J Comp Physiol B (2014) 184:729–739
DOI 10.1007/s00360-014-0832-1

ORIGINAL PAPER

Feeding and digestive responses to fatty acid intake in two South American passerines with different food habits

Juan Manuel Ríos · Gonzalo F. Barceló · Cristobal Narváez · Karin Maldonado · Pablo Sabat

Received: 26 November 2013 / Revised: 1 April 2014 / Accepted: 11 April 2014 / Published online: 6 July 2014

Abstract Specific fatty acids (FA) such as unsaturated (UFA) and saturated (SFA) fatty acids contained in foods are key factors in the nutritional ecology of birds. By means of a field and experimental approach, we evaluated the effect of diet on the activity of three esterases involved in FA hydrolysis; carboxylesterase (CE: 4-NPA-CE and a-NA-CE) and butyrylcholinesterase, in two South American passerines: the omnivorous rufous-collared sparrow (*Zonotrichia capensis*) and the granivorous common diuca-finch (*Diuca diuca*). The activity of the three esterases was measured in the intestines of freshly caught individuals over two distinct seasons and also after a chronic intake of a UFA-rich or SFA-rich diet in the laboratory. In turn, we assessed the feeding responses of the birds choosing amongst diets contrasting in the kind of specific FA (UFA- vs. SFA-treated diets). During summer, field CE activities (4-NPA-CE and a-NA-CE) in the small intestine were higher in the rufous-collared sparrow (25.3 ± 3.3 and 81.4 ± 10.8 μmol min^{-1} g $tissue^{-1}$, respectively) than in the common diuca-finch (10.0 ± 3.0 and 33.9 ± 13.1 μmol min^{-1} g $tissue^{-1}$, respectively). Two hour feeding trial test indicated that both species exhibited a clear preference for UFA-treated diets. On average, the rufous-collared sparrow consumed 0.46 g 2 h^{-1} of UFA-rich diets and 0.12 g 2 h^{-1} of SFA-rich diets. In turn, the consumption pattern of the common diuca-finch averaged 0.73 and 0.16 g 2 h^{-1} for UFA-rich and SFA-rich diets, respectively. After a month of dietary acclimation to UFA-rich and SFA-rich diets, both species maintained body mass irrespective of the dietary regime. Additionally, the intestinal 4-NPA-CE activity exhibited by birds fed on a UFA-rich or SFA-rich diet was higher in the rufous-collared sparrow (39.0 ± 5.3 and 44.2 ± 7.3 μmol min^{-1} g $tissue^{-1}$, respectively) than in the common diuca-finch (13.3 ± 1.9 and 11.2 ± 1.4 μmol min^{-1} g $tissue^{-1}$, respectively). Finally, the intestinal a-NA-CE activity exhibited by the rufous-collared sparrow was about two times higher when consuming an UFA-rich diet. Our results suggest that the rufus-collared sparrow exhibits a greater capacity for intestinal FA hydrolysis, which would allow it to better deal with fats from different sources.

Keywords Birds · Butyrylcholinesterase · Carboxylesterase · Fatty acids · Food habits

Communicated by I.D. Hume.

J. M. Ríos
Instituto Argentino de Nivología, Glaciología y Ciencias Ambientales (IANIGLA), CCT Mendoza-CONICET, Z.C. 330, 5500 Mendoza, Argentina

J. M. Ríos (✉) · G. F. Barceló · C. Narváez · K. Maldonado · P. Sabat
Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile
e-mail: jmriosrama@gmail.com

P. Sabat
Center of Applied Ecology & Sustainability (CAPES), Faculty of Biological Sciences, Pontificia Universidad Católica de Chile, 6513677 Santiago, Chile

Introduction

Evolutionary changes in trophic niche are commonly accompanied by changes in digestive physiology (Klasing 1998). Adaptive modulation hypothesis (Diamond 1993) predicts that the activities of digestive enzymes must match the relative levels of its substrates in an animal's diet, thereby no excess energy is spent in underutilized enzymes (Kohl et al. 2011). Passerine species have a wide range of food habits,

including granivores, insectivores, frugivores and omnivores (Ramirez-Otarola et al. 2011; Ríos et al. 2012a, b; Sabat et al. 2013). Therefore, what birds eat determines their physiological, morphological and behavioural traits (Klasing 1998). Several factors are involved in the shaping of food habits of birds. Some studies report positive associations between nutritional content of food and their proportion eaten by birds (Schaefer et al. 2003; Valera et al. 2005; Ríos et al. 2012a). Consumption of specific nutrients by some passerines is variable and may be influenced by season (McWilliams et al. 2004), physiological condition and ontogenetic stage (Brzęk et al. 2010), as well as the nutritional context (Schaefer et al. 2003; Ríos and Mangione 2010; Maldonado et al. 2012). For example, some wintering birds need to store dietary lipids during periods of high energy demand (Bairlein 2002; McWilliams et al. 2004), while for other species, such as the many-coloured chaco-finch (*Saltatricula multicolour*) and the zebra finch (*Taeniopygia guttata*), the dietary starch level is a key factor to properly maintain their nutritional balance (Brzęk et al. 2010; Ríos et al. 2012a). Likewise, specific fatty acids (FA) are key for energetic budget and fuelling of migrants red-eyed vireos (*Vireo olivaceous*), white-throated sparrows (*Zonotrichia albicollis*), yellow-rumped warblers (*Dendroica coronata*) (McWilliams et al. 2002, 2004; Pierce et al. 2004; Alan and McWilliams 2013) and garden warbler, *Sylvia borin* (Bairlein 2002).

Birds with broad diet breadth ingest different relative amounts of carbohydrates, proteins and lipids (Klasing 1998; Caviedes-Vidal et al. 2000; Kohl et al. 2011; Ríos et al. 2012a; Sabat et al. 2013). Protein and starch digestion is simpler. These macronutrients are directly hydrolysed by pancreatic and intestinal enzymes, and in the case of starch by-products, may be mostly absorbed by passive (non-mediated) mechanisms without additional energy expenditure in some cases (Caviedes-Vidal et al. 2007). On the other hand, the digestion of FA requires a process involving several biochemical steps including emulsification and hydrolysis by lipases or esterases before being absorbed from the distal half of the jejunum and, to a lesser extent, in the ileum into enterocytes (Griminger 1986; Klasing 1998). Once inside enterocytes, FA are resterified into triglycerides and—unlike mammals where resterified FA are packaged into chylomicrons which enter the lymphatic system—are packaged into portomicrons, which pass directly into the avian hepatic portal blood supply (Denbow 2000).

The magnitude of the esterase activities appears to be related to FA metabolism. For example, a chronic intake of unsaturated fatty acids (UFA)-rich diets, such as omega-3 (linolenic acid) and omega-6 (linoleic acid) induced a rise in intestinal esterase activity in rats (Van Lith et al. 1992). A comparative study conducted on fifty-five species of free-living birds belonging to eight orders revealed that passerines with wider dietary diversity (i.e., mixed diets) had the highest hydrolytic activity of liver esterase amongst all birds assessed (Bush et al. 1973). However, experimental studies on the functional link between diet and esterase enzymes activity during specific FA intake are needed in order to extend previous generalizations on the causal mechanisms underlying passerine's food habits.

Under certain environmental conditions (e.g., low temperature, resource depletion), the quality of FA contained in the seeds and fruits is of great nutritional value for birds (Díaz 1996; Bairlein 2002; Ríos et al. 2012a). Several seeds and fruits are rich in UFA (Bewley and Black 1982; Stiles 1993; Bozinovic and Méndez 1997; Linder 2000; Karasov and Martínez del Rio 2007), while insects contain fractions of UFA and saturated fatty acid (SFA) at different proportions depending on taxa (Bell 1990; Finke 2002). In most vertebrates, preference for FA increases with the degree of unsaturation and decreases with the chain length (Karasov and Martínez del Rio 2007), a pattern that has been confirmed in studies performed on birds. An adequate intake of UFA allows birds to optimize the flow of fat deposits and facilitate lipid mobilization under conditions that requires energy and assumes thermoregulation and flight costs (McWilliams et al. 2004; Pierce et al. 2004; Bairlein 2002; McCue et al. 2009; Alan and McWilliams 2013). Although the causes underlying birds' feeding patterns have not yet been clarified, it has been suggested that the preference for UFA over SFA may be due, at least in part, to physiological constraints to digest large amounts of SFA (McWilliams et al. 2002) as has been demonstrated for other nutrients. For example, Kohl et al. (2011) have demonstrated that the field activity of carbohydrases (maltase and sucrase) is matched with the trophic niche in five North American wild passerines. Ramirez-Otarola et al. (2011), using a phylogenetically informed approach, analysed the specific nutritional content of the diet and found that the percentage of nitrogen in diet was negatively correlated with residual maltase activity and positively correlated with the ratio aminopeptidase-N/maltase in wild passerines from central Chile. Currently, no studies have been performed on the role of FA on the birds' dietary preferences and their link with digestive capabilities such as esterase activities.

The rufous-collared sparrow and common diuca-finch are two of the most conspicuous passerines in the Mediterranean environments of central Chile (Sabat et al. 1998). The first is an omnivorous species, which includes seeds, insects and fruits in its diet, while the second is a strictly granivorous bird (Lopez-Calleja 1995; Sabat et al. 1998; Ramirez-Otarola et al. 2011). As such, these two bird species offer a suitable model for studying the role of specific FA in the shaping of food habits of South American wild passerines and their relationship with the bird's esterase activities. We hypothesized that (1) field esterase activity differs amongst species within seasons, (2) all birds will choose UFA over

SFA diets and (3) bird's esterase activity modulation will be closely related to the kind of substrate supplied; thus, species with a broader diet will have a greater digestive capacity to process dietary fats of varying quality.

Methods

Bird capture

Birds used in this study were captured at Quebrada de la Plata (33°30′S, 70°54′W), central Chile. This study site has a Mediterranean climate characterized by hot, dry summers and cold, rainy winters (mean annual precipitation = 367 mm, di Castri and Hajek 1976). All birds were captured with Ecotone mist nets from December to January (austral summer) and from July to August (austral winter) in 2012–2013. Data on diet breadth of the study species were obtained from the literature (Lopez-calleja 1995; Ramirez-Otarola et al. 2011). During the summer, eight adult individuals of both rufous-collared sparrow and common diuca-finch were captured to compare field esterase activity amongst species and seasons. In the winter, eight adult individuals of rufous-collared sparrow, and seven of common diuca-finch, were captured for the same purposes. Birds for laboratory feeding trials (adult individuals of rufous-collared sparrow: $n = 11$, and common diuca-finch: $n = 10$) were captured in March (late austral summer and early austral autumn) with mist nets at the same site. Feeding trials were conducted in autumn as passerines build energy reserves to prepare for cold temperatures, and their diet composition may be associated with lipid-rich food owing to its great energy density per unit of mass, which is needed for fattening prior to the colder season or to increase the chances of bird's overnight survival in winter (Díaz 1996; McWilliams et al. 2004; Khalilieh et al. 2012). Thus, the finely tuned discriminatory abilities to detect different specific nutrients by birds, under experimental conditions, might be more assessable in autumn (Lepczyk et al. 2000; Cueto et al. 2006; Ríos and Mangione 2010; Ríos et al. 2012a).

Bird maintenance in the laboratory

After capture, birds assigned to perform feeding trials were transported to the laboratory and housed in single cages (30 × 30 × 40 cm), and kept under a constant temperature and light regime [25 °C 12 h:12 h (light:dark)]. During the first 3 days of a captivity acclimation period lasting 20 days, birds were provided with water and a commercial seed mixture, equal parts of millet (*Panicum miliaceum*), canary (*Phalaris canariensis*), rape (*Brassica napus*) and hemp (*Cannabis sativa*) ad libitum. Two mealworm larvae (*Tenebrio molitor*) per day were supplied to each rufous-collared sparrow individual. After this three-day period, birds were fed with the same commercial seed mixture plus dry millet seeds ground into a homogeneous powder with an electric mill (particle size ≤0.5 mm, DeLonghi mill model 30, Italy) for the following 7-day period. In the last 10 days of the acclimation period, birds were fed only powdered millet seeds. Once a week, the diet was supplemented with vitamins (AEDK Holliday-Scott, #151, Argentina). We used the same powdered millet seeds as a base food to prepare treatment diets and the same individual birds to perform both feeding trials (*Experiment 1* and *Experiment 2*). Captures and experiments were conducted with permissions issued by Servicio Agrícola Ganadero, Government of Chile (SAG: Scientific Research Permits #3935). The experiments comply with the current laws of Chile in terms of animal health and care.

Reagents and chemicals

Chemicals needed for esterase assays, i.e., α-naphthyl acetate (a-NA), 4-nitrophenyl acetate (4-NPA), 4-nitrophenol, Fast Red ITR salt, 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) and butyrylthiocholine iodide (BuSCh) were purchased from Sigma-Aldrich (Santiago, Chile). In order to use ecologically realistic compounds to perform feeding trials, all specific FA used for diet formulation in the *Experiment 1* were in the pure triacylglycerols form: i.e., pure trilinolein liquid (C 18:2), pure triolein liquid (C 18:1), pure tristearin powder (C 18:0) and pure tripalmitin powder (C 16:0), all supplied by Sigma-Aldrich (Santiago, Chile). The commercial corn oil (from Arcor Group, Argentina) and coconut oil (from Droguería Pacífico, Argentina) used to prepare the UFA- and SFA-rich diets, respectively (as required for *Experiment 1* and *Experiment 2*), were purchased from a local market.

Feeding trials in the laboratory

We performed feeding trials to assess the role of specific FA on food preference for the rufous-collared sparrow and the common diuca-finch (*Experiment 1*) and to determine their digestive capabilities (measured as esterase activities) to process dietary FA (*Experiment 2*). In both experiments, we used powdered millet seeds as a base food to prepare treatment diets. The nutritional content of millet seeds is as follows: 13 % crude protein, 4.9 % total lipids, 4.2 % crude fibre and 3.6 % ash; as shown in Lorenz and Hwang (1986). All treatment diets used were isocaloric and allowed all individuals from both species to feed. Any other synthetic diets previously tested on these birds were rejected by some individuals of both species. Treatment diets and water were provided ad libitum.

Experiment 1: Feeding trials for fatty acid preference

To test the feeding responses of birds on specific FA, we used two-choice feeding trials as follows: after 3 h fast, individuals of rufous-collared sparrow ($n = 8$) and common diuca-finch ($n = 7$) were exposed to the experimental arena, choosing between a specific UFA- and a specific SFA-treated diet. In addition, we tested the feeding response to diets including oils that differed in their UFA and SFA profiles: corn oil was used to formulate an UFA-rich diet (FA content 42 % linoleic acid, 79 % total UFA and 13 % total SFA as indicated on the commercial product label), and coconut oil was used to formulate a SFA-rich diet (FA content 2.1 % linoleic acid, 12 % total UFA and 64 % total SFA, as indicated on the commercial product label). Hence, five combinations of treated diets were tested: (1) linoleic acid versus stearic acid (C 18:2 vs. C 18:0); (2) linoleic acid versus palmitic acid (C 18:2 vs. C 16:0); (3) oleic acid versus stearic acid (C 18:1 vs. C 18:0); (4) oleic acid versus palmitic acid (C 18:1 vs. C 16:0) and (5) corn oil diet versus coconut oil diet (UFA-rich diet vs. SFA-rich diet). All pure specific FA tested in this experiment were in the triacylglycerol form as mentioned before. We chose these compounds because (1) they were previously tested in preference trials for birds (Pierce et al. 2004), (2) they are commonly found in wild seeds, fruits and insects (Díaz 1996; Linder 2000; Pierce et al. 2004) and (3) all of them are commercially available. Feeders were randomly located in the cages to prevent association of feeder site with type of treatment. The measured response variable was the amount of food consumed from both diets per individual. In order to use ecologically realistic concentrations of specific FA in feeding trials that were also detectable by birds, we used each pure UFA and SFA at 5 % (% mass). Concentration of 5 % of UFA or SFA was chosen because (1) this is the total lipid concentration in *Pappophorum* spp. seeds, one of the most preferred grass seed by both bird species (Cueto et al. 2006; Ríos et al. 2012a) and (2) this is the total lipid concentration in *Panicum miliaceaum* seeds (Lorenz and Hwang 1986), the base food used to make treatment diets in our feeding trials. In the case of corn and coconut oil diets, we use a concentration of 8 % following McCue et al. (2009) who tested the chronic effect of dietary FA composition in the strict granivore zebra finch. Given that corn and coconut oil diets were used in both *Experiment 1* and *Experiment 2*, we wanted to assess them at the same concentration levels (8 %) between experiments and between the two kinds of oils for comparative purposes. To prepare each treatment diet, we weighed known amounts of the specific FA and oils and diluted them in absolute ethanol (EtOH) to facilitate the proper mixture with the dry powdered millet seeds used as the base food of the treatment diets. The EtOH was allowed to evaporate under a bell jar for 36 h at room temperature with no exposure to light. Treatment diets were stored in hermetic bags and kept at −25 °C until used. In the case of the SFA-rich diet, the bottle containing coconut oil was previously heated in a water bath (60 °C) until it became liquid, and diluted with EtOH to facilitate the proper mixture with the dry powdered millet seeds. Pure tristearin powder (C 18:0) and pure tripalmitin powder (C 16:0) were diluted with EtOH and then carefully hand-shaken until totally dissolved. Then, the ethanol solution was added to the base food as mentioned before. Because of the high proportion of the powdered seed in relation to small quantity of specific FA or oils used, all final diets had the same consistency, appearance and colour, so it was expected that the major cue in the bird's food preference would be their taste detection capabilities instead of the visual aspect (Lepczyk et al. 2000; Schaefer et al. 2003; Pierce et al. 2004).

Experiment 2: Digestive response to UFA-rich versus SFA-rich diet in chronic exposure feeding trial

During a food acclimation period lasting 30 days, we fed ad libitum with UFA-rich diet or SFA-rich diet the individuals of rufous-collared sparrow ($n = 6$ and $n = 5$, respectively) and common diuca-finch ($n = 5$ and $n = 5$, respectively). At the end of this experiment, all birds were euthanized for esterase activity determination.

Esterase activity measurement

Immediately after seasonal capture or after the end of *Experiment 2* (day 30 of whole trial, see below), birds were killed by CO_2 asphyxiation. The small intestine was quickly excised, flushed with ice-cold saline solution (0.9 % NaCl), measured (0.1 cm) and weighed (0.001 g) before storing at −80 °C. For biochemical assays, tissues were thawed and the whole small intestine was homogenized in 20 volumes of 0.9 % NaCl for 30 s at 24,000 rpm using an Ultra Turrax T25 homogenizer (Janke and Kunkel, Breisgau, Germany). The homogenates were centrifuged at 5,000 rpm for 10 min at 4 °C, and the supernatant was finally frozen and stored at −80 °C. Carboxylesterase (CE) activity was measured on a Thermo Scientific Multiskan GO at 25 °C using the substrates a-NA and 4-NPA. Hydrolysis of a-NA was measured following the method described in Thompson (1999). Briefly, the enzymatic activity was run for 10 min in a reaction medium (200 µL, final volume) composed of 0.1 M Tris–HCl (pH 7.4), 20 mM a-NA and the sample. The formation of α-naphthol was stopped by the addition of 50 mg of Fast Red ITR 0.1 % in SDS 5 %/Triton X 100 5 %, and the absorbance of the naphthol–Fast Red ITR complex was read at 530 nm after allowing the solution to stand for 30 min at 22–23 °C in the dark. The specific

activity was calculated using a molar extinction coefficient of 14×10^3 M^{-1} cm^{-1} for the naphthol–Fast Red ITR complex. Hydrolysis of 4-NPA by CE (4-NPA-CE) was determined as described by Chanda et al. (1997). The reaction mixture (200 µL, final volume) contained 20 mM 4-NPA, 0.1 M Tris–HCl (pH 7.4) and the sample. Reaction was initiated by the addition of 10 µL of 20 mM 4-NPA. The reaction was stopped after 10 min by the addition of 50 µL SDS 2 % in Tris 2 %. The liberated 4-nitrophenol was read at 405 nm and quantified by a calibration curve (5–100 µM). The specific activity was calculated using a molar extinction coefficient of 8.0×10^3 M^{-1} cm^{-1}. Butyrylcholinesterase (BChE) activity was determined according to Wheelock et al. (2005) as adapted from Ellman et al. (1961). The reaction medium (200 µL, final volume) was made by 0.1 M Tris buffer (pH 8.0) containing 10 mM DTNB and the sample. The reaction was initiated by addition of 60 mM BuSCh, and the product was monitored at 412 nm for 8 min. Specific activity was calculated using a molar absorption coefficient of 8.6×10^{-3} M^{-1} cm^{-1} (Eyer et al. 2003). Protein concentration in the small intestine tissue was determined by the Bradford method (1976) using bovine serum albumin as the standard.

Data analyses

To satisfy assumptions of parametric statistical tests, we log-transformed data on enzymatic activities (Zar 1996), since raw data did not fit normal distribution (Shapiro–Wilks W test $P < 0.05$). Normality was assessed after transformation. Pearson's correlation was used to explore relationships between enzymatic activities expressed as tissue specific and by mg of protein; as these variables were positively and significantly correlated, we only reported the tissue-specific activity of these enzymes. Esterase activities measured in birds from field and laboratory acclimation were not associated with body mass using least square regressions. Then, two-way ANOVA was performed to detect (1) differences between birds species and between seasons in the activity of three intestinal esterases (4-NPA-CE, a-NA-CE and BChE) measured in fresh caught birds, (2) differences between birds species and between diet treatment on both food intake and esterases activities in *Experiment 2* (chronic feeding trial). Fisher's least significant difference (LSD) tests were used to make multiple comparisons in enzymatic activities shown by the omnivorous and granivorous birds in the field and *Experiment 2*. Log-transformed data of food intake in the first feeding trial (*Experiment 1*), and body mass and small intestine morphometry in *Experiment 2* did not fit normality. We then used Wilcoxon's matched-pairs test to compare the bird's food intake of each specific UFA versus SFA diets. The same test was used to compare body mass at the beginning and end of *Experiment 2* within each species fed on an UFA-rich or SFA-rich diet. In *Experiment 2*, Mann–Whitney U test was used to detect differences in small intestine morphometry within each species fed on an UFA-rich or SFA-rich diet. All values reported represent averages ± SE. All statistical analyses were performed using Statistica version 6.0 (StatSoft 2001). For all statistical comparisons, a value of $P < 0.05$ was considered significant and $0.05 \leq P \leq 0.10$ was taken to indicate a trend.

Table 1 Summary of two-way ANOVA used to detect interspecific differences in field intestinal carboxylesterase activities (4-NPA-CE and a-NA-CE) and butyrylcholinesterase activity (BChE) of both birds species during two seasons

Variable	*F*	*df*	*P*
4-NPA-CE (µmol min^{-1} g $tissue^{-1}$)			
Species	14.174	1, 27	**<0.001**
Season	22.255	1, 27	**<0.001**
Species × season	1.763	1, 27	0.195
a-NA-CE (µmol min^{-1} g $tissue^{-1}$)			
Species	5.165	1, 27	**0.031**
Season	7.036	1, 27	**0.013**
Species × season	3.551	1, 27	0.070
BChE (µmol min^{-1} g $tissue^{-1}$)			
Species	9.21	1, 27	**0.005**
Season	0.00	1, 27	0.955
Species × season	6.41	1, 27	**0.017**

Significant results are shown in bold

Results

Field esterases activities of birds during summer and winter

Field CE activities (4-NPA-CE and a-NA-CE) in the small intestine differed between bird species and seasons, while BChE activity differed amongst species but not seasons (see Table 1 for statistics). When field esterase activity during summer was compared between species, the a posteriori analysis revealed that the activity of 4-NPA-CE, a-NA-CE and BChE was significantly greater in rufous-collared sparrow than common diuca-finch ($P = 0.001$, $P = 0.005$, $P = 0.0004$, respectively; Fig. 1). The a-NA-CE and BChE activities were similar between species in winter ($P = 0.789$; $P = 0.729$, respectively; Fig. 1). The activity of carboxylesterase 4-NPA-CE was apparently higher in rufous-collared sparrow than common diuca-finch, although this difference was non-significant ($P = 0.10$; Fig. 1).

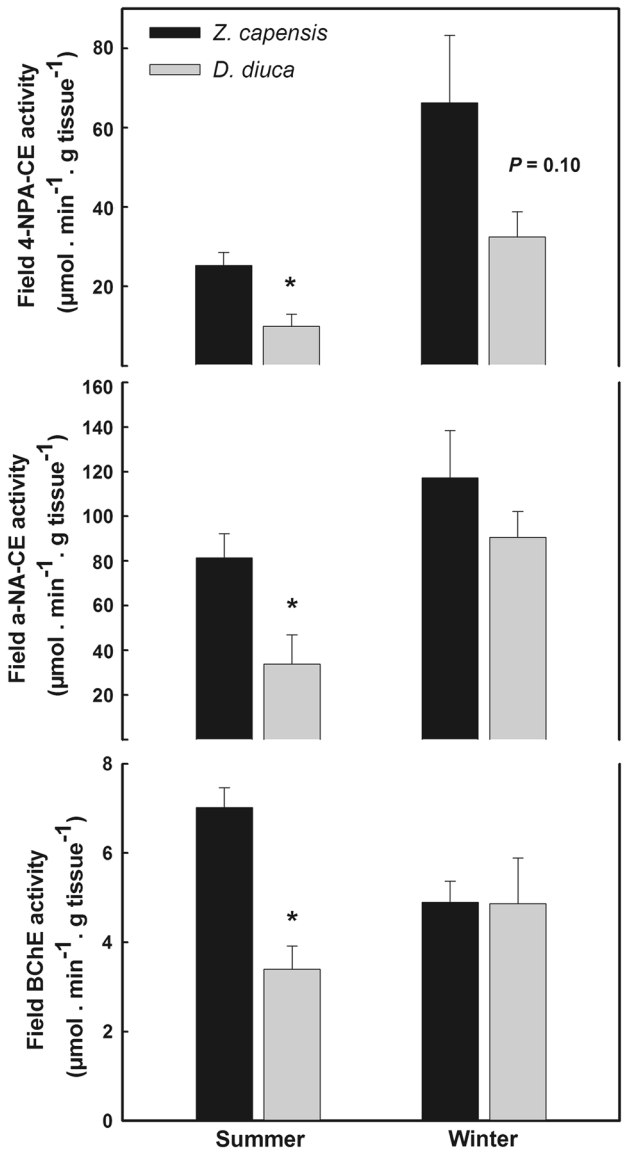

Fig. 1 Intestinal carboxylesterase activities (4-NPA-CE and a-NA-CE) and butyrylcholinesterase activity (BChE), during summer and winter season, in the omnivorous rufous-collared sparrow (summer: $n = 8$ and winter: $n = 8$) and granivorous common diuca-finch (summer: $n = 8$ and winter: $n = 7$). Bars represent a mean ± 1 standard error. *Asterisks* indicate significant differences (Fisher LSD, $P < 0.05$) between the omnivorous and granivorous within each season. Values of $0.05 \leq P \leq 0.10$ were taken to indicate a trend

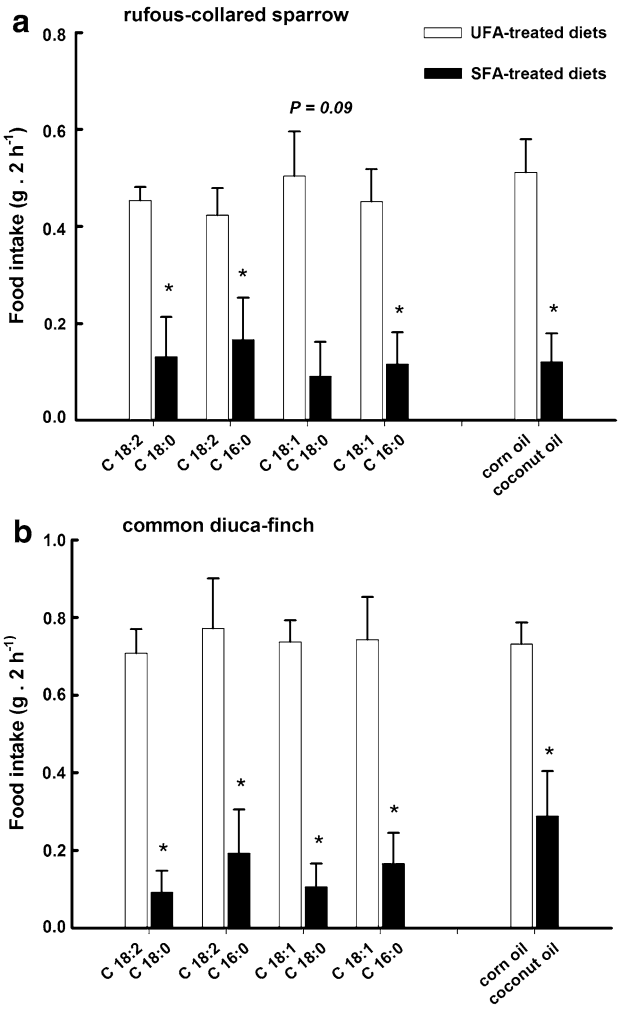

Fig. 2 Food intake by rufous-collared sparrow (**a**) and common diuca-finch (**b**) feeding for 2 h on an UFA- versus a SFA-treated diet: five combinations of treatment diets were tested: (1) linoleic acid versus stearic acid (C 18:2 vs. C 18:0); (2) linoleic acid versus palmitic acid (C 18:2 vs. C 16:0); (3) oleic acid versus stearic acid (C 18:1 vs. C 18:0); (4) oleic acid versus palmitic acid (C 18:1 vs. C 16:0); and (5) corn oil versus coconut oil diet (i.e., UFA-rich diet vs. SFA-rich diet). *Asterisks* indicate significant differences between treatment diets intake within each bird species (*$P < 0.05$). Values of $0.05 \leq P \leq 0.10$ were taken to indicate a trend

Feeding trials

Experiment 1: Feeding trials for fatty acid preference

Rufous-collared sparrow consumed more UFA than SFA diets (linoleic vs. stearic acid: $z = 2.240$, $P = 0.025$; linoleic vs. palmitic acid: $z = 1.960$, $P = 0.049$; oleic vs. palmitic acid: $z = 2.100$, $P = 0.035$) and showed a trend to consume more oleic than stearic acid diet ($z = 1.680$, $P = 0.090$) (Fig. 2a). Common diuca-finch consumed more of all UFA than SFA diets (linoleic vs. stearic acid: $z = 2.366$, $P = 0.017$; linoleic vs. palmitic acid: $z = 2.028$, $P = 0.04$; oleic vs. stearic acid: $z = 2.366$, $P = 0.017$; oleic vs. palmitic acid: $z = 2.197$, $P = 0.027$) (Fig. 2b). Finally, when the birds were able to choose between corn oil versus coconut oil diets, rufous-collared sparrow and common diuca-finch both consumed more of the corn oil diet ($z = 2.197$, $P = 0.027$ and $z = 2.520$, $P = 0.011$, respectively) (Fig. 2a, b).

Table 2 Summary of two-way ANOVA used to detect interspecific differences in intestinal carboxylesterase activities (4-NPA-CE and a-NA-CE) and butyrylcholinesterase activity (BChE) of both birds species in chronic exposure feeding trial

Variable	*F*	*df*	*P*
Food intake (g day^{-1})			
Species	0.729	1, 17	0.406
Treatment	3.772	1, 17	0.070
Species × treatment	1.194	1, 17	0.291
4-NPA-CE (μmol min^{-1} g tissue^{-1})			
Species	53.89	1, 17	**<0.001**
Treatment	0.04	1, 17	0.842
Species × treatment	0.48	1, 17	0.499
a-NA-CE (μmol min^{-1} g tissue^{-1})			
Species	4.801	1, 17	**0.042**
Treatment	0.920	1, 17	0.350
Species × treatment	1.046	1, 17	0.320
BChE (μmol min^{-1} g tissue^{-1})			
Species	4.240	1, 17	0.055
Treatment	0.066	1, 17	0.799
Species × treatment	0.146	1, 17	0.707

Significant results are shown in bold

Experiment 2: Digestive response to an UFA-rich and a SFA-rich diet in chronic exposure feeding trial

Food intake by birds did not differ significantly amongst species and diet treatments (Table 2). Rufous-collared sparrow had higher intestinal 4-NPA-CE activity than the common diuca-finch when it consumed both UFA-rich and SFA-rich diets (Table 2; Fig. 3). There was a difference in the activity of the intestinal a-NA-CE between species (Table 2), and this difference was due to a higher activity of intestinal a-NA-CE exhibited by the rufous-collared sparrow in consuming the UFA-rich diet ($P = 0.039$; Fig. 3). Instead, intestinal BChE activity did not differ between species or diet treatments (Table 2). There were no significant differences in bird body mass between the beginning and end of the experiment within each species fed on UFA-rich or SFA-rich diets (Table 3).

Discussion

The established body of knowledge has demonstrated that a broad diet breadth exposes omnivorous birds to a wide range of foods with different nutritional quality, and they appear to be better able to process a high diversity of substrates (Caviedes-Vidal et al. 2007; Kohl et al. 2011; Maldonado et al. 2012; Sabat et al. 2013). At present, this contrast between omnivorous and strictly granivorous passerines has been tested in field, and laboratory experiments where protease (aminopeptidase-N, trypsin, chymotrypsin and alanine aminotransferase) and carbohydrase (amylase, maltase and sucrase) activities were measured (Martínez del Rio 1990; Sabat et al. 1998; Brzęk et al. 2009, 2010; Kohl et al. 2011; Ramirez-Otarola et al. 2011). However, to our knowledge, no study has been made to investigate a link between a bird's FA intake and the enzymes involved in their digestion (i.e., esterases).

In *Experiment 1*, we tested feeding preferences of an omnivorous bird (rufous-collared sparrow, which consumes seeds, insects and fruits) and a strictly granivorous bird, the common diuca-finch (which only consumes seeds), against UFA- versus SFA-treated diets. Our results indicate that regardless of food habits, birds of both species clearly discriminated between specific UFA- and SFA-treated diets. In all cases, birds preferred diets with mostly unsaturated, esterified fatty acids (18:1 or 18:2) to a diet with mostly saturated, esterified fatty acids (18:0 or 16:0). Birds responded to diet types within the 2 h feeding trial, suggesting the birds have fine-tuned discrimination abilities and used cues with relatively rapid response times (e.g., taste) when making their choices. This strong feeding pattern is consistent with several studies performed with other Northern Hemisphere songbirds. For example, as with the two South American songbirds used in this study, garden warblers (*Sylvia borin*), wood thrushes (*Hylocichla*

Table 3 Means ± SE of body mass (g), small intestine mass (g) and length (cm) from birds fed on UFA-rich or SFA-rich diet

Variable	Rufous-collared sparrow		Common diuca-finch	
	UFA-rich diet	SFA-rich diet	UFA-rich diet	SFA-rich diet
Body mass (initial)	18.76 ± 0.52	19.68 ± 0.60	30.97 ± 1.67	31.20 ± 0.93
Body mass (final)	18.98 ± 0.60	19.01 ± 0.68	29.40 ± 1.62	30.10 ± 0.86
Small intestine morphometry[a]				
Mass	0.70 ± 0.04	0.76 ± 0.04	0.99 ± 0.11	0.92 ± 0.08
Length	14.0 ± 0.50	13.3 ± 0.53	16.8 ± 1.5	15.56 ± 0.41

[a] Values were calculated from an average after 30 days on UFA-rich or SFA-rich diet. Non-significant differences were detected ($P \leq 0.05$) within each species fed on UFA-rich or SFA-rich diet, as determined by Mann–Whitney *U* test

mustelina), American robins (*Turdus migratorius*), yellow-rumped warblers (*Dendroica coronata*) and red-eyed vireos (*Vireo olivaceous*) preferred diets with mostly 18-carbon unsaturated fatty acids over the diets with mostly 18-carbon saturated fatty acid (Bairlein 1991; Zurovchak 1997; McWilliams et al. 2002; Pierce et al. 2004). In addition, Alan and McWilliams (2013) reported recently that omnivorous white-throated sparrows (*Zonotrichia albicollis*) experimentally consumed relatively equal amounts of high and low UFA diets. Also, these authors found that despite antioxidant (α-tocopherol) supplementation, birds did not follow the expected feeding preference for the high versus low UFA diets (Alan and McWilliams 2013). This evidence suggests that the qualitative compositions of specific FA in a diet formulation could be more important than the quantitative properties. These dietary preferences for UFA, exhibited by many passerines, are consistent with the hypothesis that digestive constraints, in part, determine dietary preferences. One plausible explanation proposed by McWilliams et al. (2002) is that just as the inability to digest sucrose influences sugar preferences in some passerine birds (Martínez del Rio 1990), a low capacity to assimilate SFA influences birds' FA preferences (sensu McWilliams et al. 2002).

Digestive capabilities to process a UFA-rich or a SFA-rich diet may be related to bird's food habits

The field CE activity shown by the omnivorous rufous-collared sparrow become two times greater than that of the common diuca-finch in the summer season; although during winter, we did not find a similar pattern (Fig. 1). The latter could be explained, at least in part, because wintering common diuca-finch includes a high proportion of seeds from different plant families: mostly Poaceae (34 %) and Cactaceae (35 %) followed by Asteraceae (25 %). In contrast, in summer this species becomes more specialized, consuming more than 91 % of *Erodium cicutarum* (Geraniaceae) seeds (Lopez-Calleja 1995). In this sense, the common diuca-finch not only increases the diversity of seeds eaten, but likely also increases the diversity of FA consumed in winter. This phenomenon could explain, in part, the rise in the three esterases activity found in this species during winter (Fig. 1). The relationship between broad diets and a greater esterase activity, in the field results of this study, is in agreement with results reported by Bush et al. (1973). These authors found that those passerines with more diverse diets showed the highest activity of hepatic esterases amongst all species tested.

In the laboratory experiments, the chronic exposure feeding trial showed that 4-NPA-CE activity for rufous-collared sparrow was close to four times higher than for common diuca-finch after 1 month of consuming either an UFA-rich or a SFA-rich diet (Fig. 3). In turn, a-NA-CE activity was two times higher in rufous-collared sparrows but only after consuming an UFA-rich diet (Fig. 3). Although there was no difference in BChE activity between species or diet treatments, rufous-collared sparrows showed a trend towards higher BChE activity than the common diuca-finch (Table 1; Fig. 3). Biochemical processes other than hydrolysis by lipases or esterases could also limit

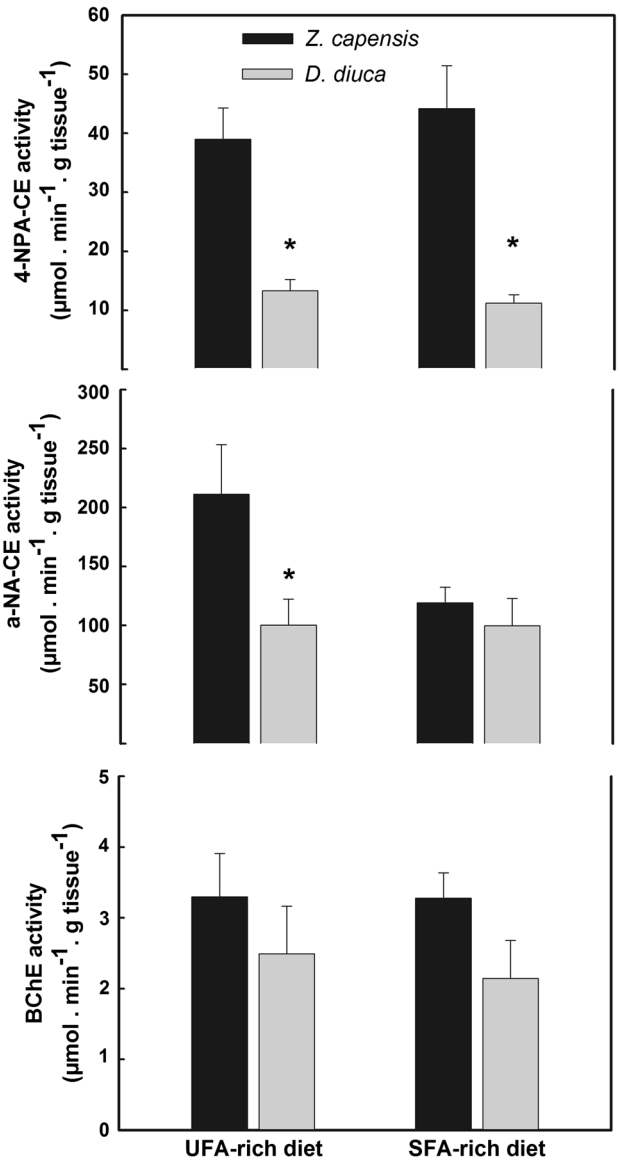

Fig. 3 Intestinal carboxylesterase activities (4-NPA-CE and a-NA-CE) and butyrylcholinesterase activity (BChE) after chronic intake of a diet with 8 % of corn oil (UFA-rich diet) by the omnivorous rufous-collared sparrow ($n = 5$) and granivorous common diuca-finch ($n = 5$) and after chronic intake of a diet with 8 % of coconut oil (SFA-rich diet, rufous-collared sparrow: $n = 6$ and common diuca-finch: $n = 5$). *Bars* represent a mean ± 1 standard error. *Asterisks* indicate significant differences (Fisher LSD, $P < 0.05$) between species within each treatment

efficient FA utilization (Hargreaves et al. 1991; McClelland 2004). For instance, protein-mediated transport of FA from the circulation to oxidation sites in the mitochondria and activities of key metabolic enzymes have shown to be increased when birds deal with higher energy demands, for example, during migrations or overnight in winter (Guglielmo et al. 2002; McFarlan et al. 2009). Although further studies including those parameters could be useful to evaluate differences in the capacity to assimilate FA in our studied species, by integrating field and laboratory approaches, we suggest that the rufous-collared sparrow has an apparently higher hydrolytic capacity—in comparison to the common diuca-finch—when processing fats.

Fatty acid preferences, digestive capabilities and the implications for the feeding ecology of austral songbirds

Why do the birds eat what they eat? The understanding of patterns of food selection by birds in their natural habitats is a challenging task. Despite more than 40 years of research conducted on this topic, little is known about the essential nutrient requirements of wild birds and their influence on selective feeding. From previous studies, we could assume that a major factor influencing the feeding behaviour of omnivorous rufous-collared sparrow is the relative abundance of food in the field (Lopez-Calleja 1995; Sabat et al. 1998; Marone et al. 2008; Ríos et al. 2012a). These findings seem to emphasize that extrinsic ecological factors are more important than the food chemistry to explain their feeding behaviour patterns (Marone et al. 2008). However, the high digestive plasticity exhibited by rufous-collared sparrow in many environmental conditions (e.g., seasonal variation in the quality of food, presence of secondary compounds in the diet, temperature changes along longitudinal gradients, amongst others; Sabat et al. 1998; Ríos et al. 2012b; Maldonado et al. 2012) demonstrates the importance of the physiological mechanisms that help to explain their food habits. Recently, McCue et al. (2011) found that the nutritional status of zebra finches affects the metabolism of exogenous nutrients. The authors found that adult birds recovering from nutritional stress had much lower rates of exogenous nutrient oxidation than fed birds, and this difference was particularly evident for FA (McCue et al. 2011). Furthermore, the authors stated that amongst the mechanisms underlying differential exogenous FA use by adult zebra finches (fasted vs. regularly fed birds), enzyme down-regulation may significantly contribute. In our field study, we found that within the species we considered, the activity of CE (both 4-NPA-CE and a-NA-CE) was lower in summer than in winter (Table 1; Fig. 1). As mentioned before, central Chile has a Mediterranean climate characterized by hot, dry summers, which coincides with food availability depletion (Sabat et al. 1998; Maldonado unpublished data). Consistent with McCue et al. (2011), due to their remarkable digestive flexibility, small passerines may reduce energetic requirements when food is limited, and the low intestinal CE activity shown by our two species during summer could be a reflection of such environmental adaptation.

In the present study, we determined that regardless of a bird's food habits, both species clearly prefer UFA- to SFA-treated diets. Thus, if high diversity FA is more easily found in mixed diets, behavioural preferences for UFA might help wild birds to better satisfy their requirements for essential nutrients. But what seems worth noting is the fact that it is unknown whether these behavioural preferences for specific fatty acids change seasonally with different energy demands (e.g., breeding) at a species-specific level. These are hypotheses that deserve experimental testing in wild austral passerine birds. The adjustment observed in the rufous-collared sparrow in the modulation of digestive enzymes involved in fat digestion, coupled with carbohydrases and proteases adjustments determined in previous studies, is a physiological trait that may explain their greater diet breadth.

Acknowledgments This work is from the postdoctoral project financed by Fondo Nacional de Desarrollo Científico y Tecnológico (Chile Proyecto No. 3130429 to JM Ríos and No. 1120276 to PS). Birds were captured with permits from SAG, Chile (No. 3935/2013). All protocols were approved by the Institutional Animal Care Committee of the Universidad de Chile, where the experiments were performed. We thank Andrés Sazo and Grabiela Píriz for their help in the field and laboratory. JMR gives special thanks to Jorgelina Altamirano and Nestor Ciocco.

References

Alan RR, McWilliams SR (2013) Oxidative stress, circulating antioxidants, and dietary preferences in songbirds. Comp Biochem Physiol B 164:185–193

Bairlein F (1991) Nutritional adaptations to fat deposition in the long-distance migratory garden warbler (*Sylvia borin*). Acta Congr Intern Ornithol 20:2149–2158

Bairlein F (2002) How to get fat: nutritional mechanisms of seasonal fat accumulation in migratory songbirds. Naturwissenschaften 89:1–10

Bell GP (1990) Birds and mammals on an insect diet: a primer on diet composition analysis in relation to ecological energetics. Stud Avian Biol 13:416–422

Bewley J, Black D (1982) Physiology and biochemistry of seeds. Springer, Berlin

Bozinovic F, Méndez MA (1997) Role of dietary fatty acids on energetics and torpor in the Chilean mouse-opossum *Thylamys elegans*. Comp Biochem Physiol A 116:101–104

Bradford M (1976) A rapid and sensitive assay of protein utilizing the principle of dye binding. Anal Biochem 772:248–264

Brzęk P, Kohl K, Caviedes-Vidal E, Karasov WH (2009) Developmental adjustments of house sparrow (*Passer domesticus*) nestlings to diet composition. J Exp Biol 212:1284–1293

Brzęk P, Lessner KN, Caviedes-Vidal E, Karasov WH (2010) Low plasticity in digestive physiology constrains feeding ecology

in diet specialist, zebra finch (*Taeniopygia guttata*). J Exp Biol 213:798–807
Bush FM, Price JR, Townsend JI (1973) Avian hepatic esterases, pesticides and diet. Comp Biochem Physiol 44:1137–1151
Caviedes-Vidal E, Afik D, Martínez del Rio C, Karasov WH (2000) Dietary modulation of intestinal enzymes of the house sparrow (*Passer domesticus*): testing an adaptive hypothesis. Comp Biochem Physiol A 125:11–24
Caviedes-Vidal E, McWhorter TJ, Lavin SR, Chediack JG, Tracy CR, Karasov WH (2007) The digestive adaptation of flying vertebrates: high intestinal paracellular absorption compensates for smaller guts. Proc Natl Acad Sci USA 104:19132–19137
Chanda SM, Mortensen SR, Moser VC, Padilla S (1997) Tissue-specific effects of chlorpyrifos on carboxylesterase and cholinesterase activity in adult rats: an in vitro and in vivo Comparison. Fund Appl Toxicol 38:148–157
Cueto VR, Marone L, Lopez de Casenave J (2006) Seed preferences in sparrow species of the Monte desert: implications for seed–granivore interactions. Auk 123:358–367
Denbow DM (2000) Gastrointestinal anatomy and physiology. In: Whittow GC (ed) Sturkie's avian physiology. Academic Press, New York, pp 299–325
di Castri F, Hajek ER (1976) Bioclimatología de Chile. Editorial Universidad Católica de Chile, Santiago
Diamond JM (1993) Logic of life: the challenge of integrative physiology. In: Noble D, Boyd CAR (eds) Evolutionary physiology, pp 89–111
Díaz M (1996) Food choice by seed-eating birds in relation to seed chemistry. Comp Biochem Physiol A 113:239–246
Eyer P, Worek F, Kiderlen D, Sinko G, Stuglin A, Simeon-Rudolf V, Reiner E (2003) Molar absorption coefficients for the reduced Ellamn reagent: reassessment. Anal Biochem 312:224–227
Finke MD (2002) Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biol 21:269–285
Gl Ellman, Courtney KD, Andres V, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95
Griminger P (1986) Lipid metabolism. In: Sturkie P (ed) Avian physiology. Springer, New York, pp 345–358
Guglielmo CG, Haunerland NH, Hochachka PW, Williams TD (2002) Seasonal dynamics of flight muscle fatty acid binding protein and catabolic enzymes in a migratory shorebird. Am J Physiol 282:1405–1413
Hargreaves M, Kiens B, Richter EA (1991) Effect of increased plasma free fatty acid concentrations on muscle metabolism in exercising men. J Appl Physiol 70:194–197
Karasov WH, Martínez del Rio C (2007) Ecological physiology: how animals process energy, nutrients and toxins. Princeton University Press, New Jersey
Khalilieh A, McCue MD, Pinshow B (2012) Physiological responses to food deprivation in the house sparrow, a species not adapted to prolonged fasting. Am J Physiol 303:551–561
Klasing KC (1998) Comparative avian nutrition. CAB International, Wallingford
Kohl K, Brzek P, Caviedes-Vidal E, Karasov WH (2011) Pancreatic and intestinal carbohydrases are matched to dietary starch level in wild passerines birds. Physiol Biochem Zool 84:195–203
Lepczyk CA, Murray KG, Winnett-Murray K, Bartell P, Geyer E, Work T (2000) Seasonal fruit preference for lipids and sugars by American robins. Auk 117:709–717
Linder R (2000) Adaptive evolution of seed oils in plants: accounting for the biogeographic distribution of saturated and unsaturated fatty acids in seed oils. Am Nat 156:442–458
Lopez-Calleja MV (1995) Dieta de *Zonotrichia capensis* (Emberizidae) and *Diuca diuca* (Fringillidae): efecto de la variación estacional de los recursos tróficos y la riqueza de aves granívoras de Chile central. Rev Chil His Nat 68:321–331
Lorenz K, Hwang YS (1986) Lipids in proso millet (*Panicum miliaceum*) flours and brans. Cereal Chem 63:387–390
Maldonado K, van Dongen WFD, Vásquez RA, Sabat P (2012) Geographic variation in the association between exploratory behavior and physiology in rufous-collared sparrows. Physiol Biochem Zool 160:117–124
Marone L, Lopez de Casenave J, Milesi FA, Cueto VR (2008) Can seed-eating birds exert top-down effects on the vegetation of the Monte desert? Oikos 117:611–619
Martínez del Rio C (1990) Dietary, phylogenetic, and ecological correlates of intestinal sucrase and maltase activity in birds. Physiol Zool 63:987–1011
McClelland GB (2004) Fat to the fire: the regulation of lipid oxidation with exercise and environmental stress. Comp Biochem Physiol B 139:443–460
McCue MD, Amitai O, Khozin-Goldberg I, McWilliams SR, Pinshow B (2009) Effect of dietary fatty acid composition on fatty acid profiles of polar and neutral lipid tissue fractions in zebra finches, *Taeniopygia guttata*. Comp Biochem Physiol A 154:165–172
McCue MD, McWilliams SR, Pinshow B (2011) Ontogeny and nutritional status influence oxidative kinetics of exogenous nutrients and whole-animal bioenergetics in zebra finches, *Taeniopygia guttata*. Physiol Biochem Zool 84:32–42
McFarlan JT, Bonen A, Guglielmo CG (2009) Seasonal upregulation of fatty acid transporters in flight muscles of migratory white-throated sparrows (*Zonotrichia albicollis*). J Exp Biol 212:2934–2940
McWilliams SR, Kearney S, Karasov WH (2002) Dietary preferences of warblers for specific fatty acids in relation to nutritional requirements and digestive capabilities. J Avian Biol 33:167–174
McWilliams SR, Guglielmo CG, Pierce BJ, Klaassen M (2004) Flying, fasting, and feeding in birds during migration: a physiological ecology perspective. J Avian Biol 35:377–393
Pierce BJ, McWilliams SR, O'Connor TP, Place AR, Guglielmo CG (2004) Diet preferences for specific fatty acids and their effect on composition of fat reserves in migratory red-eyed vireos (*Vireo olivaceous*). Comp Biochem Physiol A 138:503–514
Ramirez-Otarola N, Narváez C, Sabat P (2011) Membrane-bound intestinal enzymes of passerine birds: dietary and phylogenetic correlates. J Comp Physiol B. doi:10.1007/s00360-011-0557-3
Ríos JM, Mangione AM (2010) Respuesta disuasiva del granívoro *Zonotrichia capensis* (Passeriformes: Emberizidae) frente a fenoles comunes en las semillas. Ecol Aust 20:215–221
Ríos JM, Mangione AM, Marone L (2012a) Effects of nutritional and anti-nutritional properties of seeds on the feeding ecology of seed-eating birds of the Monte desert, Argentina. Condor 114:44–55
Ríos JM, Mangione AM, Marone L (2012b) Tolerance to dietary phenolics and diet breadth in three seed-eating birds: implications for graminivory. J Exp Zool A 317:425–433
Sabat P, Novoa FF, Bozinovic F, Martínez del Rio C (1998) Dietary flexibility and intestinal plasticity in birds: a field and laboratory study. Physiol Zool 71:226–236
Sabat P, Ramirez-Otarola N, Bozinovic F, Martínez del Rio C (2013) The isotopic composition and insect content of diet predict tissue isotopic values in a South American passerine assemblage. J Comp Physiol B 183:419–430
Schaefer HM, Schmidt V, Bairlein F (2003) Discrimination abilities for nutrients: which difference matters for choosy birds and why? Anim Behav 65:531–541
Stiles EW (1993) The influence of pulp lipids on fruit preference by birds. Vegetatio 108:227–235
Thompson HM (1999) Esterases as markers of exposure to organophosphates and carbamates. Ecotoxicol 8:369–384

Valera F, Wagner RH, Romero M, Gutiérrez JE, Rey P (2005) Dietary specialization on high protein seeds by adult and nestling serins. Condor 107:29–40

Van Lith H, Meijer GW, van der Wouw MJA, Den Bieman M, Van Tintelen G, Van Zutphen LFM, Beynen AC (1992) Influence of amount of dietary fat and protein on esterase-1 (ES-1) activities of plasma and small intestine in rats. Br J Nutr 67:379–390

Wheelock C, Eder K, Werner I et al (2005) Individual variability in esterase activity and CYP1A levels in Chinook salmon (*Oncorhynchus tshawytscha*) exposed to esfenvalerate and chlorpyrifos. Aquat Toxicol 74:172–192

Zar JH (1996) Biostatistical analysis. Prentice Hall, Upper Saddle River

Zurovchak JG (1997) Nutritional role of high-lipid fruits in the diet of migrant thrushes. Ph.D. Dissertation, Rutgers University