Journal of Wildlife Diseases, 37(3), 2001, pp. 509–522

MINERAL AND HEAVY METAL STATUS AS RELATED TO A MORTALITY EVENT AND POOR RECRUITMENT IN A MOOSE POPULATION IN ALASKA

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ABSTRACT: Moose (*Alces alces*) found dead (FD) and hunter-killed (HK) in 1995 on the north slope of Alaska (USA) in the Colville River Drainage were evaluated for heavy metal and mineral status. Compared to previous reports for moose and domestic cattle, and data presented here from Alaska moose outside the Colville River area, levels of Cu were determined to be low in hoof, hair, liver, kidney, rumen contents, and muscle for these north slope moose. Iron (Fe) was low in muscle as well. These findings, in conjunction with evidence of poor calf survival and adult mortality prompted investigation of a mineral deficiency in moose (serum, blood, and hair) captured in the spring of 1996 and 1997. Captured males had higher Ca, Zn and Cu levels in hair than captured females. Female moose hair samples were determined to be low (deficient) in Cu, Ca, Fe, and Se with mean levels (ppm) of 2.77, 599.7, 37.4, and 0.30, respectively. Serum Cu level was low, and to a lesser degree Zn was deficient as well. Whole blood (1997 only) was marginally deficient in Se and all animals were deficient in Cu. Based on whole blood, sera and hair, Cu levels were considered low for moose captured in spring 1996 and 1997 in the Colville River area as compared to published data and other populations evaluated in this study. Low levels of ceruloplasmin activity support this Cu deficiency theory. Evidence indicates that these moose are deficient in Cu and other minerals; however, the remote location precluded sufficient examination of animals to associate this apparent deficiency with direct effects or lesions. Renal levels of Cd increased with age at expected levels.

Key words: Alaska, *Alces alces*, heavy metals, minerals, moose.

INTRODUCTION

The population of Alaskan moose (*Alces alces*) north of the Brooks Range generally increased from the 1940's until 1990–95 (Karns, 1997). Although moose disperse during summer to occupy tundra habitats throughout the region, most animals congregate along rivers in winter. Aerial surveys are conducted each spring to assess population and calving trends (O'Hara et al., 1998). During 1990–95, moose populations on the north slope declined. Subsequent studies demonstrated that adult mortality and poor recruitment contributed to this population decline (O'Hara et al., 1998). Carcasses of >30 moose were located during summer 1995 on a major tributary of the Colville River. Cause of death was not determined, but did not involve obvious signs of trauma (gunshot, predation) or outright starvation (emaciation).

Causes of poor recruitment were unclear, but adverse weather, high predation rates, and inadequate forage may have interacted to produce low calf production or survivorship. The acute population decline prompted an investigation of mineral status, and infectious abortifacients (reported separately). Evidence of exposure to *Brucella suis* biovar 4 was indicated (O'Hara et al., 1998).

Malnutrition in the form of limited minerals has been well documented for many species. Essential trace elements are required as cofactors for many biochemical pathways and are therefore indispensable for metabolism. The essentiality of several trace elements (F, Si, V, Cr, Mn, Fe, Co,

Cu, Zn, Se, Mo, and I) has been well documented for many ruminants (Puls, 1988; Van Soest, 1994; National Research Council, 1996). A thorough investigation of the role of a mineral in the health of an animal should consider (1) dietary availability, (2) absorption, (3) transport to tissues, (4) storage in specific tissues, (5) mobilization to target tissue, (6) metaloprotein biosynthesis, (7) role as essential metaloenzyme, (8) catabolism of metaloenzyme, (9) recycling, transport, and reutilization of the ion, and (10) excretion (Puls, 1988; Frank et al., 1994). Although complete documentation of all these facets is rarely possible in wild animals inhabiting remote locations, a combination of approaches from tissue composition and serum metaloenzyme activities may identify aberrations of mineral metabolism.

Inadequate Cu in herbivores may result from inadequate dietary intake (simple or primary Cu deficiency), or interactions of normally adequate Cu levels with Zn, Fe, Mo and sulfide that reduce absorption of Cu or interfere with metabolic processes (complex or secondary Cu deficiency) (Suttle, 1991). Absorption of Cu is also reduced by interactions with dietary proteins, sugars and fiber (Cousins, 1985). Copper deficiency has been reported in wild cervids (Flynn et al., 1977; Flynn and Franzmann, 1987; Gogan et al., 1989). Inadequate Cu adversely affects Fe absorption, mobilization, transformation, and incorporation into hemoglobin (Owen, 1982; Suttle, 1991). A subclinical Cu deficiency in ungulates is characterized by low levels of Cu in liver and serum, but only marginal signs of poor health. Although subclinical disease (deficiency) may be the primary reason for poor health, other opportunistic factors (bacteria, viruses, predators, weather events) are the ultimate causes of mortality. Signs of Cu deficiency and molybdenosis (Mo is considered a Cu antagonist) are very similar, and careful diagnosis is required. Some populations of Alaskan moose with low levels of Cu in hair, low blood ceruloplasmin levels, low serum Cu with a Cu deficient browse displayed faulty hoof keratinization and decreased reproductive performance (Flynn et al., 1977; Flynn and Franzmann, 1987).

Geographical and seasonal variations of minerals in moose are linked to changes in behavior, biochemistry, morphology, physiology, and reproduction. For most minerals studied, concentrations in hair peak in late summer and autumn (Flynn and Franzmann, 1987). This pattern is consistent with the seasonal activity of hair follicles and the high availability of minerals in summer foliage compared with the stems available in winter (White et al., 1987). Hair analysis for the elements in moose from the Kenai Peninsula, Alaska indicated potential Cu deficiency. Many other systemic lesions and signs have been associated with Cu deficiency and include diarrhea, emaciation, enteritis, alopecia, sudden heart failure, and convulsions (Puls, 1988; Frank et al., 1994). Moose in Sweden apparently displayed a severe Cu deficiency (3.2 ppm wet weight or ww) and other nutritional deficiencies. Decreased uptake of Cu may have resulted from inadequate forage content because soil Cu was removed by acidification, and/or inadequate absorption because of viral infection and subsequent mucosal damage along the alimentary tract (Frank et al., 1994). Frank et al. (1994) determined criteria for Cu levels in moose liver as severe deficiency (<5 ppm ww), deficient (5–<10), marginal (10–<20), and adequate (>20). Serum and hair Cu reflect dietary intake when liver levels of Cu are <20 ppm ww (Puls, 1988).

We summarize levels of minerals and metals, and ceruloplasmin activity for free-ranging moose sampled in 1996 and 1997, and tissues of the FD and HK moose in 1995 from the Colville River system, Alaska. We compare these results to published studies concerning moose and cattle (when not available for wild ungulates), and to data from moose associated with areas outside the north slope of Alaska

(south of the Brooks Range, and New Hampshire).

MATERIALS AND METHODS

Aerial surveys

Aerial surveys were conducted annually in spring (prior to capture operations in 1996 and 1997) and fall (composition count) to determine changes in the total population, calf production (calves per 100 cows), and other population dynamics within a prescribed area (count area) that includes regions of the Colville, Chandler, and Anaktuvuk Rivers on the north slope of Alaska (near Umiat, Alaska) for Game Management Unit 26 (A). This prescribed area is bordered by longitudes 154°W and 151°W, and latitudes 68.5°N and 69.5°N.

Sampling of found dead (FD) and hunter killed (HK) moose

Moose carcasses (15 of a reported 30) along the Chandler River and associated drainages were examined and sampled for cause of death on August 1 and 2, 1995. The carcasses were in very poor post mortem condition and of limited diagnostic value. However, fat reserves in viscera and bone marrow indicated depleted energy reserves (Kistner et al., 1980). Predation and other signs of trauma were not obvious. Three intact moose (not scavenged) were sampled (liver, kidney, muscle, hair, lower leg, rumen contents, and feces) and remaining carcasses were visually examined grossly. Bull moose harvested in the Colville and Chandler River regions in the fall of 1995 were sampled by cooperating hunters. Hunter-collected tissues were frozen, or preserved in neutral buffered 10% formalin. Frozen samples include kidney, liver, heart, muscle, rumen contents, feces, skin and hair, and jaw. Tissues placed in formalin include liver, kidney, lung, tongue, testicle, rumen or other stomach sections, spleen, intestine, and muscle. Hunters scored the amount of subcutaneous, perirenal, and pericardial fat to comment on general condition. All hunter-killed animals were reported in good condition with fat present in all areas specified. Liver, kidney, and muscle samples were sent to Mississippi State University, College of Veterinary Medicine, Diagnostic Toxicology Laboratory for trace mineral analyses described by O'Hara et al. (1995). Samples were analyzed by atomic absorption (AA) spectrophotometry (Perkin Elmer 5000; Norwalk, Connecticut, USA) with a graphite furnace and continuous background correction. Results are reported in ppm wet weight (ww). The formalin-fixed specimens were sent to a certified pathologist (K. Burek, Alaska Veterinary Pathology Services, Eagle River, Alaska, USA).

Sampling of immobilized moose

In April 1996 and 1997, moose from the Colville River area were captured, examined, collared with VHF transmitters, evaluated for pregnancy, serologic evidence of disease, mineral status, fecal indicators of parasites, and standard blood indices. Moose were captured using a helicopter and chemical immobilization as described in O'Hara et al. (1998). Blood samples were taken by syringe from the jugular vein for serum and blood collection. Bundles of hair were removed by pulling full-length strands straight up from the dorsal midline at the shoulder.

Elemental analysis of hair, serum and whole blood

Samples of hair, serum and whole blood were submitted frozen to the Diagnostic Laboratory, College of Veterinary Medicine, University of Illinois for elemental analyses. Aliquots of 1.0 ± 0.1 g were digested with 5.0 ml of concentrated nitric acid, evaporated to 1.0 ml and diluted with 5.0 ml of concentrated HCl for 30 minutes. Hydrogen peroxide (30%) was added in 1 ml increments until effervescence ceased or until a volume of 10.0 ml was reached. The sample was quesced to a volume of 25 ml with deionized water. Tissue samples were processed in a similar fashion to hair except 2.0 ± 0.1 g was digested, without additiion of HCl or peroxide. Serum samples were prepared by adding 0.5 ml to 9.5 ml of 3 M HCl and left at room temperature for 30 minutes and the supernatant analyzed. Digests and supernatants were analyzed with the Thermo Jarrell Ash IRIS AP Inductively Coupled Plasma (ICP) Spectrometer. Quality control samples include SPEX Chemical Reference standards (SPEX Chemical, Metuchen, New Jersey, USA), Spex Chemical Spike Solutions, Spex Chemical QC Solutions, a Dade Monitrol Chemistry Control (VWR Scientific Inc., San Franciso, California, USA) and an in-house bovine serum control. Spiked samples were included with each set of digested or diluted samples. The working standard mixtures were analyzed at the beginning and end of each run, and the calibration curve determined from the first set of standards as per manufacturers instructions (Thermo Jarrell Ash). The QC samples following the standards included Spike-1 samples, monitrol samples, in-house serum control, diluted QC-21 samples (10 and 1 ppm), and QC-11 samples. For each 10 samples a QC-21 and/or QC11 samples were run.

Values for QC samples should fall within 10% of the known or historic value, if not, the samples are reanalyzed. Tissue levels for moose from Fairbanks, Nome and Nowitna, Alaska (n = 14) were determined and used for comparison to the Colville River population and were analyzed by the Environmental Trace Substances Research Center (ETSR), University of Missouri (Columbia, Missouri, USA). In a 100 ml beaker, 15 ml of HNO_3 and 2.5 ml of concentrated HCl were added to 0.5 g of sample then gradually heated. Once fumes were no longer evident the sample was cooled and 2.0 ml of HCl was added and heated, then brought to 50 ml. As and Se were measured using a Varian VGA-76 hydride generation accessory on a Perkin-Elmer Model 603 AA using appropriate standards and the manufacturer's instructions. Other element levels were determined by ICP analyses using a Jarell-Ash Model 1100 Mark III. The instrument is standardized with a series of seven standards containing 36 elements. Quality control samples were used and standards were measured every 10 to 15 samples and if measures drifted more than 5% the instrument was recalibrated.

Ceruloplasmin (CP) analysis

Ceruloplasmin activity was assayed by oxidation of o-dianisidine after the method of Schosinsky et al. (1974). Blood serum (0.05 ml) oxidation rates were determined in duplicate as the difference in absorbance between 15 min and 25 min. Activity was expressed as the rate of oxidation ($umol \cdot min^{-1} \cdot L^{-1}$ or $IU \cdot L^{-1}$). This unit of activity is equivalent to 0.2836 $mg.dl^{-1}$ of ceruloplasmin in human serum (Schosinsky et al., 1974).

Pregnancy testing

Pregnancy-specific protein B (PSPB) was determined by Biotracking Inc. (Moscow, Idaho, USA) for these moose as described in O'Hara et al. (1998) with a competitive radioimmunoassay that measures serum PSPB by determining the amount of ^{125}I-PSPB bound. If less than 93% of the ^{125}I-PSPB binds (displaced by unlabeled PSPB in the serum), then unlabeled PSPB is present and consequently the diagnosis of pregnancy (Sasser et al., 1986). This technique has been used in other moose studies (Haigh et al., 1993; Stephenson et al., 1995).

Age determination

Age determination was conducted by a contract laboratory (Matson's Laboratory, Milltown, Montana, USA) using cementum analysis of the central (primary) incisor. Pairs of matched teeth were submitted for comparison and quality control. When estimates differed between matched teeth, the estimate with the highest score of reliability was used.

Statistics and calculations

Summary statistics, simple regression analysis, and student t-tests were conducted using Microsoft Excel (7.0) for Windows. Mineral contents of serum and hair in males, non-pregnant and pregnant females were compared by ANOVA (SYSTAT 8.0, SPSS, Chicago, Illinois, USA) following transformation to square roots to meet assumptions of normality and homogeneity of variances (Zar, 1974; Wilkinson and Coward, 1998). Pairwise comparisons of transformed means were performed with Bonferroni's adjustments. Interactions between untransformed mineral contents and CP activity were tested by Pearson correlations followed by Bonferroni adjustments for pairwise comparisons. Factor analysis of the correlation matrix was used to project two principal components from the rotated matrix (SYSTAT 8.0).

Means are calculated in two forms for untransformed data: the arithmetic mean for those matrices with all samples with detectable levels (>MDL) for an element, and half the MDL level is used for samples that are <MDL to calculate the mean. "Normal" values were converted to ppm wet weight (ww) using the following formulas. Whole blood "normal" values for moose are from Bubenik (1997) and were converted: P 4.9 (±1.4) mg/dl or mg/100 ml or 10 mg/kg (assume fluid density of 1); 49 (±14) ppm; Ca 10.4 (±1.0) mg/dl, 104 (±10) ppm; Fe 150.2 (±40.3) mg/dl, 1502 (±403) ppm; Na 137.8 (±6.2) meq/l = mmol/L/0.435 = 316.8 (±14.3) mg/100 ml or 3168 ± 143 ppm; K 5.3 ± 1.4 meq/l = mmol/L / 0.2258 = 104.0 ± 6.2 mg/100 ml or 1040 ± 62 ppm.

RESULTS

The Colville River moose population declined precipitously between 1992 and 1996. Annual spring counts indicated a decline from 510 to 149 moose (regression slope = 0, $P < 0.05$), and the number of short yearlings (calves surviving their first winter) declined from 17% to <1% (regression slope = 0, $P < 0.05$) of the population in a standard trend count area. Calf production and/or summer survival of calves was very poor during 1993, 1994, and 1995 as November (fall) calf compositions were 4% (of 397 moose), 2% (of

293 moose), and 0% (of 34 moose), respectively.

The population appeared to recover during the summer of 1996 as calves made up 22% (of 161) of the fall count. Date of birth was related to calf survival in 1996–97 as 9 of 12 (75%) calves born prior to May 28, 1996 were observed with collared cows in November 1996 as compared to two of 11 (18%) born after May 28, 1996 (χ^2, $P = 0.006$). The first increase in the spring trend count in six years occurred in 1997 when 180 moose included 23% short yearlings. The increase was due to the high calf survival and low adult mortality rates as only two of 50 collared moose died during 1996–97. Pregnancy rates were 82% (23 of 28) and 74% (17 of 23) in 1996 and 1997, repectively based on PSPB. Pregnancy was significantly related to the presence of a calf ($P < 0.05$) even after accounting for prior calving as a covariate ($P < 0.001$). Similarly, the presence of a calf corresponded with prior calving (89% binary similarity coefficient; $n = 27$).

The trend of population recovery continued in 1997 and 1998. During fall counts 21% (of 102) and 18% (of 159) of the moose were calves in 1997 and 1998, respectively. During the 1998 spring count, 206 moose were counted and 26% were short yearlings. The low adult mortality has continued as only three of the 50 collared moose have died during the 3 yr of the study.

Captured Colville River moose spring 1996 and 1997

All Colville River moose hair samples had detectable levels of Ba, Ca, Cu, K, Mg, Na, S, Sn, and Zn, and we report levels for Ca, Cu, Fe and Se (Table 1). Moose had low hair levels of Ca and Fe as compared to cattle, and low Cu as compared to moose (Table 1). Males had significantly higher concentrations of Ca ($P = 0.018$), and Cu ($P = 0.003$) in hair than females (Table 1). Mineral levels in serum and hair did not differ between cows with and without calves ($P > 0.05$) or between non-pregnant and pregnant cows ($P > 0.05$). Only one male moose exceeded the minimum hair Cu level (4.3 ppm) suggested by Flynn and Franzman (1987) (Table 1). Some hair samples had levels of elements less than the minimal detectable level (<MDL) including Al, B, Bi, Cd, Co, Cr, Fe, Mn, Ni, P, Pb, Sb, Se, Si, and Tl. Only Fe and Se mean mineral levels are compared to published deficiency standards (Table 1). No hair samples had detectable levels of Mo and V. All males and 41 (84%) of 49 females were deficient of Fe in hair. Two (33%) of 6 males, 40 (82%) of 49 females, 26 (67%) of 39 pregnant moose, and 8 (89%) of 9 non-pregnant cows were deficient of Se in hair.

All serum samples had detectable levels of Ca, Fe, K, Mg, Na, P, S, and Zn. Metals for which at least one serum sample had detectable levels include Al, B, Ba, Ca, Co, Cu, Li, Ni, Se, Si, Sn, and Tl; and only Cu is listed (Table 1). Using the 1.0 ppm serum Cu deficiency level threshold for moose as suggested by Flynn et al. (1977), all moose samples are deficient in Cu (Table 1). Elements not detected in serum are Ag, As, Be, Bi, Cd, Cr, Mn, Mo, Pb, Sb and V.

Elements detected in all blood samples were Ba, Ca, Fe, K, Mg, Na, P, and Se. Some animals had deficient levels of Se in blood but the proportion deficient varied depending on comparison to either cattle or deer criteria (Table 1; Puls, 1988). Selenium was not significantly lower in non-pregnant animals ($P > 0.05$). The mean Se levels for all groups (0.12–0.13 ppm) were below the suggested marginal level of 0.16 ppm for cattle (Table 1). Elements in blood that were detected in at least one sample are Al, As, B, Co, Cr, Cu, Ni, S, Sb, Se, Si, and Sn; and Cu is listed (Table 1). Copper was low in blood of all moose sampled based on the 0.55 ppm (Puls, 1988) and 1.0 ppm (Flynn et al., 1997) threshold levels. Ag, Be, Bi, Cd, Cu, Li, Mn, Mo, Pb, Tl and V were not detected in any moose blood samples.

Ceruloplasmin (CP) activity ranged

TABLE 1. Mean, count and standard deviation for elements (ppm ww) in male, female, pregnant, and non-pregnant captured moose in spring 1996 and 1997 and compared to published deficiency levels (< minimal level).

Matrix, element	Mean	Count	Standard deviation	% moose < minimum level[a]
Hair Calcium (Ca)				<18,500 ppm
Male	970.5[e]	6	206.0	100%
Female	599.7	49	291.8	100%
PSPB+[d]	607.6	39	311.6	100%
PSPB−	572.3	9	219.1	100%
Hair Copper (Cu)				<6.7 ppm (<4.3 ppm[b])
Male	3.94[e]	6	0.42	100% (83%)
Female	2.77	49	0.72	100% (100%)
PSPB+	2.76	39	0.76	100% (100%)
PSPB−	2.75	9	0.58	100% (100%)
Hair Iron (Fe)[c]				<59 ppm
Male	36.9	6	13.0	100%
Female	38.2	48	22.7	83%
PSPB+[d]	38.4	39	23.5	85%
PSPB−	36.8	8	20.8	75%
Hair Selenium (Se)[c]				<0.5 ppm
Male	0.9	5	0.38	20%
Female	0.50	23	0.20	61%
PSPB+	0.50	17	0.17	43%
PSPB−	0.55	5	0.29	80%
Serum Zinc (Zn)				<0.8 ppm
Male	0.96	6	0.13	0%
Female	0.89	49	0.18	(33%)
PSPB+	0.88	40	0.18	(35%)
PSPB−	0.92	9	0.16	(22%)
Serum Copper (Cu)[c]				<0.8 ppm
Male	0.34	6	0.30	(83%)
Female	0.29	43	0.12	(98%)
PSPB+	0.29	34	0.13	(97%)
PSPB−	0.29	9	0.05	(100%)
Blood Selenium (Se)				<0.16 ppm cattle (<0.1 deer)
Female	0.12	22	0.03	86% (14%)
PSPB+	0.13	18	0.03	83% (6%)
PSPB−	0.12	4	0.03	100% (50%)
Blood Copper (Cu)[c]				<0.55 ppm (1.0[b])
Female	0.30	5	0.04	100%
PSPB+	0.29	4	0.05	100%
PSPB−	0.32	1	—	100%

[a] Puls (1988) minimum levels for cattle hair (ppm).
[b] Copper deficiency level described in Flynn and Franzman, 1987; and Flynn et al., 1977.
[c] At least one sample was < MDL (minimum detection limit), mean calculated using 0.5 (MDL) for samples < MDL.
[d] PSPB, pregnancy status as determined by measuring pregnancy serum protein B (+ is pregnant, − is not).
[e] Significantly different ($P < 0.05$) by ANOVA for that matrix and element by gender (male > female).
Note: one female did not have serum sampled for pregnancy test but hair was sampled.

TABLE 2. Mean hair and tissue element levels in found dead and hunter-killed Colville River moose compared to other Alaskan moose (OAM).

Matrix	Mean	SD[a]	N	OAM (n = 13)	*P* value[b]	Marginal levels[c,d]
HAIR						
Copper	2.87	1.08	7	3.24	0.082	<6.7 ppm (<4.3[d])
Zinc	59.1	22.8	7	87.1	0.0003	<50 ppm
Iron	435.6	432.1	7	52.3	0.013	<59 ppm
Age (hair)	5.2	2.17	5	4.0 (±3.7)	—	—
KIDNEY						
Cadmium	21.6	20.8	9	73.1	0.02	—
Copper	3.72	0.60	9	15.1	<0.0001	<10–25 or 20[d]
Zinc	43.0	26.5	9	117.2	<0.0001	<20–40
Iron	37.9	7.59	9	280.2	<0.0001	<30
LIVER						
Cadmium	3.13	2.5	9	11.89	0.026	—
Copper	9.80	12.66	9	103.9	0.003	<10–25 or 20[d]
Zinc	66.53	58.13	9	73.29	0.351	<20–40
Iron	120.53	51.81	9	436.00	<0.0001	<30
Molybdenum	1.54	0.09	9	3.25	0.0001	—
MUSCLE						
Copper	1.78	1.69	7	5.53	<0.0001	<1.2
Zinc	46.67	22.95	7	183.3	<0.0001	—
Iron	33.88	3.92	7	119.8	<0.0001	<45
Age (tissues)	6	2.73	8	4.0 (±3.7)	—	—

[a] Standard deviation.
[b] *P*-value for t-test of FD and HK moose versus OAM.
[c] Puls, 1988 minimal marginal level.
[d] Moose marginal level from Flynn and Franzman (1987).

from 0–10 IU and averaged 3.7 IU in 53 captured female moose. For six moose with serum Cu < MDL the average ceruloplasmin (CP) was 1.0, and for the 47 with Cu > MDL the average was 4.0 and were significantly different ($P = 0.003$). Females that were not pregnant (5.67 IU, $n = 9$) had significantly ($P = 0.009$) higher CP levels than those that were pregnant (3.24 IU, $n = 38$).

The correlation matrix produced two principal component factors that accounted for 52% of the variance in CP and the mineral contents of hair and serum (Fig. 1). Minerals associated with protein-bound systems (S, Cu and Zn) in serum and hair contributed most of the variance in the first principal component (Factor 1). This factor included positive associations between serum CP and Cu in serum and hair even though coefficients of pairwise correlation were not significant between serum CP and any minerals in serum or hair including Cu, Zn and Fe. Minerals associated with exchangeable ions (Ca, P and Na) in serum were distributed in the opposite direction and thus negatively related to Cu and Zn in Factor 1 (Fig. 1). Negative relationships between S and Fe were reflected in their opposite distribution in Factor 1 and in significant ($P < 0.05$) pairwise correlation coefficients between Fe and S in serum (−0.580), and serum Fe and S in hair (−0.549). Extracellular ions such as Na, K and Mg deposited in hair were clustered along the second factor (Fig. 1).

Found dead (FD) and hunter-killed (HK) moose in 1995

Mean Cu level in FD and HK moose hair was 2.87 ppm, with all samples below the threshold level previously described for hair of moose (4.3 ppm) (Table 2). The

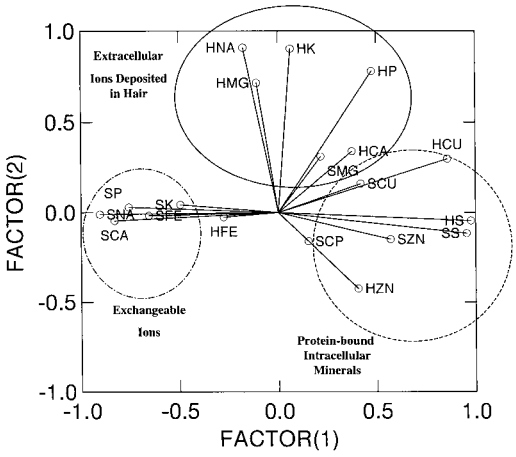

FIGURE 1. Principal component analysis of minerals in hair and serum of moose captured from the Colville River. Factors 1 and 2 account for 35% and 18%, respectively, of the variance in the data set. Circles are drawn around three groups of variables with related functions: protein-bound minerals, exchangeable ions, and extracellular ions deposited in hair. Key: The first letter indicates the tissue - hair (H) or serum (S); subsequent letters designate the element (Ca, Cu, Fe, K, Na, Mg, P, S, Zn) or ceruloplasmin activity (CP).

level was not significantly different (P = 0.082) as compared to the moose from other locations (Table 2). Linear regression analysis indicated a significant correlation for Cu versus S levels (y = 4253.8 (S/Cu)x − 726.8, r^2 = 0.814; P = 0.005); and Cu versus Zn levels (y = 18.7 (Zn/Cu)x − 5.3, r^2 = 0.782; P = 0.008) in hair. Element concentrations in liver, kidney, and muscle are listed for FD and HK moose and compared to moose from other locations in Alaska (Table 2). For muscle; mean levels of Cu, Zn, and Fe were significantly ($P < 0.05$) higher for other Alaska moose compared to the Colville River group population (FD and HK) (Table 2). The same comparison indicated significantly greater Cu (by a factor of 4.1), Cd, Zn, and Fe levels in kidney; and increased Cd, Cu (by a factor of 10.6), Fe, and Mo levels in liver of other Alaska moose as compared to the Colville River moose (Table 2). Using 10 ppm as the threshold for Cu deficiency, 5 of 6 HK moose liver and all HK moose kidney levels were deficient. All FD moose (3 of 3) were Cu deficient in liver and kidney. For muscle, two of four HK moose and two of three FD had low levels, <1.2 ppm of Cu. The mean Fe level was low in muscle (<45 ppm) for these moose (Table 2). Copper in FD moose hair (1.51 ppm) (Table 3) and hoof material (1.25 ppm) were significantly lower ($P < 0.05$) in Cu than HK (3.41 ppm), other Alaska moose (3.24 ppm), and spring (captured moose) hair (3.94 ppm) (Table 3) samples, but the sample size is small for HK and FD moose. All populations studied had average levels of Cu lower than the marginal level of 5.0 ppm (Flynn and Franzmann, 1987) for hair.

Comparisons were made for the various matrices using linear regression analyses in this study. Simple ratios (i.e., Cu:Zn) are used to express some of the relationships

TABLE 3. A comparison of mean (standard deviation) Cu and Zn levels (ppm), and Cu : Zn in liver and keratinized tissues (hair and hoof) for hunter-killed (HK), found dead (FD) and captured Colville River moose; and other Alaska moose (OAM).

	Cu ppm mean	Zn ppm mean	Cu : Zn mean
FD and HK liver (n = 9)	9.80 (12.66)	66.52 (58.13)	0.20 (0.20)
OAM liver (n = 14)	103.9 (84.0)	73.29 (24.64)	1.56 (1.22)
HK moose hair (n = 5)	3.41 (0.59)	72.00 (6.09)	0.048 (0.008)
FD hair (n = 3)	1.51 (0.59)[b]	26.73 (6.68)[b]	0.055 (0.061)
OAM hair	3.24 (0.76)	87.08 (3.50)	0.037 (0.009)
Spring 96 & 97 hair[a]	2.77 (0.72)	71.69 (15.81)	0.040 (0.014)
FD hoof (n = 4)	1.25 (0.46)	93.94 (7.29)	0.014 (0.006)

[a] Females only, n = 49.
[b] Significantly different from other hair samples ($P < 0.05$) ANOVA.

TABLE 4. Minerals analyses (ppm ww) of rumen contents of hunter-killed (HK) and found dead (FD) moose from the Colville River, Alaska.

	Mean	SD[a]	N[b]	Dietary minimal requirement[c]
Cadmium	1.71	0.84	8	—
Copper	7.93	2.89	8	10 ppm dw (5.72[d])
Zinc	220.9	65.00	8	45 ppm dw
Iron	404.9	529.3	8	<40 ppm dw
Calcium	15,142.8 (1.5%)	22,818.2	8	<0.30%
Cobalt	2.04	1.74	8	<0.07 ppm
Phosphorous	14,138.1 (1.4%)	2,973.7	8	<0.30%
Sulfur	2,146.5 (0.21%)	666.6	8	<0.1%
Cu/Zn	0.04	0.01	8	—

[a] Standard deviation
[b] Number analyzed.
[3] Puls, 1988 minimal dietary level, cattle.
[d] Flynn and Franzman (1987) for moose.

(Table 3). Linear regression analyses of Cu in hair versus Cu in serum for moose captured spring 1996 and 1997 was not significant (F = 0.19, $P = 0.66$). The Cu:Zn is lowest for the FD hoof as compared to hair for other groups (Table 3). The Zn and Cu levels were significantly ($P < 0.05$) lower for FD moose hair as compared to other hair samples. Kidney Zn and Cu levels positively correlated (y = 36.1 Zn/Cux − 91.1, $r^2 = 0.66$, $P = 0.008$) for HK and FD moose. The Cu:Zn ratio for HK and FD moose hair is not significantly different ($P > 0.05$) from the spring captured moose (Table 3). The mean Cu:Zn for liver is 1.56 and 0.20 for other Alaska moose, and the FD and HK moose, respectively, and significantly different ($P = 0.004$) (Table 3).

Rumen contents of HK and FD moose had Cu levels below the dietary cutoff of 10 ppm (dry weight) (Table 4). Ideally browse and forage would be sampled to make this assessment. Sulfur levels increased with increasing Cu concentrations (y = 143.1 (S/Cu) x + 1011.3. $r^2 = 0.38$, $P < 0.001$) in rumen contents.

Cadmium in kidney, but not liver, increased with age for Colville River moose based on linear regression for kidney ($P = 0.049$, $r^2 = 0.50$) and liver ($P = 0.12$, $r^2 = 0.35$) (Table 5). Cadmium in New Hampshire moose showed a similar age relationship for both liver ($P < 0.001$; $r^2 = 0.40$) and kidney ($P < 0.001$; $r^2 = 0.70$) (Table 5). Regression analyses indicated that Cd levels in other Alaska moose significantly increased with age for kidney ($P = 0.023$, $r^2 = 0.96$) and liver ($P = 0.009$, $r^2 = 0.93$). Based on the rates of Cd accumulation 95% confidence interval Alaska moose from Nome, Nowitna and Fairbanks ap-

TABLE 5. A comparison of cadmium accumulation rates based on linear regression analyses for liver and kidney for three groups of moose from Colville River, other Alaska moose (OAM), and New Hampshire.

Population	Slope (ppm/year)[a]	95% C.I.[a]	P value[b]	r^2
Colville River kidney	5.6	0.04–11.18	0.049	0.50
Colville River liver	0.55	−0.20–1.31	0.12	0.35
New Hampshire kidney	3.6	2.86–4.24	<0.001	0.70
New Hampshire liver	0.50	0.34–0.66	<0.001	0.40
OAM kidney	23.7	7.99–39.32	0.02	0.96
OAM liver	3.9	1.90–5.99	0.01	0.93

[a] Determined from estimate of coefficient (b1) of linear regression analyses, C.I. = confidence interval.
[b] P value that coefficient is different from 0.

pear to accumulate Cd at a faster rate than those sampled from the Colville River and New Hampshire populations (Table 5). The sample size is small for each group, thus complicating the assessment.

DISCUSSION

Mineral and heavy metal analyses were conducted on hair, hoof, rumen contents, liver, muscle and kidney in Alaska moose found dead (FD) the summer of 1995 and hunter-killed (HK) the fall of 1995. The initial assessment of FD and HK moose was limited to gross evaluation (macroscopic lesions, body condition), mineral and heavy metal analyses, and histopathology (HK only). Evidence of potential disease partly due to a mineral deficiency is based upon poor recruitment, adult mortality (30–50 animals) in 1995, unconfirmed reports of hoof lesions (hoof overgrowth), and "low" levels of Cu in liver and kidney of moose sampled in 1995. A suspected Cu deficiency is based on low levels in many tissues of moose compared to "normal" levels and to a documented mineral (Cu) deficiency occurring previously in Alaska moose (Flynn and Franzmann, 1987). Hair analysis for these elements in moose from the Kenai Peninsula, Alaska indicated potential Cu deficiency and abnormal hoof growth (keratinization) and decreased reproductive rates correlated with the Cu deficiency (Flynn et al., 1977; Flynn and Franzmann 1987). Other potential factors (i.e., viral, bacterial, predator, parasitic) were assessed in the Colville River moose, including brucellosis (O'Hara et al., 1998; Edmonds et al., 1999). We cannot conclude that a lack of Cu is the primary cause of the population decline or death, and it may actually be a small factor or a consequence of the primary cause. Despite this, the low level of Cu indicated in samples (hair, serum, and blood) of captured moose in 1996 and 1997 could predispose the moose to poor reproductive performance, immunosuppression, anemia, and other ailments that would compromise this population (Flynn and Franzmann, 1987; Puls, 1988). This is important in that subclinical disease (deficiency) may be the primary reason for poor health, but other opportunistic factors result in mortality. Currently, there is a lack of gross and histopathologic evidence to support outright clinical Cu deficiency in this population. At this point, sampling has not been adequate to make such a determination and is mostly due to the remoteness of this population.

Effects in cervids due to Cu deficiency at levels similar to those seen in moose from this Colville River population have been documented. In Texas (USA) white-tailed deer (*Odocoileus virginianus*) with stunted and twisted antlers had mean liver Cu levels of 16.7 ppm ww. Soil and vegetation were documented with low Cu content. Tule elk (*Cervus elaphus nannodes*) were reintroduced to Point Reyes (California, USA) in 1978 and showed gross signs of Cu deficiency in 1979 including antler anomalies, fractures, altered hair coats, and pica. Low levels of Cu, high levels of Mo, and overgrazing by livestock contributed to this problem (Gogan et al., 1989). Mean liver Cu levels of 5.9 ppm and serum of 0.42 ppm for elk from Point Reyes were low compared to tule elk from other areas with mean liver Cu level of 80 ppm and serum of 1.4 ppm (Gogan et al., 1989). An outbreak of paratuberculosis was concurrent with this mineral deficiency and may have affected Cu uptake (Gogan et al., 1989). Moose from the Colville River population had similarly low levels of Cu. The levels of Cu in serum of the Colville River moose in this study were deficient. Serum Cu levels considered deficient are <1.0 ppm in moose (Flynn et al., 1997) and all moose tested in the Colville River area were below the 1.0 ppm level and mean levels (range of means 0.29–0.34 ppm) for the cohorts examined were below the 0.5 ppm threshold.

In elk, demands of fetal development in the third trimester of pregnancy and periods of antler growth enhance the requirement for Cu. Copper requirements

increase by 70% for cattle beginning the third trimester and deficiencies during peak demand may result in poor reproductive performance culminating in enhanced spring mortality (Puls, 1988; Gogan et al., 1989). This same scenario may have been occurring for the moose population along the Colville River as pregnant moose showed significantly lower CP than non-pregnant moose indicating the possible increased demand of Cu during fetal development. Bovine calves are born with Cu stores in the liver and the fetus accumulates Cu at the expense of the dam (Puls 1988; Underwood 1977). The calf may exhibit inability to suckle, incoordination, stiff gait, or opisthotonus when Cu is limited; if this occurs it will likely result in death. Periparturient Cu utilization indicates that the cow and fetus could be severely affected if this essential element is lacking. However, the high correspondence between pregnancy, the presence of a calf and a history of calving suggests that poor reproductive performance in some females may be due to genetics and habitat quality in addition to nutrition.

Element interactions must be considered for these Brooks Range moose as the Cu levels were low, the Mo levels are high "normal," and the S (also a Cu antagonist) may precipitate a relative imbalance among these elements. A close examination of browse would determine if such an interaction is occurring as well as address other nutritional factors (i.e., fiber, moisture, other minerals, digestibility, protein content). The formation of insoluble inorganic or organic complexes decrease the bioavailability (absorption) of Cu and Mo, but not the actual detectable levels. Molybdenum and S are synergistic in reducing Cu availability (Puls, 1988). High dietary protein intake reduces Cu absorption while inadequate energy intake may enhance an underlying Cu deficiency. The found dead moose in our study were in poor body condition that may have exacerbated the ongoing Cu deficiency. Mean level of Cu in FD and HK moose rumen contents was 7.93 ppm. This is slightly higher than reported in browse (mean 5.72 ppm) by Flynn et al. (1977), which they considered marginal as a source of Cu and susceptible to complexation by Mo and S thus reducing bioavailability of an already marginal source. Although S exceeds the requirement for beef cattle at 0.15%, Mo is not excessive at 0–0.2 ppm and well below the level of Cu (8 ppm). Therefore thiomolybdates are possible but unlikely to be the major problem. Forage would decline in digestibility as fiber content of the diet increases during winter and may have an impact on reducing availability of the low levels of Cu in the diet (Van Soest 1994).

The diet may be "marginal" in Cu and thus any additional stressor(s) could result in the observed adult mortality and poor calf production and includes climate (prolonged winter conditions, later "green up"), intra- and interspecific competition (recent increase in snowshoe hare (*Lepus americanus*) population and highest moose population level observed prior to event; O'Hara et al., 1998), infectious disease interactions (similar to paratuberculosis and Cu in Tule elk; Gogan, 1989), ectoparasite harassment, poor caloric diet, and interactions of the above and others. We collected no data during or prior to the event related to the above and thus a retrospective analyses was not possible.

Ceruloplasmin, also known as ferroxidase I, is well known for binding Cu and is involved in Fe metabolism (Owen 1982). Puls et al. (1988) described serum ceruloplasmin levels in cattle as deficient, marginal, and adequate at 0–81, 91–122, and 132–254 $IU \cdot L^{-1}$, respectively; sheep were considered deficient below 20 $IU \cdot L^{-1}$ and adequate at 50–70 $IU \cdot L^{-1}$. This suggests a response range of 300% within each species. Samples from the Moose Research Center in Alaska averaged monthly CP levels ranging from about 4.1 to 81.2 $IU \cdot L^{-1}$ throughout the year and averaged 59.2 $IU \cdot L^{-1}$ for the year (Flynn et al., 1977). Ceruloplasmin activity ranged from

0–10 IU·L^{-1} and averaged 3.7 IU·L^{-1} in the 53 moose examined from the Colville River and was significantly lower for the <0.2 ppm serum Cu group.

Geographical and seasonal variations in minerals in moose are well known (Flynn and Franzmann 1987; Bubenik, 1997). Hair represents a matrix that is easily sampled and reflects dietary levels of minerals in moose (Flynn and Franzmann, 1987) especially during the period of molt in late summer and fall (Bubenik, 1997). The uptake and retention of mineral elements in moose hair are consistent enough to allow sequential analysis to describe prior mineralization (Flynn et al., 1975). Seasonal fluctuations of elements in moose may be due to dietary, environmental, and hormonal changes. Sexual differences in Cu content of hair may reflect differences in habitat use and diet selection underlying the seasonal cycle of food intake and productive demands on males and females (Miquelle et al., 1992; Schwartz and Renecker, 1997). Elevated Ca in hair of males compared with females may be the result of hormonal changes during antler growth and the coincident growth of hair follicles during late summer and fall (Bubenik, 1997). Serum and hair Cu reflect dietary intake when liver levels are <20 ppm ww (Puls, 1988), as has been shown for the majority of moose in this population. Serum levels of Cu are reasonably reliable (liver is ideal) as an indicator of Cu status and were consistently low in moose from the Colville River drainage. Hair proved to be a valuable matrix and we suggest more field studies include routine sampling and archiving of hair. However, moose in Alaska outside the Colville River area, with apparently adequate liver Cu levels, had levels of Cu in hair below the critical level of 4.3 ppm. Serum and hair Cu levels were associated with S and Zn in serum and hair (Fig. 1, Factor 1) but Fe was negatively associated with these minerals. This finding is unexpected because Fe transport from hepatic reserves is dependent on proteins containing Cu (Owen, 1982) and should therefore correlate positively with Cu status. However, total serum Fe may not reflect the saturation of its carrier transferrin. Similarly, the amount of other metaloproteins such as metalothionein (a participant in Cu and Zn metabolism) is unknown. This emphasizes the importance of multiple measures from hepatic, blood (serum), and other tissue samples in addition to hair to document suspected mineral deficiencies.

In our study, the mean Cu level in hair of FD moose was half (1.51 ppm) of that measured in HK moose (3.41 ppm), with both below levels considered marginally deficient for moose (4.3 ppm, Flynn and Franzmann, 1987; and 5.0 ppm Frank et al., 1994). The hoof mean Cu level in FD moose was 1.25 ppm and considered low. Abnormal hooves had 3.24 and 146.6, and normal hooves had 5.3 and 232.9 ppm of Cu and S, respectively as reported by Flynn et al. (1977).

Other minerals must be considered that are known to interact and either enhance or alleviate an ongoing Cu deficiency or act independently. For example, Se deficiency in black-tailed deer (*Odocoileus hemionus columbianus*) in California as described by Flueck (1991, 1994). Selenium is commonly deficient during an ongoing Cu deficiency and the Se status of moose in this study is marginal based on whole blood analysis as compared to cattle (0.16 ppm, Puls, 1988) and deficient based on hair (0.5 ppm, Puls, 1988). In our study, Fe was low in muscle and hair for most animals, but considered normal in liver, kidney, blood, and serum. Summer and fall Ca levels were higher than winter and spring for all age classes (Flynn and Franzmann, 1987). The Ca requirement includes annual bone development for antler growth, fetal development, and lactation costs. In Colville River moose, both hair and serum Ca were low, but hair Ca levels did not parallel serum levels. However, serum Ca level is dependent upon the analytical technique used. Hair and tissue levels do not correlate with dietary intake for

Ca and thus decrease our ability to extrapolate to a systemic status. Hair and serum may be useful matrices for determining Zn status, as tissue levels are not adequate for bovine. Zinc was low in serum for some of the female moose (<0.8 ppm), but mean levels were higher, and FD and HK moose had significantly lower Zn than OAM in tissues.

Cadmium accumulates in kidney and liver of ungulates. We compared organ accumulation in the Colville River population to others in Alaska and North America. The moose studied in the Brooks Range showed a significant age-dependent increase of Cd in kidney, while other Alaska and New Hampshire moose showed significant increases in Cd with age in both liver and kidney with possible regional differences. This indicates the wide range of Cd levels and the difficulty in comparing animals from different regions and age classes. Increased Cd burden can perturb Cu balance, resulting in a Cu deficiency.

The Colville River area, Alaska moose population has suffered from poor calf survival or recruitment, and adult mortality for which Cu has been shown to be low in multiple matrices of adults. In addition, Ca was low in hair, Se was low in hair and blood, and others were low in other samples concurrent with the Cu deficiency, which can be expected. Much evidence indicates a Cu deficiency is occurring in these moose but determining the actual impact on population performance remains elusive.

ACKNOWLEDGEMENTS

The authors thank C. Hanns, K. Burek, L. Ayres and R. Zarnke for assistance with sampling of moose. The North Slope Borough Department of Search and Rescue provided helicopter support for the examination of carcasses and transport of supplies and personnel to the field. Ray Smith of Umiat Enterprises, Inc. assisted with carcass examination. Financial support for this study was provided by the Alaska Department of Fish and Game (ADFG) and the Department of Wildlife Management of the North Slope Borough (Alaska).

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Received for publication 19 November 1999.