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DIGESTIVE ENZYMES OF A SMALL AVIAN HERBIVORE, THE RUFOUS-TAILED PLANTCUTTER[1]

CHRISTINE MEYNARD, M. VICTORIA LÓPEZ-CALLEJA AND FRANCISCO BOZINOVIC[2]
Departamento de Ecología, Facultad de Ciencias Biológicas, P. Universidad Católica de Chile, Casilla 114-D, Santiago, Chile, e-mail: fbozinov@genes.bio.puc.cl

PABLO SABAT
Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile

Abstract. We studied the activity of three intestinal membrane-bound digestive enzymes in the Rufous-tailed Plantcutter (*Phytotoma rara*), one of the smallest species of avian herbivores. We selected the disaccharidases (sucrase and maltase) as indicators of a bird's capability to assimilate carbohydrate, and the oligopeptidase (aminopeptidase-N) as an indicator of a bird's ability to digest protein. Small intestine length was 44.6% shorter than expected based on body mass. Sucrase, maltase, and aminopeptidase-N activities did not differ along the first 80% of the proximal portion of the intestine. Activities of sucrase, maltase, and aminopeptidase-N are probably matching the higher carbohydrate and lower protein concentrations of *P. rara*'s herbivorous diet. The higher and nearly constant enzyme hydrolysis observed along the intestine axis may allow the Rufous-tailed Plantcutter to compensate for and to exploit the abundant and highly diluted plant material, without sacrificing digestive efficiency and nutritional balance.

Key words: digestion, disaccharidases, herbivory, oligopeptidase, Phytotoma rara, *Rufous-tailed Plantcutter.*

The Rufous-tailed Plantcutter *Phytotoma rara* (Phytotomidae) is one of the smallest species of avian herbivores (ca. 40 g). The three members of the South American Phytotomidae are the smallest passerine herbivores (Ziswiler and Farner 1972). Of these three, the Rufous-tailed Plantcutter has the southern-most distribution, from Vallenar (28°34′S, 70°45′W) to Chiloé (42°25′S, 73°46′W), Chile. *Phytotoma rara* is found from sea level to 2,000 m above sea level (Goodall et al. 1956), and inhabits forests and scrublands, as well as crop fields and orchards (Araya and Millie 1986).

Based on field and laboratory data, López-Calleja and Bozinovic (1999) reported that *P. rara* preferred leaves to fruits and insects. In addition, when *P. rara* fed on plant material, these authors observed a higher food intake, a shorter digesta retention time, and a lower apparent digestibility in comparison to a high-quality diet. Morphohistological descriptions of the digestive tract of *P. rara* (S. Girod, pers. comm.) revealed that it is short and similar to that observed in fruit-eating birds (Ricklefs 1996). It has a narrow esophagus containing abundant mucous glands and many folds. The inner surface of the muscular stomach is rough and the intestinal ceca are small. In addition, the Rufous-tailed Plantcutter's bill is serrated (Ziswiler and Farner 1972). López-Calleja and Bozinovic (1999) postulated that chewing might increase overall digestibility through physical breakdown of cell walls, thus making available the highly digestible cell contents. These strategies may allow *P. rara* to maintain body mass and energy balance when feeding on highly energy-diluted plant material. Here we tested for digestive (enzymatic) responses that may allow the exploitation of plants by this species.

Studies of avian intestinal biochemistry revealed that birds can maintain overall extraction efficiency when confronted with poor diets by matching hydrolysis and uptake rates of nutrients with their diet contents (Karasov 1996). Here we studied the activity of three intestinal membrane-bound digestive enzymes in *P. rara*: the two disaccharidases sucrase and maltase, and the oligopeptidase aminopeptidase-N (Vonk and Western 1984, Sabat et al. 1998). We selected the disaccharidases as indicators of a bird's capability to assimilate carbohydrates and the oligopeptidase as an indicator of a bird's ability to digest protein.

In many species of birds, digestive enzymes decrease distally along the small intestine (Martínez del Río 1990, Martínez del Río et al. 1995). According to the optimization design hypothesis, a decrease in the concentration of substrates along the gut axis should be matched by a decrease in the activity of enzymes which will reduce the expensive cost of maintenance of non-utilized membrane-bound protein (Hume 1998). In the case of *P. rara,* a short intestine and retention time should decrease the efficiency of energy/matter extraction as it reduces digesta (substrates) exposure time to digestive processing (Karasov 1996). Nevertheless, and as a compensating mechanism, these birds may have higher and constant enzyme activity (McWilliams et al. 1999) along the intestine to exploit a diluted food resource and to maintain a higher overall extraction efficiency when feeding on plants.

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[2] Corresponding author.

METHODS

Birds (n = 5) were captured with mist nets during fall in Lipimavida, a coastal valley located 130 km west of Curicó, central Chile (72°08′W, 34°50′S), and killed by cervical dislocation. In the field, the small intestine was isolated, washed with ice-cold physiological saline (1% NaCl), its length measured (± 0.1 cm), and stored in liquid nitrogen for further assays (Sabat et al. 1998). Diet of captured animals was mainly composed of plant material (see López-Calleja and Bozinovic 1999). Body mass (bm) of birds averaged (± SD) 45.6 ± 5.1 g. Small intestine length was compared with expected length based on bm (g). The following allometric equation for passerine birds was used (Ricklefs 1996): log(small intestine length) = 0.68 + 0.34log(bm). Five segments of the small intestine were obtained for enzyme analysis.

To estimate disaccharidase activity, segments of the small intestine were thawed and then homogenized (30 sec in an Ultra Turrax T25 homogenizer at maximum setting) in different volumes of 0.9% NaCl. Maltase and sucrase activities were measured following Martínez del Río (1990). After incubating the solution for 5 min at 40°C, the enzymatic reaction was started by adding 100 μL of intestinal homogenate to the 100 μL solution containing the specific sugars. After 10 min of incubation, the reaction was arrested by adding 3 ml of stop-develop reagent (Trinder 315-500, Sigma Chemical Company, St. Louis, Missouri) dissolved in 500 ml of 0.5 M buffer phosphate, pH 7.0. The arrested reactions then were allowed to stand at 40°C for 18 min, and the absorbance measured at 505 nm (Sequoia Turner 390 spectrophotometer). Sugars were prepared in 0.1 M maleate-hydroxide buffer, pH 7.0, and enzyme assays were conducted at a fixed substrate concentration of 28 mM. Aminopeptidase-N assays were done using L-alanine-p-nitroanilide as a substrate. In short, 50 μL of homogenate diluted with mannitol/KOH buffer were mixed with 1 mL of assay mix (2.04 mmol L^{-1} NaH_2PO_4/Na_2HPO_4, pH 7) at 40°C. The reaction was incubated at 40°C and arrested after 10 min with 3 mL of ice-cold 2 N acetic acid, and absorbance was measured at 384 nm. Enzymatic methods used in this study are the same as those previously used by us and other authors.

Statistical analyses were performed using STATISTICA (1997) statistical package for Windows 95. Data were analyzed by a repeated measures ANOVA and by the *a posteriori* Tukey test. Data fulfilled the assumptions of the ANOVA. Results are reported as mean ± SD.

RESULTS

Small intestine length of *P. rara* was 9.7 ± 0.3 cm, which was 44.6% shorter than expected (17.5 cm) from bm. Small intestine mass was 1.8 ± 0.2 g. Sucrase, maltase, and aminopeptidase-N activities (μmol min^{-1} g-wet tissue) changed along the intestine ($F_{4,16}$ = 7.8, P = 0.001 [sucrase], $F_{4,16}$ = 7.5, P = 0.001 [maltase], and $F_{4,16}$ = 11.5, P < 0.001 [aminopeptidase-N], Fig. 1).

Nevertheless and contrary to previous reports (Martínez del Río 1990, Afik et al. 1995), in *P. rara* disaccharidase activities remained constant along the proximal 80% of the small intestine length. The Tukey test revealed that sucrase and maltase exhibited a significant decrease (P < 0.05) in the last segment (distal position, Fig. 1). In contrast, aminopeptidase-N exhibited a significant increase in activity after segment 4 (Tukey test, P < 0.05). Activities (μmol min^{-1} g-wet tissue) of the two disaccharidases sucrase (x-axis) and maltase (y-axis) were linearly, tightly, and positively correlated, being represented by the equation: y = (1.89 ± 0.59)x − (193.3 ± 72.1); r = 0.88, P < 0.05.

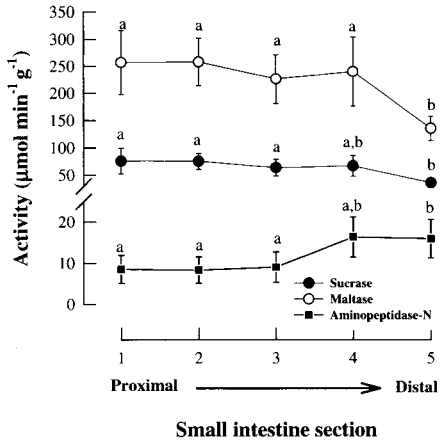

FIGURE 1. Mean ± SD digestive enzyme activity in five segments along the small intestine of *Phytotoma rara* (n = 5). Similar letters indicate nonsignificant differences among segments (*a posteriori* Tukey test) after a repeated measures ANOVA.

DISCUSSION

Vonk and Western (1984) proposed that the activities of digestive enzymes are correlated with the chemical composition of the natural diet of the species. Activity of sucrase, maltase, and aminopeptidase-N observed in *P. rara* is probably matching the higher carbohydrate and the lower protein concentrations of its herbivorous diet. Nevertheless, aminopeptidase-N increased in activity after segment 4. Similar results were reported by McWilliams et al. (1999) in Cedar Waxwings *Bombycilla cedrorum.* The increase in aminopeptidase-N activity may reflect a match with the oligopeptides released after the action of other digestive proteases at the proximal segment of the digestive tract. Perhaps the higher uptake of amino acid occurs in this segment. This hypothesis needs to be tested.

The correlation between sucrase and maltase has been reported previously by Martínez del Río (1990), Biviano et al. (1993), and Sabat et al. (1998). In many bird species, maltase activity is the result of the action of sucrase-isomaltase and maltase-glucoamilase. An increase in sucrase-isomaltase expression is translated into increase in maltase activity. Nevertheless, the lack of significance of the intercept of the regression model (t_3 = 2.68, P = 0.07) suggests that there is no maltase activity independent of sucrase activity in *P. rara.* However, the power of our test was low, so it is premature to accept that this bird has no maltase activity

TABLE 1. Hydrolytic activities (μmol min^{-1}) per unit intestinal mass and food habits in some passerines.

Species	Food habits	Maltase	Sucrase	Aminopeptidase-N	Source
Bombycilla cedrorum	F/I	45	3.6	4.4	McWilliams et al. (1999)
Cinclodes nigrofumosus	C	4.8	0	4.4	P. Sabat (pers. comm.)
Cinclodes patagonicus	C/I	4.6	0	3.4	P. Sabat (pers. comm.)
Zonotrichia capensis	S/I	26–39	2.6–3.8	5.4–9.0	Sabat et al. (1998)
Diuca diuca	S	33–51	2.8–4.6	5.4–9.4	Sabat et al. (1998)
Passer domesticus	S/F/I	72	5.6	2.5–5.1	E. Caviedes-Vidal (pers. comm.)
Dendroica coronata	I/F/S	28–36	3.3–3.5	49–64	Afik et al. (1995)
Phytotoma rara	L/F	233	66.88	11.71	This study

C = crustaceans/marine invertebrates, I = insects, F = fruits, L = leaves, S = seeds.

independent of sucrase activity. Observed total activity of sucrase, maltase, and aminopeptidase-N were 119.4 ± 17.5, 419.9 ± 37.9, and 21.8 ± 8.9 μmol min^{-1}, respectively. Mass-specific sucrase and maltase activities (μmol min^{-1} g-wet tissue) of *P. rara* were extremely higher than that previously reported in other passerines feeding on different food items (see Table 1).

Karasov (1996), through the equation: DE = (tR)/(Vn), where DE = digestive efficiency, t = digesta retention time, R = enzyme hydrolysis and nutrient absorption rates, V = volume of digesta, and n = nutrient concentration, predicted how digestive traits should interact to determine digestive efficiency. This model can also be used to explain our results. Because *P. rara* exhibited a higher food intake of nondigestible and poor foods such as plant tissues, retention time (t) is short (López-Calleja and Bozinovic 1999), and because the digestive tract of this species is shorter than expected, how does *P. rara* maintain DE? Foley and Cork (1992) pointed out that many species of small endotherms compensate for high fibrous diets by a combination of feeding and digestive mechanisms that include high food intake, rapid transit time of fibrous food, changes in digestive tract capacity, and increased nutrient uptake by the small intestine. As mentioned earlier, López-Calleja and Bozinovic (1999) indicated that chewing by *P. rara* might increase overall digestibility through physical breakdown of cell walls, making available cell contents. In addition, we found here that relatively higher and constant R values (enzyme hydrolysis) observed along the intestine axis may allow the Rufous-tailed Plantcutter to compensate for and to exploit the abundant and highly diluted plant material without sacrificing digestive efficiency and body mass balance. That is, if local regulation of enzyme activity is working, then the data suggest that substrate concentrations at the end of the small intestine are low for sugars and high for oligopeptides, and at the whole animal level the bird should be assimilating much of the soluble nutrients. Both chewing and constantly high enzyme hydrolysis in the small intestine are presumably crucial adaptations in the evolution of a herbivorous diet in the small plantcutters.

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BREEDING OF THE GRAY-WINGED TRUMPETER IN FRENCH GUIANA[1]

PIERRE DE MERCEY[2] AND MARC THÉRY[3]
CNRS—UMR 8571, Laboratoire d'Ecologie Générale, Muséum National d'Histoire Naturelle, 4 Avenue du Petit Château, 91800 Brunoy, France, e-mail: thery@mnhn.fr

Abstract. In French Guiana, a territorial group of Gray-winged Trumpeters (*Psophia crepitans*) laid three eggs in a cavity atop a *Voucapoua vouacapoua* snag, 13.5 m up. At least two individuals alternated incubation, as in White-winged Trumpeters *P. leucoptera* in Peru. One egg had a dead embryo, and the two nidifugous chicks disappeared in their first week. Some previous field records of nests or eggs are probably incorrect.

Key words: breeding, clutch size, eggs, Gray-winged Trumpeters, incubation, nesting, Psophia crepitans.

Psophiidae (Gruiformes) include only three species of trumpeters (*Psophia crepitans, P. leucoptera,* and *P. viridis*) that are allopatric in lowland rain forests of the Amazon basin and Guyana shield. The Gray-winged Trumpeter (*P. crepitans*) has the widest distribution: it occurs north of the Amazon River from northeastern Brazil through the Guyanas and southern Venezuela, to southeastern Colombia, eastern Ecuador, and northeastern Peru (Sherman 1996). Trumpeters are chicken-sized ground birds that live in groups of 6–8 individuals on large permanent territories, searching for fallen ripe fruits and arthropods (Sherman 1995b, 1996). A recent five-year study of the White-winged Trumpeter (*P. leucoptera*) showed a cooperatively polyandrous breeding system, in which one dominant female per group copulates with up to three adult males (Sherman 1995a). Otherwise, knowledge of trumpeter breeding is poorly documented. The Gray-winged Trumpeter bred in captivity in two North American zoos (Horning et al. 1988, Male 1989). Early naturalists often cited local reports (Chubb 1916), whereas other information comes from general or regional bird books in which the exact source is usually not indicated (Haverschmidt 1985, Hilty and Brown 1986, Sick 1993). This paper reports observations of Gray-winged Trumpeter breeding in French Guiana during a preliminary ecological study.

METHODS

Field work was conducted at the Nouragues Biological Station, French Guiana (4°03′N, 52°42′W), by the first author (PM) during four months at the beginning of the dry season (1 July to 13 September 1993 and 16 July to 5 September 1994), and by PM and MT during three months in the middle of the rainy season (28 February to 31 May 1995). One habituated group of Gray-winged Trumpeters was followed by sight, and a few observations were made on other groups. Groups contain an average of seven birds. Seven laying dates were calculated from sightings of chicks or juveniles, six coming from the Nouragues field station and one from another site in French Guiana (St. Eugène, on Petit Saut hydroelectric dam, 4°59′N, 53°08′W). Hatching date of the focal group was determined using morphological description of newly hatched chicks by Horning et al. (1988). Approximate laying date was defined using a mean incubation length of 28 days (Horning et al. 1988) and 2 days between successive egg layings (Sherman 1995a) subtracted from estimated date of hatching. The times of nest relief were determined by following the group to the nest area, not by watching the nest. Data on offspring production were gathered for four different groups in 1993, 1994, and 1995, giving the number of juveniles which can reach adulthood for seven group-years (4 groups × 3 years). Values presented are means ± SD.

RESULTS

The seven estimated laying dates, spread over five years (1993 to 1997), were in February and March.

One occupied nest was found on 4 April 1995 by following the group. It was located in *terra firme* or

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[2] Deceased 25 June 1999.

[3] Corresponding author.