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Percent carcass fat is often considered a primary condition indice in game bird species. Although regarded as the standard for determining fat reserves, traditional sampling methods require sacrificing animals for chemical analysis via fat extraction. Lethal methods negate the ability to track condition of individuals through time. Avian physiology studies often require the assessment of conditional changes through time and among various treatments, which necessitate the use of a non-lethal method for estimating fat levels. We were able to accurately estimate fat condition in captive ruffed grouse (*Bonasa umbellus*) utilizing total body electrical conductivity (TOBEC). We developed predictive models to estimate percent carcass fat directly from first-order regression of TOBEC and body mass values. Validation of our best model from an independent sample (*n* = 10 individuals) produced an $R^2 = 0.85 (P < 0.001)$ for determining percent carcass fat and $R^2 = 0.89 (P < 0.001)$ for determining total fat mass in ruffed grouse. Future studies investigating galliform ecology or physiology could benefit from use of TOBEC for assessment of fat condition if non-lethal sampling is desired to track changes through time.

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Key words: *Bonasa umbellus*, carcass fat, fat condition, ruffed grouse, TOBEC, total body electrical conductivity

Introduction

Investigations of nutrition often necessitate repeated measures of individual body condition. The standard method of determining fat via proximate analysis requires the death of the animal (Reynolds and Kunz 2001). Although accurate and precise, use of this method precludes repeated measures of individual fat condition through time. Few nonlethal methods are available to estimate fat condition in gamebird species. Morphometric and body size indices have been widely used but are often inconsistent, observer-biased, and generally lack the precision of other non-lethal methods (Hayes and Shonkwiler 2001, Servello et al. 2005). Isotope dilution methods accurately estimated fat condition in chukar (*Alectoris chukar*) and domestic chickens, but require expertise as well as expensive laboratory equipment for analysis (Speakman et al. 2001, Servello et al. 2005).

In contrast, total body electrical conductivity (TOBEC) technology is a non-lethal, accurate, and relatively simple method of determining body condition in animals given appropriate validation and if hydration status and gastrointestinal fill of subjects are accounted for (Walsberg 1988, Scott et al. 2001, Servello et al. 2005). Use of TOBEC for wildlife applications was first employed by Walsberg (1988) who determined lean body mass and lipid stores in various small mammals and passerine species. TOBEC has been used to determine body composition in northern bobwhite (*Colinus virginianus*) (Roby 1991, Frawley et al. 1999), American woodcock (*Philohela minor*) (Morton et al. 1991), and ring-necked pheasant (*Phasianus colchicus*) (Purvis et al. 1999).

As part of a nutritional ecology study, we used TOBEC to estimate body composition of captive female ruffed grouse (*Bonasa umbellus*). Herein we report on the efficacy of TOBEC to accurately determine fat condition in ruffed grouse.

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Study Area

Ruffed grouse used for this research were housed in the ruffed grouse facility at West Virginia University's animal research farm in Morgantown, West Virginia, USA. A ruffed grouse colony was started in 1990 with 12 fertile eggs acquired from a wild nest found near Buckhannon, West Virginia, USA (subspecies *B. u. monticola*). Between 1991 to 2001 ruffed grouse from West Virginia, Pennsylvania, and Minnesota were added to the colony to increase genetic diversity and limit the amount of genetic crossing. At the time of this research there were 190 adult ruffed grouse at the facility. All birds are kept in individual 60×60×60-cm cages with wire floors in a curtain-sided, poultry-style building. Forced-air heaters are used in winter to keep temperatures above 10° C. All ruffed grouse are kept on a natural lighting schedule and are fed a standard turkey maintenance ration, with grit and water provided ad libitum.

Methods

Sixteen female ruffed grouse were randomly sampled to develop fat condition predictive models; 7 juvenile (<1 year) females sampled in December 2003 and 9 adult females sampled in October 2005. We sampled only females because the objectives of the overall research project focused on female ruffed grouse reproductive success in relation to body condition (A. B. Proctor and J. W. Edwards, West Virginia University, unpublished data). Prior to sampling, all individuals were assumed to be in good health given normal activity and feeding behavior, had access to free water, and were maintained on a commercial turkey maintenance ration with grit ad libitum, hence all grouse sampled were assumed to be under normal gastrointestinal fill and hydration.

TOBEC sampling

We fashioned a TOBEC scanning restraint by cutting a 53×35-cm piece of soft, pliable, opaque plastic sheeting that would extend from the tail to >2.5 cm beyond the head of an adult grouse. We used 2 sets of self-adhering Velcro strips to close the restraint. We tested the dielectric properties of the empty restraint within the TOBEC scanning chamber and found it to not register a value, indicating it would not influence sampling results. For sampling, we first tared the weight of the empty restraint on an electronic balance. We then positioned the grouse dorsally onto the open restraint and held its wings folded to the body while we snugly "rolled up" the restraint and secured the Velcro strips, making sure that legs were extended posteriorly and not positioned ventrally. Grouse appeared calm once in the restraint. It was important to secure the grouse within the restraint to restrict movement and insure that they remained motionless during the TOBEC scanning process (EM-SCAN Inc 1993).

We weighed each grouse to the nearest 0.1 g on an electronic balance prior to determining a TOBEC value using an EM-SCAN Model SA-3000 small animal body composition analyzer with a 114 mm Model 3114 detection chamber (EM-SCAN, Springfield, Illinois, USA). We recorded 5 scans to obtain an average TOBEC value for each grouse. Total sampling time (mass determination, placement in restraint, and 5 TOBEC scans) averaged 8-10 min. EM-SCAN Inc (1993) recommends that the coefficient of variation of all measurements for individual subjects not exceed 3%. In preliminary trials, we found that a 3% coefficient of variation approximated a 20-unit range among 5 scans. Therefore, we would record 5 scans initially; if the range of these scans exceeded 20 units, outliers were discarded and additional scans were taken until the 3% coefficient of variation requirement was satisfied (Frawley et al. 1999, Purvis et al. 1999). Immediately following TOBEC sampling, we sacrificed grouse via carbon dioxide asphyxiation. Handling and euthanasia procedures followed West Virginia University's Animal Care and Use Committee protocol number 03-0913. Sacrificed grouse carcasses were placed in air-tight plastic bags and frozen.

Proximate analysis

Carcasses were allowed to partially thaw and prepared by removing feathers, head, legs below the tibio-tarsus-tarsometatarsus junction, and gastrointestinal and reproductive tracts (Norman and Kirkpatrick 1984). The remaining carcass was cut into 2-3 cm pieces, ground in a commercial meat grinder and frozen. Frozen ground contents were lyophilized to constant mass to determine moisture content. Lyophilized contents were homogenized in a commercial blender and subsampled for analysis. Proximate analyses of samples were performed in duplicate at West Virginia University's Rumen Fermentation Profiling laboratory. Percent fat of sacrifice homogenates was determined using ether extraction in a Sohxlet apparatus following the Association of Official Analytical Chemists (AOAC) protocol 920.39 (Association of Official Analytical Chemists 1990).

Statistical Procedures

Percent carcass fat values were arcsine transformed (Zar 1999) and tested for normality (PROC UNIVARIATE, SAS Institute, Inc. 2002-2004). A first-order polynomial regression model was expected to best explain predicted total fat mass and percent carcass fat from chemical analysis of the 16 grouse used for predictive models (Scott et al. 2001). We developed *a priori* candidate models for total fat mass and percent carcass fat using body mass and TOBEC value as predictor variables. We used a global model incorporating both body mass and TOBEC value (models 3 and 6) to explain percent carcass fat and total fat mass, as well as each predictor variable on its own (models 1, 2, 4, and 5). We used regression analysis (PROC REG, SAS Institute, Inc. 2002-2004) to develop predictive models for total fat mass and percent carcass fat. We used direct models to predict total fat mass and percent carcass fat from TOBEC value and body mass. Morton et al. (1991) and Snyder et al. (2005) recommended the use of direct models for predicting fat over 2-stage models where predicted lean mass is subtracted from total body mass due to increased relative error associated with the latter approach. Three candidate models were used to predict total fat mass (TFM) and percent carcass fat (PCF), respectively:

Model 1: TFM = Body mass

Model 2: TFM = TOBEC value

Model 3: TFM = Body mass + TOBEC value

Model 4: PCF = Body mass

Model 5: PCF = TOBEC value

Model 6: PCF = Body mass + TOBEC value

We evaluated models based on Akaike's Information Criterion (AIC) adjusted for small sample size (AIC_c), AIC_c differences (Δ_i), and Akaike weights (ω_i) (Burnham and Anderson 2002). Models with AIC_c differences ≤ 2 were considered competing models (Burnham and Anderson 2002). Akaike weight (ω_i) estimates the probability that a particular model is the best model in the candidate set (Burnham and Anderson 2002).

We validated our best models on an independent group of 10 female ruffed grouse (5 juveniles and 5 adults) that were sampled for body mass and TOBEC value and sacrificed on 18 February 2005. This validation set was sampled and processed exactly as the 16 grouse used in model development. Statistics are reported on transformed data in this manuscript while results are shown for untransformed data.

Results

Mean coefficient of variation of TOBEC values among all sacrifices was 1.08%. Total body water of 16 grouse used for predictive equation formation was 77.45 $\pm$ 0.64% (mean $\pm$ SE, range = 72.70-81.41), and 75.15 $\pm$ 1.13% (70.44-83.44) for grouse from the validation group. Percent carcass fat of grouse used in predictive models was 15.97 $\pm$ 2.19 (3.38-30.89), and 23.15 $\pm$ 3.27 (2.85-37.31) for those in the validation set. Our global models were the only supported models for predicting TFM (Model 3, ω_i = 0.98) and PCF (Model 6, ω_i = 0.98) (Table 1). In both best models, live body mass was positively related to TFM and PCF:

Model 3: TFM = -79.457 + (0.310 $\times$ BM) - (0.164 $\times$ TOBEC)

Model 6: PCF = -27.621 + (0.155 $\times$ BM) - (0.082 $\times$ TOBEC)

Table 1: Information theoretic model selection using Akaike's Second Order Criterion (AIC_c) for determining predicted total fat mass (TFM) and percent carcass fat (PCF) for both years (n = 16). Data fit using logistic regression in SAS (PROC REG, SAS Institute 2002-2004).

Model	K	Log-L[a]	AIC_c	Δ_i	ω_i	R^2
Total fat mass						
TFM = Body mass + TOBEC	4	-28.24	68.12	0.00	0.98	0.83
TFM = Body mass	3	-34.11	76.21	8.09	0.02	0.64
TFM = TOBEC	3	-41.33	90.67	22.55	0.00	0.11
Percent carcass fat						
PCF = Body mass + TOBEC	4	-16.95	45.54	0.00	0.98	0.83
PCF = Body mass	3	-22.85	53.70	8.15	0.02	0.65
PCF = TOBEC	3	-30.19	68.38	22.84	0.00	0.12

[a] Log-likelihood value

Where,

BM = Live body mass (g)

TOBEC = Average value of 5 TOBEC scans

Body mass and TOBEC were excellent predictors of fat condition in our validation samples explaining 85% ($R^2 = 0.85, P = 0.001$) of variation in percent carcass fat and 89% ($R^2 = 0.89, P = 0.001$) of variation in total fat mass among individuals. Our relative error for predicting percent carcass fat was 3.73 ± 1.62% and our absolute error was 7.62 ± 4.94 g (mean ± 95% confidence interval) (Table 2).

Discussion

We found measures of TOBEC and body mass to accurately predict carcass fat in ruffed grouse. Moreover, the addition of TOBEC as a response variable in our models substantially increased the amount of variation in carcass fat explained compared to models where body mass was the single predictor variable. Previous studies of galliform fat condition using TOBEC have reported mixed results. Roby (1991) reported TOBEC as a reliable estimator of fat condition from a sample of 52 captive and 11 wild-caught bobwhite quail, explaining 92% of the variation in total body lipid. Frawley et al. (1999) found body mass to be the best predictor of fat condition in bobwhite quail and reported limited predictive support when combining TOBEC and body mass measures. Purvis et al. (1999) reported that fat estimation from TOBEC and body mass in wild ring-necked pheasant was highly variable, and suspected that the variation in precision was due to hydration status and gastrointestinal fill. Consistent hydration status and normal feeding are important considerations to accurately assessing fat condition. Our use of captive birds with access to free water and feed likely reduced variation in these factors that might be experienced in wild populations.

Use of TOBEC for body condition studies requires a brief acclimatization period where one must become comfortable with the device and method of subject restraint. Throughout our trials, the TOBEC unit would occasionally produce obviously erroneous scan outputs. For example, where previous scans on a subject centered around 400 (TOBEC units), the next might have been 1200, which was obviously an erroneous scan and should be recorded as such. We suspected that electrical fields within the facility could have produced these results but were never certain. EM-SCAN Inc (1993) cautions that

Table 2: Validation of best models for fat mass (g) and percent carcass fat on an independent sample of female ruffed grouse (n = 10) from West Virginia University's Animal Sciences Farm, Morgantown, West Virginia, USA, 2003-2005. Differences between actual and predicted values are absolute. Body mass (g) values were recorded directly before final TOBEC sampling and sacrifice.

Age class[a]	Live mass	Actual fat mass	Predicted fat mass	Fat mass difference[c]	Actual % fat	Predicted % fat	% fat difference[d]
A	530.2	34.22	31.61	2.60	25.66	22.74	2.92
A	406.7	1.92	3.65	1.73	2.85	6.02	3.17
A	661.1	70.99	48.64	22.35	36.32	32.41	3.91
A	616.4	36.27	39.08	2.81	25.88	26.72	0.84
A	573.6	60.95	39.19	21.75	37.31	27.18	10.13
J	611.8	50.55	40.45	10.10	30.59	27.63	2.96
J	490.1	21.39	15.45	5.94	18.42	12.79	5.63
J	431.0	16.67	12.79	3.88	15.54	11.63	3.91
J	554.1	12.74	24.70	3.04	19.74	18.11	1.63
J	510.2	24.58	22.36	2.21	19.21	17.02	2.19
S.E.	25.8	6.66	4.55	2.52	3.27	2.68	0.89
Mean	538.5	34.53	27.79	7.62	23.15	20.22	3.73
95% CI[b]	±0.6	±13.04	±8.92	±4.94	±6.41	±5.25	±1.62

[a] A = adult, J = juvenile

[b] 95% confidence interval

[c] Fat mass difference = predicted fat mass − actual fat mass

[d] % fat difference = predicted % fat − actual % fat

the area chosen to use a TOBEC unit should be as far away as possible from electrical equipment and other possible sources of electrical fields. We found these erroneous scans to occur at a low rate and not effect our overall ability to arrive at an acceptable TOBEC value.

Our findings support the use of TOBEC to assess fat condition in ruffed grouse in captive studies. If hydration state and nutritional conditions are monitored and calibration procedures followed, it can provide a relatively simple method to accurately determine fat condition. Moreover, because it is nonlethal, it is possible to determine repeat measures of fat condition on individuals over time.

Management Implications

Many wildlife nutritional and conditional investigations require that captive animals be used to best represent conditions present in wild populations. In such studies where repeated measures of body condition are necessary, TOBEC can be a viable means to accurately determine percent fat (and hence lean mass) of animals if proper calibration procedures are used. We found the use of TOBEC to be a quick and easy method to determine condition of female ruffed grouse and that the different sizes of EM-SCAN scanning chambers would facilitate its use across a wide variety of galliform species.

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