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Digestive Adjustments in Cedar Waxwings to High Feeding Rate

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ABSTRACT Birds may dramatically increase their food intake during migratory periods or during winter. We tested the hypotheses that when birds are hyperphagic, (a) their digesta retention time and extraction efficiency will not change compared with that of birds feeding at reduced rates, (b) their total capacity for breakdown and absorption of nutrients will increase, and (c) the mechanism responsible for the increase in total capacity will be an increase in amount of intestine rather than an increase in intestinal tissue-specific enzyme activity or nutrient transporter activity. We measured gut anatomy, retention time of digesta, enzyme hydrolysis rates, nutrient absorption rates, and digestive efficiency in individual cedar waxwings (*Bombycilla cedrorum*) acclimated to –20°C or +21°C. Compared with cedar waxwings held at +21°C, waxwings acclimated to –20°C more than tripled their daily food intake. Mass of digestive organs increased by 22–53%, but rates of enzyme activity and nutrient uptake per unit of small intestine did not change significantly. Retention time of digesta declined slightly, and there was a small decrease in digestive efficiency. As predicted, the main adjustment to increased energy requirements and food intake was an increase in gut length, mass, and volume which largely compensated for increased digesta flow at high intake rates. However, we detected a small reduction in retention time and digestive efficiency in waxwings with high intakes which suggests that these waxwings may be unable to further increase their gut size (i.e., that the increase in gut size was maximal). If adjustments involving gut size require weeks of acclimation time, migration patterns and the pace of migration in birds could be influenced by time required for preparation of the gut. *J. Exp. Zool. 283:394–407, 1999.*

Birds may dramatically increase their food intake to satisfy the high energy requirements of migration (Blem, '76, '90; Bairlein and Simons, '95; Berthold, '96) and to offset the higher thermoregulatory costs during exposure to cold temperatures (Dawson et al., '83; Karasov, '96). The energetic gains realized by a bird when it eats more depend on interactions between food intake rates and digestive efficiency. For example, if the absorptive surface of the gut or its capacity for absorption does not change when a bird eats more, then the increased flow of digesta may cause digestive efficiency to decline and thereby directly discount the potential energetic gains provided by hyperphagia.

The main digestive adjustment to increased food intake that has been described in birds is increased surface area and volume of the gut (Savory and Gentle, '76a,b; Savory, '86; Dykstra and Karasov, '92; Piersma et al., '93; Karasov, '96; Piersma and Lindstrom, '97). Few studies have addressed whether such changes in the gut compensate for the potentially negative effects of increased food intake on digestive efficiency (e.g., Savory, '86; Dykstra and Karasov, '92). Even fewer studies have examined the occurrence or significance of other possible digestive adjustments to increased food intake, such as increased activity of enzymes or nutrient absorptive mechanisms (Jacobs et al., '75; Dykstra and Karasov, '92). No study has simultaneously measured adjustments

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in gut anatomy, retention time of digesta, enzyme hydrolysis rates, nutrient absorption rates, and digestive efficiency in response to increased food intake (see Karasov, '96; Piersma and Lindstrom, '97 for recent reviews). Only by using such an integrative approach can we begin to understand important interactions between digestive adjustments or plasticity and their ecological consequences.

We use a simple integrative model of digestion (Karasov, '96) to generate some predictions about how digestive features might respond to increased food quantity:

$$\text{digestive efficiency} \propto \frac{\text{(digesta retention time)} * \text{(reaction rates)}}{\text{(volume of digesta)} * \text{(nutrient concentration)}}$$

This model suggests that digestive efficiency is positively influenced by longer retention time of digesta in the gut and higher reaction rates (including digestive enzyme hydrolysis rates and nutrient absorption rates). Alternatively, the model suggests that digestive efficiency is negatively influenced by increased volume of digesta (as would occur with increased food intake) or increased concentration of nutrient per unit volume of digesta (which is related to food quality). An implicit assumption in this model is that there is little spare capacity in these digestive features. For example, if diet quality increases so that concentration of nutrient increases, the model assumes that digestive efficiency will decline unless compensatory changes occur in digesta retention or reaction rates. Perhaps most important, the model shows explicitly how these digestive features may interact and influence digestive efficiency and thus provides a conceptual framework from which to evaluate digestive system function in an integrated fashion.

We can use this model to generate predictions about how the gut might respond when an animal increases its food intake. Without any compensatory changes in other features, when food intake increases then digesta flow increases, and retention time decreases [i.e., retention time $\propto$ volume of digesta (ml)/digesta flow (ml/min) (Karasov, '96)]. Consequently, increased food intake will result in decreased digestive efficiency if there is no modulation of digestive features. However, the increased flow of digesta might be compensated for in a number of ways that would maintain digestive efficiency: for example, if the gastrointestinal tract lengthens, then the retention time of ingested food particles may not change and digestive efficiency could remain constant. Alternatively, if the gut doesn't enlarge and retention time of digesta shortens, if enzyme hydrolysis rates and absorption rates increase then digestive efficiency could remain constant.

We predicted that cedar waxwings would adjust to high food intakes mainly by enlarging the gut rather than by increased tissue-specific reaction rates. Specifically, we tested the hypotheses that when waxwings are hyperphagic (a) their digesta retention time and extraction efficiency will not change compared with that of birds feeding at reduced rates, (b) their total capacity for breakdown and absorption of nutrients will increase, and (c) the mechanism responsible for both (a) and (b) will be an increase in amount of intestine rather than an increase in intestinal tissue-specific enzyme activity or nutrient transport activity. We simultaneously measured gut anatomy, retention time of digesta, enzyme hydrolysis and nutrient absorption rates, and digestive efficiency in cedar waxwings acclimated to –20°C or +21°C. Cold-acclimation is an ecologically relevant way to increase food intake in cedar waxwings because the waxwing is a common latitudinal migrant that spends the winter in northern regions of the United States (Bent, '50; American Ornithologists' Union, '83).

MATERIAL AND METHODS

Capture and maintenance of birds

Six of the 20 cedar waxwings used in this study were captured in Gainesville, Florida (29° 41′ N, 82° 16′ W) and sent to us on August 8, 1994. We captured the other 14 cedar waxwings on September 30 – October 1, 1994, in Madison, Wisconsin (43° 8′ N, 89° 20′ W) using mistnets. Birds were immediately weighed and banded and then housed individually in stainless-steel cages (60 × 45 × 33 cm) under an initially constant light cycle (12L: 12D, lights on at 700 hours) and temperature (21°C). All birds were acclimated to a banana-mash diet (Denslow et al., '87) that has been used successfully for maintaining cedar waxwings and other frugivorous passerines in good health in the laboratory (Levey and Karasov, '89; Martinez del Rio et al., '89; Karasov and Levey, '90).

Temperature and diet acclimation prior to the experiment

On October 10 we randomly assigned 11 birds to a cold-acclimated group and 9 birds to a control group. Birds captured in Florida and Wisconsin were evenly distributed between treatment

and control groups. All 20 birds continued on the same daily light schedule (12L:12D). For control birds, the ambient temperature was kept constant at 21°C. For cold-acclimated birds, the ambient temperature was gradually decreased over 30 d using the following schedule: from 21°C to 1°C over 10 d (–2°C d^{-1}), held constant at 1°C for 10 d, then from 1°C to –20°C over 10 d (–2°C d^{-1}).

On November 18, we acclimated all birds to a new semisynthetic diet (see McWilliams and Karasov, '98) that simulated a fruit diet in nutrient content (65% carbohydrate:13% protein:6% fat by dry mass). Cedar waxwings select fruits that contain relatively low lipid and high carbohydrate (Witmer, '96) as in the diet we formulated. The use of such a semisynthetic diet makes the composition of the diet less ambiguous than diets compounded from raw foodstuffs (see also Murphy and King, '82).

Feeding schedules and experimental design

Birds were always presented with fresh food and water each day at 0930–1030 hours. Each day birds were provided with excess food ensuring ad libitum feeding conditions. For birds at –20°C, a small hotplate was placed in each cage to keep the food soft and palatable. Food was placed in a glass petri dish set in a clay saucer that evenly distributed the heat from the hotplate. A wooden perch attached to each hotplate enabled birds to eat while avoiding direct contact with the hotplate or clay saucer. Once each day we supplied birds in the cold with hot tap water in a plastic petri dish. This water was used by the birds mainly for bathing. Water content of the food (75%) ensured adequate consumption of water in their diet.

On the pretest day, food was removed at 1730 hours to ensure that birds would start the test day with a small energy deficit. On the test day, food was provided ad libitum beginning at 0700 hr. Then, food intake, retention time, and extraction efficiency of 10 cold-acclimated and 8 room temperature-acclimated birds were measured during a 4–5 hr test period that began at 1330 hr when the bird ingested about 0.5 g of diet containing radiolabeled nutrient and marker (see below). Food intake on a dry matter basis was estimated by drying subsamples of food collected at the start and end of the test period. We measured food intake, retention time, and extraction efficiency in only 10 of 11 treatment birds and 8 of 9 control birds because we always tested two birds simultaneously in side-by-side special observation cages (see description later in this article), which required an even number of test birds. Cedar waxwings are quite social birds and appear to behave normally as long as they have visual contact with a conspecific.

Tests on all 18 birds were conducted between December 9, 1994, and January 5, 1995. All birds were tested twice: one trial was used to measure extraction efficiency of glucose, and the other trial was used to measure retention time of digesta. Test days were always separated by at least 1 and usually 2–3 d.

Retention time and extraction efficiency

Special observation cages were used to reduce behavioral stress associated with our presence while the birds were observed and their excreta collected (see Afik and Karasov ['95] for full description). Most importantly, the cages were exactly the same as their regular cages except that the front door had one-way glass for observations and we placed a roll of plastic-coated paper (VWR Scientific Products, cat. no. 54110-527, S. Plainfield, NJ) on a roller so that sheets of paper could be pulled across the cage's floor to collect excreta with minimal disturbance to the birds. All birds were housed in these cages for at least 1 d before the test day. This one-day minimum acclimation period seemed adequate because food intake was similar on pretest and test days.

Retention time of digesta was measured using the inert marker [^{14}C] ferrocyanide (FeCN). Extraction efficiency of glucose was measured using the inert marker method (Karasov et al., '86). Separate trials were necessary for measuring retention time and extraction efficiency of glucose because in a companion study (McWilliams and Karasov, '98) we found that estimates of extraction efficiency based on tritiated nutrient underestimated actual extraction efficiency. For estimating extraction efficiency, we used [^{14}C(U) (uniformly labeled)] D-glucose (American Radiolabel Chemicals Inc., St. Louis, MO) and the inert marker polyethylene glycol ([1,2-3H] PEG (MW 4000), DuPont NEN Research Products, Wilmington, DE).

Radioisotopes were mixed into warm, unhardened food mash at a concentration of approximately 18.5 kBq of ^{14}C D-glucose and 74 kBq of ^{3}H PEG per gram of food mash for extraction efficiency trials and 18.5 kBq of ^{14}C FeCN per gram of food mash for retention time trials (for specific methods see McWilliams and Karasov, '98). The initially soupy mixture was continuously stirred as it cooled and hardened to ensure uniform labeling of food. When only extraction efficiency was measured in a trial,

excreta were collected 4–5 hr after ingestion of the labeled diet. When only retention time was measured in a trial, excreta were collected singly for the first 30 min and thereafter every 15 min for 4–5 hr. Percent recovery of inert marker 4–5 hr after ingestion was 88% ± 11% for ^{3}H PEG and 95% ± 4% for ^{14}C FeCN (McWilliams and Karasov, '98).

Mouth-to-anus total mean retention time was calculated as the sum of the products of the proportion of inert marker excreted during each time interval multiplied by the elapsed time since ingestion of marker (Warner, '81). Extraction efficiency was calculated as:

$$100 - 100[(M_f/N_f) * (N_e/M_e)],$$

where M_f is radioactivity of the inert marker (PEG) in food, N_f is radioactivity of the nutrient (D-glucose) in food, N_e is radioactivity of nutrient (D-glucose) in excreta, and M_e is radioactivity of inert marker (PEG) in excreta.

Parametric one-tailed *t*-tests were used to analyze a priori predictions regarding differences in body mass, food intake, extraction efficiency, and retention time between cold-acclimated and room temperature-acclimated birds. Percentage data were arc-sine transformed prior to analysis. Results are given as mean ± SE unless otherwise noted.

Digestive enzymes, nutrient uptake, and gut morphometrics

Nutrient uptake rates and gut morphometrics were measured and tissue samples were collected for analysis of digestive enzymes in all 20 waxwings on January 4–9, 1995. Birds were weighed and then anesthetized using methoxyflurane. The gut was exposed and then cut just proximal to the stomach and at the rudimentary caeca, cleaned of extraneous tissue, and then placed in cold (0°C) avian Ringer (solution composition in mM was 161 CaCl, 4.7 KCL, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, and 20 $NaHCO_3$). The stomach was excised, opened, rinsed of contents, blotted dry, and then weighed. The intestine was perfused with cold avian Ringer. One end of the intestine was then held against a ruler while the other end was gently pulled until the intestine was taut. After release, the length was measured. The intestine was quickly blotted dry, weighed, and placed back in cold avian Ringer.

Approximately 0.5 cm segments of the proximal and distal halves of the small intestine were collected for analysis of dissacharidases and peptidases. Segments were placed in tared cryovials, weighed, and then stored in liquid N_2. We measured activity of selected membrane-bound digestive enzymes in whole tissue homogenates (Martinez del Rio, '90; Martinez del Rio et al., '95). Intestinal segments were thawed and homogenized for 10 to 15 sec using an Omni 5100 homogenizer (setting 6, Omni International, Inc., Warrenton, VA) in 350 mM mannitol in 1 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid/KOH, pH 7 (10 µl per milligram wet tissue) at 0°C. Homogenates were stored in liquid N_2 until enzyme (maltase, sucrase, aminopeptidase-N) hydrolysis rates were measured.

We assayed disaccharidase activity using a modification of the colorimetric method developed by Dahlqvist ('84), according to the methods of Martinez del Rio ('90). Briefly, homogenates were thawed at 4°C and 100 µl aliquots were incubated at 40°C for 10 min with 100 µl of 56.0 mM sugar (maltose and sucrose) solutions in 0.1 M maleate/NaOH buffer (pH 6.5). After incubation, reactions were arrested by adding 3.0 ml of a stop/develop reagent [one bottle of Glucose-Trinder 500 reagent (Sigma Chemical, procedure 315, St. Louis, MO) in 250 ml 1.0 M tris(hydroxymethyl)aminomethane/HCl, pH 7 plus 250 ml 0.5 M NaH_2PO_4/Na_2HPO_4, pH 7 buffer]. Absorbance was measured at 505 nm.

We measured aminopeptidase-N because it accounts for all or nearly all of the peptidase activity in the brush-border membrane (Maroux et al., '73) and so provides a good indicator of protein digestion at the brush-border membrane. We assayed aminopeptidase-N using L-alanine-p-nitroanilide as a substrate for aminopeptidase-N. To start the reaction we added 10 µl (~12 mg protein/ml) to 1 ml of assay mix (2.0 mM L-alanine-p-nitroanilide in 0.2 M NaH_2PO_4/Na_2HPO_4, pH 7 buffer). After incubation for 20 min at 40°C, reactions were arrested by adding 3.0 ml of ice-cold 2N acetic acid. Absorbance was measured at 384 nm.

To determine pH optima for the disaccharidases and aminopeptidase-N, we used proximal and distal tissue sections, respectively. We used a 0.05M maleate/NaOH buffer system with pH ranging from 5.5 to 8.0 for the disaccharidases and, a 0.2 M NaH_2PO_4/Na_2HPO_4 buffer system with pH ranging from 5.5 to 8.0 for aminopeptidase-N.

On the basis of absorbance measurements and glucose and p-nitroaniline standards, we calculated standardized activities of each intestinal section by dividing enzyme activity by the wet mass of the section. Results are provided as micromoles per minute per milligram wet tissue.

We measured intestinal uptake of leucine as described in Karasov and Diamond ('83). One-centimeter everted sleeves of intestine were mounted on metal rods and kept in cold avian Ringer solution until an uptake measurement was made (always within 1 hr of anesthetization with methoxyflurane). The solution was oxygenated with 95% O_2–5% CO_2 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 for 2 min in Ringer at 37°C over a stir bar at 1,200 rpm (Karasov and Levey, '90). We measured uptake of L-[2,3-3H]leucine into the tissue across the brush-border membrane using [carboxyl-14C]inulin to correct for adherent fluid. Uptakes of 0.1 and 50 mM L-leucine were measured in the proximal and distal halves of the small intestine. We had planned also to measure mediated uptake of D-glucose but technical errors invalidated the measures.

The carcass minus the intestine was plucked, freeze-dried, and then ground in a small coffee-grinder. We refluxed 1 g dried (at 50°C) subsamples with petroleum ether for 6 hr (Dobush et al., '85) in a Goldfisch apparatus to measure fat content. Lean mass (g dry) was defined as total body mass (g dry) without feathers and intestine, minus fat content (g dry).

We used analysis of covariance (ANCOVA, lean dry mass of bird as covariate) to compare gut dimensions between cold-acclimated and room temperature-acclimated birds. We used repeated-measures analysis of variance (ANOVA) to compare hydrolysis rates and uptake rates between proximal and distal sections of the small intestine, between individuals in the groups, and between temperature treatment groups. We used parametric one-tailed *t*-tests to analyze a priori predictions regarding differences in summed hydrolysis and uptake rates between cold-acclimated and room temperature-acclimated birds. We used reduced major axis regression (Model II regression) to determine the relationship between sucrase and maltase activity because both variables vary naturally and are measured with some error, so the estimated relation between the two would be biased if determined by Model I regression (Sokal and Rohlf, '81, p. 549). Results are given as mean ± SE unless otherwise noted.

RESULTS

Effects of acclimation temperature on body mass, food intake, extraction efficiency, and retention time

Birds in the cold (–20°C) were on average 8.0% heavier than birds at room temperature (21°C) (Table 1). Birds in the cold lost more absolute mass overnight (Table 1) and a higher proportion of initial body mass than birds at room temperature (cold group: 11.0% of body mass; room temperature group: 9.0% of body mass; $t_{16} = 2.2$, $P = 0.035$).

Birds in the cold consumed about 2.5 times more food each day than birds at room temperature (Table 1). During the 5-h test period, birds in the cold ate about four times more than birds at room temperature (Table 1) at least in part because birds in the cold usually increased their food intake in the afternoon as they increased their fat depots. Associated with the higher food intake of waxwings at –20°C, waxwings in the cold had slightly lower (1.5%) extraction efficiency ($t_{16} = 3.03$, $P = 0.004$; Fig. 1) and slightly shorter total mean retention time compared to waxwings at +21°C ($t_{16} = 1.87$, $P = 0.04$; Fig. 1).

Effects of acclimation temperature on digestive organs

Comparisons of gut morphometrics between cold- and room temperature–acclimated birds is

TABLE 1. Body mass and food intake (±SE) of cedar waxwings acclimated to one of two temperature conditions [treatment group at –20°C (n = 10) and control group at +21°C (n = 8)][†]

Acclimation temperature	Body mass (g) on pretest day at 1730 hours	Body mass (g) on test day at 0700 hours	Difference in body mass (g) between pretest and test day	Mean food intake (g dry weight) per day	Food intake (g dry weight) during 5-hr test period
–20°C	39.13 ± 0.67	34.82 ± 0.56	–4.32 ± 0.18	13.98 ± 0.45	6.87 ± 0.37
+21°C	36.21 ± 1.31	32.97 ± 1.54	–3.24 ± 0.67	5.22 ± 0.50	1.63 ± 0.25
t_{16}[1]	3.06	1.98	2.98	12.97	11.02
P value*	0.004	0.033	0.004	<0.0001	<0.0001

[†]Birds had food removed at 1730 hours on the pretest day.
[1]*t*-test assuming unequal variances ($n = 18$).
*P-value for one-tailed t-test ($n = 18$).

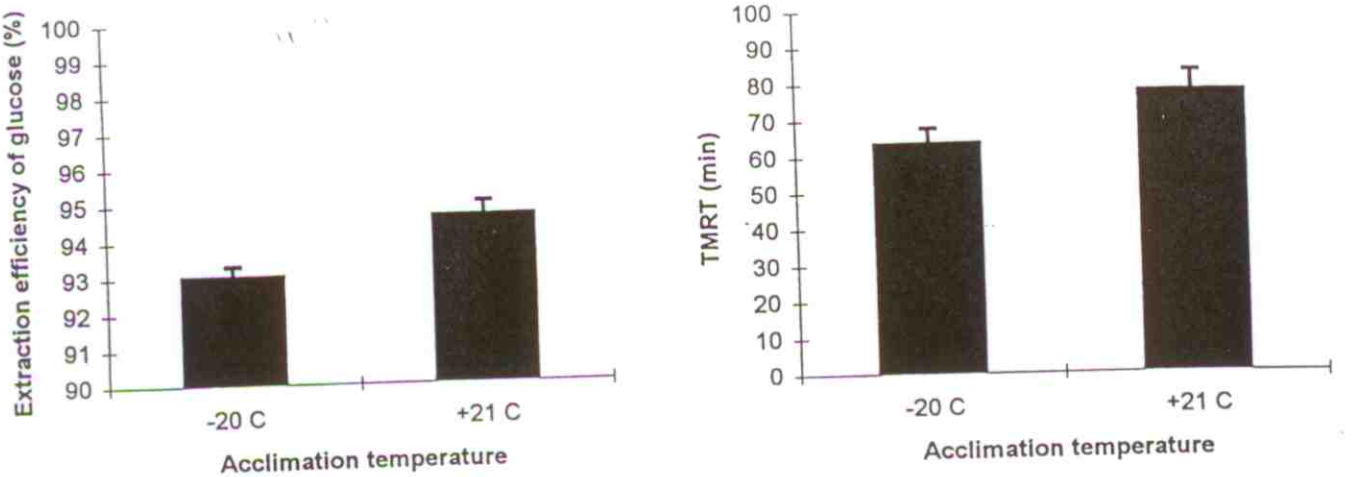

Fig. 1. Extraction efficiency of glucose (EE in %) and mouth-to-anus total mean retention time (TMRT in min) for cold-acclimated (–20°C) and room temperature-acclimated (+21°C) cedar waxwings.

complicated because birds in the cold were heavier (Table 1). However, analysis of the body composition of both groups revealed that cold-acclimated birds had only slightly higher lean mass than room-temperature acclimated birds, whereas the majority of the difference in body mass consisted of body fat (Table 2). Using lean mass to correct for body size differences between birds confirms that cold-acclimated waxwings had larger stomach and intestines compared with room-temperature-acclimated waxwings (Table 2).

Effects of acclimation temperature on enzyme hydrolysis rates

Enzyme hydrolysis rates were normalized to tissue wet mass but also can be expressed per centimeter length of intestine or per square centimeter nominal surface area of intestine using conversion factors in Table 3 or per milligram tissue protein using conversion factors in Figure 2.

Maltase activity decreased distally along the intestine whereas sucrase and aminopeptidase-N activity were similar along the intestine (Fig. 2). As predicted, acclimation temperature had no significant effect on specific activity of either carbohydrase (maltase or sucrase) or aminopeptidase-N activity (Fig. 2).

We calculated summed hydrolysis capacity for both cold- and room temperature-acclimated waxwings by averaging hydrolysis levels per cm measured in the proximal and distal small intestine and multiplying the mean by the small intestine length. As predicted, summed hydrolytic activity of both maltase and sucrase was higher in cold-acclimated waxwings than in room temperature-acclimated waxwings (Table 3). How-

TABLE 2. Body composition (g dry ± SE) and digestive organ mass (g wet ± SE) of cedar waxwings acclimated to one of two temperature conditions (treatment group at –20°C and control group at +21°C)

	Acclimation temperature		Statistical analysis (ANCOVA)[1]	
	–20°C	+21°C	$F_{1,17}$	*P*-value
n	11	9		
Body composition[2]:				
Lean (g)[3]	7.41 ± 0.24	6.66 ± 0.12		
Fat (g)[4]	7.83 ± 0.96	4.21 ± 0.46		
Small intestine (g)	2.40 ± 0.12	1.96 ± 0.04	4.38	0.053
Large intestine (g)	0.13 ± 0.01	0.09 ± 0.01	5.81	0.028
Stomach (g)	0.75 ± 0.04	0.49 ± 0.02	18.05	0.001

[1]Analysis of covariance (ANCOVA) uses lean mass as a covariate.
[2]Body composition of unfeathered waxwings includes a petroleum ether extractable component (i.e., fat) and a lean component [calculated as (whole dry mass – dry fat mass)].
[3]Waxwings at –20°C had more lean mass than waxwings at +21°C ($t_{18} = 3.31$, $P = 0.002$).
[4]Waxwings at –20°C had more fat than waxwings at +21°C ($t_{18} = 3.25$, $P = 0.002$).

TABLE 3. Intestinal measurements, summed uptake, and summed enzyme activity of cedar waxwings acclimated to one of two temperature conditions [treatment group at –20°C (n = 11) and control group at +21°C (n = 9)]

	Nominal surface area (cm^2/cm)[1]			Summed uptake (nmol/min)		Summed hydrolytic capacity (μmol/min)		
	Proximal position	Distal position	Intestine length (cm)	0.01 mM leucine	50 mM leucine	Maltase	Sucrase	Aminopeptidase-N
Treatment								
–20°C	1.68 ± 0.02	1.48 ± 0.02	15.70 ± 0.36	47.22 ± 0.8	19.8 ± 0.7	142.8 ± 16.1	13.0 ± 2.5	6.6 ± 1.3
+21°C	1.56 ± 0.04	1.38 ± 0.03	14.04 ± 0.27	38.95 ± 4.2	14.0 ± 0.8	88.7 ± 8.7	7.2 ± 0.8	8.7 ± 1.1
Statistical results[2]								
Treatment	$F_{1, 18} = 16.48$,	$P = 0.001$	$t_{17.3} = 3.57$, $P = 0.001$	$t_{15.6} = 0.98$, $P = 0.34$	$t_{12} = 5.61$, $P < 0.0001$	$t_{15.1} = 2.95$, $P = 0.01$	$t_{12.1} = 2.28$, $P = 0.04$	$t_{17.9} = 1.28$, $P = 0.22$
Position	$F_{1, 18} = 48.90$,	$P < 0.0001$						
Treatment × Position	$F_{1, 18} = 0.10$,	$P = 0.80$						

[1]Nominal surface area was calculated as the average circumference of the rods used during measurements of nutrient uptake and so does not include the contribution of villi or microvilli.
[2]Effects of temperature acclimation and intestinal position on nominal surface area of the small intestine were analyzed using repeated-measures ANOVA. Effects of temperature acclimation on intestinal length, summed uptake, and summed hydrolytic capacity were analyzed using *t*-tests with unequal variances [$n = 20$ except for sucrase ($n = 19$)].

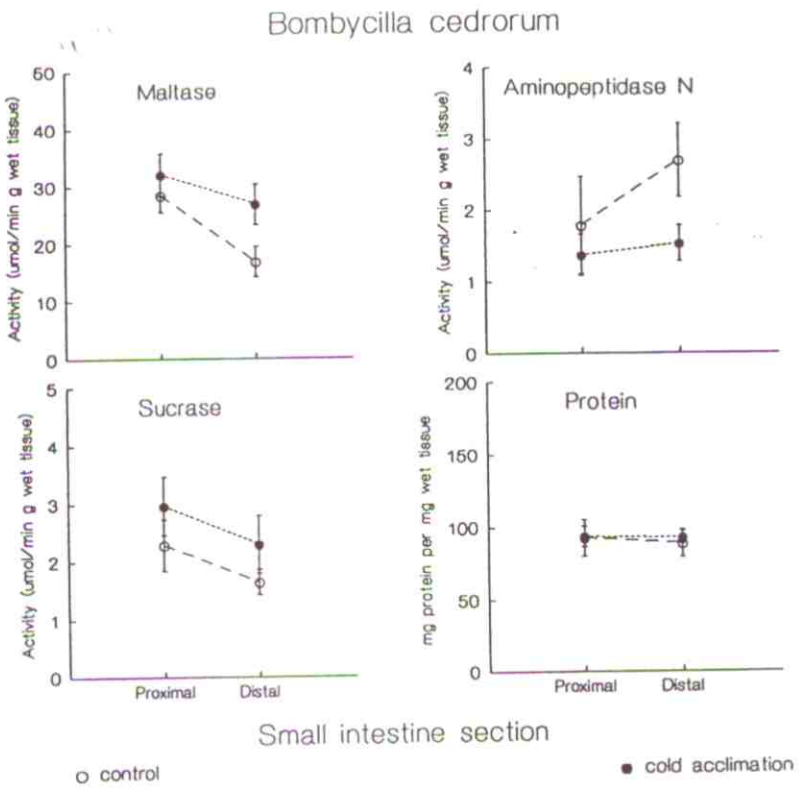

Fig. 2. Intestinal brush-border enzyme hydrolysis rates and protein content of the intestine in cedar waxwings as a function of position along the small intestine and temperature acclimation of the birds. Repeated-measures ANOVA revealed no significant effect of acclimation temperature (maltase: $F_{1,17} = 3.52$, $P = 0.08$; sucrase: $F_{1,17} = 2.28$, $P = 0.15$; aminopeptidase-N: $F_{1,17} = 3.91$, $P = 0.06$) or temperature * position interaction ($P > 0.30$ in all cases). Enzyme activity declined with distance along the small intestine for maltase ($F_{1,17} = 7.61$, $P = 0.01$) but not for sucrase ($F_{1,17} = 1.72$, $P = 0.21$) or aminopeptidase-N ($F_{1,17} = 0.82$, $P = 0.38$).

ever, acclimation temperature had no significant effect on summed hydrolytic activity of aminopeptidase-N (Table 3).

As a partial test for sucrase-independent maltase activity (Martinez del Rio, '90), we regressed maltase activity against sucrase activity (Fig. 3). Maltase and sucrase activities were tightly and linearly correlated, and the intercept of the regression line was significantly different from zero.

Measured pH optima for maltase and sucrase were 6.5 and for aminopeptidase-N was 7.0 (Fig. 4). Thus, our use of pH 6.5 for measuring carbohydrase activity and pH 7.0 for measuring aminopeptidase-N activity should provide accurate estimates of maximal enzyme activities.

Effects of acclimation temperature on in vitro intestinal uptake of nutrients

Uptake rates of L-leucine were normalized to mg wet intestine although these data can also be expressed per cm length of intestine or per cm^2 nominal surface area using conversion factors in Figure 5A or in Table 3. As predicted, specific uptake rates of leucine were similar in both cold-acclimated and control birds (Fig. 5B,C), although at 0.01 mM uptake rates increased along the small intestine in control birds but not in cold-acclimated birds.

To estimate summed uptake rate of the entire small intestine for leucine, we multiplied uptake rates per cm for each region by the length of the proximal or distal region of the small intestine (Table 3). As predicted, summed uptake in cold-acclimated waxwings was higher than in room temperature-acclimated waxwings (Table 3).

Uptake of L-leucine at 0.01 mM was mainly Na^+-dependent (i.e., active, carrier-mediated) as indicated by ratios of uptake in adjacent sleeves incubated in Na^+-free medium (Na^+ replaced by choline) or incubated in Na^+-containing medium (ratios for cold-acclimated waxwings were 0.21 ± 0.05 in proximal and 1.01 ± 0.12 in distal sections;

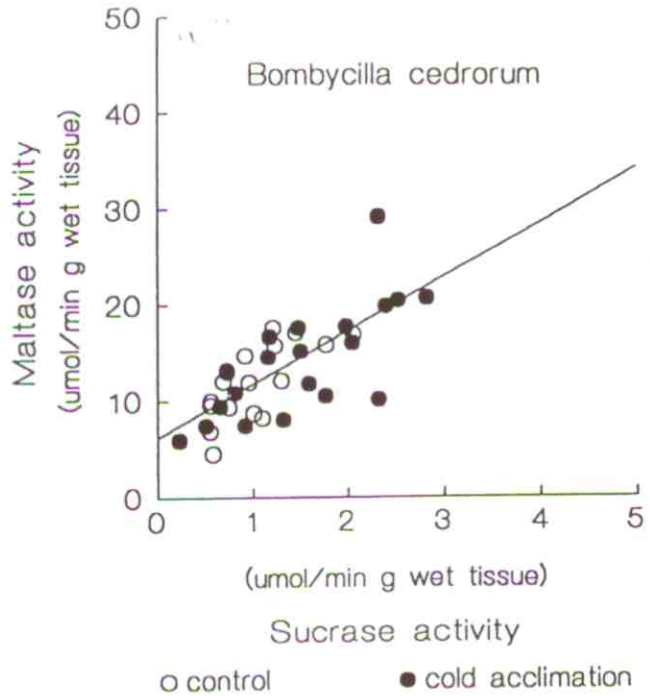

Fig. 3. Regression of intestinal maltase activity on sucrase activity in the small intestine of cedar waxwings. Model II regression (see text): maltase activity = 8.79 ± 2.57 + 7.71 ± 0.93 × sucrase activity, $r^2 = 0.569$, $P < 0.00001$.

ratios for room-temperature acclimated waxwings were 0.16 ± 0.05 in proximal and 0.34 ± 0.12 in distal sections; Treatment effect: $F_{1,8} = 12.1$, $P = 0.008$, Position effect: $F_{1,8} = 42.6$, $P < 0.0001$, Treatment * Position effect: $F_{1,8} = 16.8$, $P = 0.003$).

DISCUSSION

We begin by discussing our test of the hypothesis that hyperphagic cedar waxwings maintain digesta retention time and digestive efficiency constant, compared with waxwings feeding at reduced rates, by increasing the amount of intestine and thereby the total capacity for breakdown and absorption of nutrients. We conclude with comments about integrative gut function, spare capacity, and the time scale of digestive responses and their relevance for delineating potential digestive constraints of ecological importance.

Digestive adjustments to high feeding rate

Food intake, digesta residence time, and digestive efficiency

The higher thermoregulatory costs associated with living at cold temperatures require animals to eat more to maintain energy balance (Dawson et al., '83). Theoretically, increased food intake

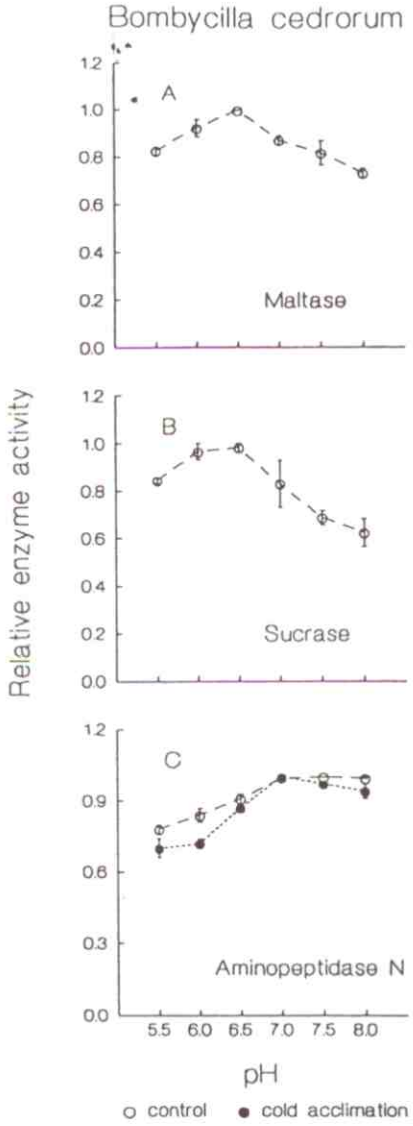

Fig. 4. Relative intestinal brush-border enzyme activity as a function of buffer pH.

alone can satisfy the increased energetic requirements within limits (Karasov, '96) but the animal's energetic gain can be enhanced by increasing or at least maintaining digestive efficiency with increased food intake.

Cedar waxwings acclimated for at least 60 days at –20°C had fourfold higher food intakes during the 4–5 hr trials compared with waxwings at +21°C. If there were no compensatory adjustments in digestive features of waxwings, the model (see Introduction) predicts that the more than fourfold increase in food intake should move food faster through the gut and result in a decrease in extraction efficiency. Extraction efficiency of glucose in waxwings at –20°C (93.0%) was lower com-

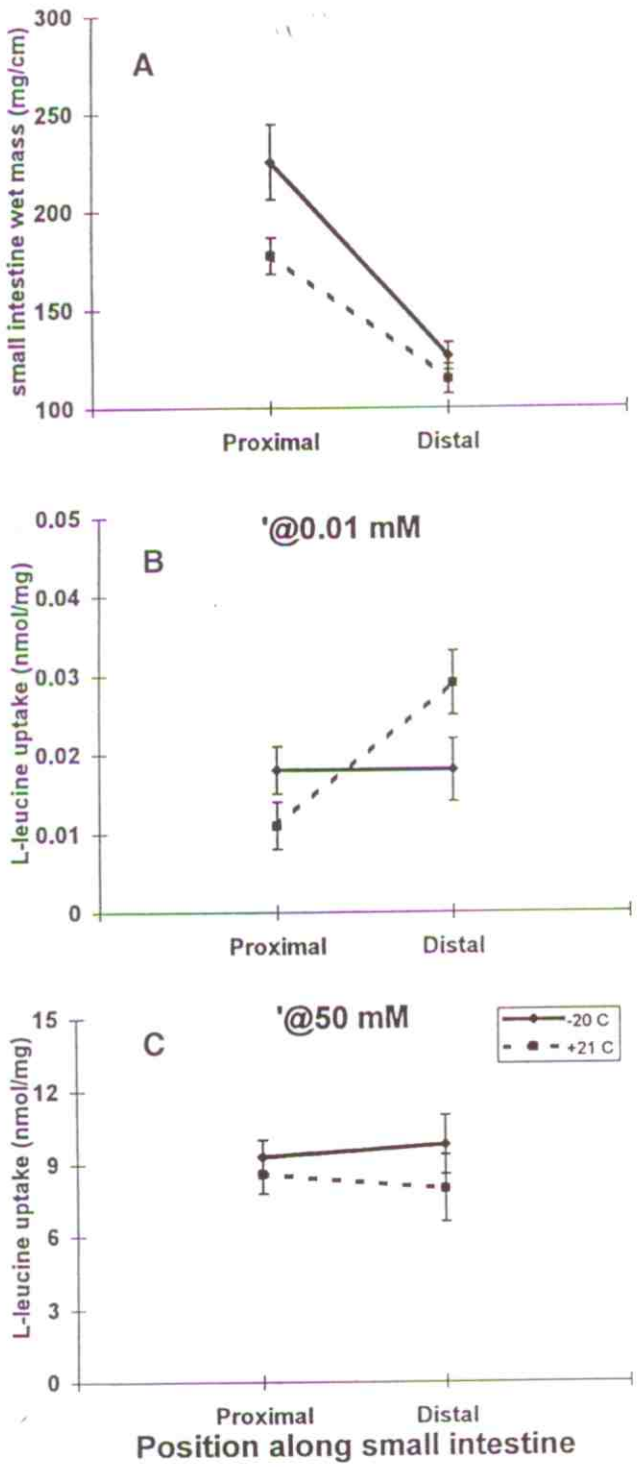

Fig. 5. Wet mass (g) of 1 cm intestinal sleeves (A) and uptake of L-leucine at 0.01 mM (B) and 50 mM (C) in the proximal and distal small intestine of cold-acclimated (–20°C) and room temperature-acclimated (+21°C) cedar waxwings. Repeated-measures analysis of variance (ANOVA) was used to compare small intestine mass and uptake rates between proximal and distal sections of the small intestine and between temperature treatment groups. Mass of the intestine declined with distance along the small intestine ($F_{1,18} = 53.7$, $P < 0.0001$) and –20°C waxwings had heavier intestines than +21°C waxwings (treatment: $F_{1,18} = 4.5$, $P = 0.04$; treatment * position: $F_{1,18} = 2.7$, $P = 0.12$). L-leucine uptake at 50 mM did not change with acclimation temperature or intestinal position ($P > 0.30$ in all cases). L-leucine uptake at 0.01 mM did not change with acclimation temperature ($F_{1,18} = 0.19$, $P = 0.66$) but did change with intestinal position (position: $F_{1,18} = 11.5$, $P = 0.003$; treatment * position: $F_{1,18} = 11.3$, $P = 0.003$).

pared with waxwings at +21°C (94.6%) although the decrease in efficiency was small and arguably ecologically irrelevant. Digestive efficiency in some birds has been reported to increase with decreasing temperature and increasing intake (Owen, '70; Stalmaster and Gessaman, '82; Dykstra and Karasov, '92) whereas in other birds, digestive efficiency has been reported to not change (El-Wailly, '66; Hamilton, '85) or decline with decreasing temperature and increasing intake (West, '68; Moss and Parkinson, '72). The lack of an increase in digestive efficiency during hyperphagia in cedar waxwings provides little support for Bairlein's ('85; Bairlein and Simons, '95) assertion that increased digestive efficiency during hyperphagia is a key mechanism by which premigratory fattening is achieved in birds.

The waxwing's gut could have accommodated the more than fourfold increase in food intake with only a slight decline in extraction efficiency of glucose in two ways: by increasing tissue-specific enzyme or uptake rates to compensate for faster food transit times; or by increasing gut size so that digesta retention time does not change, although the latter could also result in higher biochemical capacities, as well. We observed a statistically significant decline in retention time of digesta (Fig. 1), however, it was not nearly as great as the fourfold decline that one might predict if the gut were a simple, rigid fixed volume. This result suggests that the gut probably enlarged mostly to compensate for the increased food intake.

Effects of food intake on digestive organs

Small and large intestines and the stomach were 22%, 48%, and 51% heavier, respectively, in cold-acclimated birds compared with control birds. Such evidence is consistent with the hypothesis that increased digestive organ size permitted the large increase in food intake without considerable decrease in digestive efficiency or retention time.

Phenotypic plasticity of the avian gut has been reported in many birds in relation to seasonal changes in diet composition (Pendergast and Boag, '73; Moss, '74, '83; Ankney, '77; DuBowy, '85, Al-

Dabbagh et al., '87; Walsberg and Thompson, '90; Moorman et al., '92; Piersma et al., '93). There is also direct experimental evidence for an effect of diet composition (Miller, '75; Savory and Gentle, '76a,b; Kehoe et al., '88; Starck and Kloss, '95) and diet quantity (e.g., Japanese quail [Fenna and Boag, '74], domestic fowl [Savory, '86], house wrens [Dykstra and Karasov, '92]) on the avian gut. In many cases the effect of diet quality could be a direct effect of food intake because a decrease in diet quality is often associated with an increase in food intake, presumably as a result of energy dilution with decrease in diet quality.

Biochemical adjustments to high feeding rate

Another possibility suggested by the model is a simultaneous increase in reaction rates (either enzyme hydrolysis rates or nutrient uptake rates) to compensate for less contact time between digesta and gut absorptive surfaces. As predicted, we found that increased food intake in waxwings resulted in no change in carbohydrase (maltase and sucrase), aminopeptidase-N, or leucine uptake activity per unit small intestine. But, because of increased gut size, *total* enzyme hydrolysis and nutrient absorption rates were mostly higher in cold-acclimated waxwings.

Only one other study of birds has investigated the effect of increased food intake on nutrient uptake rates. Increased food intake in wrens resulted in no change in nutrient uptake per unit of intestine although, because of an increase in amount of intestine, the total capacity to absorb L-proline increased 23% compared to low-intake wrens (Dykstra and Karasov, '92). In cedar waxwings, increased food intake resulted in no change in nutrient uptake rates per unit of small intestine although, because of increased gut size, total leucine uptake rate was 41% higher in cold-acclimated waxwings.

Ours is the first study of birds to assess the direct effect of increased food intake on brush-border enzyme activity. Martinez del Rio et al. ('89) provide the only other published study of digestive enzymes (only sucrase) in cedar waxwings. Their estimates of sucrase activity in waxwings (35–40 μmol min^{-1} [g protein]$^{-1}$) were similar to our measurements in the proximal small intestine (25–38 μmol min^{-1} [g protein]$^{-1}$) but higher than our measurements in the distal small intestine (20–25 μmol min^{-1} [g protein]$^{-1}$). Summed maltase activity (89–143 μmol min^{-1}) was higher than that predicted from the allometric power relationship developed by Martinez del Rio ('90) using 11 species of passerine bird (60–68 μmol min^{-1}), although specific maltase activity (4–6 μmol [min cm^{2}]$^{-1}$) was within the same range as reported for other frugivorous passerines (Afik et al., '95). Aminopeptidase-N activity of waxwings was similar or higher than that found in yellow-rumped warblers (Afik et al., '95), one of the few other avian species in which such measurements have been made.

Intestinal enzyme activity is modulated in response to changes in diet composition in some vertebrates although relatively little work has focused on birds (Karasov, '96; Karasov and Hume, '97). The general pattern in mammals and poultry is that sucrase and maltase activity increase with dietary carbohydrate, and peptidases (e.g., aminopeptidase-N) increase with dietary protein. However, in some passerines (i.e., European starling and yellow-rumped warbler) aminopeptidase-N, but not carbohydrase, activity was modulated in response to changes in dietary substrate (protein and carbohydrates, respectively) (Afik et al., '95; Martinez del Rio et al., '95).

Granivorous chickens and turkeys fed diets with more carbohydrate increased their carbohydrase activity (Sell et al., '89; Biviano et al., '93). In contrast, omnivorous yellow-rumped warblers and starlings exposed to changes in diet composition modulated peptidase activity, but not carbohydrase activity (Afik et al., '95; Martinez del Rio et al., '95). Too few studies of modulation of digestive enzymes have been conducted to conclude whether these differences between passerines and poultry reflect important phylogenetic or ecological pattern(s).

Relationship between sucrase and maltase activity

Maltase activity is the result of both a sucrase-isomaltase complex (that also accounts for all sucrase activity) and a maltase-glucoamylase complex (Martinez del Rio, '90). Theoretically, the intercept of the regression of maltase activity against sucrase activity estimates the activity of sucrase-independent maltase (i.e., maltase-glucoamylases (Semenza and Auricchio, '89). We estimated the contribution of sucrase-independent maltase at 29–39% of the maltase activity which is higher than the 20–25% found in chickens (Biviano et al., '93) or the 0% found in yellow-rumped warblers (Afik et al., '95).

Integrative gut function: modulation of digestive features to maintain digestive efficiency?

In the only other study of birds that measured the effects of increased food intake on key digestive

features (Dykstra and Karasov, '92), insectivorous house wrens responded to cold acclimation (–9°C) as predicted here: wrens doubled their food intake, gut mass and volume increased by 25–35%, rates of nutrient uptake per unit small intestine did not change significantly, digesta retention time did not change, and digestive efficiency did not decline compared to when wrens were feeding at reduced rates. Rates of enzyme activity were not measured.

In our experiment we also used cold-acclimation to induce high food intake. Compared with cedar waxwings held at +21°C, waxwings acclimated to –20°C more than quadrupled their food intake, mass of digestive organs increased by 22–53%, rates of enzyme activity and nutrient uptake per unit small intestine did not change significantly, retention time of digesta declined slightly, and there was a small decrease in digestive efficiency. In short, for both wrens and waxwings, the amount of gut rather than the specific enzyme or nutrient absorption rates was mainly responsible for the increase in total capacity.

The principal difference between our study and the study on wrens is the degree to which food intake increased, with waxwings increasing food intake more than fourfold whereas the wrens increased food intake only twofold. The higher intake in waxwings was associated with a significant decrease in retention time and digestive efficiency in cold-acclimated waxwings despite the dramatic increase in mass of digestive organs. Such a pattern suggests that the waxwings had reached the limit of gut size increase.

Theoretically, there must be some limit to an animal's ability to enhance digestive features, increase food intake, and sustain elevated metabolic rates (see Ricklefs, '96 for a recent review). Animals at a given level of energy intake maintain some spare digestive capacity as evidenced by an ability to rapidly increase food intake without loss of digestive efficiency (Diamond, '91). As energy requirements and thus food intake increase, however, the level of spare capacity declines even after sufficient acclimation time (Toloza et al., '91). For a volant bird like a waxwing, gut size increases with energy expenditure but the increase may be limited by other physiological constraints associated with flying.

There are clear aerodynamic limits on the weight and shape of a bird given its wing design (Pennycuick, '75; Norberg and Rayner, '87). These limits are reached at times by migratory birds as indicated by their inability to fly after fattening periods (Jehl, '97). Our cold-acclimated waxwings had 17.8% of their total wet mass in gut tissue and associated digestive organs (Table 2) compared with only 14.0% for waxwings at room temperature. Presumably, heavier and longer guts also hold more digesta adding considerably more mass when the bird is full.

Time scale of digestive adjustments

Digestive adjustments are ecologically important because they help delineate constraints on feeding ecology and physiological performance of organisms (Karasov, '96; Piersma and Lindstrom, '97). In this respect, determining both the limits of digestive adjustment and the time required for these adjustments is important. We found changes in gut morphology 9–13 weeks after acclimation to cold temperatures but these changes may occur much faster than 9 weeks. Reversible changes in gut length in response to changes in diet composition have been reported to occur within 3–4 weeks in grouse and quail (Moss, '72; Savory and Gentle, '76a,b) and ducks (Miller, '75; Drobney, '84). Some of the best evidence for minimum time required for digestive adjustments comes from studies of the dynamics of cellular turnover in the avian intestine (Starck, '96). Turnover time of intestinal epithelium in adult Japanese quail was 9–17 days depending on the region of the intestine (Starck, '96) and may be as fast as a few hours in very young birds (Lilja, '87). Our acclimation period of >60 days seems adequate for the expression of gut modulation. It would be interesting to know whether a bird can support a more rapid twofold or fourfold increase in food intake without negative effects on digestive efficiency. This requires more acute challenge studies that focus on the match between digestive capacity and load at certain levels of food intake.

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LITERATURE CITED

Afik D, Caviedes-Vidal E, Martinez del Rio C, Karasov WH. 1995. Dietary modulation of intestinal hydrolytic

enzymes in yellow-rumped warblers. Am J Physiol 269:R413–R420.
Afik D, Karasov WH. 1995. The trade-offs between digestion rate and efficiency in warblers and their ecological implications. Ecology, 76:2247–2257.
Al-Dabbagh KY, Jiad JH, Waheed IN. 1987. The influence of diet on the intestine length of the white-cheeked bulbul. Ornis Scand 18:150–152.
American Ornithologists' Union. 1983. Check-list of North American birds, ed. 6. Washington, DC: American ornithologists' union.
Ankney CD. 1977. Feeding and digestive organ size in breeding lesser snow geese. Auk 94:275–282.
Bairlein F. 1985. Efficiency of food utilization during fat deposition in the long-distance migratory garden warbler, *Sylvia borin*. Oecologia 68:118–125.
Bairlein F, Simons D. 1995. Nutritional adaptations in migrating birds. Israel J Zool 41:357–367.
Bent AC. 1950. Life histories of North American wagtails, shrikes, vireos, and their allies. US Natl Mus Bull vol 197.
Berthold P. 1996. Control of bird migration. New York: Chapman and Hall.
Biviano AC, Martinez del Rio C, Phillips DL. 1993. Ontogenesis of intestine morphology and intestinal disaccharidases in chickens *Gallus gallus* fed constrasting purified diets. J Comp Physiol 163B:508–518.
Blem CR. 1976. Patterns of lipid storage and utilization in birds. Am Zool 16:671–684.
Blem CR. 1990. Avian energy storage. In: Power DM, editor. Current ornithology, vol. 7. New York: Plenum Press. p 59–113.
Dahlqvist A. 1984. Assay of intestinal disaccharidases. Scand. J Clin Lab Invest 44:69–172.
Dawson WR, Marsh RL, Yacoe ME. 1983. Metabolic adjustments of small passerine birds for migration and cold. Am J Physiol 245:R755–R767.
Denslow JS, Levey DJ, Moermond TC, Wentworth BC. 1987. A synthetic diet for fruit-eating birds. Wilson Bulletin 99:131–134.
Diamond J. 1991. Evolutionary design of intestinal nutrient absorption: enough but not too much. News in Physiol Sciences 6:92–96.
Dobush GR, Ankney CD, Krementz DG. 1985. The effect of apparatus, extraction time, and solvent type on lipid extractions of snow geese. Can J Zool 63:1917–1920.
Drobney RD. 1984. Effect of diet on visceral morphology of breeding wood ducks. Auk 101:93–98.
Dubowy PJ. 1985. Seasonal organ dynamics in post-breeding male blue-winged teal and northern shovelers. Comp Biochem Physiol 82A:899–906.
Dykstra CR, Karasov WH. 1992. Changes in gut structure and function of house wrens (*Troglodytes aedon*) in response to increased energy demands. Physiol Zool 65:422–442.
El-Wailly AJ. 1966. Energy requirements of egg-laying and incubation in the zebra finch, *Taeniopygia castanotis*. Condor 68:582–594.
Fenna L, Boag DA. 1974. Adaptive significance of the caeca in Japanese quail and spruce grouse (*Galliformes*). Can J Zool 52:1577–1584.
Hamilton KL. 1985. Food and energy requirements of captive barn owls *Tyto alba*. Comp Biochem Physiol 80A:355–358.
Jacobs LR, Bloom S, Harsoulis P, Dowling RH. 1975. Intestinal adaptation to hypothermic hyperphagia. Clin Sci Mol Med 48(abstract):14.
Jehl JR Jr. 1997. Fat loads and flightlessness in Wilson's Phalaropes. Condor 99:538–543.
Karasov WH. 1996. Digestive plasticity in avian energetics and feeding ecology. In: Carey C, editor. Avian energetics and nutritional ecology. New York: Chapman and Hall. p 61–84.
Karasov WH, Diamond J. 1983. Adaptive regulation of sugar and amino acid transport by vertebrate intestine. Am J Physiol 245:G443–462.
Karasov WH, Hume I. 1997. Vertebrate gastrointestinal system. In: Dantzler WH, editor. Handbook of physiology. Section 13: comparative physiology, vol. 1. New York: Oxford University Press. p 409–480.
Karasov WH, Levey DJ. 1990. Digestive system trade-offs and adaptations of frugivorous passerine birds. Physiol Zool 63:1248–1270.
Karasov WH, Phan D, Diamond JM, Carpenter FL. 1986. Food passage and intestinal nutrient absorption in hummingbirds. Auk 103:453–464.
Kehoe FP, Ankney CD, Alisauskas RT. 1988. Effects of dietary fiber and diet diversity on digestive organs of captive mallards (*Anas platyrhynchos*). Can J Zool 66:1597–1602.
Levey DJ, Karasov WH. 1989. Digestive responses of temperate birds switched to fruit or insect diets. Auk 106:675–686.
Lilja C. 1987. Mitotic activity of duodenal crypt cells in the young fieldfare (*Turdus pilaris*). Acta Physiol Scand 131:163–164.
Maroux S, Louvard D, Baratti J. 1973. The aminopeptidases from hog intestinal brush-border. Biochim Biophys Acta 321:282–295.
Martinez del Rio C. 1990. Dietary, phylogenetic, and ecological correlates of intestinal sucrase and maltase activity in birds. Physiol Zool 63:987–1011.
Martinez del Rio C, Brugger KE, Rios JL, Vergara ME, Witmer M. 1995. An experimental and comparative study of dietary modulation of intestinal enzymes in the European starling (*Sturnis vulgaris*). Physiol Zool 68:490–511.
Martinez del Rio C, Karasov WH, Levey DJ. 1989. Physiological basis and ecological consequences of sugar preferences in cedar waxwings. Auk 106:64–71.
McWilliams SR. Karasov WH. 1998. Test of a digestion optimization model: effects of costs of feeding on digestive parameters. Physiol Zool 71:168–178.
Miller M. 1975. Gut morphology of mallards in relation to diet quality. J Wildl Mgmt 39:168–173.
Moorman TE, Baldassarre GA, Richard DM. 1992. Carcass mass, composition, and gut morphology dynamics of mottled ducks in fall and winter in Louisiana. Condor 94:407–417.
Moss R. 1972. Effects of captivity on gut length in red grouse. J Wildl Mgmt 36:99–104.
Moss R. 1974. Winter diet, gut length, and interspecific competition in Alaskan ptarmigan. Auk 91:737–746.
Moss R. 1983. Gut size, body weight, and digestion of winter food by grouse and ptarmigan. Condor 85:185–193.
Moss R, Parkinson JA. 1972. Digestion of heather (*Calluna vulgaris*) by red grouse (*Lagopus lagopus scoticus*). Br J Nutr 27:285–298.
Murphy ME, King JR. 1982. Semi-synthetic diets as a tool for nutritional ecology. Auk 99:165–167.
Norberg UM, Rayner JMV. 1987. Ecological morphology and flight in bats (Mammalia: Chiroptera): wing adaptations, foraging strategy, and echolocation. Philos Trans Roy Soc London B 316:335–427.
Owen RB Jr. 1970. The bioenergetics of captive blue-winged

teal under controlled and outdoor conditions. Condor 72:153–163.
Pennycuick CJ. 1975. Mechanics of flight. In: Farner DS, King JR, editors. Avian biology, vol. 5. London: Academic Press. p 1–75.
Pendergast BA, Boag DA. 1973. Seasonal changes in the internal anatomy of spruce grouse in Alberta. Auk 90: 307–317.
Piersma T, Koolhaas A, Dekinga A. 1993. Interactions between stomach structure and diet choice in shorebirds. Auk 110:552–564.
Piersma T, Lindstrom A. 1997. Rapid reversible changes in organ size as a component of adaptive behaviour. Trends Ecol Evol 12:134–138.
Ricklefs RE. 1996. Avian energetics, ecology, and evolution. In: Carey C, editor. Avian energetics and nutritional ecology. New York: Chapman and Hall. p 1–30.
Savory CJ. 1986. Influence of ambient temperature on feeding activity parameters and digestive function in domestic fowl. Physiol Behav 38:353–357.
Savory CJ, Gentle MJ. 1976a. Effects of dietary dilution with fibre on the food intake and gut dimensions of Japanese quail. Br Poult Sci 17:561–570.
Savory CJ, Gentle MJ. 1976b. Changes in food intake and gut size in Japanese quail in response to manipulation of dietary fiber content. Br Poult Sci 17:571–580.
Sell JL, Kolodovsky O, Reid BL. 1989. Intestinal disaccharidases of young turkeys: temporal development and influence of diet composition. Poult Sci 68:265–277.
Semenza G, Auricchio S. 1989. Small-intestine disaccharidases. In: Cribner CR, Beaudet AL, Sly WS, Valle D, editors. The metabolic basis of inherited disease, vol. II. New York: McGraw Hill. p 2975–2997.
Sokal RR, Rohlf FJ. 1981. Biometry. W.H. San Francisco, CA: Freeman and Co.
Stalmaster MV, Gessaman JA. 1982. Food consumption and energy requirements of captive bald eagles. J Wildl Mgmt 46:646–654.
Starck JM. 1996. Phenotypic plasticity, cellular dynamics, and epithelial turnover of the intestine of Japanese quail (*Coturnix coturnix japonica*). J Zool Lond 238:53–79.
Starck JM, Kloss E. 1995. Structural responses of Japanese quail intestine to different diets. Dtsch Tierarztl Wochenschr 102:146–150.
Toloza EM, Lam M, Diamond J. 1991. Nutrient extraction by cold-exposed mice: a test of digestive safety margins. Am J Physiol 261:G608–G620.
Walsberg GE, Thompson CW. 1990. Annual changes in gizzard size and function in a frugivorous bird. Condor 92: 794–795.
Warner ACI. 1981. Rate of passage of digesta through the gut of mammals and birds. Nutr Abstr Rev 51:789–820.
West GC. 1968. Bioenergetics of captive willow ptarmigan under natural conditions. Ecology 49:1035–1045.
Witmer MC. 1996. Annual diet of cedar waxwings based on U.S. Biological Survey records (1885–1950) compared to diet of American robins: contrasts in dietary patterns and natural history. Auk 113:414–430.