Correction


The authors note that Fig. 3 appeared incorrectly. The corrected figure and its legend appear below.

Fig. 3. Fatty acid composition results for (A) brain EPA, (B) brain DHA, (C) muscle EPA, and (D) muscle DHA. Treatment means and SE bars are shown. Black circles represent our Hh treatment, gray circles represent our Hl treatment, black triangles represent our Lh treatment, and gray triangles represent our Ll treatment.

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Omega-3 long-chain polyunsaturated fatty acids support aerial insectivore performance more than food quantity

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Once-abundant aerial insectivores, such as the Tree Swallow (Tachycineta bicolor), have declined steadily in the past several decades, making it imperative to understand all aspects of their ecology. Aerial insectivores forage on a mixture of aquatic and terrestrial insects that differ in fatty acid composition, specifically long-chain omega-3 polyunsaturated fatty acids (LCPUFA) content. Aquatic insects contain high levels of both LCPUFA and their precursor omega-3 PUFAs, alpha-linolenic acid (ALA), whereas terrestrial insects contain much lower levels of both. We manipulated both the quantity and quality of food for Tree Swallow chicks in a full factorial design. Diets were either high-LCUPFA or low in LCPUFA but high in ALA, allowing us to separate the effects of direct LCPUFA in diet from the ability of Tree Swallows to convert their precursor, ALA, into LCPUFA. We found that fatty acid composition was more important for Tree Swallow chick performance than food quantity. On high-LCUPFA diets, chicks grew faster, were in better condition, and had greater immunocompetence and lower basal metabolic rates compared with chicks on both low LCPUFA diets. Increasing the quantity of high-LCUPFA diets resulted in improvements to all metrics of performance while increasing the quantity of low-LCUPFA diets only resulted in greater immunocompetence and lower metabolic rates. Chicks preferentially retained LCPUFA in brain and muscle when both food quantity and LCPUFA were limited. Our work suggests that fatty acid composition is an important dimension of aerial insectivore nutritional ecology and reinforces the importance of high-quality aquatic habitat for these declining birds.

Significance

Insect abundance is an important predictor of survival and performance for many taxa of aerial insectivores, which forage on a mixture of aquatic and terrestrial insects that differ in fatty acid composition, particularly omega-3 long-chain polyunsaturated fatty acid (LCPUFA) content. We raised Tree Swallow (Tachycineta bicolor) chicks on either a high or low quantity of feed with either high amounts of the LCPUFA found in aquatic insects or an equivalent amount of the precursor omega-3 PUFA, alpha-linolenic acid, but low LCPUFA. LCPUFA content was more important for Tree Swallow chick performance than food quantity. Tree Swallows may be timing breeding to coincide with the peak abundance of high-LCPUFA aquatic insects.


Conflict of interest statement: T.N.T. is employed at Purina Mills, LLC, which manufactures the product tested. The authors declare no other conflicts of interest.

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all EPA and DHA endogenously from ALA, whereas carnivores such as cats must obtain all of their DHA from diet (15). The ability of wild birds to synthesize DHA is not well characterized, but DHA concentrations are inversely related to mass (16). For example, DHA constitutes 12% of FAs in the muscles of House Sparrows (Passer domesticus) (16), which are similar in size to Tree Swallows, but can reach over 20% in Ruby-throated Hummingbird (Archilochus colubris) muscle (17).

In the wild, aerial insectivores consume a combination of terrestrial and aquatic insects (18), which differ in their FA composition (19). Aquatic insects contain much higher levels of LCPUFA than do terrestrial insects, a difference driven by differences in the FA composition of aquatic and terrestrial primary producers (19, 20). Aquatic primary producers, such as diatoms and dinoflagellates, are rich in EPA and DHA (21), which can be incorporated into aquatic insect tissue (22). In contrast, vascular terrestrial plants contain little to no LCPUFA but do contain their molecular precursor ALA (14), which can be either incorporated into tissue or converted to LCPUFA to a minor degree by terrestrial insects (19). As a consequence, from the perspective of LCPUFA content, aquatic insects may constitute a higher-quality food for aerial insectivores than do terrestrial insects.

However, because both aquatic and terrestrial insects contain ALA, the relative value of aquatic insects depends on the capacity of aerial insectivores to convert ALA into LCPUFA (19). The ability to elongate ALA into LCPUFA varies greatly across taxa: Strict carnivores, such as cats (23, 24), and animals from environments rich in LCPUFA, including most marine fish (25) have lost the ability to elongate ALA into LCPUFA and must obtain them directly from diet. In contrast, terrestrial herbivores appear to be relatively efficient at converting ALA to LCPUFA (26). The capacity of aerial insectivores to convert ALA to LCPUFA remains untested, but, as predators living around riparian areas with emergent aquatic insects rich in LCPUFA, they appear likely to be limited by LCPUFA content in diet.

The majority of past studies on avian FA requirements have focused on domesticated herbivorous taxa, especially chickens (e.g., refs. 27 and 28). These studies found domestic hens to be relatively efficient at elongating ALA, EPA, and DHA (e.g., refs. 27 and 28). Fewer studies have experimentally manipulated dietary FA composition for wild birds (but see refs. 29–32). These studies have found that both dietary composition and elongation capacity of individual species affect avian FA composition (32). However, with the exception of work by Pierce et al. (31) on Red-Eyed Vireos (Vireo olivaceus), these studies have either looked at seed- and fruit-eating passerines or fish-eating seabirds. To our knowledge, no studies have explicitly examined the omega-3 FA requirements of any aerial insectivores. Therefore, we sought to understand the importance of food FA composition for aerial insectivores by varying both food quality and quantity in a balanced factorial experimental design.

In nature, the effects of food quality and quantity may be confounded because parents may provide chicks with an increased quantity of food to make up for low-quality food. To address this, we experimentally manipulated both the quantity and FA composition of food for wild-hatched nesting Tree Swallow chicks. Chicks were fed one of four diets: (i) a high-LCPUFA, high-quality diet containing EPA and DHA (Hh); (ii) a low-LCPUFA, high-quality diet containing high ALA and low omega-3 LCPUFA (Lh); (iii) a high-LCPUFA, low-quality diet (Hl); and (iv) a low-LCPUFA, low-quality diet (Ll). We assessed size-specific growth rates, body condition, immunocompetence, and basal metabolic rates (BMR) as metrics of performance. We also determined the FA composition of brain and breast muscle tissue from a subset of chicks from each treatment group.

**Results**

Chicks on high-LCPUFA diets (Table S1) grew significantly more rapidly than those on low-LCPUFA diets (Table S1) regardless of food quantity (ANOVA: treatment $F_{3,128} = 59.889, P < 0.0001$; Figs. 1A and B and 2). Diet quality was more important than quantity: Even HI chicks grew significantly faster than did Lh chicks (Table S2 and Figs. 1B and 2). Among the high-LCPUFA groups, Hh chicks grew significantly faster than did Hl chicks (Table S2 and Figs. 1B and 2). Among the low-LCPUFA groups, there were no significant differences between LI and Lh chicks (Table S2). There were no significant treatment differences in head–bill or tarsus growth rates between treatments (ANOVA for head–bill: treatment $F_{3,90} = 0.099, P = 0.96$; ANOVA for tarsus: treatment $F_{3,90} = 2.091, P = 0.107$; Fig. 1C). Thus, the differences observed were in growth rates for mass, not structural size.

Chicks on high-LCPUFA diets were also in significantly better condition (reflected by the ratio of mass to head–bill length and mass to tarsus length) than those on low-LCPUFA diets regardless of quantity (ANOVA for mass to tarsus: treatment $F_{3,115} = 225.673, P < 0.0001$; ANOVA for mass to head–bill: treatment $F_{3,115} = 276.462, P < 0.0001$; Fig. 1D). Chicks on Hh were in significantly better condition than were HI chicks (Table S3), and Hh chicks were in significantly better condition than were Lh chicks (Table S3 and Fig. 1D). Among the low-LCPUFA groups, chicks on Lh were in significantly better condition than were LI chicks (Table S3).

Chicks on high-LCPUFA diets had increased immunocompetence compared with those on low-LCPUFA diets regardless of food quantity (ANOVA: treatment $F_{3,90} = 38.187, P < 0.0001$; Fig. 1E). Even Lh chicks had significantly higher phytohemagglutinin (PHA) immune response ratios than did Ll chicks (Table S4 and Fig. 1E). Among the high-LCPUFA groups, Hh chicks had significantly higher immune response ratios than HI chicks (Table S4). Among the low-LCPUFA groups, there were no significant differences between HI and Lh chicks (Table S4).

Patterns in BMR were the reverse of those in immunocompetence (Kruskal–Wallis: $\chi^2 = 7.941, df = 3, P < 0.047$; Fig. 1E and F). HI chicks had the lowest BMR whereas LI chicks had the highest BMR, and these differences were significant (Table S5 and Fig. 1F). HI chicks and Lh chicks had similar BMRRs, which were not significantly different (Table S5 and Fig. 1F). We also found that LI chicks had significantly higher BMR than HI chicks, and Lh chicks had significantly higher BMR than Hh chicks (Table S5 and Fig. 1F).

We found both treatment and tissue-based differences in FA composition (Tables S6 and 7 and Fig. 3). Hh chicks had significantly higher percentages of EPA in brain, whereas chicks on both high-LCPUFA diets had significantly higher percentages of EPA in muscle (Kruskal–Wallis for brain EPA: $\chi^2 = 7.567, df = 3, P = 0.056$; Kruskal–Wallis for muscle EPA: $\chi^2 = 9.088, df = 3, P = 0.028$; Table S6 and Fig. 3). The percentage of DHA in brain was significantly higher in Lh chicks compared with chicks on either high-LCPUFA diet (Kruskal–Wallis: $\chi^2 = 8.316, df = 3, P = 0.040$; Table S6 and Fig. 3B). In contrast, the percentage of DHA in muscle was highest in HI chicks, but it was only significantly higher than the percentage of DHA in muscle of LI chicks (Kruskal–Wallis for muscle DHA: $\chi^2 = 3.882, df = 3, P = 0.075$; Table S6 and Fig. 3D). The percentage of total omega-3 FAs in brain was also significantly higher in chicks on Lh diets compared with chicks on high-LCPUFA diets (Table S6). We found no significant differences in either the proportion of total omega-6 FAs in either brain or muscle or the percentage of total omega-3 FAs in muscle (Table S6). Muscle had significantly higher percentages of EPA and total omega-6 FAs compared with brain, but it had similar percentages of total omega-3 FAs and significantly less DHA (Table S6).
Discussion

We asked if food quality, in terms of LCPUFA, was as important as food quantity for a model aerial insectivore species, the Tree Swallow. We manipulated food quantity and FA composition in a fully factorial design and assessed performance in Tree Swallow chicks by measuring changes in size (mass, head--bill length, and tarsus length), body condition, and differences in immunocompetence and BMR at the conclusion of the experiment. Overall, we found strong evidence that LCPUFA content is as important, if not more important, than food quantity for aerial insectivores (Figs. 1 and 2). We also found significant differences in the FA composition of chicks on different diets, which suggested that the chicks preferentially retained EPA and DHA (Fig. 3).

Hh chicks grew the fastest and were in the best condition, whereas Ll chicks grew the slowest and were in the poorest overall condition (Figs. 1 and 2). Interestingly, Hl chicks performed better than did Lh chicks (Figs. 1 and 2). Increasing the quantity of low-LCPUFA food had no effect on growth rates or condition. Body mass and condition are two of the most important predictors of Tree Swallow fledgling success and survival (25). There appears to be greater pressure to quickly reach fledgling size to be ready to fledge if threatened by predation (35, 36).

We found that food quantity and quality had significant interacting effects on immunocompetence, measured as PHA ratio, across treatments (Fig. 1E). In birds, PHA ratio is an indicator of acquired T-cell proliferation and the ability to produce lymphocytes in response to pathogens (37). We found that Hh chicks had the highest PHA ratios whereas Ll chicks had the lowest ratios and Hl and Lh chicks had equivalent, intermediate PHA swelling responses. Our results suggest that wild Tree Swallow chicks with access to more food, especially high-quality aquatic insects containing EPA and DHA, may be more likely to mount an effective immune response (3, 33). In addition to predators and food deprivation, pathogens are a significant source of early mortality in nestling Tree Swallows (38), and greater immunocompetence from higher food quantity and quality likely increases Tree Swallow chick survival.

Food quality and quantity also had significant interacting effects on BMR across treatments (Fig. 1F). Hh chicks had the lowest metabolic rates and Ll chicks had the highest BMR, either mass-corrected or whole-organism (Table S5). Our low- and high-LCPUFA feeds had equal caloric content (Table S1), so differences in BMR are likely due to effects of feed FA composition and total LCPUFA content, not total energy. The negative relationship between total LCPUFA content of feed and BMR could have resulted from costs of ALA elongation and desaturation to LCPUFA. For example, although feed for Hl and Hh chicks had the same FA composition, Hl chicks consumed less total LCPUFA compared with Hh chicks, and thus may have required additional energy to convert ALA into LCPUFA. Our findings agree with those of previous studies; for example, Pierce et al. (31) found that increasing unsaturated FAs in diet decreased peak metabolic rate for Red-Eyed Vireos. Across all treatments, our findings support the inverse relationship observed by Hulbert.
et al. (16) between avian body mass and both BMR and breast muscle DHA across bird species.

The FA composition of chicks provided evidence for both dietary accumulation and preferential retention of the long-chain omega-3 FAs EPA and DHA (Fig. 3). In brain tissue, the percentage of EPA was highest in Hh chicks, whose diet contained both the highest percentage and the greatest total amount of EPA (Fig. 3A). Chicks on Hh and Hi diets had the highest percentages of EPA in muscle tissue (Table S5 and Fig. 3C). The Lh and Ll diets may not have contained sufficient amounts of EPA to accumulate dietary EPA, or Tree Swallows may be inefficient at converting ALA to EPA; this suggests that EPA accumulation in the Tree Swallow tissues may be based on dietary availability of EPA, which is consistent with findings in other taxa (28).

In brain tissue, the percentage of DHA was highest in Lh chicks (Fig. 3B). This finding could have stemmed from either increased elongation of ALA or preferential LCPUFA retention in Lh chicks. We suggest a combination of elongation and preferential retention may have been at work: Lh chicks would have had more energy to devote to elongation than did low-quantity chicks and would have had more non-LCUPFA FAs in diet to preferentially oxidize for fuel than did high-LCUPFA chicks. Tree Swallow muscle tissue had significantly less DHA than did brain (Table S6 and Fig. 3), potentially because phospholipid DHA is a key component of neural tissue (39). In muscle tissue, the proportion of DHA in muscle was highest in Hi chicks (Fig. 3D), which we suggest was due to preferential retention because Hi chicks were limited in energy but not in LCUPFA.

Chicks on low-LCUPFA and/or low-quantity diets may have either converted ALA to LCUPFA or preferentially retained LCUPFA already present in tissue. Studies suggest that chicken embryos preferentially remove LCUPFA from yolk (27). However, this does not appear to be the case with altricial chicks, such as Barn Swallows (Hirundo rustica), which contain much less DHA at hatch than do precocial birds (40). We were unable to control maternal FA investment in eggs or parental feeding during the chicks’ first few days of life, and all chicks originated from nest boxes on or near water. Thus, Hl and Lh chicks likely preferentially retained DHA from eggs and early life while oxidizing other dietary fats for energy. In contrast, Hh chicks may paradoxically have had lower tissue DHA concentrations precisely because DHA was abundant in diet, obviating the need to preferentially retain DHA.

Past work on chickens found that higher levels of LCUPFA in diet translated into increased proportions of LCUPFA in breast muscle (e.g., ref. 28). We found that increasing the concentrations of LCUPFA in diet did not necessarily result in increased LCUPFA content in Tree Swallow tissue. Instead, our findings on aerial insectivore chicks are closer to those of past studies on freshwater zooplankton, which have found preferential retention and biomagnification of LCUPFA compared with other FAs regardless of food quality (41). This suggests that there is strong pressure for aerial insectivores to obtain and retain LCUPFA in the face of poor conditions. Our performance data suggest that, when food quantity or quality are low, saving LCUPFA for future use instead of burning them as fuel may result in lower body mass and condition. Further studies using compound-specific stable isotope tracers (e.g., enriched δ13C) will be necessary to determine if Tree Swallow chicks are able to convert ALA into LCUPFA and thus whether LCUPFA are beneficial or absolutely essential components of diet.

Previous work on Tree Swallows has attempted to link Tree Swallow breeding season and nestling success with food availability (9, 11). Our findings suggest that the abundance of high-quality aquatic insects relative to Tree Swallow phenology may be a better predictor of breeding success than overall insect abundance. Aquatic insect abundance peaks earlier than terrestrial insect abundance (42), and aquatic insects are often the only available food early in the breeding season (43). Total insect abundance peaks later in the breeding season, yet Tree Swallows complete laying long before peak insect abundance, and their breeding success decreases with lay date (9). Ecologists have generally interpreted these findings to indicate that laying, although earlier than peak insect abundance, places chick rearing, thought to be the most energy-demanding phase of the breeding cycle, at a time of peak food availability (44). Our findings suggest an alternative interpretation, that Tree Swallows, and other birds, may be under selection to time their breeding seasons when insects high in LCUPFA are most available.

Our findings have significant implications for aerial insectivore conservation. Most North American aerial insectivores, including the Tree Swallow, are associated with aquatic or riparian habitats (43). We found evidence that feed containing LCUPFA representative of aquatic insects improves multiple metrics of Tree Swallow performance and that they preferentially retain these high-quality fats. Our study suggests that large quantities of terrestrial insects low in LCUPFA are, at best, no better than even small amounts of aquatic insects, even if they have high amounts of the LCUPFA precursor ALA. Land conservation is not enough for aerial insectivores to survive and thrive: Managers must conserve aquatic habitats that provide aerial insectivores with the highest-quality LCUPFA-rich aquatic insects.

**Methods**

We collected 44 wild Tree Swallow chicks from nest boxes around Ithaca, New York, from 29 May 2015 to 7 June 2015. To prevent parental abandonment of chicks, we removed all chicks from each nest box. All animal work was approved under Cornell Institutional Animal Care and Use Committee protocol 2001-0051, New York State Department of Environmental Conservation Scientific Collection Permit 1477, and United States Fish and Wildlife Service Migratory Bird Scientific Collection Permit 757670. Upon return to the laboratory, we weighed and sorted chicks into groups of three to four birds to receive one of four feeding treatments: (i) Hi; (ii) Lh; (iii) Hi; and (iv) Ll. The high- and low-LCUPFA diets were not significantly different in calories, moisture, crude protein, or crude fat (Table S1) and differed...
only in FA composition (Table S1). All diets were based upon standard commercial Mazuri nestling feed (www.mazuri.com/mazurihandfeedingdiets-1.aspx). Standard nesting diets contained soybean oil as their principal fat source. Our high-LCPUFA diets included a substitution of stabilized menhaden oil for soybean oil in a ratio of 7:3, and low-LCPUFA diets included a substitution of flax oil for soybean oil in a ratio of 1:3. The resulting high-LCPUFA diets contained \(-1.82\%\) ALA, \(3.74\%\) EPA, and \(3.44\%\) DHA, and low-LCPUFA diets contained \(-6.25\%\) ALA, \(1.47\%\) EPA, and \(1.42\%\) DHA (Table S1). Low-quantity chicks were fed \(4.5\%\) of body mass per feeding session (the point at which begging still occurred at the end of the session), and high-quantity chicks were fed \(6\%\) of body mass per session (produced chick satiation at the end of the feeding session) of body mass per feeding. We grouped chicks of similar initial sizes together in nest groups so as to avoid underfeeding or overfeeding individual chicks, and we randomly split up chicks from the same clutch to avoid genetic effects. There were multiple replicates of each food quality and quantity treatment that covered the full range of initial chick masses. Additional details on diets and care are described in SI Methods.

Each chick was weighed four times daily with an Ohaus Scout Pro balance, and the average of that mass was used for calculations. We also measured the head–bill and tarsus length of each chick to the nearest 0.01 mm a minimum of two times over the course of the experiment, with Mitutoyo Digimatic 500 calipers. Growth rates were calculated as In[mass or length on day x] – In[mass or length on day 0]/(day x – day 0), and body condition was calculated as both the ratio of mass to head–bill length and the ratio of mass to tarsus length. To measure immuno-competence, we used the simplified protocol described by Smits et al. (45) and detailed in SI Methods. To determine BMR, we used an open-flow pull-mode FoxBox respirometry setup coupled with a climate-controlled chamber at a flow rate of \(-490\) mL/min following the methods of Lighton (46) and detailed in SI Methods.

We determined the whole-tissue FA composition of brain and pectoral muscle for a subset of chicks from each treatment (n = 4). After euthanasia, we dissected and weighed out brain and pectoral muscle samples from four chicks per treatment. FA methyl esters (FAMEs) were extracted from whole tissues using a modified one-step method (47, 48) and quantified using a BPX-70 (SGE Inc.) column and an HP5890 series II gas chromatography flame ionization detector. Chromatogram data were processed using PeakSimple. Response factors were calculated using the reference standard 462a (Nucheck prep). FAMES were identified using a Varian Saturn 2000 ion trap with a Varian Star 3400 gas chromatography mass spectrometer run in chemical ionization mass spectrometry mode using Acetomihrile as reagent gas. FA composition data are expressed as percent of total FA. We also calculated total omega-3 PUFAs and total omega-6 PUFAs.

We analyzed mass, size-specific growth rates for mass, tarsus length, size-specific growth rates for tarsus length, head–bill length, size-specific growth rates for head–bill length, the ratio of mass to tarsus length, the ratio of mass to head–bill length, and PHA ratio through ANOVA, using treatment group (the interaction of LCPUFA content and food quantity: Hh, Hl, Lh, and Ll), nest, and individual as predictor variables. For all performance metrics except PHA ratio, which was only measured at the end of the experiment, we also ran analysis of covariance with experiment date as a covariate. We used post hoc Tukey tests to interpret the direction and significance of differences between treatment groups for variables that were significant as main effects and assessed relative support between models using Akaike's Information Criterion. To detect differences in the BMRs and brain and muscle FA composition, we used nonparametric Kruskal –Wallis tests and performed Dunn tests to perform pairwise comparisons between treatment groups (Hh, Hl, Lh, and Ll). We also compared differences between brain and muscle fatty acid composition using Welch’s two-sample t tests. All statistical analyses were performed in R (version 3.2.2).

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