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Source: Journal of Wildlife Diseases, 39(3) : 620-626

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-39.3.620

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Journal of Wildlife Diseases, 39(3), 2003, pp. 620–626
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ESTIMATION OF CARCASS FAT AND PROTEIN IN NORTHERN PINTAILS (*ANAS ACUTA*) DURING SPRING MIGRATION

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ABSTRACT: Foraging in stopover areas influences nutritional condition of birds during spring migration. Our purpose was to determine if body mass, percent carcass water, and serum biochemistry would predict energy reserves (carcass fat and protein) in northern pintails (*Anas acuta*) at a spring staging area, Lake St. Pierre in Québec, Canada (46°11′N, 73°08′W). Northern pintails were collected during spring 1997 (14 April–9 May). In this staging area, body mass and percent body water successfully estimated carcass protein and fat in male northern pintails, but only carcass protein in females. None of the seven blood parameters we used accurately estimated nutritional reserves in staging northern pintails. These findings suggest that investigators must use direct estimates of carcass reserves to examine nutrient reserve requirements for egg production, migration, or body maintenance during spring migration.

Key words: *Anas acuta,* carcass composition, northern pintail, nutritional status, serum chemistry, spring migration.

INTRODUCTION

Nutritional and reproductive status of waterfowl are supported by carcass fat and protein reserves (Whittow, 1986; Raveling and Heitmeyer, 1986; Harder and Kirkpatrick, 1994). This physiologic condition is partly a function of foraging in appropriate habitats (Gauthier et al., 1984; Whyte and Bolen, 1984); therefore spring staging areas may be important to allow birds to build energy reserves during and after migration to breeding areas (Olsen, 1994; Austin and Miller, 1995). Changes in body mass during spring or fall migration are common in many North American bird species (Dunn, 2001). Furthermore, the stopover allows waterfowl to build protein reserves that support egg production (Krapu, 1981; Barzen and Serie, 1990; Alisauskas and Ankney, 1992; Krapu and Reinecke, 1992; Mann and Sedinger, 1993; Esler and Grand, 1994). The use of carcass protein during migration (Marsh, 1984) would necessitate its replenishment during the stopover. Some migrants may gain carcass protein equivalent to a third of their body-mass gain during a stopover (Karasov and Pinshow, 1998). Proteins may be stored as muscle or as other functional tissue (Klaassen et al., 1997). The date that a bird arrives at each staging area, and how long it stays, may affect carcass nutrient reserves. Furthermore, the previous winter location and the ultimate nesting region could also influence nutrient reserve status (LaGrange and Dinsmore, 1988).

Lipids are the dominant fuel source during migratory flight in many bird species, and carcass fat has generally been determined by petroleum ether lipid extraction (Dobush et al., 1985; Johnson et al., 1985; Ringelman and Szymczak, 1985; Miller, 1989; Gauthier et al., 1992; Harder and Kirkpatrick, 1994; Dabbert et al., 1997). However, lipid extraction is both expensive and time consuming (Johnson et al., 1985). Because of this, easier and less expensive methods have been developed to estimate body nutrient reserves. These methods involve equations or indices based on: 1) morphometric measurements including body mass (Johnson et al., 1985;

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Ringelman and Szymczak, 1985; Miller, 1989; Smith et al., 1992; Dabbert et al., 1997); 2) amounts of fat and protein in different tissues (Chappell and Titman, 1983; Whyte and Bolen, 1984; Johnson et al., 1985; Hohman and Taylor, 1986; Miller, 1989; Smith et al., 1992; Dabbert et al., 1997); or 3) water content of the carcass (Child and Marshall, 1970; Campbell and Leatherland, 1980; Johnson et al., 1985; Miller, 1989; Conway et al., 1994; Karasov and Pinshow, 1998). For example, body mass was found to be a good predictor of nutrient reserves in northern pintails from Texas (Smith et al., 1992) and California (Miller, 1989).

Blood biochemical analyses reflecting lipid or protein metabolism seem to offer possibilities for estimating carcass reserves. For example, Ksiazkiewicz et al. (1993) found significant correlations ($P<0.05$) between cholesterol levels and abdominal fat deposition ($r=0.48$) and between intestinal fat and cholesterol ($r=0.39$) in female mallards (*Anas platyrhynchos*). Dabbert et al. (1997) found that blood triglyceride levels predicted ($r=0.57$; $P=0.007$) total body fat in mallard drakes. Measurements of plasma total protein, albumin, uric acid, urea, free fatty acids, cholesterol, β-hydroxybutyrate, and thyroid hormones may also give reliable information about nutritional status of birds (Totzke et al., 1999; Gannes, 2001; Hollmén et al., 2001; Alonso-Alvarez et al., 2002).

In this paper, we examined how well body mass, percent body water, and different biochemical compounds predicted carcass fat and protein during spring migration. We expected some blood biochemicals related to lipid metabolism (free fatty acids, triglycerides, cholesterol, and β-hydroxybutyrate) or protein metabolism (total protein, uric acid, albumin) would allow estimation of carcass fat and protein, respectively.

MATERIALS AND METHODS

Study area

We conducted the field study from 14 April to 9 May 1997 at a migratory stopover on the north shore of Lake St. Pierre (St. Lawrence River) in Québec, Canada (46°11′N, 73°08′W). This stopover (246 ha) consists of 80 agricultural fields (plowed fields, stubble fields [corn, barley, buckwheat, soybean], prairies, and abandoned fields) that are flooded during springtime. It is crossed by five drainage ditches and forms a deep basin. The staging area is managed in order to maintain water levels during spring waterfowl migration. It is part of a Ramsar wetlands site as well as a UNESCO Biosphere Reserve and is heavily used by northern pintails, which represent 80% of the 10,000 dabbling ducks present in spring. The spring staging period, which lasts nearly 5 wk, coincides with a period of high food availability for ducks (seeds and invertebrates). In addition, some female pintails nest in the Lake St. Pierre floodplain as reported by Bélanger and Couture (1989).

Specimen gathering

We collected a total of 36 male and 31 female feeding northern pintails using a .22 rifle. We sexed and weighed (hereafter termed "body mass") each bird, obtained blood samples (5–10 ml) by cardiac puncture (Donham, 1979; Hannon, 1979) with a syringe (22G×1½″ needle), and put the blood samples into additive-free glass tubes (Vacutainer®, Becton Dickinson, Rutherford, New Jersey 07070, USA). We retrieved only 30 males and 27 females rapidly enough to obtain an adequate volume of blood. We stored all blood samples at 4 C in the dark until reaching the laboratory. We centrifuged the tubes within 3 hr after their arrival at the laboratory and obtained the sera before freezing them at −20 C pending analysis. We excluded esophagi, gizzards, and ovarian follicles from carcass analysis but stored them for other studies. We distinguished yearlings from adults by first observing differences in the cloacal bursae (bursae of Fabricius), but because this criterion may be not valid during spring for waterfowl (Dane, 1968), we also used wing-feather characteristics that differentiate yearling from adult northern pintails during breeding season (discriminant functions DF and DF2 for males and females, respectively) as proposed by Duncan (1985). Our sample was therefore composed of 47 adults (25 males, 22 females) and 10 yearlings (5 males, 5 females). We stored carcasses at −30 C in plastic bags.

Measurement of body reserves

In the laboratory, we sheared all feathers from the thawed carcasses using electric clippers and scissors. We weighed carcasses again,

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TABLE 1. Variables measured on northern pintails sampled at Lake St. Pierre, Québec, in spring 1997.

	Male		Female	
	Adults (n = 25)[a]	Yearlings (n = 5)[a]	Adults (n = 22)[b]	Yearlings (n = 5)[b]
Body components				
Body mass (g)	1115 ± 110[c]	1110 ± 69[c]	984 ± 85	912 ± 74
Carcass fat (g)	169.0 ± 61.5	174.8 ± 85.0	148.6 ± 46.4	175.7 ± 55.2
Carcass protein (g)	264.5 ± 80.8	269.7 ± 72.5	247.0 ± 75.6	174.4 ± 20.9
Percent body water	52.74 ± 5.15	51.37 ± 3.34	51.25 ± 4.16	53.83 ± 3.78
Blood metabolites				
Free fatty acids (mmol/l)	1.679 ± 1.120	1.750 ± 1.052	1.465 ± 0.803	2.123 ± 1.428
Albumin (g/l)	17 ± 3	18 ± 3	18 ± 3	15 ± 2
Uric acid (μmol/l)	1039 ± 427	1134 ± 347	1005 ± 393	691 ± 82
Cholesterol (mmol/l)	7.00 ± 1.01	7.57 ± 1.15	6.90 ± 1.26	4.98 ± 0.75
Total protein (g/l)	38.0 ± 4.8	39.1 ± 4.6	43.3 ± 6.2	38.8 ± 0.8
Triglycerides (mmol/l)	4.11 ± 1.18	4.31 ± 0.78	5.78 ± 1.50	5.77 ± 2.18
β-hydroxybutyrate (g/l)	0.074 ± 0.038	0.072 ± 0.028	0.069 ± 0.030	0.040

[a] For β-hydroxybutyrate, n = 28 males (24 adults, 4 yearlings).
[b] For β-hydroxybutyrate, n = 22 females (21 adults, 1 yearling).
[c] Mean ± SD.

then ground (Hobart meat grinder, Hobart Corporation®, Troy, Ohio, USA) and homogenized them.

We dried a 100 g sample of the homogenate from each northern pintail to constant mass in a forced-air oven (60 C) to calculate percent body water ("percent water"). We ground each lyophilized sample in a high-speed grinder and weighed 1 g duplicates using an analytical balance. We extracted lipids (total body fat ["carcass fat"]) following Gauthier et al. (1992) using petroleum ether. Finally, we calculated dry lean mass ("carcass protein"): Carcass protein = fat-free dry weight − mineral ash (obtained after incinerating the lyophilized sample at 550 C for 12 hr) (Miller, 1989; Dabbert et al., 1997).

Biochemical analyses

We examined free fatty acids, triglycerides, cholesterol, and β-hydroxybutyrate, which are blood metabolites related to lipid metabolism and total protein, uric acid, and albumin, which are related to protein metabolism (Amand, 1986; Hochleithner, 1994). These serum factors could potentially be used to predict nutritional status (Ksiazkiewicz et al., 1993; Dabbert et al., 1997; Domingo-Roura et al., 2001). Because we collected variable amounts of blood, not all metabolites could be determined in all individuals (we analyzed samples from only 28 males and 22 females for β-hydroxybutyrate).

We used a Hitachi 704 automatic chemical analyzer (Boehringer Mannheim GmbH, Mannheim, Germany) to perform serum biochemical analyses with the procedures and reagents supplied by the company: albumin (Doumas et al., 1971), total protein (procedure proposed by Boehringer), free fatty acids (Shimizy et al., 1980), cholesterol (Siedel et al., 1983; Kattermann et al., 1984), triglycerides (Siedel et al., 1993), β-hydroxybutyrate (Bergmeyer and Bernt, 1965), and uric acid (procedure proposed by Boehringer). We did not conduct duplicate analyses because serum samples were too small (1–2 ml). All analyses were submitted to internal and external quality control processes (Boehringer Mannheim Canada [now Roche Diagnostics Canada], Laboratoire de santé publique du Québec [LSPQ]). Our laboratory was accredited by the LSPQ.

Statistical analyses

We analyzed all data by sex because of the differences in metabolism and the timing of physiologic events between male and female birds. However, for each sex, we grouped the yearlings and adults together due to the small sample size and because there was no significant difference (Mann-Whitney U-test, $P>0.05$) between yearlings and adults for the different variables used in the analyses, except for cholesterol in females ($P=0.003$). Table 1 presents the values (for the different variables measured on the male and female birds) separately for yearlings and adults.

We first tested all of the variables for normality and judged all as normal (albumin values were arc-tangent transformed and free fatty ac-

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TABLE 2. Correlation values (r) of body mass, percent body water, and serum chemistry variables with carcass fat and carcass protein (g) for 30 male and 27 female northern pintails sampled at Lake St. Pierre, Québec, in spring 1997. Significant values are identified with asterisks (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$).

	Males		Females	
	Carcass fat	Carcass protein	Carcass fat	Carcass protein
Body mass (g)	0.41	0.70***	0.24	0.58**
Percent water	−0.53*	−0.60**	−0.08	−0.71***
Free fatty acids (mmol/l)	0.14		−0.35	
Cholesterol (mmol/l)	0.16		0.09	
Triglycerides (mmol/l)	−0.29		−0.05	
β-hydroxybutyrate (g/l)[a]	0.35		−0.35	
Abumin (g/l)		0.29		0.42
Uric acid (μmol/l)		0.44		0.35
Total protein (g/l)		0.04		0.26

[a] Analyses of 28 males and 22 females.

ids values were natural-log transformed). We tested all null hypotheses at the $P<0.05$ significance level. We used the Pearson correlation coefficient to identify the variables (body mass, percent water, or blood metabolite) that best explained carcass fat and carcass protein. We adjusted P values using the Bonferroni method. We used SYSTAT™ software for all analyses (Geissler, 1988; White and Clark, 1994).

RESULTS

Male northern pintails collected at Lake St. Pierre during spring 1997 weighed (mean±SD) 1,114±103 g and females weighed 971±86 g (Table 1). Body mass and percent water correlated significantly with carcass protein (for both males and females), but there were no significant relationships between indices and energy reserves except for carcass fat and percent water in males (Table 2). We found no statistically significant or consistent relationships between serum metabolites and carcass fat or carcass protein in either males or females (Table 2).

DISCUSSION

At Lake St. Pierre, carcass protein was consistently predicted by body mass and percent water. Percent water reflected, at least partially, variations in carcass fat in male northern pintails. For females, no relationship was found between carcass fat and percent water; maybe there was not a sufficient range of carcass fat in the sampled birds. Lastly, we must not forget that excluding the digestive and reproductive organs might have biased the analysis. In other studies, body mass was a fair predictor of carcass fat (Miller, 1989 [r^2=0.63]; Smith et al., 1992 [r^2=0.69–0.74]) and carcass protein (Miller, 1989 [r^2=0.71]). However, carcass fat was estimated more precisely by percent water (Miller, 1989 [r^2=0.92–0.95]; Conway et al., 1994 [r^2=0.86]).

None of the seven blood parameters used in our study accurately estimated nutritional reserves in staging northern pintails. Biochemical parameters only partially reflect nutritional status, because factors other than condition influence physiology (Domingo-Roura et al., 2001). For example, age (Puerta et al., 1990), daily rhythms, starvation, stress, and method of capture can alter blood biochemistry profiles (Harder and Kirkpatrick, 1994). Stress can cause an increase in cholesterol (Franzmann and Thorne, 1970) and protein levels (Laidley and Leatherland, 1988; Marco and Lavin, 1999). Triglyceride levels decline with time between the last triglyceride-rich meal and sampling (Domingo-Roura et al., 2001). Levels of blood proteins are affected by metabolic interactions, such as tissue repair, that create demands on protein reserves and, subsequently, decrease albumin in the blood

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(Domingo-Roura et al., 2001). Moreover, protein and lipid catabolism during long-distance flight (Jenni and Jenni-Eiermann, 1998) can cause an increase in uric acid, free-fatty acids, and β-hydroxybutyrate (Gannes, 2001; Gannes et al., 2001). Blood metabolite composition is strongly affected by diet (Gannes, 2001) and varies according to age, sex, and reproductive condition (Fairbrother et al., 1990). Adults may have lower plasma uric acid and triglyceride levels than young birds (Puerta et al., 1990). Ksiazkiewicz et al. (1993) found significant correlations between cholesterol levels and fat deposition in females, but they did not find any correlation between blood cholesterol levels and fat deposition in mallard drakes.

Estimating carcass fat and protein may be useful and less costly than complete carcass analyses; however, our results suggest that investigators must use direct estimates of carcass reserves to examine nutrient reserves during spring migration or reproduction. Indeed, the destination and origin of the pintails we collected was unknown, nor did we know how long any individual pintail had been in the area. Pintail spring migration through any given area is very complicated and might affect technical results. Additional research with larger samples from additional spring staging areas, together with an understanding of the dynamics of migration of northern pintails through the areas, is needed.

ACKNOWLEDGMENTS

We were financed by La Fondation Héritage Faune (Fédération québécoise de la faune), La Fondation Universitaire du Centre du Québec, and l'Université du Québec à Trois-Rivières (UQTR). We thank the staff of La Société de la faune et des parcs du Québec for technical support and G. Gauthier (Université Laval) for kindly allowing us to use his equipment for carcass analysis. We appreciate the helpful comments of H. Glemet (*UQTR*) and G. Gauthier who reviewed a previous version of the manuscript, A. Aubin (*UQTR*) who helped in study design, and G. Daigle (Consulting Service in Statistics, *Université Laval*) for his advice on the statistical analysis. We thank the following people for reviewing the manuscript: L. Devine, B. Duval, M. R. Miller, and an anonymous Journal of Wildlife Diseases reviewer.

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Received for publication 13 March 2002.

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