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Nutritional influences on moose reproduction: relative importance of contributions from diet and body reserves

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ALASKA DEPARTMENT OF FISH AND GAME
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PROJECT TITLE: Nutritional influences on moose reproduction: relative importance of contributions from diet and body reserves

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I. PROBLEM OR NEED THAT PROMPTED THIS RESEARCH

This study investigated nutritional effects on reproduction in female Alaskan moose *(Alces alces gigas)*. We combined monitoring of browsing behavior and physiological techniques to quantify the energy and protein requirements needed to support successful gestation in female Alaskan moose foraging in pens at the Kenai Moose Research Center (MRC). In addition, we began to design and validate a technique that could allow field biologists to precisely track body composition in wild moose. Ideally, this technique would permit researchers to relate body composition to reproductive performance.

II. REVIEW OF PRIOR RESEARCH AND STUDIES IN PROGRESS ON THE PROBLEM OR NEED

Energetic and protein requirements for gestation have been measured for several ungulatespecies (e.g. White-tailed deer, Pekins et al. 1998; Roe deer, Mauget et al. 1997) but only estimated in moose. Allometric relationships predict these requirements, but do not provide detailed information that will allow biologists to better understand the complex relationships between forage availability, browse energy and protein content, body reserves, survival, and reproduction. The cost of pregnancy has typically been assessed in wild ruminants in terms of the total caloric requirements of gestation compared to those of maintenance, or expressed as the difference in metabolic rates as a result of gestation compared to maintenance. Several studies have demonstrated that maternal condition and nutrition affect reproductive success (Allaye-Chan 1991; Testa and Adams 1998; Keech et al. 2000), but the next step remains to be completed.

The interactions between dietary nutrition and body stores of fat and protein and the relative importance of diet compared to body reserves in determining overwinter survival and reproductive success remain largely unexplored. Detailed investigation of the nutritional requirements of female moose for overwinter maintenance and pregnancy in terms of energy and protein catabolized from body reserves compared to dietary energy and protein intake is necessary. Further, we will examine the contributions from body fat and protein stores independently, rather than considering all body reserves as a homogenous unit.

Wildlife managers use assessments of body condition and composition to estimate nutritional condition and body reserves of fat and protein. These metrics are typically used to describe the status of a population with respect to the carrying capacity of its habitat (K) (indicator animal concept, Franzmann 1985). Fat and protein reserves also ultimately drive reproductive rates and survival and influence recruitment. Body composition, the quantitative measurement of fat and protein levels, and body condition or body condition score (BCS), which is a qualitative assessment of an animal's general nutritional status, are currently assessed in Alaskan moose in the field by two methods, both of which have limitations. The current scheme that assigns moose a BCS between 1 and 10 (Franzmann 1977) is subjective and needs refinement. Inter-observer differences of +/- 1 are common while assessing the same animal (MRC handling records, unpublished data). In addition, inexperience may compromise comparisons among different areas of the state. For example, a moose that would be assigned a relatively low score in one area could possibly be assigned a moderate score by an investigator accustomed to animals in poorer condition. While the use of generalized assessments of body condition in moose and other species to predict reproductive potential, describe the position of a population of animals relative to K, and assess population demographics (Franzmann 1985; Gerhart et al. 1996) is a valuable concept, this tool needs to be updated. Quantitative measurement of body reserves of fat and protein is necessary to allow biologists to more completely understand the effects of nutritional change resulting from variations in habitat quality or other environmental conditions on population health. Moose total body fat content can be accurately predicted from measurements of rump fat thickness with B mode ultrasound (Stephenson et al. 1998). These measurements have provided insight about energy balance and nutrition, and have been used to describe the position of a population relative to the carrying capacity of its habitat (e.g. Testa and Adams 1998; Stephenson et al. 1998; Keech et al. 2000; Stephenson et al. in preparation). Unfortunately, this measure is of limited utility in animals in poor condition, or in animals in late winter or spring, as subcutaneous rump fat depots disappear as ungulates drop below 5-6% total body fat (Stephenson et al. 1998, Cook et al. 2001a, Stephenson et al. 2002). In addition, ultrasound measurement requires expensive equipment and highly skilled personnel to ensure precision. Bioelectrical impedance analysis (BIA) has been used to determine total body water and subsequently estimate both lean mass and total body fat in monogastric animals such as bears (Farley and Robbins 1994), but is a poor predictor of total body composition in wild moose (Hundertmark and Schwartz 2002).

There is a need for a quantitative rather than qualitative index that accurately predicts total body composition in terms of both fat and protein. This index must be easily used in a field setting, discriminate between biologically relevant amounts of energy reserves, and minimize observer error (Robbins 1993, Stephenson et al. 1998; Cook et al. 2001b). Most importantly, it must provide nonsubjective data over the wide range of body conditions observed in Alaskan moose that result from variations in habitat quality, nutritional state, or reproductive status.

III. APPROACHES USED AND FINDINGS RELATED TO THE OBJECTIVES AND TO PROBLEM OR NEED

OBJECTIVE 1: Measure total overwinter energy expenditure by pregnant and non-pregnant moose. We used the doubly-labelled water method to assess total energy expenditure in pregnant and nonpregnant moose over a 21 day period three times during gestation. Total energy expenditure was measured at the end of the first trimester, and the end of the second trimester, and and the end of the third trimester concluding with parturition. We measured total energy expenditure three times during the winter in two pregnant and two nonpregnant adult cow moose during the winter of 2005-2006, four pregnant and four nonpregnant adult cow moose in 2006-2007, and two pregnant adult cow moose in 2007-2008. This data is currently being analyzed.

OBJECTIVE 2: Assess total overwinter protein requirements in pregnant and non-pregnant moose foraging in pens at the MRC. Blood samples were obtained from eight female moose (four pregnant and four non pregnant) moose four times in 2005-2006 and again in 2006-2007. Two pregnant moose were sampled four times during the winter of 2007-2008.

Blood samples were obtained late in each trimester of pregnancy, and once immediately following parturition. These samples are currently being analyzed to obtain plasma amino acid profiles and blood chemistry measurement that will indirectly assess protein metabolism. These samples are being analyzed by the University of Missouri Experimental Station Chemistry Laboratory (amino acid profiles) and Peninsula General Hospital (blood chemistry). In addition, protein requirements of these eight female moose over two winters (2005-2006 and 2006-2007), and of two pregnant moose over winter 2007-2008 will be calculated from the total overwinter loss of whole-body protein content measured by isotopic water dilution as described under ‘mobilization of body reserves’ below.

OBJECTIVE 3: Calculate energy mobilized from body reserves of fat, and protein mobilized from labile reserves in pregnant compared to non-pregnant moose. Validate the isotope dilution method of body composition measurement to allow repeated, precise measurement of overall body composition in terms of fat and protein content in the same moose.

We validated the isotope dilution method of body composition by intravenously injecting deuterium oxide label into four moose that were later euthanized and processed for body composition analysis. Deuterium oxide dilution allows calculation of total body water content which is then used to predict body protein and fat content. We also obtained blood samples once per hour for a 12 hour period, and again at 24 and 48 hours after label administration to determine equilibration time. This allowed determination of total body water, and in turn, body fat and protein content from one post-injection blood sample rather than several. We tested equilibration time in four moose three times during pregnancy over the winter of 2005-2006, and eight moose three times during the winter of 2006-2007.

We also used the isotope dilution method to predict body fat and protein content in eight adult cow moose three times during the winter of 2005-2006, and a fourth time immediately after parturition in June 2006. Body fat and protein were again assessed in eight moose three times during the winter of 2006-2007, and a fourth time immediately after parturition in June 2007. Four of these moose were pregnant each winter, while the remaining four served as nonpregnant controls. We also used the isotope dilution method to measure body fat and protein levels in two pregnant moose three times in winter 2007-2008, and again after parturition in June 2008.

We are currently using this data to calculate the amount of total energy and protein obtained from browse compared to that obtained from body reserves over each time period. Loss of body stores of fat and protein represents energy mobilized for metabolism. When the amount of energy liberated from body stores is subtracted from total energy expenditure (measured in objective 1), the remainder will represent the dietary contribution. The dietary contribution assessed in this manner should equal calculations of dietary intake made under objective 2. Similarly, when the amount of protein liberated from body stores is subtracted from total protein requirements, the difference will represent the dietary contribution.

OBJECTIVE 4: Quantify dietary energy and protein intake of pregnant and non-pregnant moose foraging in pens assessed by observational monitoring of browsing. Generate and validate an observational technique to quantify energy and nitrogen obtained from browse by moose foraging in summer.

Techniques to visually estimate diet composition and nutrient acquisition rates of seasonal forage previously developed and validated at the Moose Research Center were used to quantify annual activity, diet composition, and nutrient intake (bite size, bite rate) of foraging moose at the Moose Research Center monthly July 2007-June 2008 (n=4, 353 hours). Representative samples of plants eaten by moose were collected for species-specific biomass regressions and analyzed for nutrient content (digestibility, protein, energy, and tannin content; n=60).

OBJECTIVE 5: Quantify dietary energy and nitrogen requirements of pregnant and non-pregnant moose in metabolic stalls assessed by comparison of forage intake and urinary and fecal losses.

Two pregnant and two nonpregnant moose were held in metabolic stalls at the MRC for a six-day feeding trial twice during winter 2004-2005, three times during winter 2005-2006 and again three times during winter 2006-2007. Trials were initiated one month from the end of the first, second and third trimesters during each winter, except during winter 2004-2005 when trials were conducted during the second and third trimesters only. During trials, moose were fed 90% of an ad lib pelleted feed diet determined during a 12-18 day pretrial feeding period (Schwartz et al 1987). Metabolic stalls allowed delivery of a known quantity of feed, and collection of all fecal and urinary waste eliminated during each trial. A measured quantity of feed was presented, and urine and fecal samples were collected, over 24-hour blocks of time for the six days of the feeding trial. We are currently measuring the energy content of feed, feces, and urine samples via bomb calorimetry, and measuring the nitrogen content of the feed, fecal material, and urine via LECO analysis. Later, subtraction of the amount of energy and nitrogen in fecal and urinary waste from the amount provided in feed will confirm energy and nitrogen requirements by these animals.

OBJECTIVE 6: Generate and validate a predictive equation for body composition and total body fat and protein content based on palpation measurement of tissue depth. Generate and validate a predictive equation for total body protein content using ultrasound-measured depth of skeletal muscles. Identify potential biopsy indicators of body condition, and identify muscle groups and organs mobilized as labile protein reserves to meet metabolic demands.

Measure tissue depth relative to underlying bone structure at withers, over ribs, and rump by palpation, and correlate with total body fat, protein, ash, and water content measures obtained via carcass body composition analysis.

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We measured tissue depth relative to underlying bone structure at withers, over ribs, and rump by palpation in four moose that were later euthanized for carcass body composition analysis, and eight moose three times each during the winters of 2005-2006 and 2006-2007, and again immediately after parturition in June 2006 and 2007. We also measured tissue depth relative to underlying bony structures in two moose three times each during winter 2007-2008, and once again after parturition in June of 2008. Measures of tissue depth relative to underlying bony structures made by palpation is currently being analyzed and correlated with total body fat, protein, ash, and water content measures obtained via carcass body composition analysis in the four euthanized animals, and also correlated with measures of body composition of fat and protein obtained by deuterium oxide dilution in the animals not euthanized.

We measured tissue depth relative to underlying bone structure at withers, over ribs, and rump via b-mode ultrasound in four moose that were later euthanized for carcass body composition analysis, and eight moose three times each during the winters of 2005-2006 and 2006-2007, and again immediately after parturition in June 2006 and 2007. We also measured tissue depth relative to underlying bony structures in two moose three times each during winter 2007-2008, and once again after parturition in June of 2008. Measures of tissue depth relative to underlying bony structures made by ultrasound is currently being analyzed and correlated with total body fat, protein, ash, and water content measures obtained via carcass body composition analysis in the four euthanized animals, and also correlated with measures of body composition of fat and protein obtained by deuterium oxide dilution in the animals not euthanized.

We obtained muscle, fat, organ and marrow biopsies, and blood samples from four animals that were euthanized and processed for body composition analysis. We are currently analyzing these biopsies for tissue protein and nitrogen content, nitrogen and carbon stable isotope signature, and fatty acid profiles. After these measurements are made, we will attempt to correlate these measures from biopsy samples with overall body fat and protein content measurements obtained via carcass body composition analysis.

Objective 7. Writing reports and manuscripts

Annual research performance progress reports were prepared and submitted for project 1.60 in both FY 05 and FY 06. During FY06, “Nutrient intake of a southeast Alaska moose population in relation to forage availability and its influence on nutritional condition”, by S.G. Crouse, K.S. White, J.A. Crouse and N.L Barten, was presented as a poster at the annual meeting of The Wildlife Society in Anchorage AK September 22-27, 2006.

IV. MANAGEMENT IMPLICATIONS

Knowledge of the interactions between diet and body reserves, and the ability to predict total body composition in terms of both fat and protein reserves, rather than just in terms of overall condition, will provide biologists the tools to better evaluate the effects and limitations that changes in habitat quality, winter severity, and subsequently nutrition have on productivity and survival in populations of wild moose. These tools are extremely important, given declining moose populations in many areas across the state. Evaluating fat and protein reserves separately may provide insight not available through standard qualitative condition assessment.

Ultimately, this study will aid in understanding how an individual animal’s health and body reserves affect its future fitness (Stephenson et al. 2002) and overall population health by delineating threshold levels of body reserves of fat and protein that predict whether a cow moose produces a single calf, twins, or experiences a reproductive pause. Detailed investigation of diet, skeletal muscle and organ tissue, and body fat reserves are also required to identify how each of these resources are utilized to meet metabolic needs, and to determine the functional consequences of nutritional or protein limitation.

For example, while not statistically valid, it is of interest to note that female moose in the Nelchina Basin and Tanana Flats areas seem to have greatly decreased reproductive output, evidenced by low parturition and extremely low twinning rates, compared with cow moose in the McGrath and Togiak areas, despite approximately equal levels of ingesta-free body fat measured in moose in all four locations. Table 1 summarizes this data. While other factors including winter severity, animal density, and habitat quality, or sampling methodology are not considered in this comparison, body fat may not be the only factor influencing reproductive rates in these animals. Separate treatment of diet, body fat, and body protein stores will be informative. Following changes in total body fat and protein reserves in the same animal across different seasons also will allow researchers to determine baseline fat and protein requirements for maintenance and survival, and then quantify the surplus that can be devoted to growth and reproduction.

V. SUMMARY OF WORK COMPLETED ON JOBS IDENTIFIED IN ANNUAL PLAN FOR LAST SEGMENT PERIOD ONLY

JOB/ACTIVITY 1: Measure total overwinter energy expenditure by pregnant and non-pregnant moose.

The doubly labeled water method was used to measure energy expenditure in two pregnant cow moose over three, three-week periods during winter 2007-2008. Energy expenditure was measured between December 1-21, 2007, March 5-26, 2008, and May 7-28, 2008.

JOB/ACTIVITY 2: Assess total overwinter protein requirements in pregnant and nonpregnant moose foraging in pens at the MRC.

Blood samples were obtained from two pregnant moose four times during the winter of 2007-2008. Blood samples were obtained late in each trimester (Dec. 1, 2007; March 5, 2008, and May 7, 2008) of pregnancy, and once immediately following parturition (June 12, 200). These samples are currently being analyzed to obtain plasma amino acid profiles and blood chemistry measurement that will indirectly assess protein metabolism. These samples are being analyzed by the University of Missouri Experimental Station Chemistry Laboratory (amino acid profiles) and Peninsula General Hospital (blood chemistry).

JOB/ACTIVITY 3: Calculate energy mobilized from body reserves of fat, and protein mobilized from labile reserves in pregnant compared to non-pregnant moose.

We also used the isotope dilution method to measure body fat and protein levels in two pregnant moose three times (Dec. 1, 2007; March 5, 2008, and May 7, 2008) in winter 2007-2008, and again after parturition on June 14, 2008.

We are currently using this data to calculate the amount of total energy and protein obtained from browse compared to that obtained from body reserves over each time period. Loss of body stores of fat and protein represents energy mobilized for metabolism. When the amount of energy liberated from body stores is subtracted from total energy expenditure (measured in objective 1), the remainder will represent the dietary contribution. The dietary contribution assessed in this manner should equal calculations of dietary intake made under objective 2. Similarly, when the amount of protein liberated from body stores is subtracted from total protein requirements, the difference will represent the dietary contribution.

JOB/ACTIVITY 4: Quantify dietary energy and protein intake of pregnant and non pregnant moose foraging in pens assessed by observational monitoring of browsing.

Generate and validate an observational technique to quantify energy and nitrogen obtained from browse by moose foraging in summer. Radio-collared moose on the Gustavus Forelands, AK were observed for activity, diet composition, and nutrient intake (bite size, bite rate) 30October – 15November 2007 (n=12, 96 hours), and 21February - 9March 2007 (n=12, 107 hours). Concurrent browse surveys (n=35) quantified willow biomass availability and utilization. Representative forage species were collected during each period for species-specific biomass regressions and analyses of nutrient content (digestibility, protein, energy, and tannin content). Moose were chemically immobilized via ground or helicopter captures 30October – 08November 2007 and 02March – 11March 2008 (n=35 and 35, respectively) for radio-collaring, collecting biological samples, and assessing nutritional condition.

Techniques to visually estimate diet composition and nutrient acquisition rates of seasonal forage previously developed and validated at the Moose Research Center were used to quantify annual activity, diet composition, and nutrient intake (bite size, bite rate) of foraging moose at the Moose Research Center monthly July 2007-June 2008 (n=4, 353 hours). Representative samples of plants eaten by moose were collected for species-specific biomass regressions and analyzed for nutrient content (digestibility, protein, energy, and tannin content; n=60).

JOB/ACTIVITY 5: Quantify dietary energy and nitrogen requirements of pregnant and non-pregnant moose in metabolic stalls assessed by comparison of forage intake and urinary and fecal losses.

This objective was inactive during the most recent reporting period.

JOB/ACTIVITY 6: Generate and validate a predictive equation for body composition and total body fat and protein content based on palpation measurement of tissue depth. Generate and validate a predictive equation for total body protein content using ultrasound-measured depth of skeletal muscles. Identify potential biopsy indicators of body condition, and identify muscle groups and organs mobilized as labile protein reserves to meet metabolic demands.

We measured tissue depth relative to underlying bony structures with both ultrasound and by the palpation method in two moose three times each (Dec. 1, 2007; March 5, 2008, and May 7, 2008) in winter 2007-2008, and again after parturition on June 14, 2008. No moose were euthanized and processed for body composition measurements during the reporting period.

JOB/ACTIVITY 7: Write reports and manuscripts.

Tentative publications are listed below.

VI. ADDITIONAL FEDERAL AID-FUNDED WORK NOT DESCRIBED ABOVE THAT WAS ACCOMPLISHED ON THIS PROJECT DURING THE LAST SEGMENT PERIOD, IF NOT REPORTED PREVIOUSLY

None

VII. PUBLICATIONS

We are currently analyzing data and preparing six manuscripts for peer review and subsequent publication in scientific journals.

Lohuis, T.D., J.A. Crouse, and S.G. Crouse. Validation of deuterium oxide dilution as a predictor of Moose *(Alces alces)* body composition.

Lohuis, T.D., J.A. Crouse, and S.G. Crouse. Nitrogen and caloric requirements of pregnant Alaskan moose.

Lohuis, T.D., J.A. Crouse, and S.G. Crouse. Nutrition and protein metabolism during pregnancy in Alaskan moose *(Alces alces):* relative importance of body reserves compared to available browse.

Lohuis, T.D., G.M Harris, T.D. McDonough, J.S. Selinger, M. A. Benoit, R. Ernst and B.J. Kraft. Comparison of nutritional condition, home range characteristics, and movements between two populations of Alaskan moose on the Kenai Peninsula.

Lohuis, T.D., G.M. Harris, T.D. McDonough, J.S. Selinger, M.A. Benoit, R. Ernst, and B.J. Kraft. Indications of protein metabolism and nutritional state in free ranging Alaskan moose.

Beckmen, K.B., T.D. Lohuis, J. A.Crouse, and S.G. Crouse. The combination of Butorphanol, azaperone, and medetomidine as an immobilizing agent in Alaskan Moose.

VIII. RESEARCH EVALUATION AND RECOMMENDATIONS

None.

IX. APPENDIX

None.

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