salt and fortified salt were provided to each herd in mineral feeders (Behlen Model HDB-3) with covers removed to ensure access. Feeders were placed near the water in both pastures to promote salt consumption. The salt was agitated periodically to prevent formation of lumps. The supplement was put in Pasture 1 in 2002 and Pasture 2 in 2003. In 2004, the supplement and the supplement group were rotated between both pastures, as was the control group. Forage levels of Cu and Mo were similar in both pastures. Cattle were visually monitored from horseback 5–6 times a week for 2–3 hours per day in the morning. Two riders alternated 4-day monitoring periods for the duration of each grazing trial. A grading system was developed by the consulting veterinarian and clinical signs were specified, quantified, and recorded on a numerical scale (Table 2) by the riders. Each cow was checked for pregnancy and gestational stage by manual palpation at the beginning and end of each trial to monitor the impact on pregnancy. Estimations of the progress of the gestation period were made by the veterinarian. Cows were bred prior to arrival at Highmont in keeping with standard management practices of the ranch the cows were sourced from.

Blood and liver samples were obtained at the home ranch at the beginning of May, on the first day at Highmont in late summer (just before the trial began) and on the last day just before departure from the mine site. Samples were taken from 15 cows in each of the control and supplement groups. The samples were treated as described previously (Majak et al. 2004). Calves were not sampled as they have shown little effect from grazing high-Mo forage (Majak et al. 2004). Average daily gains (ADG) were determined for both cows and calves, but the short grazing season of 5–6 weeks did not allow for a meaningful comparison of ADG between the 2 groups. Gains were adequate, there was no indication of weight loss in either group, and calf gains were not adversely affected by the supplement (Table 3).

The water, liver, serum, and forage samples were analyzed for minerals by inductively coupled plasma spectroscopy at Eco Tech Laboratories Ltd. (Kamloops, BC) and Griffin Laboratories (Kelowna, BC). All values for forage and liver biopsies are reported on a dry-matter basis, and all serum and water concentrations are reported on a volume basis. All cattle were cared for according to protocols of the Canadian Council on Animal Care.

Statistical Analysis

Clinical Signs. Logistic regression (Woodward 1999) was used to determine the influence of copper supplementation, previous exposure, and year on the proportion of cattle showing signs of lameness, diarrhea, and hair-coat problems. The model used was

$$P(Y=1)=1/(1+exp[-\{b_0+b_iX_i\}]) \qquad [1]$$

where Y is a binary response variable, b_0 is an estimated constant, X_i is one or more independent variables, and b_i is the associated slope for each independent variable. Binary response variables were created for each animal for each of the clinical signs of lameness, diarrhea, and hair-coat problems. For lameness, the binary variable was assigned the value 1 if the animal showed signs on 3 consecutive days of observation and was assigned the value 0 otherwise. The binary variable for diarrhea was assigned the value 1 if the animal showed signs of diarrhea on 2 consecutive days of observation and was assigned the value 0 otherwise. The binary variable for hair-coat problems was assigned the value 1 if the animal showed signs of hair-coat problems and 0 otherwise. The independent variable X_1 was assigned the value 1 if the animal received the copper supplement and 0 otherwise; X_2 was assigned the value 1 if the animals had previous exposure and 0 otherwise; X_3 was assigned the value 1 if the year was 2002 and 0 otherwise; and X_4 was assigned the value 1 if the year was 2003 and 0 otherwise. Forward selection stepwise logistic regression (SPSS Inc 2001) was used to determine which of the independent variables, as well as their two-way interactions, had significant influence on the response variable. The procedure was conducted for each response variable corresponding to lameness, diarrhea, and hair-coat problems.

Odds ratios and their 95% confidence limits were calculated from the logistic regression parameters (Woodward 1999) to facilitate estimating the effect of each independent variable on the likelihood animals showed symptoms. The odds that an animal showed symptoms are the ratio of the number of animals with symptoms to the number of animals without. Odds ratios are used to compare the odds for 2 groups of animals and indicate if symptoms are more likely in one group compared with another. An odds ratio > 1 indicates an increased likelihood of showing symptoms in one group compared with another, while an odds ratio < 1 indicates a decreased likelihood of showing symptoms.

Liver Biopsies and Serum. Liver samples were obtained from 24, 30, and 28 cows in 2002, 2003, and 2004, respectively, based on the number of successful paired liver biopsies on the first day at Highmont and at the end of the grazing period. Liver and serum Cu and Mo content were each analyzed in 2 steps. The first analysis was conducted to determine if Cu or Mo content on the first day at Highmont varied among years, supplementation, previous exposure, and all possible interactions with the use of a three-way analysis of variance (ANOVA). The second stage of the analysis was conducted to determine if changes in liver and serum Cu and Mo content between the first and last day at Highmont varied among years, supplementation, previous exposure and all possible interactions with the use of three-way ANOVA. Changes in liver and serum Cu and Mo were calculated by subtracting values from the first day at Highmont for each animal from the respective value on the last day at Highmont. This accounted for within-animal variation. Means for significant effects and interactions were resolved with the use of Fisher's least significant differences.

RESULTS AND DISCUSSION

There are only a few reports on field poisoning of cattle grazing high-Mo forage but contamination of feed with inorganic Mo has been more widespread (Ward 1978; Swan et al. 1998). Field cases of poisoning have been reported from very small areas in the western United States (Kubota 1975), England (Ferguson et al. 1943), and Canada (Cunningham et al. 1953), where Mo levels in forage ranged from 10 to 50 ppm.

Forage and Water Samples

At the Stevenson holding pasture, levels of Mo in tame grasses, Kentucky bluegrass, and white clover ranged from 6 to 64 ppm during 2002 (Table 1), which was above the acceptable maximum of 5 ppm (NRC 1996). Pinegrass ranged from 2 to 15 ppm Mo over 2 years and the range for tame grasses in 2003 was slightly narrower (3 to 11 ppm Mo). Copper levels in the forages ranged from 3 to 17 ppm over 2 years. In BC, feed levels of Cu are normally less than 17 ppm and the reported average for feeds is 6 ppm (Miltimore and Mason 1971). The Cu:Mo ratios at Stevenson ranged from 0.3 to 3.2 during 2002 and 2003 but the mean values (0.44–1.6) were slightly less than the 2:1 minimum requirement (Miltimore and Mason 1971). Although Mo levels are elevated in the area, signs of molybdenosis have not been reported in the region surrounding the mine.

At Highmont, levels of Mo in alfalfa and orchardgrass ranged from 98 to 378 ppm during 2002–2004 (Table 1), which is in agreement with previous estimates (Majak et al. 2004) as were levels of Cu (3–18 ppm). The ratio of Cu:Mo in alfalfa and orchardgrass ranged from 0.02 to 0.07, which, on average, was 2% of the minimum requirement of 2:1. The extremely low ratios imply the potential for a secondary Cu deficiency. However, acute Mo toxicity resulting in mortality or chronic poisoning with long-term disability did not occur during 2002–2004, which was also the case during 1999–2001. There is no evidence that forage Mo levels are declining at Highmont since these studies were initiated in 1999.

The Stevenson drinking water contained 5 ± 2 ppb Cu and 191 ± 72 ppb Mo (mean $\pm$ SE, $n = 5$). The Mo exceeded the recommended maximum level of 60 ppb Mo for water for cattle (Puls 1994). At Highmont, the drinking water from the seepage pond contained 11 ± 1 ppb Cu and $8{,}700 \pm 670$ ppb Mo ($n = 7$). The Mo levels in the water were exceptionally high ($\times$ 145) when compared to the acceptable maximum. It was estimated that Mo in drinking water accounted for 20% of total dietary Mo intake at Highmont (Majak et al. 2004). All other water parameters at Highmont were in the acceptable range for cattle (Puls 1994). However, the water was slightly alkaline, which would enhance soil Mo bioavailability (Goldberg et al. 1996).

Salt Consumption

Salt consumption was recorded during the 3-year study. Intake levels were estimated based on the assumption that a calf would consume one-quarter of the salt intake of a cow. On average, cows in the control group consumed approximately 90, 77, and 60 $g \cdot head^{-1} \cdot day^{-1}$ of loose cobalt iodized salt in 2002, 2003, and 2004, respectively. Cows in the supplement group consumed 90, 58, and 73 $g \cdot head^{-1} \cdot day^{-1}$ of the Cu-fortified salt during the same period. On average, range cattle consume 30 g $salt \cdot head^{-1} \cdot day^{-1}$ (Merck 1967). The animals at Highmont consumed up to 2–3 times the typical amount of salt for range cows, but were well below the maximum recommended levels of 400 g $salt \cdot head^{-1} \cdot day^{-1}$ (Radostits et al. 2000).

The supplement cows would have ingested, on average, 573, 369, and 464 mg Cu $head^{-1} \cdot day^{-1}$ during 2002, 2003, and 2004, respectively. Copper intake was not sufficient to cause toxicity problems as maximum recommended levels are estimated at 1000 mg $Cu \cdot head^{-1} \cdot day^{-1}$ based on a daily intake of 10 kg forage DM (Underwood and Suttle 1999).

Pregnancy Checks

Pregnancy checks were performed at the beginning and at the end of the trials at Highmont each year. Grazing high-Mo forage can affect conception rates (Ward 1978; Swan et al. 1998), but they should not have been affected at Highmont, as all cows should have been bred prior to arrival to the mine site.

Table 4. Proportion of animals exhibiting clinical symptoms of molybdenum toxicity while grazing at Highmont (Highland Valley Copper mine).

Year	Treatment	Previous exposure	n	Lameness	Diarrhea	Hair coat
				(Proportion ± SE)		
2002	Control	No	16	0.56 ± 0.12	0.69 ± 0.12	0.63 ± 0.12
		Yes	11	0.09 ± 0.09	0.64 ± 0.15	0.73 ± 0.13
	Supplement	No	16	0.00 ± 0.00	0.50 ± 0.13	0.56 ± 0.12
		Yes	10	0.10 ± 0.09	0.20 ± 0.13	0.20 ± 0.13
2003	Control	No	11	0.27 ± 0.13	0.18 ± 0.12	0.00 ± 0.00
		Yes	12	0.08 ± 0.08	0.00 ± 0.00	0.08 ± 0.08
	Supplement	No	12	0.08 ± 0.08	0.08 ± 0.08	0.00 ± 0.00
		Yes	11	0.00 ± 0.00	0.09 ± 0.09	0.00 ± 0.00
2004	Control	No	21	0.38 ± 0.11	0.43 ± 0.11	0.33 ± 0.10
		Yes	5	0.00 ± 0.00	0.20 ± 0.18	0.00 ± 0.00
	Supplement	No	21	0.00 ± 0.00	0.10 ± 0.06	0.05 ± 0.05
		Yes	4	0.25 ± 0.22	0.00 ± 0.00	0.00 ± 0.00

The pregnancy rate varied from 84% to 91% over the 3 years, and there was no consistent difference between supplement and control groups. Cows that were bred at the beginning of each trial were not found to be open at the end of the trial, and there was no evidence of aborted fetuses at the site.

Clinical Signs

Calves in both groups were mostly unaffected by the high-Mo forage. Only 3 calves in the control group were affected with lameness over the 3-year study, with only one case of diarrhea. Clinical signs were not observed in calves in the supplement group.

Lameness was observed in 25 of 150 cows throughout the 3 years of this study, with 0.20 (11/53), 0.11 (5/46), and 0.18 (9/51) cows showing lameness in 2002, 2003, and 2004, respectively (Table 4). For each individual animal, the duration of lameness lasted a maximum of 3–19 days (mean = 7.9 d; SE = 1.0 d). The proportion of cows that showed lameness was significantly influenced by supplementation and previous exposure with a supplementation × exposure interaction as indicated by logistic regression (Table 5). The incidence (± SE) of lameness was 0.29 ± 0.05 (22/76 cows) for animals in the control groups and 0.04 ± 0.02 (3/74 cows) in the supplement groups. The incidence of lameness in cows with previous exposure to the pasture was 0.08 ± 0.04 (4/53) and was typically lower than cattle that did not have previous exposure, where the incidence of lameness was 0.22 ± 0.04 (21/97). The significant supplementation × exposure interaction (Table 5) indicated that the effect of supplementation on reducing lameness was not consistent between cattle that had previous exposure and those that had not. The incidence of lameness in cattle with no previous exposure was 0.42 ± 0.07 (20/48) without supplementation and was 0.02 ± 0.02 (1/49) with supplementation. The odds ratio for supplementation in cattle with no previous exposure was 0.03 (95% CL = 0.004, 0.23) and indicated that the odds of lameness was greatly reduced in naïve cattle that received the supplement compared with naïve animals that did not receive the supplement. In cattle with previous exposure, the incidence of lameness was 0.07 ± 0.05 (2/28) without supplementation and 0.08 ± 0.05 (2/25) with supplementation. The odds ratio for supplementation in cattle with previous exposure was 1.13 (95% CL = 0.15, 8.68) and indicated that the odds of lameness was similar in previously exposed cattle that received the supplement compared with previously exposed cattle that did not receive the supplement. The benefits of supplementation were, therefore, mainly in reducing the incidence of lameness in animals with no previous exposure, but had little effect on animals with previous exposure.

Diarrhea lasted from 2–10 days (mean = 3.7 d; SE = 0.3 d) in individual animals. The proportion of animals with diarrhea was significantly elevated in 2002, and was reduced by both supplementation and previous exposure, as indicated by logistic regression (Table 5). The incidence of diarrhea was 0.53 ± 0.07 (28/53) in 2002 compared with 0.09 ± 0.04 (4/46) and 0.24 ± 0.06 (12/51) in 2003 and 2004, respectively. The incidence of diarrhea was reduced in animals receiving copper supplementation (Table 4) and was 0.19 ± 0.05 in the supplemented animals and 0.39 ± 0.06 in the control animals. The odds ratio for supplementation was 0.27 (95% CL = 0.12, 0.64) indicating that supplementation reduced the odds of diarrhea. The incidence of diarrhea was 0.21 ± 0.06 in animals with previous exposure and 0.34 ± 0.05 in animals grazing the site for the first time. The odds ratio for previous exposure was 0.35 (95% CL = 0.14, 0.86), indicating that previous exposure also reduced the odds of diarrhea. There were no other

Table 5. Results of logistic regression analysis[1] to determine the effect of copper supplementation, previous exposure, and year of exposure on the incidence of symptoms of molybdenosis in cattle grazing at Highmont.

	Lameness			Diarrhea			Hair coat		
X_i	$b_i \pm$ SE	χ^2	$P(b_i > 0)$	$b_i \pm$ SE	χ^2	$P(b_i > 0)$	$b_i \pm$ SE	χ^2	$P(b_i > 0)$
Intercept	− 0.34 ± 0.29	—	—	− 0.83 ± 0.33	—	—	1.17 ± 0.42	—	—
X_1	− 3.53 ± 1.05	11.3	< 0.001	− 1.30 ± 0.43	9.0	< 0.003	1.36 ± 0.48	8.0	< 0.005
X_2	− 2.23 ± 0.79	8.0	< 0.005	− 1.06 ± 0.43	5.2	< 0.02	ns	—	—
X_1X_2	3.66 ± 1.48	6.1	< 0.020	ns	—	—	ns	—	—
X_3	ns[2]	—	—	2.02 ± 0.43	21.8	< 0.0001	− 2.05 ± 0.51	16.4	< 0.0001
X_4	ns	—	—	ns	—	—	2.16 ± 1.09	3.9	< 0.05
Model		31.9	< 0.0001		36.1	< 0.0001		51.6	< 0.0001

[1]Model is $P(Y = 1) = 1/(1 + \exp[-\{b_0 + b_iX_i\}])$, where $Y = 1$ if the animal showed symptoms and 0 otherwise, b_0 is the intercept, $X_1 = 1$ if animals received supplement and 0 if not, $X_2 = 1$ if animals grazed the pasture the previous year and 0 if not, $X_3 = 1$ if 2002, 0 otherwise, $X_4 = 1$ if 2003 and 0 otherwise, and b_i is the associated slope for each independent variable.

[2]ns indicates not significant.

Table 6. Average copper (Cu) and molybdenum (Mo) levels (ppm ± SE) in liver (dry weight basis) and serum (volume basis) of cows at Highmont (Highland Valley Copper mine).

Sample	Year	Treatment	Previous exposure	*n*	Cu		Mo	
					First day	Change[1]	First day	Change[1]
					(ppm ± SE)			
Liver	2002	Control	No	5	44.5 ± 16.1	− 0.8 ± 6.3	8.8 ± 1.0	16.9 ± 2.4
			Yes	8	52.0 ± 11.1	− 11.6 ± 4.3	9.3 ± 1.1	17.2 ± 2.3
		Supplement	No	6	67.6 ± 9.0	17.4 ± 12.3	10.0 ± 1.7	14.0 ± 2.6
			Yes	6	82.5 ± 15.8	47.0 ± 19.2	8.4 ± 0.8	14.0 ± 1.6
	2003	Control	No	7	66.7 ± 7.7	− 16.2 ± 6.4	5.3 ± 0.3	18.5 ± 2.1
			Yes	8	61.3 ± 8.8	− 4.6 ± 4.0	4.9 ± 0.2	21.4 ± 1.8
		Supplement	No	7	59.7 ± 7.2	44.0 ± 14.5	4.5 ± 0.1	16.9 ± 1.5
			Yes	8	78.8 ± 18.8	84.8 ± 36.7	4.9 ± 0.2	19.7 ± 1.5
	2004	Control	No	9	45.3 ± 10.0	− 8.0 ± 3.8	7.1 ± 0.4	9.6 ± 0.7
			Yes	5	48.0 ± 12.9	− 2.9 ± 2.9	6.5 ± 0.8	10.2 ± 1.5
		Supplement	No	10	63.4 ± 12.0	77.0 ± 17.4	5.9 ± 0.2	14.5 ± 0.9
			Yes	4	42.0 ± 13.9	26.7 ± 2.1	7.0 ± 0.2	12.7 ± 0.6
Serum	2002	Control	No	4	0.64 ± 0.04	0.44 ± 0.07	0.65 ± 0.10	6.90 ± 1.51
			Yes	6	0.61 ± 0.02	0.51 ± 0.03	0.57 ± 0.11	8.32 ± 1.09
		Supplement	No	7	0.70 ± 0.03	0.22 ± 0.04	0.63 ± 0.08	7.59 ± 0.50
			Yes	7	0.72 ± 0.04	0.11 ± 0.04	0.56 ± 0.09	7.05 ± 0.64
	2003	Control	No	7	0.48 ± 0.02	0.45 ± 0.03	0.47 ± 0.08	5.29 ± 0.50
			Yes	8	0.50 ± 0.02	0.43 ± 0.04	0.39 ± 0.06	6.45 ± 0.75
		Supplement	No	7	0.46 ± 0.02	0.33 ± 0.06	0.29 ± 0.04	5.54 ± 0.66
			Yes	8	0.46 ± 0.02	0.24 ± 0.04	0.30 ± 0.06	6.39 ± 0.96
	2004	Control	No	10	0.71 ± 0.03	0.40 ± 0.04	1.05 ± 0.10	2.94 ± 0.33
			Yes	5	0.69 ± 0.06	0.43 ± 0.11	0.97 ± 0.27	2.45 ± 0.50
		Supplement	No	11	0.67 ± 0.03	0.49 ± 0.04	0.85 ± 0.14	4.74 ± 0.27
			Yes	4	0.75 ± 0.03	0.42 ± 0.04	1.12 ± 0.18	3.86 ± 0.39

[1]Difference between first day and last day at Highmont.

significant main effects or interactions, suggesting these trends were consistent across years, previous exposure, and treatment groups. Lameness, the primary clinical sign, and diarrhea were resolved in all cows by the end of each trial, and Cu supplementation was discontinued after departure from Highmont.

Hair-coat symptoms mainly consisted of fading on red portions of Hereford and British crossbred cows. The proportion of animals showing hair-coat symptoms varied among years and between the supplemented and control groups, as indicated by logistic regression (Table 5). Previous exposure had no significant effect, and none of the interaction terms were significant. The incidence of hair-coat symptoms was greatest in 2002 (0.55 ± 0.07), least in 2003 (0.02 ± 0.02), and intermediate in 2004 (0.16 ± 0.05) (Table 4). The proportion of animals with hair-coat symptoms was lower for animals in the supplemented group (0.16 ± 0.04) compared with the control group (0.34 ± 0.05). The odds ratio for supplementation was 0.26 (95% CL = 0.10, 0.66) indicating that supplementation reduced the odds of hair-coat problems. Hair-coat symptoms were only observed twice in 2003. Hair coats returned to normal by the following spring.

Liver Biopsies and Serum

The liver is the primary storage organ for Cu. Liver tissue analysis for minerals can reveal the plane of mineral nutrition in the animal (Radostits et al. 2000). The recommended hepatic levels for Cu and Mo are > 83 ppm and < 4.7 ppm, respectively, on a dry-matter basis (Puls 1994). The average hepatic levels of Cu and Mo at the home ranch in May were 90 ± 4 ppm and 4.0 ± 0.2 ppm, respectively. A comparison between these baseline levels and the values for the first day at Highmont (Table 6) indicate the effects of grazing at Stevenson. Hepatic Cu levels appear to decrease, whereas levels of Mo increase slightly as a result of grazing at Stevenson. This would decrease the interval for the onset of clinical signs from grazing forage at Highmont.

Liver Cu concentrations appeared to be in the marginal range on the first day at Highmont (Table 6) with no significant variation among years ($P = 0.17$), groups ($P = 0.09$), or previous exposure ($P = 0.70$). Thereafter, Cu levels did not increase in the control groups (mean increase ± SE = −7.4 ± 6.9 ppm), but consistently increased in the supplemented groups (mean increase ± SE = 49.5 ± 7.0 ppm), and this change was significantly different between the supplemented and control animals ($P < 0.01$). Therefore, intake of $CuSO_4 \cdot 5H_2O$ by the supplement group was obvious.

Levels of Mo in the liver on the first day varied significantly among years ($P < 0.01$) and were greatest in 2002 (mean = 9.1 ± 0.4 ppm), least in 2003 (mean = 4.9 ± 3.3 ppm), and intermediate in 2004 (mean = 6.6 ± 0.4 ppm) (Table 6). The higher level of hepatic Mo in 2002 could be linked to the higher incidence of lameness and diarrhea in that year (Table 4). Liver Mo on the first day did not vary between groups ($P = 0.63$) or previous exposure ($P = 0.80$). Increases in liver Mo varied among years ($P < 0.01$), but a significant supplementation × year interaction ($P = 0.03$) indicated that increases in liver Mo across years were not consistent among the supplemented and control groups. Increases in liver Mo were similar between control and supplemented groups in both 2002 and 2003, but were greater in the supplemented group in 2004. Liver Mo increased by 17.0 ± 1.3 and 14.0 ± 1.3 ppm in the control and supplemented groups, respectively, in 2002 and by 19.9 ± 1.2 and 18.3 ± 1.2 ppm in the control and supplemented groups in 2003. In 2004, liver Mo increased by 9.9 ± 1.3 ppm in the control group and 13.6 ± 1.3 ppm in the supplement group, a significant difference. It should be noted that increased animal variability was a factor in 2004 owing to a larger proportion (> 80%) of new cows. The increased liver Mo levels in all groups exceeded the acceptable levels of Mo 5-fold (Puls 1994). The high levels of hepatic Mo declined to baseline values by next spring. In May 2004, for example, baseline levels of Mo in previously exposed cows were 5.4 ± 0.5 ppm Mo ($n = 10$) whereas the levels were 5.3 ± 0.3 ppm Mo ($n = 19$) in new arrivals to the mine, which suggested no carryover effect of liver Mo between years.

The average serum levels of Cu and Mo at the home ranch in May were 0.57 ± 0.01 ppm and 0.13 ± 0.02 ppm, respectively. The recommended levels for Cu and Mo in the serum are > 0.6 ppm and < 0.1 ppm, respectively (Puls 1994). A comparison of the baseline levels at the home ranch and serum levels on the first day at Highmont (Table 5) indicate the effects of grazing at Stevenson. Copper levels were slightly below normal in 2003, whereas Mo levels were elevated in each year. The high levels of Mo in serum could be attributed to grazing the high-Mo forage at Stevenson.

On the first day at Highmont, serum Cu levels varied among years ($P < 0.01$) and averaged 0.66 ± 0.02, 0.47 ± 0.02, and 0.70 ± 0.02 ppm in 2002, 2003, and 2004, respectively (Table 5). Serum Cu levels increased between the first and last day at Highmont (Table 5) and the increase varied significantly among years ($P < 0.01$), supplementation ($P < 0.01$) with significant supplementation × previous exposure ($P = 0.05$), and supplementation × year ($P < 0.01$) interactions. The significant supplementation × previous exposure interaction arose because the increase in serum Cu was lower for supplemented animals with previous exposure (mean increase = 0.26 ± 0.03 ppm) compared to supplemented animals with no previous exposure (mean increase = 0.34 ± 0.03 ppm). The demand for systemic Cu may be less for supplemented animals with previous exposure, or these cows may have ingested less supplement. Increases in serum Cu in the control groups were consistent for animals that had no previous exposure (mean increase = 0.43 ± 0.03 ppm) and with previous exposure (mean increase = 0.45 ± 0.03 ppm). The significant supplementation × year interaction arose because increases in serum Cu were lower for supplemented animals compared to the control animals in 2002 and 2003, but not in 2004. Serum Cu increased by an average of 0.47 ± 0.04, 0.44 ± 0.03, and 0.41 ± 0.03 ppm in the control animals in 2002, 2003, and 2004, respectively, but increased by 0.17 ± 0.03, 0.28 ± 0.03, and 0.45 ± 0.04 ppm in the supplemented animals in 2002, 2003, and 2004, respectively. As noted above, new arrivals to the site may have increased animal variability in 2004.

Levels of serum Mo on the first day varied across years, averaging 0.60 ± 0.06, 0.36 ± 0.06, and 1.00 ± 0.06 ppm in 2002, 2003, and 2004, respectively (Table 6). Serum Mo levels were not significantly different between supplement and control groups ($P = 0.40$) or between previously exposed and naïve groups ($P = 0.92$). Increases in serum Mo varied across years, averaging 7.47 ± 0.37, 5.92 ± 0.32, and 3.50 ± 0.36 ppm in 2002, 2003, and 2004, respectively. The higher level of serum Mo in 2002 could be linked to the higher incidence of clinical signs in that year (Table 4). Neither Cu supplementation ($P = 0.25$) or previous exposure ($P = 0.54$) influenced the increase in serum Mo.

MANAGEMENT IMPLICATIONS AND CONCLUSIONS

In a 3-year (2002–2004) grazing study on forages with extremely high levels of Mo, clinical signs of lameness, diarrhea, and hair-coat problems were significantly attenuated if cows were supplemented with $CuSO_4 \cdot 5H_2O$. Only 3 cows in the supplement group (4%) showed signs of lameness, whereas 22 cows in the control group (29%) were affected over the 3-year study. Incidences of diarrhea and hair coat problems were also substantially reduced in the treated group. These findings confirm the efficacy of the supplement for cows and calves in this grazing environment. Calves were mostly unaffected by grazing high-Mo forage. Both lameness and diarrhea were reduced in animals that had previous exposure to the pasture. These results suggest that animals can develop a tolerance to grazing pastures with high levels of Mo. As a result, the benefits of supplementation were mainly in reducing the incidence of lameness in animals with no previous exposure. The primary clinical sign of lameness, manifested as a stiff shuffling gait, was resolved by the end of each trial without treatment, as was the diarrhea. Hair coats returned to normal by the following spring. Liver biopsies and serum samples showed marginal to adequate Cu levels but potentially toxic levels of Mo. The Cu supplement did not increase serum Cu levels in a consistent fashion, but had a profound effect on Cu levels in the liver. Unless total body Cu is severely depleted, serum Cu levels are tightly regulated by the animal.

Several recommendations regarding future grazing at the Highmont site can be made as a result of the supplementation study. Because of the relatively small pasture size at Highmont (56 ha), the risk of bloat, and the potential effects on conception and breeding, grazing should be carried out in late summer to early fall. Because the areas adjacent to the mine site also show elevated levels of forage Mo, supplementation should be provided at all sites to prevent any potential predisposition to the extremely high levels of Mo at Highmont. As well, cows with previous exposure to the site should be used, as they appear to develop a tolerance to the high-Mo forage. In addition, clinical signs of molybdenosis might be further reduced if the Cu supplement was increased to 3% $CuSO_4 \cdot 5H_2O$ from 2.5%. Another management option would be to formulate a slow-release bolus that would provide similar daily doses of $CuSO_4 \cdot 5H_2O$, as did the free-choice supplement (mean 469 mg $Cu \cdot head^{-1} \cdot day^{-1}$). In an earlier trial at Highmont, a commercial bolus delivered 272 mg Cu • head^{-1} • day^{-1} but it did not prevent the onset of clinical signs, nor was the configuration of the Cu defined (Majak et al. 2004). The efficacy of the $CuSO_4 \cdot 5H_2O$ supplement at Highmont should permit grazing on other areas of the HVC mine site, which show lower levels of forage Mo (Gardner et al. 2003). The supplement should counteract the high levels of Mo in forage and allow for grazing without ill effects throughout HVC.

In conclusion, this 3-year study was conducted at a reclaimed mine site in BC that permitted livestock grazing. Animal disorders, including lameness and diarrhea in cattle, were observed, but symptoms were resolved in all animals at the end of each trial without treatment. However, when a Cu supplement (2.5% $CuSO_4 \cdot 5H_2O$ in salt) was administered, it permitted normal gain and production without unnecessary distress.

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