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EFFECT OF DIET COMPOSITION ON PLASMA METABOLITE PROFILES IN A MIGRATORY SONGBIRD

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Abstract. Plasma metabolites provide information about the physiological state and fuel use of birds, and have been used for predicting refueling rates of birds during migratory stopovers. However, little is known about the effect of diet on metabolite concentrations in small songbirds. We investigated the effect of dietary macronutrient composition on lipid and protein metabolites in captive White-throated Sparrows (*Zonotrichia albicollis*). Birds fed a high-protein, low-carbohydrate insect diet had lower plasma triglyceride concentrations and higher plasma B-hydroxybutyrate concentrations than birds fed a high-carbohydrate, low-protein grain diet during feeding. The insect-fed birds also had higher plasma uric acid concentrations than grain-fed birds and birds fed a low-protein, high-fat, and high-carbohydrate fruit diet. Diet did not significantly influence plasma concentrations of glycerol or nonesterified fatty acids. After subsequent overnight fasting, birds in all three diet groups had similar concentrations of lipid metabolites, but uric acid was marginally elevated in insect-fed birds. Given that dietary macronutrient composition affected certain plasma metabolite concentrations in sparrows, investigators should consider such diet effects when using these metabolites to estimate refueling rates of free-living migratory songbirds, particularly in species that exhibit dietary plasticity during migration.

Key words: bird migration, diet composition, plasma metabolites, White-throated Sparrow, Zonotrichia albicollis.

Efecto de la Composición de la Dieta Sobre los Perfiles de los Metabolitos del Plasma en un Ave Canora Migratoria

Resumen. Los metabolitos del plasma proveen información acerca del estado fisiológico y el uso de recursos de las aves, y han sido usados para predecir las tasas de reabastecimiento de recursos de las aves durante las paradas migratorias. Sin embargo, se conoce poco acerca de los efectos de la dieta sobre la concentración de los metabolitos en las aves canoras pequeñas. Investigamos el efecto de la composición de los macronutrientes de la dieta de *Zonotrichia albicollis* sobre los metabolitos de lípidos y proteínas en condiciones de cautiverio. Tras ser alimentadas al medio día, las aves con una dieta a base de insectos rica en proteína y baja en carbohidratos tuvieron concentraciones de triglicéridos más bajas y concentraciones de B-hidroxibutirato más altas en el plasma que las aves alimentadas con una dieta a base de granos rica en carbohidratos y baja en proteína. Las aves alimentadas con insectos también tuvieron concentraciones más altas de ácido úrico en el plasma que las aves alimentadas con granos y que las aves alimentadas con una dieta baja en proteína, alta en grasas y alta en carbohidratos derivados de frutas. El tipo de dieta no afectó de manera significativa las concentraciones de glicerol ni de ácidos grasos no esterificados en el plasma. Las concentraciones de los metabolitos de lípidos fueron similares entre las aves de los tres grupos de dieta después de ser sometidas a ayunos durante la noche; sin embargo, el ácido úrico estuvo marginalmente elevado en las aves alimentadas con insectos. Dado que la composición de los macronutrientes de la dieta afectó las concentraciones de algunos metabolitos del plasma en *Z. albicollis*, los investigadores deben considerar los efectos de las dietas cuando usen dichos metabolitos para estimar las tasas de reabastecimiento de las aves canoras migratorias silvestres, particularmente en las especies que exhiben plasticidad en su alimentación durante la migración.

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INTRODUCTION

Annual migrations of small songbirds between breeding and wintering grounds involve energetically demanding nocturnal flights and frequent stops to rest and replenish energy stores. These alternating periods of fasting and feeding result in rapid depletion and deposition of both fat and protein reserves (Ramenofsky 1990, Bauchinger and Biebach 1998, McWilliams et al. 2004). The rate at which fat and protein stores are replenished at stopover sites predominantly depends on daily food intake and food quality (Schaub and Jenni 2001a, Bairlein 2002), so indices of the rate of fat and protein storage have been used to indicate the quality of stopover sites for migrating birds (Schaub and Jenni 2001b, Guglielmo et al. 2005).

A current method for estimating the physiological state of migrating birds during stopover is the analysis of plasma metabolite profiles. Certain circulating plasma metabolites increase during fat catabolism or fatty acid utilization (free fatty acids, glycerol, B-hydroxybutyrate), fat deposition (triglyceride), or protein catabolism (uric acid); thus, sampling blood from individual birds may provide information about their short-term fuel use and storage during migration (Jenni-Eiermann and Jenni 1991, Jenni and Jenni-Eiermann 1992, Landys et al. 2005). Studies of captive and free-living songbirds have demonstrated that plasma concentrations of certain metabolites, such as triglyceride and B-hydroxybutyrate, can distinguish birds that are feeding from birds that are fasting (Swain 1992, Jenni-Eiermann and Jenni 1996, 1997) and are directly related to body mass change over several hours during the day (Jenni-Eiermann and Jenni 1994, Jenni and Schwilch 2001). These metabolites have recently been used to assess refueling rates and habitat quality of free-living migratory songbirds and shorebirds (Schaub and Jenni 2001b, Ydenberg et al. 2002, Guglielmo et al. 2002, 2005, Seaman et al. 2006).

If diet composition influences plasma metabolite concentrations in birds, this could complicate interpretation of metabolites as indicators of physiological state. For example, studies on poultry demonstrated that plasma uric acid concentrations increased with dietary protein (Okumura and Tasaki 1969, Rosebrough et al. 2004), and plasma triglyceride concentrations were higher in poultry fed low-protein diets compared to low-fat or low-carbohydrate diets (Malheiros et al. 2003, Swennen et al. 2005). In addition, shorebirds fed a low-fat diet had higher plasma triglyceride, lower B-hydroxybutyrate, and higher uric acid concentrations than birds fed a high-fat diet during a period of mass gain (Seaman et al. 2005). To our knowledge, only one study has experimentally investigated the effect of dietary macronutrient content on plasma metabolite concentrations in migratory passerines (Cerasale and Guglielmo 2006). Dietary fat content did not affect the predictive relationship between lipid metabolites and mass change in Yellow-rumped Warblers (*Dendroica coronata*; Cerasale and Guglielmo 2006). It is unknown whether diet affects the fuels used during short-term fasting in these birds.

Diet composition is particularly important to consider for fall passerine migrants in temperate eastern North America and western Europe. During migratory stopovers in these regions, many songbirds consume large quantities of fruit along with insects or seeds that are more commonly consumed during spring and summer seasons (Herrera 1984, Jordano 1988, Parrish 1997, Suthers et al. 2000). Many fruits used by these birds are low in protein, but usually these fruits are high in either fat or sugar content (Herrera 1982, Johnson et al. 1985, White 1989). These high-fat or sugary fruits may enhance fat deposition in migrating birds, because dietary fatty acids can be directly incorporated into fat stores (Bairlein and Simons 1995) and high-carbohydrate diets can enhance fat deposition through increased de novo lipogenesis (Stevens 1996). Thus, diet composition may affect fattening rates and fuel use, as measured by plasma metabolite concentrations, during resting and feeding periods at migratory stopover sites.

The goal of this study was to determine the direct effect of diet composition on plasma metabolite concentrations and body mass in a short-distance temperate migrant, the White-throated Sparrow (*Zonotrichia albicollis*). We fed captive birds semisynthetic diets that differed in fat, carbohydrate, and protein content and then measured plasma metabolites during feeding and after an overnight fast. This experimental design allowed us to control the macronutrient composition of the diet and thus assess its effects on plasma metabolite concentrations.

TABLE 1. Composition of semisynthetic diets fed to White-throated Sparrows.

Ingredient	Fruit diet		Insect diet		Grain diet
	% wet mass	% dry mass	% wet mass	% dry mass	% dry mass
D-Glucose[a]	10.3	42.2	1.3	10.3	0.0
Corn starch[b]	0.0	0.0	0.0	0.0	63.4
Casein[c]	2.5	10.3	6.5	51.0	10.3
Oil[d]	7.6	30.9	2.6	20.7	8.3
Vitamin mix[e]	0.3	1.0	0.2	1.7	1.0
Salt mix[f]	1.3	5.1	0.8	6.0	5.7
Cellulose[g]	1.3	5.4	0.0	0.0	5.2
Ground silica sand	0.0	0.0	0.0	0.0	5.2
Sodium bicarbonate	0.0	0.0	0.0	0.0	1.0
Agar[h]	1.3	5.1	1.3	10.3	0.0
Water	75.5	0.0	87.3	0.0	0.0

[a] Fisher Scientific, Pittsburgh, PA.
[b] USB Corporation, Cleveland, OH.
[c] High N casein, USB corporation, Cleveland, OH.
[d] Olive oil in fruit and insect diets, corn oil in grain diet.
[e] AIN-76 vitamin and minerals mix, ICN Biomedicals, Irvine, CA.
[f] Briggs salt mixture, ICN Biomedicals, Irvine, CA.
[g] Celufil nonnutritive cellulose filler, USB Corporation, Cleveland, OH.
[h] Agar bacteriological grade, USB Corporation, Cleveland, OH.

METHODS

DIETS AND FEEDING SCHEDULE

Twenty White-throated Sparrows were captured using mist nets on Block Island, RI and in Kingston, RI between 31 October and 9 November 2003. Birds were housed indoors at the University of Rhode Island in individual stainless steel cages (59 cm × 45 cm × 36 cm) and maintained on a constant photoperiod (11 hr light:13 hr dark) and at a constant temperature (22°C). Lights turned on at 08:00 and birds were weighed each morning between 08:00 and 08:30. Birds were provided with waxworms (*Galleria mellonella*), cracked corn, and water ad libitum during the initial 3–4 week acclimation to laboratory conditions. We then randomly assigned birds to one of three semisynthetic diets that were nutritionally similar to natural grain ($n = 7$), fruit ($n = 7$), or insects ($n = 6$). The grain diet was a dry powder composed of 10% protein, 8% fat, and 63% carbohydrate (Table 1). The fruit and insect diets were an agar-based wet mash. The fruit diet contained 10% protein, 31% fat, and 42% carbohydrate, whereas the insect diet contained 51% protein, 21% fat, and 10% carbohydrate on a dry mass basis (Table 1). Passerines fed similar semisynthetic diets have been maintained in captivity for up to one year (McWilliams et al. 2002, Pierce and McWilliams 2004, 2005). Energy density of each diet was calculated given the diet composition (Table 1) and published estimates of the energy density of each macronutrient: carbohydrate = 17.6 kJ g^{-1}, protein = 17.8 kJ g^{-1}, and fat = 39.3 kJ g^{-1} (Schmidt-Nielson 1997). The energy densities of the dry diets were 16.2 kJ g^{-1} for the grain diet, 21.4 kJ g^{-1} for the fruit diet, and 19.0 kJ g^{-1} for the insect diet. Birds were offered the assigned semisynthetic diet ad libitum and 2 g cracked corn for 4–6 days while they acclimated to the new diets, and then only the semisynthetic diet ad libitum for six days prior to blood sampling. Food intake (dry mass offered – dry mass remaining ±0.1 g) between 08:30 and the time of blood sampling was measured on the sixth day of feeding on the semisynthetic diets.

BLOOD SAMPLING AND METABOLITE ANALYSES

We sampled approximately 50 μL of blood from each bird at 13:30–15:00 on the sixth day of feeding on their respective diets. Blood was sampled within 5 min of first handling the bird and within 40 min of entering the room. Blood samples were taken by puncturing the brachial vein with a 27-gauge needle and collecting blood in heparinized capillary tubes. Birds were then weighed and mass change between 08:00–08:30 and blood sampling was determined

(hereafter ''mass change'' for feeding birds). After the afternoon blood sample was taken, birds were returned to their cages and allowed to feed ad libitum until food was removed when lights were turned off. Birds fasted overnight and approximately 50 μL of blood was sampled the following morning at 08:00–09:10 and before food was offered. Birds were weighed immediately after blood samples were taken, and mass change since the previous blood sampling was calculated (hereafter ''mass change'' for fasting birds). Time of blood sampling was recorded as the time of day blood was taken during both feeding and fasting. The start of the blood sampling schedule was staggered so that birds 1–7, birds 8–14, and birds 15–20 were sampled on subsequent days.

Blood samples were centrifuged to separate plasma from red blood cells, and plasma was stored at −80°C until analysis. Plasma metabolites were assayed on a Bio-Tek Powerwave X340 microplate spectrophotometer (Bio-Tek Instruments, Inc., Winooski, Vermont) using commercial kits modified for small volumes. Triglyceride and glycerol were measured sequentially by endpoint assay (Sigma, St. Louis, Missouri; 5 μL plasma, 240 μL reagent A, 60 μL reagent B). Endpoint assays were also used to measure uric acid (WAKO Diagnostics, Richmond, Virginia; 5 μL plasma, 300 μL reagent), and nonesterified fatty acids (NEFA; WAKO Diagnostics; 3 μL plasma, 120 μL reagent A, 240 μL reagent B). B-hydroxybutyrate (BUTY) was measured by kinetic assay (R-biopharm, Marshall, Michigan; 5 μL sample, 150 μL working solution, 3 μL B-hydroxybutyrate dehydrogenase, read kinetically for 30 min) similarly to Guglielmo et al. (2005). Samples were diluted three-fold with 0.9% NaCl for all assays except NEFA. We first measured triglyceride and glycerol, followed by BUTY, and we repeated measurements until a coefficient of variation (CV) <10% was achieved between duplicates. All other metabolites were then measured with the remaining plasma and samples were used in subsequent statistical analyses if CV <10% was achieved between duplicates.

STATISTICAL ANALYSES

We used a linear mixed model to compare differences in morning body mass between the first and last days of feeding on the semi-synthetic diet. This was performed using PROC MIXED (SAS Institute 2002) with repeated measures analysis using time (day 1 or day 6) and diet as fixed effects and a compound symmetric covariance structure to account for multiple observations on individuals (Littell et al. 1996). One-way ANOVA with Tukey's HSD tests for post-hoc comparisons were used to determine differences among diet groups in body mass when blood was taken during feeding or fasting, mass change, and dry matter intake prior to blood sampling in feeding birds.

Plasma concentrations of BUTY during feeding and fasting, and glycerol during fasting, were log(metabolite + 1) transformed to satisfy the assumptions of normality and homogeneity of variance among diet groups. However, homogeneity of variance could not be corrected with transformation for glycerol during feeding and uric acid during fasting. Thus, we analyzed these metabolites nonparametrically with Kruskal-Wallis tests (exact *P*-values are reported) and follow-up Dunn's multiple comparison tests for diet group differences. We used Spearman's rank correlations to determine whether plasma metabolite concentrations were related to time of blood sampling.

We used a linear mixed model (as described above) with state (feeding or fasting) and diet as fixed effects to compare plasma concentrations of triglyceride and BUTY between feeding and fasting birds in the three diet groups. Significant main effects and interactions were further analyzed with analysis of covariance (ANCOVA) and post-hoc Tukey's HSD tests. Body mass at blood sampling and mass change prior to blood sampling were entered into the model as covariates, and nonsignificant covariates and interactions were then omitted from the model. Plasma concentrations of glycerol, NEFA, and uric acid did not conform to the assumptions of repeated measures analysis, and different transformations were required for glycerol during feeding and fasting; thus, differences among diet groups in feeding and fasting birds for these metabolites were analyzed separately. For metabolites with significant covariates (BUTY), results from ANCOVA did not differ from models without covariates. Therefore, we present the results from only the repeated measures and one-way ANOVAs. We used SAS statistical software (SAS Institute 2002) for all analyses except Dunn's tests, which were

TABLE 2. Mean ± SE body mass (g), mass change (g), and untransformed metabolite concentrations for White-throated Sparrows maintained on grain, fruit, or insect diets. Sample sizes are provided in parentheses.

State	Diet	Body mass[a]	Mass change[b]	Plasma metabolite concentrations (mM)				
				Triglyceride	Glycerol	BUTY[c]	Uric acid	NEFA[c]
Feeding	Grain	25.8 ± 0.6 (7)	1.2 ± 0.2 (7)	4.1 ± 0.4 (7)	5.0 ± 0.9 (7)	0.7 ± 0.1 (7)	0.9 ± 0.1 (7)	0.9 ± 0.2 (6)
	Fruit	25.5 ± 0.5 (7)	1.2 ± 0.2 (7)	3.1 ± 0.3 (7)	2.7 ± 0.3 (7)	1.0 ± 0.1 (6)	0.9 ± 0.1 (6)	1.1 ± 0.2 (5)
	Insect	23.9 ± 0.4 (6)	1.1 ± 0.3 (6)	2.5 ± 0.3 (6)	4.1 ± 1.5 (6)	1.5 ± 0.2 (6)	1.9 ± 0.2 (6)	1.2 ± 0.1 (4)
Fasting	Grain	24.1 ± 0.5 (7)	–1.7 ± 0.2 (7)	1.3 ± 0.1 (7)	3.7 ± 1.1 (7)	3.0 ± 0.3 (5)	0.6 ± 0.1 (6)	1.0 ± 0.2 (4)
	Fruit	24.0 ± 0.8 (7)	–1.5 ± 0.2 (7)	1.6 ± 0.2 (7)	6.6 ± 2.1 (7)	3.1 ± 0.6 (6)	0.7 ± 0.1 (6)	0.9 ± 0.2 (4)
	Insect	22.5 ± 0.4 (6)	–1.4 ± 0.2 (6)	1.3 ± 0.2 (6)	7.6 ± 2.2 (6)	3.1 ± 0.4 (5)	1.5 ± 0.3 (6)	1.0 ± 0.2 (5)

[a] Body mass when blood samples were taken at 13:30–15:00 for feeding birds, and 08:00–09:10 for fasting birds.
[b] Mass change between morning (08:00–08:30) and afternoon (13:30–15:00) for feeding birds, and between afternoon (13:30–15:00) and the following morning (08:00–09:10) for fasting birds.
[c] BUTY = B-hydroxybutyrate, NEFA = nonesterified fatty acids.

calculated by hand. Values are reported as means ± SE and the significance level was set at $P < 0.05$.

RESULTS

BODY MASS AND FOOD INTAKE DURING FEEDING AND FASTING

Morning body mass was not significantly different among birds at the start and end of the diet treatment (diet: $F_{2,17} = 1.9$, $P = 0.19$; time: $F_{1,17} = 3.0$, $P = 0.10$; diet × time: $F_{2,17} = 2.8$, $P = 0.09$). For sparrows sampled while feeding, body mass ($F_{2,17} = 3.3$, $P = 0.06$) and mass change ($F_{2,17} = 0.03$, $P = 0.97$) were not significantly different among diet groups (Table 2). Dry matter intake between morning (08:00–08:30) and afternoon (13:00–15:00) was not significantly different among diet groups ($F_{2,13} = 3.4$, $P = 0.07$) and was on average 2.3 ± 0.2 g. Although the three diets differed in energy content, energy intake between morning and afternoon was also similar for birds in all diet groups ($F_{2,13} = 0.5$, $P = 0.60$). Sparrows sampled after an overnight fast lost body mass (Table 2), although body mass ($F_{2,17} = 2.2$, $P = 0.14$) and mass change ($F_{2,17} = 0.7$, $P = 0.53$) were not significantly different among diet groups (Table 2).

EFFECTS OF FEEDING OR FASTING ON PLASMA METABOLITE CONCENTRATIONS

To facilitate comparison with other studies, we report the untransformed metabolite concentrations for feeding and fasting sparrows in Table 2, even though some statistical analyses were conducted on transformed data. Time of blood sampling was not significantly correlated with any plasma metabolite concentrations during feeding ($P \geq 0.40$) or after fasting ($P \geq 0.29$). Triglyceride concentrations were significantly higher in feeding versus fasting sparrows, although there was a significant interaction between diet and state (state: $F_{1,17} = 131.5$, $P < 0.001$; diet: $F_{2,17} = 2.8$, $P = 0.09$; state × diet: $F_{2,17} = 9.7$, $P = 0.002$). BUTY concentrations were significantly higher after overnight fasting than during feeding (state: $F_{1,13} = 46.0$, $P < 0.001$; diet: $F_{2,16} = 1.0$, $P = 0.38$; state × diet: $F_{2,16} = 0.6$, $P = 0.58$). Thus, subsequent analyses of significant main effects and interactions are presented separately for feeding and fasting birds.

INFLUENCE OF DIET ON PLASMA METABOLITE CONCENTRATIONS DURING FEEDING

Grain-fed birds had significantly higher plasma triglyceride concentrations ($F_{2,17} = 5.4$, $P = 0.02$) and significantly lower BUTY concentrations than insect-fed birds ($F_{2,16} = 10.0$, $P = 0.002$; Fig. 1). Plasma concentrations of uric acid were significantly higher in insect-fed compared to grain-fed or fruit-fed birds ($F_{2,16} = 21.4$, $P < 0.001$; Fig. 1). We detected no significant differences in glycerol ($\chi^2_2 = 2.5$, $P = 0.30$) or NEFA ($F_{2,12} = 0.6$, $P = 0.55$; Fig. 1) among diet groups.

INFLUENCE OF DIET ON PLASMA METABOLITE CONCENTRATIONS DURING FASTING

Sparrows had significantly different plasma uric acid concentrations among diet groups after an overnight fast ($\chi^2_2 = 6.0$, $P = 0.04$); however, follow-up pairwise comparisons revealed no significant differences between diet groups (Fig. 2). Plasma concentrations of all other metabolites were not significantly different among diet groups (triglyceride: $F_{2,17} = 0.6$, $P = 0.56$; glycerol: $F_{2,17} = 1.8$, $P = 0.19$; BUTY: $F_{2,13} = 0.02$, $P = 0.98$; NEFA: $F_{2,10} = 0.2$, $P = 0.85$; Fig. 2).

DISCUSSION

Diet composition did not affect short-term body mass changes, dry matter intake, or energy intake of sparrows in our study. Therefore, we focus on the direct effects of diet on plasma metabolites, independent of body mass or food intake of birds. Results from our study demonstrate that diet composition affects plasma concentrations of certain lipid and protein metabolites over short time periods in sparrows during feeding.

PLASMA METABOLITES IN FEEDING VERSUS FASTING SPARROWS

Migrating birds alternate between periods of fasting during nocturnal flights or overnight resting periods and feeding during the day at stopover sites to replenish their energy and protein stores. Therefore, understanding how plasma metabolites change during feeding and fasting is crucial for accurate interpretation of the metabolite profiles observed in free-living birds. In general, controlled studies with captive birds have found that plasma B-hydroxybutyrate is higher and plasma triglyceride is lower in birds during fasting compared to feeding birds (Emmanuel et al. 1982, Swain 1987, 1992). We also found that sparrows had significantly higher plasma B-hydroxybutyrate and lower plasma triglyceride after overnight fasting compared to feeding sparrows. Our results are consistent with these captive studies and studies of free-living migrating birds that show differences in these metabolites after even shorter fasts of $\leq$2 hr (Jenni-Eiermann and Jenni 1991, 1996, 1997) and 5 hr (Landys et al. 2005). Thus, plasma triglyceride and B-hydroxybutyrate can be used as sensitive indicators of short-term changes between feeding and fasting states of songbirds.

EFFECT OF DIET ON PLASMA METABOLITES

Diet composition had no effect on lipid metabolites after fasting, although we found some evidence that diet composition affected protein metabolites after fasting. Specifically, there was a trend toward higher uric acid levels after overnight fasting in birds that had been fed the high-protein insect diet compared to birds that had been fed the other diets. Previous research on migrating passerines suggests that protein and lipid catabolism may differ between insectivorous and frugivorous species during migratory flight (Gannes 2001). Our study is the first to experimentally demonstrate that diet composition has no effect on lipid metabolites in small migratory songbirds after an inactive overnight fast. However, when birds were actively feeding, we detected differences in plasma triglyceride and B-hydroxybutyrate between the grain and insect diet groups. The fact that these differences in lipid metabolites were not observed during fasting suggests that differences detected while birds were actively feeding can be attributed to the short-term effects of feeding on diets with different macronutrient compositions.

If plasma metabolites in feeding birds simply correspond to diet composition, we would expect plasma uric acid to be highest in birds fed high-protein diets, and plasma triglyceride to be highest in birds fed high-fat diets. As expected, sparrows fed the high-protein insect diet had higher plasma uric acid levels than birds fed the lower-protein fruit and grain diets. Uric acid is the end product of protein catabolism in birds, so plasma uric acid increases when dietary or endogenous protein

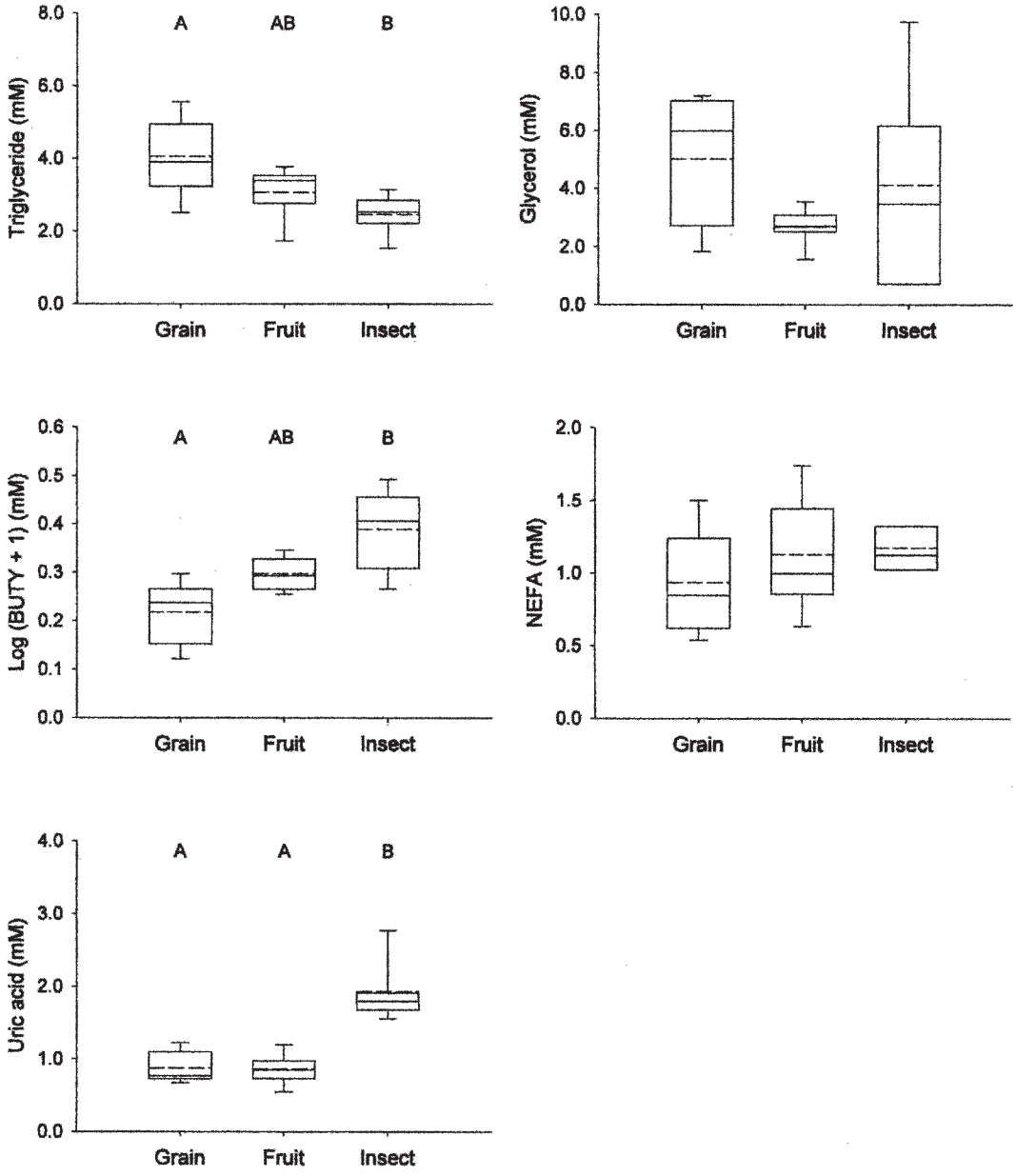

FIGURE 1. Plasma concentrations of triglyceride, glycerol, B-hydroxybutyrate (log(BUTY + 1)), nonesterified fatty acids (NEFA), and uric acid for White-throated Sparrows that were offered semisynthetic grain, fruit, or insect diets. Birds were sampled in the afternoon on the sixth day of ad libitum feeding on their respective diets. Box plots show the mean (dashed lines in box), median (solid line in box), 25th–75th interquartile range (boxes), and 10th and 90th percentiles (error bars). Diet groups with different letters are significantly different at $P < 0.05$.

is broken down (Stevens 1996). Our results are consistent with other studies of poultry and passerines (Okumura and Tasaki 1969, Goldstein et al. 2001), which suggests that increased plasma uric acid reliably indicates catabolism of dietary protein in birds.

In contrast, dietary fat levels did not correspond to lipid metabolite concentrations during

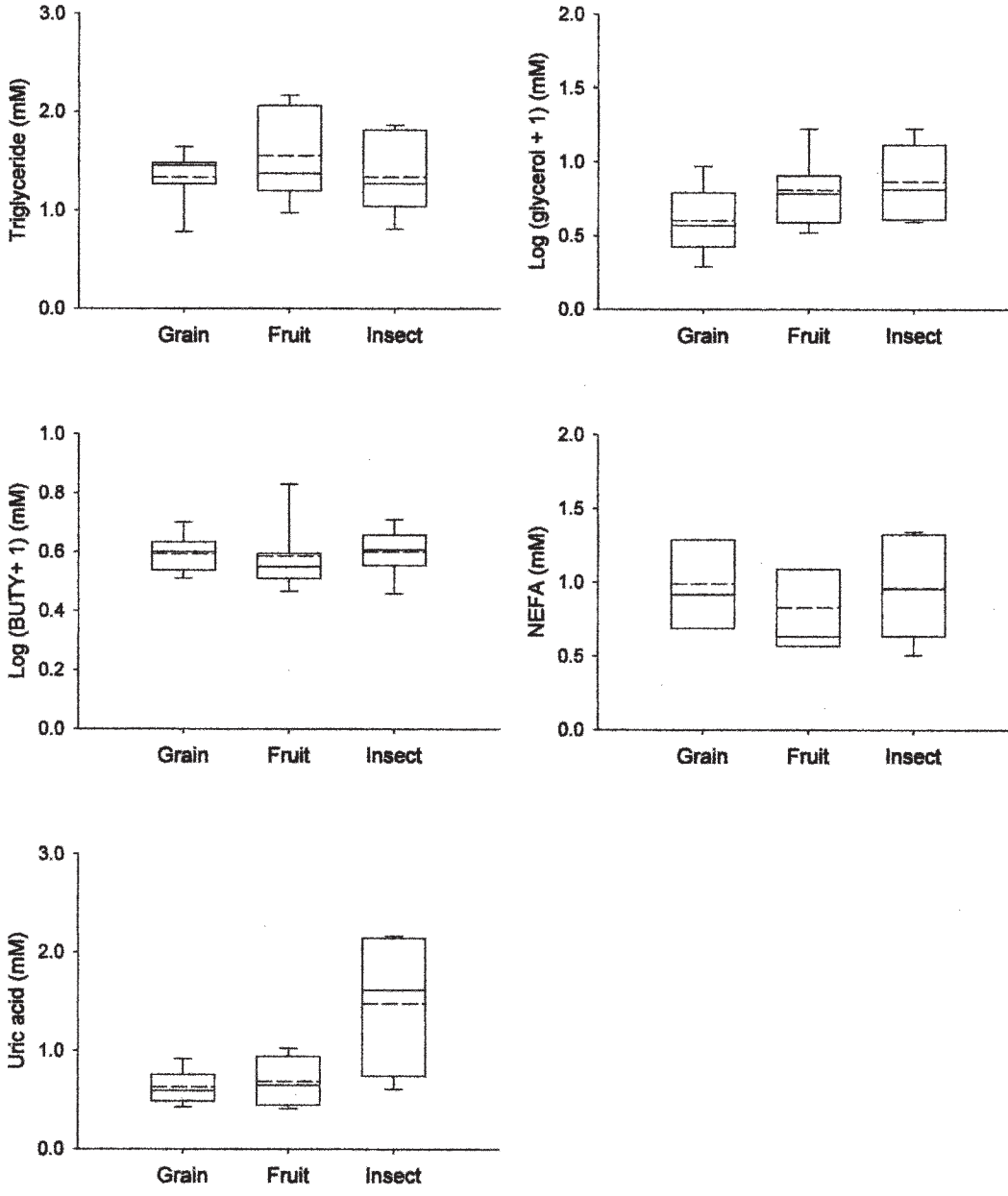

FIGURE 2. Plasma concentrations of triglyceride, glycerol (log(glycerol + 1)), B-hydroxybutyrate (log(BUTY + 1)), nonesterified fatty acids (NEFA), and uric acid for White-throated Sparrows that were offered semisynthetic grain, fruit, or insect diets prior to overnight fasting. Birds were sampled in the morning after overnight fasting on the sixth day of ad libitum feeding on their respective diets. Box plots show the mean (dashed lines in box), median (solid line in box), 25th–75th interquartile range (boxes), and 10th and 90th percentiles (error bars).

feeding; sparrows fed the high-fat fruit diet (31% fat) did not have higher plasma triglyceride than sparrows fed the other two lower-fat diets. Instead, sparrows fed the grain diet (8% fat) had significantly higher plasma triglyceride than sparrows fed the insect diet (21% fat). If the high-carbohydrate content of the grain diet led to higher rates of fatty acid synthesis and sub-

sequent fat deposition, this could explain the high triglyceride levels in birds fed the high-carbohydrate grain diet. In birds, de novo fatty acid synthesis occurs in the liver when dietary energy is higher than energetic demands, and the main substrates are carbohydrates, primarily glucose (Klasing 1998). Fatty acid synthesis proceeds at a rapid rate following carbohydrate-rich meals, whereas high-fat diets can inhibit fatty acid synthesis in the liver (Stevens 1996, Klasing 1998). In humans, low-carbohydrate diets result in lower circulating plasma triglyceride concentrations and higher B-hydroxybutyrate concentrations compared to low-fat diets (Westman et al. 2002, Yancy et al. 2004). Thus, the large difference in carbohydrate content between grain (63%) and insect diets (10%) may have contributed to the difference in plasma triglyceride concentrations. High dietary protein intake may also decrease the rate of lipogenesis and fat deposition, as demonstrated in poultry studies (Donaldson 1985, Rosebrough and Steele 1985, Rosebrough et al. 2004). However, triglyceride concentrations of sparrows in our study did not clearly follow dietary protein content, because plasma triglyceride in birds fed the insect diet (51% protein) was lower than in birds fed the grain diet (10% protein) but not the fruit diet (10% protein). This suggests interactive effects of certain macronutrients on lipid metabolites in sparrows.

Dietary fat levels also did not correspond to lipid metabolite concentrations during feeding because sparrows fed the high-fat fruit diet (31% fat) did not have the highest plasma B-hydroxybutyrate. Instead, sparrows fed the insect diet (21% fat), with intermediate levels of dietary fat, had higher plasma B-hydroxybutyrate than sparrows fed the grain diet (8% fat). These results agree with a recent study that determined that lipid metabolite concentrations in Yellow-rumped Warblers were not influenced by dietary fat content (Cerasale and Guglielmo 2006). B-hydroxybutyrate formation may be important for sparing glucose or allocating carbohydrates to glycogen synthesis, or to replace glucose during fasting or when dietary intake of carbohydrates is low (Robinson and Williamson 1980). During these times, glucose may be synthesized from glucogenic precursors, such as amino acids (Klasing 1998). Thus, higher plasma B-hydroxybutyrate concentrations in insect-fed birds could be related to low availability of dietary glucose, if gluconeogenesis from excess dietary amino acids was not sufficient to meet these needs. In contrast to our results, increased plasma B-hydroxybutyrate concentrations have been found in rats and humans consuming high-fat diets compared with high-carbohydrate diets (Askew et al. 1975, Lewis et al. 1977, Surina et al. 1993) and in shorebirds gaining mass on high-fat compared with low-fat diets (Seaman et al. 2005). High-fat diets may contribute to increased B-hydroxybutyrate formation during feeding by providing large amounts of free fatty acids as precursors for ketone body formation (Robinson and Williamson 1980). However, given the contrasting results of these studies, dietary fat does not appear to have a predominant effect on plasma B-hydroxybutyrate in sparrows.

In general, our results suggest that lipid metabolite concentrations in birds that are feeding are the product of multiple processes, whereas protein metabolites are more directly influenced by levels of dietary protein. Interestingly, our results suggest an important role for dietary carbohydrates in affecting circulating levels of lipid metabolites in feeding songbirds.

USE OF PLASMA METABOLITES TO ESTIMATE REFUELING RATES AND HABITAT QUALITY FOR MIGRATORY SONGBIRDS

Our results suggest that migrating birds captured at dawn prior to feeding after an inactive overnight fast will have higher plasma B-hydroxybutyrate and lower plasma triglyceride compared to birds captured hours later that have fed. As migrating birds feed and store fat during the day, plasma triglyceride concentrations increase and B-hydroxybutyrate concentrations decrease (Jenni-Eiermann and Jenni 1994, 1997, Jenni and Schwilch 2001, Cerasale and Guglielmo 2006). However, our results also suggest that sparrows consuming high-carbohydrate seeds will have 65% higher triglyceride concentrations and 55% lower B-hydroxybutyrate concentrations than birds consuming low-carbohydrate insects, regardless of body mass change. Likewise, sparrows in a steady state that consume high-protein insects are predicted to have higher plasma uric acid levels than birds consuming low-protein fruits and grains. The effect of dietary fat on lipid metabolite concentrations

in birds that were feeding was complex and warrants further study. In summary, investigators who use plasma metabolites as indicators of body condition and habitat quality in free-living birds must consider possible dietary effects on metabolite profiles. Such considerations are particularly relevant for songbirds during migration that exhibit dietary plasticity (Parrish 2000), and for interspecific studies that compare metabolite profiles of insectivores, frugivores, and granivores.

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