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Dietary chemistry and allometry of intestinal disaccharidases in the toad *Bufo spinulosus*

Química dietaria y alometría de discaridasas intestinales en el sapo *Bufo spinulosus*

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ABSTRACT

In amphibians, digestive physiological responses to chemical composition of food may occur through variations in the interplay between the intestinal hydrolysis of dietary substrates and their uptake in response to ontogenetic development and body size. In these animals it is expected that after metamorphosis, the intestinal hydrolysis of dietary substrates involved in energy supply, rather than in growth requirements, should decline with body mass (m_b) due to the higher mass-specific energy requirements of small-sized animals (allometric effect). In the toad *Bufo spinulosus* we test this hypothesis by measuring the activity of the intestinal disaccharidases sucrase, maltase and trehalase as a function of m_b and, the response of intestinal sucrase and trehalase by effect of dietary acclimation. We found that large-sized *B. spinulosus* feeding on the same dietary items as smaller individuals, exhibited a decreased activity of intestinal disaccharidases with m_b, probably due to the higher energetic requirements of smaller individuals, and not to dietary adaptation. Our experiments of dietary acclimation allow us to conclude that adults are unable to modulate their disaccharidase activity in response to dietary chemistry. The lack of lability in these digestive enzymes seems to support the hypothesis of digestive physiological rigidity in adult amphibians and that modulation of intestine physiological activity can cease to operate during ontogenetic development.

Key words: *Bufo spinulosus*, ontogeny, dietary chemistry, allometry, intestinal enzymes.

RESUMEN

En anfibios, las respuestas fisiológicas digestivas a la composición química del alimento pueden ocurrir a través de variaciones en la interacción entre la hidrólisis intestinal de sustratos dietarios y su absorción en respuesta al desarrollo ontogenético y al tamaño corporal. En estos animales se espera que después de la metamorfosis, la hidrólisis intestinal de los sustratos dietarios, envueltos en la obtención de energía más que en los requerimientos de crecimiento, decline con la masa corporal (m_b) debido a los altos requerimeintos energéticos masa-específicos de los individuos pequeños (efecto alométrico). En el sapo *Bufo spinulosus* se sometió a prueba esta hipótesis a través de mediciones de la actividad de las discaridasas intestinales sacarasa, maltasa y trehalasa en función de m_b, y la respuesta de sacarasa y trehalasa intestinal al efecto de aclimatación dietaria. Encontramos que los *B. spinulosus* grandes que se alimentan de los mismos ítemes dietarios que los individuos pequeños, muestran un decremento en la actividad de disacaridasas intestinales con m_b, probablemente debido a los altos requerimientos energéticos de los individuos pequeños, y no debido a adaptación dietaria. Nuestros experimentos de aclimatación dietaria nos permiten concluir que los adultos son incapaces de modular sus actividades discaridásicas en respuesta a la química dietaria. La ausencia de labilidad en estas enzimas digestivas parece apoyar la hipótesis de rigidez fisiológica digestiva en anfibios adultos y que la modulación de la actividad fisiológica intestinal deja de operar durante el desarrollo ontogenético.

Palabras clave: *Bufo spinulosus*, ontogenia, química dietaria, alometría, enzimas intestinales.

INTRODUCTION

An animal's physiological response to biotic and abiotic environmental factors can range from immediate, in acute responses, to acclimation ranging from weeks to months, to an even longer ontogenetic or developmental response; and finally, by inherited characteristics over several generations could lead to physiological evolutionary adaptation (Bozinovic et al. 1995).

Digestive physiological responses to chemical composition of food may occur through the above-mentioned physiological processes. These can include acute, chronic, ontogenetic and adaptative variations in the

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interplay between the intestinal hydrolisis of dietary substrates, their uptake or absorption, and the length of time that food is retained in the digestive tract (Martínez del Río & Karasov 1990), in response to, for example, dietary modulation, ontogenetic development and body size.

Interestingly, in animals that exhibit metamorphosis such as the bullfrog, the ratio of sugar to amino acid uptake by the small intestine is the same in adults as in tadpoles (Toloza & Diamond 1990 a, b). These authors suggested that during ontogeny the increasing ratio of carbohydrate to protein in the diet of adults (dietary modulation), is compensated by an increase in the ratio of intestinal sugar to amino acid uptake (nutritional growth requirements). If this response is general, we hypothesized that in smaller individuals preying on items with the same chemical composition as the prey of adults, the intestinal hydrolysis of dietary substrates, such as carbohydrats, involved in energy supply rather than in growth requirements (amino acids), should decline with body mass (m_b) due to the higher mass-specific energy requirements of small-sized animals (allometric effect).

Here we test this hypothesis by measuring the activity of the intestinal disaccharidases sucrase, maltase and trehalase as a function of m_b, and the response of intestinal sucrase and trehalase by effect of dietary acclimation in the toad *Bufo spinulosus* (Weigmann 1935). As demonstrated by Martínez del Río et al. (1992), the measurement of these intestinal, membrane-bound enzymes is a useful experimental tool for the study of animals' digestive responses within an ecological and evolutionary framework. Dietary sugars are hydrolised by these disacchradidases in the small intestine and then assimilated

In Chile, *B. spinulosos* inhabits desert, oceanic and Andean habitats, from sea level to up to 4,500 meters above sea level (Veloso & Navarro 1988), and feeds on aquatic insects and terrestrial worms (Cei 1962).

MATERIAL AND METHODS

Specimens of *B. spinulosus* were captured during summer in the Andean range of central Chile at 2,300 m above sea level (33° 21' S, 70° 20' W), 50 km east of Santiago. Individuals were transported to the laboratory, where one group was immediatly studied and the second group acclimated to the experimental diets. In the first group, we selected animals ranging in m_b from 3.8 g to 91.8 g, then they were sacrificed, the small intestine was isolated and washed with ice-cold physiological solution (1% NaCl), and the length (Li), mass (Mi) and nominal area (Ai) were measured. The intestine then was blotted in liquid nitrogen for further assays (see Sabat & Bozinovic 1994, Sabat et al. 1995 for details) .

For the examination of dietary acclimation, a total of eleven individuals were assigned to each of two experimental diets of mealworms (rich in trehalose) or artificial fruits (quincy jam rich in sucrose) for 21 days to determine whether disaccharidase activity alters in response to dietary substrates. When necessary, animals were forced to ingest the experimental diets. On day 21, individuals were sacrificed and the same protocol of sampling was followed.

To estimate disaccharidase activity, the small intestine was thawed and then homogenized in 10 volumes of 1% NaCl using a Potter-Elvehjem homogenizer, the homogenate was filtered through nylon of 110 mesh. Protein concentrations were determined using bovine serum as a standard (Peterson 1977). Enzyme disaccharidases activities were measured according to the method of Dahlqvist (1984) as modified by Martínez del Río (1990). After incubating the solution for 5 min at 30°C, the enzymatic reaction was started by adding 50 ml of intestinal homogenate to the solution containing the specific sugars. After 10 min of incubation the reaction was arrested by adding 3 ml of stop-develop reagent (one bottle of Trinder 315-500, Sigma Chemical Company) dissolved in 500 ml of 0.5 M buffer phosphate, pH 7.0. The arrested reactions then were allowed to stand at 30 °C for 18 min, and the absorbance measured at 505 nm (Spectronic 21 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. Activities were expressed as UI/mg protein, where UI =

number of micromoles of substrate hydrolyzed per min.

Comparisons between groups were performed by the nonparametric Wilcoxon test. The allometric relationships between Li (cm), Mi (g), and Ai (cm^2) of the digestive tract, and sucrase, maltase and threalase (UI/mg protein) activity and m_b (g) were calculated by the use of least-square linear regression ($\log Y = \log a + b \log X$) and expressed as $Y = aX^b$, where Y = digestive variables, X = m_b, a = intercept at $\log X = 0$, and b = slope. All values are given as mean ± SD (Steel & Torrie 1985).

RESULTS AND DISCUSSION

The relationship between length (Li in cm), mass (Mi in g) and area (Ai in cm^2) of the intestine and m_b (g) for all individuals (N = 8) ranging from 3.7 to 91.8 g, are shown in Fig. 1. The allometric relationships were:

$$Li = 5.67\ m_b^{0.288} \quad (1)$$
$$(r^2 = 0.879, F_{1,7} = 43.615, P = 0.0006)$$

$$Mi = 0.08\ m_b^{0.637} \quad (2)$$
$$(r^2 = 0.987, F_{1,7} = 463.019, P = 0.0005)$$

$$Ai = 1.95\ m_b^{0.552} \quad (3)$$
$$(r^2 = 0.972, F_{1,7} = 205.211, P = 0.00001)$$

On the other hand, the allometric relationships relating sucrase, maltase and trehalase activity (UI/mg protein) to m_b (g) (Fig. 2) were:

$$Sucrase = 0.045\ m_b^{-0.699} \quad (4)$$
$$(r^2 = 0.676, F_{1,7} = 12.541, P = 0.0122)$$

$$Maltase = 0.101\ m_b^{-0.354} \quad (5)$$
$$(r^2 = 0.739, F1,7 = 16.997, P = 0.0062)$$

$$Trehalase = 0.100\ m_b^{-0.619} \quad (6)$$
$$(r^2 = 0.734, F_{1,6} = 13.773, P = 0.0138)$$

Disaccharidase activites after acclimation to different dietary substrates are shown in Table 1. Body mass of animals did not differ significantly between groups (Z = 0.284, P = 0.776). Both sucrase and trehalase activity were also non-significantly different between groups after 21 days of treatment (Z = 0.477, P = 0.633 for sucrase activity, and Z = 0.473, P = 0.636 for trehalase activity).

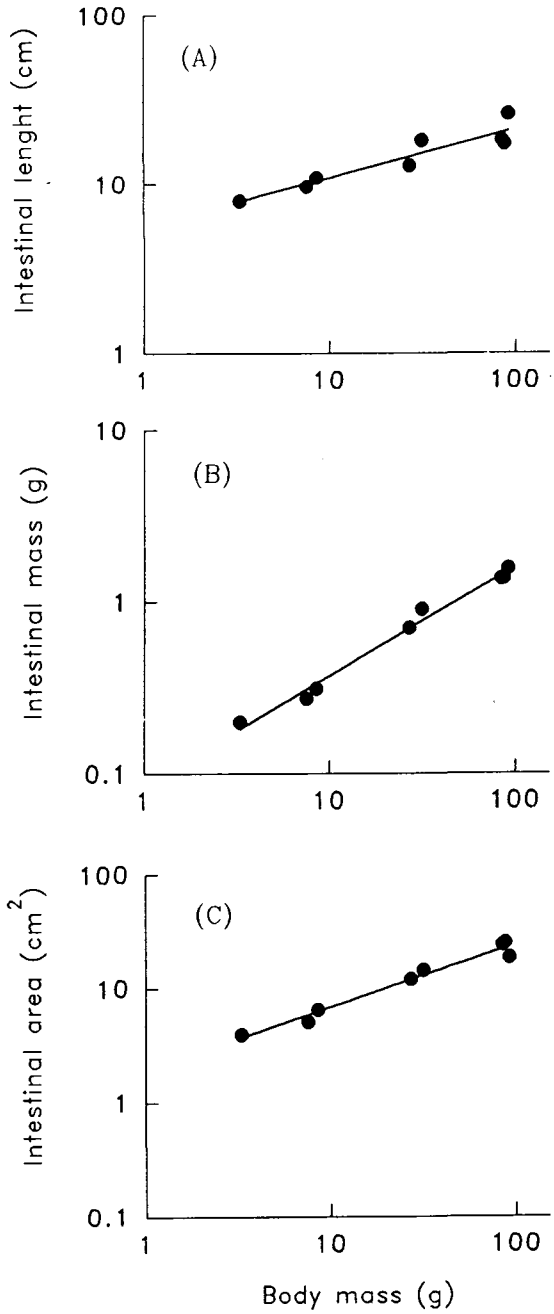

Fig. 1: Double logarithmic relationships of intestinal length (A), mass (B) and area (C) as a function of body mass in *Bufo spinulosus* (see test for allometric equations).

Relación doble logarítmica de largo intestinal (A), masa (B) y área (C) como función de la masa corporal en *Bufo spinulosus* (véase texto para las ecuaciones alométricas).

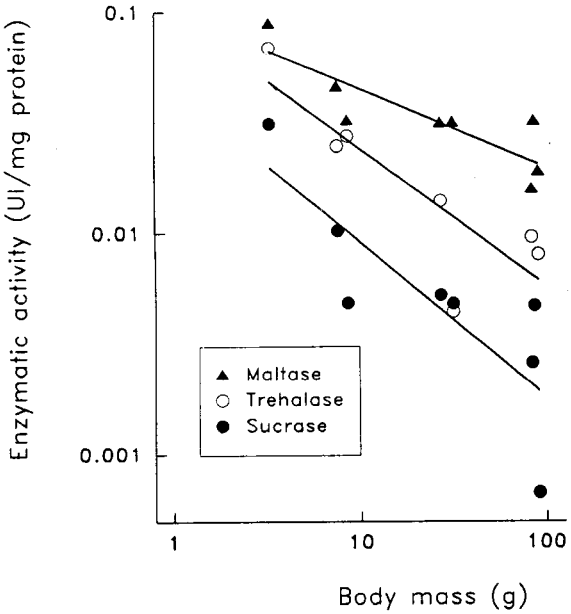

Fig. 2: Double logaritmic relationships of enzymatic activity of maltase, trehalase and sucrase as a function of body mass in *Bufo spinulosus* (see text for allometric equations).

Relación doble logarítmica de la actividad enzimática de maltasa, trehalasa y sacarasa como función de la masa corporal en *Bufo spinulosus* (véase texto para ecuaciones alométricas).

TABLE 1

Body mass (g) and activity rates of two disaccharidases (UI/mg protein) measured at 28 mM substrates in *Bufo spinulosus* acclimated to diets of artificial fruits (N = 5) and larvae of mealworms (N = 6). Values are expressed as mean ± 1 S.D., P-values after a non-parametric Wilcoxon test are shown

Masa corporal (g) y tasas de actividad de dos disacaridasas (UI/mg proteína) medidas a una concentración de sustrato de 28 mM en *Bufo spinulosus* aclimatados a dietas de frutas artificiales (N = 5) y larvas de gusanos de la harina (N = 6). Los valores se expresan como promedio ± 1 D.E., se muestran los valores de P según una prueba no paramétrica de Wilcoxon

Variable	Diet of fruit	Diet of larvae	P-value
Body mass	1.970 ± 0.705	1.817 ± 0.469	0.776
Sucrase	0.006 ± 0.008	0.001 ± 0.015	0.633
Trehalase	0.024 ± 0.014	0.040 ± 0.040	0.636

The scaling relationships of digestive morphology in this species (see equations 1 to 3) are similar to those reported previously for amphibians (see Toloza & Diamond 1990a). The morphometric relations seem to follow (approximately) classical geometrical principles, that is, Li α $m_b^{1/3}$, Mi α $m_b^{2/3}$, and Ai a $m_b^{1/2}$.

After metamorphosis, *B. spinulosus*'s diet is probably chemically constant with comparatively higher concentrations of the sugar trehalose (a storage sugar in insects), and constant and lower concentrations of sucrose (Bell 1990). According to the expected correletions between dietary chemistry and digestive enzyme activities (Vonk & Western 1984), a constant activity of trehalase and sucrase with body mass is to be expected in this species, rather than the observed decrease in activity levels of disaccharidases. As predicted, larger individuals of *B. spinulosus* feeding on the same dietary items as smaller individuals, exhibited a decreased enzymatic activity of intestinal disaccharidases with m_b (equations 4 to 6). This is probably due to the higher energetic requirements of smaller individuals, and not to dietary adaptation. In fact, our experiments of dietary acclimation allow us to conclude that adults are unable to modulate their disaccharidases activity in response to dietary chemistry. Probably both enzymes are under the same control mechanism mediated by hormones as happens in mammals (Galand 1989). In fact, Houdry et al. (1979) documented a peak in disaccharidase activity in early metamorphosed individuals of bullfrog, correlated with increased levels of the thyroid and adrenal hormones.

The lack of lability in these digestive enzymes seems to support the hypothesis of digestive physiological rigidity in adult amphibians. In fact, Toloza & Diamond (1990b) documented in *Rana catesbeina* that dietary modulation of intestine uptake activity can cease to operate during ontogenetic development, being present in tadpoles but absent in adult frogs, where it would have no function.

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