Developmental Changes in Digestive Physiology of Nestling House Sparrows, *Passer domesticus*

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ABSTRACT

Six decades of studies have speculated that digestive capacity might limit avian growth rate or that developmental changes in the gut might determine developmental changes in digestive efficiency. However, there are no studies on digestive enzymes during avian development, except for studies on mainly domestic birds that exhibit the precocial mode of development. We studied alimentary organ masses, intestinal enzyme activities (sucrase, maltase, isomaltase, aminopeptidase-N), and pancreatic enzyme activities (amylase, trypsin, chymotrypsin) during development of a wild passerine bird exhibiting the altricial mode of development. Wild nestling house sparrows were studied immediately after removal from the nest (days 0, 3, 6 of age; day 0 = hatch), whereas captives were raised in the laboratory beginning day 3 on a formulated casein/starch-based diet until fledging age (after day 12). Digestive biochemistry was dynamic. Tissue-specific activities of some digestive enzymes continued to increase through fledging, by >10 times in some cases (e.g., sucrase and maltase in midintestine). Total pancreatic amylase activity increased 100 times between hatch and day 12 through a combination of increases in tissue-specific activity and pancreas mass. House sparrows differ from poultry, in whom after about 2 wk of age the specific activity of intestinal and pancreatic digestive enzymes is generally constant or declines during development. The data on intestinal and pancreatic enzymes help explain why digestive efficiency of nestling house sparrows improves with age, and the data seem consistent with the idea that digestive capacity might limit feeding rate and hence growth rate.

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Introduction

The gastrointestinal tract has a central position in studies of avian ontogenies because of its key function in energy intake (Starck 1993). Six decades of comparative studies of alimentary tract mass (Portmann 1942; Neff 1973; Lilja 1983; Konarzewski et al. 1990; Starck 1996, 1998; Gille et al. 1999) have speculated on the possible role of the gut in determining features of avian development such as developmental changes in digestive efficiency (the proportion of ingested energy not eliminated in excreta), limitations to energy assimilation and growth, and the form of the growth curve (Konarzewski et al. 1989; Karasov 1996). However, there are no studies on digestive enzymes during avian development, except for studies on mainly domestic birds that exhibit the precocial mode of development, such as chickens (Nir et al. 1978, 1996; Obst and Diamond 1992; Biviano et al. 1993; Jackson and Diamond 1995, 1996), turkeys (Escribano et al. 1988; Krogdahl and Sell 1989; Sell et al. 1989), and ducks (King et al. 2000). We present the first data on pancreatic and intestinal enzyme activities during development of a passerine bird.

We studied house sparrows (*Passer domesticus*), which exhibit the altricial mode of development, because much is already known about their postnatal feeding, growth, and development (Weaver 1942; Summers-Smith 1967; Seel 1970; Neff 1973; Lepczyk and Karasov 2000) and because the feeding and digestion of their nestlings has been studied in captivity (Blem 1975; Lepczyk et al. 1998). Building on these earlier studies, we were able to design a study to test predictions about development of digestive biochemistry.

Digestive efficiency is apparently lower in very young birds than in older juveniles and adults, but a mechanistic explanation is lacking (Karasov 1990). It is not simply due to less gastrointestinal tract in young birds, because juveniles of altricial and precocial species generally have a greater proportion of their mass as alimentary tract compared with adults (Lilja 1983; Konarzewski et al. 1990). Both these patterns are apparent in house sparrows: efficiency of digestion increases to an adult level by day 6 (Blem 1975), but nestlings hatch with and retain proportionally more gut than adults (Neff 1973). (Here and elsewhere, day 0 is hatch day.) We predicted, therefore, that there would be notable increases in tissue-specific enzyme ac-

tivity between hatch and day 6, corresponding to the period during which digestive efficiency increases.

A major question in studies of avian development has been whether the developmental rate of the gut might limit feeding rate and the overall rate of growth and development (Ricklefs et al. 1998). At first glance, this does not seem probable in house sparrows, based on their patterns of growth. Compared with their whole body, their intestine shows an accelerated growth but then reaches an asymptotic size at about age 6 d (Neff 1973), before their feeding rate and growth rate stop increasing, which occurs by day 9 or 10 (Blem 1975; Lepczyk et al. 1998). If the gut were limiting and its digestive capacity were properly indexed by gut mass, then further increases in food intake beyond day 6 would not be possible once gut mass reached its maximum at day 6. Though intake would level off, growth might still continue, depending on shifts in allocation of energy and material to building the rest of the body once growth of the gut is completed (Konarzewski et al. 1989). To explain how food intake might still increase with no change in gut mass, we predicted that intestinal tissue-specific enzyme activity would increase until at least day 9 in growing house sparrows, and in this way biochemical digestive capacity would continue to increase. Developmental changes in pancreatic mass have not been reported, so for this tissue we cannot predict whether biochemical digestive capacity should increase by increase in pancreatic mass, tissue-specific activity, or a combination of the two. A pattern of increasing tissue-specific enzymatic activity during postnatal growth would be different from the pattern observed in precocial chickens and turkeys in which tissue-specific intestinal enzyme activity is constant or declines during most of postnatal growth (Nir et al. 1978; Escribano et al. 1988; Krogdahl and Sell 1989; Sell et al. 1989; Biviano et al. 1993; Jackson and Diamond 1995, 1996).

Material and Methods

In the wild, nestlings are fed primarily arthropods for the first few days of life and then increasing amounts of seeds beginning 2–3 d posthatch (see Summers-Smith 1967 for review). Because such diet changes could influence features of digestive biochemistry (Caviedes-Vidal and Karasov 1996; Caviedes-Vidal et al. 2000), we studied mainly birds in captivity fed a constant, formulated casein/starch-based diet (Lepczyk et al. 1998; Lepczyk and Karasov 2000). However, we were not able to maintain the very youngest nestlings (before day 3) on the diet, and so very young wild birds were collected directly from their nests and therefore may have had a variable diet. So, for the first prediction that there would be notable increases in tissue-specific enzyme activity between hatch and day 6, corresponding to the period during which digestive efficiency increases, enzyme activity was compared among wild nestlings 0, 3, and 6 d old. For the second prediction that biochemical digestive capacity would increase between day 6 and day 10 even though intestine mass would not, we brought into captivity 3-d-old nestlings, habituated them to the formulated diet, and compared enzyme activity on days 6, 9, and 12.

Study Site and Housing

All natural and artificial nest sites were located around the dairy barns of the University of Wisconsin—Madison. Cardboard blue bird nest boxes (Midland Manufacturing, Fort Smith, Ariz.) were modified by enlarging the entrances and were placed in known house sparrow breeding sites during January 1995. Beginning in mid-March 1995, all potential nesting locations were visited twice a week to note the onset of laying. From May 1 to August 8, 1995, all known nests were visited daily between 1030 and 1330 hours with rare exception, to ensure accurate and consistent aging (Burger 1988). At each visit, nestlings were weighed to the nearest 0.01 g with a portable electronic scale, marked on their back and scapulars with an indelible marker, and returned to their nest. Nestlings were removed from their nests between 1030 and 1230 hours. Nestlings for the laboratory study were removed on day 3 (hatch = day 0), which was the youngest age that we previously had success in rearing, and were transported to our laboratory on campus. Only nestlings that hatched synchronously on day 0 were removed and used in the experiment (i.e., no asynchronous nestlings were used). A total of 61 nestlings were collected from 29 nests.

All nestlings were placed into round (12 × 9 cm) tissue-lined plastic containers and housed in a custom-made environmental chamber with a 14L : 10D photoperiod and constant conditions of 35.6° ± 0.02°C and 62.0% ± 0.16% relative humidity (kept constant with a water bath system). These conditions within the chamber were similar to those found in natural nests by Blem (1975).

Feeding Protocol

The nestlings were hand-fed a synthetic liquid diet made up primarily of protein (46% casein and 3% free amino acids by dry weight), cornstarch (25% by dry weight), corn oil (8%), vitamins (1%), minerals (7%), and water (Lepczyk et al. 1998) and synthesized by ICN Biomedicals (Aurora, Ohio). Each hour, beginning at 0630 hours, nestlings were removed from the environmental chamber and fed by gavage with a 1-mL syringe for a total of 15 times a day. Before and after feeding, the nestling's body mass was recorded to account for the mass of food eaten. The volume of food consumed at each feeding was also recorded. We used a previously developed age-specific feeding schedule that yielded normal daily mass changes and 100% survival ($n = 9$ nestlings; Lepczyk and Karasov 2000): 0.3, 0.5, 0.6, 0.75, 0.85, 1.0, 1.25, and 1.5 mL food h^{-1} for nestlings of ages 3, 4, 5, 6, 7, 8, 9, and 10–16 d, respectively.

Intestinal Enzyme Assays

Stomach, intestine, pancreas, and liver were removed from each of the nestlings immediately after cervical dislocation and chilled in ice-cold saline. Organs except for intestine were cleaned of extraneous tissue, blotted, weighed, and stored in liquid N_2. The complete small intestine with its contents was measured for length, and, under ice-cold saline, 1-cm lengths of the proximal (first 20%), medial (middle 40%–60%), and distal (last 20%) regions were cut longitudinally and the contents carefully removed. After additional rinsing under ice-cold saline to remove adherent material, the segments were blotted dry from the serosal side, weighed, and stored in cryovials in liquid N_2 to measure intestinal disaccharidases and aminopeptidase-N. We measured the activity of membrane-bound enzymes in whole-tissue homogenates rather than in mucosal samples or isolated brush border preparations to avoid underestimation of activity, as previously reported by Martínez del Rio (1990).

Disaccharidase (sucrase, maltase, isomaltase) activities were assayed using a modification of the colorimetric method developed by (Dahlqvist 1984). Assays are described in detail by Martínez del Rio (1990) and in our previous study with adult house sparrows (Caviedes-Vidal et al. 2000). In brief, tissues were thawed at 4°C and homogenized (20 s, Omni 5000 homogenizer, setting 6) in 350 mmol L^{-1} mannitol in 1 mmol L^{-1} *N*-2-hydroxyethylpiperazine-*N*′-2-ethanosulfonic acid · KOH, pH 7.0. Aliquots of tissue homogenates (40 μL) were incubated twice at 40°C with 40 μL of 56 mmol L^{-1} sucrose and then 56 mmol L^{-1} maltose in 0.1 mol L^{-1} maleate · NaOH buffer, pH 6.5, for 10 min. After incubation, reactions were arrested by adding 1.2 mL of a stop/develop reagent (one bottle Glucose-Trinder 500 reagent [Sigma Chemical, procedure 315] in 250 mL of 1.0 mol L^{-1} Tris[hydroxymethyl]aminomethane HCl, pH 7, plus 250 mL of 0.5 mol L^{-1} NaH_2PO_4/Na_2HPO_4, pH 7), and absorbance was measured at 505 nm at 20 min.

We used L-alanine-p-nitroanilide as a substrate for aminopeptidase-N. To start the reaction, 10 μL of the homogenate was added to 1 mL of assay mix (2.0 mmol L^{-1} L-alanine-p-nitroanilide in one part of 0.2 mol L^{-1} NaH_2PO_4/Na_2HPO_4 buffer no. 1, pH 7, and one part of deionized H_2O) previously heated at 40°C. The reaction solution was incubated for 20 min at 40°C and then ended with 3 mL of ice-cold 2 N acetic acid, and absorbance was measured at 384 nm.

On the basis of absorbance measurements and glucose and p-nitroanilide standards, we calculated activities of each intestinal section normalized to the wet mass of the section. For comparison with other results in the literature, we also determined protein content (Bio-Rad Protein Assay, Bio-Rad, Melville, N.Y., catalog no. 500-0006) as a function of intestinal mass.

Activities of intestinal enzymes were expressed in micromoles per minute per gram wet tissue. Summed hydrolysis activity of the entire small intestine, an index of the total hydrolysis capacity, was calculated by multiplying activity per gram tissue in each region by its respective mass and summing over the three regions.

The pH optima of aminopeptidase-N was determined in the medial portion of the small intestine in 6-d-old nestlings. The assays were performed using the homogenates and a 0.05 M maleate/NaOH buffer system with pH's ranging from 4.5 to 8.5. The same homogenates were used for pH optima and for kinetics. We estimated the apparent binding constants (K_m^*), the concentration of substrate at which the rate of hydrolysis equals half the maximal hydrolysis rate (V_{max}) for maltase and sucrase. Enzyme activities were assayed at pH 7 and substrate concentration varying from 2.5 to 150 mM for disaccharidases. To minimize individual variation, we calculated relative activity (i.e., activity at test pH or concentration normalized to activity at standard pH [7.0] or standard concentration in a sample from the same bird).

Pancreatic Enzyme Assays

The pancreas was carefully excised, divided longitudinally into two parts, weighed, and immediately frozen in liquid N_2. Pancreases were later thawed and homogenized for 30 s using an Omni 5100 (setting 6) homogenizer in either (*a*) 5 mM phosphate buffer (pH 6.9, containing 7 mM NaCl, 3 mM taurocholic acid, 0.27% [w/v] Triton X-100, 1 mM benzamidine, and 2 mM hydrocinnamic acid) or (*b*) 50 mM Tris/HCl buffer (pH 8.2, containing 3 mM taurocholic acid and 0.27% [w/v] Triton X-100) at 0°C for amylase and trypsin/chymotrypsin, respectively. Homogenates were centrifuged, and aliquots of the supernatant were assayed following the procedures we used previously in studies with adult house sparrows (Caviedes-Vidal and Karasov 1995).

Amylase. Activity of amylase was measured by a modification of the 3,5-dinitrosalicylate method (Dalqvist 1962; Hjorth 1979). One-hundred-microliter aliquots were incubated with 2% potato starch (Sigma no. S2630) at 40°C for 3 min. The reaction was terminated by the addition of 200 μL dinitrosalicylate reagent. The tubes were immersed in boiling water for 10 min and cooled with tap water. Two milliliters of distilled water was added to each tube, and the absorbance was read at 530 nm. The enzyme solution was diluted so that the final reaction mixture contained less than 2.0 μU. One amylase unit (U) represents the amount of amylase that liberates 1 mol of free reducing groups in a 2% starch solution in 1 min at 40°C. Duplicates of each sample were assayed, and the activity is reported as the mean ± 1 SEM.

Trypsin and Chymotrypsin. Analysis of pancreatic trypsin and chymotrypsin requires prior activation of the zymogens. The

pancreas samples were incubated with 0.3% enterokinase (Sigma E1256) for 1 h at 25°C in 50 mM Tris/HCl buffer (pH 8.2) containing 20 mM $CaCl_2$. Preliminary studies indicated that this treatment gives reproducible maximal activation of the proteolytic zymogens (Brannon et al. 1987; Caviedes-Vidal and Karasov 1995). Aliquots of 16 μL diluted adequately were assayed to measure trypsin activity using 800 μL DL-BAPNA (benzoyl-arginine-p-nitroanilide) 1 mM solution as substrate at pH 8.2 for 10 min at 40°C. The reaction was terminated by adding 160 μL of 30% acetic acid. The liberated amount of p-nitroanilide was estimated by reading the absorbance at 410 nm (Erlanger et al. 1961) and using a p-nitroanilide standard curve. Chymotrypsin activities were also measured by the amount of p-nitroanilide released by hydrolysis of 160 μL of homogenate and 800 μL of GPNA (N-glutaryl-L-phenylalanine-p-nitroanilide) 1 mM solution at pH 7.6 and 40°C. The reaction was terminated with 160 μL of 30% acetic acid solution. The absorbance of the mixture was measured at 410 nm (Erlanger et al. 1966).

Protein. We estimated the concentration of protein in our pancreas samples using the commercial Bio-Rad Protein Assay (Bio-Rad, catalog no. 500-0006). Absorbances were read at 595 nm, and crystalline bovine serum album was used as the standard.

Standardization of Pancreatic Enzyme Activities and Calculation of Summed Hydrolysis Activity. Specific amylase activities are expressed as units normalized to measured tissue wet mass. Specific trypsin and chymotrypsin activities are expressed as millimoles of p-nitroanilide liberated per minute normalized to measured tissue wet mass. We calculated the summed hydrolysis activity of the whole pancreas, an index of the total hydrolysis capacity, by multiplying activity per gram of tissue by the pancreas mass.

Data Analysis

Results are given as means ± 1 SE (n = number of nestlings per treatment). Although 61% of nestlings had a sibling in the trial, and 16% were hatchlings from a nest that produced hatchlings at another time (e.g., a first, second, or third clutch by the same or a different parent in that nest), nestlings were treated as independent sampling units. We did this because we could not find enough nests to use only one nestling per nest, and pooling the siblings before statistical analysis reduces sample size too much. Nestlings were classified according to age and whether they were studied immediately upon field collection or were maintained in the laboratory. (Siblings were distributed into different age/site classes.) Tukey's honest significant difference multiple comparison test was used to isolate differences among the age/site groups for measures such as body and organ masses (SYSTAT software; Wilkinson 1992). Homogeneity of variance was tested (Bartlett test), and data were log-transformed if necessary. We used repeated-measures ANOVA to examine the effect of intestinal region and group on intestinal enzyme activities. Post hoc comparisons were made using the Tukey test. The F values of these and other ANOVAs are presented in the text with the relevant degrees of freedom as subscripts. In a few cases, when sample sizes were small, we made comparisons among groups using the nonparametric Kruskal-Wallis test. In all tests, the significance level was set at $P < 0.05$, and $0.05 < P < 0.1$ was taken to indicate a trend.

Kinetic parameters were determined by fitting the data (enzyme activity vs. substrate concentration) by nonlinear curve fitting (Gauss Newton routine, SYSTAT; Wilkinson 1992) to the following equation:

$$\text{relative activity} = \frac{V_{\max} \times \text{concentration}}{K_m^* + \text{concentration}}. \quad (1)$$

We used ANCOVA (Wilkinson 1992) to analyze the relationship between maltase and sucrase activity. Maltose is hydrolyzed by two independent enzymatic systems, the complex sucrase-isomaltase ("sucrase") and one or two maltase-glucoamylases (Semenza and Auricchio 1989). Caviedes-Vidal et al. (2000) found linear correlation between the intestinal activities of maltase and sucrase in adult house sparrows. Theoretically, the slope of the regression of the activities of maltase on the sucrase provides an estimate of the contribution of the sucrase-isomaltase complex to the maltasic activity, and the intercept provides an estimate of the independent activity of the maltase-glucoamylase complex. The latter activity was most apparent in the proximal region of the adult house sparrow small intestine (Caviedes-Vidal et al. 2000). Therefore, we performed this regression analysis for the proximal region of the nestling house sparrows, testing for an effect of age on the relation.

Results

Body and Organ Masses (Fig. 1)

Body mass increased to an asymptotic value of 25.4 ± 0.7 g (n = 22 captive nestlings) by day 9 (Fig. 1). The asymptotic body masses were similar to those of adult house sparrows studied previously (26.2 ± 0.5 g, n = 20; Caviedes-Vidal et al. 2000).

Stomach mass increased rapidly and was as large in 3-d-old nestlings as in 6- or 9-d-old nestlings. The mean stomach mass for all 3-, 6-, and 9-d-old nestlings (wild and captive) was 1.64 ± 0.06 g (n = 37), but 12-d-old nestlings had stomach masses 31% lower.

As expected, intestinal mass also reached its asymptotic value, 1.62 ± 0.06 g (n = 36 captive nestlings), earlier than did body mass, by day 6. Intestinal masses of wild nestlings at day 6 were significantly lower than in captives despite having similar body

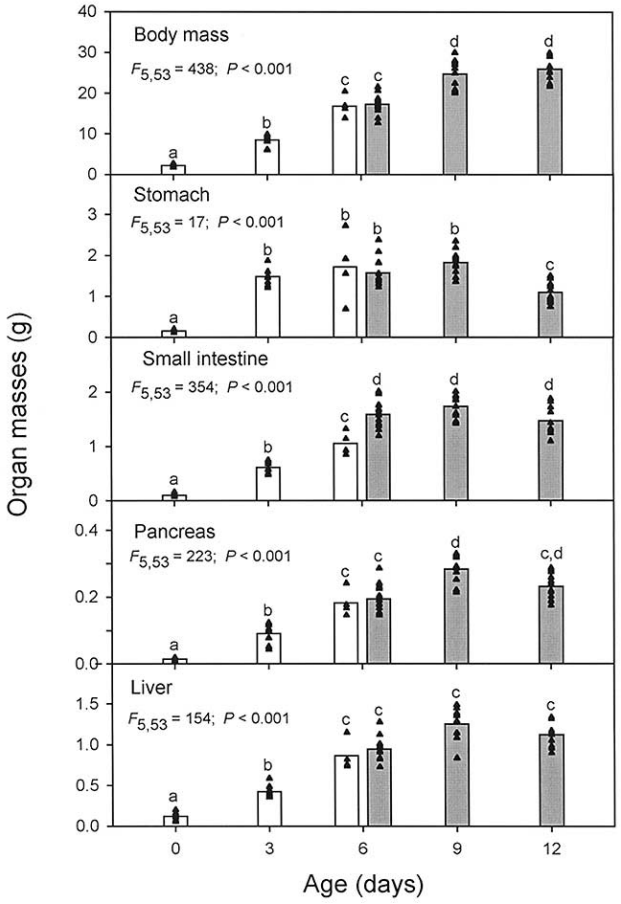

Figure 1. Body and organ masses of wild and captive nestling house sparrows as a function of age. For each variable, each point represents a separate nestling. Unshaded bars designate means for birds collected from wild nests on day of hatch (age = 0) and days 3 and 6. Shaded bars designate means for 6-, 9-, and 12-d-old birds in the laboratory. The laboratory birds were originally collected from wild nests on day 3. For each variable, log-transformed values from the groups were compared by the Tukey test, the overall F value and level of significance are given, and different letters above the bars designate significantly different means.

masses, although the sample size of wild nestlings was small ($n = 4$).

The pancreas reached its highest mass at day 9, later than the stomach and small intestine. Liver mass showed a similar pattern, with a trend for highest mass at day 9 (day 6 vs. day 9, $P = 0.07$). Pancreas and liver masses of wild nestlings at day 6 were not significantly different from those of same-age captives.

Intestinal Brush Border Enzyme Activity

Although we present enzyme activity normalized to tissue wet mass, our data can be compared with those of other studies that normalize activity to protein content through conversion using our measures of milligrams of protein per milligram wet intestine (Fig. 2). Intestinal protein contents were relatively low in hatchlings and then rose quickly with age.

Activities per gram of intestine varied significantly with intestinal position, with age, and with their interaction for aminopeptidase-N, sucrase, and maltase (all P's < 0.001) and with age and position (but not their interaction) for isomaltase (Fig. 3; Table 1). In order to compare specific activities at different ages, we performed post hoc Tukey comparisons in each region of the intestine separately because we know of no way to make such comparisons simultaneously over all the regions.

Specific aminopeptidase-N activity increased significantly in wild nestlings between day of hatch and day 3 in every intestinal position. Subsequently, the activity either remained the same as nestlings aged (medial and distal intestine) or declined slightly (proximal region).

Specific sucrase and maltase activity also increased significantly in wild nestlings between day of hatch and day 3 in every intestinal position, but in contrast to the pattern for aminopeptidase, the specific activity of these carbohydrases continued to increase with age in the proximal and medial regions.

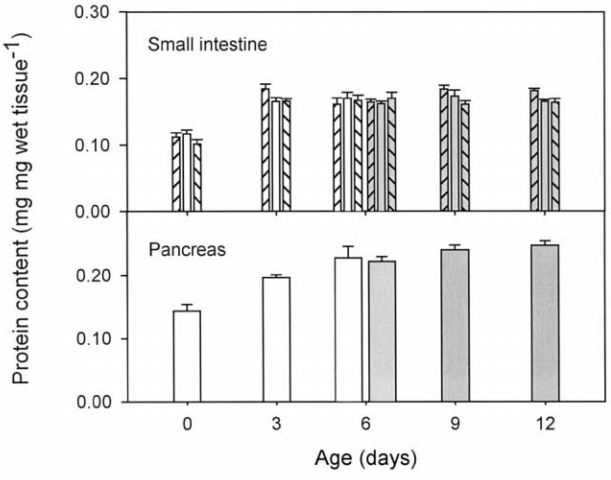

Figure 2. Intestinal and pancreatic protein contents per milligram wet tissue as a function of age in house sparrow nestlings. Unshaded bars designate means (±SE) for birds collected from wild nests (days 0, 3, 6), whereas shaded bars designate the laboratory-raised nestlings at days 6, 9, and 12. In the upper figure, for each age/site grouping, the three bars represent, from left to right, the proximal (left-tilted hatching), medial (no hatching), and distal (right-tilted hatching) regions of the small intestine. Intestinal protein content was significantly lower at hatch than in older nestlings ($F_{4,164} = 70.4$, $P < 0.001$) and declined slightly from the proximal to the distal intestinal positions ($F_{2,164} = 9.41$, $P < 0.001$; interaction of age and position, $F_{8,164} = 1.36$, $P = 0.22$). Pancreatic protein content (lower part of figure) was also significantly lower at hatch than in older nestlings and increased to a plateau value by day 6 ($F_{4,53} = 25.8$, $P < 0.001$).

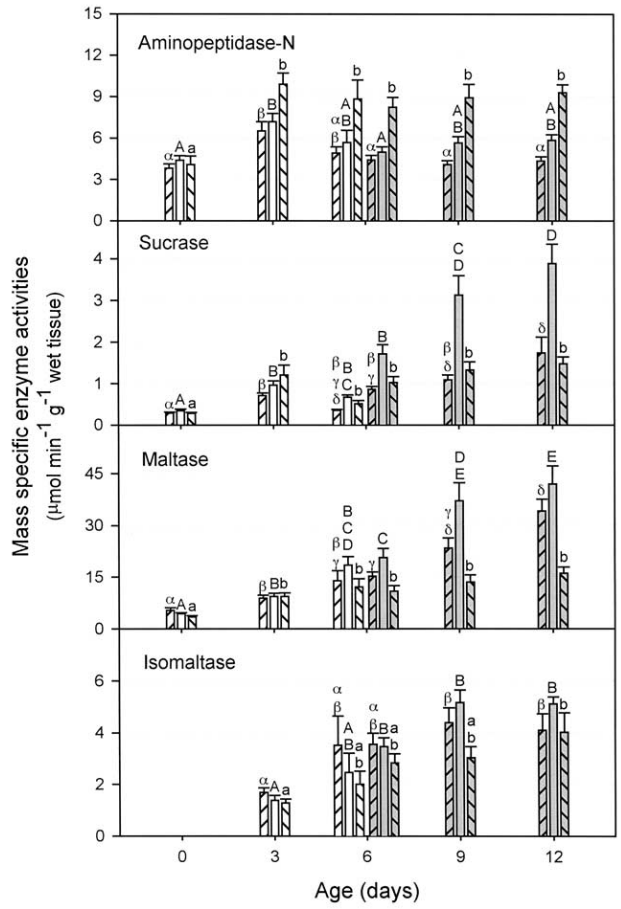

Figure 3. Tissue-specific activities of intestinal brush border enzymes of nestling house sparrows as a function of intestinal position and age. Unshaded bars designate means (±SE) for birds collected from wild nests on day of hatch (age = 0) and days 3 and 6, whereas shaded bars designate the laboratory-raised nestlings at days 6, 9, and 12. For each age/site grouping, the three bars represent, from left to right, the proximal (left-tilted hatching), medial (no hatching), and distal (right-tilted hatching) regions of the small intestine. For each enzyme, log-transformed values from the groups were compared by the Tukey test, and the overall *F* value and level of significance are given in Table 1. Different letters above the bars designate significantly different means: Greek letters for comparisons between groups in the proximal intestine, capital Roman letters for comparisons between groups in the medial intestine, and lowercase Roman letters for comparisons between groups in the distal intestine. Consult Figure 1 for the exact sample size in each group, as the measures correspond to individuals depicted there.

Isomaltase was not measured in hatchlings, but it apparently increased with age, at least based on the significantly higher values of 9- and 12-d-old laboratory birds compared with 3-d-old wild nestlings (Fig. 3). Within just the laboratory nestlings, there was a trend ($0.05 < P < 0.1$) for isomaltase to increase with age in the medial and distal regions of the small intestine.

The summed activities of aminopeptidase-N, sucrase, and maltase increased significantly between hatch and day 3 in wild nestlings (Fig. 4). Summed aminopeptidase and isomaltase activities reached asymptotic values by day 6. In contrast, summed activities of sucrase and maltase continually increased with age and were significantly higher on day 9 and/or day 12 than on day 6.

Pancreatic Enzyme Activity

Pancreatic enzyme activities were normalized to tissue wet mass but can be compared with those of other studies that normalize activity to protein content through conversion using our measures of milligrams of protein per milligram wet pancreas (Fig. 2). Pancreatic protein contents, like those for intestine, were relatively low in hatchlings and then increased with age.

Activities per gram pancreas increased significantly between hatch and day 6 for amylase, trypsin, and chymotrypsin (Fig. 5). Specific activity continued to increase significantly with age for amylase, but levels of the proteases were not significantly higher at any older age than at day 6 (Fig. 5). Six-day-old wild and captive birds did not differ significantly in enzyme activity per mg pancreas for any of the enzymes.

The summed activity per whole pancreas increased significantly between hatch and day 6 for all three enzymes (Fig. 6) and then did not increase significantly at older ages, although

Table 1: Repeated-measures ANOVA table showing effects of house sparrow age, position along the intestine, and their interaction on log-transformed intestinal brush border enzyme activity per milligram intestine

Enzyme Activity and Effect[a]	df[b]	*F*	*P*
Aminopeptidase-N:			
Age	5, 52	7.12	<.001
Position	2, 104	81.5	<.001
Age × position	10, 104	6.17	<.001
Sucrase:			
Age	5, 52	32.6	<.001
Position	2, 104	50.2	<.001
Age × position	10, 104	6.06	<.001
Maltase:			
Age	5, 52	41	<.001
Position	2, 104	43.2	<.001
Age × position	10, 104	4.48	<.001
Isomaltase:			
Age	4, 42	6.32	<.001
Position	2, 84	8.91	<.001
Age × position	8, 84	1.69	.11

[a] Position refers to 1-cm lengths of the proximal (first 20%), medial (middle 40%–60%), and distal (last 20%) region of the small intestine.

[b] Degrees of freedom in the ANOVA.

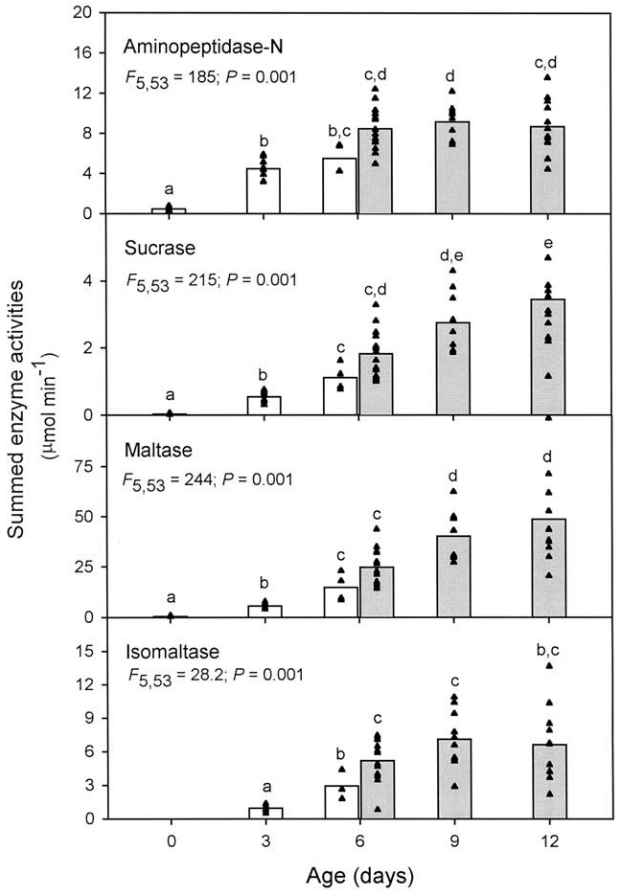

Figure 4. Intestinal summed enzyme activities (the product of tissue-specific activity and intestine mass) of wild and captive nestling house sparrows as a function of age. For each enzyme, each point represents a separate nestling. Unshaded bars designate means for birds collected from wild nests on day of hatch (age = 0) and days 3 and 6. Shaded bars designate means for 6-, 9-, and 12-d-old birds in the laboratory. For each enzyme, log-transformed values from the groups were compared by the Tukey test, the overall *F* value and level of significance are given, and different letters above the bars designate significantly different means.

the mean values for all pancreatic enzymes were (nonsignificantly) higher on day 9 than on day 6.

pH and Kinetics

We performed a limited number of measures of the pH and concentration dependence of intestinal enzyme activities to screen for any large age-dependent differences in these biochemical measures. Measured pH optimum for aminopeptidase-N was 7.5 in 6-d-old nestlings (Fig. 7), essentially the same as measured previously for aminopeptidase-N in adult house sparrows (also 7.5; Caviedes-Vidal et al. 2000).

Maltase and sucrase activity both exhibited saturable kinetics that were adequately described by Equation (1) (Fig. 8). The correlation coefficients (r^2) for the individual birds tested ranged from 0.89 to 0.99 for both substrates. The values of K_m^* reflecting apparent affinity between enzyme(s) and maltose were 11.2 ± 3.4 mM for two 3-d-old birds and 6.2 ± 0.5 mM for three 12-d-old birds ($P = 0.12$). For sucrase, the values of K_m^* were 22.0 ± 3.5 mM ($n = 2$) in 3-d-old nestlings and 32.2 ± 2.0 ($n = 3$) in 12-d-old nestlings ($P = 0.1$).

Relationship between Maltase and Sucrase

We regressed the activity per gram of maltase against that for sucrase as a partial test for sucrase-independent maltase-glucoamylase activity (Fig. 9). Intestinal maltasic activity was significantly correlated with intestinal sucrasic activity ($F_{1,51} = 67.5$, $P < 0.001$), but the relation differed according to age ($F_{4,52} = 16.8$, $P < 0.001$) with no significant interaction ($F_{4,48} = 0.5$, $P > 0.7$). The interesting pattern that emerged was that the intercept for each age progressively increased from hatch through age 12 d (Fig. 9). The values for intercept and slope of 12-d-old nestlings (17.8 and 8.6, respectively) fell within the 95% confidence intervals of the intercept and slope

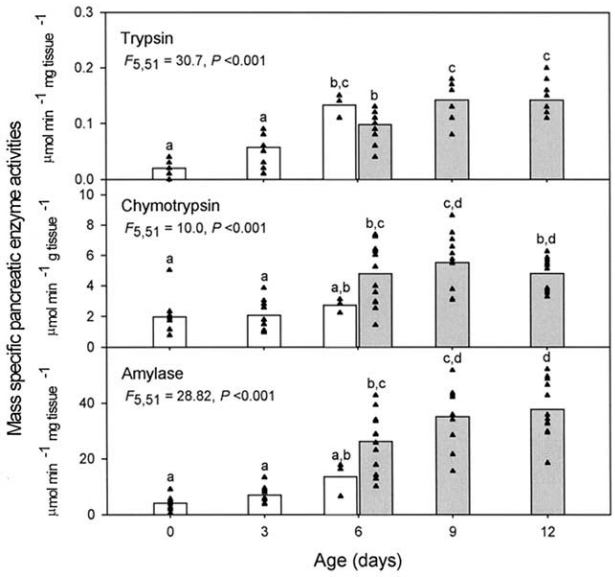

Figure 5. Tissue-specific pancreatic enzyme activities of wild and captive nestling house sparrows as a function of age. For each enzyme, each point represents a separate nestling. Unshaded bars designate means for birds collected from wild nests on day of hatch (age = 0) and days 3 and 6. Shaded bars designate means for 6-, 9-, and 12-d-old birds in the laboratory. For each enzyme, log-transformed values from the groups were compared by the Tukey test, the overall *F* value and level of significance are given, and different letters above the bars designate significantly different means.

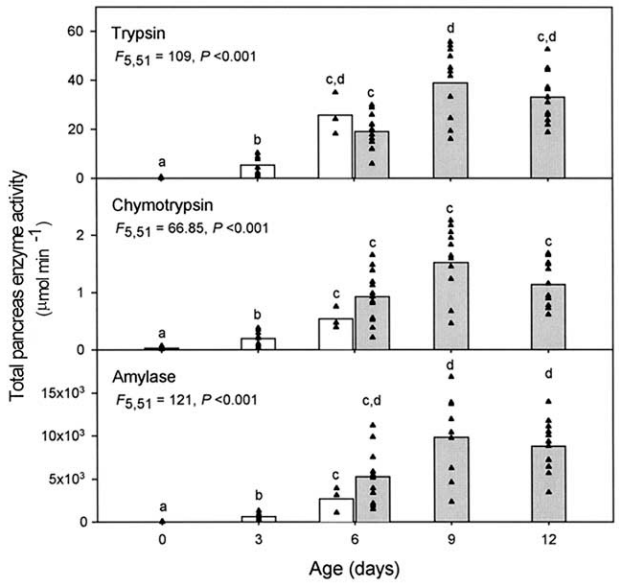

Figure 6. Total pancreatic enzyme activities (the product of tissue-specific activities and pancreas mass) of wild and captive nestling house sparrows as a function of age. For each enzyme, each point represents a separate nestling. Unshaded bars designate means for birds collected from wild nests on day of hatch (age = 0) and days 3 and 6. Shaded bars designate means for 6-, 9-, and 12-d-old birds in the laboratory. For each enzyme, log-transformed values from the groups were compared by the Tukey test, the overall *F* value and level of significance are given, and different letters above the bars designate significantly different means.

previously described for adult house sparrows (9.6–38.4 and 8.5–12.1, respectively; Caviedes-Vidal et al. 2000). The contribution of sucrase to maltasic activity in the proximal region of 12-d-old nestlings was estimated by multiplying the slope of the maltase versus sucrase relationship (8.6) by the mean sucrasic activity (1.74 ± 0.38 μmol min^{-1} g^{-1}), and sucrase-independent maltase-glucoamylase activity thus apparently accounted for 56% of the mean maltasic activity (34.2 ± 3.4 μmol min^{-1} g^{-1}). Thus, the sucrasic-independent maltasic activity increased from imperceptible levels in hatchlings to account for about half of all maltasic activity at time of fledging.

Discussion

House sparrows, like other altricial avian species, undergo rapid and dramatic anatomical and physiological changes between hatching and fledging. The astounding changes in just 12 d include increases in intestinal and total masses of >10 times, a transition from ectothermy to endothermy, and the acquisition of locomotory and sensory abilities. Our study adds changes in digestive biochemistry to this dynamic picture. Tissue-specific levels of digestive enzymes change by >10 times in some cases (e.g., sucrase and maltase in midintestine). Pancreatic amylase activity increased 100 times between hatch and day 12 through a combination of increases in tissue-specific activity and mass. Our findings offer a fresh perspective on the long-standing debate about physiological limits to avian growth (Ricklefs et al. 1998). In addition, they permit further interpretation of other data previously collected on other aspects of development of digestion in house sparrows, including changes in retention time (Lepczyk et al. 1998) and digestive efficiency (Blem 1975). Accordingly, in the sections that follow, we deal with these topics, in addition to a comparison of data in adult house sparrows (Caviedes-Vidal et al. 2000; and E. Caviedes-Vidal and W. Karasov, unpublished data).

Digestive Capacity in Relation to Feeding and Growth Rates

Comparative analysis of postnatal growth of the avian alimentary system began more than half a century ago (Portmann 1942) and has continued steadily (Neff 1973; Lilja 1983; Konarzewski et al. 1990; Starck 1996; Starck 1998; Gille et al. 1999). This activity has been spurred by interest in whether differences in digestive capacity might explain the faster growth rate of birds exhibiting the altricial rather than precocial mode of development (Ricklefs et al. 1998). These studies have relied on alimentary tract mass as a proxy for digestive capacity, assuming that relative gut size indicates relative function. While our single study cannot resolve the controversy about growth rate, it does expose the weakness of this critical assumption that had been little tested. Digestive capacity does change independent of change in gut mass because enzyme activity per gram intestine changes. Tissue-specific activity (activity per gram intestine) of maltase and sucrase, which are important in starch digestion, continued to increase after day 6 (Fig. 3), and the intestine's

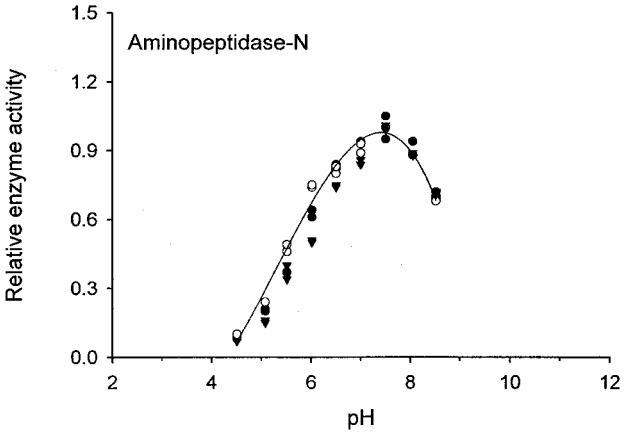

Figure 7. The effect of pH on intestinal aminopeptidase-N activity in 6-d-old house sparrows. Data are from three birds, each represented by a different symbol. The line is fitted through the mean value at each pH.

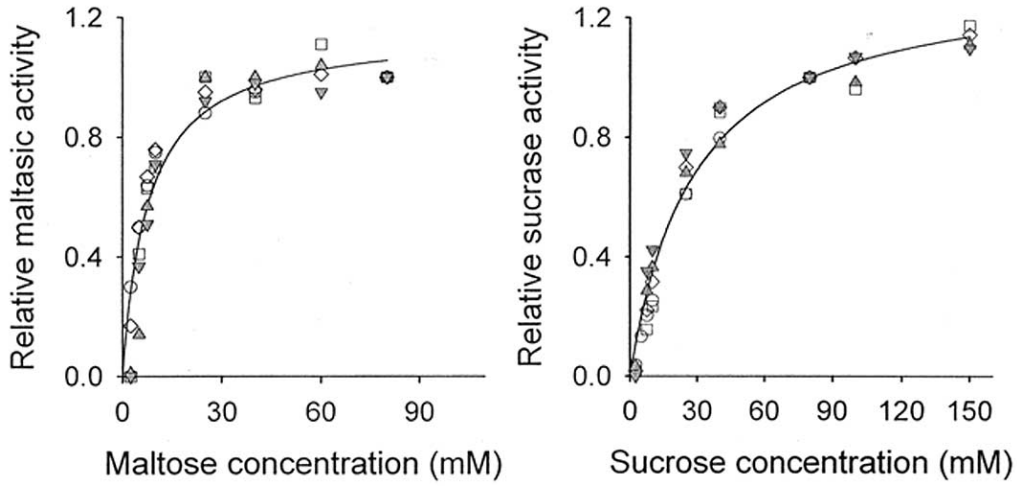

Figure 8. The effects of substrate concentration on intestinal brush border enzyme activities in 3-d-old (two nestlings, each represented by different filled symbols) and 12-d-old (three nestlings, each represented by different open symbols) house sparrows. The lines in the kinetic figures are the fits by nonlinear curve fitting to the following equation: relative activity = (V_{max} × concentration)/(K_m^* + concentration). One line is fit for each enzyme because there was no significant difference between ages 3 and 12 d for either enzyme activity, although these kinetics did appear different from those described previously for adult house sparrows (see "Results").

total maltase and sucrase capacities (the product of intestinal mass and tissue-specific activity) consequently did not reach their maxima until at least days 9–12 (Fig. 4). One of the enzymatic sources of maltasic activity, maltase-glucoamylase, apparently progressively increased from very low levels at hatch to near-adult levels by day 12 (Fig. 9). In contrast, tissue-specific activity of aminopeptidase, which is important in protein digestion, was independent of age after hatch or day 3 (Fig. 4).

Considering the house sparrow alone, some of the data on intestinal enzymes seem consistent with the idea that digestive capacity might limit feeding rate and hence growth rate. For example, house sparrow nestlings achieved full intestinal mass by day 6 but doubled feeding rate between day 6 and day 10 (Lepczyk et al. 1998). If the gut were limiting and its digestive capacity were properly indexed by gut mass, then further increases in intake would not be possible once gut mass reached its maximum at day 6. Focusing on gut mass alone or aminopeptidase activity might lead one to reject the hypothesis of gut limitation of feeding and growth, whereas one cannot reject the hypothesis upon consideration of the carbohydrases.

These are the only data to our knowledge on intestinal enzyme activities of an altricial species during development. Precocial, mainly domesticated, species have been studied more extensively. In poultry, after about 2 wk of age, the specific activity of intestinal digestive enzymes is generally constant or declines during development (Nir et al. 1978; Sell et al. 1989; Biviano et al. 1993; Jackson and Diamond 1995), although in white Pekin ducks a doubling of specific sucrase activity was

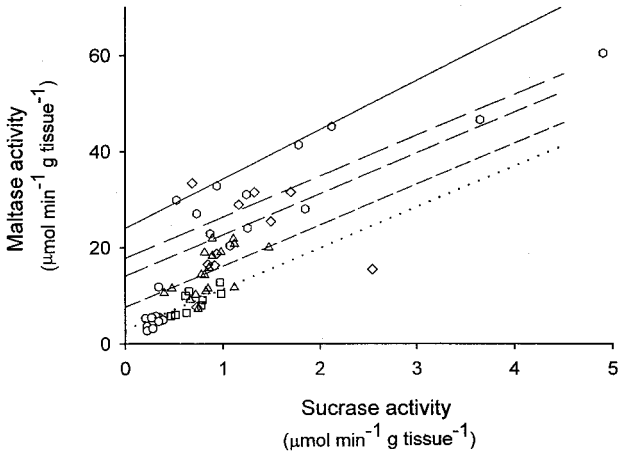

Figure 9. The relationship between intestinal maltase activity and sucrase activity in the proximal region of the small intestine. Values are coded according to age of the nestling: *circles*, day 0; *squares*, day 3; *triangles*, day 6; *diamonds*, day 9; *hexagons*, day 12. Intestinal maltasic activity was significantly correlated with intestinal sucrasic activity ($F_{1,51} = 67.5$, $P < 0.001$), but the relation differed according to age ($F_{4,52} = 16.8$, $P < 0.001$) with no significant interaction ($F_{4,48} = 0.5$, $P > 0.7$). Therefore, parallel lines were plotted for each age (*dotted line*, days 0 and 3, which had the same intercept; *short dashed line*, day 6; *longer dashes*, day 9; *solid line*, day 12). Notice that the intercept for each age progressively increased from hatch through age 12 d.

reported during the first 7 wk of age (King et al. 2000). In these precocial species, continued increase in intestinal mass during development leads to increases in total enzymatic capacity that keeps pace with increases in food intake during growth (Biviano et al. 1993).

The data available on pancreatic digestive enzymes indicate patterns somewhat similar to those for intestinal enzymes. In house sparrows, pancreatic mass and specific activity of carbohydrate-digesting amylase increased through day 9. Total pancreatic activity (the product of specific activity and pancreatic mass) appeared to increase through day 9, although mean values were not significantly above those at day 6 (Fig. 6). In poultry, pancreatic mass, but not specific activities of the pancreatic enzymes, increases with age (Escribano et al. 1988; Krogdahl and Sell 1989).

Two other points should be made regarding our analysis of digestive capacity during development. First, the finding of lower intestinal mass in wild nestlings at day 6, compared with similar-aged captives (Fig. 1), is almost certainly anomalous, possibly due to small sample size in the wild group. The difference was not due to larger size in the captives (Fig. 1; see also Lepczyk and Karasov 2000). The intestinal mass of those four 6-d-old wild individuals was a smaller percentage of body mass than in the 3-d-old wild individuals ($n = 9$), whereas Neff (1973) reported that proportional intestine mass was still increasing through day 5. Although we do not have data for day 5, our data including the captive nestlings imply a peak at day 6, which is much more similar to the data of Neff (1973) than a peak at day 3. We also note that, except for the small intestine, no other organs we measured were lower in wild nestlings at day 6 compared with similar-aged captives (Fig. 1).

As regards the second point, recall that house sparrow nestlings are fed primarily arthropods for the first few days of life and then increasing amounts of seeds beginning 2–3 d posthatch (see Summers-Smith 1967 for review). An interesting question is whether the rising levels of sucrase and maltase after day 6 are a programmed response of the nestling in anticipation of the rising proportion of carbohydrate-rich seed in its diet or a response induced by the diet change we inevitably caused when we transferred nestlings from whatever diet they received from their parents to the formulated diet in the laboratory. We favor the first answer for three reasons. First, similar programmed changes in intestinal enzymes have been described for other vertebrates that undergo normal diet changes during development (Buddington and Diamond 1989). Second, small intestine carbohydrases appear to be relatively unresponsive to dietary manipulations, at least in house sparrow adults and passerines generally (Caviedes-Vidal et al. 2000). Third, enzymatic habituation to new diets generally occurs within 1–2 d in adults (Karasov and Hume 1997), and the 6-d-old nestlings had already been eating the formulated diet for 3 d. In future studies, the question of the signal for the change in intestinal enzyme levels might be tested by manipulating the nutrient composition of the formulated diet fed to nestling house sparrows.

Digestive Capacity in Relation to Retention Time and Digestive Efficiency

In about a half dozen wild species, including house sparrows (Blem 1975), digestive efficiency apparently improves with age early in life (Karasov 1990). The results of this study provide a mechanistic explanation. The rates of chemical breakdown and nutrient absorption interact positively with digesta retention time to determine the efficiency of digestion (the proportion of ingested energy not eliminated in excreta; Karasov 1996). The biochemical rates increase through day 6 (Figs. 4, 6). There are no studies of mean retention time (MRT) during development of any bird, but we expect that it also increases, based on the following reasoning. MRT is expected to increase with increasing gut size or decrease with increasing feeding rate (Karasov 1996). It is apparent from the data on organ mass change (Fig. 1) and the data on feeding rate (Blem 1975; Lepczyk et al. 1998) that the volumetric capacity of the gut (stomach + intestine) increases more rapidly than feeding rate up through day 6 of life. Taking the quotient of gut size (in g) and intake rate (in g d^{-1}) as the index of MRT time (in d; Karasov 1996), it follows that MRT time should initially increase to a maximum at day 6. Because both the biochemical rates and the MRT increase up through day 6, one might expect a notable rise in digestive efficiency. In accord with this expectation, digestive efficiency of house sparrow nestlings rises steadily from day 1 to day 6, a total of 14 percentage points (Blem 1975). It is interesting that, overall, the various parameters measured in three studies (Blem 1975; Lepczyk et al. 1998; and this study) appear to fit together in logical fashion.

We suspect that these correlated changes will occur in nestlings of many altricial species. The alimentary tract grows rapidly, as discussed above, and early in the nestling period, its size scales with (nestling mass)$^{>1.0}$ (see Gille et al. 1999 for review). Nestling energy needs probably scale with mass$^{0.85}$ (Weathers 1996). At some point during development (e.g., day 6 in house sparrows), intestine mass ceases to increase, whereas both body mass and food intake continue to increase. At this transition point, then, the allometry changes, with gut size now scaling with mass$^{\sim 0}$ but intake still scaling positively with mass. Therefore, the scaling of MRT, which is proportional to gut size/intake, changes from positive (mass$^{>1.0}$/mass$^{0.85} \propto$ mass$^{>0.15}$) to negative (mass^{0}/mass$^{+0.85} \propto$ mass$^{-0.85}$). Before the transition point, biochemical reaction capacity almost certainly increases with gut mass and should interact with increasing MRT to yield rising digestive efficiency. This is in accord with the limited number of studies of digestive efficiency during development (Karasov 1990).

Table 2: Comparison of feeding and digestive parameters in 12-d-old nestling and adult house sparrows

Parameter	12-d-old Nestlings (Mean ± SE; $n = 12$)	Adults[a] Mean ± SE	n	Statistical Comparison
Body mass (g)	25.9 ± .9	25.9 ± .8	13	$F_{1, 23} = .01$, $P > .9$
Small intestine wet mass (g)	1.47 ± .07	1.46 ± .2	13	$F_{1, 23} = .01$, $P > .9$
Pancreas wet mass (mg)	231 ± 11	153 ± 9	23	$F_{1, 33} = 24$, $P < .001$
Aminopeptidase-A (μmol min^{-1} g^{-1} whole intestine)	5.82 ± .37	3.74 ± .36	13	$F_{1, 23} = 16.3$, $P < .001$
Maltase (μmol min^{-1} g^{-1} whole intestine)	32.9 ± 9.2	85.1 ± 8.9	13	$F_{1, 23} = 17.3$, $P < .001$
Trypsin (units min^{-1} g^{-1} pancreas)	.142 ± .010	.105 ± .008	22	$F_{1, 32} = 7.8$, $P = .009$
Amylase (units min^{-1} g^{-1} pancreas)	37.7 ± 2.6	20.6 ± 1.9	23	$F_{1, 33} = 29$, $P < .001$
Chymotrypsin (units min^{-1} g^{-1} pancreas)	4.82 ± .27	1.47 ± .19	23	$F_{1, 33} = 104$, $P < .001$
Gross energy intake (kJ d^{-1})[b]	102	85		
Energy digestibility (%)[b]	67	69.1		

[a] Unless otherwise indicated, these data are pooled from two groups of house sparrows, eating either high-carbohydrate or high-protein diet for 10 d, from Caviedes-Vidal et al. (2000) and Caviedes-Vidal and Karasov (1995). The mean of the protein contents of those two diets was 37%, and the mean of the carbohydrate contents was 38%. Lipid accounted for 8% of the mass of both diets. For comparison, the protein/carbohydrate/lipid content of the nestling diet was 49%/25%/8% (Lepczyk et al. 1998). All the diets had similar amounts of vitamins, minerals, and nonnutritive bulk (cellulose, silica).

[b] From Blem (1975) for birds fed a diet with 49% protein; sample sizes not specified.

Comparison of Digestive Biochemistry of Nestling and Adult House Sparrows

Histological study of the intestine during growth of altricial European starlings has shown that intestinal crypts and mucosal epithelium are already topographically separated from each other at hatch, and this arrangement does not change from hatchling to adult (Starck 1996). Although we have emphasized the dynamic nature of digestive biochemistry during nestling growth, comparison of many of our data on nestlings with those collected in adult house sparrows (Caviedes-Vidal and Karasov 1996; Caviedes-Vidal et al. 2000; E. Caviedes-Vidal and W. Karasov, unpublished data) shows a number of similarities.

We could discern no age-dependent differences in the pH dependence of intestinal enzymes in our limited number of tests (Fig. 7). In contrast, there were differences between nestlings (Fig. 8) and adults (Caviedes-Vidal et al. 2000) in the apparent K_m^* for disaccharidases. When compared with previously determined K_m^* for maltasic and sucrasic activity in adults (3.0 ± 0.2 and 6.7 ± 2.5 mM, respectively; $n = 3$; Caviedes-Vidal et al. 2000), it appears that the K_m^*s were higher in nestling house sparrows (for maltase: ANOVA $F_{2,5} = 8.3$, $P = 0.026$, or $P = 0.044$ by Kruskal-Wallis test; for sucrase: ANOVA $F_{2,5} = 28.0$, $P = 0.002$, or $P = 0.044$ by Kruskal-Wallis test). Interestingly, the sucrasic-independent maltasic activity apparently increased with age (Fig. 9), and perhaps this partly accounts for the observed changes in the apparent affinity for maltase (Fig. 8). These age-dependent differences in enzymatic biochemistry invite speculation about changes in isozymes during ontogeny, a suggestion made in earlier studies of proventricular and pancreatic enzymes of chickens (Yasugi et al. 1979; Yasugi and Mizuno 1981).

Both nestling and adult house sparrows appear to exhibit similar patterns of enzyme activity regionally along the small intestine. In the distal intestinal region of both, aminopeptidase activities are highest, whereas maltase and sucrase activities are the lowest, when compared with other intestinal regions (Fig. 3). Age-related changes in activity appear related to these patterns. For example, aminopeptidase activity increased with age mainly in the distal region, whereas age-related changes in maltase and sucrase activity occurred mainly in the more proximal regions of the small intestine (Fig. 3).

At time of fledging (12–15 d), the major digestive organs (small intestine, pancreas) of nestling house sparrows are similar in mass to or larger than those of adults (Table 2). Many of their tissue-specific digestive enzyme activities, especially proteases but not maltase, are somewhat higher than in adults, which in combination with the similar-sized or larger digestive organs yields higher enzymatic capacities for those enzymes. Absorption rates may also be somewhat similar, or slightly higher, as 11-d-old house sparrows had in vitro absorption rates for the amino acid L-leucine (ca. 8 pmol mg^{-1} min^{-1} at a concentration of 0.01 mM; Lepczyk et al. 1998) comparable to or slightly higher than those measured in a study of modulation of absorption in adult house sparrows (ca. 3–6 pmol mg^{-1} min^{-1}; Caviedes-Vidal and Karasov 1996).

These differences between nestlings and adults make sense

in light of the two major differences in their nutrition. At time of fledging, the nestlings are similar to adults in body mass, daily feeding rate, and diet digestive efficiency (Table 2). However, they have just completed a period of hyperphagy compared with adults (Blem 1975), and hyperphagy causes some hypertrophy of the alimentary tract in adult birds (Karasov 1996) and precocial chicks (Nir et al. 1978; Nir and Nitsan 1979), although possibly not in altricial chicks (Konarzewski et al. 1996; Konarzewski and Starck 2000). Besides relative hyperphagy, chicks also differ somewhat from adults in diet because they may ingest more invertebrate prey (see natural diet description above). Thus, the chicks' relatively higher ratios of protein- to carbohydrate-digesting enzymes in both the intestine and pancreas might reflect that the transition to the adult diet is not quite complete. Thus, in the weeks following fledging, we would predict some final modest changes in relative enzyme activities and also some regression in the size of some alimentary organs. The decline in stomach mass between day 9 and day 12 (Fig. 1) suggests that the regression has already begun for that organ at time of fledging.

Conclusions

Our study of nestlings was descriptive, but we were able to extend the interpretation of the data considerably and test some predictions by relying on a number of other studies of digestion, nutrition, and growth in house sparrows. In addition, we have generated some new predictions and some general principles that might guide future research in a topical area in which there has been little previous study and few guiding principles. We are thus eager to see whether the following patterns we observed or predicted are borne out in future research with altricial nestlings.

1. In other species, will tissue-specific levels of digestive enzymes change by >10 times during development?

2. In other species, does an increase in retention time and digestive enzyme capacity early in nestling life lead to rising digestive efficiency, as apparently occurs in house sparrow nestlings?

3. Can digestive enzymes be modulated when nestlings are raised on different kinds of diets, which might occur naturally for some omnivorous species, or are the apparent changes like those observed in house sparrows part of a fixed developmental program?

4. Will future studies of overfeeding in altricial species demonstrate hypertrophic response by the stomach and intestine and faster growth, as occurs for precocial species and possibly hinted at in house sparrows?

5. Will nutrient absorption during growth and development of an altricial species, so far unstudied except for one limited report (Konarzewski and Starck 2000), change in parallel with changes in digestive enzymes?

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