Digestive Responses during Food Restriction and Realimentation in Nestling House Sparrows (*Passer domesticus*)

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Accepted 4/28/98

ABSTRACT

We used nestling house sparrows (*Passer domesticus*) under laboratory conditions to test for modulation of digestive efficiencies during periods of low and high food intake and tested the hypothesis that nestlings would exhibit compensatory changes in digestive efficiency following a period of food restriction. During the low intake period, nestlings were held at constant body mass for 48 h beginning on either day 3 or day 6 of life by feeding them at 50% of control rations. After 48 h of food restriction, nestlings were fed as much as they could consume, allowing the nestlings restricted at day 6 (early restriction not assessed) to consume 14% more food than control nestlings. For nestlings restricted at day 6 apparent dry mass assimilation of the entire diet was found to be 5% and 8% lower during food restriction and realimentation, respectively, compared with control nestlings that were not under- or overfed. There were no significant differences in radiolabeled starch assimilation efficiencies between control and restricted nestlings. Starch assimilation efficiencies remained constant from 3 d of age onward in control nestlings. Total starch extracted was lower during food restriction but reached a rate similar to that of control nestlings during the realimentation period. Passage times (time of first defecation, mean retention time, and mode passage time) measured with an indigestible marker were longer during food restriction and shorter during realimentation, relative to control nestlings. During realimentation there was no difference in intestinal rates of hydrolysis or mediated uptake of L-leucine compared with control nestlings. The main effect of changing food intake was apparently to alter flow rate, and hence retention time, causing slight changes in digestive efficiency. Thus, nestlings did not exhibit compensatory changes in digestion rates as implied by the hypothesis. Our finding of a lower dry mass assimilation efficiency and similar total starch assimilation during realimentation (relative to controls) helps explain why nestling house sparrows do not display compensatory growth, despite higher food intake. Our results indicate that the gut has little spare capacity to deal with increased food intake during growth following food restriction.

Introduction

Ephemeral changes in food abundance are often cited as a primary extrinsic factor governing postnatal growth rates in birds (Cruz and Cruz 1990; see Martin [1987] for review). These temporary fluctuations in food abundance can result in fluctuations in food intake. Schew and Ricklefs (1998) suggested that when a nestling experiences such a fluctuation it would respond in such a way that optimizes its survival. Specifically, a nestling might exhibit compensatory changes in rates of maturation, structural growth, and/or metabolism (Schew and Ricklefs 1998). Because there are no studies of digestive physiology in nestling birds displaying the altricial mode of development, it is totally unknown what compensatory mechanisms may occur in the digestive system.

Digestive responses to changing rates of food intake during postnatal growth have classically been studied in poultry species using the food restriction–realimentation protocol (see, e.g., Wilson and Osbourn 1960; Yu et al. 1990; Santoso et al. 1995). Under this protocol, food intake, and hence energy intake, is reduced for a given duration of time followed by a period of ad lib. feeding. Recently, restriction-realimentation experiments have been applied to nestlings of wild birds, such as American kestrels, *Falco sparevicus* (Negro et al. 1994), song thrushes, *Turdus philomelos* (Konarzewski et al. 1996), and European starlings, *Sturnus vulgaris* (Schew 1995), as a means to investigate growth. However, these studies were not primarily concerned with the digestive responses to changing levels of food intake. Poultry studies, on the other hand, have shown that "feed efficiency" (i.e., gain in body mass per gram of food eaten) increases during both restriction and realimentation, as a result of lower total food intake while the growth rate is maintained or increased. For example, female broiler chicks have higher feed efficiency ratios during both restriction and realimentation than ad lib. controls (Santoso et al. 1995). Simi-

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Physiological Zoology 71(5):561–573. 1998. 0031-935X/98/7105-9709$03.00

larly, male turkeys (Plavnik and Hurwitz 1988) that were food restricted for 10 d and White Rock chickens (Plavnik and Hurwitz 1985) that were restricted for 6, 10, or 14 d have a higher feed efficiency than ad lib. controls during the realimentation period. These increases in feed efficiency may be attributable to several different mechanisms. First, higher feed efficiency may result from an increase in gut mass and length that somehow increases digestive efficiency. Such increases in gut mass were found in New Hampshire × White Leghorn crossbred chicks and White Rock chicks, which have increases of 56% and 22%, respectively, in intestinal mass after 18 d of overfeeding compared to ad lib. chicks (Nir et al. 1978). The exact time course for such increases, however, is unknown. A second mechanism by which feed efficiency could be increased is through a decrease in energy expenditure, so that relatively more absorbed energy is allocated to growth. However, because of the paucity of measurements of digestive efficiency and metabolism in growing poultry and altricial nestlings exposed to different rates of food intake, these mechanisms are open to question.

The hypothesis posed by Schew and Ricklefs (1998) suggests that nestlings exhibit compensatory rates of maturation, structural growth, and/or metabolism when faced with a change in the environment, such as a reduction in food abundance. Extending this hypothesis to include the digestive system implies that nestlings would increase digestive efficiency during periods of low food intake. Moreover, during subsequent periods of high food intake, a continued increase in digestive efficiency could provide a mechanism by which more energy is made available for growth. In turn, the growth rate might be accelerated, allowing nestlings to achieve compensatory growth (i.e., an accelerated age-specific growth rate, *sensu* Bohman [1955]).

Over the short term, however, it is unlikely that such an increase in energy could result from an increase in food intake or digestive efficiency. A primary reason for this is that the digestion of food represents a trade-off between processing rates of digesta and the thoroughness of digestion (Robbins 1993). Underlying this trade-off is the assumption that the gut has little "spare capacity" (safety margin; Diamond 1991). Spare capacity can be defined either as the excess (i.e., unused) enzymes or transporters available for hydrolysis and absorption or as an extra physical capacity that is unused in the digestive system. Studies with adult birds that experience a switch in diet support the assumption that the gut lacks a large spare capacity (Levey and Karasov 1989; Afik and Karasov 1995). Thus, if the gut acts as a chemical reactor (Penry and Jumars 1987), then increases in food intake (i.e., digesta flow) result in decreases in retention time because retention time (min) is proportional to gut volume (mL)/digesta flow (mL min^{-1}) (Karasov 1996). Similarly, decreases in food intake result in increases in retention time. Furthermore, because digestive efficiency is proportional to [retention time (min) × absorption rate (mol min^{-1})]/[concentration of digesta (mol mL^{-1}) × volume of digesta (mL)], a decrease in retention time causes a decrease in digestive efficiency (Karasov 1996). Under this paradigm, nestlings are expected to have an increased digestive efficiency during food restriction, but a decreased digestive efficiency during realimentation.

We used a restriction-realimentation protocol on nestling house sparrows (*Passer domesticus*) to change food intake in order to test the hypothesis that digestive efficiency is increased during restriction and realimentation. Assimilation efficiency is used as a proxy for digestive efficiency because, in birds, both urine and feces are excreted together (Karasov 1990). The difference between intake rates and excretory loss rates of dry matter, energy, or nutrients is more properly called an apparent assimilable fraction (apparent because it is uncorrected for endogenous losses). We measured the nestlings' assimilation efficiency of the overall diet as well as that of a specific component within the diet. Starch was the nutrient of choice as it constituted 25% of the diet and because radiolabeled proteins were prohibitively expensive. Additionally, we investigated the suborganismal aspects of digestive efficiency by measuring intestinal rates of hydrolysis and mediated uptake of L-leucine. Finally, we measured retention time so that we could interpret changes in digestive efficiency, should they occur, in the context of the chemical reactor model. All digestive measurements were made both during restriction and three days later in order to compare with other ongoing research. This study represents the first time that digestive physiology has been studied in nestling altricial birds.

Material and Methods

Study Site and Housing

All natural and artificial nest sites were located around the dairy barns of the University of Wisconsin—Madison, Madison, Wisconsin. Cardboard blue bird nest boxes (Midland Manufacturing Co., 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 insure accurate and consistent aging (Burger 1988). Nestlings were removed from their nests between 1030 and 1230 hours on day 3 (hatch = day 0), which was the youngest age that we previously had success in rearing, and 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).

To investigate the digestive responses to different rates of food intake during restriction and realimentation in the house sparrow, we set up three laboratory groups as follows: controls, early restricted, and late restricted. To control for nest effects, nestlings from the same clutch were randomly assigned to one

of the three groups (i.e., all treatments were filled once before adding a second nestling) upon being brought into the laboratory. All nestlings were placed into round (12 cm × 9 cm) tissue-lined plastic containers and housed in a custom-made environmental chamber with a 14L : 10D photoperiod and constant conditions of 35.56° ± 0.02°C and 61.91% ± 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 (casein), cornstarch, vitamins, minerals, and water (Appendix), and synthesized by ICN Biomedicals. 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 (E. Caviedes-Vidal, personal communication) age-specific feeding schedule for control nestlings: 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 (d) 3, 4, 5, 6, 7, 8, 9, and 10–16, respectively. Early restricted nestlings were fed just enough to keep body mass constant beginning on day 3 for 48 h, while late restricted nestlings were fed in a manner similar to control nestlings until day 6, at which time they were placed on a weight maintenance diet for 48 h. The weight maintenance level was found to be 0.20 mL food h^{-1} for early restricted nestlings and 0.40 mL food h^{-1} for late restricted nestlings, on the basis of a pilot group of nestlings. After 48 h of food restriction early and late restricted nestlings were fed to satiation every hour using a six-step feeding protocol. First, we tried feeding more than 125% of the age-specific level for control nestlings of the same chronological age. If the nestling could not consume more than 125%, we then tried feeding exactly 125% of the age-specific level for control nestlings of the same chronological age. As a third step, nestlings were fed either 0.06 mL h^{-1} (for early restricted) or 0.11 mL h^{-1} (for late restricted) above the age-specific control level, which provided the restricted nestlings an amount of food equal to that given to the control nestlings over the entire nestling period. The fourth step we tried was feeding the restricted nestlings at the age-specific control rate. If a nestling could not consume the control level, then it was fed whatever it was able to ingest. Finally, if the nestling refused to eat, was satiated, or regurgitated, it was skipped for that hour. Nestlings were hand-fed in this manner through day 16 to insure fledging age had been achieved (average fledging age is 14–15 d; Summers-Smith 1967; Novotný 1970).

Dry Mass Assimilation Efficiency

We used two separate groups of nestlings to measure assimilation efficiency of the whole diet and of a radiolabeled nutrient to avoid radioactive contamination of all our samples. As a result, we were limited in sample sizes and did not have enough nestlings to investigate the assimilation efficiency of the whole diet during the early restriction period. We therefore measured the whole-diet assimilation efficiency only in control ($n = 8$) and late restricted ($n = 8$) nestlings using the assimilable mass coefficient.

Because endogenous wastes mix with undigested material in the cloaca, the assimilable mass coefficient is an underestimate of the true assimilable mass coefficient (Guglielmo and Karasov 1993). As such, we expressed the values as apparent assimilable mass coefficient (AMC*), which was calculated as $1 - (Q_i/Q_e)$, where Q_i is the rate of food intake (g dry matter d^{-1}) and Q_e is the rate of excreta production (g dry matter d^{-1}; Karasov 1990). Trials began at 1230 hours and ran for a 24-h time period beginning on day 7 (time period 1) and again on day 10 (time period 2). Subsamples of food from trial days were weighed and freeze-dried to constant mass. The dry matter intake rate was then determined as wet mass ingested times the percentage dry mass. To collect excreta from nestlings, the nest containers were lined with absorbent plastic-backed paper, with the plastic lining forming the nest's surface. Excreta were collected at hourly feedings for 24 h by spraying the liner with distilled water, which washed all excreta into plastic cups. The samples were then frozen and later freeze-dried to constant mass to determine the rate of excreta production.

Starch Assimilation Efficiency and Retention Time

The assimilation efficiency of radiolabeled starch was measured on days 3, 5, 8, and 11 in control nestlings ($n = 9$), days 5 and 8 in early restricted nestlings ($n = 6$), and days 8 and 11 in the late restricted nestlings ($n = 6$) by means of the inert marker method (Karasov 1996). Experiments began at 0630 hours (day 3 control nestlings began at 1530), during a nestling's first feeding. Nestlings were fed half of their meal, gavaged with a mixture of radiolabeled starch and radiolabeled inert marker, and then fed the remainder of their meal. We used 74 kBq [1,2-^{3}H] polyethylene glycol [PEG, molecular mass of 4,000, American Radiolabeled Chemicals, St. Louis, Missouri] as the inert marker, together with 18.5 kBq of [^{14}C(U)] starch (American Radiolabeled Chemicals) and 21 μL of distilled H_2O as a carrier solution for each gavage. Excreta were collected individually for 6 h, with the last collection taking place immediately preceding the 1230 feeding. The 6-h collection period was based on time required for collections in similar sized adult wrens and warblers (4 h; Dykstra and Karasov 1992; Afik and Karasov 1995), with two extra hours added due to lack of knowledge of passage rates in nestlings. However, we subsequently found that the excreta sample collected at

hour 6 was not at background levels of radioactivity, so our absolute estimates of mean retention time and extraction efficiency may be slightly low, but comparisons between groups should still be valid. We processed the samples after Afik and Karasov (1995). In brief, samples were dissolved in 5 mL of distilled H_2O, refrigerated, and shaken periodically for a minimum of 72 h. An aliquot of the supernatant was then combined with scintillation cocktail (Ecolum; ICN Biomedical, Aurora, Ohio) and counted for disintegrations per minute (dpm) by liquid scintillation, with corrections for quench and appearance of ^{14}C counts in the ^{3}H channel. The assimilation efficiency of starch was then calculated as $100 - [100(M_f/N_f) \times (N_e/M_e)]$, where M_f is the radioactivity of the inert marker (PEG) in food, N_f is the radioactivity of the nutrient (starch) in the food, N_e is the radioactivity of nutrient (starch) in excreta, and M_e is the radioactivity of marker (PEG) in excreta. The total starch assimilated for the 6-h experiment was calculated for each individual as starch assimilation efficiency times the percent starch in the diet times the dry matter intake.

Several measures of digesta residence time were calculated for the 6-h experiment. 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 (Warner 1981). Mode passage time was the time of the defecation that contained the most PEG (Karasov 1996). Because all first defecations contained PEG, we compared elapsed time at first defecation instead of transit time (i.e., the time when marker is first detected in the excreta).

In Vitro Intestinal L-Leucine Uptake

We measured the mediated uptake by the intestine of L-[4,5-^{3}H(N)]leucine (Dupont New England Nuclear, Boston) across the brush border membrane as previously described by Karasov and Diamond (1983*b*). On day 11, intestines were removed from control and late restricted nestlings either when under Metafane anesthesia or immediately following cervical dislocation (there is no significant difference between these techniques; Caviedes-Vidal and Karasov 1996). In brief, 1-cm everted sleeves of the intestine were mounted on stainless steel rods (2–4 mm diameter) and placed in ice-cold avian Ringer solution (for composition, see Caviedes-Vidal and Karasov [1996]) until the measurement was made. After tissues were preincubated in 40°C Ringer solution for 5 min, they were suspended above a stir bar (1,200 revolutions per minute) in labeled (22,000–25,000 dpm 50 μL^{-1}) 0.01 mmol L^{-1} L-leucine for 2 min. Leucine was chosen because it is transported by the neutral amino acid carrier that many other amino acids share (Karasov et al. 1986). We determined uptake of 0.01 mmol L^{-1} L-leucine in the absence of Na^+ (replaced by choline) in adjacent tissues of the proximal region, in order to determine what proportion of the total L-leucine uptake was via Na^+-coupled active transport (Afik et al. 1995). Following incubation, the tissues were blotted, removed from the rod, weighed, incubated overnight in TS-1 tissue solubilizer (Research Products International, Mount Prospect, Ill.), and counted for disintegrations per minute. To correct for nonabsorbed leucine in adherent mucosal fluid, we used tracer concentrations of [carboxyl-^{14}C]inulin (American Radiolabeled Chemicals) or [1,2-^{14}C] PEG (American Radiolabeled Chemicals). L-leucine uptake rates were normalized to centimeter length of intestine

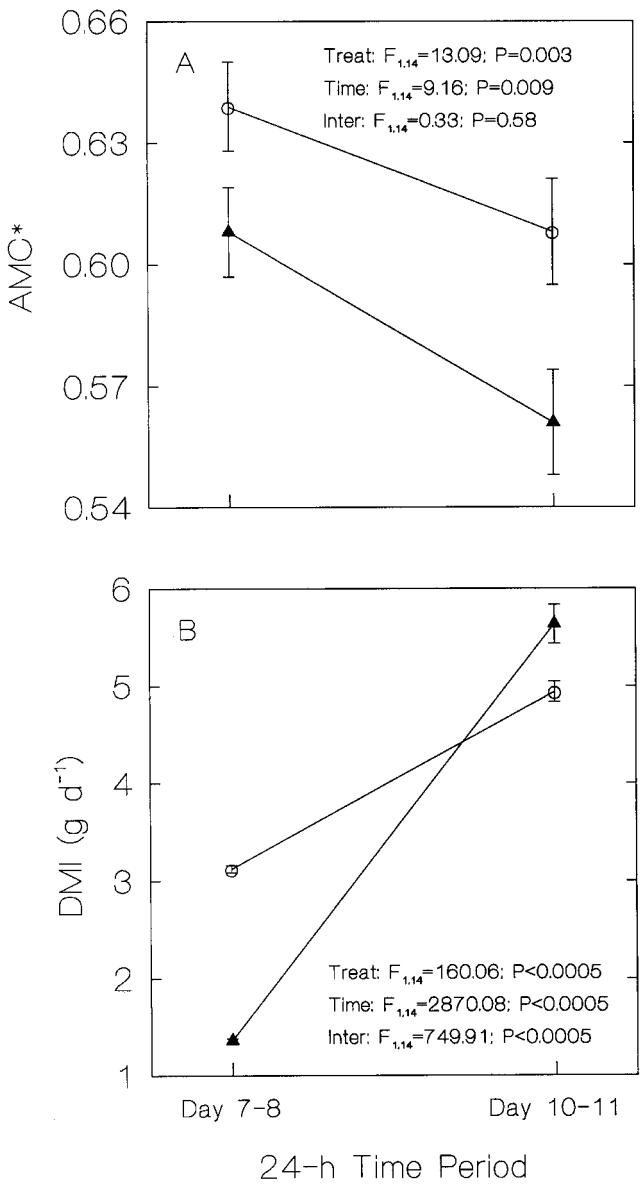

Figure 1. Mean ± 1 SE (*A*) apparent assimilable mass coefficient (AMC*) and (*B*) dry matter intake (DMI) of control (*circles; n* = 8) and late restricted (*triangles; n* = 8) nestlings at two separate 24-h intervals. Time period 1 = day 7 to day 8 and period 2 = day 10 to day 11. Dry matter intake was controlled in all instances except time period 2 in the late restricted nestlings, which were fed as much as possible. Statistics are the results of repeated-measures ANOVAs; *Treat,* main treatment effects; *Time,* time effects; *Inter,* interactions effects.

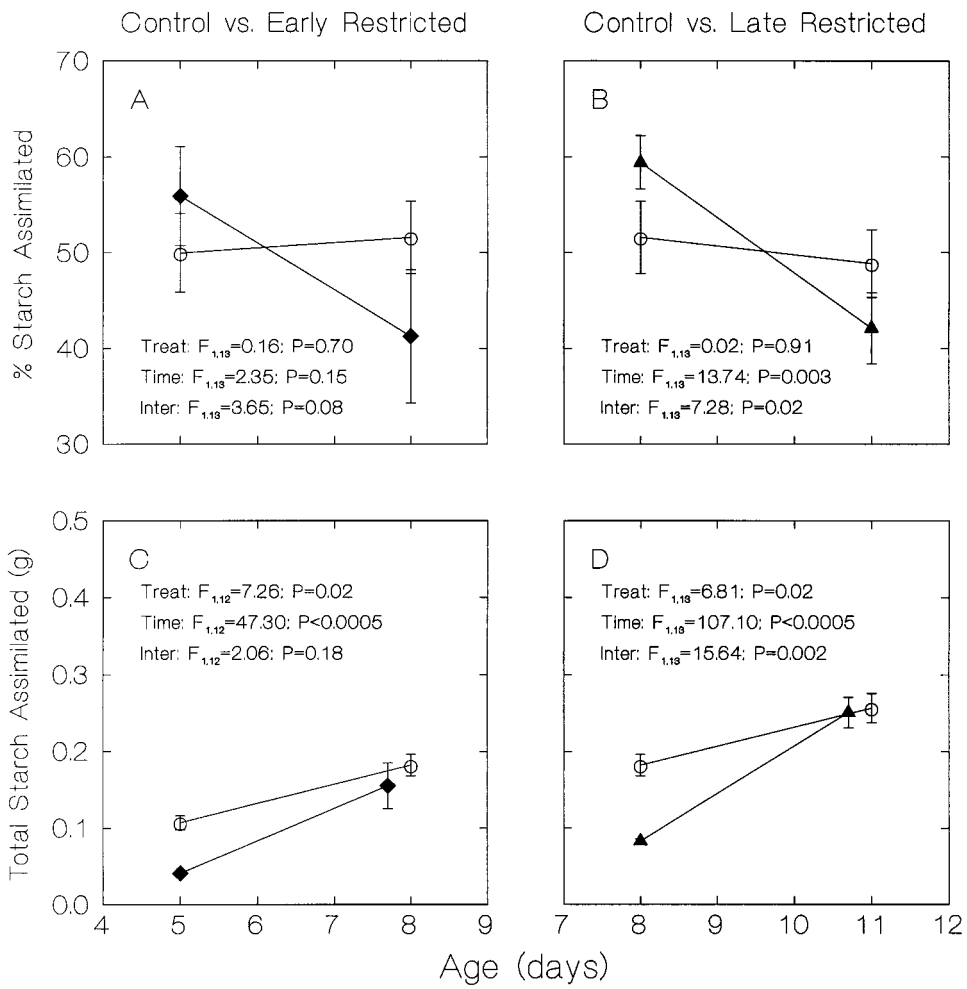

Figure 2. Assimilation efficiencies of radiolabeled starch measured by the inert marker technique and total starch extracted. *A* and *B* represent extraction efficiencies during the first (control vs. early restricted) and second (control vs. late restricted) time blocks in which nestlings were measured, respectively. *C* and *D* represent total starch extracted (g) during the 6-h trial. Mean ± 1 SE extraction efficiency for controls (*circles;* $n = 9$), early restricted (*diamonds;* $n = 6$), and late restricted (*triangles;* $n = 6$) nestlings as a function of age. Symbols are offset to display error bars. Statistical definitions are described in Figure 1.

but can also be expressed per milligram wet intestine by dividing the rates by intestinal mass.

Enzyme Assays

The small intestine, removed for L-leucine uptake measurement, was also used to measure enzymatic rates of hydrolysis. One-centimeter lengths of the proximal (first 20%), medial (middle 40%–60%), and distal (last 20%) region were cut, weighed, and stored in cryo-vials 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 (Martínez del Rio 1990).

We assayed disaccharidase (sucrase and maltase) activities using a modification of the colorimetric method developed by Dahlqvist (1984). Assays are described in detail in Martínez del Rio (1990). 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 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 of 0.2 mol L^{-1} NaH_2PO_4/Na_2HPO_4 buffer no. 1, pH

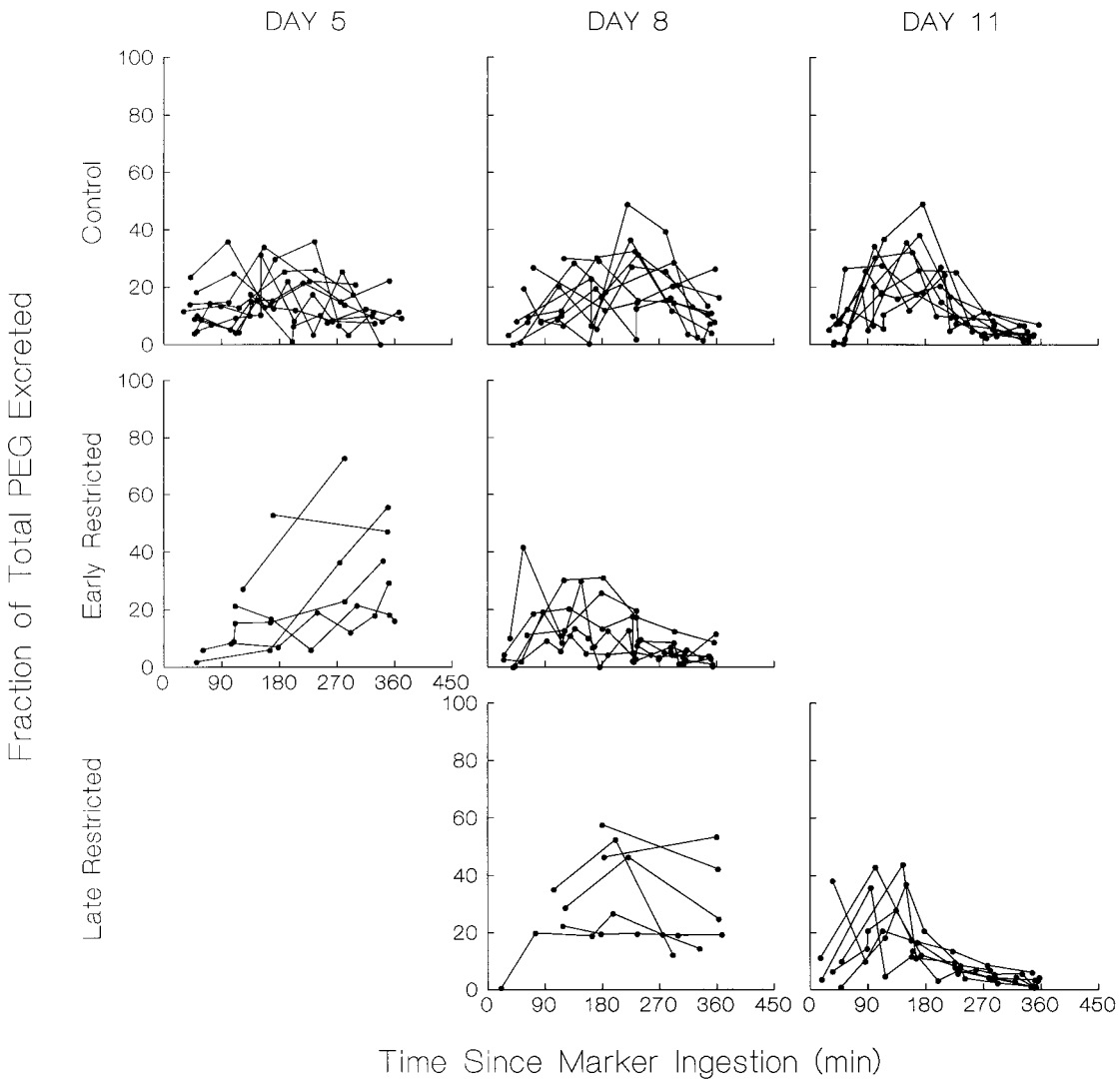

Figure 3. Output distributions of an ingested pulse of an inert marker (PEG). Plots represent the fraction of total amount of marker that came out at each time interval and were used to calculate mean retention time. Columns represent nestling ages, and rows represent the experimental group.

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., cat. no. 500-0006) as a function of intestinal mass. Thus, activities of aminopeptidase-N, maltase, and sucrase were expressed in micromoles per minute per gram wet tissue, micromoles per minute per gram protein, and micromoles per minute per centimeter.

Data Analysis

For statistical analysis, we broke the data into sets in order to test for effects of restriction and realimentation at two blocks of time. Thus, control and early restricted nestlings were compared during days 5 and 8, and control and late restricted nestlings were compared during days 8 and 11. All proportions were arcsine square-root transformed, and dry matter intake at time periods 1 and 2 were natural log transformed in order to normally distribute the data. Apparent assimilable mass coefficients, dry matter intake, starch extraction efficiency, total starch assimilated, mean retention time, time of first defecation, and mode passage time were compared at each time block with a repeated-measures ANOVA. A repeated-measures ANOVA was also used to analyze starch extraction efficiency, mean retention time, time of first defecation, and mode passage time of PEG within control nestlings on days 3, 5, 8, and 11. In vitro intestinal leucine uptakes, enzymatic rates, and intestine tissue mass (mg cm^{-1})

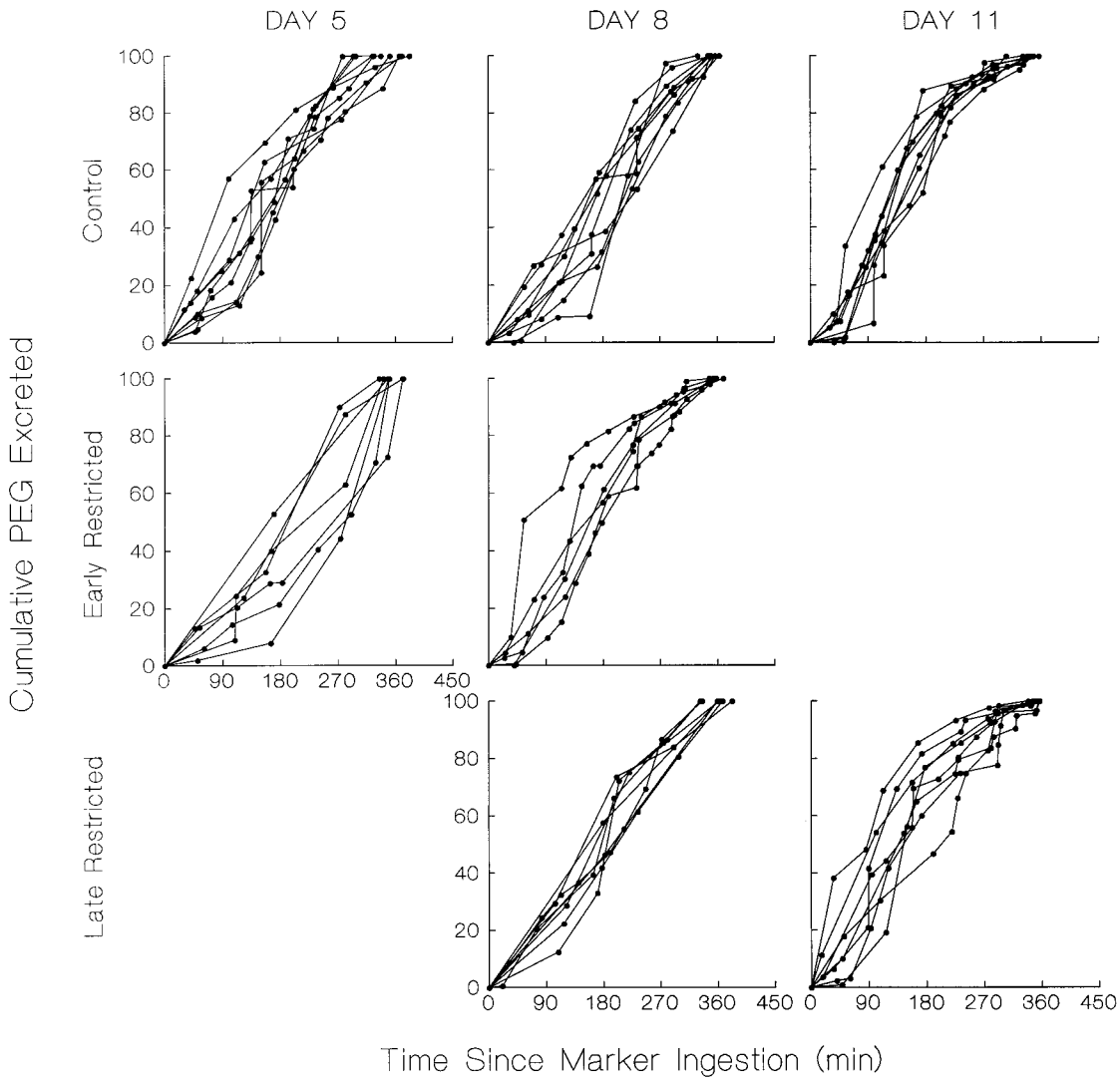

Figure 4. Cumulative excretion curves of the inert marker (PEG). Columns represent nestling ages, and rows represent the experimental group.

were compared between groups and intestinal location within nestlings using a repeated-measures ANOVA. The multivariate general linear hypothesis (MGLH) module in SYSTAT (Wilkinson 1992) was used for data analysis. All values reported are means ± 1 SE, unless otherwise noted. A value of $P < 0.05$ was considered significant, and $0.05 \leq P \leq 0.10$ was taken to indicate a trend.

Results

Digestibility and Assimilation Efficiency

Apparent assimilable mass coefficients in control nestlings were 5% and 8% higher than those of late restricted nestlings at time periods 1 and 2, respectively ($F_{1, 14} = 13.09$; $P = 0.003$; Fig. 1*A*). A decrease in apparent assimilable mass coefficient of 5% and 8% occurred in control and late restricted nestlings, respectively, from day 8 to day 11 ($F_{1, 14} = 9.16$; $P = 0.009$). Dry matter intake in late restricted nestlings was four times higher during realimentation than during restriction (Fig. 1*B*). However, compared with control nestlings, the late restricted nestlings achieved only a 14% increase in dry matter intake ($F_{1, 14} = 160.06$; $P < 0.0005$). When nestlings were fed more than this they regurgitated.

There was no significant difference in starch assimilation efficiency between control and restricted or realimented nestlings (Fig. 2*A, B*). This lack of significant differences between the treatments is likely the result of low sample sizes, which resulted in a low power. The assimilation efficiency of starch declined between the restriction and realimentation period in early and late restricted nestlings, but not control nestlings over the same time periods, as reflected in a significant or near significant interaction between time and treatment (see Fig. 2*A, B* for nestling statistics). The assimilation efficiency of starch in control nestlings on day 3 was 49.0% ± 5.0%. From day 3 to day 11, there

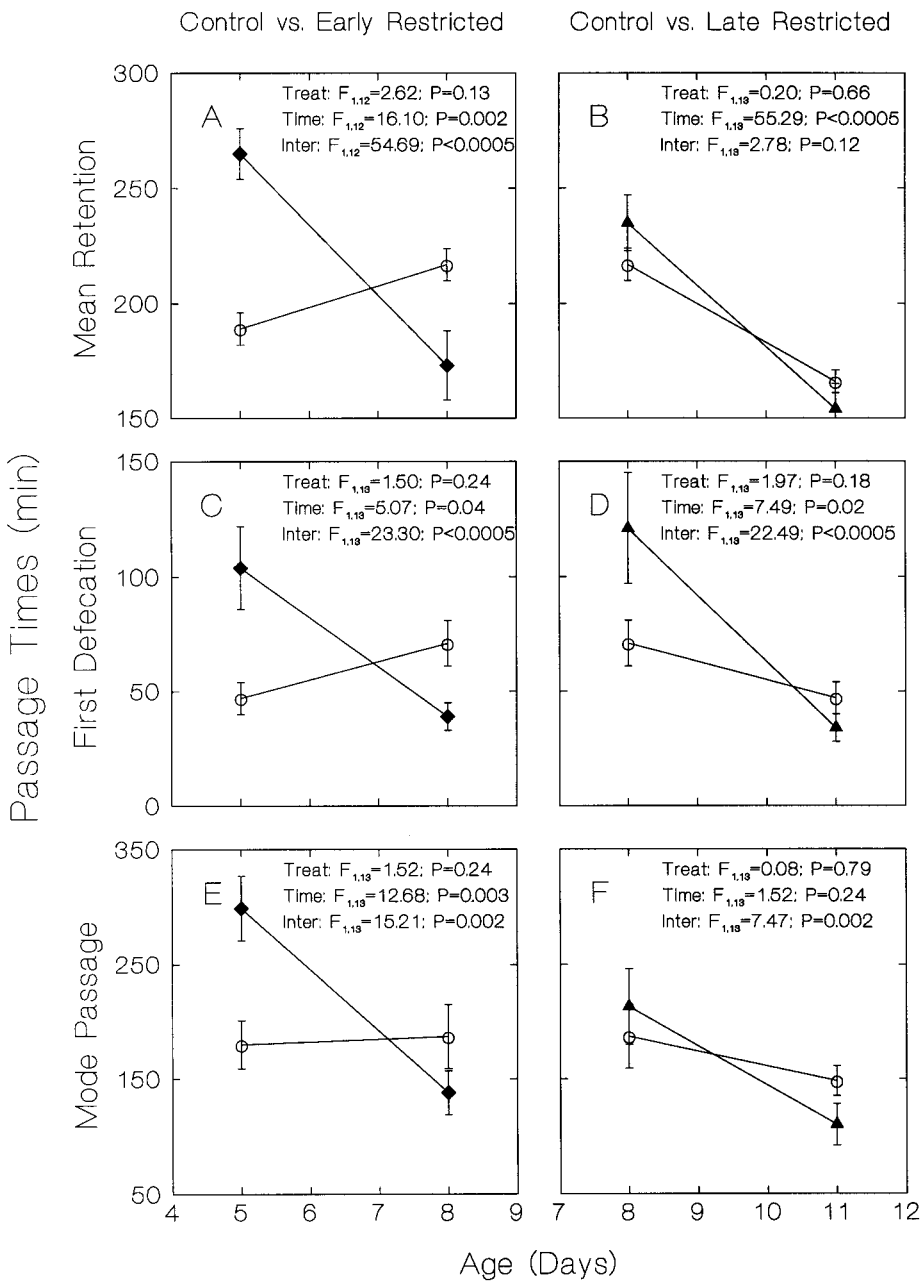

Figure 5. Passage times of digesta during restriction and realimentation. Mean retention time (min), time of first defecation (min), and mode passage time (min) of ^{3}H PEG excretion. The left-hand column represents the first time block (control vs. early restricted) while the right-hand column represents the second time block (control vs. late restricted). Mean ± 1 SE for control (*circles*), early restricted (*diamonds*), and late restricted (*triangles*) nestlings as a function of age. Statistical definitions are described in Figure 1. Sample sizes are the same as those in Figure 2, except mean retention time in early restricted nestlings, where $n = 5$.

was no change in the assimilation efficiency of starch in control nestlings ($F_{3, 18} = 0.05$; $P = 0.98$).

Control nestlings assimilated more grams of starch than early and late restricted nestlings during the restriction periods (Fig. 2*C, D*). Total starch assimilated increased in all groups over time. During realimentation, the amount of starch, and hence energy, assimilated was either lower than that in controls (for early restriction experiment) or not significantly different (for the late restriction experiment). In no case during realimentation was starch assimilation higher than the control level.

Passage Rates

PEG excretion did not follow the typical patterns displayed by adult birds (see, e.g., Afik and Karasov 1995; Karasov 1996)

Table 1: Small intestine lengths, summed hydrolysis capacity of intestinal enzymes, and summed uptake of 0.01 mmol L^{-1} leucine by the small intestine (mean ± SE)

Group	Small Intestine Length (cm)[a]	Leucine Uptake (nmol min^{-1})[b]	Enzyme Activity (μmol min^{-1})		
			Aminopeptidase-N	Maltase	Sucrase
Control ($n = 8$)	19.8 ± .8	12.58 ± 3.63	5.91 ± .77	129.32 ± 14.07	19.14 ± 2.59
Late restricted ($n = 8$)	19.7 ± .6	13.98 ± 3.40	6.14 ± .77	96.75 ± 22.23	15.25 ± 4.29
P-value	.96	.79	.84	.23	.44

[a] $n = 6$ and 5 for control and late restricted, respectively.
[b] $n = 4$ and 4 for control and late restricted, respectively.

until day 11 (Figs. 3 and 4). Specifically, the fraction excreted (Fig. 3) by nestlings on days 5 and 8 showed flat patterns rather than increases followed by declines. Similarly, the cumulative excretion curves (Fig. 4) on days 5 and 8 showed a linear increase instead of a tight sigmoidal curve.

All the measures of digesta residence exhibited a similar pattern of increased passage rates between restriction and realimentation (Fig. 5). This is indicated by significant time and/or time × treatment effects in nearly every case. There was no consistent difference between the control nestlings and those nestlings that were restricted and subsequently realimented (all treatment effects, $P > 0.12$). For control nestlings measured repeatedly on days 5, 8, and 11, there were significant changes in mean retention time ($F_{2, 16} = 16.92$; $P < 0.0005$) and time to first defecation ($F_{2, 16} = 4.18$; $P = 0.04$), but not in mode passage time ($F_{2, 16} = 1.02$; $P = 0.38$).

Intestinal Mass, Enzyme Activities, and L-Leucine Uptake

The small intestines of control and late restricted nestlings at day 11 were similar in both length (Table 1) and in length specific mass ($F_{1, 9} = 0.63$; $P = 0.45$; Fig. 6*A*). Intestinal mass decreased significantly from proximal to distal small intestine in both control and late restricted groups ($F_{7, 63} = 24.2$; $P < 0.0005$; Fig. 6*A*).

By all measures of enzyme activity there were no significant differences between the control and late restricted nestlings, except in sucrase expressed as micromoles per minute per gram protein ($F_{1, 14} = 4.65$; $P = 0.049$). There was no significant interaction between group and position ($P > 0.19$), except in maltase expressed as micromoles per minute per gram wet tissue ($F_{2, 28} = 3.42$; $P = 0.047$). However, in all cases there was a significant intestinal position effect ($P < 0.03$; Fig. 6*C–E*). We also calculated summed hydrolysis capacity for each enzyme by averaging hydrolysis levels measured in the proximal, medial, and distal small intestine and multiplying the mean by the small intestine length (Afik et al. 1995). Summed hydrolysis capacities were not significantly different ($P > 0.23$) between the control and late restricted nestlings for any enzyme measured (Table 1).

There was no significant difference between the control and late restricted nestlings in L-leucine uptake per centimeter ($F_{1, 6} = 0.06$; $P = 0.82$; Fig. 6*B*), per milligram ($F_{1, 6} = 0.33$; $P = 0.59$), or in the proportion that was Na^+ dependent ($P = 0.90$). In the proximal region, 85.3% ± 4.4% of 0.01 $mmol^{-1}$ L-leucine uptake was Na^+ dependent. Furthermore, there was no significant difference in L-leucine uptake with regard to intestinal position ($P > 0.12$). Finally, summed L-leucine uptake by the small intestine did not differ between control and late restricted nestlings (Table 1).

Discussion

Testing for Compensatory Changes in Digestion

Digestive efficiency of the entire diet, indexed as apparent assimilable mass coefficient, did not increase in nestlings during either restriction or realimentation compared with control nestlings. In fact, late restricted nestlings had lower apparent assimilable mass coefficients than control nestlings during both restriction and realimentation (Fig. 1*A*). Similarly, the assimilation efficiency of starch by early and late restricted nestlings showed no significant increase during the 48-h restriction compared with control nestlings. Moreover, the assimilation efficiency of starch declined below control levels when early and late restricted nestlings were realimented (Fig. 2*A*, *B*). Finally, intestinal rates of hydrolysis and L-leucine uptake of late restricted nestlings were not significantly different from those of control nestlings. Even though digestive efficiencies decreased in early and late restricted nestlings during realimentation, a sufficient increase in food intake could have resulted in a higher assimilable energy intake, relative to control nestlings. However, early and late restricted nestlings were at best only able to assimilate an amount of starch similar to that assimilated by control nestlings of the same age, indicating no increase in total energy assimilated from starch. Thus, we reject the notion that digestive efficiency and/or digestion rate increases during restriction or realimentation of house sparrow nestlings. Our finding of a lower digestive efficiency and similar total

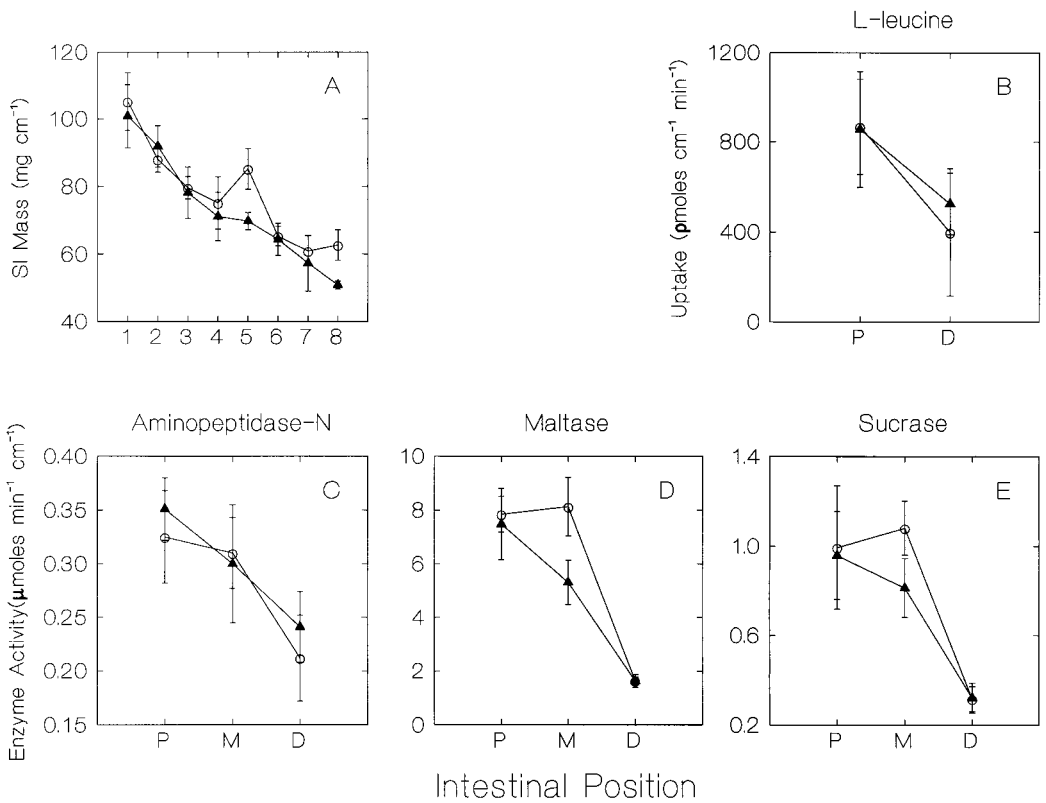

Figure 6. Parameters measured in the small intestine (SI) for 11-d-old nestlings. *A,* The small intestine mass cm^{-1} from proximal (beginning at 1) to distal (8) in control and late restricted nestlings. *B,* The uptake of L-leucine. *C–E,* The enzymatic activity of aminopeptidase-N, maltase, and sucrase (μmol min^{-1} cm^{-1}). *P,* proximal; *M,* mid; *D,* distal portions of the small intestine, respectively. Mean ± 1 SE for control (*circles*) and late restricted (*triangles*) nestlings.

starch assimilation during realimentation helps explain why nestling house sparrows do not display compensatory growth, despite higher food intake (Lepczyk 1996). Moreover, our results contrast the results of poultry studies in which restricted chicks consumed either less food (see, e.g., Yu et al. 1990) or only 6% more than controls (Plavnik and Hurwitz 1988) during realimentation and maintained or increased their growth rate.

The lack of any upward modulation in the digestive efficiency of the total diet during restriction indicates that nestlings were not adjusting digestive efficiency to compensate for low food intake. Although the mean assimilation efficiency of starch tended to increase by a small percentage (Fig. 2*A, B*), the change can be attributed to a change in flow (food intake) alone. Specifically, a decrease in food intake without a change in any other parameters results in an increase in retention time (Fig. 7) and, hence, digestive efficiency. Thus, the small increases in starch assimilation efficiency need not be explained as a compensatory response to changing food intake but, rather, can be explained as the consequence of changing digesta flow.

Implications for Spare Capacity

At first glance, many of our results appear consistent with the idea that the guts of growing birds have little spare capacity. For example, higher food intake tended to decrease both passage rates (mean retention times, time of first defecation, and mode passage of PEG; Fig. 5) and digestive efficiency (Fig. 2*A, B*). Furthermore, the data, overall, showed an inverse relationship between mean retention time and food intake (Fig. 7).

However, before concluding that these interpretations are correct one would want to make sure that the restriction-realimentation protocol did not change the gut itself. Suppose, for example, that 48 h at 40% usual food intake quickly brought a decrease in gut mass, enzyme levels, or absorptive capacity. Such a decrease is conceivable, as chickens and mammals that are fasted or semistarved demonstrate such reductions (see reviews by Karasov and Diamond [1983*a*]; Levin [1984]), though our protocol did not involve the catabolic state of those conditions. Thus, for example, the lower apparent assimilable mass coefficients in late restricted nestlings during restriction, which were not the result of shorter residence times, might be due to a decrease in enzymatic activity or gut absorptive capacity. However,

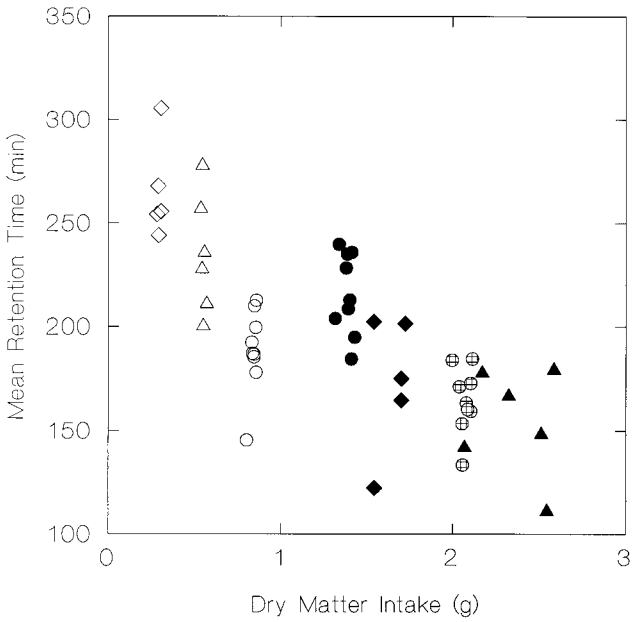

Figure 7. Mean retention time as a function of dry matter intake. Dry matter intake is the total dry mass consumed during the 6-h experiment. Symbols represent individual nestlings of a given age as follows. Control: day 5 (*open circles*), day 8 (*filled circles*), and day 11 (*hatched circles*); early restricted: day 5 (*open diamonds*) and day 8 (*filled diamonds*); late restricted: day 8 (*open triangles*) and day 11 (*filled triangles*).

even if one assumed that there had been a decrease in the gut's length or mass during restriction, there certainly was not further decrease during realimentation because after 3 d of realimentation gut lengths, mass, and enzyme and transport activity were not significantly different between controls and late restricted nestlings (Table 1; Fig. 6). Therefore, the observed decline in retention time cannot be explained as due to a decrease in intestinal volume, and the observed decline in efficiency during realimentation cannot be explained as due to decreased intestinal enzymatic or transport capacity. The best explanation is that there was no spare physical capacity, so that when food intake and digesta flow into the intestine increased, retention time declined. Because there was no spare enzymatic and transport capacity, the reduced contact time between digesta and hydrolases and transporters resulted in a lower digestive efficiency. Thus, in this particular case we do have an indication of the limitation of spare digestive capacity in house sparrow nestlings.

Our results indicate that nestling house sparrows have only a limited digestive spare capacity. For example, the mean retention time increased from day 5 to day 8 and then decreased from day 8 to day 11 while the assimilation efficiency of starch remained constant. The consistency in assimilation efficiency is similar to the previous finding that nestling house sparrows have a constant efficiency of energy use from 4 d of age on (Blem 1975). Assuming, however, that the increase and subsequent decrease in mean retention times were not statistical artifacts, it seems that the maintenance of assimilation efficiency with changing gut contact time reflects some spare capacity in the rates of breakdown and absorption. Furthermore, although nestlings had an essentially constant efficiency for starch digestion, other studies of intestinal maltase activity have shown an increase during nestling development (E. Caviedes-Vidal, unpublished data). Thus, for maltase activity there was seemingly an increasing spare capacity during development. However, by day 11 maltase spare capacity probably leveled off, as indicated by a lack of difference in rates of hydrolysis between control and late restricted nestlings (Table 1; Fig. 6). Because starch digestion was incomplete, some other step in starch breakdown and absorption may have been limiting. We suspect that because we used a low-carbohydrate diet, pancreatic amylase might not have been induced, and hence low amylase perhaps limited overall starch digestion.

Conclusions

In summary, the digestive efficiency for the whole diet by nestling house sparrows did not increase during either restriction or realimentation but remained below that of control nestlings. Although the digestive efficiency for starch did increase during restriction, it subsequently fell below that of controls during realimentation. Furthermore, during realimentation there was no difference in the suborganismal features that influence digestive efficiency (i.e., intestinal rates of hydrolysis and nutrient uptake). We thus reject the hypothesis that nestlings modulate digestive efficiency in the face of changing rates of food intake. The decline in digestive efficiency during realimentation explains why nestlings were unable to achieve compensatory growth even though food intake surpassed the level of control nestlings. Furthermore, the decline is consistent with the idea that the gut of nestling house sparrows has limited spare digestive capacity.

Acknowledgments

We are grateful to Rebecca Cook, Kris Manganaro, and Eugenia Ciminari for lab assistance and animal care. We would like to thank Juan Chediack and Erika Scheibler for help in enzyme analyses. Special thanks go to Erinn Birmingham and Meghan Saur, who volunteered to feed nestlings. Scott McWilliams assisted in experimental protocols and was always available for critical discussions of the project. Bruce Darken was instrumental in assisting with field surveys and laboratory protocol. Peter Arcese, Mark Cook, and two anonymous reviewers provided critical review of the manuscript. This manuscript was submitted in partial fulfillment of the requirements for C.A.L.'s M.S. degree at the University of

Wisconsin—Madison. Funding was provided by National Science Foundation grant IBN-9318675 to W.H.K. and the Max McGraw Wildlife Foundation.

Appendix

Table A1: Custom Nestling Diet II

Ingredient	Percent Composition
AIN vitamin mix	1
Alphacel nonnutritive bulk	4.9
Boric acid	.009
Casein (high nitrogen)	46.23
Choline chloride	.2
Cobalt sulfate $7H_2O$	.0015
Corn oil	8
Cornstarch	25.4
Glycine	.36
L-arginine	.79
L-cystine	.32
L-histidine	.18
L-isoleucine	.06
L-leucine	.18
L-lysine HCL	.41
L-methionine	.1
L-phenylalanine	.06
L-threonine	.22
L-tryptophan	.04
L-valine	.05
Salt mix-Briggs	5.5
Silica sand	5
Sodium bicarbonate	1
Sodium molybdate $2H_2O$	.009
Sodium selenite	.00002
Total	100.00197

Note. Diet was developed by E. Caviedes-Vidal and synthesized by ICN Biomedicals, Inc., Aurora, Ohio. Dry diet, wet diet is 1 : 3, diet : H_2O.

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