Clinical signs in cattle grazing high molybdenum forage

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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 84 cow-calf pairs grazed forage extremely high in molybdenum (Mo) for 11 weeks in the summer and fall for 3 consecutive years (1999–2001). The average stocking rate was 0.63 ha AUM^{-1}. The animals' diet consisted primarily of alfalfa (*Medicago sativa* L.) and orchardgrass (*Dactylis glomerata* L.) containing 100–400 ppm Mo. Both cows and calves showed adequate daily gains of 0.78 kg head^{-1} day^{-1} and 1.33 kg head^{-1} day^{-1}, respectively. Uptake of Mo was demonstrated by elevated levels of Mo in rumen contents and feces. Clinical signs of Mo toxicity were observed in less than half of the cows and in only a few calves. Clinical signs included a stiff shuffling gait, watery diarrhea, and a rough hair coat. Lameness, the primary sign, was resolved in all animals by the end of each trial. Diarrhea was also resolved by the end of the trial and hair coats returned to normal by the following spring. The onset and severity of the affliction appeared to be related to prevailing moisture conditions, which may have affected Mo availability in forage. Some affected animals were treated with Cu injections to no avail. Liver biopsies and serum samples showed marginal to adequate copper (Cu) levels but potentially toxic levels of Mo. In the third year of the trial, Cu-containing boluses were employed but they did not prevent the onset of clinical signs.

Key Words: mine tailings reclamation, liver copper status, molybdenum, copper boluses

The Highland Valley Copper mine, one of the world's largest open pit copper mines, is located in the southern interior of British Columbia, Canada near the city of Kamloops. Approximately 247,000 tons of rock are mined daily with the ore averaging 0.40% copper (Cu) and 0.006% molybdenum (Mo). The milling process recovers 87 - 91% of the Cu and 42 - 55% of the Mo (Highland Valley Copper 1996). Environmental reclamation of mine tailings is ongoing. The residual Mo content from the extraction process is of concern for reclamation especially at the Highmont tailings pond. The planned agricultural uses of the reclaimed site are cattle grazing and hay production. The forage and browse at the site are also utilized by wildlife.

Resumen

La revegetación y el apacentamiento sustentable del ganado son los principales objetivos en la restauración de las áreas explotados en la mina de cobre Highland Valley de Columbia Británica, Canadá. Un total de 84 pares de vaca-becerro apacentaron por tres años consecutivos (1999-2001), durante 11 semanas en verano y otoño, forraje con un contenido extremadamente alto en molibdeno (Mo). La carga animal promedio fue de 0.63 ha UAM^{-1}. La dieta de los animales consistió principalmente de "Alfalfa" (*Medicago sativa* L.) y "Orchardgrass" (*Dactylis glomerata* L.) conteniendo de 100-400 ppm de Mo. La vacas y los becerros mostraron ganancias de peso adecuadas 0.78 y 1.33 kg cabeza^{-1} día^{-1} respectivamente. La absorción de Mo fue demostrada por los niveles elevados de Mo del contenido ruminal y heces. Signos clínicos de intoxicación por Mo fueron observados en menos de la mitad de las vacas y en solo unos pocos becerros. Los signos clínicos incluyeron cojera por rigidez, diarrea acuosa y el pelaje áspero. La cojera, el principal signo, y la diarrea fueron corregidos en todos los animales al final de cada ensayo y el pelaje de los animales retornó a su estado normal en la siguiente primavera. El inicio y la severidad de la afección pareció estar relacionadas con las condiciones de humedad prevalecientes, las cuales pueden haber afectado la disponibilidad de Mo en el forraje. Algunos de los animales afectados fueron tratados con inyecciones de Cu para que no fueran inútiles. Las biopsias de hígado y muestras de suero mostraron niveles de cobre (Cu) de marginales a adecuados y niveles de Mo potencialmente tóxicos. En el tercer año del experimento se usaron bolos conteniendo Cu pero estos no previnieron el inicio de los signos clínicos.

Reclamation consists primarily of establishing domestic plant species with the aid of fertilizers (C.E. Jones and Associates Ltd. 1999, 2000). However, the established vegetation at Highmont contains extremely high levels of residual Mo (Majak and Steinke 2002). Animal health guidelines (NRC 1996) recommend a maximum level of 5 ppm Mo in feed for beef cattle. A Cu:Mo ratio of not less than 2:1 should be maintained in forage to avoid Cu deficiency in grazing ruminants (Miltimore and Mason 1971). Molybdenum toxicity in cattle is characterized by loss of appetite, hind limb stiffness, haircoat discoloration, severe diarrhea, recumbency and mortality (Ward 1978, Swan et al. 1998). Molybdenosis, also termed secondary Cu deficiency, can result in the reduction of biologically available Cu. In the rumen, molybdate can react with sulphide to form thiomolybdates which bind free Cu rendering it biologically unavailable (Mason 1981). However, formation of sulphide requires reduction of sulphate which is inhibited at very high levels (> 200 ppm) of dietary molybdate (Bryden and Bray, 1972).

The Highmont site at Highland Valley Copper has the highest Mo levels (> 100 ppm on a dry wt basis) in the area. The present study is a sequel to the previous grazing trial conducted at the Bethlehem tailings pond of Highland Valley Copper where clinical disorders were not observed in cattle, but the forage levels of Mo were much lower averaging 32 ppm (Gardner et al. 2003). The objectives of the present study were to determine the Mo and Cu levels in alfalfa (*Medicago sativa* L.) and orchardgrass (*Dactylis glomerata* L.) at Highmont, to determine the number of cattle showing clinical signs of poisoning, to monitor the Cu and Mo status of the liver and serum, and to determine whether cattle could graze the forage without ill effects.

Materials and Methods

Before the commencement of each trial, cattle were held at Witches Brook, which is a natural meadow adjacent to the mine along highway 97C but at a lower elevation (1300 m) than Highmont. Kentucky bluegrass (*Poa pratensis* L.), smooth brome (*Bromus inermus* Lessyer) and sedge (*Carex rostrata* Stokes) are the major forage components at Witches Brook. Witches Brook is primarily fed by the periodic discharge of mine water. Seven samples of drinking water were obtained from the brook and 2 from a dugout on the site. The dugout was sampled when the brook was dry. The 1 litre samples were obtained during May to July in 2000 and 2001.

Site Description

The experimental site was located at the Highmont tailings site (latitude 50° 26' N., longitude 120° 55' W., elevation 1,500 m) at Highland Valley Copper near Logan Lake, B.C. Reclamation of the tailings site was initiated in 1989 on soils derived from milling wastes containing residual amounts of chalcocite (Cu_2S), chalcopyrite ($CuFeS_2$) and molybdenite (MoS_2). The forage cover consisted mainly of red fescue (*Festuca rubra* L., 40% cover), alfalfa (24%) and orchardgrass (15%) with 10% bare ground (Gizikoff 2002). Drinking water was pumped from a local seepage pond (S-2). During the trial water samples (n = 9) were obtained from the feed line going to the stock tank. The 11 week grazing period for each year extended from the middle of July to the beginning of October 1999 to 2001.

Forage Samples

Aerial portions of plant samples were randomly clipped during the grazing trials at Witches Brook 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. Kentucky bluegrass, smooth brome and sedge were collected at Witches Brook (n = 32 over 2 years). Alfalfa and orchardgrass were collected at Highmont (n = 36 over 3 years). Red fescue was not collected because it was overmature and desiccated during the grazing period and it was not a preferred species. 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 using a 1mm screen.

Grazing Trials

The 1999 trial was a preliminary study mainly conducted to observe clinical signs, if any. The 2000 and 2001 trials were conducted to reproduce the symptoms in cattle that were first observed in 1999 and to collect tissue samples. The herd consisted of Hereford and Red Baldy cows 3 to 10 years old with Charolais sired calves at foot and 2 bulls. Cattle were confined in the study area with a permanent fence. The study area at Highmont was expanded from 34 to 63.5 ha during the 3-year grazing trial to accommodate increases in the size of the herd from 20 to 34 cow-calf pairs. The average stocking rate at Highmont was maintained at 0.63 ha AUM^{-1}. Different cows were used in 1999 and 2000, but 19 of the cows in 2000 were used again in 2001 to determine carryover effects, if any. Cattle at Highmont were periodically monitored in 1999 for signs of toxicity but were visually monitored on a daily basis from horseback in 2000 and 2001.

During 1999 and 2000, two rumen fistulated Jersey steers accompanied the herd for part of the trial to provide rumen contents for the determination of ingested Mo and feces for the estimation of excreted Mo. Whole rumen contents were obtained from the dorsal sac and feces were collected from the rectum. The grab samples, approximately 500 g, were obtained before, during and after the Highmont trial. All samples were frozen and freeze-dried.

In 2001, a treatment option was also tested. The cow-calf pairs at Highmont were randomly assigned to 1 of 2 treatment groups. Half of the cows received slow release Cu boluses (ALLTRACE, trace element boluses for cattle, Agrimin Ltd., UK, 136 mg Cu head^{-1} day^{-1}) and the others served as a control group. Two boluses were administered to each cow with an oral balling gun at spring turnout in May to provide mineral supplementation for 8 months. The corresponding calves also received 2 boluses (smALLTRACE, trace element boluses for calves, 62 mg Cu head^{-1} day^{-1}) once they were large enough to be safely administered. Some affected animals were also treated with Cu injections (Majak and Steinke 2001, 2002).

Table 1. Range of values for Cu and Mo levels (ppm dry wt) in Kentucky bluegrass, smooth brome, and sedge at Witches Brook, for alfalfa and orchardgrass at Highmont and for the Cu:Mo ratios.

Location	Year	Species	n	Cu	Mo	Cu:Mo
				--------- (ppm) ---------		
Witches Brook						
	2000[1]	Kentucky bluegrass	5	7 – 20	15 – 43	0.3 – 0.8
		Smooth brome	5	8 – 10	10 – 39	0.3 – 0.9
		Sedge	4	20 – 24	3 – 16	1.3 – 7.0
	2001[1]	Kentucky bluegrass	6	10 – 45	7 – 29	0.8 – 1.6
		Smooth brome	6	6 – 13	9 – 17	0.5 – 1.5
		Sedge	6	11 – 24	2 – 12	1.0 – 9.0
Highmont						
	1999[2]	Alfalfa	3	9 – 14	128 – 311	0.03 – 0.11
		Orchardgrass	3	7 – 9	99 – 283	0.03 – 0.08
	2000[3]	Alfalfa	7	9 – 15	170 – 460	0.02 – 0.08
		Orchardgrass	7	10 – 14	164 – 341	0.03 – 0.07
	2001[3]	Alfalfa	8	4 – 11	187 – 433	0.02 – 0.04
		Orchardgrass	8	4 – 7	95 – 262	0.02 – 0.04

[1]Samples were collected approximately every 3 weeks from May to July.
[2]Samples were collected approximately every 25 days from July to October.
[3]Samples were collected approximately every 10 days from July to October.

Table 2. Average daily gains (kg head^{-1} day^{-1} ± S.E.) in affected and unaffected cows and calves at Highmont.

Year		Cows				Calves			
		n	Unaffected	n	Affected	n	Unaffected	n	Affected
			------ (kg) ------		----- (kg) -----		------ (kg) -----		----- (kg) ------
1999**	no bolus	15	0.99 ± 0.09	4	0.93 ± 0.13	18	1.48 ± 0.03	1	1.48
2000	no bolus	8	0.56 ± 0.05	20	0.43 ± 0.11	25	1.29 ± 0.04	4	1.11 ± 0.09
2001	no bolus	9	0.74 ± 0.15	4	0.87 ± 0.17	14	1.24 ± 0.04	1	1.09
	bolus	11	0.85 ± 0.05	4	0.87 ± 0.21	15	1.31 ± 0.05	0	---
Mean		43	0.79 ± 0.09	32	0.78 ± 0.16				

** Significant (P < 0.01) year effect for cows.

Blood samples were obtained at week 1 in July (just before the trial began) and at the end of week 6 and week 11 during the trial in 2000 and 2001. Jugular blood samples were obtained by venipuncture using 3 red/gray Vacutainers per cow followed by temporary storage on ice. Anti-coagulants or preservatives were not used. Serum was collected after low speed centrifugation (2000 rpm for 20 min. at 5° C) for mineral analysis. Liver biopsies were performed to obtain liver samples (Buckley et al. 1986) which were rinsed in saline containing 2.5% heparin. Liver sample acquisition was facilitated by withholding water and fasting animals 12–18 hours before the biopsy procedure. Liver samples were obtained at the beginning of week 1 and at the end of week 11 in 2000 and 2001. Liver and blood samples were also obtained at the home ranch in May 2000 and 2001. The cattle were over-wintered on alfalfa-grass silage.

The water, liver, serum and forage samples were analyzed for minerals by ICP (Eco-Tech Laboratories, Kamloops, B.C.; Griffin Laboratories, Kelowna, B.C.). The Mo content of freeze-dried rumen digesta and feces were determined spectrophotometrically using the method of Marczenko (1976) after samples were ashed and solubilized (Surridge et al. 2001). Forage crude protein content was determined by the Kjeldahl method (AOAC 1990). All cattle were cared for according to protocols of the Canadian Council on Animal Care.

Statistical Analysis
Clinical Signs
Treatment effects on the animals were tested in an unbalanced, completely randomized design with repeated measures. Main effects were year, affected status (affected animals showed signs of lameness, unaffected animals did not) and sampling week. Individual animals were the experimental units. Annual changes in the frequency of clinical signs were analysed only for cows because relatively few calves were affected. The proportion of affected cows was calculated for each year.

A binary variable was created for each cow and assigned the value 1 if the cow was affected and displayed lameness and 0 if not. Contrast variables were created to compare 2000 and 2001. Logistic regression (Hosmer and Lemeshow 1989) was used to determine if the proportion of animals affected varied between 2000 and 2001.

Average Daily Gain
Cows were classified as affected or unaffected and average daily gain (ADG) was calculated for each animal in each year. For 2001, only cows that did not receive the copper bolus were used in the analysis. General linear model (GLM) was used to determine if ADG varied among years and among affected and unaffected cattle. Calf ADG was not analyzed statistically since only a few calves were affected.

The effect of bolus application on ADG was determined for cows using data from 2001. PROC GLM (SAS Institute 1999) was used to determine if ADG of cattle treated with a copper bolus were similar to untreated cattle at Highmont.

Rumen Digesta and Feces
Levels of Mo in rumen and fecal samples collected from fistulated steers before the trial began were compared with samples collected in late August, after 3 weeks of grazing the Highmont study area using paired t-tests. Data were pooled across years since only 2 rumen fistulated animals were used per year in 1999 and 2000.

Liver Biopsies and Serum
Changes in liver Cu and Mo were examined in cattle grazing Highmont during 2000 and 2001 using repeated measures mixed model (SAS Institute 1999) with year and affected status as the between-subject effects and week (week 1 and week 11 for liver biopsies and week 1, 6 and 11 for serum) as the within subjects effect. Cows treated with boluses were not included in this analysis.

The effect of treatment with Cu bolus on hepatic Cu and Mo was determined using data collected from Highmont in 2001. Data were analysed using repeated measures mixed model (SAS Institute 1999) with treatment (bolus or control) and affected status as the between-subject effects and week as the within-subjects effect.

Results and Discussion

Forage Samples
At Witches Brook, levels of Mo in Kentucky bluegrass and smooth brome ranged from 7–43 ppm during 2000–2001 (Table 1), which were above the acceptable maximum of 5 ppm (NRC 1996). The range in sedge was from 2–16 ppm. Copper levels in Kentucky bluegrass, smooth brome, and sedge ranged from 6 – 45 ppm during 2000–2001. In B.C., feed levels of Cu are normally < 17 ppm and the reported average for feeds is 6 ppm

Table 3. Average Mo levels (ppm ± S.E.) in rumen contents and feces of fistulated steers (n = 4) at Highmont during 1999 and 2000.

Sampling Date		Rumen Contents	Feces
1999	2000	------------------ Mo ppm -------- ---------	
19 July	31 July	4 ± 3**[1]	7 ± 4**[1]
26 Aug	23 Aug	209 ± 42	264 ± 43
10 Sept	6 Sept	123 ± 22	274 ± 28
23 Sept	20 Sept	139 ± 14	287 ± 32
7 Oct	5 Oct	10 ± 3 [2]	15 ± 1 [2]

** P < 0.01 for July vs Aug
[1] Before Highmont
[2] After Highmont

Table 4. Average Cu and Mo levels (ppm ± S.E.) in liver (dry wt basis) and serum (volume basis) of cows at Highmont.

Sample	Year	n	Cu Week 1	Week 6	Week 11[1]	Mo Week 1	Week 6	Week 11
					(ppm + S.E.)			
Liver								
	2000[2]	14	15.2 ± 4.3	—	18.4 ± 3.8	3.7 ± 0.2	—	23.9 ± 1.1
	2001							
	(Control)	15	49.7 ± 9.5	—	51.2 ± 8.6	1.8 ± 0.2	—	18.4 ± 1.1
	(Bolus)	12	227.9 ± 16.6[3]	—	170.2 ± 12.3	2.7 ± 0.3[3]	—	18.3 ± 0.7
Serum								
	2000[2]	13	0.62 ± 0.03	1.12 ± 0.06	1.00 ± 0.06	0.26 ± 0.02	8.01 ± 1.24	9.17 ± 0.75
	2001							
	(Control)	15	0.51 ± 0.02	1.08 ± 0.05	1.15 ± 0.03	0.09 ± 0.02	4.73 ± 0.37	5.60 ± 0.29
	(Bolus)	15	0.52 ± 0.02[3]	1.19 ± 0.04	1.15 ± 0.04	0.09 ± 0.03[3]	5.10 ± 0.29	5.49 ± 0.34

[1]In 1999, 64.9 ± 13.5 ppm Cu for liver (n = 6) and 1.19 ± 0.06 ppm Cu for serum (n = 11).
[2]Significant ($P < 0.05$) week effect for liver Mo, serum Cu, and week by year effect for serum Mo.
[3]Significant ($P < 0.05$) bolus effect for liver Cu, bolus by week for liver Cu, week effect for liver Mo and week effect for serum Cu and Mo.

(Miltimore and Mason 1971). The Cu:Mo ratios at Witches Brook ranged from 0.3 to 9.0 during 2000–2001. The ratio in Kentucky bluegrass and smooth brome ranged from 0.3–1.6 due to their elevated Mo levels, and was slightly less than the 2:1 minimum requirement. The Cu:Mo ratios in sedge were more acceptable. The reduced ratios in Kentucky bluegrass and smooth brome compared to sedge occurred in both years. Forage mineral levels at Witches Brook are representative of the forages in the general area of the Highland Valley (unpublished data, K. Gizikoff).

At Highmont, levels of Mo in alfalfa and orchardgrass ranged from 95–460 ppm during 1999–2001 (Table 1). On average, this exceeded the norm of 1.7 ppm (Miltimore and Mason 1971) by 130-fold. Copper levels in alfalfa and orchardgrass ranged from 4–15 ppm during 1999–2001 (Table 1). The ratio of Cu:Mo in alfalfa and orchardgrass ranged from 0.02–0.11 at Highmont during 1999–2001, which, on average, is less than 5% of the minimum requirement of 2:1. The low ratio could imply the potential for a secondary Cu deficiency. At Highmont, Cu:Mo ratios were similar between forages and between years. These observations suggested that cattle would be subjected to exceptional risk at the Highmont site, but acute poisoning resulting in mortality did not occur. Chronic poisoning with long-term residual effects did not occur either.

The molybdate (MoO_4^{2-}) anion can cause Mo toxicity in cattle (Swan et al. 1998). Surridge et al. (2001) reported on the isolation of the molybdate anion from alfalfa grown at Highmont. However, in the isolation of Mo from alfalfa, spectroscopic detection of the Mo complex required alkaline conditions, which may have generated the MoO_4^{2-}. More recently, we have shown that at physiological pH the Mo in alfalfa is bound as a complex (unpublished data, D. Steinke). It is unclear at this time as to the biological availability of the bound form of Mo.

Water
The Witches Brook drinking water contained 23 ± 9 ppb Cu and 122 ± 13 ppb Mo (n = 7). The dugout contained 204 ppb Cu and 338 ppb Mo (n = 2). The elevated levels in the dugout might be explained by seepage from Witches Brook and subsequent evaporation. Both sources of drinking water showed elevated levels of Mo that exceeded the recommended maximum level of 60 ppb Mo (Puls 1994).

At Highmont, the drinking water contained 14 ± 2 ppb Cu and 7,200 ± 300 ppb Mo (n = 9). The Mo levels in the water were exceptionally high (x 100) when compared to the recommended level (Puls 1994). If the water contained 7,200 ppb Mo (7.2 ppm Mo), the daily consumption of the Mo could be 0.6 g head^{-1} day^{-1} based on an estimated water intake of 80 liters day^{-1}. The MoO_4^{2-} anion is the only form of Mo present in the drinking water at Highmont (unpublished data, D. Steinke). If the forage contained 221 ppm Mo, the daily consumption of Mo as the complex could be 2.6 g head^{-1} day^{-1} based on a daily forage intake of 12 kg (dry wt). Forage probably accounts for at least 80% of the Mo intake by cattle.

Clinical Signs
Animals that showed signs of lameness were deemed affected as lameness was the major affliction at the site. The observed diarrhea and depigmentation of haircoat, which was most apparent at the end of the trial, were considered secondary signs. The proportion of cows that were affected and displayed clinical signs of lameness was 0.2, 0.7 and 0.3 in 1999, 2000, and 2001 respectively. The proportion of affected cattle were similar between 1999 and 2001 (P = 0.51) but were significantly different in 2000 (P < 0.01).

Clinical abnormalities were first detected in 1999 in 4 cows and a calf after 4 - 5 weeks of grazing at Highmont and the transient condition persisted for up to 3 weeks. The affected animals displayed a stiff shuffling gait and favoured their tiptoes when travelling. This appeared to be the only abnormality as all other functions including temperature, pulse, respiration, and body condition appeared to be normal. Each animal had fully recovered by the end of the trial.

More animals showed clinical signs in 2000 as lameness was observed in 21 cows, 4 calves, and 2 bulls. The stiff shuffling gait occurred within a week of arrival at Highmont and tended to be more severe and lasted longer with occasional relapses as compared to conditions in 1999. In severe cases, the animals were reluctant to move and were recumbent much of the time. The severity of the signs seemed to wax and wane with ultimate recovery from the stiff gait occurring without treatment such as a Cu injection (Majak and Steinke 2001, 2002). Calves seemed to be less affected and for a shorter period of time than adults. It is unlikely that the clinical signs were due to Se deficiency because whole blood levels of Se were in the adequate range (Majak and Steinke 2001).

Radiographs were taken of the knee joint, cannon bone, fetlock, pastern and hoof of 2 animals to look for signs of osteoporosis, flaring of the metaphysis of

the bone, or laminitis that can be associated with Cu deficiency or Mo toxicity. No abnormalities were noted on the radiographs. An affected cow was euthanized and a complete post mortem was performed on the carcass. Tissue samples, bone, joint, and hoof were histologically examined at the Pathology Department, Western College of Veterinary Medicine, University of Saskatchewan. No lesions were found in any of the tissues that could be associated with the cause of lameness.

In 2001, signs of lameness were reproduced at Highmont in 9 cows, a calf and a bull but the signs occurred 3 weeks after arrival at the site. The delayed occurrence and lesser frequency of clinical signs in 2001 compared to 2000 could be related to moisture availability for plant growth. The precipitation for the growing season (May to September) was threefold greater in 2000 (323 mm) compared to 2001 (93 mm) (Highland Valley Copper 1999, 2000, 2001). The increased moisture in 2000, especially during August and September (54 mm in 2000 compared to 8 mm in 2001), would enhance forage growth and biomass thereby increasing Mo availability and intake with the resultant increase in clinical abnormalities. The rainfall for the growing season was twofold greater in 2000 than in 1999, which also yielded a lower incidence of clinical abnormalities. Four cows receiving the copper bolus suffered mild to severe lameness in 2001. The Cu bolus did not eliminate clinical signs even when the risk was reduced in 2001.

A 57% pregnancy rate at Highmont in 1999 was attributed to the selection of late calvers for the grazing trial. The rate increased to 100% and 87% in 2000 and 2001 respectively. Any effects on pregnancy rates were difficult to study because most of the cows were bred prior to arrival at the mine site. There appeared to be no negative effects on early pregnancy as cows that arrived pregnant remained pregnant and delivered normal calves in the following spring.

Average Daily Gains

Despite these abnormalities, ADG (Table 2) was similar between affected and unaffected cows (P = 0.70) but varied among years (P < 0.01). Differences between years probably reflect random variation rather than affected status since ADG was lower for both affected and unaffected cows in 2000 compared with other years. The ADG of affected cows and calves was acceptable. The forage crude protein content (CP) was adequate

at Highmont. For example, in 2000 and 2001 the average CP in alfalfa was 17 ± 1.0% (n = 15) and in orchardgrass it was 7.0 ± 0.6% (n = 14) during the grazing trial. Average daily gains did not differ between the bolus and control cows (P = 0.69) in 2001. Bolus cows gained 0.86 ± 0.06 kg head^{-1} day^{-1} (n = 15) compared with 0.78 ± 0.22 kg head^{-1} day^{-1} (n = 13) for the control cows. Bolus-treated calves gained 1.31 ± 0.05 kg head^{-1} day^{-1} (n = 15) compared with 1.23 ± 0.04 kg head^{-1} day^{-1} (n = 15) for the control calves (P = 0.19).

Rumen Digesta and Feces

Levels of Mo in rumen digesta and fecal samples increased significantly (P < 0.01) after cattle were placed at Highmont (Table 3). Levels of Mo escalated 50-fold by late August. Thereafter, there was a decrease in the Mo content of the rumen contents probably because the forage biomass was declining in September. Conversely, there was a dramatic decrease in the Mo content within 2 weeks of removal of the steers from the trial. It appears that the feces are a major route of Mo excretion (Table 3). Urine samples that were collected in 1999 contained < 10 ppm Mo.

Liver Biopsies

The liver is the storage organ for Cu. Liver tissue analysis for minerals can reveal the plane of nutrition in the animal (Blood and Radostits 1989). The average hepatic levels of Cu and Mo at the home ranch in May were 73.0 ± 5.5 ppm and 4.0 ± 0.2 ppm respectively. The recommended levels for Cu and Mo in liver are > 83 ppm and < 4.7 ppm respectively on a dry matter basis (Puls 1994).

Copper levels in the livers of control cattle at Highmont (Table 4) were similar between affected and unaffected animals (P = 0.90), years (P = 0.12) and between weeks 1 and 11 (P = 0.66). Copper levels in the liver were marginal at Highmont in 2001 but deficient in 2000 (Table 4), which could explain, in part, the larger proportion of affected cows in 2000 and the lower ADG (Table 2). Liver Cu levels were determined in 1999 at week 11 and they were similar to those in 2001 (Table 4).

Hepatic levels of Mo did not vary according to affected status (P = 0.36) or among years (P = 0.59) but varied between week 1 and week 11 (P < 0.0001). The escalation from week 1 to week 11 was dramatic in 2000 and 2001 when 6-fold and 10-fold increases were observed in hepatic Mo. However,

the elevated levels of Mo in 2000 declined to baseline levels by next spring. The baseline levels of Mo in the 19 returning cows (3.5 ± 0.2 ppm) were similar to the baseline levels (2.5 ± 0.2 ppm) in the 11 new arrivals in the spring of 2001 suggesting no long-term accumulation of Mo. Only 3 of the 19 returning animals showed clinical signs but 6 of 11 cows new to the study were affected.

The efficacy of the Cu bolus to elevate hepatic Cu is clearly evident from Table 4. Hepatic Cu did not vary between affected and unaffected animals (P = 0.29) but was higher in the bolus-treated group compared with the control group (P < 0.0001) (Table 4). The significant treatment by week interaction (P < 0.01) indicated that changes in hepatic Cu across time were not consistent between treatments. Hepatic Cu declined between weeks 1 and 11 in the bolus-treated group but remained threefold greater than levels in the untreated group at the end of the trial. Hepatic Cu levels remained relatively consistent between weeks in the control group. Levels of Mo were similar between affected and unaffected animals (P = 0.22) and between the bolus-treated and control groups (P = 0.40) but were elevated at week 11 compared with week 1 (P < 0.0001). This elevation was consistent between groups (P = 0.43). Although the bolus elevated hepatic Cu, levels of Mo still increased during the study.

Serum

The average serum levels of Cu and Mo at the home ranch in May were 0.65 ± 0.05 and 0.08 ± 0.01 respectively. The recommended levels for Cu and Mo in the serum are > 0.6 ppm and < 0.1 ppm respectively (Puls 1994). Serum Cu levels in cattle grazed at Highmont during 2000 and 2001 did not vary between years (P = 0.12) but varied between weeks (P < 0.001) increasing from week 1 to week 6. At week 1 (Table 4), the serum levels of Cu were acceptable, but almost doubled between weeks 1 and 6 and showed little change between weeks 6 and 11. Serum Cu levels did not vary between affected and unaffected animals (P = 0.52) nor between the bolus and control groups (P = 0.64). The Cu bolus did not enhance serum Cu levels, but it had a profound effect on Cu levels in the liver (Table 4). In the face of adequate total body Cu, serum Cu levels are tightly regulated by the cow.

Molybdenum levels did not vary between affected and unaffected animals (P = 0.38). There was a significant year by week interaction (P < 0.02) indicating that

changes in serum Mo across weeks was not consistent between years. Serum Mo increased between weeks 1 and 6 during both years and remained high between weeks 6 and 11, however, serum Mo levels were higher in 2000 when clinical signs were aggravated. Serum Mo did not vary between the bolus and control groups ($P = 0.71$)

Conclusions

Clinical signs of lameness were documented in less than half of the cows and in only a few calves in a 3 year grazing study on forage with extremely high levels of Mo (> 100 ppm) on reclaimed mine tailings. Extended periods of grazing high Mo forage produced clinical signs primarily manifested as a stiff shuffling gait, and secondarily as diarrhea and haircoat problems mainly in cows. The signs were observed within a week in 2000 and persisted for up to 4 weeks with the exception of haircoat problems, which were resolved by next spring. The signs were delayed in 2001, probably due to reduced moisture availability for plant growth. The abnormalities had little impact on animal performance at the site as average daily gains were adequate and the animals recovered fully by the end of each grazing trial. Liver biopsies showed marginal to deficient Cu but toxic levels of Mo. Clinically affected animals did not necessarily show highest hepatic Mo or lowest hepatic Cu indicating that liver Mo and Cu status did not directly correlate to clinical signs. Serum levels of Cu appeared adequate. Average levels of Mo in alfalfa and orchardgrass exceeded 200 ppm, which would inhibit sulfide formation in the rumen and the resultant formation of thiomolybdates. The Cu bolus did not enhance serum Cu levels but it had a profound effect on Cu levels in the liver. Copper boluses did not prevent the onset of clinical signs. Decreasing the duration of exposure to high Mo forage and supplementation with alternative forms of dietary Cu should be investigated to alleviate clinical disorders at Highmont and to ensure cattle grazing without ill effects.

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