ULTRASONIC FAT MEASUREMENT OF CAPTIVE YEARLING BULL MOOSE

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ABSTRACT: The ability to measure fat thickness in live moose offers potential as an index of population condition. Consequently, we evaluated the feasibility of using portable real-time ultrasound to measure body fat in five captive yearling bull moose (*Alces alces*). 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 thebeginning 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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A logical approach for assessing the nutritional status of ungulate populations is the use of condition indices. Techniques to assess body condition include body mass (Riney 1955), skeletal measurements (Kirkpatrick 1980), peroneus muscle group fat (Huot and Goodreault 1985), 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 (DelGiudice *et al.* 1989), twinning rates (Franzmann and Schwartz 1985), physical condition class (Franzmann 1977), tritiated-water (Torbit *et al.* 1985, Schwartz *et al.* 1988), bioelectrical impedance analysis (Hundertmark *et al.* 1992), whole body composition (Torbit *et al.* 1985, Huot and Picard 1988), and ultrasound (Houghton and Turlington 1992).

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 measureof 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 relatively insensitive to short term changes in condition.

A number of techniques are limited in their use because they cannot be applied to live animals. Hundertmark *et al.* (1992) found that peroneus fat was a poor predictor of body fat in moose because of the high standard error of estimates. Bone marrow fat (Mech and DelGiudice 1985) is useful for identifying animals in poor condition but is invalid for individuals in better condition. Similarly, kidney fat index and kidney fat mass are useful primarily for evaluation of the middle range of condition (Depperschimdt *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 duringcapture, 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), phosphorous (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 for monitoring nutritional condition over time. Urinary urea nitrogen, usually expressed as a ratio to creatinine (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 in 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 to chemical estimates of fat content (R^2 = 0.961), 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 lean 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 ofelectrode 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.

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 reproduction study; an additional set of fatmeasurements was obtained for 4 of these bulls during April 1993. The rump region was scanned using an Aloka model 210 portable ultrasound device (Corometrics Medical Systems, Inc., Wallingford, Conn.) 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 (Fig. 1) at multiple sites along a longitudinal line between the spine, at its closest point to the tuber coxae, and the tuber ischii, 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 hand-held animal clipper with a surgical blade; hollow moose hair prevents scanning through the 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.

Validation of the ultrasound for measuring fat thickness was conducted on 2 adult female moose which were euthanized. Pre-mortem ultrasonic measurement of fat thickness at multiple sites and post-mortem caliper measurements via an incision were compared.

Repeated measures Analysis-of-Variance was used to test for a significant difference in fat thickness over time. Because of missing observations during several of the immobilizations, we were able to compare all 5 moose only for the beginning and end of the rut. In a second analysis, fat thicknesses of 4 of the 5 moose were compared among the beginning and end of the rut and April; tests for linear and non-linear relationships among means over time were performed using orthogonal polynomials. 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, and fat thickness at site 3, located at approximately 60% of the distance posterior from the tuber coxae,

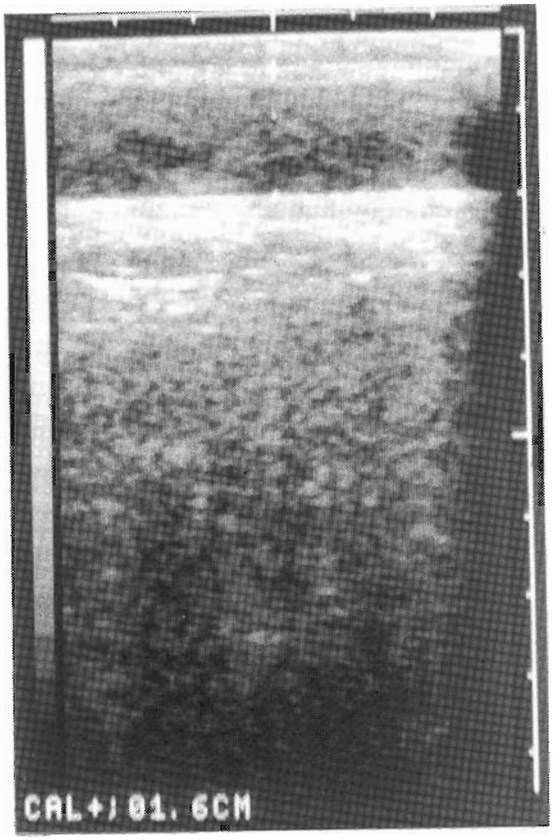


Fig. 1. Portable real-time ultrasound image illustrating fat thickness in the rump region of a moose. Shown is a longitudinal cross-section of a portion of the subcutanous fat layer; the left of the screen is in the cranial direction of the animal and the right side is in the caudal direction. The photographed segment is only 8 cm of a 60 cm region that was measured on 14 October 1992. Although the thickness (1.6 cm as indicated by calipers on screen) is uniform over this 8 cm length, thicknesses measured over the entire region ranged between 0 - 2.4 cm. 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 internal to the fat.

differed between 30 September and 14 October 1992 (F = 15.44, df = 1 and 8, $P = 0.0044$).

A second analysis, using only the observations from the 4 moose sampled in April, was conducted (Table 1). Fat thickness at sites 2 and 3 differed significantly among 30 September 1992,14 October 1992, and 26 April 1993 (F = 6.34, df = 2 and 5, $P = 0.0425$). Orthogonal polynomials revealed a significant curvilinear relationship (F = 14.68, df = 1 and 6, $P = 0.0086$), indicating a decline in fat thickness during the rut and an increase 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 (Fig. 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 zero early in the experiment and showed no indication of change.

Comparison of pre-mortem ultrasonic measurement of fat thickness and post-mortem measurement by incision indicated that the difference between the 2 methods averaged ± 1 mm. Variability between the 2 methods probably resulted more from the difference between a sternally recumbent animal and a hanging carcass, as well as shrinkage of the carcass fat following skinning prior to measurement, than because of equipment precision.

Mean (se) body mass of the 5 bulls was 331 (10.6) kg, 336 (9.7) kg, and 348 (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 = 9.01, df = 2 and 3, $P = 0.0539$). 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. Hall *et al.* (1988) stated that as-

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

Period	Date	N	Mean fat thickness (cm) Site 2	Site 3
Rut				
	30 September 1992	5	0.68 (0.13)[1]	1.18 (0.26)[1]
	14 October 1992	5	0.30 (0.12)	0.78 (0.14)
Annual				
	30 September 1992	4	0.70 (0.17)[2]	1.05 (0.28)[2]
	14 October 1992	4	0.32 (0.15)	0.80 (0.17)
	26 April 1993	4	0.35 (0.16)	1.00 (0.16)

[1] Means between dates during the rut are significantly different (p=0.0044) according to repeated measures Analysis-of-Variance.

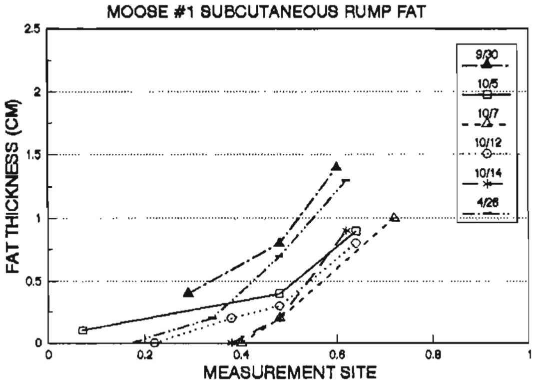
[2] Means among dates during the annual period are significantly different (p=0.0425) according to reapeated measures Analysis-of-Variance. In addition, fat thickness exhibited a significant curvilinear relationship based on orthogonal polynomials (p=0.0086).

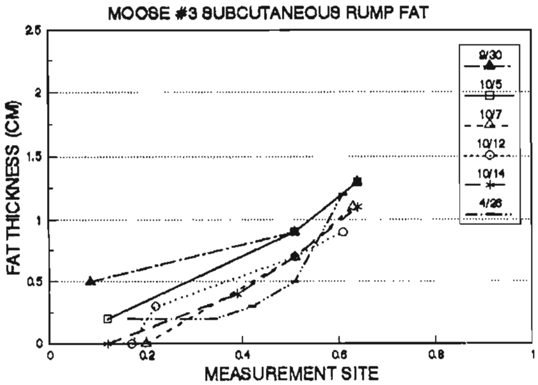
sessment of body composition is important in evaluating nutritional status. Since the animal is a product of its environment we should be able to use individuals to monitor shifts in the environment (Franzmann 1985).

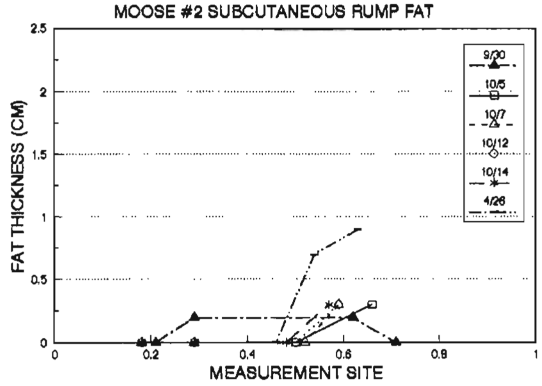
Regelin *et al.* (1987) proposed that determination of body condition of moose will become "a valuable tool for measuring habitat quality and carrying capacity". Accurate body condition indices may reduce the need for expensive and time-consuming vegetation measurements. Schwartz *et al.* (1988) discussed the dynamic nature of fat metabolism in northern cervids and noted 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 beexpected as population density increases, or habitat quality declines, and the condition of individuals could be monitored to assess the nutritional quality of diets.

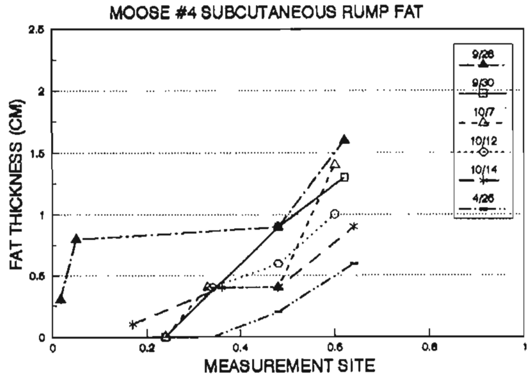
In modelling mule deer survival, Hobbs (1989) determined that starvation mortality was extremely sensitive to changes in prewinter fatness. Model application was dependent on acquiring estimates of fat levels in deer populations.

Riney (1955) noted that at any instant during metabolism, fat is being deposited or removed from most of the depots simultaneously. Fat reserves are depleted in the following order: subcutaneous fat first, then abdominal cavity fat, and finally bone marrow fat. The order of catabolism refers only to the order of disappearance, which is related to the original size of the deposit. 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









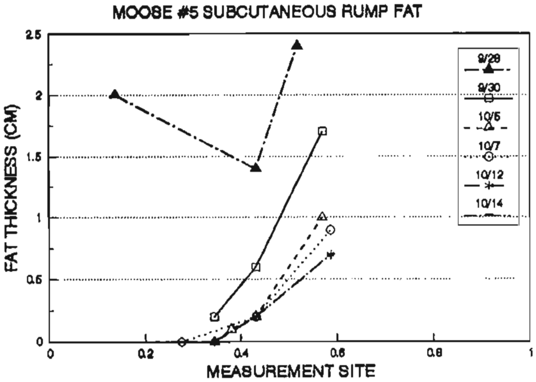


Fig. 2-6. Subcutaneous fat thickness (cm) of 5 yearling bull moose at multiple measurement sites between the tuber coxae (0 on the x-axis) and the tuber ischii (1 on the x-axis). Measurements were obtained during 28 September 1992 - 26 April 1993.

useful in differentiating individuals in the poor range of 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 were 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 (± 1 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 an *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 ofindividuals in pre- and post-treatment sampling such as in studies of disturbance, habitat degradation, or habitat enhancement effects.

The high precision of ultrasound for measuring fat depots enables detection of subtle changes in condition, thus refining the biologists ability to assess management actions. The range of condition that subcutaneous fat can differentiate may preclude obtaining measurements from some individuals in poor condition. 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. Interestingly, under conditions of a dense moose population, rump fat did not decline below measurable levels. This further indicates that measuring rump fat may be a technique suitable for evaluating animals in a variety of circumstances. Fall measurements would be useful to assess condition entering winter and thus reflect quality of the previous summer's range. Post-winter measurements would indicate winter habitat quality and availability. 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 measurable fat but a percentage of cows with 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 the ultrasound unit to enable recording of all images that are scanned. This potentially allows for complete measurement of fat depots on an area basis.

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