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COMMENTS ON ESTIMATING TOTAL BODY LIPIDS FROM MEASURES OF LEAN MASS[1]

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Physiological condition of wild birds generally is considered to be a function of total body lipids relative to body size. Lipid has twice the caloric density of protein (Ricklefs 1974) and only 0.2–0.3 g lipid/g nonlipid tissue are needed to maintain functional homeostasis in birds (Odum et al. 1964). Lipid levels can be measured accurately by ether extraction of the homogenized carcass, but this requires sacrificing the bird. Alternatively, body mass alone or body mass expressed in some allometric relationship with external structural measurements (to account for individual differences in body size) can be used as an index of lipid levels in live birds (see Johnson et al. 1985 and references therein).

Total body electrical conductivity (TOBEC) recently has been used to index lean body mass of several wild avian species (Walsberg 1988, Castro et al. 1990). Because the electrical conductivity of lipids is only 4–5% that of nonlipid tissues (Pethig 1979; cited in Walsberg 1988), currently used commercial devices primarily measure lean mass. Regression analyses have shown that 95–99% of the variation in TOBEC values are attributable to differences in lean body mass (Walsberg 1988, Castro et al. 1990); predicted lean body mass can then be subtracted from total body mass to estimate lipid mass. Because the technique is simple, noninvasive (as opposed to lipid extraction methodologies), and appears to be more accurate than conventional indices for measuring physiological condition of live birds, it significantly expands the possibilities of conducting nutritional studies under field situations.

While studying the applications of TOBEC, we found that R^2 values for lean mass regressions may misrepresent the precision with which lipid mass can be estimated. Lipid and lean masses sum to body mass by definition. It has been implicitly assumed in the literature that prediction of one component should permit prediction (with the same confidence) of the other. In this paper, however, we use body composition data from dead American Woodcocks (*Philohela minor*) to show that although the absolute value of the error associated with predicting lean mass is identical to that associated with calculating lipid mass (i.e., from predicted lean mass), the relative error is much greater for the latter. Furthermore, body mass and TOBEC values regressed on lipid mass directly predict lipid mass better than subtracting predicted lean mass from body mass. The fact that TOBEC is lower in dead birds than live birds (Castro et al. 1990) is irrelevant because the problem we identify in this note is fundamentally statistical and not methodological.

METHODS

Fourteen Woodcocks were collected in December 1988 and January 1989 on the Eastern Shore National Wildlife Refuge, Virginia. Specimens were double-bagged in plastic and frozen. After completely thawing, Woodcocks were sexed and aged by plumage characteristics (Larson and Tabor 1980) and weighed to the nearest gram.

TOBEC was measured with an EM-SCAN SA-1 Small Animal Body Composition Analyzer using the procedures outlined in Walsberg (1988). Each Woodcock was placed on its dorsum with spine straight, aligned along the long axis of the chamber, and centered in the middle of the measurement chamber. An index of lean body mass (I_{LM}) was calculated as

$$I_{LM} = (S - E)/R; \quad (1)$$

where S = average of four chamber measurements with sample, E = average of four empty chamber measurements, and R = average of two calibration measurements (Walsberg 1988, Castro et al. 1990).

The carcass was plucked, and bill, tarsi, and contents of esophagus, proventriculus, and viscera were discarded. The carcass was then sectioned, freeze-dried for ≥48 hr, ground in a commercial Waring blender, and freeze-dried again for 24 hr to obtain dry carcass mass. Lipids in one 7–10 g sample from each carcass homogenate were extracted for ≥12 hr using ethyl ether in a Soxhlet apparatus after oven-drying samples at 55°C for 12 hr. Lean mass (LM) was the difference between whole body and lipid masses.

Lean and lipid masses were regressed on log-transformed I_{LM} (Castro et al. 1990) using simple linear regression techniques (PROC REG, SAS Institute 1985). Relative error was expressed as $|\hat{y} - y|$ divided by y. Body mass (BM) and log I_{LM} were subsequently regressed on lipid mass using multiple regression tech-

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niques (PROC REG, SAS Institute 1985). Lipid mass predicted from the multiple regression model was denoted by $\hat{LI}$, whereas lipid mass estimated from the difference between BM and $\hat{LM}$ was denoted by $\hat{LI}^*$. Significance was defined as $P \leq 0.05$ for all statistical inference.

RESULTS AND DISCUSSION

Our sample of Woodcocks included 10 juvenile males and 4 juvenile females; $n = 14$ for all subsequent analyses. Mean body mass was 163 g (SE = 8.5) and ranged 106–215 g. Mean lipid mass was 13.2 g (SE = 1.6), ranged 1.0–21.6 g, and represented 7.8% (SE = 0.9) of whole body mass.

Lean mass regressed on I_{LM} yielded the following equation:

$$\log I_{LM} = 4.6572 + 0.0087\ \hat{LM}. \quad (2)$$

The R^2 value for this regression model was 0.725 ($P = 0.0001$). In contrast, the regression between lipid mass and log I_{LM} yielded an R^2 of 0.317 ($P = 0.036$).

Lean mass was subsequently predicted from the inverted regression equation shown above (Eq. 2):

$$\hat{LM} = -532.85 + 114.42(\log I_{LM}). \quad (3)$$

As expected, Eq. 3 predicted lean mass well; the mean difference in lean mass between predicted and laboratory values was 11.8 g (SE = 3.1), which is within 8.9% (SE = 2.9) of laboratory values. Similarly, estimates of lipid mass derived from predicted values of lean mass ($\hat{LI}^* = BM - \hat{LM}$) differed 11.8 g (SE = 3.1) from laboratory values; however, this translates to estimates that are only within 362% (SE = 236) of laboratory values.

It is apparent from these data that the absolute error (11.8 g) associated with $\hat{LM}$ is identical to the error associated with $\hat{LI}^*$. However, because lipid always represents a smaller proportion of avian body mass than nonlipid tissue (Griminger 1976), the proportional error will be correspondingly larger for estimates of the lipid fraction than the nonlipid fraction. This disparity is reflected empirically in the very different coefficients of determination for regressions of lipid and lean masses on log I_{LM}.

This difference can be further explored theoretically by examining the sources of error for parameter estimation and by considering an alternative multiple regression model for predicting lipid mass. The model implied by subtracting $\hat{LM}$ from BM is a two-stage model. The first stage of the model is derived from Eq. 3:

$$LM = \gamma_0 + \gamma_1(\log I_{LM}). \quad (4)$$

The regression parameters are estimated from the simple linear regression between LM and log I_{LM} (Eq. 2), and from the inverted regression (Eq. 3). The second stage of the model is given as the following:

$$LI^* = BM - LM. \quad (5)$$

Combining the two stages results in the following model:

$$LI^* = BM - \gamma_0 - \gamma_1(\log I_{LM}). \quad (6)$$

This model has a fixed slope for BM of 1.0 and the parameters γ_0 and γ_1 are estimated from the association between LM and log I_{LM}; this is not an efficient way to estimate lipid mass.

In contrast, body mass and log I_{LM} can be used to predict lipid mass ($\hat{LI}$) in the following general multiple regression model:

$$LI = \beta_0 + \beta_1(BM) + \beta_2(\log I_{LM}). \quad (7)$$

The power of BM and log I_{LM} to predict lipid mass is indicated by the reduction of the sum of squares for lipid mass. The sum of squares error,

$$SSE = \Sigma\ (LI - \hat{LI})^2, \quad (8)$$

relative to the sum of squares for lipid mass gives the proportion of variation unexplained by the model used to predict lipid mass. For the two-stage model, however, SSE is equal to the sum of squares error for lean mass because

$$SSE^* = \Sigma\ (LI - \hat{LI}^*)^2 = \Sigma\ (LM - \hat{LM})^2, \quad (9)$$

and because $LI = BM - LM$ and $\hat{LI}^* = BM - \hat{LM}$. The problem with the two-stage model is that if lean mass has a high SSE then it is possible that the SSE for lipid mass is actually larger than the total sum of squares for the model! This situation could occur when lean mass is poorly estimated or when lean mass has a much larger variance than lipid mass (even though LM may be well estimated).

As the estimated coefficient of determination using SSE is not appropriate in this application (i.e., it could be negative), another approach would be to compare the squared correlation between LI and $\hat{LI}^*$ with the coefficient of determination for the multiple regression model (Kleinbaum et al. 1988:330). The two-stage model yielded $r = 0.416$ ($P = 0.139$) or $R^2 = 0.173$. In contrast, the multiple regression model yielded an R^2 of 0.692 ($P = 0.002$); partial correlation coefficients for BM and log I_{LM} were 0.549 ($P = 0.004$) and 0.115 ($P = 0.257$), respectively. The higher coefficient of determination represents a 300% increase in association between lipid mass and the explanatory variables.

Multiple regression models also are more flexible than the two-stage approach because they allow the addition of covariates. For example, with the addition of sex into the above model (Eq. 7), the R^2 increased to 0.828 ($P = 0.0004$) and sex explained 44% of the variation in lipid mass ($P = 0.018$).

The multiple regression model confirms several points made both empirically and theoretically in the previous discussion. Because the variation in lipid mass is primarily reflected in body mass and not lean mass, body mass should be incorporated into the regression model rather than being used externally to the model to calculate lipid mass from predicted lean mass. Secondly, although body mass and log (I_{LM}) are highly correlated with each other ($r = 0.83$, $P = 0.0002$), these variables do not contain completely redundant information (see Hamilton 1987); in this case, the inclusion of log I_{LM} explained 11% more of the variance in lipid mass than body mass alone. Thirdly, TOBEC values do not explicitly measure lean mass and need not be used to calculate lean mass directly; rather, TOBEC values can be used to correct for individual differences in body size using multiple regression techniques in

much the same way as more conventional lipid indices have been calculated in the past (e.g., Whyte and Bolen 1984, Ringelman and Szymczak 1985).

Our intent is not to disparage use of TOBEC or other measures of lean body mass (e.g., Connell et al. 1960, Child and Marshall 1970, Pasco and Rutishauser 1985). To the contrary, we believe that TOBEC does estimate body lipids in live birds better than most conventional condition indices, albeit only slightly. However, the current practice of reporting R^2 values for regression models of lean mass on TOBEC grossly overestimates the precision with which body lipids are being estimated. We suggest that investigators using measures of lean mass, such as TOBEC, should apply cross-validation techniques (Kleinbaum et al. 1988:330) or confidence intervals (Castro et al. 1990) to investigate the appropriateness of equations for predicting lipid mass. Furthermore, we suggest using TOBEC within multiple regression models to predict lipid mass, rather than using TOBEC to predict lean mass.

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