MOLYBDENOSIS AND MOOSE AT HIGHLAND VALLEY COPPER

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ABSTRACT

In the fall of 2003, a study was conducted at Highland Valley Copper Mine (HVC) to determine if moose (*Alces alces*) grazing reclaimed mine tailing sites were at risk of molybdenosis – a molybdenum induced copper deficiency that affects ruminant animals. Past research at HVC indicates that the mine site has vegetation with higher molybdenum and copper concentrations then sites that do not have mining potential. To conduct this study, five $50m^2$ plots were selected at each treatment site, HVC and the reference site (Wentworth Creek), and feces and vegetation were collected. All feces deposited (within a one-year period) were collected and the current years' growth of shrubs, forbs, and grasses were sampled from five $1m^2$ quadrats within each plot. The vegetation and feces were then analysed at the Agriculture and Agri-Food Canada research facility via the Dithiol Method for molybdenum and the Atomic Absorption Spectrophotometer Method for copper. Statistical analysis of the data indicated that molybdenum concentrations in feces and molybdenum and copper levels in the vegetation were higher at HVC then at the reference site. The copper concentration in the feces did not differ between sites. Thus, as moose are consuming more molybdenum and copper at HVC then at the reference site, they are at potential risk of molybdenosis. However, based on the extrapolation of results from the cattle studies conducted at HVC and the study on mule deer at Brenda Mines (as literature stating safe levels of molybdenum for moose is unavailable), moose are unlikely to display symptoms of molybdenosis from foraging at HVC.

INTRODUCTION

Highland Valley Copper (HVC) is a copper and molybdenum mine located in the interior of British Columbia and has been in operation for over 30 years. HVC has over 6000ha of disturbed land; of which 33% has been revegetated since reclamation began in 1983 (Jones et al. 2003). Due to the mining process, which only recovers 50% of the molybdenum content (HVC 1996), molybdenum is elevated in the mine tailings (Steinke and Majak 2003); thus, HVC is concerned with the effects that molybdenum may have on wildlife. Therefore, this study was conducted to determine if moose (*Alces alces*) have a potential risk of contracting molybdenosis by consuming forage high in molybdenum at HVC.

Reports from HVC have indicated that of the portion of the mine that has been revegetated, the majority of it is composed of grasses and forbs, with shrub islands dispersed throughout. Forage quantities reaching up to 4343kg ha^{-1} were reported in 2000 (Jones & Associates Ltd 2001). While moose are browsers, they will vary their diet depending on forage availability (Franzmann and Schwartz 1997); however, they prefer to consume forage at a ratio of 45:4:1 of shrubs, forbs, and grasses, respectively (Franzmann and Schwartz 1997). In addition, to continually utilize an area moose must be able to obtain, consume and digest food at a rapid rate (Franzmann and Schwartz 1997), with the quantity of foraged needed reaching up to 20kg per day during the winter (MELP 2000). This indicates that while there is not a substantial amount of shrubs present at HVC, there is enough forage available to sustain their energy requirements and thus moose continue to forage on vegetation that has high molybdenum concentrations.

Molybdenosis

Molybdenosis is a form of molybdenum toxicity that creates a copper deficiency in ruminant animals (Mills 1982 in: Stark and Redente 1990, Stone et al. 1983, Ward 1994). It is a problem that has been reported world wide (Stone et al. 1983) and is caused by ruminant animals consuming forage high in molybdenum (Mills 1982 in Stark and Redente 1990, Stone et al. 1983). Once the molybdenum has been consumed, it reacts with sulphur in the rumen to form thiomolybdate complexes (Fausto da Silva and Williams 1991, Mills 1982 in Stark and Redente 1990, Ward 1994), which causes copper to become biologically unavailable (Stone et al. 1983, Ward 1994, Steinke and Majak 2003). Symptoms of molybdenosis include diarrhea (Frank 1998, Cunningham et al. 1953, Huber et al. 1971, Stone et al. 1983, Ward 1994), weight loss, hair depigmentation (Frank 1998, Stone et al. 1983, Ward 1994), hair loss, ulcers, lesions (Frank 1998), lameness (Frank 1998, Ward 1994) and even death (Ward 1978).

Research by Miltimore and Mason (1970) has suggested that a forage copper to molybdenum ratio of 2:1 be maintained, as they believe that molybdenosis will occur in ruminant animals below this level; however, this ratio is very controversial with many researchers believe that this ratio cannot reliably predict molybdenum toxicity (Gardner et al. 2003, Ward 1994). However, the 2:1 ratio can be used as a general guideline to distinguish between safe and hazardous feed (Ward 1994)

Previous Research on Ruminant Animals and Molybdenosis

The effects of molybdenum on domestic ruminant animals have been studied and it has been found that cattle, followed by sheep, are most susceptible to molybdenum toxicity (NRC 1980, Ward 1994). In sheep, it has been found that fecal and urinary excretion of molybdenum increased linearly with increasing dietary molybdenum concentration, whereas the copper excretion in feces and urine was not influenced (Pott et al. 1999). Studies conducted at HVC between 1994 and 1996 have found that symptoms of a copper deficiency did not develop at the reclaimed Bethlehem mine tailings site even though the average molybdenum concentration was 34ppm and the copper to molybdenum ratio in forage ranged between 0.35:1 and 0.62:1; thus the copper to molybdenum ratio of 2:1 did not apply to this site (Gardner et al. 2003). In addition, studies at the HVC Highmont site found that no symptoms of molybdenosis were evident in 1998 even though elevated levels of molybdenum were present in the forage ($\bar{x}$ = 286.3ppm), rumen digesta, liver, blood serum, and feces ($\bar{x}$ = 238.02ppm) (Stager 1998, Majak and Steinke 2003). However, in 1999 and 2000 clinical signs of molybdenosis (lameness, diarrhea, and hair depigmentation) were observed at the Highmont site, with the molybdenum concentration in forage ranging between 100 and 410ppm (Majak and Steinke 2003); all cattle recovered with treatment

(Majak and Steinke 2003). Thus, molybdenosis is a treatable condition that can occur in cattle at the Highmont tailings site of HVC.

Wild ruminant animals have been studied to a lesser extent; in British Columbia two studies have been conducted. A study at Endako Mine sampled hair from moose and deer (*Odocoileus spp*) and analysed it for total copper and molybdenum (Riordan 1999). It was found that moose within 10km of the mine had molybdenum concentrations that were significantly higher then moose harvested greater then 10km away. It is thought that moose were exposed to higher concentrations of molybdenum due to the consumption of riparian vegetation and aquatic plants, which were found to contain higher concentrations of molybdenum than terrestrial vegetation at Endako Mine (Riordan 1999). The copper concentration in moose and the molybdenum and copper concentrations in deer were not significantly different based on the location of harvest (Riordan 1999). A second study, which was conducted at Brenda Mines, was preformed on mule deer (*Odocoileus hemionus*). This study found that the forage had an average molybdenum concentration of 130ppm and an approximate copper to molybdenum ratio of 0.05:1; mule deer had a feces copper to molybdenum ratio that ranged between 0.1:1 and 4.2:1 and a mean molybdenum concentration of 140ppm (Taylor and McKee 2003). Taylor and McKee (2003) concluded that mule deer appeared normal and healthy with no health problems due to molybdenosis at Brenda Mines; however this study is limited as the feces sample size was relatively small (N = 17), only two feces samples were taken from the reference site and the reference site was only 4km away from Brenda Mines.

METHODS

I conducted this study in the interior of British Columbia at HVC and a reference site north of Kamloops (Wentworth Creek). These sites were selected based upon location and the known elevated presence of moose. The location of the treatments sites was critical, as they need to have the same general site characteristics, and contain moose that did not have overlapping home ranges. Moose in British Columbia have seasonal home ranges of 5-10km^2 (Blood 2000) and moose in Canada have annual home ranges that can vary between 28 and 938km^2 (Franzmann and Schwartz 1997). The sites has several topographical obstacles between them and were approximately 80 linear kilometres apart (i.e. as the crow flies), which equates to approximately 800km^2. The elevated presence of moose populations at the sites was mandatory for data collection, as the goal of the research was to determine if moose are at potential risk, not the abundance of moose in the area. The HVC plots were mainly composed of grasses and forbs. The plots in the reference site were primarily vegetated with shrubs and large trees, with forbs and some grasses in the understory; many of these plots were located in previously harvested sites.

All plots within the study areas were randomly chosen so that the entire area of each site was represented in they study and so that each sample collected could act as a replicate. At each site, five plots were chosen, with each plot measuring 50m^2. Each plot was extensively searched for feces and every pellet group collected was considered one sample. The feces were analyzed before collection to determine the approximate age of the sample (Table 1), this allowed samples in which leaching had occurred to be avoided. Within each plot, five 1m^2 quadrats were randomly placed to collect subsamples of vegetation. All current years' growth was clipped from within the quadrats and the vegetation was separated based on vegetation type (shrubs, forbs and grasses); subsamples of like-vegetation from each plot were pooled.

Table 1: Fecal pellet age analysis

Age	Description
fresh	moist, dark brown pellets
< 1 year	moist to dry, dark brown pellets which may have started to fade
> 1 year	dry, cracked and faded/light brown pellets, possibly crumbling or containing fungi

The fecal and vegetation samples were dried at an average temperature of 86^{O}C until they reached a constant weight and ground through a 1mm sieve using a Thomas-Wiley Laboratory Mill. Two grams of each sample were ashed at 475^{O}C overnight using a Thermolyne Type 30400 Automatic Furnace and then digested with 25mL of 0.5 molar hydrochloric acid. The samples were analyzed for molybdenum using the Dithiol Method (Marczenko 1976) and copper using an atomic absorption spectrophotometer (Allen 1961, Baker and Smith 1974, David 1958, Isaac and Johnson 1975, Perkin-Elmer 1976, Price 1974, Varian Techtron 1979). Upon completion of the lab analysis all negative numbers obtained for the concentration of molybdenum and copper were converted to zero, as a negative value for the amount of an element in a substance is biologically impossible. Statistical analysis were preformed using a Mann-Whitney U-test to determine if there was a difference in the concentrations of molybdenum and copper found between the sample sites. The data samples were pooled based on the site they were collected from and analyzed in categories: all vegetation, shrubs, forbs, grasses, and feces.

It is recognized that pseudo replication has occurred in this study as there was only two sites considered, HVC and the reference site, and there was no replication of the treatment sites. However, this issue has been addressed as the sample plots were spread out over the entire site to get an even representation of the entire site. Therefore, the results of this study are specific to HVC and care should be taken when extrapolating this data to other studies.

RESULTS

It was found that the concentrations of molybdenum and copper in the vegetation, shrubs, grasses, and forbs at HVC were higher then the concentrations at the reference site (Table 2). The copper to molybdenum ratio of the vegetation at HVC was below the 2:1 ratio recommended for forage (Table 3), whereas the ratio at the reference site was above the recommended 2:1 ratio in all vegetation, forbs and grasses. However, the shrub ratio at the reference site was 0:1 as there was no copper present in the shrubs at the reference site (Table 3).

Upon analysis of the fecal pellet samples it was found that molybdenum concentrations in the feces at HVC were significantly different then the concentrations in feces at the reference site (Table 2). However, the copper concentrations in the feces were not different between the treatment sites (Table 2). The copper to molybdenum ratio was below 2:1 ratio at HVC and above 2:1 at the reference site (Table 3).

Table 2: Mean concentrations of molybdenum and copper found in vegetation and feces at Highland Valley Copper and the reference sites

	Molybdenum			Copper		
	HVC	Control	*P*-value	HVC	Control	*P*-value
All Vegetation	48.35	0.19	< 0.01	19.91	0.47	< 0.01
Shrubs	28.77	0.35	0.052	16.92	0	0.005
Forbs	58.32	0.15	0.008	24.07	0.78	0.034
Grasses	57.96	0.04	0.013	15.76	0.66	0.013
Feces	66.35	1.23	< 0.01	38.58	17.23	0.974

Table 3: Sample size and copper to molybdenum ratio at Highland Valley Copper and the reference sites

	Highland Valley Copper		Reference	
	N	Cu:Mo	N	Cu:Mo
All Vegetation	15	0.41:1	14	2.46:1
Shrubs	5	0.59:1	5	0:1
Forbs	5	0.41:1	5	5.20:1
Grasses	5	0.27:1	4	18.09:1
Feces	62	0.58:1	54	13.97:1

DISCUSSION

Due to visual evidence (feces and sightings) of moose presence and the low quantity of shrubs at HVC, it is logical to conclude that moose are consuming all three vegetation types (shrubs, grasses and forbs) because if moose were only eating shrubs they would not be able to meet their energy requirements. As such, by eating all three types of vegetation, moose were able to obtain enough food to meet the energy requirements needed to make it beneficial to stay in the HVC area. The percentage of the different forage types being consumed cannot be determined, as moose were not observed while foraging.

The concentrations of molybdenum at HVC in all vegetation types were above 10 ppm (Table 2), which is the lower limit of toxicity for forage (Britton and Goss 1946 in Cunningham et al. 1953). In addition, the suggested copper to molybdenum ratio for forage is 2:1 (Miltimore and Mason 1971), and the ratio of all of the vegetation types at HVC is below this (Table 2), thus indicating that a copper deficiency due to molybdenum could occur. However, as noted above, the 2:1 ratio is a very general guideline and there is research that opposes the relevancy of this ratio (Gardner et al. 2003, Mason 1981, Ward 1994). In particular research by Gardner et al. (2003) at HVC has found that cattle being intensively grazed on the HVC Bethlehem site did not have any signs of a copper deficiency even though the cattle were consuming an average of 34ppm molybdenum and the average copper to molybdenum ratio in the forage was 0.47:1. Gardner et al. (2003) concluded that the 2:1 ratio did not apply to the HVC Bethlehem site. However, Majak and Steinke (2003) did observe clinical signs of molybdenosis at the HVC Highmont site during cattle studies carried out in the 1999 and 2000 seasons. In 2000 the forage the cattle were consuming at the Highmont site contained an average of 234ppm molybdenum and had a copper to molybdenum ratio that ranged between 0.17:1 and 0.04:1. Thus as cattle are considered to be the domestic ruminant at the highest risk of molybdenosis (NRC 1980, Ward 1994), the same level of precaution should be applied to animals in which the safe level of molybdenum in feed is unknown. Therefore, through extrapolation of results from the cattle studies conducted at HVC, it can be stated that moose consuming forage from various locations on the mine are unlikely to display signs of molybdenosis.

The concentration of molybdenum was significantly higher in the feces at HVC then at the reference site; the copper to molybdenum ratio also reflects this being 0.58:1 at HVC and 14.00:1 at the reference site. The feces concentrations of these elements are higher then the vegetation concentrations, as excretion concentrates bodily wastes; the higher feces concentrations are indicative of the high forage concentrations (Majak and Steinke 2002, Pott et al. 1999). While Majak and Steinke (2000) found that 90% of molybdenum consumed is excreted in cattle, Puls (1994) found that normal molybdenum levels in feces are less then 10ppm, thus while most of the molybdenum consumed is excreted, the feces molybdenum concentration at HVC (66.35ppm) is well over the normal level. However, Taylor and McKee (2003) found that mule deer with a feces ratio as low as 0.1:1 did not display signs of molybdenosis; thus as moose at HVC have a ratio of 0.58:1 they may not be affected by molybdenosis.

In conclusion, based on the vegetative and fecal analysis conducted in this study, it can be stated that moose at HVC were consuming more copper and molybdenum from any of the vegetation types than moose at the Wentworth Creek reference site and that moose were excreting more molybdenum at HVC then moose at the reference site. Thus, as the moose are exposed to higher levels of molybdenum at HVC, they are susceptible to molybdenosis. However, based on past research conducted on cattle at HVC and on mule deer at Brenda Mines, it is unlikely that moose will display symptoms of molybdenosis.

As little is known on the tolerance level moose have for molybdenum and as they are susceptible to molybdenosis, it is recommended that further analysis of molybdenosis in moose at HVC be investigated. Several options are possible for future research, including collecting specimen samples (such as liver and blood serum samples), observing moose and their activity, and conducting research on un-mined land that has a naturally high-underlying concentration of molybdenum.

ACKNOWLEDGEMENTS

I would like to acknowledge Highland Valley Copper for their financial contribution and overall support of this project. In addition, I would like to thank Agriculture and Agri-Food Canada for allowing me to conduct the lab analysis at their research facility in Kamloops, and all other individuals that made this project possible.

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