Alaska Department of Fish and Game Division of Wildlife Conservation

> Federal Aid in Wildlife Restoration Research Progress Report 1 July 1992 - 30 June 1993

Estimation of Body Composition in Moose

by Kris J. Hundertmark Charles C. Schwartz and Curtis C. Shuey



Study 1.42 Project W-24-1 December 1993

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PROGRESS REPORT (RESEARCH)

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Cooperator: Kenai National Wildlife Refuge, Soldotna, Alaska

Project No.:	<u>W-24-1</u>	Project Title:	Wildlife Research and Management
Study. No.:	<u>1.42</u>	Job Title:	Estimation of body composition in moose

Period Covered: <u>1 July 1992 - 30 June 1993</u>

SUMMARY

We evaluated techniques for estimation of body composition in moose under field conditions. Body water was estimated via bioelectrical impedance analysis (BIA) for 2 moose, and via urea dilution for 1 of those individuals. These animals were slaughtered and tissue samples were analyzed for protein, water, fat, and ash content. Additionally, the peroneus muscle group was dissected from 1 of these individuals and submitted to the same analyses. Chemically-determined ingesta-free body (IFB) fat measurements were 15.4 and 13.1% on a fresh weight basis, and IFB water ranged from 58.6 and 62.0%. With the addition of one more sample to our previous estimates, we determined that our estimates of peroneus fat were related to IFB fat, with the exception of two samples that were collected in a different manner than the remaining samples. Empty body water space (EBWS) determined by urea dilution proved to be an imprecise estimator of IFB water, and we are terminating further investigations of this technique. Percentage fat in the shaved skin, skinless empty carcass, and empty viscera declined linearly with % IFB fat, which indicated that the fat in these body components was utilized simultaneously, contradicting the long-held belief of a sequence of fat mobilization in moose. Chemically-determined fat and water content of the IFB were significantly related to a number of factors, including BIA parameters, live weight (LW), total length (TL), and packed cell volume (PCV). However, BIA parameters were not included in all models, and it seems at this point in our analysis that LW and TL may be the most important predictors of body composition. Live weights (LW) of moose were predicted best by a linear model incorporating total length, heart girth, and condition class score.

<u>Key Words</u>: *Alces alces*, BIA, bioelectrical impedance analysis, body composition, body fat, body water, moose, urea dilution, weight.

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BACKGROUND

Body condition was identified as a critical variable within the moose carrying capacity model (Hubbert 1987, Schwartz et al. 1988<u>a</u>, 1988<u>b</u>), and body fat is a major driver of the moose submodel. Body fat must be accurately measured in moose. A proposal was prepared to test methods for estimating body composition of moose (Schwartz et al. 1988c), focusing primarily on measurement of urea space (Preston and Kock 1973), as an *in vivo* technique, and measurement of composition of the peroneus muscle group

(peroneus tertius, extensor digitorum longus, and extensor digiti III proprius, Huot and Goodreault 1985), as a technique for use on dead animals.

Over the last decade a body composition estimation technique known as bioelectrical impedance analysis (BIA) has been demonstrated to be a precise and unbiased predictor of human body composition (Lukaski 1987) and is being investigated for potential use in animal applications (Hall et al. 1989, Jenkins et al. 1988, Swantek et al. 1991). This technique works on the principle of measuring the impedance (resistance to alternating current) of hydrated body tissues to an alternating current of known frequency. Nyboer et al. (1943) demonstrated that

$V = rL^2/Z$

where V = body water volume, r = volume resistivity and is constant for a given conductor, L = conductor length, and Z = impedance. Impedance is computed by $(R_{s2} + Xc^2)^{0.5}$ where R_s = resistance and Xc = reactance, but as Xc is small in relation to R_s the equation can be reduced to Z = R_s ; however, as R_s and Xc are both easily measured we decided to use Z as an estimator. This technique was tested as a potential indicator of moose body composition.

Implicit in the estimation of body composition by the aforementioned techniques is an accurate measurement of body weight. Obtaining weights of free-ranging moose is difficult because they are often too heavy to be lifted and weighed by a helicopter. Franzmann et al. (1978) and Haigh et al. (1980) reported high correlations between certain body measurements and body weight of moose; however, body measurements are insensitive to changes in the fat depots of moose. Franzmann et al. (1976) developed a subjective 11-point scale for assessing condition class (CC) of moose based on physical appearance that may, when combined with body length, more precisely predict body weight.

OBJECTIVES

To determine the relationship between urea space measurements, impedance measurements, chemically determined composition of the peroneus muscle group, and chemically determined body composition in moose.

To determine if moose body weight can be predicted accurately from measurement of body length and heart girth, and appraisal of physical appearance.

To determine if these techniques have potential to estimate moose body composition in field applications.

METHODS

Job 1. Acquire and maintain experimental animals

Experimental animals were obtained from the pool of surplus animals of the Kenai Moose Research Center (MRC) herd, and included 2 adult females. These animals were kept in captivity at the MRC and were fed a controlled ration (Schwartz et al. 1985). The first cow (Zumu) was killed on 1 December and the second cow (Deneki) was killed on 15 December. Both animals were in good physical condition at the time of sampling.

Job 2. Determine the body water content of experimental animals via urea dilution

Experimental animals were weighed prior to immobilization whenever possible and were immobilized with either xylazine hydrochloride and/or carfentanil by means of hand-injection or darting. A polyethylene catheter was inserted into the jugular vein from which blood samples were drawn into a heparinized and a non-heparinized vacutainer. A solution containing 20% urea in physiological saline was administered through the catheter at a rate of 66 ml/100 kg live weight (130 mg/kg). Non-heparinized blood samples were drawn at 10, 15, 20, 30, 40, 50, 60, 75 and 90 min post-infusion (time 0 was defined as the midpoint of the duration of the infusion, which took approximately 2 min to complete). The non-heparinized blood samples were centrifuged at the MRC immediately after collection, and serum was stored frozen until analyzed by an independent veterinary pathology lab for serum urea nitrogen (SUN). Whole blood sampled prior to urea administration was analyzed for packed cell volume (PCV) and hemoglobin (Hb) (Franzmann et al. 1987) at the MRC.

Empty body water space (EBWS, the volume of water in the body not including ingesta) was calculated as:

$EBWS = D(S_e - S_b)^{-1} - V_d$

where D = dose of urea nitrogen (UN) administered (mass UN = mass urea * 0.4667); S_e = equilibrium-specific concentration of SUN, S_b = background SUN naturally occurring in the animal (from the sample taken at t=0); and V_d = the volume of urea solution infused. As V_d is negligible in comparison to EBWS the equation can be reduced to

$$EBWS = D(S_{e} - S_{b})^{-1}$$
.

We estimated S_e by a least squares model:

$$S_0 = S_t (e^{-kt})^{-1} = S_t e^{kt}$$

where S_0 = the extrapolated specific concentration of SUN, which approximates S_e ; and $S_t = SUN$ at time t, provided t occurs after equilibration (Holleman et al. 1982). As urea does not infuse significantly into the rumen space over the duration of our sampling (Bartle and Preston 1986) we will confine ourselves to analysis of the relationship between ingesta-free body (IFB) water (which we define as the chemically determined water content) and EBWS.

Job 3. Determine the body water content of experimental animals via BIA

A plethysmograph (Model BIA-101, RJL Systems, Inc. Detroit, MI) was used to estimate electrical impedance of moose. The animals were allowed to assume a sternally-recumbent position after immobilization. Any variation in positioning of animals was corrected so that all animals were tested in similar positions. Electrodes were constructed from trocars removed from 18ga spinal needles and were bent to an angle of 90–13mm from the tip. A "source" electrode was inserted subdermally at the carpal joint on the foreleg and at the joint between the metatarsus and the hoof on the hind leg on the side of the body most exposed while the moose was sternally-recumbent. A "detector" electrode was placed 7.5 cm proximal to each source electrode. The tips of the electrodes were oriented distally. Electrodes were connected to the plethysmograph via alligator clips on the end of 10-ft cables. Resistance and Xc were recorded as well as total body length (TL) and heart girth (HG). Electrodes were removed and re-inserted, and R_s and Xc measured, a minimum of 3 times per animal to ascertain variation associated with electrode placement.

Job 4. Determine body composition of experimental animals

The animals were killed within 24h after final urea dilution and BIA measurements were obtained. The animals were eviscerated and skinned (with as much fat as possible being left on the carcass). The empty carcasses were bisected along the spinal column, with one half of the carcass frozen for analysis. The peroneus muscle groups were dissected from the carcass halves that were not used in the analysis and were frozen. The digestive tract was emptied, and the ingesta and viscera were weighed separately. Entire viscera and samples of ingesta and shaved hide were frozen. The frozen side of the carcass and the visceral mass were cut into 51- and 25-mm slices, respectively, on a commercial band saw. The sawdust that collected at the base of the blade was collected for each component, thoroughly mixed and refrozen, and shipped along with ingesta, hide and peroneus samples to the Wildlife Habitat Laboratory of Washington State University for determination of crude fat, crude protein, ash, and water content (Huot and Picard 1988). Crude fat was determined by methanol-chloroform extraction, crude protein content was determined by the Kjeldahl procedure (AOAC 1975), ash content was determined by burning in a muffle furnace at 550C for 2h, and percent organic dry matter (1.00 moisture content) was determined by drying samples in a 100C oven for 12-16h and subtracting ash content. Three replicates of each sample were analyzed. Peroneus and hide samples were freeze-dried and ground in a Wiley mill before being subject to chemical analysis.

Job 5. Determine if body weight of moose can be predicted accurately from total body length and visual appraisal

Moose at the MRC that were immobilized and weighed during this reporting period were subject to visual appraisal of CC. Total body length (TL), HG and CC were used in a multiple regression analysis to predict live weight (LW).

Job 6. Examine the relationship between urea space, impedance values, peroneus fat, and body composition estimates

Empty body water space estimates, peroneus composition, and BIA values (TL²/Z and Z) were compared to body composition estimates by simple linear and/or stepwise multiple regression with LW and TL as additional predictors. Swantek et al. (1991) demonstrated that R_s and/or Xc (and by extension their product Z) were occasionally better predictors of IFB fat and water than the traditional parameter TL²/Z. Nyboer (pers. comm.) suggested that conductance (C) and susceptance (S), which are the reciprocals of R_s and Xc, respectively, were truly the parameters of interest. Packed cell volume (PCV) was also used as a predictor because it served as an index of dehydration, which accompanies malnutrition and would have an effect on BIA measurements (Brodie et al. 1991). We constrained the stepwise regression procedure to minimize multicollinearity by instructing the software to not enter predictors that were highly correlated with predictors already in the model (Wilkinson 1990). Adjusted coefficients of multiple determination (R_{a2}) were reported for multiple regressions rather than raw R² values because, unlike R², R_{a2} is not influenced by the number of independent variables in the model (Neter and Wasserman 1974:229).

RESULTS AND DISCUSSION

Job 2. Determine the body water content of experimental animals via urea dilution

Empty body water space was estimated for Deneki only. The least squares method yielded an estimate of S_e of 152.6.

Job 3. Determine the body water content of experimental animals via BIA

Mean (SE) R_s estimates were 446.7 (2.0) ohms for Zumu and 373.8 (3.5) ohms for Deneki, and mean (SE) Xc estimates were 34.0 (1.1) ohms and 39.3 (0.7) ohms, respectively. These estimates were within the range of those obtained from animals in prior years (Table 1).

Job 4. Determine body composition of experimental animals

The two subjects had IFB fat of 13.1% (Zumu) and 15.4% (Deneki), which rank them as two of the fattest moose we have examined. These animals were killed in December, which is the month when cows normally reach their peak body condition (Schwartz et al. 1987). The IFB water content of these animals was 62.0% and 58.6%, respectively.

We observed linear relationships between IFB fat, visceral fat, empty carcass (skinned and eviscerated) fat and shaved skin fat expressed as percentages of weight (Fig. 1). This illustrated that mobile fat depots (with the likely exception of marrow fat, which was not

measured in this study) were used simultaneously and that the sequence of fat mobilization described by Harris (1945) referred only to the sequence of disappearance of these depots based upon visual appraisal, which was dependent upon their original size. When weight of depot fat was expressed as a percentage of the weight of IFB fat we computed means (SE) of 70.9% (2.4), 27.1% (2.5), and 2.1% (0.5) for carcass, viscera, and skin, respectively. When fat depot percentages (independent variables) were regressed against % IFB fat (dependent variable) we concluded that the slopes of the regression lines were not significantly different from 0 (Table 3), which indicated that the amount of fat in each depot (expressed as a percentage of total fat) did not change across the range of body condition represented in our sample. If this relationship holds with additional sampling it would have a profound effect as a management tool, as a skin sample could be taken from dead moose from which % IFB fat could be estimated. Least squares estimation of % IFB fat (Y) using % shaved skin fat (X) as the independent variable resulted in the following predictive equation and regression parameters: Y =0.087 + 9.25(X), R² = 0.78, SEE = 2.60, F = 43.56, P < 0.0001. The constant in this model was not significantly different from zero (t = 0.065, P = 0.95), and regression through the origin yielded a slope of 9.33 with no change in \mathbb{R}^2 .

Job 5. Determine if body weight of moose can be predicted accurately from total body length and visual appraisal

Data collected from the pair of animals this year were added to data reported last year (Hundertmark et al. 1992). Of the various linear regression models applied to the data, the model using TL, HG and $1/CC^2$ continued to offer the most precision (Table 4). This model also was selected as the best predictor of TL using a backward stepwise regression program (Wilkinson 1990).

There were some notable differences between our results and those reported elsewhere for weight-length relationships (Franzmann et al. 1978, Haigh et al. 1980). The correlation (r) between TL and LW for our data was 0.85, which was not as high as that reported by Franzmann et al. (1978) (0.94) but was higher than that reported by Haigh et al. (1980) (0.71). These differences may be attributable to the considerably greater sample size reported by Franzmann et al. (1978) and the fact that Haigh et al. (1980) measured weight by suspending moose from a helicopter and recording weight only to the nearest 5 kg. Haigh et al. (1980) also subtracted a subjective estimate of antler weight from LW of males, which would increase the variation of the estimate.

Regression equations presented in these publications for predicting LW as a function of TL differ from that reported here (Fig. 2). A major difference between our study and theirs is that we have no data from animals less than 1.5 yr old. Also, Haigh et al. studied a smaller subspecies (A. a. andersoni) than that studied by Franzmann et al. (1978) and this study (A. a. gigas). The relative positions of the regression lines (Fig. 2) indicated that representatives of A. a. andersoni are shorter than representatives of A. a. gigas of

the same weight. We can only speculate that as our sample size increases the value of our parameters will approach those of Franzmann et al. (1978).

During this reporting period we assisted T. Stephenson (Univ. of Idaho) in testing the effectiveness of a portable ultrasound unit in measuring subcutaneous rump fat depths (Appendix A). This technique proved to measure fat depths accurately and also delineated the size of the rump fat depot. This exercise demonstrated to us that rump fat can exist on an animal even though visual appraisal and/or palpation could not detect it. As the presence/absence of rump fat is a criterion in determining CC, use of this technique may provide a less biased alternative to CC in appraising animal condition.

Job 6. Examine the relationship between urea space, impedance values, peroneus fat, and body composition estimates

The peroneus muscle group was collected from only one of the two moose sampled this year. Adding this observation to those collected in previous years, we determined that percent peroneus fat was significantly related to percent IFB fat (Fig. 3). Two of our observations were obvious outliers and were eliminated from the analysis. On these two samples, the connective tissue lying between the muscle and the skin was removed before analysis, and we believe this biased the results toward lower fat levels. Huot and Goodreault (1985) reported a high correlation between peroneus fat and total body fat (r=0.96) for caribou (*Rangifer tarandus*), which is similar to that for our data (r=0.91). Also, their predictive equation (Y = 1.55 + 3.43(X)) was similar to ours (Y = 1.70 + 3.34(X)). However, our data have a larger standard error than do theirs.

When all animals were included in the analysis, impedance parameters were not included in any of the stepwise regression models (Table 5). Live weight was included in all 4 models, which indicated that variation in LW accounted for the greatest proportion of variation in the dependent variable. Similar results were reported for caribou and reindeer (Gerhart et al. 1992).

We computed separate models with Angel removed from the analysis because of our suspicions that the plethysmograph was not working correctly at the time we sampled her. However, a BIA parameter (S in this case) was included in only one model (Table 5).

Estimation of body composition components for all animals without using LW as an independent variable (see Job 6) resulted in TL being entered into all models and PCV being entered into all models with the exception of IFB water (%). Removing Angel from the analysis improved predictive power (greater R_{a2} and lesser SEE) of all models with the exception of IFB water (kg), which remained the same (Table 5), and a BIA parameter was included in one of the models.

We realize that our sample sizes are extremely small for this type of analysis, and that regression parameters could change significantly with the addition of more animals. We

present these data as preliminary, and caution against drawing conclusions from the information presented here. Indeed, Gerhart et al. (1992) concluded that BIA was inferior to traditional body composition indices in estimation of body composition in caribou and reindeer. However, we believe our data indicate that further research is warranted.

RECOMMENDATIONS

Based upon the data reported herein, we will discontinue Job 2 (urea dilution). We plan to continue the study, focusing our efforts on BIA and the peroneus muscle group. Six animals will be sampled in the coming year to improve the reliability of our estimates. Historic records of moose measurements from the MRC will be analyzed for relationships to predict weight.

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Figure 1. The relationship between percent IFB fat and percent fat in the shaved skin, skinless carcass and viscera components.



Figure 2. The relationship between LW and TL. Lines were calculated from predictive equations from (a) Franzmann et al. (1978), (b) Haigh et al. (1984), and (c) this study.



Figure 3. The relationship between peroneus fat and IFB fat. The line was calculated from the relationship between these 2 parameters in caribou (Huot and Goodreault (1985).

			R			Xc		
Animal	N	Mean	SE	CV	Mean	SE	CV	
Angel	5	235.6	3.5	3.3	14.0	0.0	0.0	
Brooks	3	302.0	1.2	0.7	21.0	0.0	0.0	
Oly	5	581.6	8.2	3.2	42.0	2.8	14.7	
Luke	3	363.0	0.6	0.3	30.7	0.3	0.3	•
Hydro	3	365.3	6.1	2.9	29.3	0.3	2.0	
Sol	4	317.0	1.1	0.7	30.0	0.7	4.7	
Stripes	3	411.7	2.3	1.0	30.0	0.6	3.3	
Kobuk	3	365.3	7.1	3.3	34.3	0.3	1.7	
Deneki	4	373.8	3.5	1.9	34.0	1.1	6.4	
Zumu	3	446.7	2.0	0.8	39.3	0.7	2.9	

Table 1. Mean values of R and Xc and associated estimates of variation for 10 moose used in the body composition study, Moose Research Center.

Table 3. Regression (Y = a + bX) parameters for percentage of IFB fat contained in each of 3 depots (Y) and percentage IFB fat (X) for 12 moose.

	Regression parameters				
Y	a	b	Y		
Carcass	75.6ª	-0.62 ^b	70.9		
Viscera	21.4ª	0.75 ^b	27.1		
Skin	3.28ª	-0.16 ^b	2.1		

^a $\underline{P} > 0.01$, <u>t</u>-test. ^b $\underline{P} < 0.10$, <u>t</u>-test.

Table 4. Regression equations, coefficients of determination (\mathbb{R}^2), standard errors of the estimate (SEE), and error degrees of freedom (DF) for prediction of moose live weight (LW) from total length (TL), heart girth (HG), and condition class (CC), Moose Research Center, <u>N</u> = 18.

Regression equation	R_a^2	SEE	DF
LW = 3.91(TL)-782	0.70ª	35.0	16
= 3.24(TL)+0.45(HG)-671	0.66ª	32.7	12 ^b
= 3.04(TL) + 15.8(CC) - 622	0.89ª	21.7	15
= 3.84(TL)-132.0(1/CC)-733	0.88ª	22.0	15
$= 3.98(TL)-114.5(1/CC^{2})-791$	0.88°	22.7	15
= 2.70(TL)+0.18(HG)+13.7(CC)-546	0.87°	20.0	11 ^b
= 3.13(TL)+0.63(HG)-123.7(1/CC)-647	0.93ª	14.7	11 ^b
$= 3.21(TL)+0.76(HG)-110.8(1/CC^{2})-709$	0.94ª	14.1	11 ^b

а <u>Р</u><0.01

^b Heart girth measurements were not available for 3 animals.

Regression equation	Error df	R, ²	SEE	Pª
All animals, all parameters:				
IFB water $(kg) = 51.55 + 0.43(LW)$	8	0.90	10.49	>0.0001
IFB water (%) = $85.70 - 0.05(LW)$	8	0.64	2.88	0.0035
IFB fat (kg) = $-222.44 - 0.50(TL) + 0.28(LW)$	7	0.87	10.04	0.0003
IFB fat (%) = $-47.77 - 0.05(LW) + 0.12(TL)$	7	0.75	2.96	0.0034
Angel removed, all parameters:				
IFB water (kg) = $26.91 + 1.16(PCV) + 0.40(LW)$	5	0.90	9.65	0.0016
IFB water (%) = $124.68 - 0.21(TL)$	7	0.69	2.15	0.0033
IFB fat $(kg) = -413.75 + 1.35(TL) + 1520.92(S)$	6	0.93	7.20	0.0001
IFB fat (%) = $-66.51 - 0.02(Z) + 0.29(TL)$	6	0.85	2.31	0.0016
All animals, LW removed as a predictor:				
IFB water (kg) = $-330.27 + 1.56(TL) + 2.54(PCV)$	6	0.75	15.63	0.0067
IFB water (%) = $125.20 - 0.23(TL) + 181.12(S)$	7	0.57	3.14	0.0221
IFB fat (kg) = -548.41 + 1.81(TL) + 1.27(PCV)	6	0.80	12.78	0.0036
IFB fat (%) = -111.84 + 0.37(TL) + 0.29(PCV)	6	0.75	3.00	0.0063
Angel removed, LW removed as a predictor:				
IFB water $(kg) = -321.61 + 1.61(TL) + 1.91(PCV)$	5	0.75	14.95	0.0139
IFB water (%) = $124.68 - 0.21(TL)$	7	0.69	2.15	0.0033
IFB fat $(kg) = -413.75 + 1.35(TL) + 1520.92(S)$	6	0.93	7.19	0.0001
IFB fat (%) = $-66.51 + 0.29(TL) - 0.02(Z)$	6	0.85	2.31	0.0016

Table 5. Estimates of ingesta-free body composition components using TL, LW, PCV and BIA parameters (Z, TL^2/Z), fit by stepwise regression, and associated regression parameters.

^a Significance level of <u>F</u> statistic from regression ANOVA.

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RH: Ultrasonic Fat Measurement

ULTRASONIC FAT MEASUREMENT OF CAPTIVE YEARLING BULL MOOSE

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<u>Abstract</u>: Five captive yearling bull moose (*Alces alces*) were evaluated to determine the feasibility of using portable real-time ultrasound to measure body fat. The rump region of the bulls was scanned, twice weekly for 3 weeks during the rut, using a 5 MHz transducer; an additional set of measurements was obtained in April 1993. Ultrasonic fat thickness was measured at multiple sites along a line between the spine, at its closest point to the tuber coxae (hip bone), and the tuber ischii (pin bone), as well as along a second line perpendicular to the first line at its midpoint. The range of maximum subcutaneous fat thickness at the beginning of the study was 0.3-2.4 cm. Fat thickness declined significantly during the rut. The range of fat loss during the rut at the intersection of the 2 measurement lines was 0.2 - 1.2 cm. This *in vivo* technique exhibits potential to monitor body condition.

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Key words: ultrasound, moose Alces alces, body condition, nutritional status.

Techniques studied to assess body condition of ungulates include body mass (Riney 1955), skeletal measurements (Kirkpatrick 1980), femur marrow fat (Riney 1955), kidney fat index (Riney 1955, McGillis 1972), kidney fat mass (Anderson et al. 1990), rump fat (Mitchell et al. 1976, McGillis 1972), blood (Franzmann et al. 1987, DelGiudice and Seal 1988), urine (Del Giudice et al. 1989), twinning rates (Franzmann and Schwartz 1985), physical condition class (Franzman 1977), tritiated-water (Torbit et al. 1985), bioelectrical impedance analysis (Hundertmark et al. 1992), whole body composition

(Torbit et al 1985, Huot and Picard 1988), and ultrasound (Houghton and Turlington 1992). A logical approach for assessing the nutritional status of ungulate populations is the use of condition indices.

Kirkpatrick (1980) reviewed indices of nutritional status and noted that there is much variability in the accuracy of some of the present techniques. Body mass was considered as a measure of condition, however, many factors such as early development of the animal and rumen fill result in highly variable values. Skeletal indices such as the femur/hind foot ratio may be of value in monitoring long-term nutritional status (Kirkpatrick 1980); however, skeletal indices are only gross indicators of condition.

A number of techniques are limited in their use because they cannot be applied to live animals. Bone marrow fat (Mech and DelGiudice 1985) is useful for identifying animals in poor condition. Similarly, kidney fat index and kidney fat mass are useful primarily for evaluation of the middle range of condition (Depperschmidt et al. 1987). Although whole body composition is the most accurate technique for determining total body fat, it is not an *in vivo* procedure and is expensive.

Recently, much research has concentrated on potential *in vivo* techniques. Blood has been evaluated as a physiologic monitor of the nutritional status of ungulates (Franzmann et al. 1987). To effectively use blood parameters to assess the status of a population, one must know sources of variation in the data, baseline values, effects of environmental changes on blood values, and the resilience of blood parameters to environmental perturbation (Franzmann et al. 1987). Franzmann et al. (1987) stressed the importance of standardization in techniques during capture, collecting, handling, and analysis.

Kirkpatrick (1980) notes that although blood urea nitrogen (BUN) is a good indicator of protein intake on a diet of constant energy intake, high dietary energy levels may depress it, whereas at very low energy levels BUN may rise as a result of tissue catabolism. The use of blood parameters also is complicated by the influence of stress of collection and the daily rhythms of many blood characteristics (Kirkpatrick 1980).

Franzmann et al. (1987) found packed cell volume (R=0.35), hemoglobin (R=0.22), total serum protein (R = 0.22), phosphorus (R = 0.22) and calcium (R = 0.17) to be the highest ranking blood values, using Pearson correlation coefficients, for condition evaluation. It is evident, however, that the validity of these blood values as condition indices may be limited by their weak correlation with condition.

Franzmann (1977) developed a series of condition classes for moose based on general appearance which have been widely used in Alaska. However, the precision of this technique is limited because it is subjective, affected by observer bias, and subject to difficulties associated with isolating the effect of age on appearance (unrelated to actual fatness).

DelGiudice et al. (1988) discussed the use of blood and urine constituents as indices of deer nutritional status. Blood urea nitrogen (BUN) is the end-product of protein (dietary and endogenous) metabolism. BUN was used to differentiate between deer on low and high protein diets, as well as to monitor nutritional condition over time. Urinary urea nitrogen (UUN), usually expressed as U:C, is strongly correlated with BUN. There appeared to be conflicting use of U:C ratios to assess crude protein intake; in cases of

rising protein intake, U:C remained low because of retention but in other cases U:C was low because it directly reflected protein intake.

In vivo determination of mule deer body composition was estimated by dilution of tritiated water into the total body water pool (Torbit et al. 1985). The volume of rumen water was approximated and subtracted from total body water estimates when using the tritiated water technique to restrict water estimation to that present in tissue. Although tritiated-water based estimates of fat were strongly related ($R^2 = 0.961$) to chemical estimates of fat content, all animals had to be switched to an "equilibrium" diet 3 days prior to estimating body composition. This would not be possible in a field situation.

Bioelectrical impedance analysis uses the conductance (e.g., 1/resistance) of an applied current to determine the volume of total body water (Hall et al. 1988) and exhibits potential as an *in vivo* condition index. Conductance is limited to the fat-free body component since fat contains minimal water and thus lean body mass can be estimated. Total body weight minus lead body mass is total body fat. Hall et al. (1988) found a high correlation (R = 0.848) between lean body mass (LBM) as determined by bioelectrical impedance and LBM from chemical analysis of sacrificed rat carcasses. Standardization of electrode placement and body orientation are essential to accurate estimates of body composition (Hundertmark et al. 1992). Furthermore, this technique also may be limited by difficulty in subtracting rumen water from calculations.

For this study bulls during the rut were selected because we wanted to assess the ability of ultrasound to detect changes in fat depots at a time (the rut) when bull moose were known to exhibit a substantial mass loss (Schwartz et al. 1984) and presumably fat as well. Thus, bulls provided the greatest potential for fat and mass loss, and the ability to monitor changes in fat composition, over a short period of time. The purpose of this study was to evaluate the feasibility of using portable real-time ultrasound to repeatedly measure body fat as an estimate of animal condition.

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MATERIALS AND METHODS

We used five yearling bull moose to test portable real-time ultrasound to measure body fat. Bulls were reared at the Moose Research Center and fed a formulated ration (Schwartz et al 1985) *ad libitum* during the rut and fed primarily on natural browse, with supplemental feed available, the remainder of the year. Animals were rotated between pasture and handling pens and weighed weekly during the rut and again in April. Bulls were immobilized with a carfentanil/xylazine hydrochloride mixture twice weekly for 3 weeks during September/October 1992 as part of a simultaneous reproductive study; an additional set of fat measurements was obtained for 4 or these bulls during April 1993. The rump region was scanned using an Aloka 210 with a 5 MHz 8 cm transducer. Because the rump region exhibits the largest deposits of subcutaneous fat, it was the preferred region for obtaining a range of condition. Ultrasonic fat thickness was measured (Figure 1) at multiple sites along a longitudinal line between the spine, at its closest point to the tuber coxae (hip bone), and tuber ischii (pin bone), as well as along a transverse line perpendicular to the first line at its midpoint. Prior to scanning, hair on the measurement lines was shaved, wide enough (2.5 cm) to permit scanning, using a handheld animal clipper with a surgical blade; hollow moose hair prevents scanning through hair. Vegetable oil was applied frequently to the exposed skin in the shaved areas to assure air-free contact of the transducer with the skin. Subcutaneous fat thickness, to the nearest 0.1 mm, was measured at 3-6 sites along the longitudinal line and 3 sites along the transverse line including a reading at the intersection of the lines. Location of measurement sites was variable and selected to measure fat at points of maximum and minimum thickness along the lines, as well as at intermediate points to allow identification of shrinkage and expansion of fat deposits both horizontally and vertically. Measurement sites were recorded by measuring distance, to the nearest 1 cm, along the longitudinal or transverse measurement lines.

Because of missing observations during several of the mobilizations, we were able to compare all 5 moose only for the beginning and end of the rut; a paired t-test was used to test for a significant difference in fat thickness over time. Fat thicknesses of 4 of the 5 moose were compared among the beginning and end of the rut and April using a repeated measures Analysis-of-Variance. Repeated measures Analysis-of-Variance also was used to test for differences in body mass among the 5 bulls.

RESULTS

Mean subcutaneous rump fat declined significantly during the rut at both sites that were compared statistically (Table 1). Thickness at site 2, located at the intersection of the longitudinal and transverse lines, differed between 30 September and 14 October 1992 (t = -4.75, df = 1, $\underline{P} = 0.009$). Similarly, fat thickness at site 3, located at approximately 60% of the distance posterior from the tuber coxae, differed significantly among periods (t - -2.20, df = 1, $\underline{P} = 0.0925$).

A second analysis, using only the observations from the 4 moose sampled in April, was conducted (Table 1). Fat thickness at site 3 clearly showed a pattern of decline during the rut and, although, not significant, began to increase in the spring (F = 0.76, df = 2, $\underline{P} = 0.508$). Site 2 did show a significant decline (F = 4.76), df = 2, $\underline{P} = 0.058$) during the rut but a significant increase was not detected in the spring. Measurement sites at the anterior end of the longitudinal line were not compared statistically because their location varied greatly in an effort to follow the disappearance of fat (Figures 2-6). The longitudinal measurement sites proved to be more useful than the transverse sites for monitoring changes in fat because they included the locations of greatest fat thickness and thus provided a greater range over time. Most of the transverse measurement sites decreased to 0 early in the experiment and showed no indication of change.

Validation of the ultrasound for measuring fat thickness was conducted on 2 moose which were euthanized. Ultrasonic measurement of fat thickness at multiple sites occurred pre-mortem and caliper measurements via an incision occurred post-mortem. The difference between the 2 methods averaged ± 1 mm.

Mean (se) body masses of the 5 bulls were 331 (10.6) kg, 336.4 (9.7) kg, and 348.6 (8.3) kg for 28 September 1992, 12 October 1992, and 26 April 1993, respectively. Although there was no significant mass loss during the rut, body mass did differ significantly between the beginning of the rut and April (F = 7.45, df = 2, $\underline{P} = 0.0149$). Subcutaneous fat thickness during the rut was more sensitive to changes in condition than body mass. Mass is affected not only by metabolism of fat but by rumen fill as well.

DISCUSSION

Evaluation of animal body composition exhibits potential as a means to assess habitat condition. Since the animal is a product of its environment we should be able to use individuals to monitor shifts in the environment. Hall et al. (1988) stated that assessment of body composition is important in evaluating nutritional status.

Regelin et al. (1987) proposed that determination of body condition of moose will become "a valuable tool for measuring habitat quality and carrying capacity." Accurate body condition indices may reduce the need for expensive and time-consuming vegetation measurements. Schwartz et al. (1988) discuss the dynamic nature of fat metabolism in northern cervids and note that gains and depletions are directly related to forage quantity and quality. Thus, indices of total body fat could be used to assess the nutritional quality of ungulate habitat. As a population approaches carrying capacity, average body condition would be expected to decline due to increased competition for forage resources. Hobbs and Swift (1985) further point out that as population density increases, the upper limit on nutritional quality of diets obtainable will decline progressively. A deterioration in the nutritional status of individuals would be expected as population density increases, or habitat quality declines, and the condition of individuals could be monitored to assess the nutritional quality of diets.

Riney (1955) noted that at any instant during metabolism, fat is being deposited or removed from most of the depots simultaneously. Thus, the following order of catabolism refers only to the beginning of the process. Fat reserves are depleted in the following order, subcutaneous fat first, then abdominal cavity fat, and finally bone marrow fat. Hundertmark et al. (1992) determined that skin, carcass, and visceral fat of moose all declined linearly with ingesta-free body fat; this further supports simultaneous utilization of fat depots. Thus, low bone marrow fat indicates that an animal is in prolonged stages of malnutrition (Mech and DelGiudice 1985). Depperschmidt et al. (1987) determined that although kidney fat indices correlate with total body fat content, they are not sensitive when fat levels are at their lowest. Although rump fat may not be useful in differentiating individuals in the poor range condition, it has the greatest sensitivity in differentiating individuals in the fair to good range (Riney 1955). A valuable condition index to body composition should have a linear or nearly linear relationship, with only slight variation (Robbins 1983). The most useful indices provide a continuous and accurate indication of body composition.

Even with the low sample sizes obtained during this project, ultrasound was able to detect a statistically significant decline in fat thickness of yearling bulls during the rut. Furthermore, although these animals lost fat, they are still in a growth phase and did not lose the magnitude of fat expected in mature bulls. The technique illustrated considerations for selection of a condition assessment technique which include accuracy, precision, repeatability, sensitivity, range, potential for use *in vivo*, and field application.

Before total body fat can be effectively predicted by an index, accurate measurement of the index must be achievable. Ultrasound is both accurate $(\pm 1 \text{ mm})$ and precise (< 1 mm) at measuring the thickness of the layer of subcutaneous rump fat. The accuracy and precision of measuring rump fat to predict total body fat must be further validated. However, ultrasonic fat measurement has been used in animal science to predict beef carcass chemical composition (Houghton and Turlington 1992). Rouse and Wilson (1993) found a correlation of 0.84 between ultrasound and carcass measurements for fat cover.

Use of *in vivo* technique such as ultrasound enables repeated measurements to be taken from individuals over time. This will reduce sampling variation and lower required sample sizes. Furthermore, this enables continuous monitoring of individuals in pre- and post-treatment sampling such as in studies of disturbance, habitat degradation, or habitat enhancement effects.

The high precision of ultrasonic fat measurement for measuring fat depots enables detection of subtle changes in condition, thus refining the biologists ability to assess management actions. The range of condition for particular individuals that subcutaneous fat can differentiate may exclude the poor range. However, McGillis (1972) found that subcutaneous rump fat thickness ranged from 1-30 mm during sampling of adult cow moose in Alberta between December and February. Under conditions of a dense moose population in Alberta, rump fat did not decline below measurable levels. This further supports that measuring rump fat may be a technique suitable for evaluating animals in a variety of circumstances such as in the fall to assess condition entering winter and thus relate the quality of the previous summer's range or post-winter during the spring to indicate winter habitat quality. Although ultrasonic measurement of subcutaneous fat may be unable to assess the condition of individuals below a certain level, the technique could be used on a population-wide basis by calculating the proportion of the population with no subcutaneous fat in relation to the animals with measurable fat. In late winter, a likely scenario in harsh climates is that cows without calves will have no measurable fat.

This technique exhibits potential as a reliable means of assessing ungulate body condition. The range of the procedure could be improved on smaller ungulates by the ability to scan kidney fat; this was not consistently possible in moose due to their large size and the limited penetration of ultrasound. Until we are able to accurately and precisely estimate total body fat *in vivo* we must continue to rely on indices. A further improvement to our methodology is the addition of a recording unit to the ultrasound equipment. A camcorder, video cassette recorder, or digital recorder can be linked to enable recording of all images that are scanned. This potentially allows for complete measurement of fat depots on an area basis.

Additionally, ultrasound enables pregnancy detection. Thus, fat variability can be isolated based on pregnancy status, as well as allowing for the use of twinning rates as an additional measure of nutritional status. Rectal scanning enables pregnancy determination as early as 20 days into gestation (Beal et al. 1992). This makes possible

categorization of scanned cows as non-pregnant, single calf pregnancies, or twin pregnancies.

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Period	Date	N	Mean Fat Thickness (cm) Longitudinal Site 2Longitudinal Site 3	
Rut	20 Santambar 1002	E	0.68(0.12) 11 18(0.26)	
	30 September 1992	3	0.08(0.13)a 1.18(0.20)a	
	14 October 1992	5	0.30(0.12)0.78(0.14)b	
Annual				
	30 September 1992	4	$0.70(0.17)a^21.05(0.28)a$	
	14 October 1992	4	0.32(0.15)b0.80(0.17)a	
	26 April 1993	4	0.35(0.16)b1.00(0.16)a	

Table 1. Mean (se) subcutaneous fat thickness of yearling bull moose a 2 longitudinal measurement sites (50% and 60% [posterior of the tuber coxae towards the tuber ischii).

¹ Means within the same column and during the rut that share the same letter are not significantly different (p>0.1) according to paired t-test. ² Means within the same column and during the annual period that share the same letter are not significantly different (p>0.1) according to repeated measures Analysis-of-Variance.



Figure 1. Portable real-time ultrasound image illustrating fat thickness in the rump region of a moose. Shown is a longitudinal cross-section of a portion of the subcutaneous fat layer with the thinnest portion of the fat depot on the left of the screen in the cranial direction of the animal and the thickest portion on the right in the caudal direction. The skin of the animal is the narrow uniform band at the top of the screen, and the lower portion of the screen below the fat is a muscle layer. The two white cross-hairs mark the upper and lower bounds of the fat layer which measures 1.6 cm. Scales on top and right side of image are marked in 1 cm increments.



Figure 2. Jammers subcutaneous fat thickness (cm) of 5 yearling bull moose at multiple measurement sites between the tuber coxae (0 on the x-axis) and the tuber ischii (1 on the x-axis). Measurements were obtained during 28 September 1992 - 26 April 1993.



Figure 3. Los subcutaneous fat thickness (cm) of 5 yearling bull moose at multiple measurement sites between the tuber coxae (0 on the x-axis) and the tuber ischii (1 on the x-axis). Measurements were obtained during 28 September 1992 - 26 April 1993.







Figure 5. Soccer subcutaneous fat thickness (cm) of 5 yearling bull moose at multiple measurement sites between the tuber coxae (0 on the x-axis) and the tuber ischii (1 on the x-axis). Measurements were obtained during 28 September 1992 - 26 April 1993.



Figure 6. Wasilli subcutaneous fat thickness (cm) of 5 yearling bull moose at multiple measurement sites between the tuber coxae (0 on the x-axis) and the tuber ischii (1 on the x-axis). Measurements were obtained during 28 September 1992 - 26 April 1993.

Table 1. Mean (se) subcutaneous fat thickness of yearling bull moose at 2 longitudinal measurement sites (50% and 50% posterior of the tuber coxae towards to tuber ischii.

	MEAN FAT THICKNESS (CM)					
DATE	LONGITUDINAL SITE 2	N	LONGITUDINAL SITE 3	N		
28 September 1992	1.15 (0.25) a	2	2.00 (0.4) a	2		
30 September 1992	0.68 (0.13) b	5	I.42 (0.09) b	4		
5 October 1992	0.38 (0,19) bở	4	0,88 (0.21) c	4		
7 October 1992	0.30 (0.12) c	5	0.94 (0.18) c	5		
12 October 1992	0.36 (0.13) bc	5	0.74 (0.12) c	5		
14 October 1992	0.30 (0.12) c	5	0.78 (0.14) c	5		
26 April 1993	0.35 (0.16) bc	4	1.00 (0.16) c	4		

Means within the same column that share the same letter are not significantly different (P<0.05) according to protected LSD.

Alaska's Game Management Units



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ment of Fish and Game uses the funds to help restore, conserve, manage, and enhance wild birds and mammals for the public benefit. These funds are also used to educate hunters to develop the skills, knowledge, and attitudes necessary to be reponsible hunters. Seventy-five percent of the funds for this project are from Federal Aid. The Alaska Department of Fish and Game administers all programs and activities free from discrimination based on race, color, national origin, age, sex, religion, marital status, pregnancy, parenthood, or disability. The department administers all programs and activities in compliance with Title VI of the Civil Rights Act of 1964, Section 504 of the Rehabilitation Act of 1973, Title II of the Americans with Disabilities Act of 1990, the Age Discrimination Act of 1975, and Title IX of the Education Amendments of 1972.

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