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SOME FACTORS AFFECTING PRECISION OF THE TOTAL BODY ELECTRICAL CONDUCTIVITY TECHNIQUE FOR MEASURING BODY COMPOSITION IN LIVE BIRDS

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ABSTRACT.—Measurement of total body electrical conductivity (TOBEC) is a simple nondestructive method for estimating total body fat in live birds. Some published validations of the TOBEC technique have been promising, but other results, especially from species less than 100 g live mass, have indicated that TOBEC measurements add little to the accuracy of body fat estimates obtained with other nondestructive methods. We examined the accuracy of TOBEC body fat estimates for small birds by validating the technique on two passerine species, House Sparrows (*Passer domesticus*) and European Starlings (*Sturnus vulgaris*). Lean mass explained only 57% of the variation in TOBEC for sparrows (average mass: 28.4 g) and 74% of the variation for starlings (average mass: 83.7 g). TOBEC measurements were not sufficiently precise to detect even large (i.e., 100%) changes in fat reserves of either sparrows or starlings. These results, when compared with validations for larger species, indicate that the precision of body composition estimates from TOBEC is very sensitive to subject size in relation to chamber size (coil diameter) of the TOBEC instrument; precision is greatest for subjects that nearly fill the chamber. We confirm that accuracy of TOBEC estimates of body composition in a variety of bird species depends on developing species-specific calibration curves; precision of estimates depends on use of a TOBEC chamber size appropriate to the study species. *Received 13 May 1993, accepted 10 Jan. 1995.*

The total body electrical conductivity (TOBEC) method is a noninvasive technique for estimating body composition in live animals (Walsberg 1988). Recently, the TOBEC technique has gained popularity among field ornithologists as a method for measuring fat reserves of free-ranging birds, either as a means of monitoring temporal changes in fat reserves of individuals or of assessing the relationship of fat reserves to subsequent survival and reproduction (Castro et al. 1990, Morton et al. 1991, Scott et al. 1991, Roby 1991, Skagen et al. 1993). Fat reserves can serve as a measure of physiological condition and can provide a useful index to habitat quality, efficacy of habitat management programs, and impacts of environmental contaminants (Blem 1990).

Research on energetic constraints for birds has been limited by the lack of a simple field technique for nondestructive measurement of fat re-

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serves. TOBEC analysis is attractive because the instrument can be used in the field, is simple to operate, and because measurements can be taken rapidly without invasive procedures. Although TOBEC analysis does not measure fat directly, total body fat can be estimated by subtracting TOBEC-estimated lean body mass from total body mass, determined by weighing the subject (see Harrison 1987, Malina 1987, Fiorotto et al. 1987, Boileau 1988, and Walsberg 1988 for details of operating principles). Alternatively, TOBEC has been used as an independent variable in multiple regression models to enhance the accuracy of body fat predictions from body mass and morphometrics (Morton et al. 1991, Skagen et al. 1993).

Although the SA-1 and SA-2 TOBEC analyzers (Em-Scan, Inc., Springfield, Illinois) are thought to be useful for estimating body composition of subjects as small as 10 g live mass (Walsberg 1988, Castro et al. 1990, Scott et al. 1991), body fat estimates for species at the lower end of this range (10–125 g) could be imprecise. With smaller subjects, there is less interaction between body water volume and the electromagnetic field (Em-Scan, Inc. 1989). Also, small subjects produce small TOBEC values, suggesting lower measurement precision.

The objective of this study was to evaluate the usefulness of the SA-1 TOBEC analyzer for measuring body composition in a wide range of passerines and other small birds. We sought to determine the precision of estimates of body fat and lean body mass for two passerines, European Starling (*Sturnus vulgaris*) and House Sparrow (*Passer domesticus*) and some of the factors that influence the precision and accuracy of these estimates. We wanted to identify a range of subject body sizes where estimates of body fat from the SA-1 TOBEC analyzer were sufficiently accurate to provide a useful index to body condition.

METHODS AND MATERIALS

We mist-netted both House Sparrows and European Starlings in the wild near Carbondale, Illinois and measured TOBEC using the SA-1 Small Animal Body Composition Analyzer (Em-Scan, Inc., Springfield, Illinois, USA). We measured TOBEC for 35 adult House Sparrows; 12 were measured during 4 February–4 March 1990 and 23 during 11–27 April 1991. We measured TOBEC for 63 European Starlings caught between 20 July and 9 August 1990 as they went to roost in the evening. Juvenile starlings were distinguished from adults by plumage color (Kessel 1951). We sexed live House Sparrows using plumage and live adult starlings using iris color and hackle feather morphology (Kessel 1951). Subjects were selected for TOBEC analysis so as to provide a wide range of body masses and approximately equal numbers of the two sexes.

We brought subjects indoors immediately after capture and weighed them to the nearest 0.01 g prior to TOBEC measurement. Subjects were inserted head first into a nonconductive nylon mesh stocking to restrict movement and hold the head and legs close to the body. The beak was inserted through a small hole in the end of the stocking to allow easy res-

piration. Consistent subject placement and restriction of movement are critical for repeatability of TOBEC measurements (Em-Scan, Inc. 1989). We positioned the subject near the center of the vertical axis of the measurement chamber using non-conductive acrylic spacing strips (Walsberg 1988, Em-Scan, Inc. 1989). A rubber band around the center of the body and the acrylic spacing strip provided additional restraint.

We used the protocol described by Roby (1991) to measure TOBEC of live subjects. The SA-1 (unlike the SA-2) continuously displays the impedance of the coil in real-time so that the subject can be centered in the electromagnetic field by moving it slightly in or out and recording the smallest value displayed. Immediately following TOBEC measurements, we humanely sacrificed subjects by cervical dislocation (AOU 1988), placed them in double plastic bags, and froze carcasses at −20°C. Prior to proximate analysis, carcasses were partially thawed, weighed, plucked, and reweighed to determine feather mass. The sexes of European Starlings were verified by inspection of gonads. Carcass analysis procedures followed those described by Roby (1991), except petroleum ether was used as the solvent system (Dobush et al. 1985).

We used a Lilliefor's test to test for normality of the data. We validated the TOBEC technique for each species by regressing TOBEC value against lean body mass determined by proximate analysis. Residuals about the regression line were used to investigate effects of other variables on TOBEC values (e.g., sex, total body fat, feather mass, total body water, % body water of lean mass). Least squares linear regression was used to predict lean mass from TOBEC value. We predicted body fat from TOBEC using the regression equation to estimate lean body mass and subtracting predicted lean body mass from live body mass. We used the inverse regression procedure (Sokal and Rohlf 1981:496) to establish 95% confidence limits for estimates of lean mass and body fat obtained by TOBEC analysis alone. Finally, we used multiple regression analysis with body fat as the dependent variable in order to evaluate the contribution of TOBEC, body mass, and sex for predicting total body fat (Morton et al. 1991, Skagen et al. 1993). Statistical analyses were conducted using SYSTAT 4.0 (Systat, Inc., Evanston, Illinois).

RESULTS

Average body mass, lean body mass, total body fat, and % body fat in the sample of House Sparrows used for the validation were normally distributed ($P > 0.05$, N = 35). Average total body mass for males (28.2 g, SD = 1.50, N = 22) and females (28.6 g, SD = 2.19, N = 13) was not different ($t = -0.65$, $P = 0.52$). Also, average body fat for males (0.99 g, SD = 0.33, N = 17) did not differ from females (1.26 g, SD = 0.51, N = 8; $t = 1.76$, df = 33, $P = 0.09$). The regression of % body fat as a function of total body mass was not significant ($r^2 < 0.02$, $F_{1,23} = 0.35$, $P > 0.05$, N = 25, $b = 0.094$).

Lean body mass explained 54% of the variation in TOBEC ($F_{1,33} = 38.3$, $P < 0.0005$, N = 35, $b = 1.37$). The coefficient of variation in TOBEC (a function of variation in the subject's position within the measurement chamber) for the six trials averaged 6.5% (SD = 2.80, range = 3.2–13.0%, N = 35). For 10 subjects, the standard deviation of % fat among aliquots was greater than 1.0%. When these 10 cases were eliminated, the significance of the regression ($r^2 = 0.55$, $F_{1,23} = 30.0$, $P <$

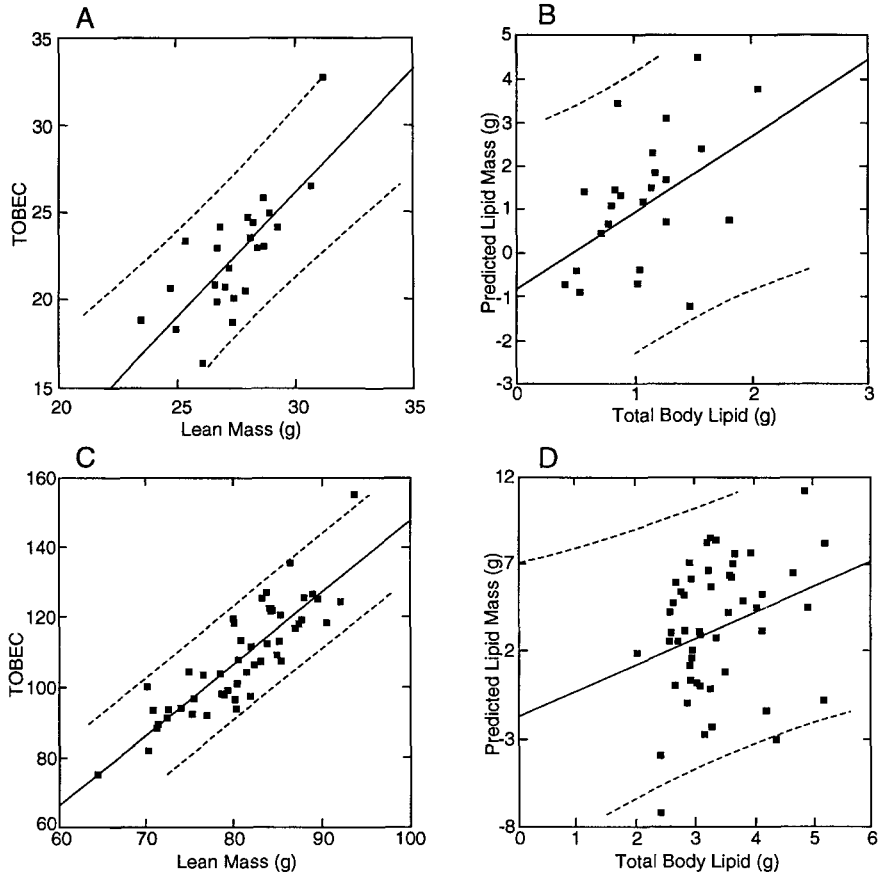

FIG. 1. (A) TOBEC as a function of lean body mass in House Sparrows. (B) TOBEC-estimated body fat as a function of extracted body fat in House Sparrows. (C) TOBEC as a function of lean body mass in European Starlings. (D) TOBEC-estimated body fat as a function of extracted body fat in European Starlings. Dotted lines represent 95% confidence intervals of the estimate of body fat from TOBEC in all graphs.

0.0005, N = 25, b = 1.43) was only slightly higher. For House Sparrows, TOBEC was related to lean body mass by: TOBEC value = −16.657 + 1.427(lean mass). The average residual of the regression of TOBEC on lean mass was 1.68 TOBEC units (SD = 1.33, range = 0.14–4.90, N = 25). The 95% confidence limits for TOBEC-estimated lean mass (derived by inverse regression) were 18.2–26.3 g for 23 g estimated lean mass, 23.4–30.5 g for 27 g estimated lean mass, and 19.0–44.2 g for 31 g estimated lean mass (Fig. 1A).

TOBEC-estimated body fat and solvent-extracted body fat were linearly-related, but the latter explained only 19% of the variance ($r^2 = 0.19$, $F_{1,23} = 5.26$, $P = 0.03$, N = 25, $b = 1.75$). The 95% confidence limits for TOBEC-estimated body fat were −7.37–2.96 g for 0 g estimated body fat, −1.04–8.82 g for 2 g estimated body fat, and 1.46–24.61 g for 5 g estimated body fat (Fig. 1B).

Several variables (% water of lean mass, body fat, % fat of live mass, feather mass, sex) were regressed against the residuals of the regression of TOBEC on lean mass in order to identify factors that affect the accuracy of TOBEC-estimated lean mass; none were correlated with the residuals ($P > 0.05$).

The contribution of TOBEC for predicting body fat was examined using multiple regression analysis, with body fat as the dependent variable and live body mass, TOBEC, and sex as independent variables. The model was significant ($F_{3,21} = 3.85$, $P = 0.024$) but explained only 36% of the variation in body fat. Live body mass ($P = 0.01$) and TOBEC ($P = 0.028$) both contributed significantly to explaining the variation in body fat, but sex did not ($P > 0.05$). However, live body mass was a better predictor of body fat than TOBEC, and live body mass and TOBEC together could explain less than half of the variation in body fat.

Average body mass and lean body mass of starlings used in the validation were normally distributed ($P > 0.05$, N = 63). However, body fat ($P = 0.011$) and % body fat ($P = 0.007$) were not distributed normally. Average body mass of adult males (88.5 g, SD = 3.23, N = 27) was greater than for adult females (79.4 g, SD = 5.04, N = 27; $t = -4.801$, df = 61, $P < 0.0005$). Average juvenile body mass (81.7 g, SD = 8.57, N = 9) was not different from average adult body mass (84.0 g, SD = 6.22, N = 54), but the sample size of juveniles was small. Body fat as a percent of live mass and total body mass were not related ($r^2 < 0.01$, $F_{1,50} = 0.47$, $P = 0.497$, N = 52, $b = 0.012$). Average % body fat of males (4.00%) and females (3.93%) were not different ($t = -0.276$, df = 50, $P = 0.784$). However, lean mass was greater for adult males (85.1 g, SD = 3.18, N = 25) than for adult females (76.3 g, SD = 4.28, N = 20; $t = 7.9$, df = 43, $P < 0.0005$).

The coefficient of variation for the six TOBEC measurements for each subject averaged 1.5% (SD = 1.269, range = 0.0–8.6%, N = 63). Eleven individuals were excluded from the validation because of high variance in % fat of extracted aliquots. The regression of TOBEC as a function of lean body mass was significant ($r^2 = 0.66$, $F_{1,50} = 97.2$, $P < 0.0005$, N = 52, $b = 1.97$). Removal of an outlier caused by an individual with wet plumage during TOBEC analysis increased the correlation ($r^2 = 0.75$, $F_{1,49} = 142.4$, $P < 0.0005$, N = 51, $b = 2.06$). TOBEC was related to

lean body mass by: TOBEC value = −58.143 + 2.063(lean mass). The average residual of the regression of TOBEC on lean mass was 6.03 TOBEC units (SD = 4.60, range = 0.01–19.93 TOBEC units, N = 51). The 95% confidence limits for TOBEC-predicted lean mass were 56.4–72.7 g for 65 g estimated lean mass, 72.3–87.7 g for 80 g estimated lean mass, and 87.3–103.5 g for 95 g estimated lean mass (Fig. 1C).

The regression of TOBEC-estimated body fat on solvent-extracted body fat was only marginally significant ($r^2 = 0.084$, $F_{1,49} = 4.456$, $P = 0.040$, N = 51, $b = 1.473$) and body fat explained only 8% of the variation in TOBEC-estimated body fat. The 95% confidence limits for body fat estimated from TOBEC were −33.5–22.4 g for 0 g estimated body fat, −8.4–25.7 g for 4 g estimated body fat, and 2.9–101.6 g for 10 g estimated body fat (Fig. 1D). Feather mass, water mass, body fat, sex, % water of lean mass, and % body fat of live mass did not explain a significant portion of the variance in the residuals of the regression of TOBEC on lean mass ($P > 0.05$).

The multiple regression model with body fat as the dependent variable and live body mass, TOBEC, and sex as independent variables was significant ($F_{3,47} = 4.585$, $P = 0.007$), but explained only 23% of the variation in body fat. Live body mass ($P = 0.003$) and TOBEC ($P = 0.044$) both contributed significantly to explaining variation in body fat, but sex did not ($P > 0.05$). As with House Sparrows, live body mass of starlings better predicted body fat than did TOBEC, and live body mass and TOBEC together could explain less than half of the variation in body fat.

DISCUSSION

TOBEC was highly correlated with lean mass in the two study species. However, estimates of lean mass from the SA-1 TOBEC analyzer were not sufficiently precise for estimation of body fat in either species. For House Sparrows, the average residual of the regression of TOBEC on lean body mass was 1.678 TOBEC units, which corresponds to 1.18 g of lean body mass. Average body fat was only 1.08 g (SD = 0.407, range = 0.42–2.26 g, N = 25), so the average error in TOBEC-predicted lean mass exceeded average fat reserves. For European Starlings, the average residual was 6.03 TOBEC units which corresponds to 2.92 g lean mass. Average body fat was 3.34 g (SD = 0.743, range = 2.05–5.20 g, N = 52). Consequently, a large change in fat reserves for either sparrows or starlings was not detectable using the SA-1 and the instrument was not precise enough for monitoring temporal variation in body fat in these two species. The large 95% confidence intervals for the estimation of lean mass or fat mass from TOBEC indicate the lack of precision of the SA-1 when used with small birds weighing less than 100 g.

Scott et al. (1991) developed a TOBEC calibration curve for European Starlings based on a sample of 10 individuals. The 95% confidence interval for TOBEC-predicted lean mass (80 g estimated lean mass) was ±2.5 g in that study, compared with ±7.7 g in the present study. Scott et al. (1991) obtained a smaller 95% confidence interval, at least in part, because they treated TOBEC as the dependent variable and lean mass as the independent variable in the regression model. This statistical approach results in smaller confidence intervals (inverse regression need not be used to calculate confidence intervals), but it violates the assumptions of linear regression. Intraspecific variation in TOBEC is primarily a function of variation in lean mass, not the reverse. Linear regression assumes that the independent variable (x) is measured without error and the dependent variable (y) is normally distributed for any fixed value of x (Sokal and Rohlf 1981:496). Lean mass can be measured with reasonable accuracy using proximate analysis techniques, but TOBEC values are subject to substantial error related to the subject's posture and position in the measurement chamber. Consequently, lean mass should be the independent variable and TOBEC the dependent variable.

Linear models best fit the relationship between TOBEC and lean mass for a single species, but a quadratic model best describes curves composed of several species (Walsberg 1988, Scott et al. 1991). A quadratic function fitted to the House Sparrow and European Starling data had an r^2 of 0.98 ($F_{2,83} = 2252$, $P < 0.0005$). TOBEC data for Northern Bobwhite (*Colinus virginianus*) (Roby 1991) were combined with House Sparrow and European Starling data to provide an even wider range of lean body mass. TOBEC was significantly correlated with lean mass, using a simple linear model ($r^2 = 0.95$, $F_{1,121} = 2589$, $P < 0.0005$), but a quadratic function provided a better fit ($r^2 = 0.996$, $F_{2,120} = 12{,}152$, $P < 0.0005$, Fig. 2). The quadratic prediction equation was: TOBEC value = $-2.152 + 0.084$(lean mass) + 0.018(lean mass)^2. However, predicting lean mass of a previously unvalidated species from such a multiple species quadratic curve is likely to result in large errors for two reasons. First, body shape varies considerably among bird species of similar mass, and this variability will influence TOBEC readings. Also, it violates the assumptions of regression (Sokal and Rohlf 1981) to use quadratic prediction equations derived from several species to predict lean mass of a species with a different lean mass. Data for each species are clustered and behave as a point defining the regression line. Fig. 2 illustrates how inaccurate previously derived quadratic prediction equations can be for estimating lean mass of other species. TOBEC should, therefore, be validated for each species being studied (or a closely-related species of similar body mass and shape) and a species-specific regression formulated.

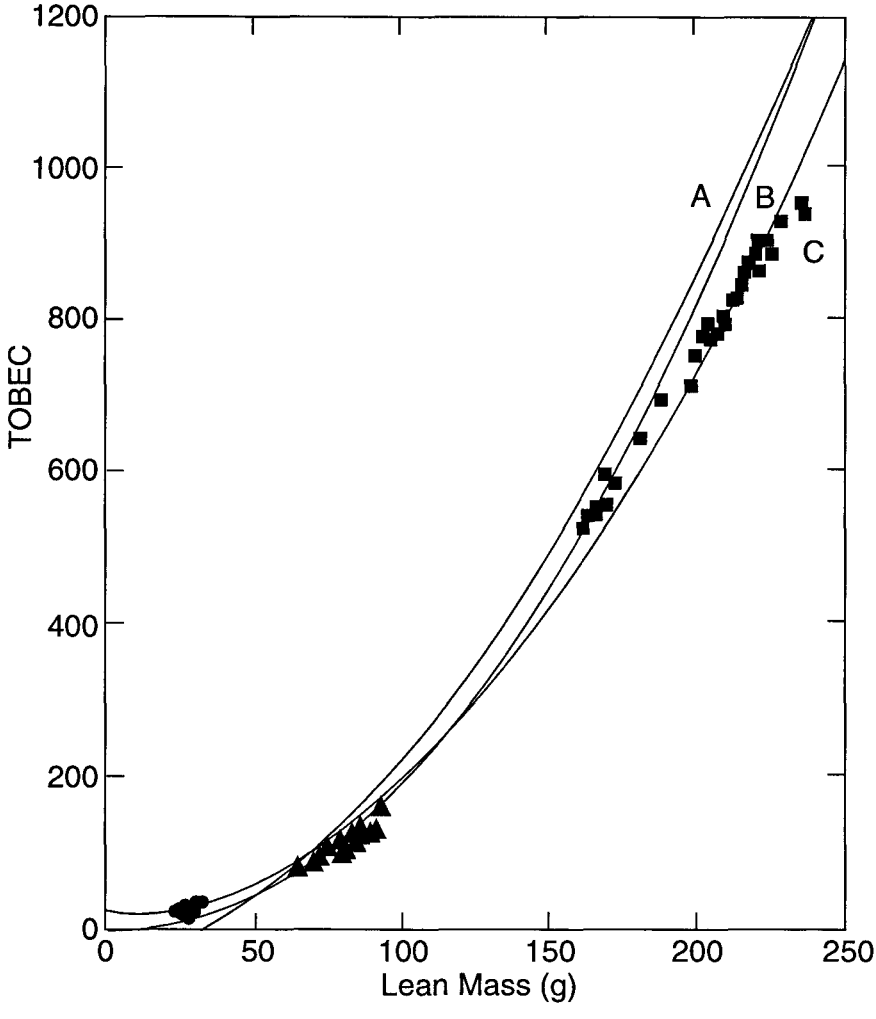

FIG. 2. Quadratic prediction curves of TOBEC vs lean mass derived by (A) Walsberg (1988), (B) Scott et al. (1991), and (C) this study. Circles represent House Sparrows (present study), triangles represent European Starlings (present study), and squares represent Northern Bobwhites (Roby 1991).

The slopes of the regression of TOBEC on lean mass increased with an increase in lean mass for House Sparrows (b = 1.43), European Starlings (b = 2.06), and Northern Bobwhite (b = 5.85). The trend of increased TOBEC as a function of increased subject size can best be described by a quadratic curve (Fig. 3). The curvilinear relationship between

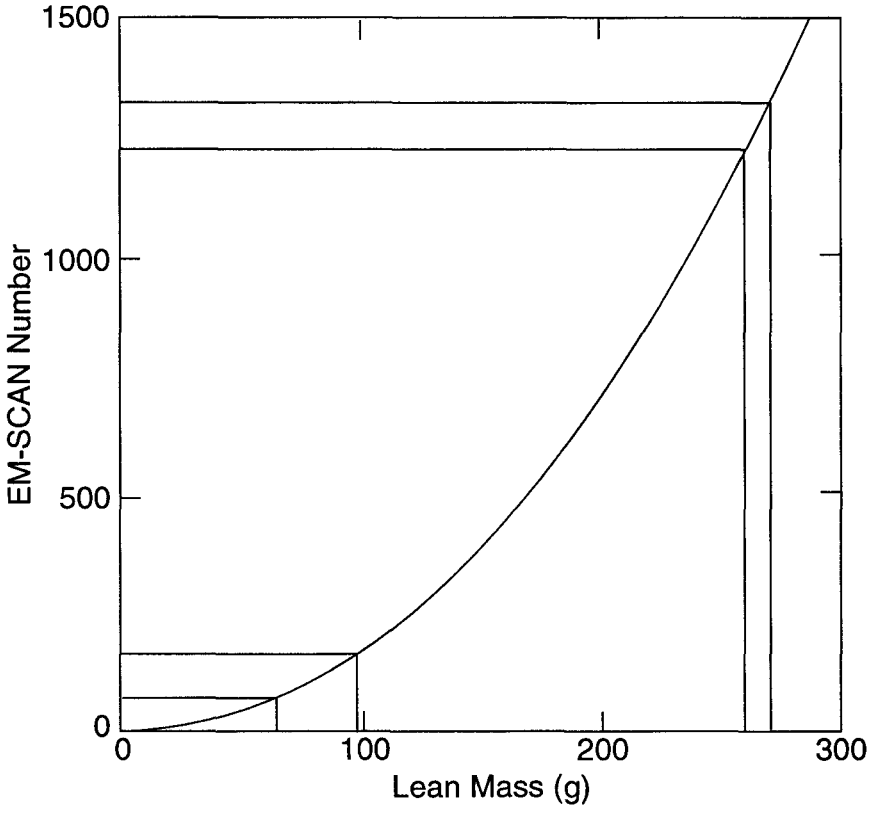

FIG. 3. Quadratic curve fitted to data on TOBEC as a function of lean mass for House Sparrows, European Starlings, and Northern Bobwhites. The upper pair of horizontal lines is separated by the same number of TOBEC units as the lower pair.

TOBEC and lean mass indicates that the SA-1 and SA-2 are less precise for determining lean mass of small birds, such as sparrows and starlings. Precision of the prediction equation would be lower for smaller species because TOBEC values correspond to a wider range of lean mass (Fig. 3). TOBEC values that fall high on the curve will correspond to a relatively narrow range of lean body mass; thus precision will be higher for large subjects. Scott et al. (1991) also found that prediction of lean mass was more affected by error associated with TOBEC value in birds weighing 40–60 g lean mass than larger birds weighing 150–200 g lean mass.

Measurements of a calibration standard varied as much as five TOBEC units from day-to-day. This drift is well within the limits established for proper functioning of the instrument (Em-Scan, Inc., pers. comm.). How-

ever, we can use this error rate to illustrate the instrument's lack of precision for measuring birds <100 g live mass. Using species-specific prediction equations, a change of five TOBEC units corresponds to a change in predicted lean mass of 0.85 g for bobwhite (Roby 1991), 2.42 g for European Starlings, and 3.51 g for House Sparrows. Consequently, a slight change in the accuracy of the TOBEC instrument associated with normal day-to-day variation in measurement of a calibration standard would be of little consequence when measuring a large subject (e.g., bobwhite), but important when measuring smaller subjects. Also, the converse indicates that a small change in lean mass is easily detectable in >200 g subject, but not in a subject <100 g.

This study demonstrates important constraints for using the SA-1 or SA-2 TOBEC analyzers for measuring small amounts of body fat in passerines and other small birds weighing less than about 100 g. Large changes in body fat (such as a doubling of fat reserves) of species the size of House Sparrows or European Starlings would not be detectable by the SA-1 or SA-2 due to the error in estimation of lean body mass from TOBEC. Despite these limitations, we think that TOBEC analysis is a promising technique for research that requires nondestructive measurement of body composition in live birds, particularly when temporal variation in body composition is considerable. Previous validations of the SA-1 and SA-2 indicate it is precise enough for measuring body composition in species approaching the maximum size that can be inserted in the measurement chamber (about 175–275 g body mass; Roby 1991). Precision of body fat estimates in smaller subjects would be correspondingly improved by using a smaller subject chamber equipped with a smaller coil. Development of new instruments with smaller and larger coil diameters than the SA-1 and SA-2 should extend the usefulness of this technique to include the study of a much wider range of subject sizes. A new generation of Small Animal Body Composition Analyzers (SA-3000) are currently being developed that will include a range of measurement chamber sizes (Em-Scan, Inc. 1993). The SA-3000 could make the TOBEC technique more versatile and potentially meet the need for a simple, accurate, and nondestructive method for measuring body composition of birds weighing 10–3000 g.

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