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Diet preferences for specific fatty acids and their effect on composition of fat reserves in migratory Red-eyed Vireos (*Vireo olivaceous*)

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Abstract

Fatty acid composition of body fat in birds often differs between bird species and between seasons, and changes in diet may be responsible for this variation. We tested two related hypotheses using Red-eyed Vireos, a long-distance migratory songbird: (1) birds prefer diets with certain fatty acids, and (2) fatty acid composition of the diet primarily determines the composition of lipid reserves. During paired-choice experiments, vireos preferred semi-synthetic diets with triolein (81% digestive extraction efficiency) over diets with tristearin (54% digestive extraction efficiency) and, in general, ate more when offered diets with unsaturated fats compared to saturated fats. These results demonstrate that vireos can discriminate between diets differing only in fatty acid composition and prefer diets with long-chain unsaturated fatty acids. When vireos were fed one of two diets for 1 month, the primary fatty acids in each diet also predominated in the tissues of birds fed each diet. However, some fatty acids that were absent in the diet occurred in bird tissues (e.g., 22:4, 22:5) suggesting that selective metabolism of fatty acids along with diet composition determine the fatty acid composition of lipid reserves in migratory birds.

Keywords: Diet preferences; Extraction efficiency; Fatty acid composition; Migratory bird

1. Introduction

Body mass and composition of migratory birds changes seasonally. For example, lipid and protein stores increase especially during migratory periods (Marsh, 1984; Piersma, 1990; Lindström and Piersma, 1993; Karasov and Pinshow, 1998; Bordel and Haase, 2000). Birds increase their energy and protein stores by eating more food (Blem, 1990; Bairlein and Gwinner, 1994) and by selective feeding (Wheelright, 1988; Whelan and Willson, 1994). Since most migrating birds accumulate large lipid stores as the primary source of energy for their flights, and choose their diets based in part on total lipid content (Stiles, 1993; Schaefer et al., 2003), it is important to understand whether birds prefer diets with certain types of lipids.

During periods of fat storage (e.g., during migration or in the cold), birds store large amounts of fats comprised mostly of 16- and 18-carbon, unsaturated fatty acids (Bower and Helms, 1968; Caldwell, 1973; Blem, 1976; Egeler and Williams, 2000). Some studies have shown that birds eat foods with more long-chain unsaturated fatty acids (LCUFA) during migration (Heitmeyer and Fredrickson, 1990; Bairlein and Gwinner, 1994; Zurovchak et al., 1999). Selectively feeding on LCUFA may be advantageous because such fatty acids may be absorbed and/or metabolized more efficiently than saturated fats (e.g., migratory birds, Johnston, 1973; rats, Leyton et al., 1987; mallards, Heitmeyer and Fredrickson, 1990). Thus, the fatty acid composition of body fat in birds during migration may result at least in part from their dietary preferences for LCUFA.

Diet preferences are most easily investigated by allowing an animal equal access to certain food types and measuring relative use (Johnson, 1980). However, only two studies have directly tested whether migratory songbirds prefer diets with specific fatty acids (Bairlein, 1991; McWilliams

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et al., 2002). Bairlein (1991) found that Garden Warblers (*Sylvia borin*) offered semi-synthetic diets with certain purified triglycerides (TGs) preferred diets containing 18-carbon unsaturated fatty acids over diets containing 18-carbon saturated fatty acids and preferred diets containing 16-carbon over 18-carbon saturated fatty acids. Interestingly, these warblers preferred diets containing the fatty acids that predominated in their depot fat during migration. McWilliams et al. (2002) found that Yellow-rumped Warblers (*Dendroica coronata*) preferred diets with unesterified long-chain unsaturated fatty acids to diets containing unesterified long-chain saturated fatty acids. They suggested that this preference was at least in part due to the warblers' limited ability to assimilate some long-chain saturated fatty acids in energy-demanding situations. This finding is consistent with the hypothesis that dietary preferences are in part determined by digestive constraints (Martínez del Rio et al., 1988). However, our knowledge of absorption efficiency and metabolizability of certain fatty acids in songbirds is scant (Place and Stiles, 1992; Zurovchak et al., 1999).

Understanding how preferences for specific fatty acids influence diet selection is ecologically important because fatty acid composition of an animal has performance consequences (McKenzie et al., 1995, 1997, 1998; Ayre and Hulbert, 1997), and a birds' preference for specific fatty acids may influence its body composition. Past studies provide contradictory evidence about how diet influences the fatty acid composition of body fat in birds (Bower and Helms, 1968; West and Meng, 1968a, b; Morton and Liebman, 1974; Napolitano and Ackman, 1990; Egeler and Williams, 2000). For example, Bower and Helms (1968), West and Meng (1968a), and Morton and Liebman (1974) found that dietary fatty acid composition influenced the depot fat composition in three different migratory birds. However, West and Meng (1968b) found that diet did not influence depot fat composition in redpolls (*Acanthis flammea*), and Napolitano and Ackman (1990) and Egeler et al. (2003) found that endogenous (de novo) lipid synthesis played a key role in determining the fatty acid composition of adipose tissue in the Semipalmated Sandpiper (*Calidris pusilla*) and Western Sandpiper (*Calidris mauri*), respectively.

We studied diet preferences for specific fatty acids in Red-eyed Vireos (*Vireo olivaceous*) using diets with purified forms of TGs. Red-eyed Vireos are abundant, medium-sized (13–25 g) neotropical migrants that are primarily insectivorous throughout the year, except during fall when they eat mostly fruit (Cimprich et al., 2000). In addition, they store relatively large fat reserves in preparation for their long-distance migration. Thus, Red-eyed Vireos are an excellent species in which to study food preferences and composition of fat reserves. We tested the following specific hypotheses: (1) given that 16:0 and 18:1 fatty acids typically predominate in depot fat of migratory songbirds, Red-eyed Vireos will prefer these fatty acids to ones that are less common in their depot fat; (2) fatty acids preferred by Red-eyed Vireos are digested more efficiently than dietary fatty acids that are avoided by vireos; and (3) the fatty acid composition of certain tissues in Red-eyed Vireos will be influenced by the fatty acid composition of their diet.

2. Materials and methods

2.1. Capture and maintenance of birds

Red-eyed Vireos (*V. olivaceous*; n=22) were captured using mist nets during October 1999 on Block Island, Rhode Island (41°10′ N, 71°34′ W) (USFWS permit #MB-003201, R.I. DEM permit #99-27). All vireos captured were hatch-year birds and sex was unknown. Birds were immediately banded and weighed (±0.1 g). In the laboratory, birds were housed individually in stainless-steel cages (59×45×36 cm), at constant temperature (23 °C), and on a light cycle that simulated the natural light cycle at time of capture (11L:13D light/dark cycle, lights on at 0700 h). During the 30-day acclimation period, birds were offered equal amounts (ca. 30 g wet) of the same semi-synthetic diet (Table 1, Acclimation diet) along with four waxworms (*Galleria mellonella*) in two adjacent Petri dishes. This protocol ensured that birds visited each dish and, given that at least 10 g of food always remained in the dishes, were fed ad libitum. Nutrient content of the acclimation diet was similar to that of insects (10% carbohydrate, 52% protein, 20% fat; Table 1; Bairlein, 1987). Use of such semi-synthetic diets makes the composition of the diets less ambiguous than diets compounded from raw foodstuffs

Table 1
Composition of two semi-synthetic diets fed to Red-eyed Vireos (*Vireo olivaceous*) during acclimation and during digestibility trials

Ingredients	Acclimation diet		Digestibility trial diet	
	% Wet	% Dry	% Wet	% Dry
Glucose	1.31	10.04	10.25	41.00
Casein[a]	6.44	49.54	2.50	10.00
Amino acid mix[b]	0.36	2.76	0.70	2.80
Vitamin and minerals mix[c]	0.22	1.67	0.25	1.00
Salt mix[d]	0.76	5.86	1.25	5.00
Cellulose[e]	–	–	1.30	5.20
Olive oil[f]	2.61	20.08	7.00	28.00
Tristearin[g]	–	–	0.50	2.00
Agar[h]	1.31	10.04	1.25	5.00
Water	87.00	–	75.00	–

[a] Casein (high N): U.S. Biochemical, Cleveland, OH.

[b] Amino acid mix: Murphy and King (1982); all amino acids supplied by Fisher Scientific, Pittsburgh, PA.

[c] AIN-76 Vitamin and Mineral Mix, ICN Biomedicals.

[d] Salt mix: Briggs-N Salt mixture, ICN Biomedicals.

[e] Celufil-hydrolyzed: U.S. Biochemical.

[f] Rienzi-brand Olive oil.

[g] Tristearin: 90+% pure tristearin powder supplied by Sigma-Aldrich.

[h] Agar bacteriological grade: U.S. Biochemical.

(Murphy and King, 1982). All birds were provided ad libitum water. Each day, we measured body mass (±0.1 g) and food intake (±0.1 g wet) of each bird. All bird husbandry procedures used during this experiment conformed to Gaunt and Oring (1997) and were approved by URI IACUC (#AN01-04-029).

2.2. Diets and preference experiments

After the 30-day acclimation period, we conducted paired-preference trials using six different semi-synthetic diets (Table 2). All six diets were composed of 10% carbohydrates, 52% protein, and 20% fat, but the fatty acid composition of the dietary fat in each diet was different (Table 2). Purified forms of TGs were used in each diet except for the 18:2 diet where pure safflower seed oil was used to reduce costs (Table 2). We used the TG form of the fatty acids because fatty acids in foods eaten by wild birds are mostly in the TG form (Zurovchak, 1997). The “standard” diet contained similar proportions of the five primary fatty acids in the diets, whereas the other diets contained predominantly one of these five fatty acids. All birds were acclimated to the standard diet for 1 week prior to preference tests.

We used a 4×4 Latin square experimental design, with four groups of birds and four paired-preference feeding trials (Table 3), so that we could directly assess order of treatment or time effects on bird preferences. Birds were randomly assigned to one of four groups (n=6 per group for A, B, and C and n=4 for D). Birds in each group were given a series of paired-choice feeding trials in a specified order. All birds were provided one paired-choice for three consecutive days. A paired-preference trial involved providing the birds each morning with ad libitum amounts of two different diets, along with four waxworms, in adjacent Petri dishes. We use the term “trial” to describe the 3-day period for each paired-preference given to the birds. After each 3-day preference trial, birds were given standard diet (in both Petri dishes) for 2 days prior to the next preference trial.

We measured dry matter food intake (±0.1 g) each day. We dried (100 °C) and weighed (±0.1 g) samples of food offered and remaining to estimate water content and then we calculated dry matter intake (DMI) for each vireo as DMI=dry mass offered−dry mass of uneaten food.

2.3. Retention time and extraction efficiency

Eleven Red-eyed Vireos were captured using mist nets between 2 October and 7 October 2000 on Block Island, Rhode Island (41°10′ N, 71°34′ W) (USFWS permit #PRT-003201, R.I. DEM permit #2000-95, IACUC #AN01-04-029). Birds were immediately banded and weighed (±0.1 g). In the laboratory, birds were housed individually in stainless-steel cages (59×45×36 cm), at constant temperature (23 °C), and on a light cycle that simulated the natural light cycle at time of capture (11L:13D light/dark cycle, lights on at 0700 h). Each day, birds were provided ad libitum water and a semi-synthetic diet with nutrient content similar to a high-lipid fruit diet (41% carbohydrate, 13% protein, 30% fat; Table 1) along with eight waxworms.

Special observation cages with one-way glass and plastic-coated paper on a roller were used to observe birds

Table 2
Fatty acid composition (%) of the lipid portion of the experimental diets used for the paired-preference trials and the 1-month feeding trial of Red-eyed Vireos (*Vireo olivaceous*)

Fatty acids[a]	Fatty acid composition (%) of seven experimental diets and waxworms							
	Standard[b]	14:0[c]	16:0[d]	18:0[e]	18:1[f]	18:2[g]	Mixed[h]	Waxworms[i]
14:0	18.8	92.7	2.8	0.3	0.4	1.1	0.5	0.4
16:0	20.0	5.0	91.7	2.7	1.6	7.1	23.6	28.4
16:1n7	0.1	0.1	–	–	0.1	0.1	0.1	1.3
18:0	15.6	1.0	4.9	96.5	0.6	3.1	21.2	1.9
18:1n9	24.2	0.1	0.0	0.0	93.8	17.0	29.4	48.0
18:2n6	19.9	0.8	0.1	0.1	1.0	70.2	23.8	10.7
18:3n6	–	–	–	–	–	–	–	1.5
20:1n9	0.2	–	–	–	0.8	0.2	0.3	6.1

All diets were 10% carbohydrate, 52% protein, and 20% fat and were formulated like the acclimation diet (Table 1) except olive oil was replaced with either a triacylglycerol or safflower oil. The fatty acid composition of the seven diets and waxworms was directly measured using gas chromatography.

[a] Other fatty acids that were found in <1% of the lipid portion of at least one diet were 18:1n7, 18:3n3, 18:4n3, 20:4n6, 20:4n3, 22:4n6, 22:5n6, and 22:6n3.

[b] Lipid in standard diet was composed of indicated amounts (% dry mass) of pure triacylglycerols—14:0 (4.1), 16:0 (3.7), 18:0 (3.7), 18:1 (3.7), and safflower oil (4.9), all supplied by Sigma-Aldrich, except 14:0 supplied by Fisher Scientific.

[c] Lipid in 14:0 diet was 90+% pure trimyristin powder supplied by Fisher Scientific.

[d] Lipid in 16:0 diet was 85+% pure tripalmitin powder supplied by Sigma-Aldrich.

[e] Lipid in 18:0 diet was 90+% pure tristearin powder supplied by Sigma-Aldrich.

[f] Lipid in 18:1 diet was 95+% pure triolein liquid supplied by Sigma-Aldrich.

[g] Lipid in diet was pure safflower seed oil supplied by Sigma-Aldrich.

[h] Lipid in mixed diet was composed of equal amounts (4.7% dry mass) of pure triacylglycerols—16:0, 18:0, 18:1, and 6.1% dry mass of safflower oil, all supplied by Sigma-Aldrich.

[i] Waxworms (*Galleria mellonella*), Grubco, Hamilton, OH.

Table 3
Latin-square experimental design for fatty acid paired-preference trials

Group	n	Fatty acid diet choices			
		16:0 vs. 18:0	18:0 vs. 18:1	16:0 vs. 14:0	18:1 vs. 18:2
A	6	1	2	3	4
B	6	2	3	4	1
C	6	3	4	1	2
D	4	4	1	2	3

Numbers denote sequence of trials for each of four groups (A–D) (n=number of birds per group).

and to collect their excreta during digestibility trials (as described in McWilliams and Karasov, 1998). On a given test day, treatment birds were weighed (±0.1 g) and provided fresh food and water at 0800 h. Food intake, retention time, and extraction efficiency were measured during a 4-h test period which began when the bird was gavaged at 0900 h with a solution containing a radiolabelled nutrient and marker (see below). Birds were randomly assigned to three groups, and trials were performed over a 3-week period between 17 March and 1 April 2001. Each group was given a different radiolabelled nutrient for 1 day each week for a 3-week period. Thus, each group received each of the three radiolabelled nutrients 1 week apart; however, no two groups received the same nutrient in the same week. This allowed us to control for any effect of time over the 3-week period.

Retention time of digesta was measured using the inert lipid-phase marker glycerol triether (GTE, from A. Place, University of Maryland). Extraction efficiency of lipids was measured using the inert marker method (Karasov et al., 1986; Jackson and Place, 1990; Place and Stiles, 1992) with GTE used as the inert marker and either glycerol trioleate (GTO), glycerol tripalmitate (GTP), or glycerol tristearate (GTS) (Amersham, Arlington Heights, IL, USA) used as the digestible lipid.

A mixture of 74 kBq of [^{3}H] GTE and 18.5 kBq of one of the [^{14}C] digestible lipids in 15 μl of carrier solution was gavaged into a bird's esophagus at the start of each 4-h test period. All birds were returned to the observation cages and began feeding soon after being gavaged; thus, a small volume of marker was inserted into the flow of food in the bird's digestive system.

Excreta were collected every 15 min for 4 h. Each excreta sample was placed in a pre-weighed sealed vial, reweighed, and 10 ml of a 2:1 chloroform/methanol mixture was added to each vial (Place and Stiles, 1992). The vials were vortexed daily for 3 days to ensure solubilization of probes and then processed following protocols in McWilliams and Karasov (1998).

Total mean retention time (TMRT) was calculated as the sum of the products of the proportion of inert marker excreted during each time interval multiplied by the elapsed time since ingestion of marker (Warner, 1981). Extraction efficiency was calculated as $100-100[(M_f/N_f)*(N_e/M_e)]$, where M_f is radioactivity of the inert marker (GTE) in food, N_f is radioactivity of the nutrient in food, N_e is radioactivity of nutrient in excreta, and M_e is radioactivity of inert marker in excreta.

2.4. Influence of diet on fatty acid composition of body fat

After the completion of the preference trials, all birds were fed with the standard diet for 2 weeks and then switched to one of two diets with different fatty acid compositions. To determine how dietary fatty acids influenced fatty acid composition of vireos, we randomly assigned nine vireos to either the triolein diet (n=5; "18:1 diet" in Table 2) or a mixed fatty acid diet (n=4) (Table 2). All birds were also provided with 1.0 g live waxworms each day. We were able to successfully maintain five birds on the triolein diet and two birds on the mixed diet for 28–32 days. These birds were then killed and their liver and breast muscles were removed, rinsed in distilled water, blotted dry, and weighed (±0.1 mg). The intestines were removed, perfused with distilled water, blotted dry, and weighed (±0.1 mg). We collected and weighed (±0.1 mg) fat from the furcular region and the abdominal region when such fat was present. Each tissue sample was placed into individual glass scintillation vials and stored at −20 °C for later analysis. Two birds fed with the mixed diet died after only 9 and 13 days on this diet. These birds were included in the following analysis because the fatty acid composition of their tissues was similar to the other two birds fed with the mixed diet for 28–32 days.

2.5. Fatty acid composition analysis

All organic solvents used were of HPLC grade and were from Fisher Scientific (Pittsburgh, PA, USA). All bird tissues and diet samples were freeze-dried, weighed (±0.1 mg), and ground. Lipids were extracted from ca. 100 mg of sample using a modified version of Folch et al. (1957). Briefly, samples were homogenized with 3.0 ml methylene chloride/methanol (2:1 CH_2Cl_2:MeOH), centrifuged for 15 min at 2000 rpm, and the supernatant was transferred to a large test tube. This procedure was repeated with 1:1 CH_2Cl_2:MeOH and 2:1 CH_2Cl_2:MeOH. Lipid extract was first washed with 0.88% potassium chloride water solution and then with methylene chloride/methanol/water (3:48:47). Samples were dried under nitrogen, weighed (±0.1 mg), resuspended in 500 μL 1:1 methylene chloride/methanol, and capped under nitrogen.

Quantification of fatty acid methyl esters was achieved by hydrolyzing ca. 500 μg of extracted lipid with methanolic HCl, adding 25 μg of internal standard mixture [equal amounts of nonadecanoic acid (C19:0) and heneicosanoic acid (C21:0) (Nu-chek PreP) suspended in methylene chloride] to each sample and extracting the methyl esters into methylene chloride. An aliquot of the methylene chloride extract was subjected to gas chromatography directly on a Hewlett-Packard model 5890A instrument equipped with a flame ionization detector using a J&W DBWAX fused silica capillary column (30 m×0.25 mm i.d., J&W Scientific, Folsom, CA, USA). The oven temperature was programmed from an initial temperature of 50 °C for 2 min to 200 °C in 16 min, from 200 to 210 °C in 11 min, and from 210 to 220 °C in 18 min. Helium was used as the carrier gas with a flow rate of 1.0 ml min^{-1}. Peaks were identified by comparison with retention times of known standards and expressed as percentages of fatty acid methyl esters.

2.6. Statistical analysis

Latin square general linear model analysis of variance (GLM ANOVA) (Systat) was used to assess whether order of treatment affected bird preferences. Student's paired *t*-tests were used to compare vireo preferences for specific fatty acids on each trial day. Repeated-measures analysis of variance (RMANOVA) was used to determine if total food intake and body mass of vireos changed during the 1-month feeding experiment and to compare retention times and extraction efficiencies of each fatty acid. Fatty acid composition (%) of the diets and tissues were arcsine-transformed, and analysis of variance (ANOVA) was used to compare the fatty acid composition of diet and various body tissues (Austin, 1993; Käkelä and Hyvärinen, 1996; Peng et al., 2003; Rollin et al., 2003; Thil et al., 2003). As we compared both the fatty acid composition of diet and tissue by conducting multiple ANOVAs, one for each fatty acid, we report statistical significance at both $P<0.01$ and $P<0.001$. All statistical analyses, except the Latin square general linear model ANOVA, were performed using the general linear model in SPSS 11.0 (SPSS) and Tukey's honest significant difference (HSD) was used for all post hoc comparisons. Results are reported as means±standard error (S.E.M.).

3. Results

3.1. Preferences for specific fatty acids

Total food intake was not influenced by order of treatment during any of the paired-preference trials [Latin-square ANOVA: Day 1: $F_{(3,78)}=0.13$, $P=0.94$; Day 2: $F_{(3,78)}=0.73$, $P=0.73$; Day 3: $F_{(3,78)}=1.00$, $P=0.40$]. In general, vireos ate more total food when given choices with mostly unsaturated fat diets (18:2 vs. 18:1, 18:1 vs. 18:0) than with saturated fat diets (18:0 vs. 16:0, 16:0 vs. 14:0), and total food intake of vireos was relatively stable within each 3-day paired-preference trial [RMANOVA: Choice: $F_{(3,84)}=5.03$, $P=0.003$; Time: $F_{(2,168)}=0.16$, $P=0.85$; Choice*Time: $F_{(6,168)}=1.45$, $P=0.20$; Fig. 1]. Vireos ate significantly more 18:1 than 18:0 on 2 of the 3 days (Day 1: $t=-3.34$, $P=0.002$; Day 2: $t=-1.58$, $P=0.06$; Day 3: $t=-3.40$, $P=0.001$; Fig. 1). Vireos ate significantly more 14:0 than 16:0 on Day 1 ($t=-2.46$, $P=0.01$; Fig. 1), but showed no significant preferences on Day 2 or Day 3 ($t=-1.51$, $P=0.07$ and $t=-1.60$, $P=0.06$, respectively; Fig. 1). In general, vireos ate similar amounts of 16:0 and 18:0, although they ate more 16:0 than 18:0 on Day 3 ($t=-1.67$, $P=0.055$; Fig. 1).

Vireos maintained body mass when given paired choices with unsaturated fat diets, whereas body mass of vireos

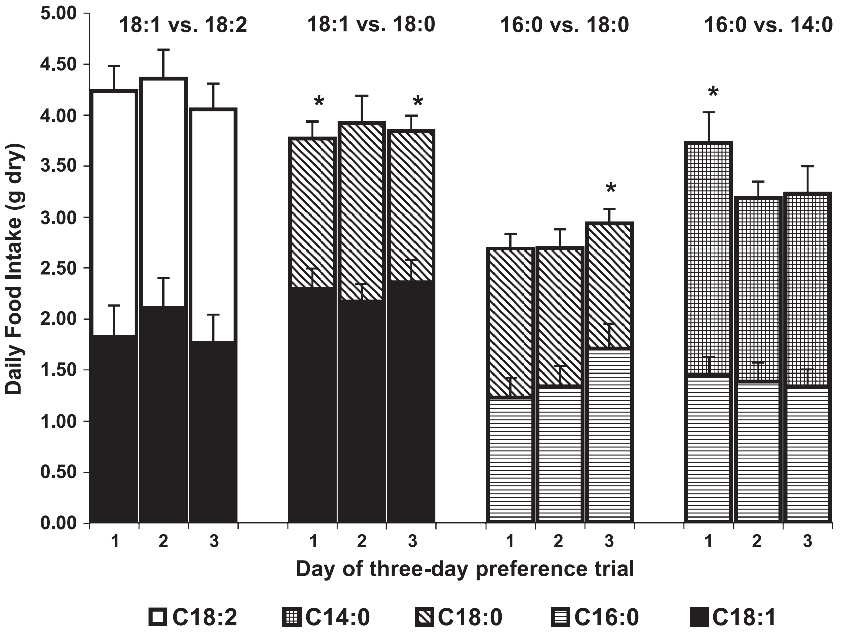

Fig. 1. Food intake (g dry)±S.E.M. of vireos (*V. olivaceous*; $n=22$ per trial) during each of four paired-preference trials. Asterisks above the bars denote significant differences ($P<0.05$) in food intake between the two diets.

decreased when given paired choices with only saturated fat diets [RMANOVA: Choice: $F_{(3,84)}$=0.43, P=0.74; Time: $F_{(2,168)}$=1.46, P=0.24; Choice*Time: $F_{(6,168)}$=2.70, P=0.02; Fig. 2].

3.2. Retention time and extraction efficiency

Body mass of all birds was stable during the GTO (17.36±0.29 g), GTS (17.74±0.35 g), and GTP (17.65 g±0.26 g) feeding trials [RMANOVA: $F_{(2,20)}$=0.83, P=0.45]. In addition, total food intake of vireos was similar during all three trials [RMANOVA: $F_{(2,20)}$=1.5, P=0.25; Fig. 3]. Total mean retention time (TMRT) of digesta was similar for all three labeled lipids [RMANOVA: $F_{(2,20)}$=0.04, P=0.96; Fig. 3]. Extraction efficiency of 18:0 was significantly lower than that of 18:1 or 16:0 [RMANOVA: $F_{(2,20)}$=28.63, P<0.001; Fig. 3).

3.3. Influence of diet on fatty acid composition of body fat

Comparison of fatty acid composition of the diet with that of bird tissues requires estimating the fatty acid composition of the dietary fat. Waxworms comprised 8% of the total daily dry intake (17% of the total daily lipid intake) of vireos during the 1-month feeding trial. We estimated the fatty acid composition of a “combined” diet of semi-synthetic diet and waxworms by adding 83% of the amount of each fatty acid in the semi-synthetic diet to 17% of the amount of each fatty acid in waxworms. We then compared the fatty acid composition of the combined diets to that of tissues from vireos fed with the diet. The fatty acid compositions of the combined mixed diet and of tissues taken from birds fed with that diet are depicted in Table 4. The fatty acid compositions of the combined triolein diet and of tissues taken from birds fed with that diet are depicted in Table 5.

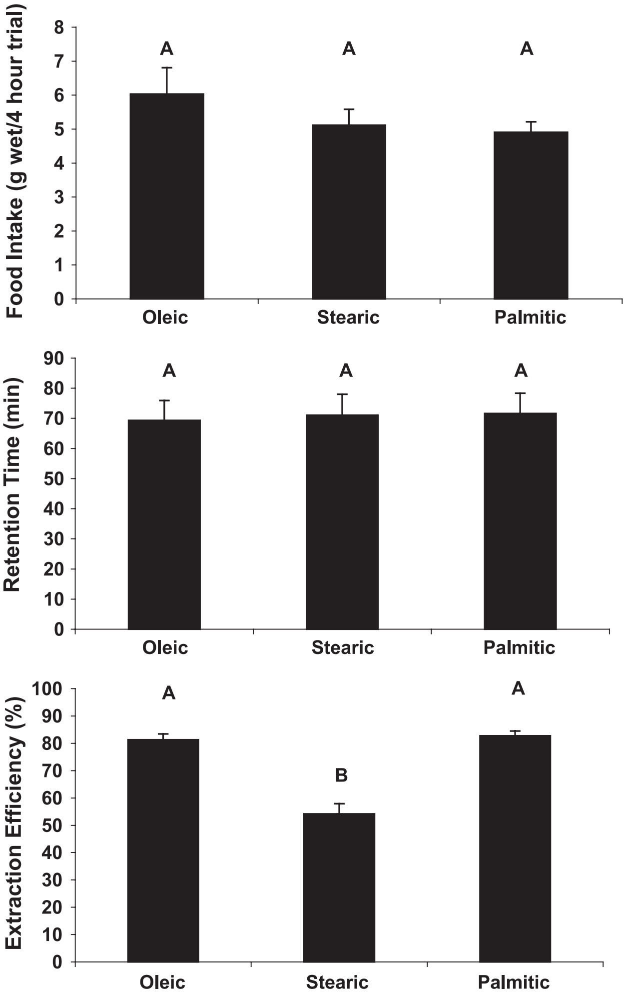

Fig. 3. Food intake±S.E.M. of vireos (*V. olivaceous*) during the 4-h digestibility trials, mean retention time±S.E.M., and extraction efficiency±S.E.M. of glycerol trioleate (Oleic, 18:1), glycerol tristearate (Stearic, 18:0), and glycerol tripalmitin (Palmitic, 16:0). Differences in letters above each bar denote significant differences (P<0.05) between extraction efficiencies for a given fatty acid.

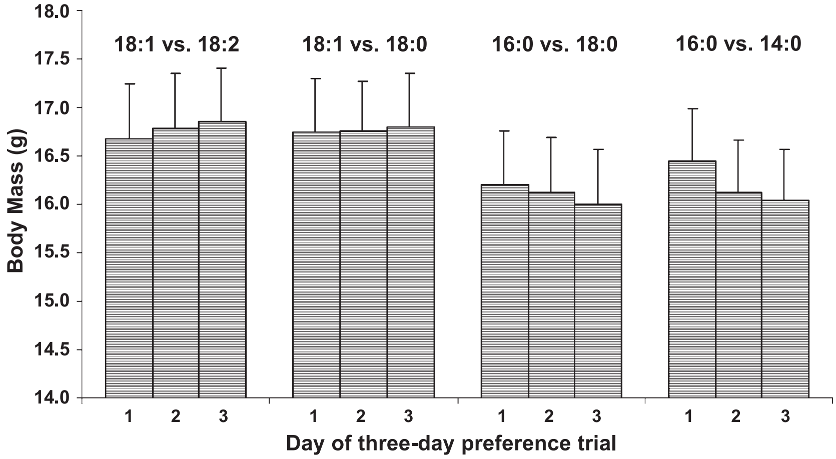

Fig. 2. Body mass±S.E.M. of vireos (*V. olivaceous*) during each of four paired-preference trials. Body mass of vireos increased slightly when fed only with unsaturated diets and decreased when fed only with saturated diets.

Table 4
Fatty acid composition (%±S.E.M.) of the mixed diet plus waxworms and of selected tissues from Red-eyed Vireos (*Vireo olivaceous*) fed with the mixed diet

Fatty acid	Mixed/worm diet (*n*=3)	Tissues from vireos fed with the mixed diet			
		Fat (*n*=1)	Breast (*n*=4)	Intestine (*n*=4)	Liver (*n*=4)
14:0	0.51±0.01	1.42	0.70±0.10	0.74±0.28	0.99±0.19
16:0	24.41±0.41	28.24	18.90±1.76	27.28±1.24	27.16±1.06
16:1n7	0.31±0.01	0.45	0.42±0.14	0.56±0.14	0.42±0.08
18:0	17.90±1.09	11.06	21.30±2.30	19.11±4.77	25.47±2.55
18:1n7	0.61±0.00	0.47	1.53±0.14*	0.92±0.24	1.36±0.29
18:1n9	32.56±0.00	47.92	22.59±7.07	35.22±5.49	30.08±4.82
18:2n6	21.57±1.48	–	–**	–**	–**
18:3n3	0.15±0.00	–	0.12±0.05	0.07±0.05	0.03±0.03
18:3n6	0.26±0.00	0.12	–	0.09±0.06	0.21±0.09
18:4n3	0.13±0.03	–	0.06±0.06	–*	–*
20:1n7	–	0.64	0.23±0.82	2.56±2.08	0.50±0.17
20:1n9	1.26±0.00	9.16	2.20±0.72	2.33±1.67	2.37±0.69
20:4n3	–	–	0.05±0.05	1.65±1.65	0.09±0.85
20:4n6	0.01±0.00	–	13.65±3.06*	4.35±2.24	6.25±1.39*
20:5n3	–	–	0.12±0.12	0.44±0.44	–
22:4n6	–	–	2.09±0.66	0.56±0.34	0.51±0.13
22:5n3	–	0.15	1.41±0.46	0.20±0.13	0.09±0.09
22:5n6	–	–	5.39±1.41	1.70±1.55	1.62±0.77
22:6n3	0.01±0.00	–	8.29±2.05	1.19±0.97	2.16±1.02

* Denotes significant differences ($P<0.01$) between tissue and diet for a given fatty acid.

** Denotes significant differences ($P<0.001$) between tissue and diet for a given fatty acid.

The primary fatty acids in tissues of birds fed with the mixed diet included 16:0, 18:0, and 18:1n9 (Table 4). The proportions of these three fatty acids in the tissues of birds were similar to those in the diet. However, 18:2n6 comprised 22% of the fatty acids in the diet, yet it was not detected in the tissues of vireos fed with this diet. Conversely, 20:4n6, 22:4n6, 22:5n6, and 22:6n3 which were scarce (<0.01% each) in the diet comprised approximately 30% of the fatty acids in breast, 8% of the fatty acids in intestine, and 11% of the fatty acids in liver tissue.

The primary fatty acids in tissues of birds fed with the triolein diet included 18:1n9 (42–65%) and 16:0 (17–23%) even though the diet contained primarily 18:1n9 (86%) and relatively little 16:0 (6%; Table 5). Birds fed with the triolein diet had more 18:0 in their liver than in their diet, and all tissues had significantly less 18:1n9 and 18:2n6 than the diet. In addition, both 18:3 fatty acids were less common

Table 5
Fatty acid composition (%±S.E.M.) of each fatty acid in the triolein diet plus waxworms and of selected tissues from Red-eyed Vireos (*Vireo olivaceous*) fed with the triolein diet

Fatty acid	Triolein/worm diet (*n*=4)	Tissues from vireos fed with the triolein diet			
		Fat (*n*=4)	Breast (*n*=5)	Intestines (*n*=5)	Liver (*n*=5)
14:0	0.40±0.03	0.47±0.03	0.44±0.03	0.51±0.04	0.67±0.08
16:0	6.21±0.02	19.00±0.18**	18.62±0.47**	17.36±0.29**	22.70±00.01**
16:1n7	0.33±0.01	0.92±0.12*	0.74±0.14	0.68±0.08*	0.41±0.06
18:0	0.79±0.03	3.94±2.18	8.98±2.39	5.32±1.67	15.75±2.00**
18:1n7	0.68±0.38	1.57±0.92	2.59±0.66	2.55±0.65	2.93±0.12**
18:1n9	85.93±0.40	64.96±6.17*	49.78±7.23**	64.64±3.77**	42.73±4.81**
18:2n6	2.71±0.01	–**	–**	–**	–**
18:3n3	0.15±0.00	0.13±0.03	0.20±0.03	0.13±0.01*	0.06±0.02**
18:3n6	0.26±0.00	0.06±0.04**	–	0.19±0.12	0.12±0.03*
18:4n3	0.06±0.03	–	–	–	–
20:1n7	–	0.53±0.07**	0.53±0.06**	0.82±0.10**	0.60±0.03**
20:1n9	1.73±0.02	4.06±0.06*	2.96±0.95	3.45±0.50	1.44±0.33
20:4n3	–	–	–	–	–
20:4n6	0.03±0.01	1.79±1.74	6.71±2.13	2.84±1.32	7.56±1.13**
20:5n3	–	–	–	–	–
22:4n6	0.03±0.03	0.15±0.15	0.58±0.29	0.36±0.19	0.36±0.12
22:5n3	–	0.16±0.16	0.69±0.31	0.11±0.10	0.13±0.08
22:5n6	0.15±0.03	0.49±0.49	1.90±0.82	0.21±0.12	1.36±0.45
22:6n3	0.001±0.07	1.33±1.30	4.85±2.00	0.40±0.27	2.64±1.33

* Denotes significant differences ($P<0.01$) between tissue and diet for a given fatty acid.

** Denotes significant differences ($P<0.001$) between tissue and diet for a given fatty acid.

Table 6

F-values and P-values from an ANOVA model that compares the proportion of a specific fatty acid in three tissues of Red-eyed Vireos (n=9) fed with either a Mixed diet (n=4) or Triolein diet (n=5)—see Table 1 for diet composition. Actual fatty acid compositions of the two diets and three tissues are in Tables 4 and 5. The only fatty acid with a significant Diet*Tissue interaction was 16:0 [$F_{(2,21)}$=11.34, P<0.001]

Fatty acids	Diet		Tissue		Tissue comparisons[a]
	F-value df=(1,21)	P-value	F-value df=(2,21)	P-value	
16:0	34.95	<0.001	18.62	<0.001	L>I>B
18:0	30.02	<0.001	5.06	0.02	L>I, L=B, B=I
18:1n7	14.75	0.001	0.46	0.64	
18:1n9	25.35	<0.001	4.10	0.03	I>L, L=B, B=I
18:3n3	4.25	0.05	5.90	0.01	B>L, L=I, B=I
20:4n6	2.31	0.14	5.85	0.01	B>I, L=B, L=I
22:5n3	1.84	0.19	10.38	0.001	B>L, B>I, L=I
22:6n3	1.16	0.29	8.79	0.002	B>L, B>I, L=I

[a] Results of Tukey's HSD post hoc comparison of the proportion of a specific fatty acid in breast (B), intestine (I), and liver (L) of Red-eyed Vireos.

in certain tissues than in the triolein diet. Several fatty acids that were uncommon in the triolein diet, including 16:1n7, 18:1n7, 20:1n7, 20:1n9, and 20:4n6, were significantly more common in certain tissues of vireos fed with this diet (Table 5).

The fatty acid composition of tissues from birds fed with the mixed diet was different than that of birds fed with the triolein diet (Tables 4–6). The tissues from birds fed with the mixed diet had significantly more 16:0 and 18:0 and significantly less 18:1n9 and 18:1n7 than the tissues from birds fed with the triolein diet (Table 6). In general, breast muscle had significantly more long-chain polyunsaturated fatty acids (i.e. 18:3n3, 20:4n6, 22:5n3, and 22:6n3) than intestine or liver (Table 6). However, liver had more saturated fatty acids (i.e. 16:0 and 18:0) than breast muscle or intestines (Table 6).

4. Discussion

4.1. Micronutrient preferences of birds for diets with specific fatty acids

Only three previous studies have focused on micronutrient preferences for specific fatty acids (Bairlein, 1991; Zurovchak, 1997; McWilliams et al., 2002). Zurovchak (1997) offered Wood Thrushes (*Hylocichia mustelina*) paired-choices between diets that were similar in gross lipid content but had different fatty acid compositions because different oils (e.g., olive vs. soy) were used. McWilliams et al. (2002) used unesterified "free" fatty acids. In contrast, we used the TG form of each fatty acid because fatty acids in fruits eaten by wild birds are mostly in the TG form (Zurovchak, 1997).

Despite differences in the form of fatty acids used in these preference studies, the behavioral preferences of songbirds were consistent across studies. We found that Red-eyed Vireos preferred diets with long-chain unsaturated fatty acids (18:1) over diets with long-chain saturated fatty acids (18:0) and, in general, ate more when offered diets with unsaturated fat than when offered diets with saturated fat. Like Red-eyed Vireos in our study, Garden Warblers (Bairlein, 1991) and Yellow-rumped Warblers (McWilliams et al., 2002) preferred diets with 18:1 over diets with 18:0. Zurovchak (1997) showed that wood thrushes preferred diets with 18:1 (olive oil) over diets with 18:2 and 18:3 (soy oil). In contrast, we found that Red-eyed Vireos did not have a specific preference for diets with 18:1 over diets with 18:2 (safflower oil). When given choices of diets with only saturated fatty acids, vireos ate more of the diets containing the shorter-chained fatty acid. Similarly, Garden Warblers preferred diets with 16:0 over diets with 18:0 (Bairlein, 1991). Thus, migratory birds clearly discriminate between diets based on only fatty acid composition, and they prefer monounsaturated fatty acids over similar chain saturated fatty acids.

4.2. Determinants of micronutrient selection in birds

Birds may prefer diets with certain nutrient compositions because the preferred food satisfies specific nutrient requirements or because the alternative food contains components that are avoided. McWilliams et al. (2002) reported that Yellow-rumped Warblers in energy-demanding situations were unable to maintain body mass when given a diet with 50% of the fat comprised of unesterified stearic acid. They suggested that Yellow-rumped Warblers avoided diets with stearic acid because they were unable to efficiently digest such saturated fats. In our Red-eyed Vireos, the extraction efficiencies (EE) of triolein (81%) and tripalmitin (83%) were significantly higher than tristearin (54%). Our estimate of triolein digestibility is similar to estimates for Yellow-rumped Warblers (83%; Afik and Karasov, 1995) but lower than estimates for Wood Thrushes (90%; Zurovchak et al., 1999). Our estimate of digestibility for tripalmitin is higher than estimates found by Place and Stiles (1992) for wax ester tripalmitin (77%) fed to Tree Swallows (*Tachycineta bicolor*). Only Renner and Hill (1961) measured EE for all three fatty acids in the same species of bird. For adult chickens (*Gallus gallus*), they found that EE of unesterified

16:0 and 18:0 was 12% and 4%, respectively, and that EE of both these saturated fatty acids was significantly lower than that of unesterified 18:1 (94% EE).

A possible explanation for the lower extraction efficiency of tristearin in Red-eyed Vireos is that tristearin was relatively uncommon in the diet (10% of dietary fat). However, the fat portion of the diet contained an equal amount of tripalmitin (10%) and the extraction efficiency of tripalmitin was ca. 1.5× greater than tristearin. In addition, the diet also contained ca. 68% triolein and 7% trilinolein. These two fatty acids in the diet can improve the assimilation efficiency of saturated fats such as tristearin (Scott et al., 1982). Nonetheless, TMRT of each of the fatty acids was similar and our estimates of TMRT of all three fatty acids for vireos eating a 20% fat diet are similar to those reported for American Robins (*Turdus migratorius*) fed with a 26% fat diet (Zurovchak et al., 1999). So, why do vireos not increase digesta retention time in order to increase the extraction efficiency of tristearin? McWilliams et al. (2002) found that Yellow-rumped Warblers fed with diets that had 50% of their dietary fat composed of unesterified stearic acid had difficulty maintaining body mass and showed signs of steatorrhoea possibly caused by incomplete digestion of the unesterified stearic acid. This suggests that tristearin may be incompletely digested by such birds even when retention time of digesta is relatively long.

These results provide some support for the hypothesis that birds consume more 18:1 vs. 18:0 to avoid consumption of the less digestible stearic acid (18:0). In the same way, an inability to digest sucrose influences sugar preferences in some passerine birds (Martínez del Rio et al., 1988; Martínez del Rio et al., 1992). We emphasize that whereas we have evidence that digestive constraints, in part, determine dietary preferences, the alternative hypothesis (nutrient preference for certain fatty acids to satisfy specific nutrient requirements) has not been adequately addressed in this or any other study.

4.3. Influence of diet on fatty acid composition of body fat

We found that the fatty acid composition of the diet clearly influenced the fatty acid composition of the body fat of birds. The four predominant fatty acids in the diets (16:0, 18:0, 18:1n9, and 20:1n9) also predominated in the body fat of birds fed with these diets. When vireos were fed with a diet with mostly 16:0, 18:0, and 18:1n9 for 1 month, their tissues (fat, breast, intestines, liver) were also comprised mostly of these same three fatty acids. Likewise, when vireos were fed with a diet with mostly 18:1n9 for 1 month, their tissues were also comprised mostly of this 18:1n9 fatty acid.

Nonetheless, selective metabolism (preferential deposition and/or utilization) appears to play a role in determining the fatty acid composition of body fat in vireos. The triolein diet had relatively little 16:0 (6%), yet this fatty acid comprised 17–23% of fatty acids in the tissues of birds fed with this diet. In addition, 18:2n6 comprised 22% of the fatty acids in the mixed diet, yet this fatty acid was absent from the tissues of vireos fed this diet. Instead, the tissues of vireos fed with the mixed diet contained as much as 13% 20:4n6, 2% 22:4n6, and 5% 22:5n6, even though these fatty acids comprised <1% of their diet. Since all n-6 series fatty acids (e.g., 18:3n6, 20:4n6, 22:4n6, 22:5n6) are produced from the essential fatty acid 18:2n6 (Watkins, 1991), the absence of 18:2n6 from the tissues of vireos fed with a diet containing 18:2n6 and the presence of 20:4n6, 22:4n6, and 22:5n6 in the tissues of these same vireos suggest that selective metabolism of 18:2n6 produced these other n-6 series fatty acids in vireos. This same selective metabolism of the 18:2n6 fatty acid is evident in vireos fed with the triolein diet, although the pattern is less obvious because the triolein diet contained relatively little 18:2n6 (2.7% of all fatty acids).

Although we found that 18:2n6 was absent from the tissues of vireos despite its presence in the diet, other studies have found this fatty acid in the fat stores of Red-eyed Vireos (Walker, 1964; Caldwell, 1973). The relative amount of a given fatty acid in a bird's fat stores is likely related to (1) the amount of the given fatty acid in the diet relative to the nutritional requirements of the bird, (2) the relative abundance of other fatty acids in the diet as well as the bird, and (3) the specific metabolism of the given fatty acid. Presently, we know little about the selective metabolism of fatty acids, or the fatty acid requirements of migratory birds, and we know even less about the fatty acid composition of natural diets of free-living migratory birds.

4.4. Fatty acid composition of specific tissues

Although TGs make up the majority of fatty acids in adipose tissue, the majority of fatty acids in other tissues (e.g., breast, intestine, liver) are structural lipids with large amounts of long-chain polyunsaturates and the saturate, stearic acid (Mead et al., 1986). Since our analysis of fatty acid composition did not segregate between the different lipid classes, it is likely that the majority of the long-chain polyunsaturated n-6 series fatty acids in the breast, intestines, and liver are in the phospholipids of the cell membranes. The results of our study suggest that vireos were utilizing all the dietary 18:2n6 to maintain their structural lipids.

Few studies of migratory birds have examined the fatty acid composition of tissues other than fat (Napolitano and Ackman, 1990; Surai et al., 2000). Napolitano and Ackman (1990) found that lipids of muscle, liver, and intestines from Semipalmated Sandpipers contained more 18:2n6 (4.3%, 3.1%, and 6.8%, respectively), less 20:4n6 (4.37%, 4.02%, and 2.10%), and less 22:4n6 and 22:5n6 (<1% for each tissue) than that found in tissues from Red-eyed Vireos. Surai et al. (2000) found that the phospholipid fraction of liver and breast muscle from free living gulls (*Larus fuscus*) contained more 18:2n6 (7.1% and 16.8%, respectively), more 20:4n6 (23.1% and 18.9%, respectively), less 22:4n6 (0.9% and 1.6%, respectively), and less 22:5n6 (absent from both tissues) than that found in tissues from Red-eyed Vireos. We are unable to determine if these differences in fatty acid composition of vireos, sandpipers, and gulls are due to differences in diet composition because the specific fatty acid composition of the diets eaten by the sandpipers and the gulls was not reported.

Several studies have examined the influence of diet composition on the fatty acid composition of body fat in migratory birds (Bower and Helms, 1968; West and Meng, 1968a, b; Landau, 1970; Morton and Liebman, 1974; Yom-Tov and Tietz, 1978; Heitmeyer and Fredrickson, 1990). However, to our knowledge, no investigations have examined the influence of diet on the fatty acid composition of tissues other than fat in a migratory songbird, although it has been examined extensively in other vertebrates of commercial importance (e.g., poultry: Olomu and Baracos, 1991; Vilchez et al., 1991; Sanz et al., 1999; Wang et al., 2002; fish: Sheikh-Eldin et al., 1996; Bruce et al., 1999; Fontagne et al., 1999; Parpoura and Alexis, 2001; Ballantyne et al., 2003).

4.5. Fatty acid preferences, digestibility, and the composition of body fat: implications for the ecology of migratory birds

Little is known about the specific nutrient requirements of wild birds and their influence on selective feeding. Murphy and King (1984a,b,c) showed that during periods of molt, White-crowned Sparrows (*Zonotrichia leucophrys gambelii*) required more sulfur amino acids in order to maintain body mass and shorten molt duration. Moreover, the sparrows displayed significant dietary preferences for sulfur amino acids during molt (Murphy and King, 1987). Since lipids are the primary source of fuel for migratory flight, it is possible that specific fatty acids are of particular nutritional importance to birds during migration. If these fatty acids are rare in natural foods, behavioral preferences for these specific fatty acids might help wild birds satisfy nutrient requirements. It is unknown whether these behavioral preferences for specific fatty acids change seasonally with different energy demands (e.g., migration, breeding). In addition, given that birds can significantly influence the fatty acid composition of their body fat by selectively feeding, what remains to be demonstrated is whether migratory birds benefit from having certain fatty acid compositions of their body fat.

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