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Essential amino acid requirements of granivorous and omnivorous songbirds and the provision of natural foods

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Abstract

Wild birds must consume certain amounts of protein and an appropriate balance of amino acids while inhabiting environments where foods often differ in the quantity and quality of available protein. The requirements for amino acids are well documented for domestic bird species but are largely unknown for wild birds, which makes it impossible to reliably assess the nutritional adequacy of foods eaten by wild birds. We measured the maintenance requirements for three essential amino acids (lysine, methionine, and arginine) in two species of songbird, the omnivorous Hermit Thrush (*Catharus guttatus*) and granivorous White-throated Sparrow (*Zonotrichia albicollis*). Hermit Thrushes and White-throated Sparrows had similar requirements for lysine (20.02 and 19.95 mg/day, respectively) and methionine (12.3 and 10.85 mg/day, respectively), whereas thrushes had lower requirements for arginine (18.07 mg/day) compared to sparrows (34.5 mg/day). Consistent with previous studies, most birds fed diets with inadequate essential amino acid concentrations reduced food intake and fecal output, lost body mass, and had lower, but not negative nitrogen balance. However, we provide the first evidence that songbirds overcompensate when they consume diets very deficient in lysine. Available data on amino acid concentrations in natural foods suggests that most insects contain relatively high concentrations of all essential amino acids, seeds likely satisfy requirements of lysine and arginine but not methionine for Hermit Thrushes and White-throated Sparrows, whereas fruits generally contain inadequate amounts of all essential amino acids. Therefore, birds that eat mostly fruit may consume enough protein but likely must eat other types of foods to satisfy their essential amino acid requirements.

Keywords Essential amino acids · Songbirds · Protein quality · Bird food

Zusammenfassung

Bedarf an essentiellen Aminosäuren von granivoren und omnivoren Singvögeln und deren Verfügbarkeit in der natürlichen Nahrung

Wildlebende Vögel müssen bestimmte Mengen an Proteinen und ein ausgewogenes Gleichgewicht an Aminosäuren zu sich nehmen, während sie bestimmte Habitate bewohnen, in denen sich die Nahrung oft in Bezug auf die Quantität und Qualität der verfügbaren Proteine unterscheidet. Der Bedarf an Aminosäuren ist für domestizierte Vogelarten gut dokumentiert, für wildlebende Vögel jedoch weitgehend unbekannt. Dies macht es unmöglich, den Nährstoffbeitrag der von Wildvögeln gefressenen Nahrung zuverlässig zu beurteilen. Wir untersuchten den Grundbedarf für drei essentielle Aminosäuren (Lysin, Methionin und Arginin) bei zwei Singvogelarten, der allesfressenden Einsiedler-Musendrossel (*Catharus guttatus*) und der körnerfressenden Weißkehlammer (*Zonotrichia albicollis*). Einsiedler-Musendrosseln und Weißkehlammern hatten einen ähnlichen Bedarf an Lysin (20,02 mg/day bzw. 19,95 mg/day) und Methionin (12,3 mg/day bzw. 10,85 mg/day), während

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die Drosseln einen geringeren Bedarf an Arginin (18,07 mg/day) im Vergleich zu den Ammern (34,5 mg/day) hatten. In Übereinstimmung mit vorherigen Studien reduzierten die meisten Vögel, die Nahrung mit unzureichenden Konzentrationen an Aminosäuren zu sich nahmen, die Nahrungsaufnahme und die Kotabgabe, verloren Körpermasse und hatten eine niedrigere, aber nicht negative Stickstoffbilanz. Wir liefern jedoch den ersten Beweis dafür, dass Singvögel überkompensieren, wenn sie Nahrung mit einem hohen Lysinmangel aufnehmen. Verfügbare Daten über Aminosäurekonzentrationen in natürlicher Nahrung lassen vermuten, dass die meisten Insekten und Samen wahrscheinlich den Bedarf an Lysin und Arginin, aber nicht an Methionin für Einsiedler-Musendrosseln und Weißkehlammern decken. Früchte hingegen enthalten im Allgemeinen unzureichende Mengen aller essentiellen Aminosäuren. Daher nehmen Vögel, die hauptsächlich Früchte essen, vermutlich genug Proteine auf, müssen aber andere Arten von Nahrung zu sich nehmen, um den Bedarf an essentiellen Aminosäuren zu decken.

Introduction

Proteins are a major component of an animal's body and serve a variety of key functions (Robbins 1993). Some proteins can be produced by animals but a continual dietary supply is necessary to enable important bodily functions (Robbins 1993; Murphy 1993a; Klasing 1998). Proteins are composed of 20 different amino acids. Birds lack the enzymes to create ten amino acids in vivo and these are thus referred to as essential amino acids (EAAs) and must be obtained via diet (Robbins 1993; Murphy 1996). In the case of birds, these EAAs include arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Glycine, serine, and proline are frequently referred to as semi-essential for growing birds because they can be synthesized in vivo, but not at sufficient rates (Scott et al. 1982; Robbins 1993; Murphy 1996; Klasing 1998). The breadth of research conducted on the topic of nutritional requirements for amino acids in birds is extensive for domesticated species but is scant for wild birds (Robbins 1993; Murphy 1993b; Levey and Martínez del Rio 2001). Due to the vital role amino acids play in nutrition, acquiring a better understanding of the amino acid needs of wild birds is of great importance (Klasing 1998; Sales 2007) and makes possible a more complete assessment of the nutritional adequacy of foods eaten by wild birds.

Food resources vary in protein quantity and quality (Murphy and Pearcy 1993), the latter referring to the balance of amino acids in the food source relative to that required by the consumer (Klasing 1998; Izhaki 1998). Few studies have quantified the amino acid content of foods available to birds throughout their annual cycle (Peoples et al. 1994; Boren et al. 1995; Izhaki 1998; Levey and Martínez del Rio 2001; Foeken et al. 2008; McCusker et al. 2014). However, the few studies conducted suggest that the amino acid composition varies considerably among fruits, invertebrates, seeds, and nectar (Borroughs 1970; Peoples et al. 1994; Izhaki 1998; Wendeln et al. 2000; Finke 2002; Nicolson 2007) which may make it difficult for birds to obtain adequate protein quality given their requirements.

Amino acid deficiencies in organisms can lead to a host of complications including malnutrition, stunted growth, physical deformities, and even death (Murphy et al. 1988; Murphy and King 1991; Harper and Skinner 1998; Klasing 1998) and so animals must select foods to avoid such deficiencies. The deficiency of one EAA can result in increased overall protein requirements (Maynard et al. 1979; Klasing 1998). Specifically, a shortage of EAAs can lead to a distortion of the quantity of free amino acids in the body, reduce rates of protein synthesis in body tissues, increase rates of oxidation of non-deficient amino acids, and increase rates of ingestion of nutrient-deficient food (Murphy and Pearcy 1993). Birds seem able to discriminate between diets that differ only in their amino acid composition and this likely helps them to avoid these micronutrient deficiencies. For example, White-crowned Sparrows (*Zonotrichia leucophrys gambelii*) discriminated between diets differing in sulfur amino acids (SAA) and predominately chose diets with high SAA levels during peak molting periods (Murphy and King 1987). White-crowned Sparrows were also able to distinguish between diets deficient in lysine and valine and they preferred diets with adequate EAA levels during the winter maintenance period (Murphy and King 1989). Furthermore, birds in both experiments detected EAA deficiencies in diets in a relatively short period of time (less than 18 h).

We quantified the requirements of three of the ten EAAs for two species of passerine bird, the Hermit Thrush (*Catharsus guttatus*) and the White-throated Sparrow (*Zonotrichia albicollis*). We selected these two model species due to their known differences in food habits (Dellinger et al. 2020; Falls and Kopachena 2020) and the likelihood they evolved different nutritional needs (Klasing 1998). The following EAAs were selected for examination in our study because they cause the most common dietary deficiencies in birds: lysine, methionine, and arginine (Klasing 1998). Lysine and arginine concentrations (on average 0.20% DM, 0.25% DM, respectively) in European wild fruits were 30–37% below that required by North American White-crowned Sparrows (Izhaki 1998), and we measured and present here the dry matter intake for Hermit Thrush and

White-throated Sparrows in this experiment. Lysine is often a major limiting amino acid in Gramineae grass seeds (Parrish and Martin 1977), which is the primary winter food of granivorous songbirds. Lysine and methionine were the most limiting amino acids during growth in domestic Bobwhite Quail (*Colinus virginianus*) (Scott et al. 1982). Methionine was the most limiting EAA for birds that eat wild fruits (Izhaki 1998) and for gallinaceous birds consuming seeds of preferred forages (Boren et al. 1995), and wild fruits were deficient in arginine relative to bird requirements (Izhaki 1998). We compared the requirements for lysine, methionine and arginine of Hermit Thrushes and White-throated Sparrows to those of other wild birds and to that in natural foods to determine the likelihood that selected natural foods satisfy the EAA requirements of wild birds.

Methods

Capture and maintenance of birds

We used mistnets to capture White-throated Sparrows ($n=21$) and Hermit Thrushes ($n=20$) during fall migration in 2006 in Kingston, Rhode Island (41°28' N, 71°31' W). The birds were transferred to indoor facilities and housed individually in stainless-steel cages (59 × 45 × 36 cm) at constant temperature (23 °C) and a constant photoperiod representative of natural photoperiod at capture (12 h light:12 h dark, lights on at 0800 h). Following capture, a 10-week acclimation period provided birds with ad libitum food and water along with about 8–10 mealworms (*Tenebrio molitor*) each day. All experiments were run within 6 months of the initial capture date.

The nutrient composition of the acclimation diet was similar to that of many natural high-carbohydrate fruits (59.5% carbohydrates, 12.8% protein (casein) and 8.0% fats; see Table 1 Langlois and McWilliams 2010). Songbirds fed similar semi-synthetic diets have been successfully maintained for more than 1 year (Murphy and King 1982; McWilliams et al. 2002; Pierce and McWilliams 2004, 2005; Casagrande et al. 2020). The acclimation diet was formulated so that the essential amino acid concentrations satisfy the maintenance requirements of White-crowned Sparrows (Online Resources Tables 1, 2) (Murphy 1993b). Body mass was measured daily (± 0.1 g) and birds remained in good health.

Diets and experimental design

After the 10-week acclimation period, we offered birds only the casein-based acclimation diet (no mealworms) for the next 6 weeks. Birds were then used in a protein requirement experiment (Langlois and McWilliams 2010) for 9 days. Thereafter, birds were switched to a crystalline amino acid-based diet (diet A for all EAA experiments; Online Resources Table 1). Diet A was nearly identical in composition to the acclimation diet except L-crystalline amino acids replaced casein as the sole source of dietary protein. This allowed for the adjustment of minute quantities of individual EAAs to create diets with varying amounts of EAAs, which was not possible with the casein-based diet.

We randomly assigned birds to one of four initial diet groups (A, B, C, D) that differed in EAA concentration. Therefore, birds were fed one of the four diets during the first EAA trial but rotated to a different dietary level (A–D) in subsequent trials to reduce potential carryover effects. Diets were isocaloric and nearly isonitrogenous because we replaced the EAA (lysine, methionine, or arginine) with reciprocal amounts of glutamic acid (Online Resources Table 2). The dietary EAA concentrations were chosen so that birds in some groups (diets A and B) were fed diets intended to provide adequate EAA whereas birds in other groups (diets C and D) were fed diets intended to provide inadequate EAA given the EAA requirements of White-crowned Sparrows (Murphy 1993b; Fig. 1). We used Murphy (1993b) as a guide to determine the exact EAA concentrations in our experimental diets.

We conducted 3-day total-collection trials (Murphy 1993b) for each diet group of each EAA trial for both species during the 18th through 21st week of captivity in the following order: lysine, methionine, arginine, and lysine repeated (lysine-repeat). Each trial was separated by 3–4 days on the maintenance diet (diet A). Two identical lysine trials (lysine and lysine-repeat) were conducted with each bird to confirm that there were no changes in the bird's estimated EAA requirements over time. For the first trial (lysine), we randomly assigned 4–6 birds of each species to one of the four diet groups (A–D) that differed in EAA concentration. To reduce potential carryover effects between subsequent trials, we reciprocally switched birds fed with the highest EAA amount in the first trial (diet A) to the diet (D) with lowest EAA amount in the next trial and vice versa. We also reciprocally switched birds fed diet B in the first trial to diet C in the second trial and vice versa. For the third and fourth trials, birds returned to their original randomly selected diet group (A–D). Two Hermit Thrushes were removed from the experiment after the first lysine trial because they were behaving abnormally, although there were still 4–6 birds in each diet group. At 0800 h, each day during these 3-day trials, we measured each bird's body mass, provided each bird with ad libitum fresh food and water, and weighed the food that remained from the previous day. We also collected all excreta produced by each bird during the previous 24 h, along with samples of food offered and remaining. All samples were stored frozen at − 20 °C for later analysis.

We dried food remaining at 47 °C until sample mass was constant (~ 1 week). The samples of food offered and

Table 1 Regression equations and associated statistics for estimating essential amino acid (EAA) requirements (mg/day and % DM intake) of White-throated Sparrows ($n=21$) and Hermit Thrushes ($n=20$ for lysine; $n=18$ for methionine and arginine) given measured daily food intake, change in body mass, and nitrogen (N) balance for birds fed one of four diets (A–D) with decreasing dietary concentration of each EAA

Species	Change in body mass	Daily food intake	N balance
White-throated Sparrow			
Lysine			
Diet: B, C ($n=10$)			
Y	$0.174x-3.471$	$17.23x+0.363$	$1.503x-0.514$
R^2	0.80	0.66	0.59
p value	<0.001	0.005	0.010
mg/day (% DM diet)	19.95 (0.30%)	24.29 (0.37%)	29.15 (0.44%)
Methionine			
Diet: C, D ($n=10$)			
Y	$0.253x-2.745$	$7.32x+3.536$	NS
R^2	0.57	0.33	
p value	0.012	0.082	
mg/day	10.85 (0.16%)	28.61 (0.43%)	
Arginine			
Diet: B, C ($n=10$)			
Y	$0.052x-1.794$	$2.897x+4.419$	$0.668x+17.11$
R^2	0.69	0.54	0.45
p value	0.003	0.015	0.034
mg/day	34.5 (0.52%)	51.24 (0.77%)	39.21 (0.59%)
Hermit thrush			
Lysine			
Diets: B, C ($n=10$)			
Y	$0.149x-2.981$	NS	NS
R^2	0.38		
p value	0.058		
mg/day	20.02 (0.29%)		
Methionine			
Diets: C, D ($n=10$)			
Y	$0.213x-2.619$	$8.920x+3.796$	NS
R^2	0.47	0.60	
p value	0.029	0.009	
mg/day	12.3 (0.18%)	23.37 (0.34%)	
Arginine			
Diets: C, D ($n=10$)			
Y	$0.208x-3.759$	$10.967x+2.598$	$1.741x-2.172$
R^2	0.84	0.45	0.44
p value	<0.001	0.033	0.037
mg/day	18.07 (0.26%)	26.80 (0.39%)	22.10 (0.32%)

These regressions used the subset of diets ($n=$number of birds) for which birds had lower than average N balance or they had lost significant body mass as shown in Figs. 2, 3, 4 for each of the three EAAs and two species. EAA requirements were estimated by solving for EAA intake given the grand average N balance or change in body mass for birds fed adequate diets (Hermit Thrush N balance: 36.3 mg/day, change in body mass: − 0.10 g; White-throated Sparrow N balance: 43.3 mg/day, change in body mass: 0.06 g) or dietary EAA concentration given the grand average daily food intake for birds fed adequate diets (Hermit Thrush food intake: 6.87 g; White-throated Sparrow food intake: 6.65 g)

NS indicates non-significant results for regression analysis

collected excreta that were used for measuring nitrogen content were freeze-dried until constant mass (2 days) to ensure that nitrogen was not lost during drying. Dried samples were homogenized using mortar and pestle. Methods for measuring nitrogen concentration of food and excreta are described in Langlois and McWilliams (2010).

We estimated nitrogen balance (mg day^{-1}) as the N intake minus N lost in excreta; the equation is as follows: N

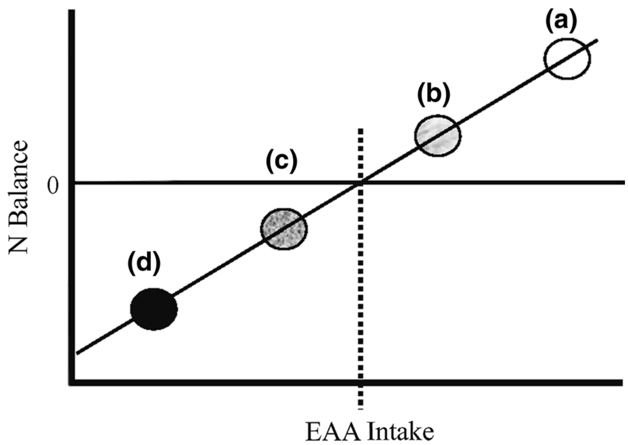

Fig. 1 Visual depiction of the experimental design where the dietary essential amino acid (EAA) concentrations of diets A and B provide adequate EAA (to the right of dotted line), whereas diets C and D provide inadequate EAA (to the left of dotted line) given the requirements of White-crowned Sparrows (Murphy 1993b). EAA concentrations for the experiments are as follows: lysine (diet A, 0.89%; diet B, 0.3%; diet C, 0.16%; diet D, 0.10%); methionine (diet A, 0.4%; diet B, 0.3%; diet C, 0.2%; diet D: 0.1%); and arginine (diet A: 0.85%, diet B: 0.63%, diet C: 0.3%, diet D:0.18%)

Balance = (F × FN) – (D × DN) where F is amount of food consumed (g DM day^{-1}), FN is nitrogen content of the food (mg N/g DM), D is the amount of excreta (g DM day^{-1}), DN is the nitrogen content of the excreta (mg N/g DM) (Murphy 1993a). Because all EAA diets were nearly isonitrogenous, we did not expect energy density in food or excreta to change during the trials and we confirmed this by measuring energy density of excreta in the lysine trials (Langlois 2008).

Statistical analysis

We conducted three analyses to confirm key aspects of our experimental design (see Online Resources for details): (1) adequate acclimation time within the 3-day collection trials, (2) no change over time between the two lysine trials conducted at the start and end of the experiment, and (3) confirming that diet A was nutritionally adequate. After confirming these key aspects of the experimental design (results in Online Resources), we used ANOVA for Hermit Thrushes and White-throated Sparrows fed diets A–D to determine the effect of EAA intake on N balance, food intake, amount of excreta, and percent nitrogen in excreta for each species on day 3 of each EAA trial. We used a conservative *P* value (0.01) for Levene's Test of Homogeneity of Variance due to the robustness of the ANOVA model; all reported statistical analyses satisfied the assumptions of normality and homogeneity of variance among treatment groups.

We used linear regression to estimate birds' requirements for each of the three EAAs. For each species and EAA, we regressed change in body mass and N balance on EAA intake, and food intake on dietary EAA concentration for birds fed deficient diets on day 3 of each trial. Diets were considered deficient when birds had significantly lower N balance than the grand average N balance for birds fed adequate EAA (see Figs. 2, 3, 4), or when birds lost significant body mass. We used *t* tests to compare slope and elevation (y-intercept) parameters from these regression equations between Hermit Thrushes and White-throated Sparrows in all EAA trials (Sokal and Rohlf 1981). Values are reported as means ± SD and the significance level was set at $P \leq 0.05$. All statistical analyses were conducted using R version 4.0.0 (R Core Team 2020).

Results

Nitrogen balance, food intake, and body mass for birds fed less essential amino acids

We first report the results that show how Hermit Thrush and White-throated Sparrow responded to decreasing concentrations of each dietary EAA. We then use these results in the next section to estimate the EAA requirements of each bird species.

Lysine—birds fed diets with less lysine generally lost body mass, ate less, and were in lower N balance (Fig. 2). Hermit Thrush and White-throated Sparrow lost on average 2.7 ± 1.0 g and 2.6 ± 0.8 g, respectively, when fed the lowest dietary lysine whereas birds gained or maintained body mass when fed the higher dietary lysine ($F_{3,16} = 3.67$, $P = 0.035$; $F_{3,17} = 21.18$, $P < 0.001$, respectively). Birds fed less dietary lysine generally ate less food (Hermit Thrush: $F_{3,16} = 25.98$, $P < 0.001$, White-throated Sparrow: $F_{3,17} = 20.00$, $P < 0.001$) and had lower N balance (Hermit Thrush: $F_{3,16} = 14.14$, $P < 0.001$; White-throated Sparrow: $F_{3,17} = 21.06$, $P < 0.001$). However, White-throated Sparrow fed the lowest dietary lysine diet ate more food and thus had a more positive N balance than White-throated Sparrows fed slightly more dietary lysine (Tukey HSD: $P < 0.001$, $P < 0.001$, respectively). Birds fed the lowest dietary lysine produced less excreta (Hermit Thrush: $F_{3,16} = 14.16$, $P < 0.001$; White-throated Sparrow: $F_{3,17} = 14.05$, $P < 0.001$), and Hermit Thrush had higher percent N in excreta ($F_{3,16} = 8.43$, $P = 0.001$) whereas percent N in excreta was similar for White-throated Sparrow fed each diet ($F_{3,17} = 1.18$, $P = 0.346$). Therefore, White-throated Sparrows fed the most deficient lysine diets to some extent compensated by increasing food intake and this improved their N balance although they still lost body mass.

Methionine—Hermit Thrush and White-throated Sparrow fed the lowest dietary methionine lost on average 1.6 ± 1.1 g and 1.6 ± 1.1 g, respectively, whereas birds gained or maintained body mass when fed the higher dietary

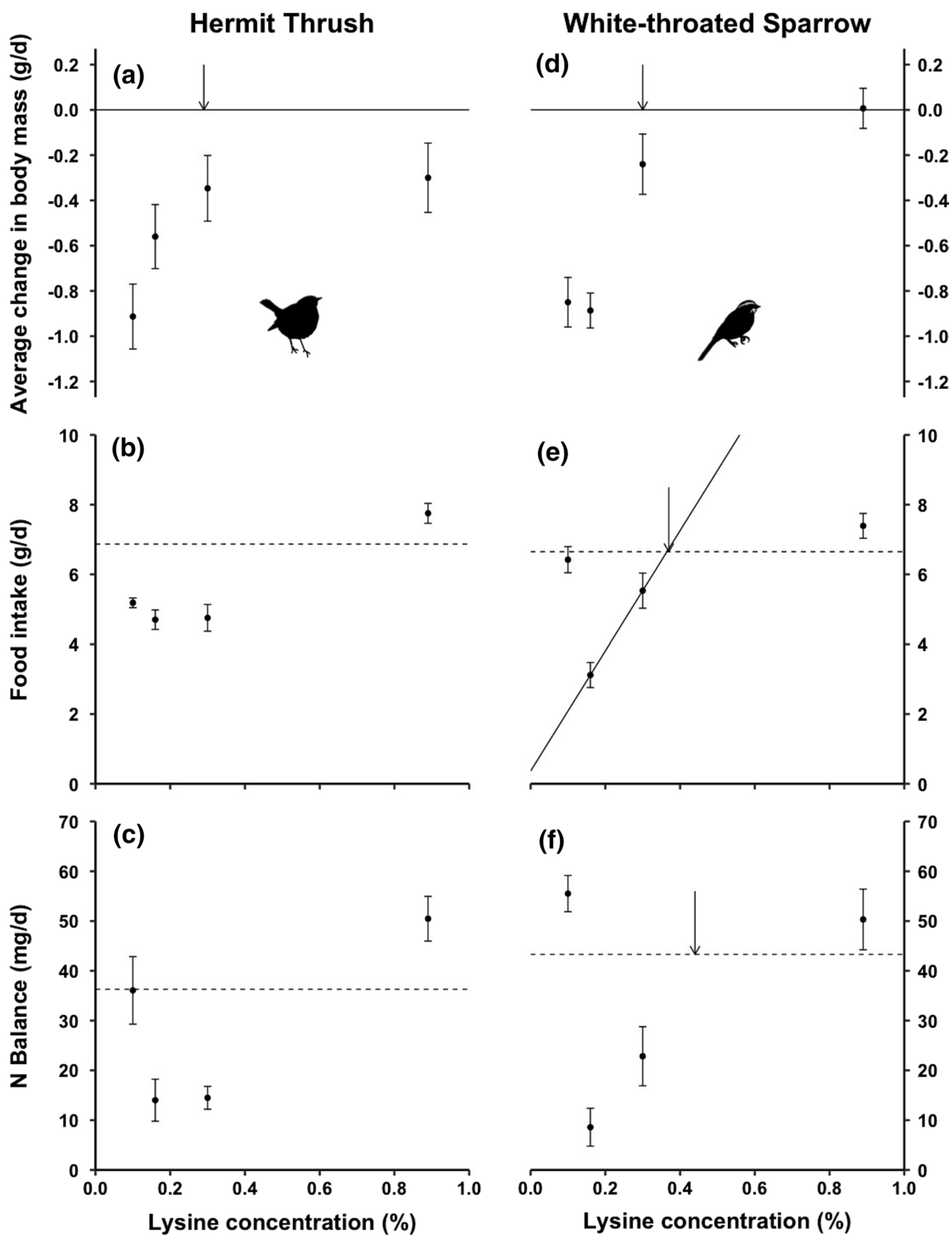

Fig. 2 Average (± SE, $n = 4$–6 birds per diet) changes in body mass, food intake, and nitrogen (N) balance in Hermit Thrushes and White-throated Sparrows ($n = 20$, $n = 21$, respectively) that ingested different lysine concentrations (diet A, 0.89%; diet B, 0.3%; diet C, 0.16%; diet D, 0.10%) on day 3 of the 3-day trials. Horizontal dashed line depicts average food intake (panels B and E) and N balance (panels C and F) of birds that consumed adequate essential amino acid (EAA) (Hermit Thrush, $n = 17$; White-throated Sparrow, $n = 21$) during the 3-day collection trials. EAA requirements of birds (shown by arrows) were estimated from regressions fit to only those data for which birds had lower than average N balance or when birds lost significant body mass; only significant regressions for food intake are depicted (see Table 1 for all equations and estimates of requirements)

methionine resulting in significant changes in body mass across the four diets ($F_{3,14} = 3.29$, $P = 0.052$; $F_{3,17} = 9.44$, $P < 0.001$, respectively; Fig. 3). White-throated Sparrow ate less when fed the lowest dietary methionine ($F_{3,17} = 4.01$, $P = 0.025$), whereas food intake was more similar across diets for Hermit Thrush ($F_{3,14} = 2.98$, $P = 0.068$). Birds fed the lowest dietary methionine produced less excreta (Hermit Thrush: $F_{3,14} = 4.35$, $P = 0.023$; White-throated Sparrow: $F_{3,17} = 12.25$, $P < 0.001$) while percent N in excreta remained the same (Hermit Thrush: $F_{3,14} = 2.51$, $P = 0.101$; White-throated Sparrow: $F_{3,17} = 0.79$, $P = 0.516$). N balance of Hermit Thrush and White-throated Sparrow did not change across diets ($F_{3,14} = 0.69$, $P = 0.573$; $F_{3,17} = 2.07$, $P = 0.142$, respectively).

Arginine—Hermit Thrush and White-throated Sparrow fed the lowest dietary arginine lost on average 1.9 ± 1.0 g and 1.5 ± 0.7 g, respectively, whereas birds gained or maintained body mass when fed the higher dietary arginine resulting in significant changes in body mass across the four diets ($F_{3,14} = 13.80$, $P < 0.001$; $F_{3,17} = 15.36$, $P < 0.001$, respectively; Fig. 4). Hermit thrush ate less when fed the lowest dietary arginine ($F_{3,14} = 3.74$, $P = 0.037$), whereas food intake was more similar across diets for White-throated Sparrow ($F_{3,17} = 2.26$, $P = 0.119$). White-throated Sparrow fed the lowest dietary arginine also produced less excreta ($F_{3,17} = 3.34$,

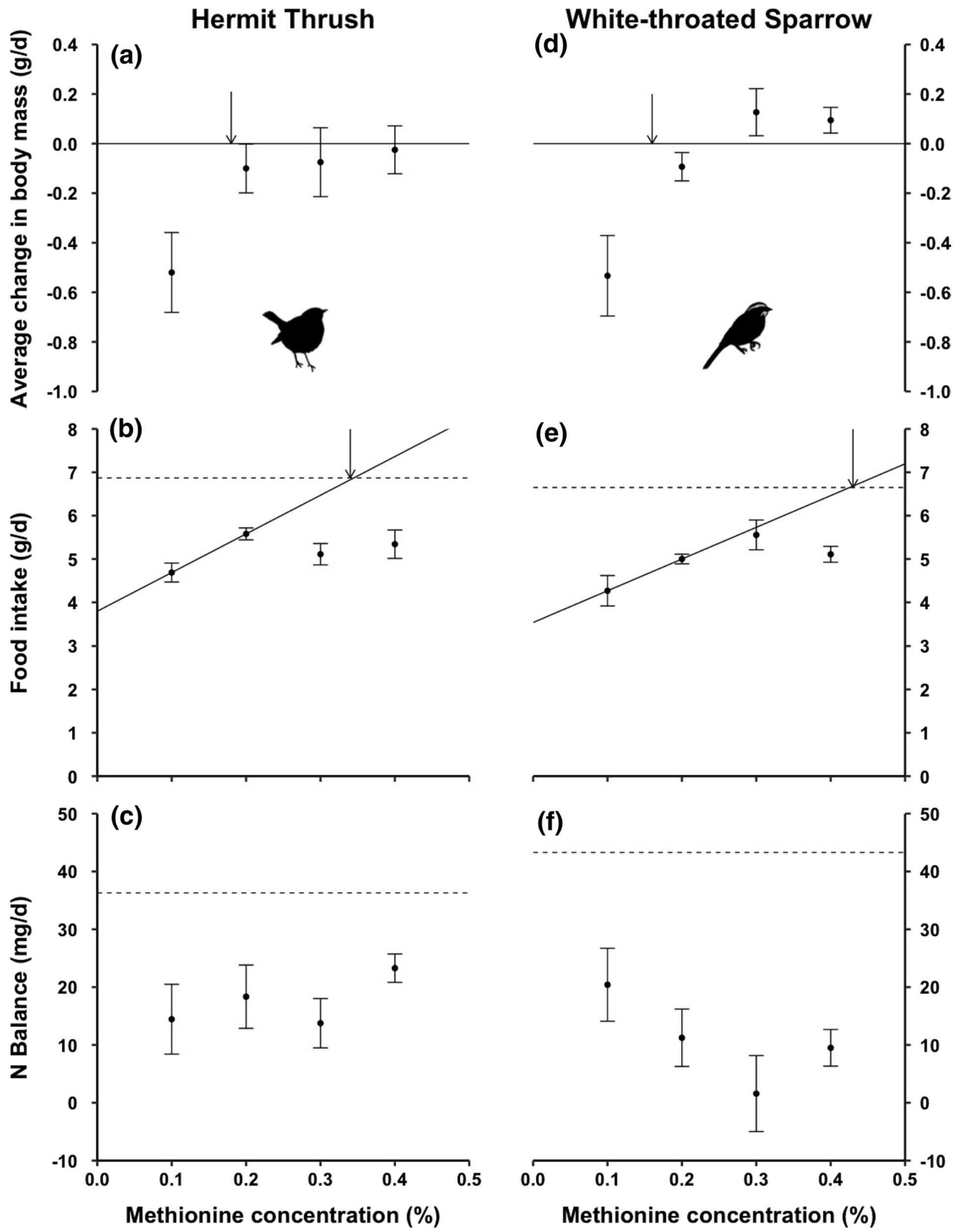

Fig. 3 Average (± SE, $n=4$–6 birds per diet) changes in body mass, food intake, and nitrogen (N) balance in Hermit Thrushes and White-throated Sparrows ($n=18$, $n=21$, respectively) that ingested different methionine concentrations (diet A, 0.4%; diet B, 0.3%; diet C, 0.2%; diet D: 0.1%) on day 3 of the 3-day trials. Cystine was replaced with glutamic acid in all diet groups because these two amino acids functionally complement one another (Murphy 1993b; Klasing 1998). See Fig. 2 for explanation of symbols

$P=0.044$), while Hermit Thrush produced the same amount of excreta across diets ($F_{3,14}=2.83$, $P=0.079$). Percent N in excreta remained the same across diets (Hermit Thrush: $F_{3,14}=1.94$, $P=0.169$; White-throated Sparrow: $F_{3,17}=0.37$, $P=0.773$). Birds fed less dietary arginine had lower N balance (Hermit Thrush: $F_{3,14}=4.00$, $P=0.030$; White-throated Sparrow: $F_{3,17}=3.39$, $P=0.042$, respectively).

Essential amino acid requirements and interspecies comparisons

Dietary EAA requirements were estimated from the regression of change in body mass and N balance on N intake, and the regression of food intake on dietary EAA concentration for birds fed deficient diets on day 3 of each trial (Table 1). In general, lysine requirements for both species were 20–29 mg/day, methionine requirements for both species were 10–23 mg/day, and arginine requirements for White-throated Sparrow were 34–51 mg/day whereas those for Hermit Thrushes were 18–27 mg/day depending on the dependent variable used to estimate requirements. We detected no significant difference in regression parameters for Hermit Thrushes and White-throated Sparrow in the lysine and methionine trials (lysine slope: $t_{19}=0.27$, $P=0.79$; elevation: $t_{19}=0.46$, $P=0.65$; methionine change in body mass—slope: $t_{19}=0.33$, $P=0.74$; elevation: $t_{19}=0.45$, $P=0.66$; methionine food intake—slope: $t_{19}=0.33$, $P=0.66$; elevation: $t_{19}=2.04$, $P=0.06$). We detected significant differences in regression

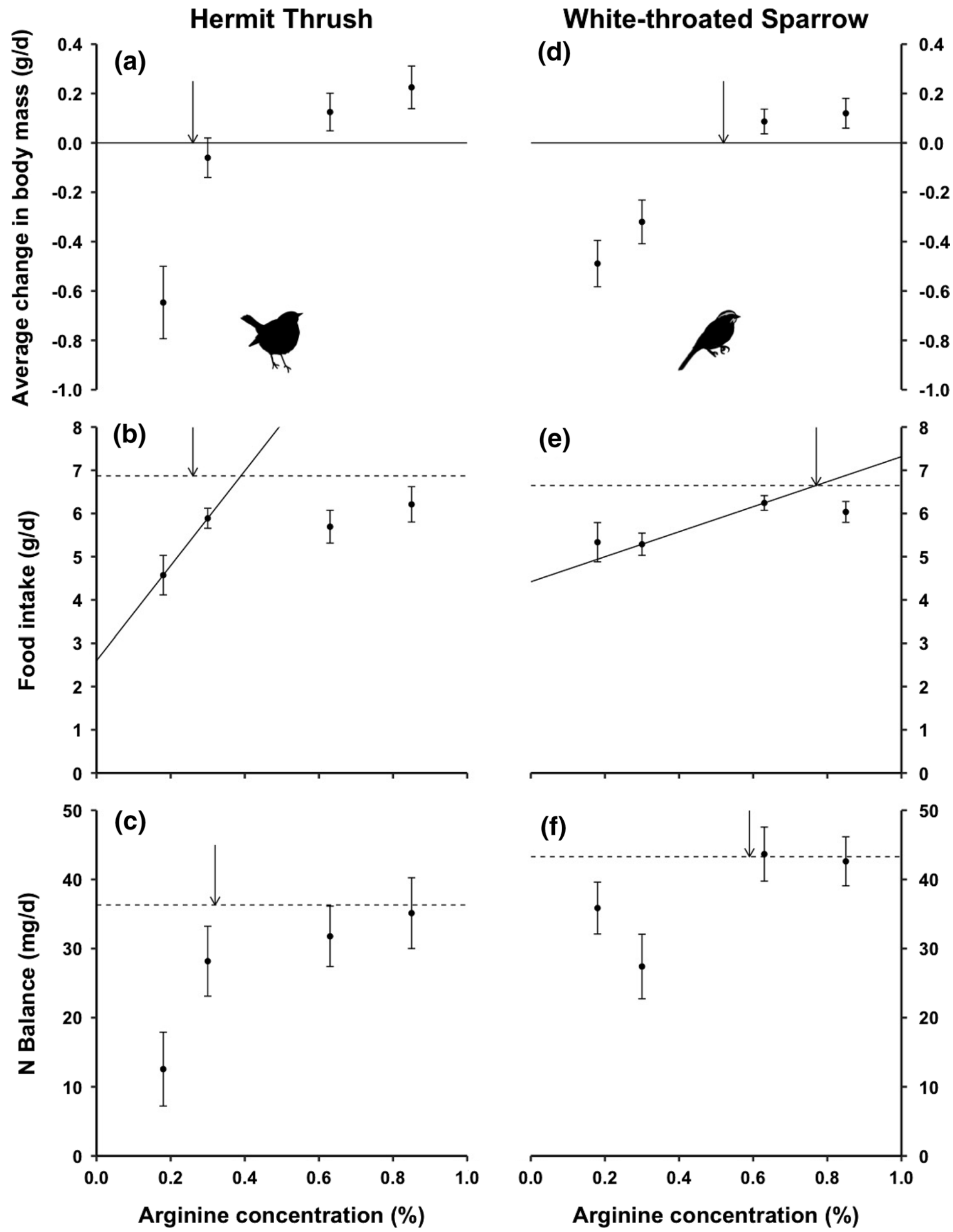

Fig. 4 Average (± SE, $n = 4$–6 birds per diet) changes in body mass, food intake, and nitrogen (N) balance in Hermit Thrushes and White-throated Sparrows ($n = 18$, $n = 21$, respectively) that ingested different arginine concentrations (diet A: 0.85%, diet B: 0.63%, diet C: 0.3%, diet D: 0.18%) on day 3 of the 3-day trials. See Fig. 2 for explanation of symbols

parameters for Hermit Thrushes and White-throated Sparrow in the arginine experiment. The intercept was higher in White-throated Sparrow compared to Hermit Thrush, and the slope was higher in Hermit Thrushes compared to White-throated Sparrow when change in body mass was regressed against arginine intake (slope: $t_{19} = 4.34$, $P < 0.01$; elevation: $t_{19} = 2.62$, $P = 0.02$). We detected no significant difference in regression slopes for Hermit Thrush and White-throated Sparrow when daily food intake was regressed against dietary arginine concentration ($t_{19} = 2.01$, $P = 0.06$), although the elevations from these regressions were significantly different ($t_{19} = 2.10$, $P = 0.05$). We detected no significant difference in regression equations for Hermit Thrush and White-throated Sparrow when N balance was regressed against dietary arginine intake (slope: $t_{19} = 1.40$, $P = 0.18$; elevation: $t_{19} = 0.37$, $P = 0.72$). These overall significant differences in regression parameters in the arginine experiment provide further evidence that Hermit Thrush had lower arginine requirements than White-throated Sparrow.

Discussion

Birds respond to diets deficient in essential amino acids

Birds fed diets with inadequate lysine, methionine, and arginine concentrations generally reduced food intake and fecal output, lost body mass, and had lower, but not negative N balance (N intake – N output), which is consistent with previous studies (Leveille et al. 1960; Murphy 1993b). However, White-throated Sparrow fed the most deficient lysine diets overcompensated by eating more food and thus had more positive N balances than birds fed less deficient diets. Similar results were found in growing chickens where they ate more to compensate for diets slightly inadequate in EAAs (Solberg et al. 1971). Ours is the first report of which we are aware that demonstrates such compensatory food intake in captive wild birds in response to EAA deficiency, in this case, the deficiency of lysine but not methionine and arginine. Omnivores and granivores such as Hermit Thrush and White-throated Sparrow may overcompensate for deficiencies in lysine because it is a major limiting amino acid for seed eaters (Parrish and Martin 1997) as well as other birds (Klasing 1998). However, very few published studies have provided the EAA profile of seeds eaten by a variety of wild songbirds (Table 3) and even fewer have documented the response of wild songbirds to dietary deficiencies in lysine and other key amino acids.

Hermit Thrush and White-throated Sparrow fed the methionine diets had lower N balance on average than when fed the other EAA diets suggesting these diets were more nutritionally deficient (see Online Resources). Furthermore, birds fed the highest methionine concentration (diet A) ate significantly less than birds fed diet A in other EAA experiments. These results suggest that even the highest concentration methionine diet (diet A) had insufficient sulfur amino acids. Methionine is the precursor for cystine and the lack of dietary cystine results in an increase in the requirement of methionine (Klasing 1998). However, birds were able to maintain body mass on diets with higher methionine concentration despite eating less and having lower but still positive N balance. Therefore, if sulfur amino acids were limiting to some extent, it was not significant enough to reduce body mass in diet A birds.

Our results are consistent with other studies in that birds were able to detect diets deficient in EAAs and usually responded by reducing food intake (Murphy and King 1987, 1989; Murphy 1993b). If birds can modify food intake and fecal output in response to EAA deficiency, then it shows they have some capacity to compensate for such dietary deficiencies, although to a limited extent. Merritt (1986) demonstrated this ability in Dark-eyed Juncos (*Junco hyemalis*) with radio-labeled methionine where birds fed deficient methionine concentrations were better able to incorporate methionine into their tissues, reduce methionine turnover time, and reduce fecal loss compared to birds fed adequate levels of methionine. Birds' ability to alter their metabolism in response to EAA deficiency may help them conserve limiting amino acids at least in the short term.

Accuracy of estimates for essential amino acid requirements

Of the three dependent variables we used to estimate amino acid requirements, change in body mass was the most sensitive variable in that regression analyses were more often statistically significant and explained a greater portion of the variance. Regressions based on food intake often estimated higher EAA requirements than change in body mass. Nitrogen balance was an effective means of estimating requirements for lysine and arginine, but not methionine, which may be a result of the methionine diets being more deficient in sulfur amino acids than the other EAA diets. Our estimates of amino acid requirements are likely minimal given that the captive birds were relatively sedentary compared to free-living, wild birds.

Essential amino acid requirements of songbirds

Comparisons with other avian species are limited because direct measurements of EAA requirements have only been conducted on several species of wild birds to date (Murphy 1996). After adjusting for differences in basal metabolic rate across species, we estimated that Hermit Thrushes and White-throated Sparrow require similar amounts of dietary lysine and more than White-crowned sparrows (Table 2). Lysine requirements of Dark-eyed juncos (0.20% of diet) were similar to White-crowned sparrows although the Dark-eyed juncos were maintaining body mass while in negative N balance (Parrish and Martin 1977). The granivorous Budgerigar (*Melopsittacus undulates*) required 2.0% lysine as a percentage of crude protein in their diet (Earle and Clarke 1991), which is similar to our estimates of requirements for

Table 2 Comparison of estimated essential amino acid (EAA) requirements for maintenance in passerine birds (mg EAA/kJ basal energy expenditure)

EAA	Hermit thrush	White-throated Sparrow	White-crowned Sparrow[1]
Lysine	0.67	0.74	0.37
Methionine	0.41	0.40	0.48
Arginine	0.61	1.27	0.49

EAA requirements for Hermit Thrush and White-throated Sparrow were based on regressions of change in body mass on EAA intake (Table 1). The basal metabolic rates (BMR) for all species were based on McKechnie and Wolf's (2004) conventional allometric estimates of BMR (W; $\log BMR = -1.461 + 0.669 \log M_b$)

[1]EAA requirements for White-crowned Sparrows were calculated from Murphy (1993b)

Table 3 Concentration (% DM) of essential amino acids (EAAs) in various foods available to songbirds throughout the annual cycle

	Compared to Hermit Thrush and White-throated Sparrow (this study)			Compared to White-crowned Sparrow (Murphy 1993b)						Source
	Lys	Met (plus Cys)	Arg	His	Ile	Leu	Phe	Thr	Val	
Fruit										
Acai berry puree (*Euterpe oleracea*)	0.65	**0.12**[a]	*0.50*	0.21	0.40	0.76	0.49	0.49	0.53	McCusker et al. (2014)
Blueberry (*Vaccinium cyanococcus*)	**0.20**	**0.04**[a]	*0.41*	**0.07**	**0.11**	**0.21**	**0.13**	**0.12**	**0.14**	McCusker et al. (2014)
Cactus fruit (*Ferocactus* spp.)	**0.38**	0.32[a]	1.41	0.28	0.33	0.65	0.45	0.34	0.40	McCusker et al. (2014)
Cherimoya (*Annona cherimola*)	**0.38**	**0.08**[a]	**0.26**	0.12	0.20	0.42	**0.26**	0.21	0.26	McCusker et al. (2014)
Figs of Panama (average of 14 *Ficus* spp.)	**0.17**	**0.02**	**0.15**	**0.06**	**0.12**	**0.19**	**0.12**	**0.10**	**0.15**	Wendeln et al. (2000)
Fruits of Israel (average of 27 spp.)	**0.20**	**0.16**	**0.25**	0.09	**0.18**	0.28	0.45[b]	0.17	0.25	Izhaki (1998)
Plum (*Prunus* spp.)	**0.14**	**0.02**[a]	**0.07**	**0.05**	0.22	**0.17**	**0.11**	**0.10**	**0.12**	McCusker et al. (2014)
Pomegranate (*Punica granatum*)	**0.18**	**0.08**[a]	*0.63*	0.15	**0.18**	0.36	**0.21**	0.19	0.21	McCusker et al. (2014)
Prickly pear (*Opuntia ficus-indica*)	**0.21**	**0.05**[a]	**0.16**	0.12	**0.11**	**0.24**	**0.14**	**0.12**	**0.15**	McCusker et al. (2014)
Rimu (*Dacrydium cupressinum*)	*0.40*	0.26	*0.51*	0.22	0.32	0.49	0.31	0.27	0.41	Cottam et al. (2006)
White sapote (*Casimiroa edulis*)	**0.24**	**0.05**[a]	**0.16**	0.08	**0.12**	0.28	**0.15**	0.15	**0.16**	McCusker et al. (2014)
Arthropods										
American cockroach (*Periplaneta americana*)	3.49	0.89[a]	3.37	1.54	1.88	3.59	1.97	2.10	3.10	McCusker et al. (2014)
Beetles (*Coleoptera* spp.)	2.90	1.38	2.84	1.75	3.55	4.49	1.68	2.16	3.15	Ramsey and Houston (2003)
Black soldier fly, larva (*Hermetia illucens*)	2.86	0.69[a]	2.33	1.56	1.73	2.88	1.60	1.65	2.60	McCusker et al. (2014)
Caterpillars (*Lepidoptera* spp.)	3.28	1.46	2.88	1.48	2.08	3.36	1.89	1.97	2.58	Ramsey and Houston (2003)
Flesh fly, larva (*Sarcophaga bullata*)	5.61	1.60[a]	3.57	2.35	2.74	4.49	4.06	2.72	3.45	McCusker et al. (2014)
Flesh fly, adult (*Sarcophaga bullata*)	6.14	1.99[a]	4.43	2.49	3.21	5.18	3.24	2.90	4.04	McCusker et al. (2014)
Pallid-winged grasshoppers (*Trimerotropis pallidipennis*)	1.18	0.54	1.76	0.44	0.95	1.64	0.70	0.82	1.39	Finke (2015)
Rhinoceros beetles (*Oxygrylius ruginasus*)	1.11	0.51	1.50	0.51	0.88	1.24	0.66	0.72	1.21	Finke (2015)
Spiders (*Arachnida* spp.)	4.28	2.30	4.26	1.76	2.84	4.67	2.47	2.43	3.22	Ramsey and Houston (2003)
Western harvester ant (*Pogonomyrmex occidentalis*)	2.84	0.80[a]	2.57	1.51	3.03	4.69	1.62	2.44	3.79	McCusker et al. (2014)
White-lined sphinx moth (*Hyles lineata*)	1.30	0.70	1.50	0.74	0.98	1.67	0.84	0.93	1.41	Finke (2015)
Seeds										
Forbs of USA (average of 8 species)	0.57	**0.16**	1.09	0.21	0.40	0.67	0.49	0.38	0.55	Peoples et al. (1994)
Grass of USA (average of 4 species)	**0.28**	**0.10**	**0.24**	0.11	0.26	0.78	0.33	0.23	0.33	Peoples et al. (1994)
Legumes of USA (average of 6 species)	1.51	*0.23*	1.70	0.42	0.78	1.48	0.91	0.68	0.83	Peoples et al. (1994)
Pumpkin (*Cucurbita* spp.)	2.20	1.91	9.32	1.38	2.30	4.09	3.14	1.84	2.82	Glew et al. (2006)
Woody plants of USA (average of 3 species)	0.72	**0.08**	1.21	0.23	0.38	0.79	0.44	0.35	0.46	Peoples et al. (1994)

Bolded values denote foods that have insufficient concentrations of EAAs to satisfy birds' requirements assuming they ate 5 g DM/day of each food type. For lysine (Lys), methionine (Met) plus cystine (Cys), and arginine (Arg), the amount eaten for a given food was compared to the requirements estimated for Hermit Thrushes and White-throated Sparrows (this study). For histidine (His), isoleucine (Ile), phenylalanine (Phe), threonine (Thr) and valine (Val), the amount eaten for a given food was compared to the requirements for White-crowned Sparrows (Murphy 1993b). Italic values denote foods that have insufficient concentrations of Lys, Met, or Arg to satisfy the requirements of either Hermit Thrush or White-throated Sparrow

[a]Denotes methionine only

[b]Denotes phenylalanine plus tyrosine

Hermit Thrush and White-throated Sparrow (2.4% and 2.5% per crude protein, respectively).

Requirements for methionine were similar in Hermit Thrush and White-throated Sparrow and were slightly lower than that for White-crowned Sparrows (Table 2). Methionine requirements of Dark-eyed Juncos were 0.12% of the diet when 0.10% cystine was provided (Merritt 1986), which is higher than our estimates of methionine requirements of

Hermit Thrush and White-throated sparrow. Requirements for budgerigars were 3.5% (methionine plus cystine) of dietary crude protein (Earle and Clarke 1991), which was nearly double that for Hermit Thrush and White-throated Sparrow (1.5% and 1.3% per crude protein, respectively).

Requirements for arginine in White-throated Sparrow were approximately twice that for Hermit Thrush, and both species had higher requirements than White-crowned sparrows (Table 2). The requirement for arginine in Dark-eyed juncos was estimated at 0.80% of the diet (Westerhaus 1983), which is greater than estimates for Hermit Thrush, White-throated Sparrow, and White-crowned sparrows. Requirements for budgerigars were 3.5% arginine of dietary crude protein (Earle and Clarke 1991), which was higher than that for Hermit Thrush (2.2% per crude protein) and lower than that for White-throated Sparrow (4.3% per crude protein).

Differences in birds requirements for EAAs can result from differences in dietary composition, environment, and the age, health, nutritional, and physiological state of the bird (Murphy 1993b; Koutsos et al. 2001). All experiments on EAA requirements of wild birds were conducted on adults, and the composition of our semi-synthetic diets containing 12% protein were very similar to that in Murphy (1993b) and to the diets used to measure SAA and arginine requirements for Dark-eyed juncos (Westerhaus 1983; Merritt 1986). However, Parrish and Martin (1977) used wheat-based diets to measure lysine requirements for Dark-eyed juncos, which may have rendered the amino acids less available than semi-synthetic diets. Furthermore, their experimental diets contained only 8% protein which may partially explain why Dark-eyed juncos were in negative N balance on the low lysine diets (Parrish and Martin 1977). Overall, requirements for lysine and methionine in birds were less variable between species compared to arginine requirements. The role of arginine in metabolism appears to differ between species and the reasons for this are unclear. Arginine requirements and metabolism are known to vary considerably across vertebrates (Ball et al. 2007) and this appears to be the case across bird species as well although many more such studies are needed.

Availability of essential amino acids in natural foods

A seasonal shift in diet occurs in many songbirds where birds eat much more fruit during fall migration and then eat mostly or entirely insects or seeds during nonmigratory periods (Bairlein 1990; Parrish 1997; McWilliams et al. 2004; Smith et al. 2007). The limited information available on the amino acid composition of foods available to birds (Peoples et al. 1994; Izhaki 1998; Özcan 2006) suggests that these foods vary considerably in amino acid composition and so likely differ in how well they satisfy the nutritional requirements of birds (Table 3). Most arthropods contain relatively high concentrations of all essential amino acids and likely satisfy avian requirements even when small quantities are consumed (Krapu and Swanson 1975; Finke 2002, 2015; Ramsay and Houston 2003; McCusker et al. 2014; Boulos et al. 2020). Likewise, most seeds contain relatively high concentrations of essential amino acids and so should satisfy avian requirements (Table 3), although the amounts of lysine, methionine, and arginine in seeds of grasses appears to be insufficient to satisfy the requirements of Hermit Thrush and White-throated Sparrows. Methionine was also in low concentrations in seeds of some forbs and woody plants, which would likely not satisfy the requirements for both Hermit Thrush and White-throated Sparrow (Peoples et al. 1994).

In general, fruits contain lower concentrations of EAA compared to invertebrates and seeds (Table 3). However, the EAA composition of fruits range from relatively high concentrations in Acai Berry (*Euterpe oleracea*), Cactus Fruit (*Ferocactus* spp.), and Rimu (*Dacrydium cupressinum*) (Cottam et al. 2006; McCusker et al. 2014) to others (e.g., blueberry, plums, and figs; Table 3) that were among the most deficient in EAA of the food resources analyzed (Wendeln et al. 2000; McCusker et al. 2014). The three EAA we focused on in our study (lysine, methionine, arginine) were most often deficient in the fruits and seeds available to wild birds.

The ability of food resources to satisfy birds' requirements for amino acids largely depends on the quantity of food eaten. Given birds generally eat to fulfill their energy requirements (Robbins 1993), they may not necessarily eat enough to satisfy amino acid requirements if foods contain marginal amounts of EAAs. Knowing the amino acid composition of individual food items allows an assessment of whether birds can satisfy their requirements while eating exclusively that food or whether some combination of complementary foods must be consumed. However, we found that White-throated Sparrows increased their food intake when fed diets most deficient in lysine. Therefore, amino acid concentrations in foods should be considered in relation to the energy density of food and its digestibility, although our results suggest that wild birds can at least partially compensate for EAA deficiency in some foods by increasing their food intake. Unfortunately, our ability to determine the extent to which natural foods satisfy wild bird requirements is limited because too little is known about the EAA composition of natural foods and the EAA requirements of wild birds. Furthermore, requirements for amino acids vary within a birds' annual cycle and are greater in times of protein accretion, such as during growth, molting, and reproduction (Murphy and King 1991, 1992; Klasing 1998; Ramsay and Houston 1998; Koutsos et al. 2001), for which further cross-seasonal studies of EAA requirements are needed.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was conducted on adult Hermit Thrush and White-throated Sparrows procured from the wild. The procurement of animals from the wild was done under a master bander permit from the U.S. Geological Survey Bird Banding Laboratory. All aspects of the research were in full compliance with the Guidelines to the Use of Wild Birds in Research (Fair et al. 2010). All the experimental procedures were approved by the University of Rhode Island Institutional Animal Care and Use Committee (no. AN07-01-019).

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