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Developmental adjustments of house sparrow (*Passer domesticus*) nestlings to diet composition

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SUMMARY

House sparrow nestlings are fed primarily on insects during the first 3 days of their life, and seeds become gradually more important afterwards. We tested whether developmental changes in size and functional capacity of the digestive tract in young house sparrows are genetically hard-wired and independent of diet, or can be modified by food type. Under laboratory conditions, we hand-fed young house sparrows with either a starch-free insect-like diet, based mainly on protein and fat, or a starch-containing diet with a mix of substrates similar to that offered to older nestlings in natural nests when they are gradually weaned from an insect to a seed diet. Patterns of overall development in body size and thermoregulatory ability, and in alimentary organ size increase, were relatively similar in house sparrow nestlings developing on both diets. However, total intestinal maltase activity, important in carbohydrate breakdown, was at least twice as high in house sparrow nestlings fed the starch-containing diet (P<0.001). The change in maltase activity of nestlings was specific, as no change occurred in aminopeptidase-N activity in the same tissues. There was no significant diet effect on digesta retention time, but assimilation efficiency for radiolabeled starch tended to be higher (P=0.054) in nestlings raised on starch-containing diet. Future studies must test whether the diet-dependent increase in maltase activity during development is irreversible or reversible, reflecting, respectively, a developmental plasticity or a phenotypic flexibility.

Key words: developmental flexibility, digestive physiology, diet composition, digestive enzymes, house sparrow, *Passer domesticus*.

INTRODUCTION

Several recent studies have addressed the question how an animal's digestive system copes with challenges imposed by varying environmental factors (e.g. Brzęk and Konarzewski, 2001; Brzęk and Konarzewski, 2004; Ciminari et al., 2005; McWilliams and Karasov, 2005; Naya and Bozinovic, 2006; Naya et al., 2007). The phenotypic variability that can be expressed by a single genotype is called phenotypic plasticity (Pigliucci, 2001), and phenotypic flexibility if it is reversible (Piersma and Drent, 2003). Phenotypic plasticity of the gastrointestinal tract is important in feeding ecology because it can influence the magnitude of the energy budget and the dietary niche (Karasov and Martinez del Rio, 2007). In the first case, it can permit an animal to increase its food intake beyond its typical digestive capacity when expenditures (e.g. thermoregulation) or allocations (e.g. storage, reproduction) increase. Adult birds may have spare digestive capacity allowing them to increase food intake by about 50% (Karasov and McWilliams, 2005), but increases of 100% or more are generally accompanied by simultaneous increases in mass of the small intestine and biochemical digestive capacity (McWilliams et al., 2002; Karasov and McWilliams, 2005; McWilliams and Karasov, 2005). In the second case, digestive plasticity may permit an animal to eat a larger variety of foods and thus increase its dietary niche. Not all adult birds exhibit such plasticity to the same extent [c.f. *Dendroica* species (McWilliams and Karasov, 2005)], but in some birds biochemical changes in digestive enzymes and intestinal nutrient transport, as well as changes in digesta retention time, help match substrate flow to rates of breakdown and absorption (Karasov and Martinez del Rio, 2007).

Our understanding of the digestive plasticity of nestling birds is more limited. A few studies have tested for nestlings' abilities to adjust to higher rates of food intake, which could be important in adjusting growth rates after periods of food shortage. Those studies indicate relatively low plasticity relative to adult birds. Nestlings challenged to eat more barely accomplished increases greater than 20% above normal level without associated declines in digestive efficiency, and they failed to increase their intestine mass and total biochemical digestive capacity above that of nestlings fed at normal rates (Lepczyk et al., 1998; Konarzewski and Starck, 2000). However, we are not aware of any studies that tested abilities of altricial nestlings to digestively accommodate changes in food composition. The goal of this study was to evaluate this capacity in an omnivorous species, the house sparrow (*Passer domesticus* Linnaeus 1758). House sparrows, like many bird species, change their diet during ontogeny. Whereas adult house sparrows are mostly granivorous, nestlings are fed primarily on insects during the first 3 days of their life and plant material becomes gradually more important afterwards (Anderson, 2006). While it might be hypothesized that they adjust their digestive physiology to the different types of food, there is also the question of whether those adjustments are a programmed component of the developmental schedule or are responsive to whatever diet is consumed. To illustrate, house sparrow nestlings' conversion to a higher carbohydrate plant-based diet coincides with a marked increase in the specific activity of key carbohydrate digesting enzymes, maltase–glucoamylase and sucrase–isomaltase complexes (Caviedes-Vidal and Karasov, 2001), but it is not known whether

this change is entirely programmed [hard-wired, *sensu* Toloza and Diamond (Toloza and Diamond, 1992)] or is directly responsive to the increase in consumption of carbohydrates as parents begin to deliver more plant material. Would the pattern of change in intestinal enzyme activity levels change if nestlings remained entirely insectivorous? The capacity of nestlings to adjust their digestive development could be ecologically important in species, such as house sparrows, that are generally opportunistic and for whom the food type brought to their nestlings reflects variation in its temporary abundance in environment (Anderson, 2006).

We studied the growth, development, and digestive physiology of young house sparrows hand-fed to fledging with either a starch-free insect-like diet, based mainly on protein and fat, or a starch-containing diet with a mix of substrates more similar to that offered to nestlings in natural nests that are gradually weaned from an insect to a seed diet. This experimental design allowed us to test whether developmental changes in size and functional capacity of the digestive tract in young house sparrows are genetically hard-wired and independent of diet, or can be modified by food type. Our null hypothesis was no developmental plasticity; for example, we expected that young house sparrows would not alter the development schedule of maltase activity because this activity in adults did not differ significantly in those fed diets with high (61%) *versus* low (14%) starch levels (Caviedes-Vidal et al., 2000). We also tested for diet-related differences in other key features of digestive structure and function such as intestine mass and aminopeptidase-N activity, digesta retention time, and overall ability at the whole-animal level to digest starch. Also, in order to test the nestlings' overall plasticity in processing the different diets, we tested for diet-based differences in growth, in both mass and structural size, sizes of other organs important in digestive processing (pancreas, liver), and the development of thermoregulatory capacity.

MATERIALS AND METHODS

Most of our methods were developed during previous experiments with hand-fed house sparrow nestlings (Lepczyk et al., 1998; Lepczyk and Karasov, 2000; Caviedes-Vidal and Karasov, 2001).

Study site and collection of nestlings

Natural and artificial nest sites were located in close vicinity to the Department of Forest and Wildlife Ecology on the campus of the University of Wisconsin, Madison. House sparrows bred in wooden nest boxes placed in the dairy barns, and in cavities inside and outside of the dairy barns. Beginning in late March 2007, all potential nesting locations were visited twice per week to note the onset of laying. Broods were checked daily around the expected time of hatching to ensure accurate aging. Nestlings were marked on their back with an indelible marker, and returned to the nest. The day when hatchlings were found was subsequently counted as day 0.

Nestlings selected for the experimental treatments were removed from their nests between 10:30 and 12:30 h on day 3 and transported to our laboratory at the University of Wisconsin. Only nestlings that had hatched synchronously on day 0 were used in the experiment. In most cases two nestlings were collected from one clutch. To control for nest effect, nestlings from the same clutch were randomly assigned to different diets. When nestlings from the second brood from the same nest were collected, they were assigned to trials of different length (see below) than individuals from the first brood. All nestlings used in the present experiment were collected between May 18 and July 18. Nestlings were placed in round (12 cm×9 cm) tissue-lined plastic containers and housed in an environmental chamber under constant conditions of 15 h:9 h light:dark photoperiod, 35°C, and 40–45% relative humidity using a water bath system. Body mass was measured three times per day, tarsus length at days 3, 8, and 12, and wing length at days 8 and 12.

Feeding protocol

Based on their random assignment, nestlings were hand-fed with either of two synthetic liquid diets developed by E. Caviedes-Vidal (Lepczyk et al., 1998). Both diets were composed of protein (casein), corn oil, free amino acids, and vitamin and microelements (Table 1). The diet intended to mimic insects consumed by young house sparrows during the first 3 days post-hatch contained no starch at all, 20% corn oil and 59.63% casein in dry mass, and is hereafter referred to as '0 starch'. The other diet, intended to mimic the mixture of insects and plant (seed) material, contained 25.4% corn starch, 8% corn oil and 46.23% casein, and is hereafter referred to as '+starch'. Both diets contained 75% water on a wet-mass basis, which provided an adequate amount of water for hydration.

Nestlings were removed from the environmental chamber every hour and fed by gavage using a 1-ml syringe for a total of 15 times per day, beginning at 06:30 h. Syringe mass (±0.01 g) was recorded prior to and following feeding to calculate the exact meal mass. The age-specific feeding schedule for was 0.3, 0.5, 0.6, 0.75, 0.85, 1.0, 1.25 and 1.5 ml of food per hour for nestlings of ages 3, 4, 5, 6, 7, 8, 9 and 10–12 days, respectively. Measured wet mass intakes were corrected to dry mass based on daily aliquots of both diets that were weighed, dried and reweighed. Age-specific dry matter intake rates never differed between diets by more than 5%.

Experimental schedule

Performance of nestlings was tested at three time points during their development which were selected on the basis of previous knowledge of the ontogeny of digestive anatomy and physiology in house sparrow nestlings (Caviedes-Vidal and Karasov, 2001). They were: day 4 (the phase of rapid development of gastrointestinal tract); day 6 (the day of the peak in relative intestine mass), and day 12 (2–3 days before normal fledging, when adult body mass is already reached). Thus, we created six experimental groups: nestlings fed with 0 starch or +starch diets, and analyzed at day 4, 6 or 12. Total number of nestlings dissected in each group was for the +starch diet: 7, 14, 11 and for the 0 starch diet: 7, 15, 10. Nestlings assigned to different diets and analyzed at different time points did not differ in their initial body mass at day 3 (two-way ANOVA; effect of age P=0.27; effect of diet: P=0.7, interaction n.s.).

Several trials, which could be completed without sacrificing nestlings, were carried out at days 4 and 6 on individuals that were subsequently used in later time points. Because we could usually carry out only one trial per day, for older chicks we had to spread

Table 1. Composition of diets used in the present study

	0 starch (% of dry mass)	+starch (% of dry mass)
Corn starch	0	25.4
Casein (protein)	59.63	46.23
Corn oil	20	8
Alphacel non-nutritive bulk	4.9	4.9
Silica sand	5	5
Aminoacids, vitamins, mineral salt etc.*	10.47	10.47

Diets were mixed with distilled water in the ratio by mass of 1:3 (diet:water).
*Content as described by Lepczyk et al. (Lepczyk et al., 1998).

our protocols over 2 or even 3 days. A detailed description of our schedule is given in Table 2.

Tracking of body mass and structure measures

Body mass (±0.01 g) was recorded daily before the first feeding at 06:30 h, again after the 13:30 h feeding, and after the last feeding, 20:30 h. Tarsus (±0.01 mm) and wing length (±1 mm) were measured at approximately 13:30 h on days 3 (tarsus), 8 (tarsus and wing), and 12 (tarsus and wing) of nestling growth. At days 4, 6 and 12, nestlings were dissected; at which time the masses of internal organs were recorded (±0.1 mg).

Tracking of thermoregulatory ability

Prior to the 06:30 h feeding, ≥5-day-old nestlings underwent a cooling challenge trial (Seel, 1969) daily, in order to determine at what age the nestlings were able to regulate their body temperature. Their cloacal temperature (±0.1°C) was measured immediately after removal from the environmental chamber with a thermocouple thermometer (BAT-12; Physitemp Instruments, Clifton, NJ, USA). They were then placed into a water jacketed tissue-lined beaker (6.5 cm×12 cm) the temperature of which was controlled at 20±0.1°C. After 15 min, their cloacal temperature was measured again and they were returned to the environmental chamber.

Organ sizes

Nestlings were euthanized with CO_2 in the evening (between 17:00 h and 21:00 h), and dissected to remove the intestines, stomach, liver, pancreas and pectoral muscles. Intestines were flushed with ice-cold avian Ringer solution, weighed, cut into three sections, corresponding to proximal, middle and distal regions, and immediately preserved in liquid nitrogen. Similarly, the stomach was emptied, and all organs cleaned of external fat and tissues, rinsed with ice-cold avian Ringer solution, and weighed.

Intestinal enzyme assays

Lengths (1 cm) of the proximal (first 20%), medial (middle 40%–60%) and distal (last 20%) regions of intestine were cut, weighed, opened longitudinally and their length and width measured (±0.1 mm), and stored in cryo-vials in liquid N_2, for later measurement of intestinal disaccharidase 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 (Martinez del Rio, 1990).

We assayed maltase activity using a modification of the colorimetric method developed by Dahlqvist (Dahlqvist, 1984). Assays are described in detail elsewhere (Martinez del Rio, 1990; Fassbinder-Orth and Karasov, 2006) and in our previous studies with nestling house sparrows (Caviedes-Vidal and Karasov, 2001), including details about pH optima and the apparent binding constants (K_m^*), the concentration of substrate at which the rate of hydrolysis equals half the maximal hydrolysis rate (V_{max}). Briefly, tissues were thawed at 4°C and homogenized (Omni 5000 homogenizer, Omni International, Waterbury, CT, USA; 20 s, setting 6) in 350 mmol l^{-1} mannitol in 1 mmol l^{-1} Hepes-KOH, pH 7.0. Gut homogenates (30 µl) diluted with 350 mmol l^{-1} mannitol in 1 mmol l^{-1} Hepes-KOH were incubated with 30 µl of 56 mmol l^{-1} maltose in 0.1 mol l^{-1} maleate and NaOH buffer, pH 6.5, at 40°C for 20 min. Next, 400 µl of a stop-develop reagent (GAGO-20 glucose assay kit; Sigma Aldrich, St Louis, MO, USA) was added to each tube, vortexed, and incubated at 40°C for 30 min. Lastly, 400 µl of 6 mol l^{-1} H_2SO_4 was added to each tube, and the absorbance was read at 540 nm.

We used L-alanine-*p*-nitroanilide as a substrate for aminopeptidase-N. To start the reaction we added 10 µl of the homogenate to 1 ml of assay mix (2.0 mmol l^{-1} L-alanine-*p*-nitroanilide in one part 0.2 mol l^{-1} NaH_2PO_4/Na_2HPO_4 buffer no. 1, pH 7 and one part deionized H_2O) previously heated to 40°C. The reaction solution was incubated for 20 min at 40°C and then ended with 3 ml of ice-cold 2 mol l^{-1} 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 and expressed them in micromoles per minute per gram wet mass of tissue or per nominal surface area (area of smooth bore tube). We calculated the summed hydrolysis activity of the entire small intestine, which is an index of the total hydrolysis capacity, by multiplying average activity per gram tissue in the first and last halves of intestine by their respective masses, and summed over the two regions.

Table 2. Experimental schedule

Days post-hatching	Protocols
3	Nestlings collected
4	Starch assimilation efficiency, passage times, dissection
6	Starch assimilation efficiency, passage times, dissection
10	Starch assimilation efficiency, passage times.
12	Dissection

Starch assimilation efficiency and digesta retention time

The assimilation efficiency of radiolabeled starch was measured by means of the inert marker method. Each trial began at 12:30 h. Nestlings were fed half of their typical meal, gavaged with a solution containing radiolabeled starch and inert marker, and then fed the remainder of their meal. We used 5 µCi [U-^{14}C]starch (ARC-142, American Radiolabeled Chemicals, St Louis, MO, USA), and 20 µCi of [^{3}H]polyethylene glycol (PEG; ART-502, American Radiolabeled Chemicals) as the inert marker, dissolved in 1 ml of distilled water as a carrier solution. Each nestling was gavaged with about 0.1 g of that solution, measured by mass change of the syringe. Excreta were collected individually for 2 h before gavage and for 8 h after gavage. Even though we collected excreta for the rest of the day, the last samples were still not at background level of radioactivity. Thus, our absolute estimates of mean retention time and extraction efficiency may be too low, but all comparisons between groups should be valid.

Samples were dissolved in 3 ml of distilled H_2O, refrigerated, and shaken periodically for a minimum of 72 h. An aliquot of 0.5 ml was then sampled, combined with scintillation cocktail (EcoLume, MP Biomedicals, Solon, OH, USA), and counted (d.p.m.) using a liquid scintillation counter (Wallac 1414 WinSpectral, Turku, Finland), with correction for quench and spill of ^{14}C into ^{3}H counts. The assimilation efficiency of starch was then calculated as $100-[100(M_f/N_f)\times(N_e/M_e)]$, where M_f and M_e are the radioactivity of the inert marker in, respectively, food and excreta, and N_f and N_e are the radioactivity of starch in the food and excreta, respectively.

Mean retention time was calculated by multiplying the proportion of PEG excreted at each defecation by the elapsed time since ingestion and then summing over all intervals.

Data analysis

Results are given as means ± 1 s.e.m. (N=number of nestlings per treatment). All tests were carried out using SYSTAT (Wilkinson,

1992) and SAS software. We tested for effects of age and diet on body mass, tarsus and wing lengths, and cloacal temperatures on all nestlings held until age 12 days, using repeated measures analysis of variance (ANOVA). Calendar day of measurement had a strong effect on nestlings' cloacal temperature, reflecting presumably difference in handling by different people. To control for this effect, we re-analyzed our data for change in cloacal temperature separately for each day of nestling's life by means of two-way ANOVA (factors=calendar day of measurement and diet). Organ sizes were analyzed with two-way ANOVA or ANCOVA (factors=diet [+starch and 0 starch] and age at dissection [4, 6 and 12 days], initial body mass as covariate). We could not use body mass at dissection as a covariate in these analyses, because its range differed significantly between ages, which violates an assumption of ANCOVA. Rather, we re-analyzed our data with ANCOVA separately for each age, with diet as a main effect and with body mass at dissection as a covariate. Starch assimilation and digesta retention time were analyzed with two-way ANOVA (factors=diet, age), and also using ANCOVA with percentage recovery of PEG as a covariate, because recovery was <100% and varied among individuals. To analyze intestinal enzyme activities we used two-way ANOVA (factors=diet, age) on each of the three intestinal positions (proximal, mid, distal) and on activity summed over the entire length of the intestine. The *F*-values of analyses of variance are presented in the text with the relevant degrees of freedom as subscripts. In all tests, the significance level was set at $P<0.05$, and $0.05<P<0.1$ was taken to indicate a trend.

RESULTS

Our results can be grouped into two categories: those estimating general performance (growth and development) of young house sparrows on two different diets and those comparing their digestive function.

Effect of diet on growth and development of whole body and body parts

We analyzed body mass measured in the morning, before first feeding, to avoid potential confounding effect of changes in content of gastrointestinal tract (Fig. 1A). Diet had no overall significant effect on body mass ($P=0.11$, repeated-measures ANOVA). However, there was a significant interaction between age and diet ($P=0.016$), which reflected slightly lower body mass in nestlings fed on the 0 starch diet compared with the +starch diet, a difference that increased with age (Fig. 1A). This diet effect was not significant when analyzing body mass measures made at midday (data not shown).

Diet had no significant effect on tarsus length measured at day 3, 8 and 12 (Fig. 1B), or on wing length (Fig. 1C) measured at days 8 and 12 (repeated measurement ANOVA; for both parameters effect of diet $P>0.8$ and diet *versus* age interaction $P>0.2$).

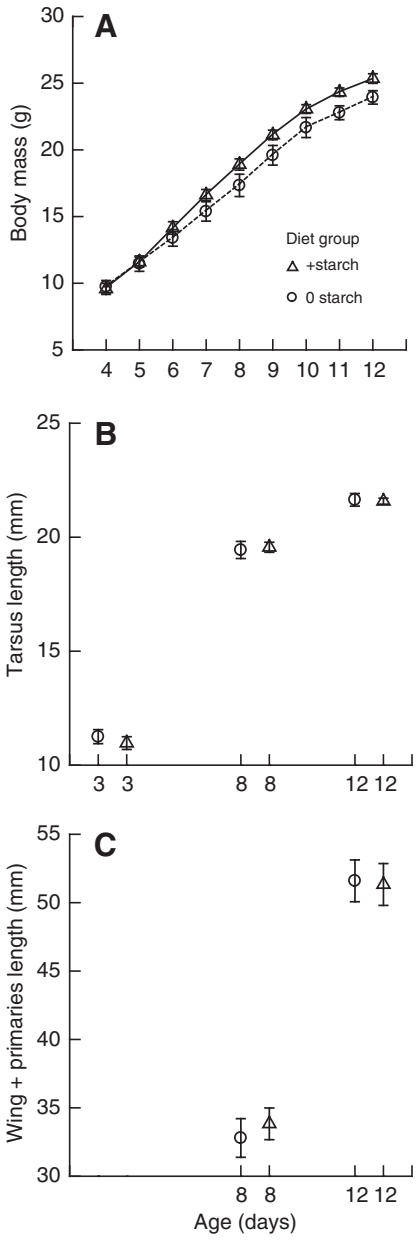

Fig. 1. Repeated measures over ages on house sparrow nestlings for (A) body mass, (B) tarsus length, and (C) wing length. Data are presented as means ± s.e.m. Circles, nestlings fed on the 0 starch diet ($N=10$); triangles, nestlings fed on +starch diet ($N=12$). There was a significant interaction between diet and age for body mass, reflecting a difference between diet groups that increased with age (see Results).

Thermoregulatory ability

Cloacal temperature measured in nestlings first removed in the morning from their nest cups in the environmental chamber (initial T_b; Fig. 2A) increased with age (repeated measurement ANOVA; $P<0.0001$) but was not affected by diet ($P=0.13$). Nestlings cooled when transferred to the water-jacketed chamber at 20°C, but the change in cloacal temperature (ΔT) declined as they aged up to about 10 days (Fig. 2B; repeated measures ANOVA; effect of age $P<0.0001$), with no significant difference by diet (effect of diet $P=0.33$; interaction age *versus* diet $P=0.23$). We found very large differences in the change in cloacal temperature between calendar days, presumably reflecting differences in nestlings' handling or undetected variation in measurement conditions. To control for this variation, we analyzed our data independently for each nestling age by means of two-way ANOVA, with diet as the main factor and the calendar day as a random factor. Diet again had no significant effect on $T_{initial}$ for all nestling ages. However, ΔT was significantly higher in nestlings fed on 0 starch diet at day 6 ($P=0.011$), day 10 ($P=0.0004$), and tended to be higher at day 11 ($P=0.07$).

Organ sizes

Age had significant effect on the mass and length of intestine, as well as mass of pancreas, liver and pectoral muscle (Fig. 3, Table 3).

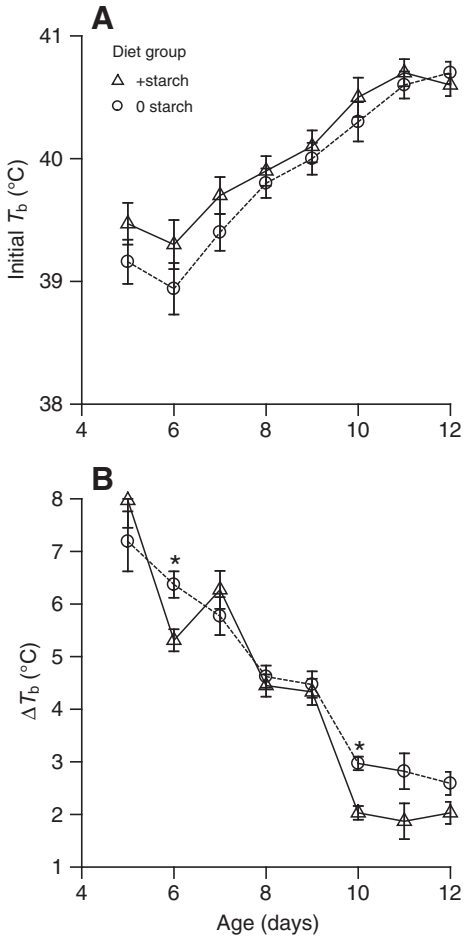

Fig. 2. Cloacal temperatures of captive house sparrow nestlings *vs* age (in days) and diet. (A) Line graphs of cloacal temperatures initially when removed from the environmental chamber. (B) Line graphs of the change (Δ) in cloacal temperature after 15 min at 20°C. These are adjusted least squares mean values (±s.e.m.) from a statistical analysis which revealed that diet had a significant effect on ΔT_b at days 6 and 10 (asterisks).

For all these organs, all comparisons between different age points were significant. Gizzard was the only studied organ that did not change size with age. However, diet had little effect on the size of studied organs. The only significant difference was a larger liver mass in nestlings fed on 0 starch diet. The same group tended also to have larger pancreas mass and lower pectoral muscle mass though both effects were only marginally significant or non-significant (Table 3). Interaction of age *vs* diet was non-significant for all studied organs.

Additionally, we compared size of all organs between both diets independently for every time point by means of ANCOVA using body mass at dissection as a covariate. The only significant difference between diets was found for liver mass at day 12, which was heavier in nestlings fed on 0 starch diet (P=0.025).

Intestinal enzyme activities

Diet had a highly significant effect on mass-specific maltase activity in the middle and distal part of the intestine but no significant effect on mass-specific activity of aminopeptidase-N (Fig. 4, Table 4). Patterns were the same when data were expressed per cm^2 nominal area (Fig. 4; statistical analysis not shown).

Both age and diet had highly significant effects on summed maltase activity along the entire length of intestine (Fig. 5, Table 4). Nestlings fed the on +starch diet showed significantly higher summed maltase activity. Interaction between age and diet was also significant, revealing that the difference between diets increased as nestlings grew older. Activity of aminopeptidase-N, both on a mass- and area-specific bases (Fig. 4) and summed over the entire intestine (Fig. 5) also increased with age but was not dependent on diet composition (Table 4).

Starch assimilation efficiency and digesta retention time

Assimilation efficiency of radiolabeled starch (Fig. 6A) was not affected by nestlings' age (ANOVA, P=0.46) or by diet, although it tended to be higher in birds fed on +starch diet (P=0.054; percentage of recovered PEG was significant as covariate).

Mean retention time (Fig. 6B) was significantly affected by nestlings' age (ANOVA, P<0.0001; percentage of recovered PEG was significant as covariate). There was no difference between mean retention time at days 4 and 6 (P=0.9), but retention time at day 10 was significantly shorter than for both earlier time points (P<0.0001). Diet had no significant effect on mean retention time (P=0.22).

DISCUSSION

An emerging research frontier is integrated physiological and developmental studies that increase understanding of how an organism's ontogeny is influenced by environmental and genetic background (Burggren and Warburton, 2005). In general, any response to environmental changes, such as changes in feeding level or nutrient composition in the case of the gut, can take one of two forms (Smith-Gill, 1983; Schew and Ricklefs, 1998; Schlichting and Pigliucci, 1998). Environment can have a direct effect on processes that underlie development and physiology by limiting availability of substrates and co-factors [phenotypic modulation *sensu* Smith-Gill (Smith-Gill, 1983); imposed variation *sensu* Schew and Ricklefs (Schew and Ricklefs, 1998)]. An example of this is the slowing of growth in zebra finch (*Taeniopygia guttata*) nestlings fed low protein diet (Boag, 1987). Environment can also have indirect effects by activating alternative developmental program(s) [developmental conversion *sensu* Smith-Gill (Smith-Gill, 1983); induced variation *sensu* Schew and Ricklefs (Schew and Ricklefs, 1998)], presumably by modulation of gene expression through changes in hormonal secretions and/or other physiological processes.

The rapid turnover of intestinal cells and high rate of enterocyte proliferation of hatchling birds suggested to Starck (Starck, 1996) that the gut might easily be adjusted in size and function to the changing needs of growing birds. However, after experiments with altricial song thrushes (*Turdus philomelos*), Konarzewski and Starck (Konarzewski and Starck, 2000) concluded that nestling songbirds had limited plasticity of the developmental program of their guts. In our study we tested for developmental adjustments of house sparrow nestlings to change in diet composition. We did not dramatically alter diet composition to directly limit growth (i.e. an imposed variation), seeking instead evidence of alternative outputs of the house sparrow developmental program. Young house sparrows face variable and, presumably, unpredictable diet composition (Anderson, 2006). The exact timing of their switch from insectivory to granivory can be affected by temporary variation in abundance of different types of food (Anderson, 2006). Therefore, considering the feeding ecology of this species, there might be strong selection for plasticity of their digestive physiology. We tested for this plasticity with regard to alimentary organ size and functional

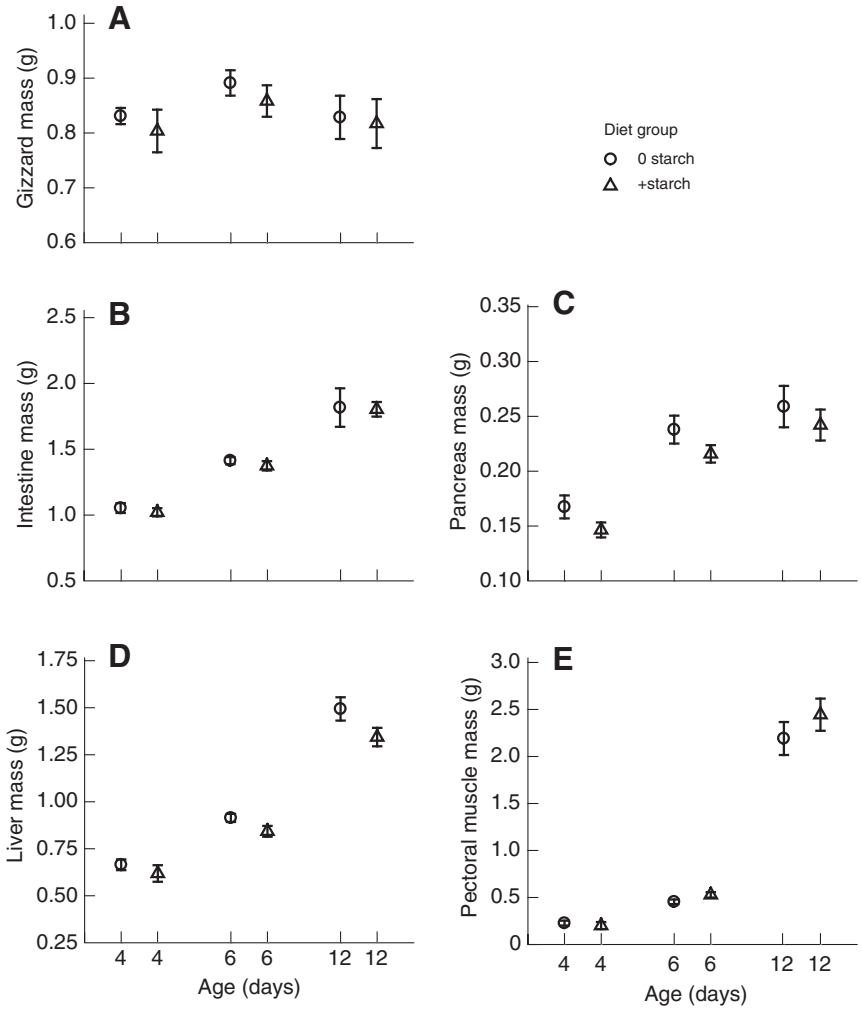

Fig. 3. Mass of internal organs of captive house sparrow nestlings as a function of age and diet. Circles, mean organ mass of nestlings fed on the 0 starch diet (N=32); triangles, mean organ mass of nestlings fed on the +starch diet (N=32). Age had a significant effect on all organs except gizzard, and there was a significant effect of diet group only on liver mass at day 12 (see Results and Table 3 for statistical analyses).

activity of the gastrointestinal tract, as well as with regard to overall growth and development (body mass increments, development of wing, tarsus, pectoral muscle, and physiological maturity defined as endothermic capacity). To what extent was development of these features relatively fixed or influenced by diet?

Effect of diet on growth and development of whole body and body parts

Our experiment revealed that patterns of overall development and organ size increase were relatively similar in house sparrow nestlings developing on diets with different composition. Young house sparrows seemingly developed normally at least until day 12 on a carbohydrate-free diet, which is very different from the common food of such nestlings in the wild. Moreover, an earlier study showed that young house sparrows were not able to compensate for periods of food shortage by increasing their growth rate (no apparent 'catch-up growth'), although they did extend the period over which they grew (Lepczyk and Karasov, 2000). Thus, for these features (growth and development of whole body and body parts) the developmental program seems relatively fixed in relation to the nutritional challenge we imposed.

However, some clues suggest that nestlings' development was slightly impaired on the 0 starch diet. First, their body mass measured in the morning was lower than in those fed on +starch, and this difference increased with age (Fig. 1A). However, this difference was so small that it became non-significant for body mass measured later during the day, when it was presumably blurred by variation in the content of gastrointestinal tract. Second, pectoral muscles tended to be slightly smaller in the group fed 0 starch. Finally, although all the nestlings apparently attained their thermoregulatory independence at the typical time (10.5 days) (Seel, 1969), the nestlings fed on 0 starch seemed to attain theirs slightly later (significantly higher ΔT at day 10; Fig. 2B). By contrast, birds on the 0 starch diet had larger livers and the pancreas tended to be larger than those on +starch diet. This difference presumably reflected a necessity to process higher protein content in the diet of the former group. However, all these differences between diets were relatively small and perhaps have no significant effect on nestlings' fitness in the wild.

Effect of diet on functional activity of the digestive tract

In mammals, there are examples of changes in digestive enzymes during development that are genetically programmed and less influenced by diet or hormones [hard-wired, *sensu* Toloza and Diamond (Toloza and Diamond, 1992)], and also examples of changes that can be influenced or induced by diet changes (Henning, 1985; Henning et al., 1994). In chickens after hatching, tissue-specific intestinal carbohydrase activity (sucrase, maltase) initially rises and then is constant with age after day 17, and the plateau level is twice as high in chicks fed a high-carbohydrate *versus* carbohydrate-free diet (Biviano et al., 1993). In this regard, chickens appear similar to mammals in whom diet modulates expression

Table 3. Summary of results of ANOVA or ANCOVA for the effects of diet and nestling age on size of internal organs

	Age			Diet			Initial body mass		
	F	d.f.	*P*	*F*	d.f.	*P*	*F*	d.f.	*P*
Intestine mass	63.44	2,60	<0.0001	0.34	1,60	0.6	n.s.	n.s.	n.s.
Intestine length	58.41	2,59	<0.0001	1.02	1,59	0.32	n.s.	n.s.	n.s.
Pancreas mass	23.92	2,60	<0.0001	4.05	1,60	0.049	n.s.	n.s.	n.s.
Gizzard mass	2.23	2,60	0.12	0.92	1,60	0.34	n.s.	n.s.	n.s.
Liver mass	195	2,60	<0.0001	8.89	1,60	0.0041	n.s.	n.s.	n.s.
Pectoral muscle mass	374	2,59	<0.0001	3.76	1,59	0.057	24.69	1,59	<0.0001

levels of many intestinal hydrolases during ontogeny but it does not seem to influence expression timing, which is determined by both corticosteroids and an intrinsic ontogenetic program (Henning, 1985; Henning et al., 1994). No such tests have been performed in altricial avian species previously. As a starting hypothesis, it seemed reasonable to expect that house sparrow nestlings, like chickens, would show rising levels of carbohydrases during development, but, unlike chickens and turkeys [*Meleagris gallipavo* (Sell et al., 1989)], the magnitude of expression would be diet-independent in house sparrows. This is because higher carbohydrate diet content did not increase maltase activity in almost all adult altricial birds studied: European starlings [*Sturnus vulgaris* (Martinez del Rio, 1990)], yellow-rumped warblers [*Dendroica coronata* (Afik et al., 1995)], rufous-collared sparrow (*Zonotrichia capensis*) and common diuca finch (*Diuca diuca*) (Sabat et al., 1998), house sparrow (Caviedes-Vidal et al., 2000) and pigeons [*Columba livia* (Ciminari et al., 2005)]; however see Levey et al. (Levey et al., 1999) for an apparent exception.

We found that maltase activity increased twofold in house sparrow nestlings fed a starch-containing compared with a starch-free diet, which is in contrast to the apparent lack of dietary adjustment to high carbohydrate diet in adult house sparrows and most other passerines. The twofold increase in maltase activity may be an underestimate, because the tissue sample we used for enzyme assays to represent the proximal part of the intestine was collected from the area immediately adjacent to the gizzard, where activity was relatively low compared with more distal regions (Table 4). The change in maltase activity of nestlings was specific, as no change occurred in aminopeptidase-N activity in the same tissues. Activity of aminopeptidase-N in birds and mammals is usually more diet-dependent than maltase activity (Martinez del Rio, 1990; Afik et al., 1995; Martinez del Rio et al., 1995; Sabat et al., 1998; Sabat et al., 1999). However, we found no significant effect of diet on activity of aminopeptidase-N, although it tended to be higher in 0 starch nestlings, which consumed more protein. However, the relative difference in protein content between diets was low and this could explain the lack of significant modulation of aminopeptidase-N activity in our study.

Was the change in maltase activity a plastic response of a genetic program in nestlings that is expressed in response to dietary

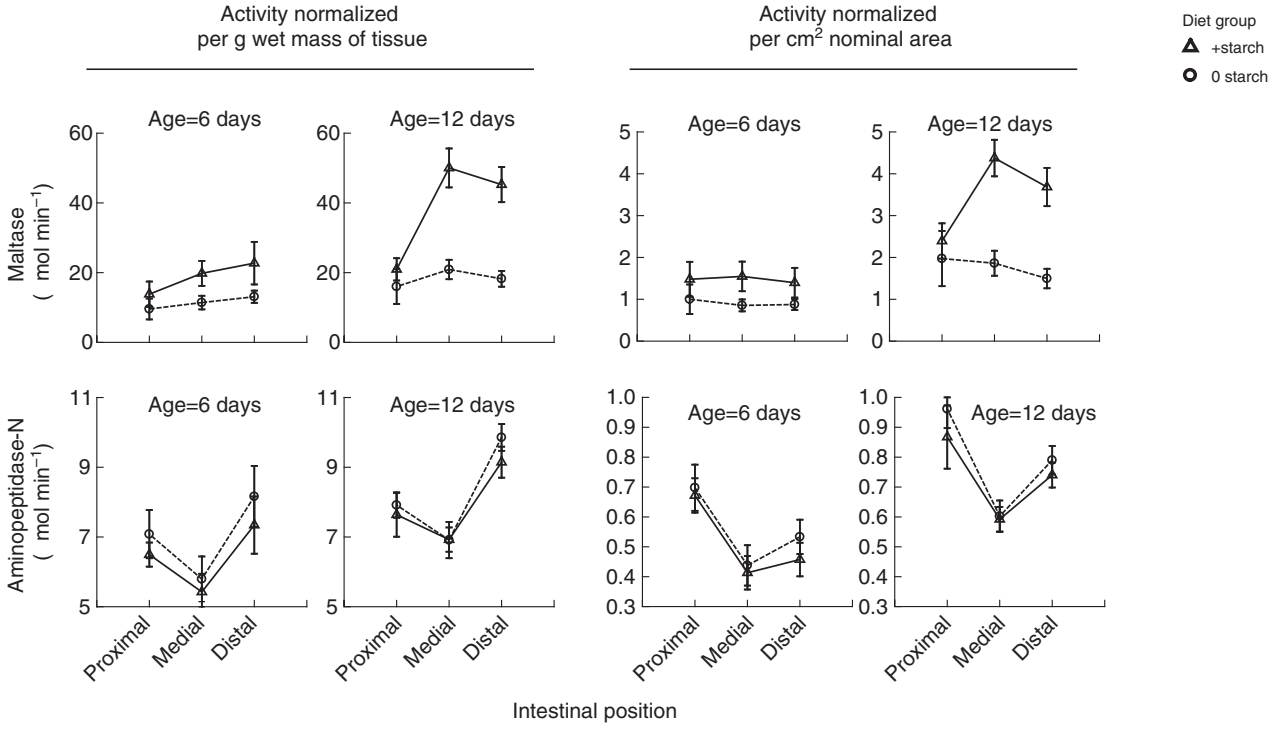

Fig. 4. Intestinal enzyme activities of captive house sparrows as a function of age and diet. Maltase activity (top row) and aminopeptidase-N activity (bottom row) are expressed per g wet mass of tissue or per cm^2 nominal area in three intestinal positions. Error bars are s.e.m. (N=7–8 individuals in each case). Diet had a significant effect only on maltase activity (see Results; Table 4).

Table 4. Summary of results of ANOVA for the effects of diet and nestling age on maltase and aminopeptidase-N activity (mass-specific activity in proximal, middle, and distal part of intestine, and total enzyme activity summed over intestine length)

	Age			Diet			Interaction age *vs* diet		
	F	d.f.	*P*	*F*	d.f.	*P*	*F*	d.f.	*P*
Maltase									
Proximal	3.96	1,26	0.057	1.82	1,26	0.19	n.s.	n.s.	n.s.
Middle	34.81	1,25	<0.0001	31.05	1,25	<0.0001	9.49	1,25	0.005
Distal	13.33	1,25	0.0012	23.37	1,25	<0.0001	5.26	1,25	0.03
Total (summed)	58.03	1,25	<0.0001	27.42	1,25	<0.0001	8.96	1,25	0.006
Aminopeptidase-N									
Proximal	3.88	1,26	0.06	0.76	1,26	0.39	n.s.	n.s.	n.s.
Middle	7.15	1,26	0.013	0.14	1,26	0.7	n.s.	n.s.	n.s.
Distal	7.73	1,26	0.01	1.49	1,26	0.23	n.s.	n.s.	n.s.
Total (summed)	50.87	1,26	<0.0001	1.29	1,26	0.27	n.s.	n.s.	n.s.

carbohydrate level (i.e. an induced variation), but which is silent in adults? We cannot yet deduce this. In adult house sparrows, maltase activity can be reduced – high dietary oil content but not high protein content resulted in significantly lower maltase activity (Caviedes-Vidal et al., 2000). In the present experiment, the carbohydrate-free diet also had higher oil content than the starch-containing diet (Table 1). In mammals, dietary lipid content and composition influences the lipid composition and fluidity of the intestinal brush border and influences enzyme activities in some species (Dudley et al., 1994; Kaur et al., 1996; Drozdowski et al., 2004). If the same occurs in birds, then the change we observed in nestling house sparrows might be considered an imposed variation. To clarify the dietary signal (carbohydrate or lipid) that modulates intestinal maltase activity, future studies with nestling house sparrows must change dietary carbohydrate content while keeping dietary lipid content constant. Future studies must also test whether the diet-dependent increase in maltase activity during development is irreversible or reversible, reflecting, respectively, a developmental plasticity or a phenotypic flexibility that is lost later in life. It will also be interesting to learn whether the change in maltase activity is due to mRNA transcriptional or post-transcriptional control of maltase–glucoamylase and/or sucrase–isomaltase complexes.

It has been hypothesized that reversible regulation of enzyme activity, modulated by the presence of substrates, should occur in generalist and omnivorous animals but not in dietary specialists, because natural selection should eliminate presumably costly mechanisms of regulation of enzymes when little variation in diet type is expected [‘adaptive modulation hypothesis’ (Karasov and Diamond, 1988; Diamond and Hammond, 1992)]. In many

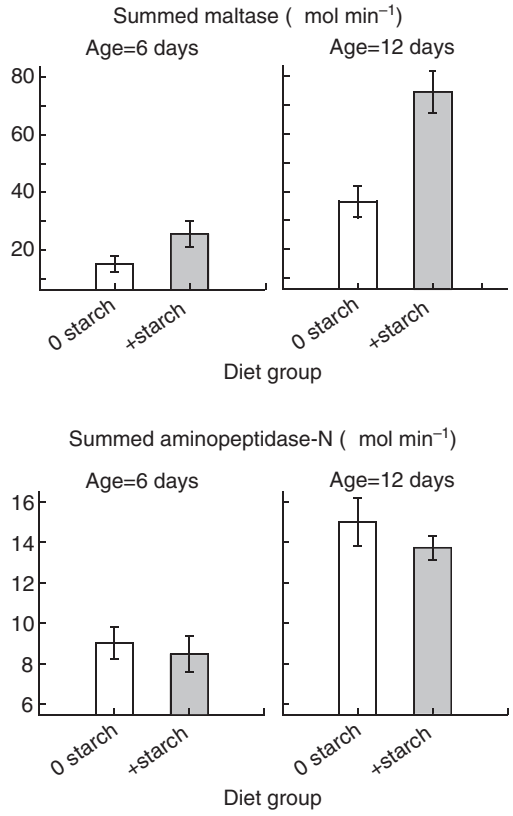

Fig. 5. Enzyme activities summed over the entire small intestine as a function of age and diet in house sparrow nestlings. Values are means ± s.e.m. (*N*=7–8 individuals in each case). Summed maltase activity was significantly higher in nestlings fed on the +starch diet (Table 4).

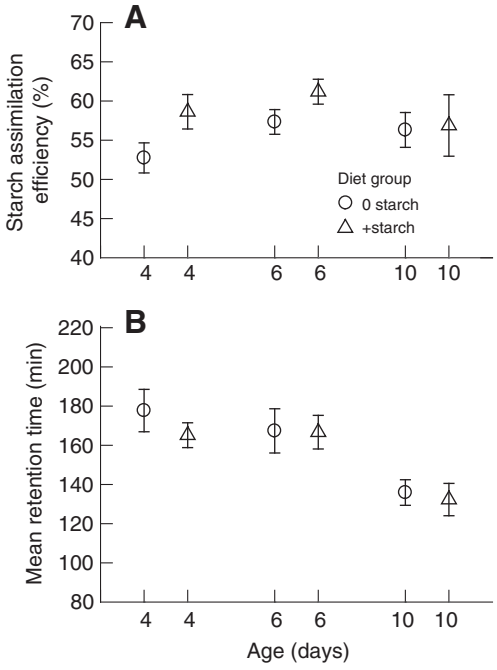

Fig. 6. (A) Efficiency of assimilating radiolabeled starch, and (B) mean retention time of digesta as a function of age in captive house sparrows. Mean retention time declined with age, and there was a trend for higher assimilation efficiency in sparrows raised on the +starch diet (see Results).

organisms, changes in enzyme activity during ontogeny correspond to the natural diet shift (Caviedes-Vidal and Karasov, 2001; Moran and Clements, 2002; Drewe et al., 2004; German et al., 2004). However, experimental manipulation of diet composition in growing organisms has offered only weak evidence for a link between diet variation and digestive plasticity. Enzymatic activity of tadpoles was unaffected by diet, even though amphibians show remarkable plasticity in their development, and commonly change their diet composition during ontogeny (Sabat et al., 2005; Castañeda et al., 2006). Similarly, digestive plasticity in fish species that change their diet during development was not significantly greater than in their close relatives that rely on one food source (German et al., 2004). Remarkable variation in maltase activity that we observed in young house sparrows agrees with predictions of the 'adaptive modulation hypothesis' because house sparrow nestlings change their diet during ontogeny. However, the full test of this hypothesis would also require the evidence that nestlings' digestive physiology is less flexible in species that rely on the same diet from hatching through adulthood [e.g. the wholly granivorous zebra finch (Zann, 1996)].

Whole-animal significance of programmed- or diet-induced changes in features during development

The increased maltase activity in nestlings fed a starch-containing diet is probably important in permitting house sparrow nestlings to efficiently digest their diet, because several lines of evidence suggest that nestlings have relatively little spare digestive capacity. First, between days 6 and 12 digesta flow per hour increases *ca*. 100% (see Materials and methods) through organs whose volumetric capacities increase not at all (stomach) or <30% (intestine). This probably explains the significant decline in digesta mean retention time (Fig. 6B) and hence contact time between digesta and digestive enzymes. A simultaneous increase in substrate (carbohydrate) concentration as diet shifts to granivory would exacerbate the mismatch between substrate and capacity to break it down, which is the product of amount of enzyme and contact time. Consistent with this picture of operating at the limit, when house sparrows were force fed extra food to capacity, they exhibited declines in digestive efficiency (Lepczyk et al., 1998). In our data there are similar hints of stretched capacity. Assimilation efficiency for radiolabeled starch tended to be higher (P=0.054; Fig. 6A) in nestlings that had higher maltase activity (i.e. those raised on starch-containing diet). Our starch digestion trial was actually a very conservative test for differing abilities, for the following reason. During the trial, nestlings were fed on their routine diets. Therefore, nestlings fed on the +starch diet had to digest and absorb both the pulse of radiolabeled starch and the starch from their normal diet. On the other hand, in nestlings fed on 0 starch diet, radiolabeled starch did not compete with other carbohydrates for interaction with enzymes. Thus, the observed small difference in starch assimilation efficiency probably underestimates the true difference between the two groups of nestlings in their capacity to digest starch. Because diet type seemed to have no effect on glucose absorption (P.B., E.C.-V. and W.H.K., unpublished), differences in starch absorption efficiency resulted presumably mostly from differences in breakdown activity. Whereas we know that maltase activity differed, it remains to be seen whether pancreatic amylase levels also differed.

In summary, young altricial birds are characterized by a very fast rate of body mass growth and general development, the requirements for which must be fulfilled by changes in the gastrointestinal tract. In house sparrows, there are substantial increases in both organ size and mass-specific enzyme activity during the nestling period (Caviedes-Vidal and Karasov, 2001). It is of interest to know whether these changes are controlled mainly by a relatively fixed genetic program or affected also by environmental factors (primarily food type). Our results suggest that changes in mass of internal organs are largely diet independent. Maltase activity was greatly influenced by diet, although some increase in maltase activity is diet independent (there was increase in mass-specific maltase activity between 6- and 12-day-old nestlings fed on both diets). The changes in maltase activity seem important for maintaining digestive efficiency and rate at the whole animal level. Because young house sparrows face variable and, presumably, unpredictable changes in diet composition (Anderson, 2006), the high developmental plasticity of their digestive physiology is probably important ecologically. Moreover, because adaptability of the gastrointestinal tract may determine species food niche (Karasov, 1996), we hypothesize that house sparrows' plasticity during development could also help in colonization of new areas with new food sources.

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