

1 **Title:** Aminopeptidase activity is related to the amino acids composition of the food in
2 passerine birds

3 Cintia Garro^{1,2‡}, Antonio Brun^{4‡}, Enrique Caviedes-Vidal^{1,2,3}.

4 ¹*Laboratorio de Biología Integrativa, Instituto Multidisciplinario de Investigaciones*
5 *Biológicas de San Luis, Consejo de Investigaciones Científicas y Técnicas, San*
6 *Luis, 5700 San Luis, Argentina.*

7 ²*Laboratorio de Biología “Profesor E. Caviedes Codelia”, Universidad Nacional de San*
8 *Luis, San Luis, 5700 San Luis, Argentina.*

9 ³*Departamento de Bioquímica y Ciencias Biológicas, Universidad Nacional de San Luis, San*
10 *Luis, 5700 San Luis, Argentina.*

11 ⁴*Department of Forest and Wildlife Ecology, University of Wisconsin, Madison, Wisconsin*
12 *53706*

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14 ‡ These authors share first authorship on this work.

15 * Corresponding author:

16 Enrique Caviedes-Vidal

17 Laboratorio de Biología Integrativa

18 Instituto Multidisciplinario de Investigaciones Biológicas de San Luis (IMIBIO-SL)

19

20 CONICET – Universidad Nacional de San Luis

21 Chacabuco 917. San Luis 5700, Argentina

22 Ph: +54 (266) 4520300 ext. 6611

23 e-mail: enrique.caviedes@gmail.com

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31 **ABSTRACT**

32 **Background.** Passerine birds exploit different kinds of feeding habits and they have to
33 face seasonal changes in food availability. Therefore, the composition of the principal nutrient
34 in their food differs from the usual. In consequence the digestive function – enzyme
35 hydrolysis and absorption – have to adapt to these nutrients. These changes in digestive
36 physiology could respond to the adaptive modulation hypothesis which postulated that the
37 activities of digestive enzymes should match the levels of their substrates in their diet so
38 energy is not wasted on enzymes that are no need. Thus, we decide to measure intestinal
39 enzymes activities of two species of passerine birds that differ in natural diet. Overall we
40 hypothesized that species with different feeding habits present enzyme activity according to
41 the mainly component of the diet (e.g., carbohydrates, proteins). Our prediction is that the
42 individuals will present enzyme activity proportionally to the primary components of the
43 diets.

44 **Methods.** We select for study: red ovenbirds (*Furnarius rufus*), which are strict
45 insectivores and zebra finches (*Taeniopygia guttata*), which are specialist granivores. We
46 complete the analysis with publish data for house sparrows (*Passer domesticus*) feed on high
47 starch from the literature. To examine intestinal enzyme activities, we measured the activity
48 of two disaccharidases (sucrase-isomaltase and maltase-glucoamilase) and one dipeptidase
49 (aminopeptidase-N).

50 **Results.** The average intestinal activity of sucrase shows that the omnivorous *P.*
51 *domesticus* presents almost 4 times more activity than the granivorous *T. guttata* and more
52 than 11 times than the insectivorous *F. rufus*. This difference is also reflected in the total
53 sucrase hydrolytic capacity where *P. domesticus* has roughly 10 times more than the other two
54 birds. Surprisingly in *F. rufus* we found maltase and aminopeptidase activity while sucrase
55 activity was close to zero. In the case of the average activity of maltase for the omnivorous *P.*
56 *domesticus* is approximately 40 % more than the granivorous *T. guttata* and more than 5 times
57 than the insectivorous *F. rufus*. Although the total maltase hydrolytic capacity of *P.*
58 *domesticus* is 5 times more than *T. guttata* and *F. rufus*. The average of aminopeptidase-N
59 activity for *F. rufus* and *T. guttata* almost doubled the *P. domesticus* ones. Also *F. rufus*
60 roughly doubles the other two birds in total aminopeptidase hydrolytic capacity.

61 **Discussion.** This study has shown that exist a relationship between the levels of amino
62 acids in the diet and the total aminopeptidase capacity, but in the case of carbohydrates this
63 relationship is not evident.
64

65 **INTRODUCTION**

66 Birds possess the capacity to exploit a broad diversity of resources and they have to
67 face seasonal changes in food availability. The switch to available food modifies the
68 predominant nutrients in their food intake. Consequently the consumption of food with

69 different nutrients requires that the optimization of the digestive function – enzyme hydrolysis
70 and absorption – is adapted to these nutrients (Karasov & Martínez del Río 2007). In several
71 vertebrates was reported a modulation of disaccharidases activity correlated with substrates in
72 their diets (Biviano et al. 1993; Harpaz & Uni 1999; Hernández & Martínez del Río 1992;
73 Sabat et al. 1995). These changes in digestive physiology could respond to the adaptive
74 modulation hypothesis which postulated that activities of digestive enzymes should match the
75 levels of their substrates in their diet so energy is not wasted on enzymes that are no need
76 (Karasov 1992; Karasov & Diamond 1988). In birds, at the interspecific level it has been
77 observed that the hydrolytic capacity of the individuals is related with the level of the
78 substrate in the feeding habits (Kohl et al. 2011; Ramírez-Otarola & Sabat 2011). In
79 consequence, we decide to measure intestinal enzymes activities of two species of passerine
80 birds that differ in natural diet.

81 To examine intestinal enzyme activities, we measured the activity of two
82 disaccharidases and one dipeptidase. Digestion of carbohydrates implicates glucosidase
83 enzymes located on the brush border of the small intestine; between them we can find
84 sucrase-isomaltase (EC 3.2.1.10) and maltase-glucoamilase (EC 3.2.1.3) (Hunziker et al.
85 1986; Palmer 1971). Maltase-glucoamilase hydrolyzes maltose in two molecules of glucose,
86 while sucrase-isomaltase hydrolyzes sucrose in one molecule of fructose and another of
87 glucose. In reference of protein digestion we have chosen to measure aminopeptidase-N (E.C.
88 3.4.11.2) that account for almost all peptidase activity in the brush border membrane (Maroux
89 et al. 1973). This enzyme cleaves oligopeptides to produce dipeptides and amino acids
90 (Sjostrom et al. 1978).

91 We select for study: red ovenbirds (REDO; *Furnarius rufus*), which are strict
92 insectivores (Fraga 1980b) and zebra finches (ZEBF; *Taeniopygia guttata*), which are
93 specialist granivores (Zann 1996a). We complete the analysis with publish data for the house

94 sparrows (HOSP; *Passer domesticus*) feed on high starch from the literature (Caviedes-Vidal
95 et al. 2000). We choose the high starch because represent most likely the natural diet
96 (Anderson 2006).

97 Overall we hypothesized that species with different feeding habits present enzyme
98 activity according to the mainly component of the diet (e.g., carbohydrates, proteins). Thus
99 we predicted that the individuals will present enzyme activity proportionally to the primary
100 components of the diets.

101 MATERIALS AND METHODS

102 Animals

103 Zebra finches (ZEBF) were purchased in San Luis, Argentina and house sparrows
104 (HOSP) and red ovenbirds (REDO) were captured on the campus of Universidad Nacional de
105 San Luis (UNSL), San Luis. HOSP and ZEBF were housed in cages indoors under constant
106 environmental conditions ($25 \pm 1^\circ\text{C}$, relative humidity of $50 \pm 10\%$) on a photoperiod of
107 14:10 (L:D) with water and food ad libitum (alpist, millet, vitamins and minerals). REDO
108 were used on the same day of capture in order not to alter their eating habits. All animal
109 procedures adhered to institutional animal use regulations and approved animal use protocols
110 by the Animal Care and Use Committee of the UNSL, protocol number B212/15. Captured
111 animals were approved by the Environmental Office of the state of San Luis, resolution
112 number 75-PBD-2015.

113 Intestinal Enzyme Assays

114 Disaccharidases activities, sucrase-isomaltase and maltase-glucoamilase were assayed
115 using the colorimetric method developed by Dahlqvist (Dahlqvist 1984) and modified by
116 Martínez del Río (Martínez del Río 1990). Briefly, tissues were thawed at 4°C and
117 homogenized for 30 s using a manual homogenizer (Fisher Scientific™ Laboratory

118 Homogenizer, Model 125) in mannitol buffer (350 mM for birds) in 1 mM Hepes-KOH, pH
119 7.0. Aliquots of 40 μ L of diluted intestinal homogenates were incubated with 40 μ L of 56
120 mM sucrose or 56 mM maltose in 0.1 M maleate/NaOH buffer, pH 6.5, at 40 °C for 20 min.
121 After 20 min of incubation the reaction was stopped by adding 1 mL of enzymatic glucose
122 assay (Glucosa Liquid plus reagent-GT Laboratorios S.R.L.). Sample solutions were allowed
123 to stand for 5 min at room temperature and the absorbance was measured at 505 nm and
124 activity was determined using a glucose standard curve.

125 Aminopeptidase-N activity was assayed using L-alanine-p-nitroanilide as a substrate
126 (Maroux et al. 1973). Aliquots of 10 μ L of the intestinal homogenate were added to 1 mL
127 assay solution (2.0 mM L-alanine-p-nitroanilide in 0.2 M phosphate buffer
128 ($\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$, pH 7). The reaction was incubated for 20 min at 40 °C and then stopped
129 with 3 mL of chilled 2 M acetic acid. Absorbance was measured at 384 nm, and activity was
130 determined using a p-nitroanilide standard curve.

131 On the basis of absorbance measurements and glucose and p-nitroanilide standard
132 curve we calculated activities of each intestinal section normalized to the wet mass of the
133 section. Activities of intestinal enzymes were expressed in micromoles per minute per gram
134 of wet tissue.

135 We calculated the summed hydrolysis activity of the entire small intestine, an index of
136 the total hydrolysis capacity, by multiplying activity per gram of wet tissue in each region by
137 its respective mass, and summed over the three regions.

138 **Statistics**

139 Statistical analyses were conducted with SPSS and results are expressed as means \pm 1
140 s.e.m. Total hydrolytic capacity were determined using ANOVA with Tukey's post hoc tests.
141 As for enzymatic activity we use a repeated measures ANOVA with a between subject factor.

142 The F-values of these and other analyses of variance are presented in the text with the relevant
143 degrees of freedom as subscripts. Significance was determined at $P < 0.05$.

144 RESULTS

145 The intestinal sucrase activity significantly varies among species and intestinal
146 segments ($F_{2,13} = 30.54$, $P < 0.001$, $F_{2,26} = 5.07$, $P = 0.014$, respectively; Fig.1 A). *P.*
147 *domesticus* presents significantly higher levels of enzyme activity all along the intestine
148 compare with the other two species ($P < 0.001$, for the three segments). Also only for this
149 specie the activity decays towards the distal segment ($P < 0.05$). In addition no differences
150 between *T. guttata* and *F. rufus* were detected ($P = 0.604$) despite the fact that *T. guttata* more
151 than doubles the activity for proximal and medial segments (See discussion).

152 The pattern of maltase activity also varies among species and intestinal segments ($F_{2,13}$
153 $= 8.51$, $P < 0.001$, $F_{2,26} = 13.54$, $P < 0.001$, respectively; Fig.1 B). *P. domesticus* and *T.*
154 *guttata* presents no differences between all segments ($P > 0.05$, for the three segments) and
155 also significantly higher levels of enzyme activity compare with the *F. rufus* ($P < 0.05$, for the
156 three segments for both species). Also only in *P. domesticus* the activity decays towards the
157 distal segment ($P < 0.05$).

158 The activity of aminopeptidase-N differs from the other two enzymes, the model
159 shows no differences between segments but significantly differences among species ($F_{2,26} =$
160 1.163 , $P = 0.301$, $F_{2,13} = 4.897$, $P = 0.026$, respectively; Fig.1 C). The activity along the
161 intestine for *T. guttata* decays on the distal segment ($P < 0.05$), the opposite happens to *F.*
162 *rufus* where the activity rises on the distal segment ($P < 0.05$) and for *P. domesticus* the activity
163 stays without changes ($P > 0.05$). For the proximal and medial segment *T. guttata* shows
164 higher activity than the *P. domesticus* ($P < 0.01$ for both) but not different than *F. rufus*
165 (proximal $P = 0.108$, medial $P = 0.152$) and at the same time no differences were detected

166 between *P. domesticus* and *F. rufus* (proximal $P = 0.319$, medial $P = 0.553$). For the distal
167 segment *F. rufus* present higher activity than *P. domesticus* ($P > 0.05$) but no different from *T.*
168 *guttata* ($P = 0.106$) and no differences was detected between *T. guttata* and *P. domesticus* (P
169 < 0.05).

170 The total hydrolytic capacity for sucrase significantly varies among species ($P <$
171 0.001). *P. domesticus* presents more than 9 times higher capacity than *T. guttata* and *F. rufus*
172 ($P < 0.001$ for both species) and no variation was detected between this last two species ($P =$
173 0.994 ; Fig. 2 A). For maltase the pattern is the same ($P < 0.001$). *P. domesticus* presents more
174 than 4 times more capacity than *T. guttata* and *F. rufus* ($P < 0.001$ for both species) and no
175 variation was detected between this last two species ($P = 0.981$; Fig 2 B). As for
176 aminopeptidase-N capacity the pattern is different than the two others enzymes. *F. rufus*
177 presents more than 2 times more capacity than *T. guttata* and *P. domesticus* ($P < 0.05$ for both
178 species) and no variation was detected between this last two species ($P = 0.632$; Fig.2 C).

179 DISCUSSION

180 The average intestinal activity of sucrase shows that the omnivorous *P. domesticus*
181 presents almost 4 times more activity than the granivorous *T. guttata* and more than 11 times
182 than the insectivorous *F. rufus*. This difference is also reflected in the total sucrase hydrolytic
183 capacity where *P. domesticus* has roughly 10 times more than the other two birds.
184 Surprisingly in *F. rufus* we found maltase and aminopeptidase activity while sucrase activity
185 was close to zero, so its biological significance is unclear. In our first enzyme assay we did
186 not find activity of sucrase in the samples of *F. rufus*. In consequence we examine the sucrase
187 activity from new homogenates with different dilutions and we did not obtain activity. In
188 order to evaluate if the reagents were in good conditions we determine sucrase and maltase
189 activity in samples of rats, house sparrows and zebra finchs. Also in this assay we added a

190 standard solution from glucose standard curve as a positive control. For these enzyme assays
191 we found activity of both disaccharidases in the three samples. Finally, we decided to assay
192 the three enzymes (sucrase, maltase, aminopeptidase) in samples of *F. rufus* and we obtained,
193 again, values close to zero of sucrase activity in the three intestinal segments. These results
194 are consistent with those of other studies which found little or no activity of sucrase in
195 different species of passerine birds. Several studies focus on omnivorous and insectivorous
196 passerines birds have described very low or total absence of activity of sucrase and/or maltase
197 compared with other species of birds as shown in this study (Malcarney H.L. 1994; Martínez
198 del Río 1990; Martínez del Río & Stevens 1989; Ramírez-Otarola et al. 2011; Sabat 2000;
199 Sabat & Gonzalez 2003) (see Table 2). This phenomenon has been related to feeding
200 preferences or to evolutionary process. In the case of feeding preferences, it refers that diet is
201 determined by the digestive available machinery. In this context, our results may be
202 explained that insectivorous diet of *F. rufus* is a consequence of the low activity of sucrase.
203 However, Martínez del Río et al. (Martínez del Río & Stevens 1989) showed that when birds
204 that lack of sucrase activity were fed with sucrose solutions developed signs of discomfort
205 and osmotic diarrhea. Thus it can be suggested that the association between the absence of
206 sucrase activity and diets is not merely a preference matter. It could be hypothesized that the
207 illness observed in birds that lack of sucrase activity in consequence of sucrose feeding is an
208 important factor to determinate their eating habits. Nonetheless more research on this topic
209 needs to be undertaken. Another possible explanation for this might be that in birds take place
210 evolutionary events that cause the lack of sucrase activity. For example, in
211 hummingbirds occurred a transformation of a taste receptor function after their divergence
212 from an insectivore ancestor. This evolutionary adaptation contributed to the acquisition of
213 nectar-feeding behavior (Baldwin et al. 2014). In passerine birds, the lack of sucrase activity
214 has been reported for species belonging to the Sturnidae-Muscicapidae lineage (Martínez del

215 Río 1990). Nevertheless, the data available add 8 families of *Passeriformes* birds that have
216 zero or little activity of sucrase with different feeding habits (Table 2.). It can be therefore
217 assumed that the lack of sucrase activity is not only observed in insectivorous passerine birds,
218 but also affect species of birds that do not belong to the Sturnidae-Muscicapidae lineage. A
219 further comparative work will reveal the relationship between feeding habits and evolutionary
220 process that is governing the sucrase activity among birds.

221 The average activity of maltase for the omnivorous *P. domesticus* is approximately 40
222 % more than the granivorous *T. guttata* and more than 5 times than the insectivorous *F. rufus*.
223 This shrink in the difference of the activity between *P. domesticus* and *T. guttata* (~400% to
224 ~40%) could be associated to diet. Millet and alpist contain considerable amount of starch
225 therefore *T. guttata* needs maltase to final breakdown of the starch. Although the total maltase
226 hydrolytic capacity of *P. domesticus* is 5 times more than *T. guttata* and *F. rufus*. Also even
227 though that *T. guttata* presents 3 times more activity of maltase than *F. rufus*, the total
228 hydrolytic capacity is the same mainly due the differences in intestinal mass (see Table 1).
229 The average of aminopeptidase-N activity for *F. rufus* and *T. guttata* almost doubled the *P.*
230 *domesticus* ones. Also *F. rufus* roughly doubles the other two birds in total aminopeptidase
231 hydrolytic capacity, this is mainly due to differences in activity and differences in intestinal
232 mass (see Table 1).

233 As for the activity of three enzymes throughout the intestine we observed a tendency
234 to diminish towards distal portion in birds that present disaccharidases activity (*P. domesticus*,
235 *T. guttata*). While aminopeptidase activity does not present a uniform pattern among the
236 species of birds studied. In the case of *F. rufus* aminopeptidase activity increases towards the
237 distal portion and an opposite tendency is observed for the other two species. Aminopeptidase
238 activity does not exhibit a predictable pattern throughout the intestine, as if it has been found
239 for disaccharidases. These patterns may be related to feeding habits. Because, in insectivorous

240 birds or protein-rich diets they exhibit an increase aminopeptidase activity towards the distal
241 portion, whereas frugivorous, granivorous or omnivorous species show a pattern that
242 decreases towards the distal portion or remains constant throughout the entire intestine. The
243 present findings seem to be consistent with other research which found several pattern of
244 aminopeptidase activity among birds (Afik et al. 1995; Meynard et al. 1999; Witmer &
245 Martínez del Río 2001).

246 These findings suggest that exist a trend between L-proline and total hydrolytic
247 capacity. The reason for this is not clear but it may have something to do with the adaptive
248 modulation hypothesis which postulated that enzyme activity is related to the diet (Karasov
249 1992; Karasov & Diamond 1988). Moreover, there are several studies that support that the
250 intestinal enzyme activity responds to changes in the diet (Afik et al. 1995; Brzęk et al. 2010;
251 Caviedes-Vidal et al. 2000).

252 CONCLUSIONS

253 In conclusion, this study has shown that exist a relationship between the levels of
254 amino acids in the diet and the total aminopeptidase capacity, but in the case of carbohydrates
255 this relationship is not evident.

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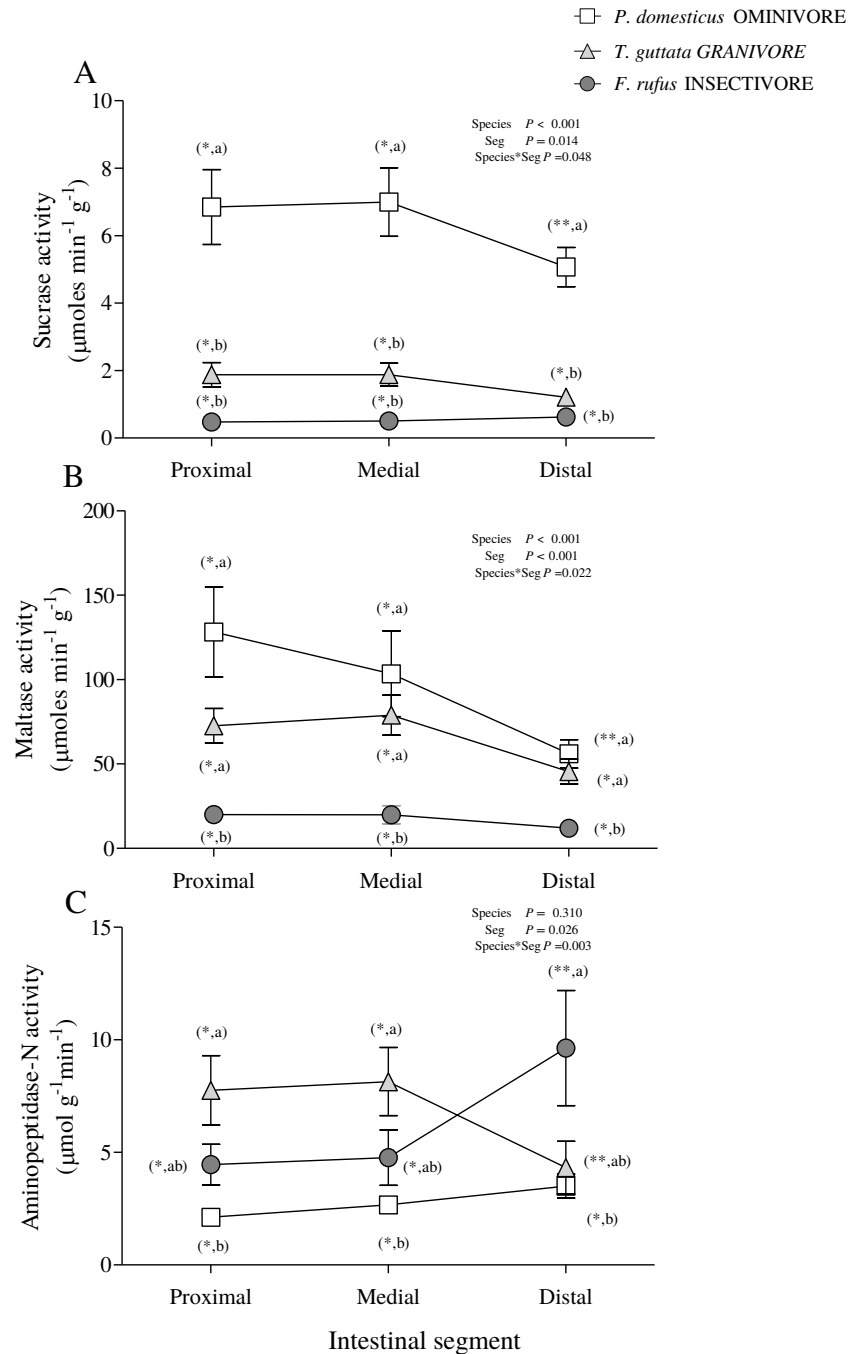
359

360 **Tables and Figures**361 **Table 1.** Animals attribute.

362 Letters P = proximal, M = medial, D = distal, intestinal segments.

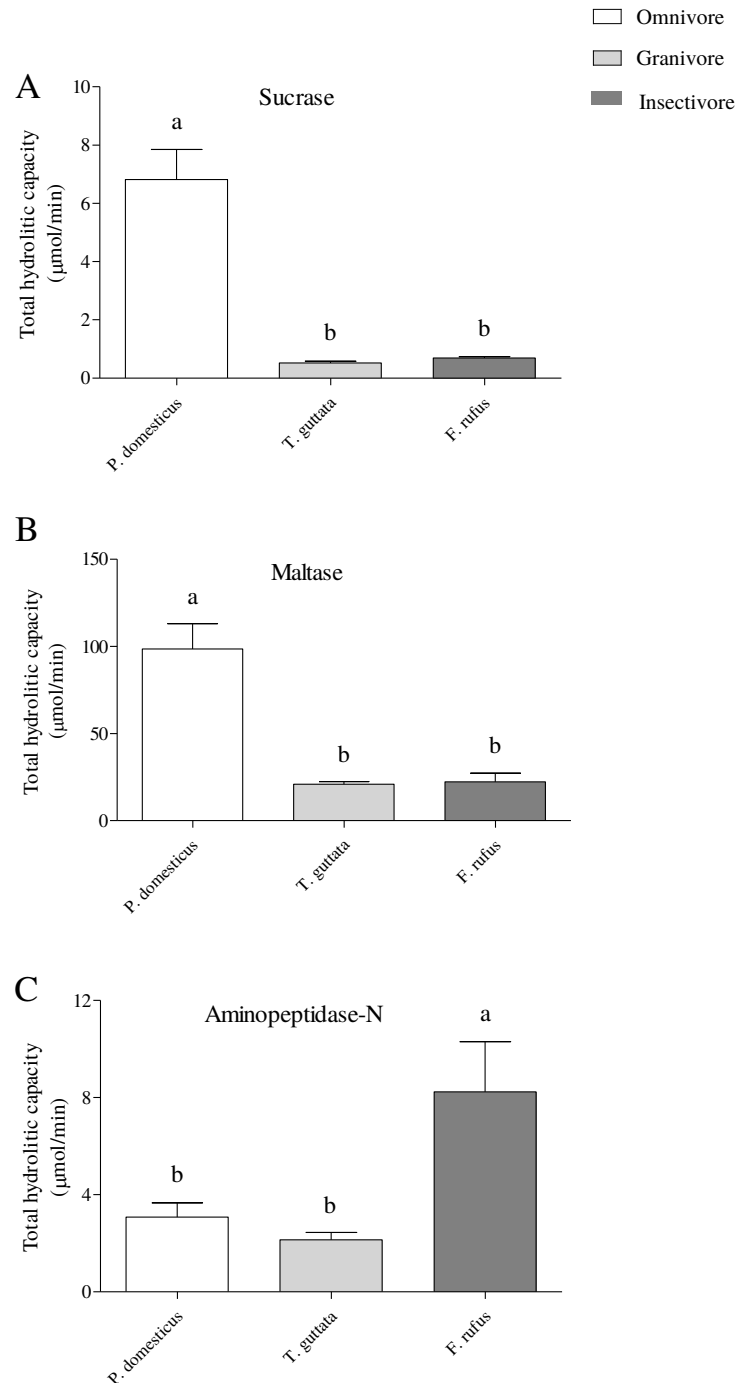
Enzyme Assay Animals	<i>P. domesticus</i>	<i>T. guttata</i>	<i>F. rufus</i>
N (male/female)	4/2	4/1	2/3
Body mass (g)	26.0 ± 0.82	12.9 ± 0.40	46.7 ± 0.82
Small intestinal length (cm)	18.6 ± 0.9	9.7 ± 0.32	18.7 ± 0.55
		P: 0.63 ± 0.02	P: 0.97 ± 0.02
Small intestinal width (cm)	None	M: 0.55 ± 0.03	M: 0.89 ± 0.004
		D: 0.52 ± 0.04	D: 0.82 ± 0.02
Small intestinal mass (g)	1.4 ± 0.28	0.3 ± 0.02	1.2 ± 0.04
Diet	Omnivore	Granivore	Insectivore
	(Anderson 2006)	(Zann 1996b)	(Fraga 1980a)
References	(Caviedes-Vidal 2000)	This study	This study

363 Values are means ± 1 s.e.m.



364

365 **Figure 1.** Intestinal enzymes activity of (A) sucrase, (B) maltase and (C) aminopeptidase-N
 366 for the three intestinal segments. Sample sizes were as follows: *P.domesticus* $n=5$, *T.guttata* n
 367 $= 4$, *F.rufus* $n=5$ (See table 1). P -values in the figures are for comparisons of species or
 368 intestinal regions. When species within an intestinal segment present the same letter they do
 369 not differ significantly. When segments within a species share an equal number of (*) they do
 370 not differ significantly. Data are means \pm s.e.m.



371

372 **Figure 2.** Total hydrolytic capacity for (A) sucrase, (B) maltase and (C) aminopeptidase-N
373 for the three species *P. domesticus* $n=5$, *T. guttata* $n=4$, *F. rufus* $n=5$ (See table 1). Data are
374 means \pm s.e.m.; bars that share letters indicate no statistically significant difference ($P > 0.05$).

375 **Table 2.** Summary of sucrase ($< 1 \text{ mol min}^{-1}$) and maltase activity in passerine birds.

Order	Family	Genus	Scientific name	Diet	Sucrase Activity		Maltase Activity		Ref.
					Mmin ⁻¹ gprot ⁻¹	Mmin ⁻¹	Mmin ⁻¹ gprot ⁻¹	Mmin ⁻¹	
Passeriformes	Sturnidae	<i>Sturnus</i>	<i>S. vulgaris</i>	O	0		276±23	-	(Martínez del Río & Stevens 1989)
Passeriformes	Sturnidae	<i>Onychognathus</i>	<i>O. morio</i>	F	0		-	0.24±0.08	(Bizaaré et al. 2012)
Passeriformes	Tyrannidae	<i>Empidonax</i>	<i>E. difficilis</i>	I	-	0.33±0.12	-	7.15±2.51	(Martínez del Río 1990)
Passeriformes	Tyrannidae	<i>Anairetes</i>	<i>A. parulus</i>	I	-	0.16±0.04	-	3.56±0.73	(Ramírez-Otarola & Sabat 2011)
Passeriformes	Mimidae	<i>Dumetella</i>	<i>D. carolinensis</i>	O	0.50±0.43	0.04±0.05	102.03±31.03	28.96±14.12	(Malcarney et al. 1994)
Passeriformes	Mimidae	<i>Mimus</i>	<i>M. thenca</i>	O	0		-	116.55±26.21	(Gatica et al. 2006; Ramírez-Otarola & Sabat 2011)
Passeriformes	Emberizidae	<i>Zonotrichia</i>	<i>Z. capensis</i>	S/I	-	0.99±0.18	-	39.81±10.09	(Ramírez-Otarola & Sabat 2011)
Passeriformes	Troglodytidae	<i>Troglodytes</i>	<i>T. aedon</i>	I	-	0.10±0.05	-	8.06±2.31	(Ramírez-Otarola & Sabat 2011)

Passeriformes	Estrildidae	<i>Taeniopygia</i>	<i>T. guttata</i>	S	-	0.52±0.06	-	20.90±1.59	This study
Passeriformes	Furnariidae	<i>Cinclodes</i>	<i>C. nigrofumosus</i>	C	0		4.78±0.60	-	(Sabat 2000)
Passeriformes	Furnariidae	<i>Cinclodes</i>	<i>C. patagonicus</i>	C/I	0		4.65±2.34	-	(Sabat 2000)
Passeriformes	Furnariidae	<i>Cinclodes</i>	<i>C. oustaleti</i>	C/I	0			-	(Sabat & Gonzalez 2003)
Passeriformes	Furnariidae	<i>Asthenes</i>	<i>A. humicola</i>	I	-	0.38±0.07	-	27.90±7.95	(Ramírez-Otarola et al. 2011)
Passeriformes	Furnariidae	<i>Leptasthenura</i>	<i>L. aegithaloides</i>	I	-	0.21±0.06	-	13.23±0.82	(Ramírez-Otarola et al. 2011)
Passeriformes	Furnariidae	<i>Furnarius</i>	<i>F. rufus</i>	I	-	0.69±0.04	-	22.29±4.92	This study
Passeriformes	Pycnonotidae	<i>Pycnonotus</i>	<i>P. tricolor</i>	F	-	0.26±0.03	-	0.13±0.16	(Bizaaré et al. 2012)
Passeriformes	Turdidae	<i>Catharus</i>	<i>C. aurantirostris</i>	I/F	-	<0.05	-	5.47±1.12	(Martínez del Río 1990)
Passeriformes	Turdidae	<i>Catharus</i>	<i>C. minimus</i>	I/F	-	0.2	-	23.6	(Witmer & Martínez del Río 2001)
Passeriformes	Turdidae	<i>Catharus</i>	<i>C. guttatus</i>	I/F	-	0.2	-	26.9	(Witmer & Martínez del Río 2001)

									Río 2001)
Passeriformes	Turdidae	<i>Catharus</i>	<i>C. ustulatus</i>	I/F	-	0.05	-	31.1	(Witmer & Martínez del Río 2001)
Passeriformes	Turdidae	<i>Hylocichla</i>	<i>H. mustelina</i>	I/F	-	0.3±0.04	-	29.9±4.6	(Witmer & Martínez del Río 2001)
Passeriformes	Turdidae	<i>Turdus</i>	<i>T. migratorius</i>	I/F	-	0.4±0.2	-	60.0±15.2	(Witmer & Martínez del Río 2001)
Passeriformes	Turdidae	<i>Turdus</i>	<i>T. rufopalliatus</i>	I	-	<0.05	-	9.45±2.15	(Martínez del Río 1990)
Passeriformes	Turdidae	<i>Turdus</i>	<i>T. falcklandii</i>	O		0	-	228.61±110.1 9	(Gatica et al. 2006; Ramírez-Otarola & Sabat 2011)
Passeriformes	Zosteropidae	<i>Zosterops</i>	<i>Z. virens</i>	F	-	0.34±0.15	-	0.17±0.04	(Bizaaré et al. 2012)

376 Values are means ± s.e.m

377 I= Insects, F= Fruits, S=Seeds, C= crustaceans and marine invertebrates, O= Omnivorous.