MONITORING MOOSE MINERAL METABOLISM VIA HAIR ELEMENT ANALYSIS. [1]

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Abstract: Three hundred seventeen Alaskan moose (Alces alces gigas) hair samples were analyzed for 10 elements by atomic absorption spectroscopy between May, 1972 and May, 1973. Results demonstrated seasonal variation associated with general moose condition. Peak levels occurred in the fall and the low levels occurred during late winter and early spring. Some element levels were lower than domestic animal lower limits of "normal values" over extended periods of time. Moose from different geographical locations demonstrated significant differences with certain elements, suggesting geochemical or range differences. Significant differences in three elements (magnesium, copper and manganese) were noted between winter-killed calves and live moose from the same area. These results indicated the potential of using the hair sampling

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technique to monitor mineral element metabolism in the moose. Based upon this, studies at the Kenai Moose Research Center (MRC) have been broadened to: 1. analyze additional elements, 2. collect moose hair from more diverse areas, 3. analyze plants and soils for the same elements, 4. determine the lag-time from ingestion to deposition of elements in the hair, and 5. determine the role of mineral deficiency as a factor in the etiology of the hoof overgrowth syndrome on the Kenai Peninsula. This paper reports our findings to date relative to these projects.

The application of the indicator species concept (Franzmann 1971) with physiologic values obtained from wildlife populations has as many facets as there are values to measure. Most physiologic values share a common asset in that they are easily obtained and do not require sacrificing the animal. Many values, particularly those obtained from blood, are subject to sources of variation which confound interpretation (Franzmann 1972, Seal et al. 1972, and LeResche et al. 1973). Tissues that would not be subject to sources of variation related to daily activity, capture and short term handling would be useful if they reflected changes in the environment.

Researchers in human and animal medicine have investigated hair sampling and analysis from several aspects. Some have studied protein metabolism (Bradfield 1968, Bradfield et al. 1967, Bradfield and Jelliffe 1970, Crounse et al. 1970, Godwin 1959, Lowry et al. 1951 and Sims 1968) whereas others have investigated mineral metabolism (Klevay 1970a, Klevay 1970b, Schroeder and Nason 1969, Strain et al. 1966, and Strain et al. 1972b). The utilization of hair analysis to monitor

systemic exposure to radioisotopes and toxic elements has also been demonstrated (Hammer et al. 1971, Kopito et al. 1967, Strain et al. 1965, and Strain et al. 1972a). When we recognized the potential of hair as a stable material to use as a physiologic monitor of moose, hair sampling and analysis were initiated at the Kenai Moose Research Center (MRC). We thank the Department of Surgery, Cleveland Metropolitan General Hospital, Case Western Reserve University School of Medicine, Cleveland, Ohio for providing analyses of hair samples. We appreciate the help from J.W. Coady who assisted by collecting hair samples from the Alaska Range. Other Alaska Department of Fish and Game personnel are collecting samples for future analysis and we appreciate their help. The MRC is a cooperative project between the Alaska Department of Fish and Game and the U.S. Fish and Wildlife Service, Kenai National Moose Range.

PAST STUDIES ON MOOSE HAIR AT MRC

The procedure for collecting and analyzing moose hair was outlined by Franzmann and Arneson (1973). Essentially, a hair bundle approximately 2cm in diameter was plucked from the shoulder hump of the moose. The samples were analyzed by atomic absorption spectroscopy for 10 elements [calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), and zinc (Zn)].

Results of the initial year of sampling (May 1972 to May 1973) were reported by Franzmann and Arneson (1973) and Franzmann et al. (1974). This study, based upon samples from 317 moose, demonstrated seasonal variation, in all elements except iron, associated with general moose condition. Peak levels occurred in the fall and low levels occurred during late winter and early spring. Some element levels were

lower than domestic animal lower limits of "normal values" over extended periods of time. Moose from different geographical locations demonstrated significant differences with certain elements, suggesting geochemical or range differences. Significant differences in three elements (magnesium, copper and manganese) were noted between winter-killed calves and live moose from the same area.

The results indicated the potential of using the hair sampling technique to monitor mineral element metabolism in the moose. Based upon this, we have broadened our studies in several ways. We have added additional elements for analysis, collected from more diverse areas, collected browse and will collect soil for analysis of the same elements, set up a study to determine the lag-time from ingestion to deposition of elements in the hair, and tentatively established that the hoof overgrowth syndrome, which occurs sporadically on the Kenai Peninsula, is a copper deficiency disease.

Unfortunately, we have not completed all of these projects, but there are sufficient data from several to warrant reporting.

PRESENT STUDIES ON MOOSE HAIR AT MRC

Additional Elements Analyzed

Cobalt (Co) was added to our list of elements analyzed and results since May 1973 are listed in Table 1. The sample size is not adequate, nor is the distribution over the seasons sufficient at this time to warrant discussion.

All hair samples collected to date have been retained and we are presently preparing to add aluminum (Al), arsenic (As), beryllium (Be), chromium (Cr), mercury (Hg), molybdenum (Mo), nickel (Ni), phosphorus (P), and selenium (Se) to our analysis of these and future samples.

Table 1. Alaskan moose hair cobalt values, 1973.

Month Sampled	Location	Sample Size	Cobalt (ppm) Mean	S.D.
June	MRC[1]	1	2.8	
July	MRC	2	2.8	
August	MRC	15	2.4	0.8
September	MRC	20	2.5	0.8
October	MRC	20	2.9	0.4
October	Alaska Range	21	3.4	0.8

1 MRC - Kenai Moose Research Center

Hair Analysis Since May 1973

Since the initial study we have completed analyses of 191 additional moose through October 1973 (Tables 2 and 3). Comparing these results with those of the previous year (Franzmann et al. 1974) on a monthly basis we observed, in general, similar trends (Figs. 1,2, and 3). Calcium, potassium, manganese, sodium and cadmium values peaked the same month each year (calcium in August, potassium, manganese, sodium and cadmium in October). Copper values in 1973 peaked in September and magnesium peaked in August. In 1972 both peaked in October. Iron values in 1973 had no particular trend as noted in 1972. Lead values in 1973 peaked in August not in November as in 1972. Zinc levels did not follow a general year to year pattern. The highest 1973 zinc values were in June, whereas the 1972 values peaked in October.

Elements were tested (.005 level) on a monthly basis for significant differences between years. Zinc and copper were significantly different in both June and July comparisons with the 1973 values being the greater. All other June and July comparisons were not significantly different. The August comparisons indicated significant differences between years for copper, manganese, calcium and sodium. The September comparisons indicated significant differences between years for calcium, sodium and iron. The October comparisons indicated a significant difference between years for magnesium only.

The greater number of values demonstrating significant differences through August and September are likely due to this being the period of most rapid increase in these values. Following this dynamic period the values stabilized at their peaks in October and only magnesium was significantly different from the previous year.

Table 2. Mean and standard deviation of monthly hair mineral macro element values from Alaskan moose, 1973.

Month Sampled	Location	Sample Size	Elements (ppm)			
			Ca	Mg	K	Na
June	MRC[1], Kenai Peninsula	23	257.2±262.5	36.4±30.0	1060.9±535.7	680.8±308.4
July	MRC, Kenai Peninsula	20	270.1±206.7	55.3±52.4	949.0±511.4	764.1±161.4
Aug.	MRC, Kenai Peninsula	15	361.1±158.1	191.8±65.2	1669.9±611.8	1037.2±170.9
Sept.	MRC, Kenai Peninsula	20	283.0±126.1	166.2±45.8	1653.1±343.4	1025.4±230.7
Oct.	MRC, Kenai Peninsula	20	137.0± 48.6	150.5±31.8	1759.2±185.4	1126.0±148.4
Oct.	Alaska Range	21	129.6± 37.6	102.5±34.3	854.9±324.0	1374.6±381.4

1. MRC - Kenai Moose Research Center

Table 3. Mean and standard deviation of monthly hair mineral micro element values from Alaskan moose, 1973.

Month Sampled	Location	Sample Size	Elements (ppm)					
			Cd	Cu	Fe	Pb	Mn	Zn
June	MRC[1], Kenai Peninsula	23	0.6±1.4	7.4±3.7	34.8±33.8	5.5±5.0	0.1±0.4	81.5±21.5
July	MRC, Kenai Peninsula	20	0.3±0.3	7.3±3.2	67.1±75.4	6.9±7.2	0.3±0.6	72.7±15.4
Aug.	MRC, Kenai Peninsula	15	0.9±0.4	11.2±2.4	51.2±15.9	17.6±1.1	1.3±0.4	67.1± 7.1
Sept.	MRC, Kenai Peninsula	20	0.3±0.3	7.3±3.2	62.7±3.4	15.7±7.7	0.9±0.4	68.1± 5.9
Oct.	MRC, Kenai Peninsula	20	1.4±0.4	10.6±3.7	67.3±4.6	11.4±3.4	4.0±3.6	63.0± 6.5
Oct.	Alaska Range	21	1.2±0.8	13.0±3.5	68.0±7.0	14.8±5.8	10.3±4.3	60.1± 6.6

1. MRC - Kenai Moose Research Center

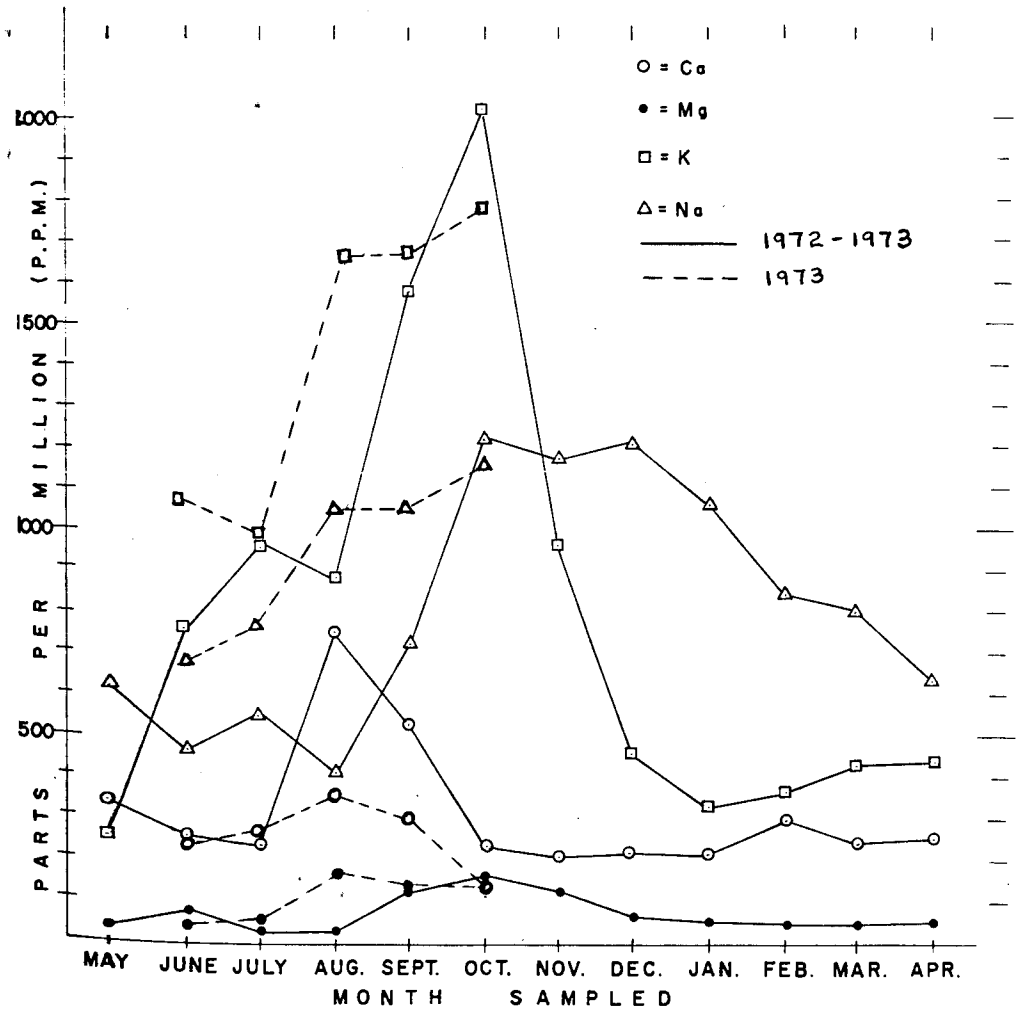

Fig. 1. Monthly means of Kenai Peninsula moose hair Ca, Mg, K, and Na levels, May 1972 to November 1973.

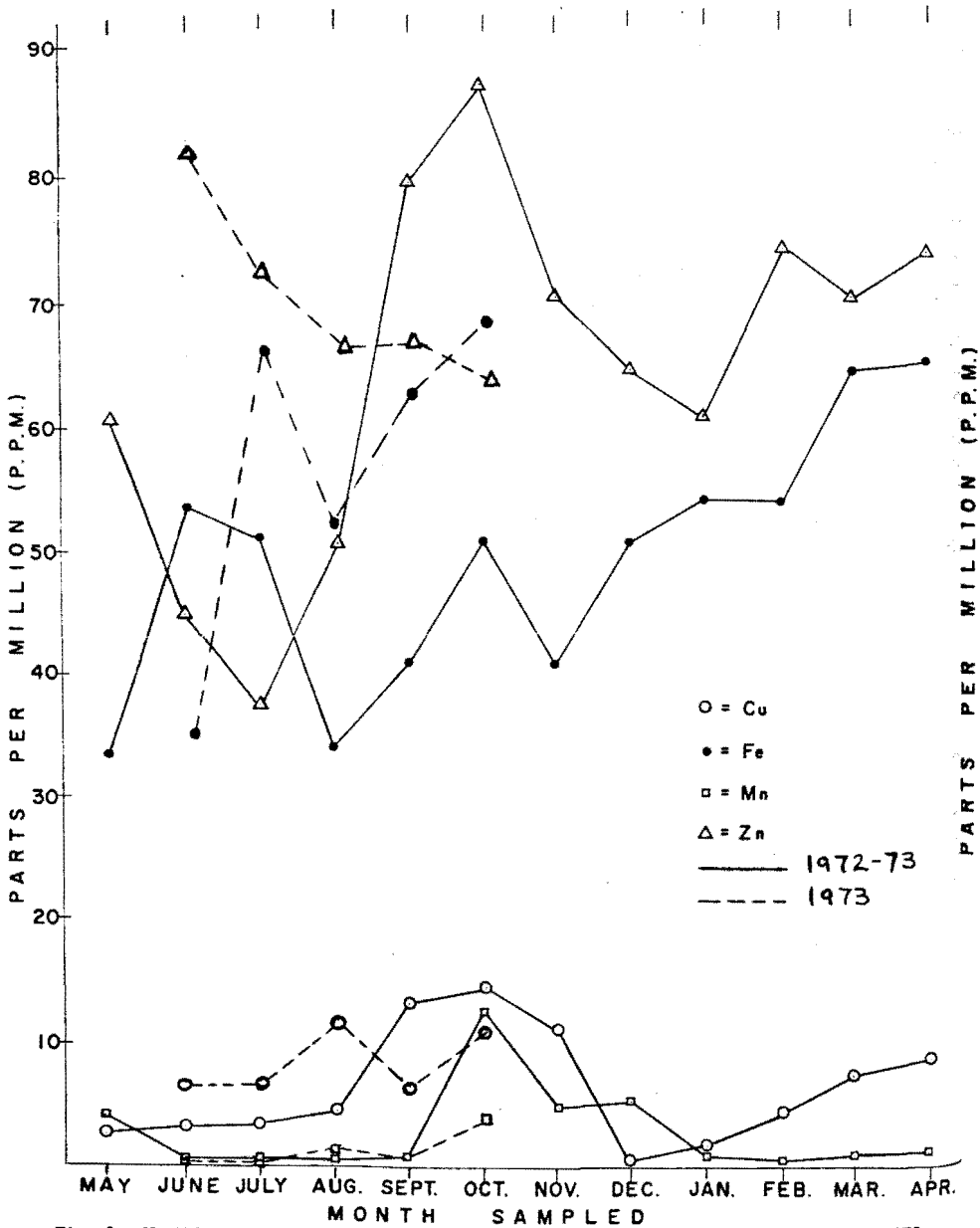

Fig. 2. Monthly means of Kenai Peninsula moose hair Cu, Fe, Mn, and An levels, May 1972 to November 1973.

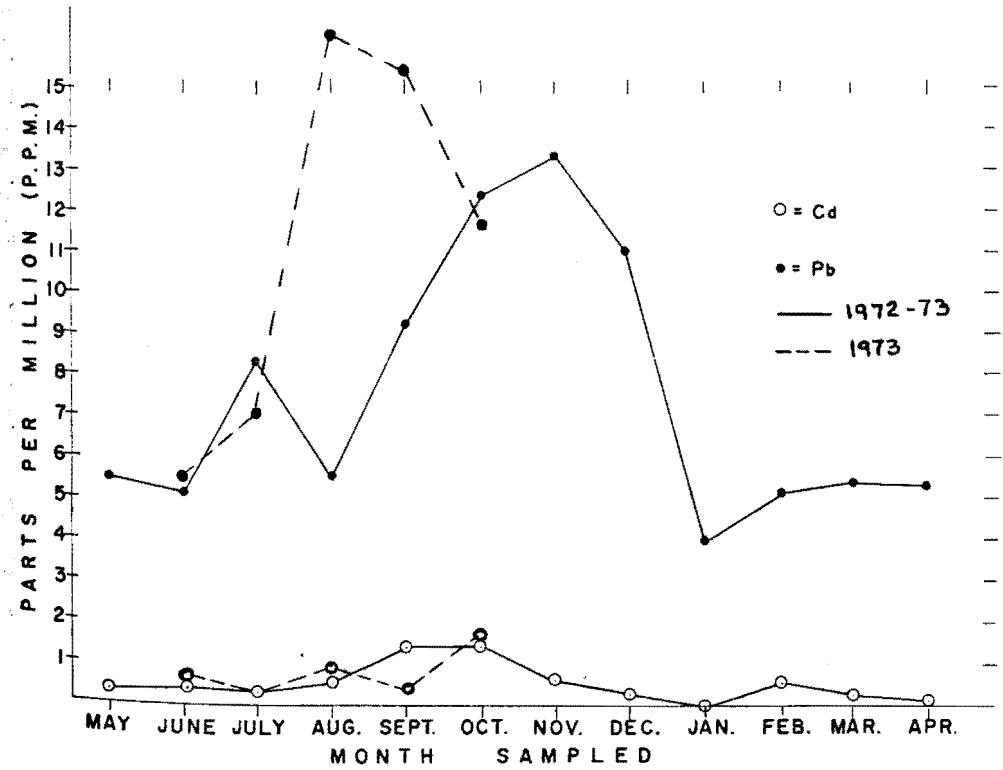

Fig. 3. Monthly means of Kenai Peninsula moose hair Cd and Pb levels, May 1972 to November 1973.

Franzmann et al. (1974) noted that for at least 9 of the 12 months studied three minerals (magnesium, copper, and manganese) were particularly low in relation to domestic animal low "normal" limits on the Kenai Peninsula. Values obtained for these elements from June through October, 1973, from Kenai Peninsula moose were again at these comparatively low levels. The part that copper may play in this will be discussed separately in this paper. The possibility of magnesium and manganese deficiency in moose has not been clinically demonstrated, but the potential exists.

Hair Analysis from Diverse Areas

Because of demonstrated differences in hair element values from different areas (Franzmann et al. 1974), we have initiated collections from various parts of Alaska. Since May, 1973, we have received samples from the Alaska Range, Southeast Alaska, Copper River Delta and Fort Richardson. We have plans to obtain samples from other areas. The Alaska Range samples are the only ones analyzed at this time (Tables 2 and 3).

The Alaska Range samples were collected in October, 1973. Magnesium and manganese levels were significantly higher (.005 level) in the hair samples from the Alaska Range than those from the October, 1973, Kenai Peninsula samples. Conversely, the potassium levels were significantly lower.

The differences in levels between regions are perhaps not meaningful when values fall within "normal" ranges, but they are meaningful when we are comparing and utilizing values that are potentially in the "sub-normal" or deficiency range.

The need is for more samples from more areas throughout the season, in order to further demonstrate the potential of monitoring mineral metabolism in moose.

Browse and Soil Analysis

To trace the relationship between the element levels in plants and soil to hair levels, we began collecting plants in September of 1973. Samples were collected within the MRC enclosures, just outside the enclosures and in the Kenai Mountain foothills. In each area the samples were collected from the same soil types. Another collection inside and outside of the MRC enclosures was made in January, 1974.

Analysis of the September collection was completed for 11 elements (Ca, Mg, K, Na, Cd, Co, Cu, Fe, Pb, Mn, and Zn). Plants sampled were Betula nana, Salix commutata, Populus tremuloides, Alnus sinuata, Betula papyrifera, Vaccinium vitis-idaea, and Salix arbusculoides (Table 4). No conclusions can be drawn from this single period sample, but an obvious observation is that at each site there was tremendous variation in values between the plant species sampled. Kubota et al. (1970) collected moose browse for mineral analysis in Alaska, and their results showed less variation between plants than between leaves and twigs of the same plant. They concluded that these differences due to seasonable availability of parts and kinds of plants, were larger than were the differences due to plants growing in different kinds of soils.

Manganese values which were low throughout the year in moose hair were not detectable in ten of eighteen plants analyzed (Table 4).

We will continue to obtain plant analysis throughout the year and will supplement this with soil analysis.

Table 4. Mineral analysis of selected moose browse plants on the Kenai Peninsula, Alaska, September, 1973.

Plants Analyzed	Elements (ppm)										
	Zn	Cu	Mg	Mn	Ca	Na	K	Cd	Fe	Pb	Co
Group 1 inside MRC[1]											
Betula nana	52.5	10.0	1560	7.7	4430	55	4950	All	61	2.5	0.3
Salix commutata	55.0	27.5	2165	ND[2]	10155	70	7740	Values	99	ND	ND
Populus tremuloides	17.5	5.0	2190	ND	9390	75	7005	less	48	1.2	ND
Alnus sinuata	17.5	25.0	2105	ND	6260	70	4890	than	79	0.3	0.1
Betula papyrifera	12.5	5.0	2060	22.7	5215	90	6305	0.5	103	ND	ND
Vaccinium vitis-idaea	12.5	5.0	1450	20.7	4565	65	4550		74	ND	0.2
Salix arbusculoides	22.5	15.0	2020	ND	8410	65	7050		92	ND	0.1
Group 2 outside MRC											
Populus tremuloides	65.0	10.0	2170	ND	9155	70	7640		40	ND	0.3
Alnus sinuata	12.5	15.0	2010	ND	5470	65	6530		81	ND	ND
Betula papyrifera	60.0	7.5	2020	7.7	3840	55	6725		75	ND	ND
Vaccinium vitis-idaea	7.5	2.5	1075	18.4	4705	60	4550		37	ND	ND
Salix arbusculoides	15.0	10.0	2100	ND	8330	60	7295		80	ND	0.2
Group 3 Kenai Mtns.											
Betula nana	37.5	12.5	1900	1.99	3410	55	6150		57	1.0	0.1
Populus tremuloides	67.5	0.5	2080	ND	7270	50	7005		65	ND	0.3
Alnus sinuata	40.0	12.5	1950	ND	6460	65	6320		102	ND	0.1
Betula papyrifera	75.0	10.0	2035	6.4	4770	55	6530		70	ND	0.1
Salix arbusculoides	65.0	10.0	2075	ND	10945	65	7060		84	ND	0.1
Vaccinium vitis-idaea	5.0	10.0	1520	13.6	5540	40	4050		34	ND	0.1

1 - MRC - Kenai Moose Research Center
2 - ND - Not detectable

Lag-time Study Plan

Two determinations that aid in better interpretation of hair mineral element analysis are the growth rate of hair and the time-lag associated with ingestion of an element and its deposition in the hair. The growth rate of moose hair was 0.8 to 1 cm/mo based upon monthly measurements of hair sampled from tame moose at the MRC (Franzmann et al. 1974).

The time-lag from ingestion to deposition of elements in moose hair is a more difficult problem and in an attempt to obtain this information we will use heavy pellets that are impregnated with selected mineral elements and placed in the recticulum of the moose for subsequent slow release. This procedure has been used to provide cobalt and selenium to domestic sheep in deficient areas of Australia (Dewey et al. 1958, and Kuchel and Buckley 1969). Given the known time of pellet ingestion and by using periodic monitoring of the potential uptake in moose hair via sampling and analysis, we hope to establish this lag-time.

Moose Hoof Overgrowth Syndrome and Copper Deficiency

Moose with hoof overgrowth have been observed on the Kenai Peninsula, Alaska. Hunters have reported this condition and we have trapped several moose with this abnormality at the MRC. G.K. Davis, University of Florida (personal communication), indicated frequent observations of this phenomenon have been made of cattle in the Everglades and deer in the area around Perry in Taylor County, Florida. Both areas are recognized as being copper deficient.

Copper deficiency is known to interfere with the establishment of a proper matrix in the formation of hard keratin due to the absence of cross-linking between peptide chains in the stabilizing material.

Normal cross-linking results from condensation reactions following the oxidative deamination of amino groups allowing the creation of the strengthening disulfide bonds. Straight, stringy wool from copper deficient sheep has fewer disulfide groups than normal wool suggesting that copper is required for the formation or incorporation of disulfide groups in keratin synthesis (Underwood 1971).

Hair and hoof samples were taken from a moose with abnormal hoof growth and a control moose in September, 1973. Hair copper level for the deformed moose was 5.7 ppm and 12.0 ppm for the control moose. Hoof copper level for the deformed moose was 3.3 ppm and 5.3 ppm for the control moose. In December another deformed moose was sampled and the hair copper level was 5.2 ppm and the hoof level was 2.9 ppm. Low limits of "normal" hair element copper levels from domestic ruminants reported by Anke (1973) were 8.5 ppm.

Mean hair copper levels from other areas in Alaska varied from the deformed moose sample, as well as from the Kenai Peninsula sample, during the same month sampled. In January, 1973 the mean hair copper level of Kenai Peninsula moose was 2.5 ppm and from Fort Richardson, Alaska, moose was 7.1 ppm (Franzmann et al. 1974). In April, 1973 the mean hair copper level of Kenai Peninsula moose was 7.9 ppm and from Tanana Flats (Fairbanks area) moose was 10.5 ppm (Franzmann et al. 1974). In October, 1973 the mean hair copper level of Kenai Peninsula moose was 10.6 ppm and from Alaska Range moose was 13.0 ppm (Table 3). The preliminary conclusion from this was that the Kenai Peninsula is a copper deficient area. Some copper levels in plants sampled in September, 1973 (Table 4) indicate this potential since a September plant sample should represent the peak in mineral levels and several had

copper levels below 5 ppm. Additional plant analyses during critical times of year are required to substantiate a copper deficiency. The potential of other mineral elements influencing the hoof overgrowth syndrome was recognized and values for sulfur, cadmium, iron, molybdenum, and zinc were also obtained from the deformed and control moose in September, 1973 (Table 5). High molybdenum intake can depress copper retention (Underwood 1971), but in our sample molybdenum levels were normal and not significantly different between control and abnormal samples. Cadmium, iron and zinc levels demonstrated no particular trends and were in the normal range. Sulfur levels were considerably higher in the control hair and hoof samples reflecting the increase in disulfide groups. Conversely, a loss of elemental sulfur was detected in the copper deficient sample due to the decrease in disulfide groups.

The tentative conclusion was made that copper deficiency was responsible for the hoof overgrowth syndrome on the Kenai Peninsula, Alaska.

Hair mineral element analyses demonstrated the copper level discrepancies and seasonal and geographic differences of other elements. Based upon these findings, we consider monitoring moose mineral metabolism via hair element analysis a beneficial tool applicable to moose management and ecologic studies.

Table 5. Comparison of moose hair and hoof samples from deformed and control animals, September, 1973. (ppm)

Hair Sample

Element	Control	Abnormal
Copper	12.0	5.7
Sulfur	327.5	192.4
Cadmium	1.6	1.0
Iron	94.9	112.3
Molybdenum	0.3	0.6
Zinc	90.2	74.9

Hoof Sample

Element	Control	Abnormal
Copper	5.3	3.3
Sulfur	232.9	130.9
Cadmium	0.8	0.6
Iron	74.0	60.3
Molybdenum	0.3	0.3
Zinc	116.8	116.8

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10th NORTH AMERICAN MOOSE CONFERENCE AND WORKSHOP

Duluth, Minnesota March 1974

91 DELEGATES TO THE 1974 CONFERENCE
ATTENDED FROM ALL PARTS OF THE MOOSE
RANGE OF NORTH AMERICA