Changes in Gut Structure and Function of House Wrens (Troglodytes aedon) in Response to Increased Energy Demands

Cheryl R. Dykstra
William H. Karasov
Department of Wildlife Ecology, University of Wisconsin—Madison,
226 Russell Labs, 1640 Linden Drive, Madison, Wisconsin 53706
Accepted 9/16/91

Abstract
We compared digestive tract structure and function in house wrens (Troglodytes aedon) feeding on crickets at two intake levels (5.18 g/d and 2.42 g/d). We increased the birds' intake through a combination of lowered air temperature and forced exercise. Apparent metabolizable energy coefficient (1 - (energy excreted/energy ingested)) was .771 in high-intake birds adapted to -9°C and 4 h exercise/d and .716 in low-intake unexercised birds at 24°C (P = 0.016). Small intestine length was 21% greater in the high-intake birds than in low-intake birds (11.4 cm vs. 9.4 cm, P < 0.001). Stomach size, mouth-to-cloaca digesta retention time of aqueous digesta, and intestinal absorption of I-proline/cm did not vary between groups. A simple digestion model from chemical reactor theory is used to analyze these results and to deduce that an additional important adjustment was an increase in digesta retention time in the absorptive region of the gut, the small intestine. This would explain the observed increased digestive efficiency in the high-intake birds. The near-maximal rate of intake and digestion that we measured was among the highest ever recorded for a passerine. It exceeded by about 50% the field metabolic rate of parents during the reproductive season. Therefore, we reject the hypothesis that rate of digestion limits reproductive effort in house wrens.

Introduction

Digestive adaptation is well-known in endotherms subjected to changes in energy demands or different diets (Fenna and Boag 1974; Sibly 1981; Gross, Wang, and Wunder 1985). When energetic demands are increased, as occurs during cold winters or reproduction, endotherms generally increase their energy intake. Increased food intake, however, may cause decreased di-
gestive efficiency unless there is accompanying digestive acclimation (Sibly 1981).

Digestive acclimation might be ecologically important if digestion and maximal intake set the limit on metabolizable energy intake and thus on rates of growth and reproduction (Drent and Daan 1980; Masman et al. 1989) or limit geographic distribution (by limiting sustainable long-term heat production and hence survival in cold climates) (Peterson, Nagy, and Diamond 1990).

The primary digestive adjustment that has been described in mammals and birds is increased surface area and volume of the absorptive region of the gut (Savory and Gentle 1976; Gross et al. 1985). Other digestive adjustments might include an increase in activities of enzymes or nutrient absorptive mechanisms (Jacobs et al. 1975) or changes in retention time (passage time of the food through the gut), but these have been little studied.

We tested for adjustments in several facets of digestion (anatomy, digesta retention, and nutrient absorption rate) in house wrens (Troglodytes aedon) with elevated food-intake rate. As a device for increasing food intake, we increased the wrens’ energy expenditure through a combination of lowered ambient temperature and forced exercise. We analyze the responses of the various facets of digestion in relation to each other and in relation to intake and digestive efficiency with a simple digestion model from chemical reactor theory (Penny and Jumars 1987). We also compare the birds’ near-maximal digestion rate to energy flow during a period of possible energetic stress, that is, reproduction, as a test of the proposal that digestion may set limits on the ecological parameter of reproduction.

Material and Methods

Experimental Animals: Thirty-six wrens, seven adults and 29 chicks, age 7–15 d, captured in June and July 1989 at the Leopold Memorial Reserve in central Wisconsin, were divided into two groups, hereafter called high intake and low intake. Initially all birds were housed in cages 55 cm x 77 cm x 58 cm, which contained branches for perches and cover, a food plate, and a water bowl. In addition, cages with chicks contained their nest, or a substitute “nest,” a box lined with paper toweling. Chicks were caged with one of their parents or an unrelated wren willing to feed them. Adult birds not associated with chicks were caged singly or in pairs. Photoperiod was 12L:12D.

Wrens were fed frozen 3-wk-old crickets (Fluker’s Crickets, Baton Rouge, La.), a component of wrens’ natural diet (Bent 1948), and mealworms
(Rainbow Mealworms, Compton, Calif.) ad lib. Birds raised from chicks also ate frozen “hamburger mash” (R. Greenberg, personal communication), a mix of 45 kg very lean ground meat, one-half parboiled carrot, one to two hard-boiled eggs and shells, 375 ml dry baby cereal (rice), 30 mL avian vitamins (we used Chirp, Lambert Kay, Cranbury, N.J.), two to three 500-mg tablets CaCO₃, 5 mL paprika, and enough milk to form sausage shapes (about 20 mL). Ingredients were ground, mixed together, formed into sausage shapes 5 mm in diameter and 20 mm long, and frozen.

**Temperature and Exercise Acclimation.** The high-intake birds, two adult males and 17 unsexed younger birds age 5–7 mo, were transferred to a climate-controlled room at the Biotron, University of Wisconsin—Madison, where they were housed individually in the cages described and fed ad lib. Air temperature, initially 24°C, was lowered in 5°C–6°C steps, over the course of 16 wk, to −9°C, allowing 1–2 wk for acclimation at each temperature (fig. 1; air temperature remained at 8°C for a few weeks so we could conduct a feeding trial). The low-intake group, 13 birds age 5–7 mo, remained at room temperature and were fed ad lib.

While being acclimated to lower temperatures, the high-intake birds also were forced to exercise, in increasing amounts. The exercise apparatus, modified from that described by Kontogiannis (1968), was a motorized “cylinder within a cylinder;” horizontal along its axis, which revolved 2.3 times/min, by means of belts, reducing gears, and a motor. The outer cylinder was screen, 61 cm in diameter and 84 cm in length. The inner cylinder consisted only of six dowels (perches) spaced equally along its perimeter. The inner cylinder of perches, with a 46-cm diameter, was centered inside the screen cylinder. The birds flew or hopped from one perch to the next as the apparatus revolved. A curtain hanging from a central bar prevented the wrens from riding one perch through an entire rotation, and perforated plastic that lined the screen prevented them from clinging to the screen. The birds began exercising 5 h/d, and we gradually increased their exercise time to 4 h/d; when the exercise period exceeded 2 h, we allowed the birds a 1-h break for feeding. All birds were exercised 3 d of every four, and exercise typically took place during the afternoon hours.

The photoperiod, originally 12L:12D, was gradually shifted to 16L:8D (fig. 1). The light period was lengthened not only to allow the birds to feed longer but also to avoid long nighttime fasts (because of our interest in intake and digestion rather than fat storage and utilization).

The lowering of temperature and increase in exercise were ceased at −9°C and 4 h/d, respectively, by which point 67% of the original high-intake birds had died (including one of the two adults). Of the 13 low-
intake birds, only one died during the same time. This difference in mortality was significant (log-rank test, $P = 0.001$).

**Feeding Rate and Digestive Efficiency.** Diet was switched to 100% crickets at least 2 wk before a feeding trial began. After the birds’ acclimation at −9°C and 4 h/d forced exercise, we measured feeding rate and digestive efficiency with a 2- or 3-d total collection feeding trial. Trials were conducted in the same cages, but all branches except one leafless perch were removed. Exercising birds during the trial was impossible. Excreta were collected from plastic sheets lining the cage floor and surrounding the cage sides, as in Levey and Karasov (1989).

Daily dry-matter intake was calculated by subtracting the dry weight of uneaten crickets from the calculated dry weight of the crickets given to the bird. The calculated dry weight for the frozen crickets was based on wet
and dry weights of three or four 5–10-g samples of the crickets used in each trial.

Energy content of the food and excreta was measured on a Phillipson microbomb calorimeter (Gentry Instruments). Excreta samples for each bird were pooled over the entire feeding trial for energy analysis. At least two replicates were run on each sample, and coefficients of variation averaged 5.1%.

For ash determinations, samples (2–3 g) of dried excreta and of food were weighed,ashed at 500°C at least 16 h, and reweighed. The proportion of ash-free material in each sample was used to determine the ash-free apparent assimilable mass coefficient, AMC* (apparent because uncorrected for endogenous losses):

\[
\frac{I(AF) - E(AF_x)}{I(AF)}
\]

where \( I = \) intake (g dry matter/d), \( E = \) excreta produced (g dry matter/d), \( AF = \) percent of ash-free material in the crickets, and \( AF_x = \) percent of ash-free material in the excreta. Apparent metabolizable energy coefficient, MEC* (or assimilation quotient), was calculated as

\[
\frac{I(K) - E(K_x)}{I(K)}
\]

where \( K = \) energy content of the crickets in kJ/g and \( K_x = \) energy content of the excreta in kJ/g.

Behavior. To determine whether the increase in feeding rate was due to an increase in meal size or meal frequency, we observed the wrens' feeding behavior. Behavioral observations were made in 2-h periods between 0900 and 1300 hours and were replicated for each bird a few days later. For the high-intake birds (\( n = 8 \)) the observer sat outside the animal room and watched through a small window. For the low-intake birds (\( n = 9 \)), the observer sat at the far end of the room. The observer recorded the time of each meal and the number of crickets eaten at each meal, which was defined as a feeding period that ended when the bird left the cage floor or performed some other activity. (Typically all the crickets in a meal were eaten within a 2-min period.) The observer also recorded the length of time the bird was either "resting" or "active," which included feeding, drinking, dust bathing, flying, hopping, and singing. Results from the two observation periods were averaged for each bird.
Digesta Retention. Retention time was measured using a radio-labeled water-soluble marker, [1,2-3H]-polyethylene glycol (PEG, molecular weight 4,000, DuPont New England Nuclear). (Although we chose a water-soluble marker, we recognize that the diet contained significant amounts of fat in which transit kinetics might differ from water-soluble material and might be better traced with a lipid-soluble marker.) We injected 10 μl (5 μCi) PEG into a half-thawed cricket for each bird.

Trials were begun 2–4 h after the lights came on. Each wren was confined to a cage 28 cm × 48 cm × 20 cm that was lined with plastic sheets. To begin each trial, we placed one injected cricket in the cage of each bird and checked the birds every 5 min to determine when each ate the labeled cricket. After the cricket was consumed, we gave each bird unlabeled crickets and collected total excreta by periodically removing the plastic sheets beneath the birds. Excreta collections were made 4 times/h during the first 2 h and 2 times/h in the next 2–6 h, for a total of 4–8 h. (Earlier pilot studies in which collections were continued to 72 h showed that wrens excreted 95% of the recovered PEG within the first 4 h after ingestion [W. H. Karasov and C. R. Dykstra, unpublished data].) Spots of excreta and the underlying plastic were collected, mixed with 10–20 ml distilled water, and counted for 3H activity (in Ecolume, ICN Schwarz/Mann, Cleveland) as in Karasov and Levey (1990).

In some birds (n = 6 high-intake birds and n = 4 low-intake birds), we measured the time of the first defecation that contained the marker. During the first 45–60 min, we observed the birds and recorded the time of each defecation. Each spot of excreta was dissolved in water and counted. After 45–60 min, we continued the collections as described above.

Cumulative marker excretion was plotted against time, and the time of excretion that made the cumulative total exceed 50% was calculated from the graph. Mean retention time was calculated as in Warner (1981) and Karasov and Levey (1990), and mode passage time was the time of excretion that contained the most PEG.

Gut Measures: High-intake birds were transported in paper bags from the Biotron to the laboratory, with a transportation time of about 5 min. Low-intake birds were also placed in bags for 5–7 min before the procedures, so they went without food for a similar amount of time as the high-intake birds. Birds were anesthetized with methoxyfurane and weighed. The gastrointestinal tract was removed, and the intestine was cut from the stomach to the rudimentary caeca. The intestine was quickly blotted dry and weighed with its contents; then the intestine was perfused and the contents were collected in a tared beaker, weighed, dried at 50°C, and reweighed. The
empty intestine was everted over a glass rod. With one end tied to the rod, the other end was gently pulled until the intestine was taut. After release, its length was measured, and then sleeves were cut for measurement of uptake (below). The muscular stomach (gizzard) was cut from the glandular stomach/esophagus. The stomach containing its contents was weighed; then the stomach was cut open, and its contents were collected. The empty stomach was blotted, and both organ and contents were weighed, dried, and reweighed.

**Nutrient Uptake.** We measured intestinal uptake of glucose and proline as described in Karasov and Diamond (1983). One-centimeter everted sleeves of the intestine were mounted on 2-mm-diameter metal rods for all birds and kept in ice-cold avian Ringer solution until the measurement was made. Solution composition in mM was 161 NaCl, 4.7 KCl, 2.5 CaCl₂, 1.2 KH₂PO₄, 1.2 MgSO₄, and 20 NaHCO₃. The solution was oxygenated with 95% O₂–5% CO₂ to yield pH 7.3–7.4 at 37°C, and osmolarity was 350 mosm. After a 5-min preincubation in Ringer solution at 37°C, tissues were incubated 1 min for glucose or 2 min for proline in Ringer at 37°C over a stir bar at 1,200 rpm (Karasov and Levey 1990). We measured uptake of D-[1,3-H]glucose and L-[2,3-3H]proline into the tissue across the brush-border membrane. In studies of carrier-mediated D-[1,3-H]glucose uptake, L-[1,4-C]glucose at tracer concentration was used to correct simultaneously for D-glucose in adherent mucosal fluid and for D-glucose taken up passively. In studies of total L-[2,3-3H]proline uptake, [carboxyl-14C]inulin was used to correct for adherent fluid. Uptakes of 50 mM L-proline were measured in the proximal, mid, and distal small intestine; uptake of 50 mM D-glucose was measured in midgut only.

Numerical results are given as means ± SEM (n = number of animals). Statistical tests are described below. The *P < 0.05* level was considered significant.

**Results**

**Feeding Rate, Digestive Efficiency, and Mass Balance.** As expected, feeding rate of high-intake birds increased with decreasing temperature (fig. 2). At the lowest temperature, high-intake birds consumed 2.15 times what the low-intake birds did, whether expressed as g dry matter/d (5.18 ± 0.30 vs. 2.42 ± 0.07, *t*-test, *P < 0.001*) or on a per-gram-body-mass basis (table 1). (Whether the increased intake was due solely to decreased temperature or also due to the effects of activity or altered photoperiod is not critical to our subsequent analysis, which is focused on digestive adjustment to increased intake.)
Fig. 2. House wrens' consumption of crickets (A) and AMC* (B) as a function of air temperature. Birds at 24°C are the low-intake group mentioned in the text while the wrens of the high-intake group are represented at both 8°C and −9°C. (There are fewer high-intake birds at −9°C because of losses while acclimating to lower temperatures.) Each symbol, except open circles, represents an individual bird. Open circles represent all birds for which only one measurement was made. Symbols have been displaced horizontally to clarify graph; measurements were made at 24°C, 8°C, and −9°C.

At −9°C, AMC*, on an ash-free basis, was 24% higher in high-intake wrens than in the low-intake wrens; MEC* was 8% higher (table 1). At 8°C, AMC* (.497 ± .012) was not significantly different from that at 24°C, whether measured on day 121 (table 1) or day 224 (.459 ± .048, n = 3) (P = 0.918); MEC* at 8°C was not determined.

As a result of both the increased intake and the increased MEC* of high-intake birds, the energy assimilated per day increased even more than did
### Table 1

**Dry-matter intake, energy assimilated, AMC*, and MEC* for high-intake wrens at −9°C and low-intake house wrens at 24°C**

<table>
<thead>
<tr>
<th></th>
<th>High-Intake Wrens (n = 6)</th>
<th>Low-Intake Wrens (n = 7)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (g)</td>
<td>10.8 ± .2</td>
<td>11.0 ± .2</td>
<td>.334</td>
</tr>
<tr>
<td>Mass change (g/d)</td>
<td>.02 ± .06</td>
<td>−.11 ± .02</td>
<td>.069</td>
</tr>
<tr>
<td>Food intake (mg/g body mass/d)</td>
<td>480 ± 24</td>
<td>223 ± 7</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>kJ/d assimilated (kJ/g dry mass)</td>
<td>92.7 ± 6.6</td>
<td>40.0 ± 1.7</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>AMC* (ash-free basis)</td>
<td>.633 ± .024</td>
<td>.511 ± .008</td>
<td>.003</td>
</tr>
<tr>
<td>MEC*</td>
<td>.771 ± .014</td>
<td>.716 ± .013</td>
<td>.016</td>
</tr>
</tbody>
</table>

Note. Mean dry mass of one cricket was .038 ± .001 g. Crickets were 22.8 kJ/g dry mass and 9±10.6% ash. Dry matter content was 254 ± .005 g/g wet mass (n = 4) for high-intake birds and 237 ± .002 (n = 3) for low-intake birds. Feeding trials lasted 2 d for high-intake birds and 3 d for low-intake birds. Included here are only those wrens used in subsequent measures of retention time, gut morphology, and nutrient absorption. P values are for significance of difference between the two groups by the t-test. Data are means ± SEM.

The dry-matter intake. The kJ/d assimilated by high-intake wrens was 2.3 times that of low-intake birds (table 1). High-intake birds maintained body mass during the feeding trials (mean change .04 ± .11 g; not significantly different from 0). Low-intake birds lost mass over the 3-d trial (mean change, −.34 ± .06 g, differs significantly from 0, P < 0.001). Apparent mass loss in low-intake birds may be explained by the fact that birds were initially weighed after eating their first meal of the day but were weighed before eating on the final day of the trial. But even were the mass loss real, the associated small energy imbalance would not appreciably alter the calculated assimilated energy or MEC*, as demonstrated by Levey and Karasov (1989) for other passerines.

**Behavior.** High-intake birds increased their daily food intake by increasing meal size (1.77 ± .11 crickets/meal in high-intake birds vs. 1.13 ± .05 in low-intake birds, P < .001, t-test) but not meal frequency (respectively, 12.4 ± 1.2 min between meals vs. 11.6 ± .6, P = .591). High-intake wrens were active only 3.5 ± 1.0 min/h, compared with 29.4 ± 3.2 min/h for low-intake wrens (P < .001); practically all the activity exhibited by the high-intake birds was feeding. The difference in activity levels was not caused by changes in high-intake birds' exercise schedule, since birds exercised...
as usual on the day observations were made, as well as on the preceding day.

**Retention Time.** None of the retention measures were significantly different between high-intake birds and low-intake birds. All measures show high variability among individuals as commonly observed in such studies (Warner 1981). Figure 3A shows the percent PEG excreted at each time interval. Figure 3B shows the cumulative excretion curves used to calculate the time to excrete 50%, which was 52.1 ± 5.8 min in high-intake wrens and 55.4 ± 8.8 min in low-intake birds ($P = 0.759$, $t$-test). Mean retention time was 65.3 ± 5.9 min in high-intake birds versus 70.8 ± 9.2 min in low-intake birds ($P = 0.620$). Mode retention time was 35.0 ± 8.2 min in high-intake birds versus 41.5 ± 9.5 min in low-intake birds ($P = 0.334$). Finally, the time of the first excretion containing the marker was 5.5 ± 1.5 min in high-intake birds and 10.7 ± 4.3 min in low-intake birds ($P = 0.324$). In every bird tested, the first defecation containing the marker was also the first defecation of the trial.

**Gut Measures.** Body mass was slightly higher in high-intake birds than in low-intake birds for the gut measures and uptake measurements (table 2), so we also compared some measures on a per-gram-body-mass basis. Intestinal length was 21% greater in high-intake birds (table 2) and 14% greater on a per-gram-body-mass basis (1.03 ± 0.17 cm/g vs. 0.906 ± 0.26, $P = 0.003$). Intestine mass, calculated by interpolating mass per centimeter between the proximal, mid, and distal intestine and summing over the total length, was significantly higher, whether compared directly (table 2) or on a per-gram basis (56.2 ± 4.4 mg/g vs. 44.9 ± 9.4, $P = 0.036$). Correspondingly, the dried mass of the intestinal contents also varied significantly (table 2).

There were no significant differences in measures of the mass of the stomach or its contents (table 2). Although there was a trend toward larger stomachs in high-intake wrens, the differences were smaller when considered on a per-gram-body-mass basis ($t$-test, $P = 0.526$ for wet-mass stomach per gram body mass and $P = 0.522$ for dry-mass stomach per gram body mass).

**In Vitro Intestinal Uptake of Nutrients.** Uptake rates were normalized to centimeter length of intestine (fig. 4B) but also can be expressed per milligram wet intestine by dividing those rates by intestinal masses (fig. 4A) or per square centimeter nominal surface area by dividing by 0.63 cm²/cm (the surface area of our 2 mm rods). There was no significant difference in l-proline uptake per centimeter between the two experimental groups ($F = 0.55$, $df = 1, 8$, $P > 0.5$, repeated measures ANOVA, general multivariate
Fig. 3. A, Excretion of $^3$H PEG by house wrens, 0–4 h after ingestion. Percent shown is the portion of the recovered PEG. B, Cumulative excretion of $^3$H PEG by house wrens, 0–4 h after ingestion. Percent shown is the portion of the recovered PEG. Values are means for six high-intake and 11 low-intake birds. Standard errors were omitted to avoid clutter; in A SEM ranged .1%–5.2%; in B SEM ranged .2%–7.8%. See text for statistical comparisons.

model), but there was a significant linear trend in proline uptake rate with position in the gut ($F = 6.94$, df = 1.8, $P = 0.03$).

To estimate the summed uptake rate of the entire length of the small intestine for proline at 50 mM we interpolated uptake rates linearly between successive positions along the intestine (fig. 4B) and then summed over


<table>
<thead>
<tr>
<th></th>
<th>High-Intake Wrens (n = 5)</th>
<th>Low-Intake Wrens (n = 6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (g)</td>
<td>11.05 ± .02</td>
<td>10.44 ± .22</td>
<td>.040</td>
</tr>
<tr>
<td>Stomach:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wet mass:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organ and contents (g)</td>
<td>.358 ± .016</td>
<td>.300 ± .025</td>
<td>.080</td>
</tr>
<tr>
<td>Organ (g)</td>
<td>.251 ± .008</td>
<td>.229 ± .008</td>
<td>.087</td>
</tr>
<tr>
<td>Contents (g)</td>
<td>.107 ± .016</td>
<td>.071 ± .021</td>
<td>.202</td>
</tr>
<tr>
<td>Dry mass:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organ (g)</td>
<td>.071 ± .002</td>
<td>.065 ± .002</td>
<td>.068</td>
</tr>
<tr>
<td>Contents (g)</td>
<td>.031 ± .006</td>
<td>.020 ± .006</td>
<td>.255</td>
</tr>
<tr>
<td>Intestine:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wet mass:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organ and contents (g)</td>
<td>.603 ± .027</td>
<td>.512 ± .034</td>
<td>.072</td>
</tr>
<tr>
<td>Organ (g)</td>
<td>.621 ± .022</td>
<td>.459 ± .005</td>
<td>.012</td>
</tr>
<tr>
<td>Dry mass contents (g)</td>
<td>.026 ± .002</td>
<td>.018 ± .001</td>
<td>.017</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>11.4 ± .2</td>
<td>9.4 ± .2</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Note. The P values are for significance of difference between the two groups by the t test. Data are means ± SEM.

the small-intestine length (from table 2). Summed uptake in high-intake wrens was significantly higher than in low-intake wrens whether expressed on a whole-animal basis (respectively, 3.10 ± .12 μmol/min [n = 5] vs. 2.52 ± .10 [n = 5]; P = 0.007, t test) or per gram body mass (respectively, 28 ± .01 μmol min⁻¹ g⁻¹ vs. 25 ± .01; P = 0.052).

In the midgut uptake of L-proline at 50 mM appeared to be primarily by a Na-independent pathway on the basis of ratios of uptake by sleeves incubated in Na⁺-free medium (Na⁺ replaced by choline) normalized to uptake by adjacent sleeves incubated in Na⁺-containing medium. The ratios did not differ significantly from 1.0 in high-intake birds (1.065 ± .05) or low-intake birds (1.02 ± .03).

Glucose-uptake rates were an order of magnitude lower than proline-uptake rates. Carrier-mediated D-glucose uptake in the midgut of high-intake birds was 20 ± 2 nmol min⁻¹ cm⁻¹ (n = 5) (cf. fig. 4B), whereas it was
Fig. 4. A, Small-intestinal mass in high-intake and low-intake house wrens. B, Uptake of L-proline at 50 mM by the intestine of high-intake and low-intake house wrens; Prox, proximal; Mid, mid; Dist, distal small intestine. Values are means ± SE; n = 5 high-intake and 5 low-intake birds.

immeasurably low in two low-intake birds and averaged 8 ± 3 nmol min⁻¹ cm⁻² in three others. Four tissues from two low-intake wrens were incubated in medium with radio-labeled L-glucose, the stereoisomer of D-glucose that is not actively transported (Karasov and Diamond 1983), to measure the apparent passive permeability coefficient of glucose, 1.2 ± .1 μL min⁻¹ cm⁻². The product of this and 50 mM equals the passive uptake at 50 mM, which
can be added to the carrier-mediated uptake (which it exceeds) to calculate total uptake at 50 mM.

**Discussion**

Most of our results seem consistent with changes that occur naturally in wild animals rather than simply pathological responses in the laboratory. For example, increases in feeding rate were expected at low temperatures, and the observed changes in gut length were no larger than observed in some wild birds (see below; Sibly 1981; Karasov 1990). Differences in intestine lengths were not solely attributable to selection favoring birds already possessing long intestines and thus best adapted to cold conditions, since the ranges of intestine lengths do not overlap (thus, it was unlikely the high-intake group was simply a selected subset of the low-intake group [chi-square, $P < 0.001$]). Body mass may have changed (cf. tables 1, 2), but other studies indicate that passerines may regulate body mass in relation to air temperature (Kendeigh et al. 1969).

We now discuss our results at three levels of biological integration, in order of increasing complexity. We begin by considering the responses of individual facets of digestion (i.e., gut morphology, retention, nutrient absorption) to increased food intake. Then we use a simple reactor model to integrate these responses in relation to the whole-animal digestive features of food-intake rate and digestive efficiency. Finally, we consider whether digestion limits energy flow in wild wrens.

**Adaptation of Gut Structure and Function**

1. **Gut Structure.** Increase in food intake and small-intestine length after acclimation to cold, as seen in the wrens (tables 1, 2), was also observed in Japanese quail held at 2°–10°C (Fenna and Boag 1974) and in prairie voles held at 5°C (Gross et al. 1985). The increase in intestinal length and surface area in these situations did not exceed 25%.

   The gut enlargement is thought to be related to a direct effect of increased luminal nutrient flow on mucosal growth (Karasov and Diamond 1983). But one cannot rule out some kind of physical effect of increased volume flow, because when food intake increases after diet dilution (e.g., with indigestible fiber) intestine length sometimes increases even if the flow of digestible nutrients is little changed (e.g., Savory and Gentle 1976; but also see Fenna and Boag 1974).
2. Digesta Retention Time. When normalized to grams (Karasov 1990) the mean retention time of 10-g wrens eating crickets, 38 min, is longer than European starlings (19 min) and American robins (22 min) eating the same. This might relate to the fact the latter species are more frugivorous than wrens, and more frugivorous species of birds tend to have short gut retention times (Karasov and Levey 1990). (Conditions for the measures were fairly similar to those used for wrens. The primary difference was that we removed the plastic sheets more frequently, possibly disturbing the birds more. Nonetheless, the wrens’ mean retention time was longer.)

3. Intestinal Nutrient Uptake. The body mass–normalized summed L-proline uptake of house wrens held at room temperature, 79 ± 0.3 μmol min⁻¹ g⁻¹, was comparable to that in five other species of passerines, 1.01 ± 0.14 μmol min⁻¹ g⁻¹ (range 0.5–1.3; Karasov and Levey 1990). It is surprising that all of the L-proline uptake at 50 mM in wrens could be accounted for by Na⁺-independent pathways. In other passerines (Karasov and Levey 1990) and vertebrates studied (Karasov 1988) there is an Na⁺-dependent pathway that usually accounts for at least a third of L-proline uptake at this high concentration.

The increase in summed L-proline uptake in high-intake wrens was probably important in permitting efficient absorption at high-intake rate, as suggested by the following calculations. Considering the L-proline content of crickets (3.83% of dry mass; Nakagaki, Sunde, and Defoliart 1987), low-intake and high-intake wrens ingested, respectively, 0.9 and 2.0 μmol L-proline/d. Assuming digestion and absorption for 12 h/d and a temperature correction (Q₁₀ = 2.0; Karasov 1988) since the incubation temperature (37°C) was below wren body temperature (40.4°C; Kendeigh 1934), the wrens’ summed uptake rates at 50 mM would be 2.3 and 2.9 μmol/d, respectively. If the high-intake wrens did not have longer intestines then their L-proline intake would have nearly equaled their uptake capacity, leaving little margin for subsequent increase in intake (i.e., a safety margin, sensu Toloza, Lam, and Diamond 1991). We hasten to point out, however, some of the complications inherent in these calculations: the in vitro technique might not yield absorption rates similar to those in vivo (but see Karasov and Debnam 1987), the lumen concentration of proline and other amino acids competing for its carriers (Karasov 1988) may not be near 50 mM, and other routes of proline absorption not accounted for might be significant (e.g., peptide absorption, solvent drag).

It is not surprising that, for an insect-eating bird, intestinal uptake rate for D-glucose was an order of magnitude below that for the amino acid L-proline (ratio of glucose uptake to proline uptake in adjacent tissues in
the same birds equaled 0.45 ± 0.01, n = 8). There is a positive correlation between dietary carbohydrate level and glucose-uptake rate in birds (Karasov and Levey 1990) and other vertebrate classes (Karasov and Diamond 1988). The glucose/proline ratio in house wrens is low as in carnivorous shrikes and insectivorous European starlings; values are higher in more frugivorous passerines and highest in nectarivorous hummingbirds (Karasov and Levey 1990). The adaptational explanation for this pattern is that the costs (biosynthetic and other) of possessing many sugar transporters on a high carbohydrate diet are more than met by the resulting additional energy absorption while having fewer transporters of nonessential sugar on a low carbohydrate diet would be safe and would save transporter costs.

The apparent passive permeability coefficient to glucose, 1.9 ± 0.2 μL min⁻¹ cm⁻², was comparable to that in three other species of passerines, 1.9 ± 0.2 μL min⁻¹ cm⁻² (range 1.3–2.3; Karasov and Levey 1990).

Whole-Animal Digestive Response and Integration

Responses in digestive efficiency to cold acclimation vary from species to species. Our high-intake house wrens at −9°C were more efficient than their low-intake counterparts at 24°C, as were cold-exposed blue-winged teal (Owen 1970) and bald eagles (Stalnaker and Gessaman 1982). In far more bird species digestive efficiency was either unchanged (El-Wailly 1966; Hamilton 1985) or declined with decreasing temperature and increasing intake (West 1968; Moss and Parkinson 1972).

Because digestive responses to cold-exposure and increased intake vary greatly, we feel that the data can be better understood if they are analyzed within the framework of an integrative digestion model.

House wrens probably have guts that approximate a stirred-tank reactor (the stomach) in series with a plug-flow reactor (small intestine) (Penny and Jumars; 1987). Within each reactor, retention time is the quotient of digesta volume and flow rate (Penny and Jumars 1987; fig. 5). We found that high-intake birds had food-intake rates (i.e., flow rates) 115% larger than low-intake birds (see Results) and intestinal digesta volumes 44% greater (i.e., mass of digesta; table 2). Total throughput times and stomach digesta volumes were not significantly different (table 2). The model in figure 5 relates these findings and shows deductively that retention time in the small intestine was longer in the high-intake wrens than in the low-intake wrens.

Because throughput time in the absorptive region determines the extent of absorption or digestion (Penny and Jumars 1987), digestive efficiency (AMC* or MEC*) should be greater in high-intake birds, assuming that the reaction rate (i.e., hydrolysis and absorption) was unchanged (Penny and
LOW-INTAKE
\[ t = \frac{V_1 + V_2}{v_o} \]

HIGH-INTAKE
\[ t' = \frac{V_1' + V_2'}{v_o} \]

We found \( t = t', \ V_1 = V_1', \ v_o' > v_o. \)

Since \([t = t'] = [V_1/v_o + V_2/v_o = V_1'/v_o' + V_2'/v_o']\)

then \( V_2/v_o < V_2'/v_o'. \)

Fig. 5. A model from chemical reactor theory relating various aspects of digestion in house wrens with small intestines of different lengths; \( v_o \) is flow rate of the digesta (g/d), \( V_1 \) is volume of the stomach in g capacity, \( V_2 \) is volume of the small intestine in g capacity, and \( t \) is digesta retention time (d). Values \( V_1/v_o \) and \( V_2/v_o \) represent digesta retention time in the stomach and small intestine, respectively. As discussed in the text, a deduction from the model is that retention time in the small intestine was greater in the high-intake wrens than in the low-intake wrens.

Jumars 1987). This assumption may be correct because the absorption rate per unit length for the amino acid l-proline was not significantly altered (fig. 4B).

Thus, the model (fig. 5) offers an explanation for how intake rate could be increased in concert with an increase in digestive efficiency if small intestine volume is increased, even if whole-animal retention time and absorption rate per centimeter remain unaltered. We conclude that the most important gut change that permitted the increased rate of food intake was the increase in intestine volume and absorptive surface area (via the increase in length). Total-animal retention time did not change to permit increased food-intake rate. Instead, the retention time in the stomach was decreased while retention in the small intestine was increased. The increase in intestinal retention time allowed an increase in digestive efficiency (without requiring an increase in absorption per centimeter), which further raised...
the rate of metabolizable energy (\textit{ME}) intake since \textit{ME} intake = intake × MEC*.

We note that our analysis depends on a deduction from the data at hand rather than a direct measurement of the retention time in the stomach and small intestine. Although the time of first appearance of the marker may be a good index to retention time in the intestine (Martínez del Río and Karasov 1990), our experiment could not differentiate between the two groups because all first excretions contained the marker. One result does support the idea of shorter stomach retention: stomach fill in the two groups was not significantly different (table 2), although meal size was greater in the high-intake wrens.

\textit{Does Digestion Limit Energy Flow in Wild House Wrens?}

Physiological limits to sustained energy flow may be ecologically important in limiting production (i.e., growth and reproduction). Kirkwood (1983) suggested an interspecies ceiling for maximum metabolizable energy intake of 3–6 times the fasting metabolic rate. Karasov (1990) found that metabolizable energy intake ranged 3–5 times the standard metabolic rate (SMR) in birds (13 species) exposed to temperatures near or at the lower limit of their long-term temperature tolerance.

To express our metabolizable energy intake in this fashion, we calculated the house wren’s inactive phase SMR (or basal metabolic rate) as the active-phase SMR (1.14 kJ/h; Kendeigh 1939) divided by 1.24 (Aschoff and Pohl 1970). During the feeding trial, the high-intake birds metabolized energy at a rate 4.2 times the inactive-phase SMR (22.1 kJ/d). The rate might have been higher still when they were also forced to exercise 4 h/d if all the heat produced during the activity did not replace that for thermoregulation. We doubt that we could have increased metabolizable energy intake any more by our manipulations because some birds were lost, implying stress. We note that we are not certain that the birds’ deaths were due to reaching an intake limitation; death may have been due to inability to store enough fat to survive the night, modified feeding behavior due to exhaustion or stress, and so forth. In any case, the house wrens metabolized energy at a rate 27%–50% higher than that predicted from Kirkwood’s (1983) and Karasov’s (1990) allometric equations for near-maximal metabolizable energy intake and 15% higher than the highest value cited by Kirkwood (2,200 kJ/kg”.

In a related field study in central Wisconsin, we tested whether house wrens’ reproductive output might be constrained by limits to energy flow. We found field metabolic rate (FMR) in male and female house wrens feed-
ing broods aged 7–14 d averaged 61 kJ/d, slightly correlated with a brood size of 1–10 chicks (C. R. Dykstra and W. H. Karasov, unpublished data). This FMR was substantially lower than the near-maximum 93 kJ/d the wrens digested and assimilated in the laboratory. Even if the energetic cost of laying one egg per day, 8.6–9.0 kJ/d (Kendeigh, Kramer, and Hamerstrom 1956; Kendeigh, Dołnik, and Gavrilov 1977) is added to the wrens’ FMR, their daily expenditure is still well below the maximum we found in the laboratory. Therefore, we conclude that digestive processes and structures determining the physiological limits to maximum sustained energy flow were not limiting wrens’ reproductive effort in the field during laying or the feeding of nestlings. A caveat to this argument is that we assume summer-acclimated birds could make digestive adjustments to increase their sustained energy flow. Masman et al. (1989) showed that breeding kestrels had the ability to increase their sustained energy flow.

Acknowledgments

We would like to thank D. Keith Warnke for field and lab assistance, Sandra Wendel for field assistance, and Deborah PENNY for suggesting the model. Supported by NSF BSR8452089 and the Sand County Foundation.

Literature Cited


