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Moose Research Center Report

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Overview of the Kenai Moose Research Center

The concept for the Moose Research Center (MRC) came from the Alaska Interagency Moose Meetings during the early 1960s which were informal gatherings of persons representing the agencies in Alaska having land tenure or management responsibilities (ADF&G, USFWS, USFS, BLM, USPS, US Army, and US Air Force). Participants agreed that a facility should be constructed to obtain a more thorough and specific knowledge of the interrelationships of moose and their environment. It was agreed that the MRC operate as a cooperative venture of ADF&G and USFWS. ADF&G was assigned the responsibility for research on animals and USFWS was assigned responsibility for habitat studies. This was the operational arrangement until 1981 when the USFWS research staff position was eliminated by the Denver Wildlife Research Center. Since that time ADF&G has continued research activities; the Kenai National Wildlife Refuge continues to provide support for facilities maintenance as the facility is located on refuge land.

Construction of the facility began in 1965 and was partially operational by 1968. The facility is 30 air miles northeast of Soldotna, Alaska, and presently consists of 4 1mi^2 enclosures constructed from approximately 14 miles of 8-ft high woven wire fence. Four moose populations can be maintained in the large enclosures at density and composition dependent upon research needs. Twenty-two corral type traps were constructed along fence lines to facilitate capture and transfer of animals between pens. We also constructed 5 vegetation exclosures. Two smaller enclosures (10 and 15 acres) are used to maintain a population of hand-reared moose used for studies appropriate for tame animals. Associated with the smaller enclosures are holding pens, feeding facilities, a scale house, an open-circuit respiration chamber, and a log building that serves as a laboratory and storage facility. In 1989 3 small enclosures were constructed adjacent to the 15-acre pen with electrified fence to extend holding facilities and to test this type of fencing for its ability to hold moose. Two log cabins provide living quarters for employees, students, and guests. A 13Kw generator provides electricity, but efforts are underway to upgrade to solar and wind power.

The initial objective of MRC studies was to obtain a more thorough and specific knowledge of how moose affect vegetation and conversely, how vegetation affects moose. This is accomplished by direct and indirect assessment of the qualitative and quantitative aspects of the moose and its environment. During the first decade (1968 to 1977) studies were separated into those primarily aimed at moose (ADF&G) and vegetation (USFWS). Moose studies placed much emphasis upon using the animal indicator concept, whereby morphometric, physiologic, reproductive, and behavioral characteristics of moose yield information about its environment. The MRC staff was also involved in studies of moose migration (population identity) studies on the Kenai Peninsula. Vegetation studies concentrated on measuring productivity and utilization of browse

In the mid-1970s interest on the Kenai Peninsula focused on predator impacts on the moose population and the Kenai Peninsula Predator-Prey Study was initiated with ADF&G (MRC) staff responsible for the Moose Calf Mortality and Black Bear Ecology study and the USFWS responsible for the Kenai Peninsula Wolf Study.

In 1978 the focus of studies at the MRC began to change in light of the need to better integrate the moose and vegetation studies. This was the beginning of high-priority nutrition studies. In this process an animal model and a vegetation model were developed which guided our emphasis as to study priorities. An overall carrying capacity model was used to integrate the information base as it was produced. The final phase of this study—nondestructive estimation of moose body composition—is underway at this time.

In 1990 a portion of the facility was modified to house a small number of caribou. These animals are being used to study the effect of maternal nutrition on caribou reproduction. The impetus for this research was the population decline observed in the Southern Alaska Peninsula caribou herd.

(Continued on inside back cover)

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Physiological Ecology of Moose: Nutritional Requirements For Reproduction with Respect to Body Condition Thresholds

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RESEARCH PROGRESS REPORT

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AUTHORS: Thomas R. Stephenson, Kris J. Hundertmark, John A. Crouse and Stephanie R. Rickabaugh

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SUMMARY

We conducted feeding trials with adult female moose (*Alces alces*) on high- and low-quality diets to assess the influence of nutrition and reproductive success on body condition and future reproduction. Although dry matter intake rates did not differ between animals on the 2 treatments during January–April 1998, metabolizable energy intake was reduced for the low-quality diet and was reflected in greater loss of body fat and body mass. We conducted additional experiments to quantify the relationship between dietary energy and dynamics of nutritional reserves. We handled neonatal calves within 24 hours of birth to determine mass and collect morphometric measurements and serum (to assay for immunocompetency). We further supplemented our pelleted diets with vitamin E and, by doing so, eliminated in utero and neonatal losses associated with this deficiency. We developed a quantitative PSPB test, using blood serum that predicts twinning in utero with >90% accuracy. We continued collecting body condition and in utero litter sizes among free-ranging populations within the state of Alaska in an effort to compare habitat quality and density effects among statewide moose populations.

Key words: *Alces alces*, body condition, energy intake, fat reserves, PSPB, reproduction, selenium, ultrasound, vitamin E.

CONTENTS

BACKGROUND

To facilitate intensive management of moose populations, wildlife managers need to predict survival and reproductive success of individuals within these populations. Although population size is dictated by numerous factors such as weather and predation, ultimately habitat quality defined by the nutritional quality of diets will determine the maximum number of moose that an area can support. Reproductive performance of cow moose is related to their body condition. We intend to refine the use of an individual animal's condition as an indicator of the nutritional quality of its habitat and as a predictor of its potential for reproduction and survival.

Recently, methodology for applying the "animal indicator concept" (Franzmann 1985) was validated. Stephenson et al. (1998) developed equations to predict total body fat in moose from ultrasonographic fat measurements. Hundertmark et al. (1994) also developed equations to predict body composition using bioelectrical impedance analysis. The animal indicator approach assumes that because the animal is a product of its environment, it represents the quality of its environment. Thus, rather than define carrying capacity in numbers of animals, this approach provides a relative indication of the proximity of the population to K. Recently, Grubb (1995) defined nutritional condition as "the state of body components controlled by nutrition and which in turn influence an animal's fitness." Saltz et al. (1995) noted that Grubb's definition clearly identifies the role of nutrition in determining an animal's condition and ultimately its reproductive success.

Because body fat is the primary energy store of the body (Price and White 1985), measurement of lipid reserves has been the focus of much research aimed at estimating nutritional condition (Stephenson et al. 1998, Chan-McLeod et al. 1995, Franzmann and Ballard 1993, Harder and Kirkpatrick 1994, Gerhart 1995). Assessment of body condition provides insight into the ability of individuals in a population to survive and reproduce. However, in order to evaluate the role of body condition in determining an animal's reproductive fitness, biologists also must be able to assess reproductive performance including ovulation, conception, fetal numbers and survival, and natal survival.

Although summer twinning rates have been used to indicate the quality of moose habitats (Franzmann and Schwartz 1985), undetected predation may lead to biased postpartum estimates (Stephenson et al. 1995). Knowledge of reproductive status is critical to understanding both reproductive performance and the costs of reproduction. Ultrasonography has been used to successfully determine in utero pregnancy and twinning in moose during both early (Stephenson et al. 1995) and late gestation (Testa and Adams, in press). Because ultrasonography requires specialized equipment and expertise, a serum assay that diagnoses twinning is of interest. Willard et al. (1995) recently developed a quantitative pregnancy-specific protein B assay for domestic sheep that permitted detection of fetal twins with up to 82% accuracy.

Although the existence of threshold "set points" of body condition have been hypothesized for ungulates (Schwartz et al. 1988; Renecker and Samuel 1991; Gerhart 1995), their existence relative to reproduction in moose has only recently begun to be quantified (Sand 1998, Testa and Adams 1998). An understanding of thresholds required for ovulation, gestation, neonatal calf survival, and identification of the mechanisms of reproductive failure will enhance our insight into the importance of different seasonal habitats and the management of these habitats.

Poor maternal nutrition may lead to failure in the passive immunity process between mother and offspring and increase susceptibility to diarrhea, septicemia, and other diseases in neonates. Sams et al. (1996) identified a relationship between serum immune parameters and neonatal mortality of white-tailed deer fawns. Low neonate serum levels of colostral antibodies may occur from inability to efficiently nurse, poor colostral absorption, or depressed colostrum production (Sams et al, 1996). Indices of fawn viability such as immunocompetency or maternal condition may provide insight relative to the additive or compensatory nature of predation.

To validate the animal-condition approach and define density effects on body condition and reproduction, we will conduct experiments with animals foraging on natural browse in addition to animals on trials using pelleted rations. As a population approaches carrying capacity, increased competition for forage resources should reduce average body condition. Hobbs and Swift (1985) hypothesized that as population density increases, the upper limit on nutritional quality of diets obtainable will progressively decline. Deterioration in the nutritional status of individuals would be expected as population density increases. The condition of individuals could be monitored to assess diet quality. However, ruminants may be able to increase intake rate in response to declining forage quality. Determining the ability of moose to compensate as density changes will enable us to understand the limitations of using the animal-condition approach to assess habitat quality and the mechanisms of density dependence.

OBJECTIVES

1 Determine overwinter nutritional requirements for reproductive success in female moose.

2 Determine thresholds in body condition at which reproductive performance declines.

3 Evaluate the existence of cumulative effects in female moose relative to body condition, reproductive performance, and nutrition.

4 Refine estimation of moose body composition using ultrasonography.

5 Using ultrasonography and a quantitative serum assay, develop and refine methodology for diagnosing twinning in moose.

6 Evaluate effects of density dependence on body condition, reproductive performance, and diet quality of moose on natural browse.

STUDY AREA

This research was conducted at the Moose Research Center located on the Kenai Peninsula, Alaska (60°N, 150°W).

METHODS

JOB 1 CONDUCT FEEDING TRIALS TO EVALUATE THE RELATIONSHIP BETWEEN MOOSE NUTRITION, BODY CONDITION, AND REPRODUCTIVE PERFORMANCE

During January 1998, 10 adult female moose were randomly assigned to 1 of 2 treatment groups (5 per group). Treatment groups consisted of a high-quality, pelleted moose feed (Schwartz et al. 1985) and a poorer quality submaintenance ration developed during this study (Tables 1–2). During January–April 1998, rations were offered ad libitum. Animals, confined together in a 4-ha fenced enclosure, accessed feed, using individual-specific feed gates (American Calan, Inc., Northwood, New Hampshire USA) developed for controlled-access feeding trials. The system utilizes a feed container, accessible only through a neck slot controlled by a 24-volt electronically locking gate that is unlocked by an individual-specific sensing "key" collar worn by the animal (Mazaika et al. 1988). Known amounts of feed were offered and orts were collected daily to permit calculation of daily energy and protein intake for each animal. Subsamples of feed offered and orts were collected daily and frozen for subsequent dry matter determination. During October–December 1997 and May 1998, animals were maintained on the high-quality ration ad libitum. During the remainder of the year (June-September), animals were maintained on natural browse.

A second trial was conducted during 16 November 1998–30 April 1999. In this trial we used 9 of the 10 cows from the previous trial and the same assignment to treatment groups. The tenth cow's replacement received the poorer diet. The 5 cows in the high quality treatment were fed ad libitum throughout the entire trial. The 5 cows fed a low-quality diet received feed ad libitum during November. During December the low-treatment animals feed intake was restricted to a maintenance energy level determined using body mass. During January–April, low-treatment cows were fed at a submaintenance level. During the remainder of the year, all animals were maintained as indicated for the previous year's trial.

We immobilized moose during September, November, January, March, and April, using carfentanil hydrochloride/xylazine hydrochloride, reversed with naltrexone/tolazoline. Portable, real-time ultrasound was used to measure fat reserves of adult females. The rump region was scanned using an Aloka model 500 ultrasound device (Aloka, Inc., Wallingford, Connecticut USA) with a 5-MHz 8-cm linear-array transducer. Ultrasonic fat thickness was measured at 2 sites along a line between the spine, at its closest point to the coxal tuber (hip bone), and the ischial tuber (pin bone). Subcutaneous fat thickness was measured with electronic calipers to the nearest 0.1 cm at the midpoint and point of maximum thickness (immediately adjacent to the cranial process of the ischial tuber) along the line. Two fat thickness indices were determined: 1) the maximum fat thickness detected along the line (MAXFAT), and 2) the sum of the maximum thickness and the thickness at the midpoint (SUMFAT). Stephenson et al. (1998) developed an equation to predict percent ingesta-free body fat from rump fat thickness ($R^2 = 0.96$, SEE = 1.09). Ingesta-free body fat was calculated using the equation: ingesta-free body fat (%) = 5.61 + 2.05 (maximum fat thickness).

In addition, moose were weighed in September and weekly during feeding trials. Serum was collected during all immobilizations for determination of PSPB and serum urea nitrogen levels.

Transrectal ultrasonography was used to detect the presence, viability, and number of fetuses (Stephenson et al. 1995).

Feed samples were analyzed using sequential detergent fiber analysis to obtain estimates of NDF, ADF, and lignin. We also determined in vitro digestible dry matter, using cattle innocula. The Kjeldahl method was used to determine total nitrogen (N) converted to crude protein (6.25 x N). Samples were analyzed by Washington State University's Wildlife Habitat Analysis Laboratory, Colorado State University's Department of Range Science Analytical Laboratory, and the Institute of Arctic Biology's Nutritional Analysis Laboratory.

We calculated metabolizable energy intake (MEI) as the product of dry matter intake, gross energy, digestible energy, and metabolizable energy. We used t-tests to determine differences in body condition relative to diet quality. Linear regression was used to evaluate relationships among diet quality, body fat, and body mass. Analyses were conducted using program SAS (SAS Institute, Cary, North Carolina USA) and SYSTAT.

JOB 2 EVALUATE RELATIONSHIP BETWEEN CALF HEALTH AND THE DAM'S NUTRITION AND BODY CONDITION

Newborn calves located by ground surveillance of cows were captured by hand. Calves were handled after >12 hours had elapsed since birth to avoid abandonment by the mother. Captured calves were equipped with expandable breakaway radio collars and numbered ear tags. We recorded sex, body mass, total body length, and hind foot length at capture.

Trophic relations as determined by nitrogen isotopes of mother-neonate pairs

To quantify isotope enrichment, we collected and froze (-20C) paired plasma- , red blood cell- , and milk samples from 3 mother/neonate pairs of moose and caribou. Samples were obtained <24 hours postpartum, every 2 weeks thereafter for the first 6 weeks, and then monthly until November. In addition, we bottle-raised 5 caribou and 4 moose calves to quantify digestible energy and protein intake of neonates. We conducted 7-day total collection digestion trials of milk and the pelleted feed at the beginning and end of the bottle-raising period, respectively.

JOB 3 VALIDATE APPROACHES FOR DETERMINING BODY FAT AND BODY PROTEIN IN LIVE MOOSE

Captive moose on various nutritional planes and during different seasons were further evaluated for body composition. To estimate fat reserves, the rump region of immobilized moose was scanned using an Aloka model 210 portable ultrasound device (Corometrics Medical Systems, Inc., Wallingford, Connecticut USA) with a 5-MHz, 8-cm linear-array transducer (Stephenson 1995). Ultrasonic fat thickness was measured at 2 sites along a line between the spine, at its closest point to the tuber coxae (hip bone), and the tuber ischii (pin bone). Subcutaneous fat thickness was measured with electronic calipers to the nearest 0.1 mm at the midpoint and point of maximum thickness along the line. Two fat thickness indices were further evaluated: 1) the maximum fat thickness detected along the line (MAXFAT), and 2) the sum of the maximum thickness and the thickness at the midpoint (SUMFAT). To estimate protein reserves, ultrasonic muscle thickness of the biceps femoris and gluteus medius were recorded directly under the hip and pin bones, respectively. In addition, longissimus dorsi muscle thickness was measured at the 12th/13th rib (Johns et al. 1993).

Further evaluation of bioelectrical impedance analysis to determine body composition (particularly protein reserves) was conducted in conjunction with ultrasonography. Electrodes from a plethysmograph (Model BIA-101, RJL Systems, Inc. Detroit, Michigan USA) were placed in the hindleg and foreleg of sternally recumbent moose. Resistance and conductance were recorded.

Animals were euthanized immediately following ultrasonic and BIA measurements while still chemically immobilized. Whole body mass was determined and then each animal was eviscerated and skinned (subcutaneous fat will remain on the carcass). The carcass was bisected longitudinally along the vertebral column, with one half frozen for chemical analysis. The gastrointestinal tract was emptied of ingesta (Hundertmark et al. 1994). The fetus(es) and amniotic fluid of pregnant females were removed and their mass determined to permit fetus-free calculations. Kidney fat mass was recorded as the mass, to the nearest 1-g, of trimmed fat attached to the kidney. Marrow samples were collected and frozen for determination of percent marrow fat. The entire viscera and samples of shaved hide were frozen for analysis. The frozen carcass half and visceral mass was sliced at 51- and 25-mm intervals, respectively, on a commercial band saw. The homogenate at the base of the blade was collected for each component, mixed, and refrozen. Hide samples were freeze-dried and ground in a Wiley mill to create a homogenate. Chemical analysis of frozen samples was conducted at Washington State University's Wildlife Habitat Laboratory. Crude fat was determined by ether extraction (AOAC 1975). Samples were analyzed in duplicate.

Additional samples will be used to validate existing predictive equations. Regression analysis will be used to develop additional predictive equations for body composition.

JOB 4 DEVELOP SERUM ASSAY TO DETECT TWINNING

This is a cooperative project with the University of Idaho, Department of Animal and Veterinary Sciences. A graduate student who worked closely with MRC personnel recently completed a master's thesis (Huang 1998). Serum samples collected at regular intervals in association with feeding trial immobilization enabled establishment of gestational PSPB profiles.

JOB 5 MONITOR DENSITY EFFECTS ON BODY CONDITION AND REPRODUCTIVE PERFORMANCE

In preparation for this study, we constructed a 30 x 120-m pen at the intersection of pens 2, 3, and 4. This large pen will be used to contain rutting animals and hold animals for handling. In addition, we built 5 30 x 30-m enclosures/exclosures for use in foraging trials. We also repaired fences of existing exclosures in Pens 3 and 4 to ensure their continued exclusion from historical plots. Stocking of pens 3 and 4 will occur in November 1999.

Considerable data from wild populations have been collected through collaborative projects during this and previous reporting periods. During November, March, and April, free-ranging (wild) moose in Denali National Park, Yukon Flats National Wildlife Refuge, and Togiak National Wildlife Refuge, Alaska, were immobilized from a helicopter (Bell 206B) by administering carfentanil citrate-xylazine hydrochloride with Palmer Cap-Chur equipment using 3-cc darts. Carfentanil was reversed with naltrexone. We radiocollared captured moose; we collected sera by jugular venipuncture and froze samples (-80°C) for pregnancy-specific protein B assay (Stephenson et al. 1995; Huang 1998). We used portable ultrasound to measure subcutaneous rump fat reserves and to predict total body fat as described under job 2. We also recorded the number of calves at heel during capture as a measure of cost of lactation.

RESULTS AND DISCUSSION

JOB 1 CONDUCT FEEDING TRIALS TO EVALUATE THE RELATIONSHIP BETWEEN MOOSE NUTRITION, BODY CONDITION, AND REPRODUCTIVE PERFORMANCE

Moose successfully used the Calan feed gates to obtain their feed during 1 November 1999–30 April 2000. Appropriate height of the gates relative to the "key" collar was essential for proper functioning of the gate. The mean height above ground of the electronically active section of the door was 50.5 inches (range = 49–54 inches). Gate slot width and base height were 9.5 and 40.5 inches, respectively. The edge of the feed bowl was 13 inches behind the gate, and the maximum depth of the bowl was 22 inches below the base of the door.

Diet composition and quality differed markedly among high and low quality diets used in our trials (Tables 1–2). In vitro dry matter digestibility was 70% and 54% for the high and low quality diets, respectively. The high and low diets contained 10.5% and 6.5% crude protein, respectively. We were able to boost aspen sawdust in the low quality diet to 45% and maintain pellet integrity by adding bentonite clay.

The individualized feeding system effectively permitted measurement of intake rates for each animal in the trial. Fluctuations in daily intake rate did occur as a result of locking all animals out of feeding stations periodically to permit weighing. However, the Calan feeding system eliminated experimental bias associated with the stress of individually confining animals during feeding trials. Furthermore, experimental treatments were assigned to individual animals contained within a common pen.

For this preliminary analysis we evaluated intake and response parameters for most of the trial, rather than focusing on daily variation (e.g., intake rates). Although Schwartz et al. (1988) observed that animals on poorer quality diets compensated by eating more food and thus maintained energy intake, we did not observe this during January-April 1999. Analysis of November 1999–April 2000 data is pending. We observed that intake rates were similar between treatments and that metabolizable energy intake was less for animals consuming poorer quality feed. The lower energy intake was reflected in greater loss of fat and body mass. One notable difference in comparing our study animals to those of Schwartz et al. (1988) is that Schwartz used males and nonpregnant females and we used pregnant females during mid to late gestation.

Job 2 Evaluate relationship between calf health and the dam's nutrition and body condition

Vitamin Deficiency

Manuscript for submission to Alces is presented in the Appendix.

Trophic relations as determined by nitrogen isotopes of mother-neonate pairs

Manuscript by in cooperation with graduate student is in preparation.

Job 3 Validate Approaches for determining body fat and body protein in live moose

We continued to evaluate measuring longissimus dorsi and biceps femoris thickness using ultrasonography. Measurement of these muscles indicates potential for estimating protein reserves that may be important as additional energy reserves.

Job 4 Develop serum assay to detect twinning

Published manuscript abstracts are presented in the Appendix.

Job 5 Monitor density effects on body condition and reproductive performance

To date, we have collected data on fat reserves and reproductive performance from 5 free-ranging moose populations during late winter (March–early April) within the state of Alaska (Table 3). Median rump fat thickness varied between 0.9 cm and 2.8 cm in Unit 20A and the Copper River Delta, respectively. Median, in contrast to mean, values probably represent population body fat levels more accurately because of the high number of individuals with 0 cm of rump fat in some populations (e.g., Unit 20A during 1997). During early March 1997, 7 of 30 (23%) adult cows possessed <5.6% ingesta-free body fat in Unit 20A. Survival and reproductive performance of these individuals could be compromised, particularly if winter were prolonged. Although in utero twinning rates in Denali and Fort Yukon were comparable to those seen at calving, they tended to be higher than those observed at calving in Unit 20A (Boertje et al., in press). This suggests that either the accuracy of the test in Unit 20A is questionable or in utero and early neonatal losses were higher in this population. The latter may be plausible given the poorer condition of cows in this population in late winter. Testa and Adams (1998) and C. C. Schwartz (pers. commun.) observed substantial in utero or neonatal losses in moose, particularly those in poor condition. Adult cows in Yukon Flats National Wildlife Refuge possessed similar fat reserves (median IFBFAT = 7.8%) with Unit 20A, yet twinning rates were much higher. Lactational costs were higher for most cows in Unit 20A, given the higher percentage of cows with calves at heel. This and density-dependent competition for forage may have reduced ovulation and/or conception rates relative to other populations. Low fat reserves for cows in the Yukon Flats indicate winter nutritional limitation. However, high twinning rates in the Yukon Flats may indicate better summer nutrition but could also relate to reduced lactational costs as a result of high neonatal mortality. High fat reserves observed in Denali National Park and the Copper River Delta result from a combination of excellent summer and winter nutrition provided by abundant willow (*Salix* spp.) forage and reduced lactational costs from high neonatal mortality. As expected, in utero twinning rates were high in Denali. The moderate fat reserves (median IFBFAT = 8.3%)

observed on the Togiak National Wildlife Refuge are reasonable, given that animals were sampled in April, the number of calves at heel was moderately high, the population has recently moved into this area, and snow was relatively deep. Testa and Adams (1998) observed that mean rump fat during November in Unit 13A in cows with calves and cows without calves at heel was 2.9 and 4.2 cm, respectively. During November 1998 captures in Denali National Park, we recorded that mean rump fat in cows with calves (n = 3) and cows without calves (n = 12) was 0.55 and 4.55 cm, respectively. Moose handled in Denali National Park spanned the Park from East to West and included a broad range of habitats varying in their potential nutritional adequacy.

CONCLUSIONS AND RECOMMENDATIONS

Relative to the identification of a vitamin E deficiency at the MRC, we are boosting selenium levels in our feeds to 0.65–0.7 ppm. Because of a sparing interaction that occurs between selenium and vitamin E in the diet, higher selenium levels in feed may aid in preventing vitamin E deficiencies as well. Furthermore, vitamin E levels in moose feeds will be boosted to 220 IU/kg.

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Table 1 Formulation of pelleted moose rations fed at the Moose Research Center, Alaska, December 1997–May 1998

Ingredient	Feed Type (%)	
	MRC High[a]	MRC Low[b]
Aspen sawdust	25	45
Corn, ground yellow	30	25
Ground barley	29	
Beet pulp		14
Soybean meal	6.5	
Dry cane molasses	7.5	9
Dicalcium phosphate	1.1	1.1
Vitamin premix	0.3	0.3
Trace mineral salt	0.5	0.5
Protein Plus	0.1	0.1

[a]Original ration formulated by Schwartz et al. (1985).

[b]Formulated specifically for this trial.

Table 2 Mean chemical composition of pelleted moose rations fed at the Moose Research Center December 1997–May 1998

Nutrient	Feed Type	
	MRC High[a]	MRC Low[b]
Dry matter (%)	91	92
Gross energy (kcal/g)	4450	4031
NDF (%)	35.725	49.68
ADF (%)	20.035	33.39
Lignin (%)	2.74	6.65
Crude protein (%)	10.59375	5.15
In vitro DMD (%)	70.64	56.085
Selenium (ppm)	0.257	0.257
Vitamin E (IU/kg)	5.62	5.62

[a]Original ration formulated in Schwartz et al. (1985).

[b]Formulated specifically for this trial.

Table 3 Body fat and reproductive characteristics (calves "at heel" and in utero as determined by PSPB at date of capture) of Alaskan moose populations during 1993–1998

Population	Date	Median maximum rump fat thickness	Median ingesta-free body fat	Calves "at heel" (%)			Calves in utero (%)		
				0	1	2	0	1	2
CRD	Mar 1993	1.6 cm	8.9%	67	28	5			
	Mar 1994	2.8 cm	11.2%	81	19				
GMU 20A	Mar 1996	1.2 cm	8.1%	51	49		4	62	34
	Mar 1997	0.9 cm	7.4%	50	43	7	21	52	27
DNP	Mar 1998	2.6 cm	10.9%	65	29	6		40	60
YFNWR	Mar 1998	1.0 cm	7.8%	70	22	6		30	70
TNWR	30 Mar–6 Apr 1998	1.3 cm	8.3%	44	30	26		60	40

APPENDIX A. Published abstract.

Huang, Fan, Diane C. Cockrell, Thomas R. Stephenson, James H. Noyes, and R. Garth Sasser. 1999. Isolation, Purification and Characterization of Pregnancy-Specific Protein B from Elk and Moose Placenta. Biology of Reproduction 61:1056-1061.

Pregnancy-specific protein B (PSPB) has been isolated, purified and partial characterized from elk and moose placenta, respectively. The procedure, which was monitored by bovine PSPB (bPSPB) radioimmunoassay, included homogenization and extraction in aqueous solution, acidic and ammonium sulfate precipitation and ion exchange, gel filtration and affinity chromatographies. The estimated molecular weights of moose PSPB (mPSPB) were 58 kD and 31 kD and of elk PSPB (ePSPB) were 57 kD, 45 kD and 31 kD by SDS-PAGE. The isoelectric points (pI) of mPSPB were 4.8, 6.6, 6.7, and of ePSPB were 4.8, 4.9, 6.1, 6.2 determined by IEF and two-dimensional gel electrophoresis. The carbohydrate content of mPSPB and ePSPB was approximately 3.15% and 4.98%, respectively. Ouchterlony double immunodiffusion test showed when recognized by anti-bPSPB, ePSPB and mPSPB shared identical and both had partial identities compared to the bPSPB. After treatment at different temperatures (20-60°C) for 1 h, the immunoreactivities of ePSPB and mPSPB in serum were very stable. Only ePSPB in serum treated at 60°C lost some immunoreactivity. After pH treatment of serum (pH 3-11) for 2 h, the immunoreactivities of ePSPB and mPSPB became lower at acid conditions, remained stable at neutral conditions and became higher at base conditions. These data show that moose and elk PSPB have properties similar to those of bovine and ovine PSPB.

APPENDIX B. Published abstract.

Huang, Fan, Diane C. Cockrell, Thomas R. Stephenson, James H. Noyes, and R. Garth Sasser. 2000. A serum pregnancy test with a specific radioimmunoassay for moose and elk pregnancy-specific protein B. Journal of Wildlife Management 64:492-499.

A double antibody radioimmunoassay (RIA) specific for elk and moose pregnancy-specific protein B (PSPB) was established. Sheep anti-moose (m) PSPB was used for the first antibody and placental mPSPB was used as a standard. This assay was shown to quantify moose and elk PSPB in serum. When this assay was used to detect pregnancy in elk near 40 days after artificial insemination, there was agreement with a bovine RIA at 96%. Accuracy of both RIA's was 93% compared to calving observation. Regardless of whether cows were bearing single or twin fetuses, PSPB concentration in serum increased steadily from 40 to 100 to 150 days in gestation; but near day 190, PSPB amount in serum increased slightly over the 150 day level in some and decreased slightly in others. During the different periods of gestation, the mean amount of PSPB in serum of moose bearing twin fetuses was much higher than that of moose bearing a single fetus, resulting in a significant difference in PSPB concentration in serum of moose bearing single or twin fetuses at mid-gestation. When this mPSPB RIA was used to detect fetal numbers in moose at approximately 10 weeks before parturition, a cut off point at 365 ng/ml PSPB concentration in serum was chosen to separate moose bearing single or twin fetuses. The accuracy of this detection was 90.5%. Based on this RIA, pregnancy can be detected in elk and moose and prediction of single or twin pregnancies in moose is possible.

APPENDIX C. Manuscript for submission to Alces.

VITAMIN E, SELENIUM, AND REPRODUCTIVE LOSSES IN ALASKAN MOOSE

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Abstract: We recently identified a severe vitamin E deficiency in a semi-captive moose (*Alces alces*) population that was maintained on a pelleted ration during 9 months per year. Vitamin E acts as an essential antioxidant and is generally abundant in fresh vegetation. During 1998 only 8 of 17 calves identified in utero using ultrasonography at the Moose Research Center (MRC), Alaska, were born alive. Furthermore, an additional 3 calves exhibited symptoms of white muscle disease (e.g., posterior lameness) within 3 weeks following birth and 2 subsequently died. During 1998, we observed both previously identified clinical patterns in neonatal ruminants of white muscle disease, a primary symptom of vitamin E and selenium deficiencies. The first is a congenital form of muscular dystrophy in which young are stillborn or die within a few days postpartum. Secondly, we observed the delayed form which manifested itself at about 3 weeks of age in otherwise healthy, large, rapidly growing calves. However, whole blood and liver selenium levels were 0.16 μg/g and 1.8 μg/g, respectively in 3 animals with white muscle disease that were sampled; both are above recommended levels. In contrast, mean serum vitamin E (α-tocopherol) level was 0.63 μg/ml (range 0.53 - 0.8 μg/ml) for MRC calves which is significantly lower than levels observed in free-ranging neonatal calves (2.36 μg/ml) in interior Alaska (Tanana Flats). Furthermore, mean serum vitamin E levels in adult cows during March at the MRC (0.08 μg/ml) were alarmingly lower than free-ranging Tanana Flats moose (2.8 μg/ml). We observed vitamin E deficiencies in animals being fed diets supplemented with 5 IU/kg feed. Our data suggest that clinical symptoms of vitamin E deficiencies in adult moose may be difficult to detect, unless animals are reproducing. Following supplementation of vitamin E to 220 IU/kg in our pelleted ration during 1999, we observed no abortions and only 1 cow had still born calves but this was attributed to dystocia. Indeed, during 1999 only 2 of 16 calves identified in utero died of nonpredation causes. A vitamin E deficiency in free-ranging moose is unlikely, however, low selenium levels have been observed in free-ranging ungulate populations. We determined that mean whole blood selenium levels in Tanana Flats moose (0.12 μg/g) were significantly lower than MRC adult cows (0.16 μg/g), fed a supplemented diet. However, 8 of 10 animals from the Tanana Flats had selenium levels ≤ 0.085 μg/g and as such were below recommended levels for domestic cattle. We suggest that, given the lack of data on soil selenium levels in Alaska, deficiency-related neonatal losses may occur that are attributed to other causes of mortality. In utero and neonatal calf losses resulting from selenium and vitamin E deficiencies may be difficult to quantify if blood or tissue samples from study locations are not tested.

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Key words: *Alces alces*, antioxidants, captive wildlife, moose, mortality, nutrition, selenium, vitamin E, white muscle disease

Essential dietary nutrients for proper growth and survival include numerous vitamins and minerals. Vitamin E and selenium (Se) protect biological membranes from oxidative degeneration and deficiencies in them result in the breakdown of tissues. Vitamin E largely functions as a lipid antioxidant by protecting membranes in most cells from oxidative degradation (Combs 1992). Se is an essential constituent of the enzyme glutathione peroxidase that destroys peroxides before damage to lipid membranes occurs (McDowell 1992).

Free-ranging ungulates obtain vitamin E by consuming plants that synthesize it, and in particular the green parts of plants that contain α-tocopherol. A labile pool of vitamin E occurs in tissues such as plasma and liver; a fixed pool for long-term storage occurs in adipose tissue (Combs 1992). Se concentrations in natural forages are determined by levels of available selenium in the soil and are generally low in the northeast, southeast, and northwest United States, as well as areas adjoining the Great Lakes. Storage of Se occurs in the kidney, liver, and other glandular tissue (McDowell 1992).

Dierenfeld (1989) reviewed vitamin E deficiencies in reptiles, birds, and ungulates housed in zoos. Deficiencies of vitamin E may be manifested, among other means, through various forms of reproductive failure in mammals. In particular, vitamin E deficiencies result in fetal death and abortion, as well as white muscle disease (WMD) in neonates. Similarly, selenium deficient young may exhibit WMD whereas retained placenta (Julien et al. 1976, Hurley and Doane 1989) are uniquely a consequence of females suffering from selenium deficiencies.

We describe a vitamin E deficiency in a population of captive moose in Alaska. In addition, we sampled a free-ranging moose population in Alaska and quantified vitamin E and selenium levels.

STUDY AREA AND METHODS

Captive moose research was conducted at the Moose Research Center located on the Kenai Peninsula, Alaska (60°N, 150°W). Samples from free-ranging moose were collected on the Tanana Flats and in the foothills of the Alaska Range (64°N, 147°W); this area was described in detail by Keech et al. (2000) and Gasaway et al. (1983).

MOOSE RESEARCH CENTER

We conducted feeding trials with captive moose as part of a larger study on the effects of nutrition on body condition and reproductive performance (Stephenson et al. 1999). Beginning in November 1997, ten adult female moose were fed in trials that included a high digestibility pelleted moose feed (Schwartz et al. 1985) and a lower digestibility ration (Stephenson et al. 1999). Both rations contained 5 IU vitamin E/kg. During November – April 1998, rations were offered ad libitum. Animals, confined together in a 4-ha fenced enclosure, accessed feed, using individual-specific feed gates (American Calan, Inc., Northwood, New Hampshire USA) developed for controlled-access feeding trials. The system utilizes a feed container, accessible only through a neck slot controlled by a 24-volt electronically locking gate that is unlocked by an individual-specific sensing "key" collar worn by the animal (Mazaika et al. 1988). Known amounts of feed were offered and orts were collected daily to permit calculation of daily energy and protein intake for each animal. During September/October 1997 and May 1998, animals were maintained on the high quality ration ad libitum. During the September-May trial period, diets of trial moose consisted almost entirely of pelleted feed except for minimal amounts of spruce. During the remainder of the year (June-September), animals browsed entirely on native shrubs, grasses, and forbs. A second feeding trial was conducted during 16 November 1998 – 30 April 1999 but all feeds were supplemented with 220 IU vitamin E/kg.

During both trial years, moose were immobilized during September, November, January, March, and April using carfentanil hydrochloride/xylazine hydrochloride and reversed with naltrexone/tolazoline (). Portable, real-time ultrasound was used to diagnose initial reproductive condition during November 1997 and 1998. We transrectally scanned cows using an Aloka model 500 ultrasound device (Aloka, Inc., Wallingford, Conn.) with a 5 MHz 8 cm linear-array transducer to detect the presence, viability, and number of fetuses (Stephenson et al. 1995). Serum was collected during all immobilizations for determination of pregnancy-specific protein B (PSPB) levels (Huang et al. 1999, 2000). In addition, moose were weighed in September and weekly during feeding trials.

Newborn calves located by ground surveillance of cows were captured by hand. Calves were handled during 12 – 48 hours postpartum. Captured calves were equipped with expandable break-away radio collars and numbered eartags. Sex, body mass, total body length, and hind foot length were recorded at capture. Serum was collected and evaluated for determination of vitamin E (α-tocopherol). In addition when available postmortem, for animals that exhibited white muscle disease, we submitted whole blood or liver samples for determination of selenium, vitamin, and mineral. Analyses were conducted by Washington State University's Washington Animal Disease Diagnostic Laboratory.

Assays of serum vitamin E and whole blood selenium were conducted by the Washington Animal Disease and Diagnostic Laboratory, Pullman. Vitamin E (alpha-tocopherol) was determined by high performance liquid chromatography. Total selenium was quantified by ICP atomic emission. T-tests were used to test for differences in vitamin E and selenium between the MRC and the Tanana Flats. Paired t-tests were used to test for differences in cow vitamin E levels between March 1998 and 1999. Analyses were conducted using program SAS (SAS Institute, Cary, NC) and program SYSTAT (SPSS, Inc., Chicago, Illinois).

TANANA FLATS

In association with a larger study of moose ecology on the Tanana Flats (Keech et al. 2000), we immobilized adult female moose during March 1996 and 1997. A mixture of carfentanil hydrochloride/xylazine hydrochloride was administered by dart rifle (Palmer Cap-Chur Equipment) during helicopter pursuit. Blood was collected by jugular venipunture and serum and whole blood were stored frozen at –20C. Cows were collared with frequency-specific VHF transmitters.

Neonatal calves of radio-collared cows were located and captured within 48 hours postpartum using a helicopter. Calves were weighed and blood was collected by jugular venipunture and serum was stored frozen at –20C.

RESULTS

During 1998 only 8 of 17 calves identified in utero using ultrasonography at the Moose Research Center (MRC), Alaska, were born alive; 5 were aborted and 3 were stillbirths. PSPB profiles

(Figure 1) indicate that of the 4 cows that aborted, 1 aborted early in gestation and the remaining 3 were late term abortions. Furthermore, an additional 3 calves exhibited symptoms of white muscle disease (e.g., posterior lameness) within 3 weeks following birth and 2 subsequently died. Necropsy of one of these calves revealed multifocal, severe, myofiber degeneration and fibrosis during histological inspection of skeletal muscle indicative of white muscle disease. Hence during 1998, 59% of fetuses or calves died from nonpredation mortality. By contrast during 1999, 2 of 16 (12.5%) calves died of from nonpredation sources and both of these were due to dystocia during birth in the same cow.

Whole blood and liver selenium levels were 0.16 μg/g and 1.8 μg/g, respectively in 3 animals with white muscle disease that were sampled; both are above recommended levels. In contrast, mean serum vitamin E (α-tocopherol) level was 0.63 μg/ml (range 0.53 - 0.8 μg/ml) for MRC calves which was significantly lower ($t = 6.3$, $df = 4$, $P = 0.003$) than levels observed in free-ranging neonatal calves (2.36 μg/ml, SE = 0.27) in interior Alaska (Tanana Flats).

Mean serum vitamin E levels in adult cows during March at the MRC (0.08 μg/ml, SE = 0.02) were alarmingly lower ($t = 9.8$, $df = 4$, $P = 0.0006$) than free-ranging Tanana Flats moose (2.8 μg/ml, SE = 0.28). However, paired samples obtained from MRC cows during 1999, following increased vitamin E supplementation, had increased mean serum vitamin E levels to 0.78 μg/ml (SE = 0.09)

Mean whole blood selenium levels in Tanana Flats moose (0.12 μg/g, SE =) were significantly lower ($t = 2.5$, $df = 22$, $P = 0.02$) than MRC adult cows (0.16 μg/g, SE =), fed a supplemented diet. However, 8 of 20 (40%) animals from the Tanana Flats had selenium levels $\leq$ 0.085 μg/g.

DISCUSSION

McDowell (1992) described 2 clinical patterns in neonatal ruminants of white muscle disease, a primary symptom of vitamin E and selenium deficiencies, and we observed both at the MRC. One is a congenital form of muscular dystrophy in which young are stillborn or die within a few days postpartum. Our high incidence of late term abortions/still births is likely a manifestation of a deficiency especially given the decline in these losses in year 2 of the study. Secondly, we observed the delayed form which manifested itself at about 3 weeks of age in otherwise healthy, large, rapidly growing calves. Two maternally-raised calves, one of whom died, exhibited substantial daily mass gains initially but began to falter by day 8 (Stephenson et al. 1999); we contend that this was related to the onset of white muscle disease. We successfully treated one these 2 calves with a selenium/vitamin E injection and its condition improved markedly. The second calf died and necropsy confirmed white muscle disease.

Although selenium levels in MRC calves and cows appear normal, vitamin E levels in calves at the MRC fell in the low range observed for cervids in zoos and well below the mean of 2.09 μg/g. (Dierenfeld 1989). Vitamin E levels in adult females at the MRC were orders of magnitude lower than those observed in free-ranging moose on the Tanana Flats during the same time of year. Dierenfeld (1989) recommends that zoo ungulate feeds contain >200 IU vitamin E/kg total diet to avoid deficiencies. In contrast, prior to 1999 MRC moose feeds contain 5 IU vitamin E/kg.

We hypothesize that the effects of a vitamin E deficiency were manifested during this project because of the high productivity of these animals relative to previous MRC projects. In the past, although female moose at the MRC are routinely bred, they often were not permitted to breed in successive years while maintained primarily on pelleted feeds. High reproductive effort by females increases their vitamin and mineral costs. Furthermore, the duration (October through May) that our cows were on only pelleted feed is not typical of past studies with reproductive females at the MRC. In this study pregnant cows did not have access to browse during gestation or the first 2 weeks postpartum; thus, they were not able to consume lush green vegetation with high vitamin E in spring when supplementation at the end of gestation and beginning of lactation may be critical. Furthermore, animals were observed consuming limited quantities of spruce, which is generally avoided by moose. Although this may be attributed to "cribbing" behavior, the terpenes present in spruce may have increased vitamin E requirements (Dierenfeld 1989). Consequently in contrast to this study, ungulates with access to abundant natural browse are unlikely to be suspected of vitamin E deficiencies. Furthermore, because vitamin E storage occurs in lipid reserves, seasonal deficiencies are less suspect.

Relative to the identification of a vitamin E deficiency at the MRC, we increased selenium levels in our feeds to 0.65–0.7 ppm. Because of a sparing interaction that occurs between selenium and vitamin E in the diet (Dierenfeld 1989), higher selenium levels in feed may aid in preventing vitamin E deficiencies as well. Furthermore, vitamin E levels in our moose feeds were boosted to 220 IU/kg.

Predation is routinely identified as the proximate cause of mortality in neonatal moose in Alaska. Factors that contribute to predation vulnerability are rarely considered but may be the ultimate causal factor associated with predation losses in some cases (Sinclair and Arcese 1995, Keech et al. 2000). The apparent selenium deficiencies that we observed in cow moose from the Tanana Flats indicate that levels are below recommended levels for livestock and could be a contributing factor related to neonatal losses.

Flueck (1991) illustrated that whole blood selenium levels were representative of glutathione peroxidase activity in black-tailed deer (*Odocoileus hemionus*) erythrocytes. Two previous studies in Washington (Hein et al. 1994) and Sweden (Galgan and Frank 1995) determined deficient selenium levels in free-ranging moose. Hein et al. (1994) determined that whole blood selenium levels in moose in Washington averaged 0.015 ppm (μg/g), an order of magnitude below that which we observed in calves at the MRC. Selenium levels in our moose on the Tanana Flats, Alaska, generally fell between the MRC and Washington values but many were deficient. Selenium levels in domestic cattle are considered adequate at >0.1 ppm in whole blood. However, Robbins et al. (1985) hypothesized that wildlife evolved in low selenium environments and may be better adapted to them. In contrast, Flueck (1994) suggested that wild ruminants may be equally susceptible to deficiencies compared to domestics. Flueck (1994) documented increases in recruitment in a California deer population following supplementation with selenium and established a causal link between selenium deficiencies and depressed reproduction in free-ranging ungulates.

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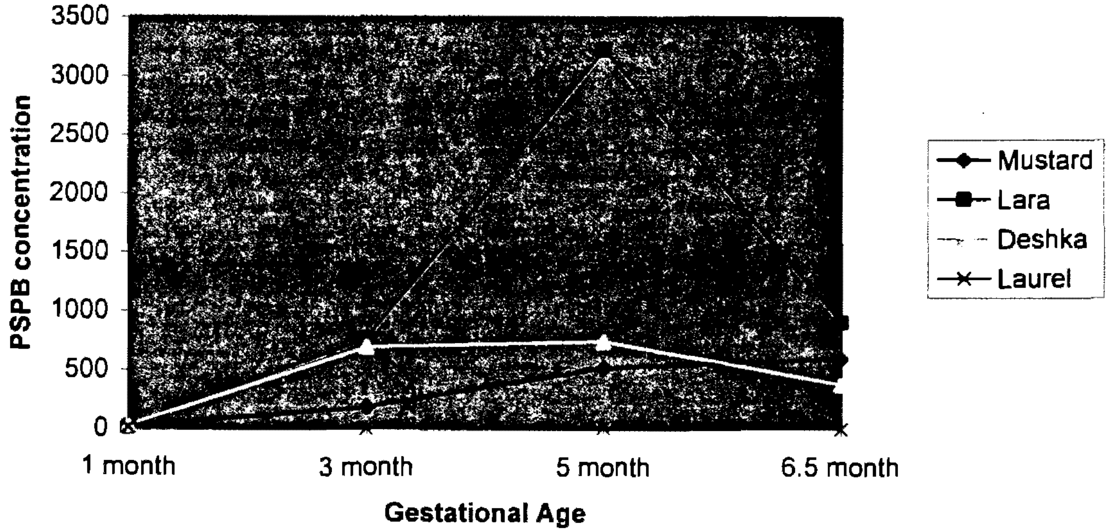

Figure 1. PSPB concentration plotted against gestational age in 4 female moose that either aborted or delivered stillbirths at the Kenai Moose Research Center, Alaska, November 1997 – June 1998. All were diagnosed with a severe vitamin E deficiency.

Overview of the Kenai Moose Research Center

Over the past decade MRC staff have been involved in activities not directly related to moose. These include the testing of the drug Ivermectin to rid wolves of lice, implementation and testing of a field technique to estimate lynx densities, and involvement in interagency efforts to study local populations of brown bear and wolverine and help develop management plans for these species.

The MRC is designed to facilitate long-term studies as well as address problems of immediate concern. As new management issues and techniques arise, the MRC is able to address them without a large expenditure of time and money.

Other recent projects include:

- Study moose reproduction, with a focus on describing the estrous cycle, predicting pregnancy and parturition via analysis of female reproductive hormones in urine and feces, comparing growth and development of first- vs second-estrus calves, monitoring male reproductive hormones and investigating a potential pheromone produced by rutting bulls. With this information, we hope to gain more knowledge about appropriate bull:cow ratios in managed populations.

- Monitor the effects on population dynamics of recently imposed hunting regulations that limit the harvest of bulls to those with spike/fork or 50"+ antlers.

- Test new techniques for measuring body fat in moose. The amount of body fat that a moose produces during the summer has a tremendous influence on its ability to survive the winter and, in females, its reproductive potential. Being able to measure this parameter accurately in a non-destructive manner will allow us to evaluate the nutritional status of populations. Ultrasound methodology is being developed and applied to moose populations statewide for quantitative assessment of condition and reproductive status.

- Evaluate genetic diversity of moose and its potential relationship to moose management.

- Determine genetic aspects of the wolf–louse problem on the Kenai Peninsula.

- Study the growth and development of antlers.

- Evaluate new immobilizing drugs. As new immobilizing drugs become available, they can be tested at the MRC to determine their usefulness to moose managers.

- Study the effect of restricted nutrition and prior reproductive success on future reproductive performance and body condition.

- Participate in a project using satellite imagery and GIS technology to map vegetation and land cover on the Kenai Peninsula.

- Develop a blood test to determine if a moose is pregnant with single or twin calves.

To date, over 95 refereed publications, in addition to numerous proceedings, book chapters, dissertations, and reports have resulted from work at the Moose Research Center.

The Federal Aid in Wildlife Restoration Program consists of funds from a 10% to 11% manufacturer's excise tax collected from the sales of handguns, sporting rifles, shotguns, ammunition, and archery equipment. The Federal Aid program allots funds back to states through a formula based on each state's geographic area and number of paid hunting license holders. Alaska receives a maximum 5% of revenues collected each year. The Alaska Department of Fish and Game uses federal aid funds to help restore, conserve, and manage wild birds and mammals to benefit the public. These funds are also used to educate hunters to develop the skills, knowledge, and attitudes for responsible hunting. Seventy-five percent of the funds for this report are from Federal Aid.

ADF&G