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USE OF BODY MEASUREMENTS AND SERUM METABOLITES TO ESTIMATE THE NUTRITIONAL STATUS OF MALLARDS WINTERING IN THE MISSISSIPPI ALLUVIAL VALLEY, USA

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ABSTRACT: We collected mallards (*Anas platyrhynchos*) from bottomland hardwood habitats on the Bayou Meto Wildlife Management Area and the White River National Wildlife Refuge, Arkansas County, Arkansas during the winter of 1990 to 1991 to determine if measures of physiological condition could be predicted from structural size, serum metabolite levels, or from direct measures of carcass composition. Serum triglyceride levels were correlated ($r = 0.57$, $P = 0.007$) with total body fat in males and slightly increased the value (from $R^2 = 0.64$ to 0.76) of intact body mass alone for predicting total body fat in males. Overall, however, serum metabolites appeared to be poor indicators of the magnitude of nutrient masses in mallards. Three potential indices of nutritional status were developed from carcass composition data: protein/total ash, fat/total ash, and fat/fat-free body mass. Protein masses of male mallards changed over winter ($P = 0.02$). Consequently, fat-free masses are not constant and represent poor indicators of structural size for mallards wintering in the Mississippi Alluvial Valley.

Key words: Mallards, *Anas platyrhynchos*, serum, chemistry, nutritional status, carcass composition.

INTRODUCTION

Differences in nutritional status among individuals can influence survival and reproduction of waterfowl (Krapu, 1981; Hepp et al., 1986). Alternatively, variation in the magnitude of nutrient masses among individuals may simply reflect differences in structural size (Blem, 1990). Consequently, some researchers have attempted to develop indices of physiological condition to allow comparisons of nutrient masses among individuals while correcting for differences in structural size (Johnson et al., 1985).

Most indices of physiological condition are a ratio of lipid mass to a variable representing structural size. Biological relevance of these condition indices (Robbins, 1983) and their validity among different races, populations, or geographic areas is uncertain at best. For example, an index for estimating total body fat of mallards (*Anas platyrhynchos*) wintering in Colorado (Ringelman and Szymczack, 1985) poorly predicted total body fat of mallards wintering in the Mississippi Alluvial Valley (MAV) (Heitmeyer, 1988). Thus, an accurate index of physiological condition should be developed for mallards in the MAV, where the largest proportion of the continental mallard population winters (Reinecke et al., 1987).

Condition indices should incorporate nutrient measures other than lipids alone to accurately reflect nutritional status. Protein is seldom measured via prediction equations of condition, yet it is necessary to maintain proper body function in birds (Robbins, 1983); and protein stores may be catabolized when daily protein requirements are not met by diet (Heitmeyer, 1988). Protein constituted an average of 88% of the dry, fat-free body mass for five species of birds (Robbins, 1983). Fat-free body mass is often used to adjust for structural size differences when calculating indices of nutritional condition (Johnson et al., 1985; Ringelman and Szymczack, 1985). However, if protein mass changes,

then fat-free body mass will not accurately reflect structural size. Skeletal volume or mass may be a more appropriate indicator of structural size, because skeletal volume is constant during winter based on studies with Canada geese (*Branta canadensis*) (Moser and Rusch, 1988).

Serum metabolites may provide an index of fat and protein status that is less biased by structural size. Serum metabolites have been useful in tracking body composition changes in many bird species during certain seasons and conditions (Griffin et al., 1982; Robin et al., 1987; Cherel et al., 1988; Bacon et al., 1989). Serum assays could be performed to potentially quantify fat and protein composition nonlethally. Concurrent use of blood indices and morphological measurements may be more accurate than using a single measure (Lochmiller et al., 1988), but such approaches are uncommon.

Our objectives were to determine if mallard body protein mass changes over winter, to calculate a predictive equation of mallard total ash mass from morphological measures available on live birds, to develop predictive indices of total body fat and protein for live mallards wintering in the MAV, and to determine if serum metabolites can be used to predict nutritional status and increase predictive ability over equations using only intact body mass and morphological measures.

MATERIALS AND METHODS

Mallards were collected by trapping and shooting from bottomland hardwood habitats on the Bayou Meto Wildlife Management Area and the White River National Wildlife Refuge, Arkansas County, Arkansas (USA). Characteristics of the study areas were described by Christman (1984). Birds were collected during two periods. Collection periods were limited to 2 wk to reduce temporal biases in nutrient storage. Birds in the early winter sample were captured with rocket-nets on 21 November 1990 (Dabbert and Powell, 1993). Birds were collected for the late winter sample from 13 to 17 February 1991 by shooting. Nine morphological measurements were taken after collection: intact body mass to the nearest gram, body length, wing chord length, length of ninth primary, skull length, culmen length, tarsal length, midwing length (Moser and Rusch 1988) and flattened-straightened wing length (FS wing length) (Ringelman and Szymczack 1985). Birds were aged using the criteria of Krapu et al. (1979).

Following measurement, gut contents were removed and the birds sealed in plastic bags and frozen at −20 C until processed. All feathers were sheared from thawed carcasses except primaries and retrices which were plucked by hand. Down and contour feathers missed by the shears were trimmed with scissors. Sheared carcasses were weighed and then processed twice through a Hobart meat grinder (Hobart Corporation, Troy, Ohio, USA). Five hundred gram samples of homogenates were dried to constant weight at 90 C to determine percent carcass moisture. Dried samples were then processed once through a Moulinex coffee grinder (Moulinex-Canada, Concord, Ontario, Canada) to reduce the homogenate to powder. Approximately 10 g samples were placed in cellulose thimbles which had been previously dried to constant weight. Filled thimbles were weighed again and then extracted using petroleum ether in a modified Soxhlet apparatus to remove lipids. After lipid extraction, thimbles containing lean sample material were dried to constant weight as previously described. Grams of body fat for each bird were calculated by multiplying percent body fat by the dry carcass weight. Total carcass lean was calculated by subtracting the grams of body fat from the dry carcass weight. Lean samples were heated at 550 C in a muffle furnace overnight and the remaining ash was weighed. Ash-free lean values were calculated by subtracting the mineral ash component from the total carcass lean. Ash-free lean values were considered to represent protein. The mineral ash component values were considered to represent carcass ash (skeletal mass). Fat-free wet mass was calculated by subtracting the grams of body fat from the intact body mass (Ringelman and Szymczack, 1985).

The November 1991 sample of birds was bled via the venous metatarsus (Dabbert and Powell, 1993) to measure serum metabolite levels. Serum total proteins, albumin, amylase, uric acid, cholesterol, and triglyceride concentrations were determined by the Washington Regional Medical Hospital Lab (Fayetteville, Arkansas, USA) using a Beckman CX4 chemistry analyzer and Beckman reagents including Beckman Multical for calibration (Beckman Instruments, Inc., Fullerton, California, USA). Volunteer Hospitals of America positive controls levels 1 and 2 (Baxter Diagnostics, Inc., McGraw Park, Illinois, USA) were used to standardize these assays. Serum D-beta-hydroxy-

TABLE 1. Mean body mass and carcass constituent masses (g) for mallards collected within two week periods during November 1990 (early winter) and February 1991 (late winter) in Arkansas.

Mass	Males		Females	
	Early (n = 18)[a]	Late (n = 15)[b]	Early (n = 12)[c]	Late (n = 15)[d]
Intact body[e]	1,164 ± 117.9[f]	1,394 ± 126.5	992 ± 85.9	1,209 ± 122.4
Sheared body	1,045 ± 118.3	1,167 ± 99.2	894 ± 79.0	986 ± 27.2
Fat	129 ± 50.5	176 ± 47.2	106 ± 51.0	127 ± 29.5
Protein	254 ± 27.1	272 ± 22.4	209 ± 20.0	225 ± 23.0
Ash	47.1 ± 4.7	47.9 ± 5.3	38.9 ± 4.2	39.2 ± 4.2

[a] One juvenile and 17 adults.
[b] Four juveniles and 11 adults.
[c] Five juveniles and 7 adults.
[d] Five juveniles and 10 adults.
[e] Field measurement.
[f] Mean ± SD.

butyrate (B-HBA) concentrations were determined using an Abbott VP Chemistry Analyzer (Abbott Laboratories, Irving, Texas, USA) and Sigma reagents including a B-HBA calibrator solution and a B-HBA normal control (Sigma Chemical Company, St. Louis, Missouri, USA).

All variables were evaluated for normality (Norusis, 1990); all variables were judged normal after arc tangent transformation of triglyceride values and natural log transformation of uric acid values. Forward selection multiple regression analyses were used to determine the best variables to predict total body fat, protein, and ash (Norusis, 1990). Spearman rank correlation was used to determine relationships between serum metabolites and nutrient masses (Norusis, 1990). Serum metabolite variables with significant relationships with total body fat or protein were used as independent variables along with structural measurements in the multiple regression analyses of the related nutrient mass (Norusis, 1990).

Both analysis of covariance and condition indices were used to test changes in mallard body composition between collections. Two indices of fat were calculated: fat/fat-free body mass (Ringelman and Szymczack, 1985), and fat/total ash. One index of protein was calculated: protein/total ash. Differences in the condition indices, intact body mass, sheared body mass, and fat, protein, and ash masses between early versus late winter collection periods were tested using separate t-tests (Norusis, 1990). Differences in total body fat and protein masses between early and late winter collections were also tested using an analysis of covariance with carcass ash as a covariate (Statistical Package for the Social Sciences, 1988). Differences in total body fat and protein masses between adult and juvenile mallards were tested using the same analysis of covariance with carcass ash as a covariate. All analyses of covariance had homogenous slopes (Statistical Package for the Social Sciences, 1988). Differences in ash masses between adult and juvenile mallards were tested using a separate t-test (Norusis, 1990) to determine if age influences body size of wintering mallards. All data were analyzed by sex because of differences in metabolism and timing of physiological events between male and female birds (Bacon et al., 1989; Heitmeyer, 1988).

RESULTS

Mean intact body mass of both males and females were greater ($P < 0.001$) in late than early winter (Table 1). Mean sheared body mass of both males and females were also greater ($P \leq 0.02$) in late than early winter (Table 1). Carcass ash was not different between collection periods for either sex ($P \geq 0.65$) (Table 1) or between age classes ($P \geq 0.51$). Mallard carcass ash was best predicted as: carcass ash in grams = (5.575 × skull length in mm + (0.109 × intact body mass in grams − (2.714 × culmen length in mm) − 315.375, ($R^2 = 0.854$ $P < 0.0001$). Total body fat and protein mass were not different between adult and juvenile male mallards (Table 2). Total mass of body fat was higher in adult than juvenile female mallards, but total body protein mass was not different between adult and juvenile female mallards (Table 2).

Based on all analyses except fat/fat-free wet mass for males, mean total body fat of

TABLE 2. Use of condition indices and analysis of covariance to detect age related or seasonal differences in nutritional status.

Condition index	Covariate	Test	*P*-value	
			Males[a]	Females[b]
Fat[c]	Carcass ash	ANCOVA[d]	0.88	0.009
Protein[c]	Carcass ash	ANCOVA	0.75	0.14
Fat[c]	None	*t*-test	0.009	0.24
Fat/fat-free wet mass	None	*t*-test	0.14	0.82
Fat/carcass ash	None	*t*-test	0.006	0.32
Fat	Carcass ash	ANCOVA	0.009	0.22
Protein	None	*t*-test	0.043	0.07
Protein/carcass ash	None	*t*-test	0.045	0.13
Protein	Carcass ash	ANCOVA	0.023	0.08

[a] Five juveniles and 28 adults.
[b] Ten juveniles and 17 adults.
[c] Test for differences between age classes.
[d] ANCOVA, analysis of covariance.
[e] All subsequent analyses were used to test for differences between early winter and late winter samples.

females did not differ between early and late winter, but mean total body fat of males was less in early than late winter (Tables 1 and 2). The condition index using fat-free wet mass as a denominator to calibrate fat among different body sizes did not differ between the two collections of male mallards. In all analyses, mean total body protein of males increased ($P \leq 0.045$) from the early to the late winter collections (Tables 1 and 2). For females, total body protein was not different ($P \geq 0.074$) between sampling periods (Tables 1 and 2).

Highly significant proportions of the variances in total body fat and protein of males and females were best predicted by combinations of morphological measurements (Table 3). No serum metabolites were correlated with total body fat or protein except serum triglycerides which were correlated with total body fat of males (Table 4). Serum triglycerides increased the value (from $R^2 = 0.64$ to 0.76, $P < 0.0001$) of intact body mass alone for predicting total body fat of males. The combined equation was: total body fat in grams = (0.317 × intact body mass in grams) + (7,352.3 × arc tangent of serum triglycerides in mg/dl) − 11,736.9. Serum triglycerides were not correlated ($P = 0.42$) with total body fat of females.

DISCUSSION

Body masses of both males and females were comparable to those reported for

TABLE 3. Regression of total body protein (BP) and total body fat (BF) on selected morphological measures (X) for male and female mallards.

Sex	Coefficient of determination (R^2)[a]	Equation[b]
Female (n = 27)	0.610	BF = −12.743 + 0.225 (BDMS) + 5.426 (FSWL) −3.107 (MDWL)
	0.645	BP = −128.599 ± 0.095 (BDMS) + 2.263 (SKLL)
Male (n = 33)	0.636	BF = −184.815 + 0.265 (BDMS)
	0.918	BP = −380.968 + 0.103 (BDMS) + 2.336 (MDWL) + 0.471 (WCDL) + 1.547 (SKLL)

[a] All coefficients of determination significant ($P \leq 0.0001$).
[b] BDMS = intact body mass (g), FSWL = flattened-straightened wing length (mm), MDWL = midwing length (mm), SKLL = skull length (mm), WCDL = wing chord length (mm).

TABLE 4. Correlation of serum chemistry values of 18 male and 12 female mallards with total body fat and protein mass (g).

	r^a			
	Body fat		Body protein	
Assay	Male	Female	Male	Female
Total protein (g/dl)	0.018	0.118	−0.004	−0.057
Albumin (g/dl)	0.026	0.246	−0.512	0.034
Uric acid (mg/dl)	−0.168	0.229	−0.121	0.067
Triglycerides (mg/dl)	0.570*	0.256	0.164	0.285
Cholesterol (mg/dl)	0.052	0.117	−0.244	0.031
D-beta-hydroxybutyrate (mg/dl)	−0.156	0.414	−0.287	0.169

[a] $P > 0.05$ unless otherwise denoted.
* $P = 0.007$.

mallards in the MAV (Delnicki and Reinecke, 1986; Heitmeyer, 1988) and in Texas, USA (Whyte and Bolen, 1984; Whyte et al., 1986). Equations to predict protein content have not been developed previously for mallards. From our results, protein content is highly predictable and possibly more easily predicted than body fat (Table 3). Equations for predicting fat had slightly lower coefficients of determination than those developed by Ringelman and Szymczack (1985), but higher coefficients of determination than developed by Whyte and Bolen (1984). All regressions included morphological measures in addition to intact body mass, except male mallard total body fat (Table 3). Heitmeyer (1988) found that external body measures did not increase the value of intact body mass alone as a predictor of total body fat of female mallards.

In comparing our data with those of Heitmeyer (1988), we agree with Sparling et al. (1992) that predictive equations of waterfowl total body fat and protein are highly specific to location and season.

Serum metabolites were poor indicators of diet in canvasbacks (*Athya valisineria*) (Perry et al., 1986) and they appear to be poor indicators of the magnitude of nutrient masses in mallards. Serum total protein and albumin levels of mallards were maintained even after consuming diets for 9 wk that were potentially more than 50% below recommended protein concentrations (Dabbert, 1991). The correlation coefficient for male mallard serum triglycerides and body fat was similar to those reported for poultry (r = 0.50 to 0.33) (Griffin et al., 1982). Serum triglycerides, however, only slightly improved the predictive ability of equations for male body fat. Serum triglycerides were not correlated with total body fat of females. This lack of correlation for females may have been caused by small sample size; however, lipid metabolism can be different between male and female northern bobwhite (*Colinus virginianus*) during the breeding season (Mcrae and Dimmick, 1982). Growing (16 wk old) domestic turkeys (*Meleagris gallopavo*) have similar sex-related differences in relationships between male and female body fat and serum triglycerides (Bacon et al., 1989). Female turkeys may be able to maintain plasma triglyceride levels longer after fasting than males (Bacon et al., 1989).

Carcass ash used as a covariate to correct for skeletal mass appears to be the best method to calibrate for structural size in analysis of the nutritional status of some waterfowl species (Rave and Baldassarre, 1991; Moorman et al., 1992). Mean total carcass ash in our data set was not different between collections for either sex or between age classes, but mean intact body mass was higher for the late winter collection of both sexes. Thus, either mean total body fat or protein increased from the first

to second collection. Both fat and protein gains contributed to the intact body mass increase of males. Neither mean female total body fat nor protein were different between samples. Thus, the significant increase in intact body mass of females may have been caused by insignificant increases in each of total body fat and protein, because sheared carcass mass was different between samples.

We had low power to detect differences between age classes, because of small sample sizes. Some data indicate juvenile mallards generally have a lower body mass than adults during winter (Delnicki and Reinecke, 1986). Thus samples containing a higher proportion of juveniles would be expected to have lower mean body masses. Heitmeyer (1988) found body fat, protein, and ash masses of wintering adult and juvenile female mallards of 11 different status groups were the same with only two exceptions. Fat masses of adults of middle prebasic molt status were higher than fat masses of juveniles of the same status. Ash masses were higher in early migrant adults which were in alternate plumage and unpaired as compared to juveniles of the same status (Heitmeyer, 1988). We also found higher fat masses in adult females as compared to juveniles. Thus it is possible our comparison of fat masses between early and late winter female mallards is biased, because of a slightly higher proportion of juveniles in the early versus late winter samples. It is unlikely this problem affected our analysis of male mallards, because we found no differences in body mass or carcass constituents between the two male age classes. Further, the late winter sample of male mallards contained the highest proportion of juvenile birds but also had the highest mean intact body and carcass constituent masses.

Change in the protein masses of male mallards over winter confounds the use of lean body mass as an indicator of structural size during winter (Ringelman and Szymczack, 1985). Data similar to ours for protein masses have been found for mallards in other studies (Heitmeyer, 1988) and for other species of waterfowl (Rave and Baldassarre, 1991). Thus, fat-free masses should not be used as indicators of structural size for mallards wintering in the MAV. Alternatively, we provide predictive equations for mallard total carcass ash. If sample sizes are large enough, calibrators of body size may not be required (Heitmeyer, 1988; Miller, 1989). The *P*-values in our study were almost identical between *t*-tests of uncalibrated total body fat and protein and analysis of covariance of total body fat and protein using carcass ash as a covariate for our small sample size. Use of ratios (condition indices) can encourage type II errors (Blem, 1984). Thus, analysis of covariance may be a better test of differences in nutritional status (Blem, 1990) if calibration of the data according to body size appears warranted.

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