Fatty acid composition of depot fat of shorebirds collected at mid-continental stopover sites during spring migration

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ABSTRACT. Despite the critical role of fat in providing energy for large-scale seasonal movements, little is known about the fatty acid composition of shorebird depot fat. Fatty acid composition is important because it may impact flight performance and seasonal migratory movements. We analyzed the fatty acid composition of depot fat of 12 species of shorebirds collected during spring migration at stopover sites in Kansas from two different subcutaneous fat depots (furcular and saddle depots). Five fatty acids (palmitate [16:0], palmitoleate [16:1], stearate [18:0], oleate [18:1n-9], and vaccenate [18:1n-7]) accounted for 70–96% of the total composition of depot fat despite the diverse foraging behaviors of the species sampled. This similarity in fatty acid profiles may be due to a limited availability of high-lipid food items in the shallow water and mudflat habitats where migratory shorebirds forage in the southern Great Plains. In addition, shorebird depot fat was composed primarily of long-chain saturated and monounsaturated fatty acids (i.e., 16- and 18-carbon), fatty acids thought to be more easily converted to energy during migration. Depot fat in the furcular depot was similar in composition to fat from the saddle depot, and we found no differences in fatty acid composition of the adipose tissue of males and females. Thus, our results suggest that representative shorebird depot fat fatty acid profiles can be obtained even if investigators are limited to sampling one sex, and that adipose tissue collected from saddle depots provide representative samples so biopsies can be limited to areas of the body with the least impact on flight performance.

RESUMEN. La composición de ácidos grasos en los depósitos de grasa de playeros colectados en sitios de parada en el medio del continente durante la migración pre-reproductiva

Aunque la grasa juega un papel crítico en la provisión de energía para movimientos estacionales de grande escala, poco se conoce sobre la composición de ácidos grasos de depósitos de grasa en playeros. La composición de los ácidos grasos es importante porque podría impactar el desempeño de vuelo y los movimientos migratorios entre temporadas. Analizamos la composición de ácidos grasos en depósitos de grasa de 12 especies de playeros colectados durante la migración pre-reproductiva en sitios de parada en Kansas. Analizamos la grasa de dos sitios de depósitos de grasa: la fúrcula y el fémur. Aunque las especies muestreadas demuestran diversos comportamientos de forrajeo, el 70–96% de la composición total de los depósitos de grasa fue compuesto por cinco ácidos grasos (palmitólico [16:0], palmitoleico [16:1], estéreo [18:0], oleico [18:1n-9], y vaccínico [18:1n-7]). Esta similitud en los perfiles de ácidos grasos podría ser un producto de una limitada disponibilidad de items de comida con altos niveles de lipidos en los hábitats de agua pando y humedales donde forrajean los playeros migratorios en el sur de las Grandes Llanuras de Norteamérica. Adicionalmente, los depósitos de grasa de los playeros fueron compuestos principalmente por ácidos grasos saturados de cadena larga y monounsaturados (de 16 y 18 carbonos), cuales se cree son más fáciles de ser convertidos a energía durante la migración. Los depósitos de grasa en la fúrcula fueron similares en su composición al de la grasa del fémur y no encontramos diferencias en la composición de ácidos grasos del tejido adiposo entre machos y hembras. Entonces, nuestros resultados sugieren que los perfiles representativos de los ácidos grasos en los depósitos de grasa de playeros pueden ser obtenidos aún si los investigadores están limitados a muestrear un solo sexo, y que el tejido adiposo colectado del fémur provee muestras representativas para que biopsias pueden ser limitadas a las áreas del cuerpo con el mínimo impacto al desempeño de vuelo.

Key words: depot fat, fatty acids, Kansas, shorebird, spring migration, vaccenate

Long-distance movements of migratory birds are fueled by oxidation of fatty acids stored in adipose tissue (Blem 1990, McWilliams et al. 2004, Price 2010). Fatty acids stored in adipose triglycerides can be derived from the diet, from de novo biosynthesis, or from biochemical processes such as desaturation, beta-oxidation, or elongation (Williams and Buck 2010). The relative contribution of fatty acids from exogenous (dietary) and endogenous (de novo biosynthesis or other processes) sources to adipose stores can be influenced by multiple

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factors. However, Podlesak and McWilliams (2007) demonstrated that the isotopic signature of adipose fatty acids of Yellow-rumped Warblers (*Dendroica coronata*) fed a high-fat diet was similar to that of their diet, whereas the signature for birds fed a low-fat diet was not. Other studies have shown that migrating songbirds preferred foods containing fatty acids that were easier to extract and store relative to foods that were otherwise identical in nutritional content (Pierce et al. 2004). In addition, Klaiman et al. (2009) found that seasonal changes in the fatty acid composition of adipose tissue of White-throated Sparrows (*Zonotrichia albicollis*) were heavily influenced by diet. Such findings support the hypothesis that storage lipids of migratory birds largely reflect the lipids in their diet, at least for birds whose diets contain significant amounts of lipid.

Research has also shown that particular lipids derived from the diet can be used as biomarkers, allowing quantitative assessment of the contributions of particular dietary items to the storage lipid pool (Thiemann et al. 2009) and providing important information about foraging habitat quality. Moreover, fatty acid analysis can also improve our understanding of trophic interactions and seasonal movements of animals when combined with other analytical techniques (Thiemann et al. 2009, Williams and Buck 2010). Therefore, a number of investigators have focused on documenting variation in fatty acid composition and understanding the functional significance of this variation in the storage lipids of migratory birds (Morrison and Hobson 2004, McWilliams et al. 2004, Podlesak and Williams 2007, Price et al. 2008, Klaiman et al. 2009, Price 2010). However, much remains to be understood about the role of fatty acids in fueling migratory flights (Guglielmo 2010).

**METHODS**

Shorebirds were collected from 6 to 18 May 1999 at playa wetlands located on private land in Meade County, Kansas (37°18'N, 100°35'W), and from 18 to 26 May 1999 at wetlands located on Quivira National Wildlife Refuge in Stafford County, Kansas (38°10'N, 98°40'W), a period that encompasses the peak of shorebird migration during spring in this area (Thompson and Ely 1989). We collected individuals representing 12 species opportunistically in shallow wetlands during daylight hours with a shotgun. Because birds were collected as part of a broader investigation of shorebird ecology (see Rivers and Briskie 2003), sample sizes were dictated by other objectives. Immediately after collection, individuals were weighed using a spring scale and bill length was measured as the distance between the tip of the bill and the edge of the feathering at the base of the upper bill; body condition was measured via body mass alone and body mass corrected for size. We did not determine shorebird age, but did determine sex by internal examination of gonadal tissue.

Fat samples were taken from two subcutaneous regions (King and Farner 1965): the claviculo-coracoid depot region on the breast (hereafter the furcular depot) and the sartorial and femoral region of the leg (hereafter the saddle depot). Within an hour of collection, samples were placed in a scintillation vial in a cooler with dry ice. In the lab, samples were
purged with a stream of dry N₂ to prevent oxidation of polyunsaturated fatty acids, and moved to a –20°C freezer for additional extraction and analysis.

Lipids were extracted from frozen samples and separated into neutral lipids and phospholipids on short silicic acid columns (Unisil, Sigma Chemical Co., St. Louis, MO; see Florant et al. 1993 for details). Fatty acid methyl esters (FAME) of neutral lipid extracts were prepared as described in Florant et al. (1993), and separated by gas chromatography using a gas chromatograph (HP5890A, Hewlett-Packard, Wilmington, DE) with a Supelco 30-m Omegawax 250 capillary column (Restek, Bellefonte, PA) run isothermally at 220°C, and detected with a flame ionization detector. FAME were identified by comparison to known standards (Nu-Chek Prep, Elysian MN). To confirm that the fatty acid composition of the total lipid fractions was primarily determined by the fatty acid composition of the triglycerides in those fractions, at least one sample from each species was further fractionated by thin-layer chromatography on silica gel (Florant et al. 1993). The triglyceride fraction was identified, extracted from the silica gel, and FAMEs from these purified triglycerides were prepared and analyzed using the methods described above.

Because modest sample sizes for most species precluded robust statistical analyses, we focus primarily on describing variation in fatty acid profiles among species via mean values and their associated 95% confidence intervals (CIs). Presenting mean values with 95% CIs provides more information than P values from null hypothesis tests because 95% CIs also provide an estimate of effect size and a measure of its uncertainty while providing qualitative results regarding hypothesis tests (Johnson 1999). Importantly, collecting additional birds for a more robust statistical analysis did not appear justified given the consistent pattern found with the samples we obtained. In addition to describing variation in fatty acid composition, we used Spearman’s correlations to examine the relationship between body condition (i.e., both body mass and body mass corrected for bill size) and the relative proportion of saturated and monounsaturated fatty acids in depot fat. We used the following standard calculation for calculating a double bond index (DBI):

\[ \text{DBI} = \left[ 1^\prime \right] \left( \text{percentage of monoenoic fatty acids} \right) + \left[ 2^\prime \left( \text{percentage of dienoic fatty acids} \right) \right] + \left[ 3^\prime \left( \text{percentage of trienoic fatty acids} \right) \right] ; \text{no fatty acids with more than three double bonds were detected in the analysis (Rivers and Rintoul, unpubl. data).} \]

**RESULTS**

With the exception of the Killdeer whose depot fat was enriched in oleate and very low in 18:1n-7, fatty acid composition of shorebird depot fat was similar among the 12 species sampled (Table 1). We found no differences in fatty acid composition of males and females or in samples from the two fat depots (as evidenced by overlapping 95% CIs for all comparisons where \( N_1 > 1 \) and \( N_2 > 1 \)). Across individuals of all species, 70% to 96% of the total composition of depot fat consisted of five fatty acids: palmitate (16:0), palmitoleate (16:1), stearate (18:0), oleate (18:1n-9), and vaccenate (18:1n-7). We found a similar proportion of saturated and monounsaturated fatty acids in depot fat across species, with <10% of the total fatty acids typically being polyunsaturated (Table 2). We also fractionated some total lipid extracts using thin-layer chromatography and compared the fatty acid composition of the total extracts with that of the purified triglycerides from those extracts. We found no significant or reproducible differences in the fatty acid composition of the total extracts and the purified triglycerides (Rivers and Rintoul, unpubl. data), indicating that the fatty acid composition of total lipid extracts was a reasonable proxy for the triglyceride fraction.

Most correlations between the two aspects of body condition (i.e., body mass and body mass corrected for bill size) with two types of fatty acids (i.e., proportion of saturated fatty acids and proportion of monounsaturated fatty acids) were not significant (\( P > 0.12 \)). The two exceptions were the correlation between body mass corrected for bill size and the mean proportion of saturated fatty acids for Least Sandpipers (Spearman’s \( r = 0.77, P = 0.03, N = 8 \)) and Semipalmated Sandpipers (Spearman’s \( r = -0.61, P = 0.01, N = 16 \)).

**DISCUSSION**

We found that the composition of fatty acids in depot fat collected at mid-continental
Table 1. Mean percentage fatty acid (±95% CI) composition of depot fat collected from shorebirds at stopover sites in Kansas during spring migration. Values were calculated by averaging the mean percentage of fatty acids in the furcular and saddle depots for each individual, and then averaging across individuals of both sexes within each species.

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>14:0 ± CI</th>
<th>16:0 ± CI</th>
<th>16:1 ± CI</th>
<th>18:0 ± CI</th>
<th>18:1n-9 ± CI</th>
<th>18:1n-7 ± CI</th>
<th>18:2 ± CI</th>
<th>18:3 ± CI</th>
<th>Other ± CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Avocet (Recurvirostra americana)</td>
<td>8</td>
<td>1.7 (1.2, 2.3)</td>
<td>23.8 (21.8, 25.7)</td>
<td>10.8 (7.1, 14.5)</td>
<td>11.8 (9.9, 13.7)</td>
<td>24.9 (18.7, 31.2)</td>
<td>6.5 (4.3, 8.7)</td>
<td>6.2 (4.1, 8.2)</td>
<td>4.6 (1.4, 7.8)</td>
<td>9.8 (4.8, 14.8)</td>
</tr>
<tr>
<td>Wilson’s Phalarope (Phalaropus tricolor)</td>
<td>11</td>
<td>1.4 (1.1, 1.8)</td>
<td>27.9 (24.2, 31.5)</td>
<td>10.8 (9.0, 12.6)</td>
<td>13.1 (12.0, 14.2)</td>
<td>28.8 (25.7, 31.8)</td>
<td>5.2 (4.2, 6.2)</td>
<td>4.7 (2.3, 7.2)</td>
<td>2.8 (1.4, 4.2)</td>
<td>5.3 (4.1, 6.5)</td>
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<tr>
<td>Killdeer (Charadrius vociferus)</td>
<td>1</td>
<td>0.4</td>
<td>25.2</td>
<td>3.4</td>
<td>8.3</td>
<td>52.2</td>
<td>0.0</td>
<td>5.0</td>
<td>1.1</td>
<td>4.3</td>
</tr>
<tr>
<td>Sanderling (Calidris alba)</td>
<td>5</td>
<td>2.7 (2.2, 3.3)</td>
<td>24.9 (22.6, 27.1)</td>
<td>13.6 (9.7, 17.6)</td>
<td>10.7 (9.1, 12.2)</td>
<td>27.5 (22.9, 32.1)</td>
<td>4.8 (3.7, 5.9)</td>
<td>6.9 (4.1, 9.7)</td>
<td>2.4 (1.0, 3.7)</td>
<td>6.5 (4.5, 8.6)</td>
</tr>
<tr>
<td>Semipalmated Sandpiper (C. pusilla)</td>
<td>18</td>
<td>2.1 (1.8, 2.5)</td>
<td>26.1 (24.5, 27.8)</td>
<td>14.8 (12.6, 16.9)</td>
<td>9.9 (9.0, 10.8)</td>
<td>26.8 (25.3, 28.3)</td>
<td>4.0 (3.4, 4.7)</td>
<td>6.1 (4.7, 7.6)</td>
<td>3.6 (3.1, 4.1)</td>
<td>6.5 (5.3, 7.7)</td>
</tr>
<tr>
<td>Western Sandpiper (C. mauri)</td>
<td>1</td>
<td>2.3</td>
<td>25.1</td>
<td>11.1</td>
<td>13.3</td>
<td>31.6</td>
<td>5.4</td>
<td>6.6</td>
<td>2.6</td>
<td>2.0</td>
</tr>
<tr>
<td>Least Sandpiper (C. minutilla)</td>
<td>7</td>
<td>1.5 (0.8, 2.2)</td>
<td>24.5 (23.0, 26.1)</td>
<td>8.7 (6.4, 11.1)</td>
<td>9.9 (8.4, 11.5)</td>
<td>30.3 (28.5, 32.2)</td>
<td>4.6 (2.5, 6.8)</td>
<td>11.7 (9.1, 14.2)</td>
<td>4.0 (2.4, 5.6)</td>
<td>4.6 (2.0, 7.2)</td>
</tr>
<tr>
<td>White-rumped Sandpiper (C. faucoontii)</td>
<td>17</td>
<td>2.1 (1.5, 2.6)</td>
<td>25.8 (24.4, 27.3)</td>
<td>12.5 (10.5, 14.5)</td>
<td>12.3 (11.2, 13.4)</td>
<td>30.2 (27.8, 32.6)</td>
<td>5.2 (4.2, 6.1)</td>
<td>5.2 (4.0, 6.4)</td>
<td>2.0 (1.6, 2.5)</td>
<td>4.6 (3.4, 5.8)</td>
</tr>
<tr>
<td>Baird’s Sandpiper (C. bairdii)</td>
<td>2</td>
<td>1.2 (0.9, 1.7)</td>
<td>22.1 (16.2, 28.0)</td>
<td>14.5 (28.0, 57.0)</td>
<td>11.0 (-21.3, 43.3)</td>
<td>24.8 (-21.0, 70.7)</td>
<td>4.2 (4.2, 12.6)</td>
<td>9.2 (-16.6, 35.1)</td>
<td>8.6 (-64.6, 81.8)</td>
<td>4.3 (-32.3, 40.9)</td>
</tr>
<tr>
<td>Pectoral Sandpiper (C. melanotos)</td>
<td>1</td>
<td>2.2</td>
<td>30.0</td>
<td>11.2</td>
<td>9.6</td>
<td>27.5</td>
<td>5.7</td>
<td>5.0</td>
<td>6.8</td>
<td>2.1</td>
</tr>
<tr>
<td>Stilt Sandpiper (C. himantopus)</td>
<td>10</td>
<td>2.0 (1.6, 2.5)</td>
<td>27.7 (25.5, 29.9)</td>
<td>9.2 (7.7, 10.8)</td>
<td>11.8 (10.7, 13.0)</td>
<td>28.7 (25.7, 31.8)</td>
<td>5.4 (4.5, 6.4)</td>
<td>5.9 (4.5, 7.3)</td>
<td>2.5 (1.9, 3.0)</td>
<td>6.6 (4.8, 8.4)</td>
</tr>
<tr>
<td>Long-billed Dowitcher (Limnodromus scolopaceus)</td>
<td>5</td>
<td>1.4 (1.2, 1.7)</td>
<td>26.7 (24.7, 28.8)</td>
<td>6.4 (4.5, 8.4)</td>
<td>11.2 (9.5, 12.9)</td>
<td>27.1 (23.5, 30.7)</td>
<td>5.9 (5.4, 6.4)</td>
<td>5.8 (4.3, 7.2)</td>
<td>4.8 (2.1, 7.5)</td>
<td>10.6 (8.3, 13.0)</td>
</tr>
</tbody>
</table>
stopover sites was similar among the taxonomically diverse shorebird species sampled, with 70 to 96% of the total composition of depot fat consisting of only five fatty acids. We found a similar proportion of saturated and monounsaturated fatty acids in depot fat across species and, with the exception of the Killdeer, <10% of the total fatty acids were polyunsaturated fatty acids. The fatty acid composition of depot fat of the Killdeer was enriched in 18:1n-9 and depleted in 18:1n-7. Based on the collection date and gonadal data (Rivers and Briskie 2003), the Killdeer in our study was a local breeder and may have consumed different prey than birds still migrating and restricted to wetland habitats.

With the exception of the Killdeer, most species in our study had similar fatty acid profiles. If fatty acid composition of depot fat reflects diet in migratory birds (Egeler and Williams 2000, Pierce and McWilliams 2005), such results are unexpected because the species in our study differed markedly in body size and, as a corollary, would be expected to differ in foraging ecology. One explanation for the similarity in fatty acid profiles of the different species is a limited diversity of high-lipid food items available for fueling migratory flights (Maillet and Weber 2006). In support of this hypothesis, Davis and Smith (2001) documented a limited availability of invertebrate prey during spring in the shallow water and mudflat habitats where migratory shorebirds forage in the southern Great Plains, south of where birds were collected in our study. Examining the foraging behavior of four species that vary in size and foraging niches (American Avocet, Long-billed Dowitcher, Least Sandpiper, and Western Sandpiper; see Table 1 for scientific names), Davis and Smith (2001) found that all four species preferred smaller prey and that the abundance of prey items in their diets was not correlated with nutritional and energetic quality, but with the abundance of prey in playa lakes. Thus, similar fatty acid profiles of different species in our study may be due to foraging on similar prey items at our study sites, at more southern stopover sites visited prior to arrival at our study sites, or both. However, we cannot rule out an endogenous source for many of these fatty acids, which would also explain the similarity across species. Regardless, this topic has important implications for assessing the quality of stopover habitats in the interior of North America, and additional work to determine the dietary or endogenous sources of depot lipids of shorebirds is clearly warranted.

As also reported for Western and Semipalmated sandpipers collected in coastal habitats (Napolitano and Ackman 1990, Egeler and Williams 2000, Maillet and Weber 2006), we found that shorebird depot fat was composed primarily of long-chain saturated and unsaturated fatty acids.
monounsaturated fatty acids (i.e., 16- and 18-carbon). These types of fatty acids likely dominate because they are thought to be more easily converted to energy during migration than longer-chain fatty acids (McWilliams et al. 2004, Price et al. 2008). In addition, we detected vaccenate (18:1n-7) in all shorebird species we examined. Although this fatty acid was most likely the cis isomer based on identification using a cis 18:1n-7 standard, our chromatographic methods did not allow us to consistently discriminate between cis and trans isomers. However, the trans isomer is primarily a product of ruminant bacterial metabolism and is found predominantly in the tissues of ruminants and in dairy products, whereas the cis isomer is a major component of many bacterial phospholipids (but can also be synthesized de novo by mammals; see Holloway and Wakil 1964). Levels of this fatty acid in our shorebird samples were higher than previously reported for adipose tissue lipids of shorebirds (Napolitano and Ackman 1990, Egeler and Williams 2000) and other migratory birds (Pierce and McWilliams 2005, Klaiman et al. 2009). Stanton et al. (2001) reported de novo synthesis of cis 18:1n-7 by birds so it seems likely that birds, like mammals, can generate small amounts of this fatty acid endogenously. Importantly, the percentage of 18:1n-7 in purified triglyceride fractions from the adipose lipid extracts was identical to that of the total neutral lipid extract, indicating that the presence of this fatty acid was not due to bacterial contamination. Thus, our results and those of previous studies indicate that fatty acid composition is similar across a diverse group of migratory shorebirds and this similarity could be due to the need for easy mobilization of fat stores during migratory flights (Egeler and Williams 2000).

We found no significant differences in the fatty acid composition of males and females. Such differences might be expected given that females undergo markedly different physiological processes than males shortly after arrival on the breeding grounds (e.g., egg laying and incubation) and the energetic resources used for these processes appear to be based, in part, on fatty acids obtained during migration (Morrison et al. 2004, Tulp et al. 2009, Price 2010). A likely explanation for similarities between males and females, as noted above, is the limited diversity of food resources available at spring stopover areas. Regardless of the reason, similarities between males and females in fatty acid composition indicate that investigators seeking representative shorebird depot fat fatty acid profiles can sample shorebirds opportunistically, facilitating the sampling of species with intersexual variation in migration phenology.

We also found no differences in fatty acid composition of different fat depots, a finding consistent with other data for shorebirds (Egeler and Williams 2000) and in contrast to some mammals (Calder et al. 1992). Our observations, along with those from previous studies, indicate that investigators could take adipose tissue biopsies from the saddle depot instead of the furcular depot, limiting the invasive procedure to areas least likely to impact flight performance.

In sum, we found that the composition of adipose tissue collected at spring migration stopover sites was similar among shorebird species with diverse foraging styles, likely due to a limited diversity of available prey at stopover sites. In addition, fatty acid composition of adipose tissue was similar between furcular and saddle regions and for males and females. Our results provide the basis for additional studies that will allow a better understanding of variation in fatty acid composition and the possible effects of such variation on the migration behavior and breeding performance of birds undergoing seasonal migratory movements.

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LITERATURE CITED


