

Does Gut Function Limit Hummingbird Food Intake?

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ABSTRACT

Many nectar-feeding bird species decrease food intake when sugar concentration in food is increased. This feeding response can be explained by two alternative hypotheses: compensatory feeding and physiological constraint. The compensatory feeding hypothesis predicts that if birds vary intake to maintain a constant energy intake to match energy expenditures, then they should increase intake when expenditures are increased. Broad-tailed hummingbirds were presented with sucrose solutions at four concentrations (292, 584, 876, and 1,168 mmol L⁻¹) and exposed to two environmental temperatures (10° and 22°C). Birds decreased volumetric food intake in response to sugar concentration. However, when they were exposed to a relatively sudden drop in environmental temperature and, hence, to an acute increase in thermoregulatory energy expenditures, they did not increase their rate of energy consumption and lost mass. These results support the existence of a physiological constraint on feeding intake. A simple chemical reactor model based on intestinal morphology and *in vitro* measurements of sucrose hydrolysis predicted observed intake rates closely. This model suggests that intestinal sucrose hydrolysis rates were near maximal and, thus, may have imposed limits to sugar assimilation. Although sugar assimilation was high (95%), the proportions of excreted sucrose, glucose, and fructose found in excreta differed significantly. The monosaccharides glucose and fructose were about eight and three times more abundant than sucrose, respectively. Broad-tailed hummingbirds are small high-altitude endotherms that face unpredictable weather and the energetic expense of premigratory fattening. Digestive processes have the potential to impose severe challenges to their energy budgets.

Introduction

To fuel the energetic demands of hovering flight (Suarez 1992) and temperature regulation of tiny bodies (Pearson 1950; Lasiewski 1963), hummingbirds feed on sugar-containing floral nectars (Martínez del Río 1990*b*). The expensive metabolic life of hummingbirds is matched by efficient and rapid sugar processing in the gastrointestinal tract. Hummingbirds display remarkably high rates of sucrose hydrolysis (Martínez del Río 1990*a*) and the highest levels of carrier-mediated glucose absorption recorded in a vertebrate (Karasov et al. 1986). Although hummingbirds have powerful digestive systems, it is conceivable that they are insufficient to provide adequate fuel when metabolic demands are exceptionally high. Here we explore the idea that digestive processes can impose constraints on the rate at which hummingbirds ingest food.

When sugar concentration in food is experimentally increased, many nectar-feeding bird species, including hummingbirds, decrease volumetric food intake (Collins 1981; Downs 1997; López-Calleja et al. 1997). The inverse relationship between volumetric intake and sugar concentration often leads to relatively constant sugar intake. By modulating volumetric intake with sugar concentration, birds appear to defend a constant rate of energy intake (López-Calleja et al. 1997). A reciprocal relationship between nutrient density and food intake is not exclusive to nectar-feeding birds. Similar relationships have been observed in a variety of animal species, ranging from blowflies to herbivorous mammals (Montgomery and Baumgardt 1965; Batzli and Cole 1979; Simpson et al. 1989; Nagy and Negus 1993; Castle and Wunder 1995). The negative relationship between intake and food quality has been called an "intake-response relationship" (Castle and Wunder 1995), and we adopt this terminology here.

The widespread occurrence of an inverse relationship between caloric/nutrient density and food intake often has been attributed to compensatory feeding (Simpson et al. 1989). According to this explanation, animals regulate food intake to maintain a constant flux of assimilated energy or nutrients (Montgomery and Baumgardt 1965; Slansky and Wheeler 1992). If the energy/nutrient density in food is decreased, animals compensate by increasing intake. An alternative hypothesis to compensatory feeding is that intake is constrained by the ability of animals to process the nutrients contained in food (Levey and Martínez del Río 1999). The question that we address here is whether the intake-response relationship found in hummingbirds is the result of constraints on the intake rate imposed by digestive processes or whether it is the result of compensatory feeding.

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We hypothesized that if we varied both sugar concentration in food and ambient temperature in captive broad-tailed hummingbirds, *Selasphorus platycercus*, (1) the birds would show the typical negative intake-response relationship between volumetric intake and sugar concentration in food and (2) for a given food energy density, birds exposed to lower temperatures and, hence, higher energy demands would show higher rates of food intake. An increase in sugar intake with increased energetic demands would provide evidence for compensatory feeding. Conversely, no changes in sugar intake with increased demands would support the notion that some physiological process constrains the rate of sugar intake. Chronic cold exposure can be accompanied by increased digestive and metabolic capacities (Konarzewski and Diamond 1994 and references therein). Thus, testing between constraint and compensatory feeding requires that animals be exposed to the cold under short-term, acute conditions (see López-Calleja et al. 1997). The experiments described here to detect the role of digestive traits in shaping the intake response of hummingbirds were conducted under acute cold exposure.

To investigate the potential physiological processes that could impose limits to sugar intake, we measured the efficiency with which broad-tailed hummingbirds assimilated sugars, the time that food was retained in the gastrointestinal tract, the rate of sucrose hydrolysis in the intestine, and the content of different sugar types in their excreta. We emphasized sucrose and its assimilation because this sugar is the primary constituent of the floral nectars preferred by hummingbirds (Martínez del Río 1990b; Martínez del Río et al. 1992). To be assimilated, sucrose must first be hydrolyzed by membrane-bound sucrase (Martínez del Río 1990a). The products of sucrose hydrolysis, the monosaccharides glucose and fructose, are then transported into intestinal cells (Karasov and Diamond 1988). Therefore, the ability of hummingbird intestines to rapidly hydrolyze sucrose is critical to the maintenance of high rates of energy assimilation. To assess the importance of sucrose hydrolysis relative to the transport of its hydrolysis products (glucose and fructose) as a limiting factor in sugar assimilation, we measured the relative composition of sugars in hummingbird excreta. A large proportion of sucrose, relative to glucose and fructose, in excreta would indicate hydrolysis as a limiting step, whereas a relatively large fraction of glucose and fructose would indicate that uptake is limiting.

Broad-tailed hummingbirds are the major breeding hummingbirds in the southern and central Rocky Mountains, the mountains of eastern California, and the Sierra Madre of Mexico (Calder and Calder 1992). The mountainous environment inhabited by these hummingbirds is characterized by unpredictable and often harsh weather that frequently exposes them to spells of cold temperature (Calder and Calder 1992). The challenges that broad-tailed hummingbirds normally face (described in detail for congeneric *Selasphorus rufus* by Gass and Lertzman 1980) make our investigation ecologically pertinent

and make these birds suitable subjects for research on the effects of low temperatures on food intake.

Material and Methods

Bird Capture and Maintenance

Eight broad-tailed hummingbirds (body mass = 3.3 ± 0.1 g, mean \pm SE) were captured with mist nets in Albany County, Wyoming (lat. $41^{\circ}20'N$, long. $106^{\circ}15'W$), and housed individually in wire-mesh cages ($0.75 \times 0.75 \times 0.75$ m). During experiments, birds were housed individually in opaque Plexiglas cages ($0.5 \times 0.5 \times 0.5$ m) with individual light sources. The front of these cages was a one-way mirror that permitted observation of birds with minimal disturbance. Birds were allowed to acclimate to experimental cages for 2–3 d before the experiments began. The study was conducted using a constant natural photoperiod from the time of bird capture (16L : 8D). Birds were fed Roudybush Nectar 3 for adult hummingbirds between experiments (Roudybush, Templeton, Calif.). Birds maintained body mass in captivity during these periods. During experiments, birds were fed synthetic diets modified from Brice and Grau (1989).

Intake, Mean Retention Time, and Sugar Assimilation

Each bird was randomly assigned to one of four sugar concentrations (292, 584, 876, and 1,168 mmol sucrose L^{-1}) and exposed to 22° and $10^{\circ}C$ in two different experiments. These sugar concentrations span the range of energy densities found in floral nectars of hummingbird-pollinated species (Baker 1975). Birds were tested at $22^{\circ} \pm 2^{\circ}C$ and $10^{\circ} \pm 1^{\circ}C$ in a walk-in environmental chamber. The resting metabolic rate of broad-tailed hummingbirds at $10^{\circ}C$ is about 75% higher than that of hummingbirds at $20^{\circ}C$ (Bucher and Chappel 1988). Birds were weighed to the nearest 0.1 g before and after experimental treatments. Each experiment consisted of 1 d during which the birds were acclimated to experimental diets and two treatment days. Mean retention time (MRT) was estimated on treatment day 1 and sugar assimilation was estimated on treatment day 2. Because the bird holding room was at $22^{\circ}C$, birds exposed to $22^{\circ}C$ in the environmental chamber were subject to the same conditions for several months. In contrast, birds exposed to $10^{\circ}C$ were only exposed to this temperature for a day before measurements were made. Birds in both treatments were held in experimental cages and in the environmental chamber for a day before measurement to standardize experimental conditions. Food was provided ad lib. throughout experiments in small glass feeding tubes placed through a hole in the back wall of experimental cages. Perches were situated so that birds were forced to fly in order to feed.

On the first treatment day, birds were fed an unlabeled experimental diet immediately after lights were turned on. After an hour, the unlabeled diet was removed and birds were offered

a diet containing a radiolabeled marker. Birds all fed on the labeled diet within 5 min of the diet shift. After one labeled meal, birds were shifted back to the unlabeled diet. Radiolabeled diets were prepared as described above but with the addition of $5 \mu\text{Ci mL}^{-1}$ ^{14}C sodium ferrocyanide ($\text{Na}_4\text{Fe}[\text{CN}]_6$; NEN Research Products, DuPont, Wilmington, Del.). Sodium ferrocyanide was chosen as a marker to measure MRT because it is not absorbed across the intestine and because it shows high recovery in excreta (Levey and Martínez del Rio 1999). Time and volume of all meals were recorded for a minimum of 3.5 h, starting with the single meal of radiolabeled diet. This time period allowed near complete elimination of radioactivity ingested. Plastic-coated paper was drawn through slots in the bottom of the cages to facilitate excreta collection while minimizing disturbance. Microcapillary tubes ($50 \mu\text{L}$) were used to collect excreta and quantify volume. All excreta produced after birds were fed radiolabeled diets was immediately collected and placed in separate scintillation vials. Liquid scintillation cocktail (Ecolume, ICN Research Products, Costa Mesa, Calif.) was added to excreta samples, which were counted correcting for quench and lumex (model LS 6000IC liquid scintillation counter, Beckman Instruments, Fullerton, Calif.). Mean retention time was estimated as $\text{MRT} = \sum f_i t_i$, where f_i is the fraction of total $\text{Na}_4\text{Fe}(\text{CN})_6$ excreted at time t_i since ingestion of radiolabeled diet (Levey and Martínez del Rio 1999). At the end of the experimental period, total volume consumed was measured.

On the second treatment day, unlabeled experimental diets were placed in the cages immediately after lights were turned on. Intake was measured hourly and excreta was collected quantitatively from nonstick metal pans for 24 h. Excreta samples and experimental diets were assayed for total sugar (Yemm and Willis 1954). The apparent sugar assimilation coefficient (SAC^*) was estimated as the percentage of sugar ingested that was not excreted, $\text{SAC}^* = 100 \times (\text{sugar ingested} - \text{sugar excreted})/\text{sugar ingested}$.

We determined the relative composition of sugars in excreta samples using an HPLC system (model 655A-11 liquid chromatograph, Hitachi, Tokyo) with a refractive index detector. Excreta samples were homogenized by sonication and filtered before being eluted ($20 \mu\text{L}$ loading volume) on a carbohydrate column (CHO-620, Interaction Chemicals, Mountain View, Calif.) at a flow rate of 0.6 mL min^{-1} using 0.5% CaNa_2 EDTA (Sigma, St. Louis) solution as the vehicle.

Intestinal Sucrase Activity Measurements

Two birds were killed by halothane overdose. The small intestines were immediately excised, flushed clean with ice cold 1.02% saline, divided into four sections, and stored in liquid nitrogen. Intestinal sections were thawed at 5°C and homogenized (30 s at setting 6, model 5100 homogenizer, Omni, Waterbury, Conn.) in nine volumes of 350 mmol L^{-1} mannitol

in 1 mmol L^{-1} Hepes/KOH, pH 7.5. Disaccharidase activities were measured according to Dahlqvist (1984) as modified by Martínez del Rio et al. (1995). Briefly, tissue homogenates ($100 \mu\text{L}$) diluted with 350 mmol L^{-1} mannitol in 1 mmol L^{-1} Hepes/KOH were incubated at 40°C with $100 \mu\text{L}$ of 56 mmol L^{-1} sugar (sucrose or maltose) solutions in 0.1 M maleate/NaOH buffer, pH 6.5. After a 10–20 min incubation, reactions were arrested by adding 3 mL of a stop/developing Glucose-Trinder (one bottle of Glucose-Trinder 500 reagent in 250 mL 1.0 mol L^{-1} TRIS/HCl, pH 7, plus 250 mL of 0.5 mol L^{-1} $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$, pH 7; Sigma, St. Louis). After 18 min at 20°C , absorbance of the resulting solution was measured at 505 nm with a spectrophotometer (model DU-64, Beckman Instruments, Fullerton, Calif.). In our preparation, disaccharide hydrolyses were linear even after 30 min. Apparent Michaelis constant (K_m^*) and pH optima for intestinal sucrase activity were $52.4 \pm 2.7 \text{ mmol L}^{-1}$ (mean \pm SE) and 6.5, respectively. On the basis of absorbance standards constructed for glucose, we calculated total intestinal hydrolytic activities and activities standardized per intestinal length and volume (Biviano et al. 1993). Intestinal volume was estimated from the average circumference of the small intestine of two broad-tailed hummingbirds measured at 0.5-cm intervals along the length of the intestine. Because the internal diameter of the small intestine in this species tapers distally (McWhorter and Martínez del Rio 1999), we estimated total intestinal volume as that of the sum of a series of cylinders with decreasing radius.

Statistical Analysis

In order to compare the relationship between volumetric intake and food energy density at the two experimental temperatures, we used ANCOVA. ANCOVA was performed on log-transformed data because we found that the relationship between intake and food energy density was best described by a power function. Correlations were assessed using Spearman rank correlation coefficients (r_s). Repeated measures ANOVA was used to determine differences in the mean proportions of sugars (sucrose, glucose, and fructose) found in excreta. One-sample t -tests were used to determine the significance of body mass changes. Values for percent mass loss, SAC^* , MRT, and sugars in excreta are reported as means \pm 1 SD, unless otherwise indicated.

Results

Feeding Trials

Because volumetric sugar intake was tightly and linearly correlated when measured at 3.5 and 16 h ($r = 0.90$), we present data gathered for 16 h exclusively. Because volumetric food consumption did not differ significantly between treatment days 2 and 3 (paired $t = 0.043$, $P > 0.5$, $N = 16$), we present data for treatment day 2. Volumetric food intake decreased signifi-

icantly with increased energy density. The relationship between volumetric intake and sugar concentration was well described by a power function (Fig. 1). Although intake was negatively correlated with sugar concentration (ANCOVA_{concentration}, $F_{1,12} = 25.2$, $P < 0.001$), the relationship between intake and sugar concentration in food did not vary between temperatures (ANCOVA_{slopes}, $F_{1,12} = 0.03$, and ANCOVA_{intercepts}, $F_{1,12} = 0.02$, $P > 0.8$). Ingested sugar was positively correlated with food sugar concentration (ANCOVA_{concentration}, $F_{1,12} = 9.0$, $P < 0.01$; Fig. 1), but it was independent of temperature (ANCOVA_{slopes}, $F_{1,12} = 0.15$; ANCOVA_{intercepts}, $F_{1,12} = 0.04$, $P > 0.7$; Fig. 1).

Birds lost significant mass at both temperatures (Fig. 2; one-sample t -tests, $t > 3$, $P < 0.05$, $N = 8$, respectively). However, the percent mass lost daily was significantly higher at 10°C ($3.4\% \pm 1.5\%$ per 24 h) than at 22°C ($1.2\% \pm 1.1\%$ per 24 h; ANOVA, $F_{1,15} = 11.0$, $P < 0.01$). There was no significant cor-

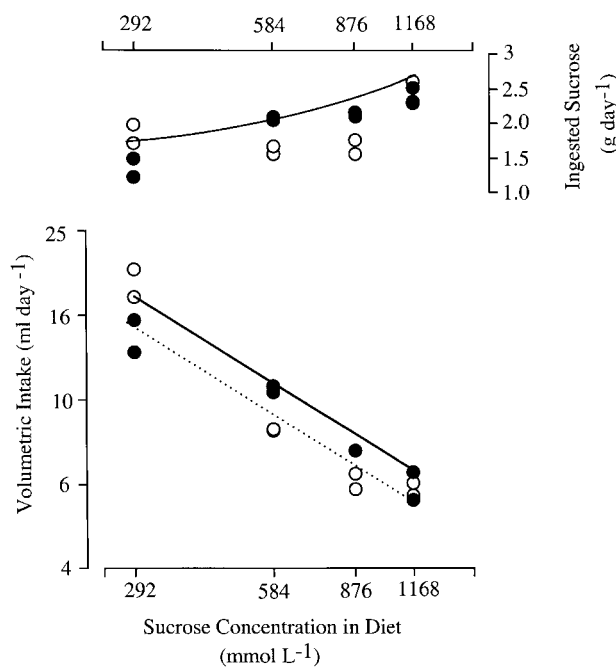


Figure 1. Volumetric food intake (lower panel) and sugar ingestion (upper panel) as a function of sugar concentration in broad-tailed hummingbirds. Filled circles represent data for birds at 22°C and unfilled circles represent birds at 10°C. Intake (I) decreased significantly with increased sugar concentration (C), whereas sucrose ingestion (S) increased significantly. The relationship between volumetric food intake and sugar concentration was adequately described by a power function ($I = 1000C^{-0.77}$, $r^2 = 0.87$) represented by a dotted line in the lower panel. Note that the scales of both axes on the lower panel and the X-axis of the upper panel are logarithmic. There were no significant differences in slope or intercept between the two temperatures (ANCOVA on log-transformed data, $P > 0.1$). The solid curves represent volumetric and sugar intake rates predicted by a model of gut function. Both curves are power functions ($I = 653C^{-0.67}$ and $S = 0.22C^{0.33}$, respectively).

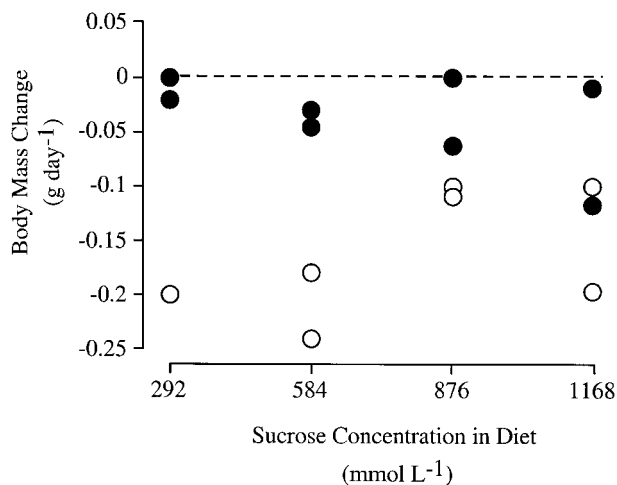


Figure 2. Body mass change at two environmental temperatures and four dietary sugar concentrations in broad-tailed hummingbirds. Filled circles represent data for birds at 22°C and unfilled circles represent birds at 10°C. Body mass change was independent of sucrose concentration but differed between environmental temperatures (ANOVA, $P < 0.01$).

relation between mass loss and concentration of sugar in food ($r_s < 0.35$, $P < 0.1$, for both temperatures; Fig. 2).

Mean Retention Time

Mean retention time (MRT) was not significantly correlated with sugar concentration ($r_s = 0.13$, $P > 0.6$; $N = 16$) or volumetric intake ($r_s = -0.31$, $P > 0.2$, $N = 16$). Temperature had no effect on MRT (ANOVA, $F_{1,15} = 0.08$, $P > 0.7$). The average MRT for *Selasphorus platycercus* was 74.6 ± 18.29 min ($N = 16$). Plots of the proportion of ^{14}C dpm excreted versus time (i.e., retention time distributions; Martínez del Rio et al. 1994) were variable in shape. For illustration, we present four retention time distribution curves selected at random for birds feeding on each of our experimental concentrations (Fig. 3). Differences in the qualitative shape of retention time distributions among sugar concentrations were not readily apparent by visual examination. Most trials showed jagged multi-peaked distributions of marker retention (Fig. 3).

Sugar Assimilation

Apparent sugar assimilation was high (mean SAC* for all trials = 0.95 ± 0.02 , $N = 16$) and independent of sugar concentration ($r_s = 0.18$, $P > 0.4$; $N = 16$) and temperature (ANOVA, $F_{1,15} = 0.1$, $P > 0.5$). However, the proportions of sucrose, glucose, and fructose found in excreta differed significantly (repeated measures ANOVA, $F_{2,14} = 96.81$, $P < 0.001$; Fig. 4). Fructose was the most abundant sugar in excreta

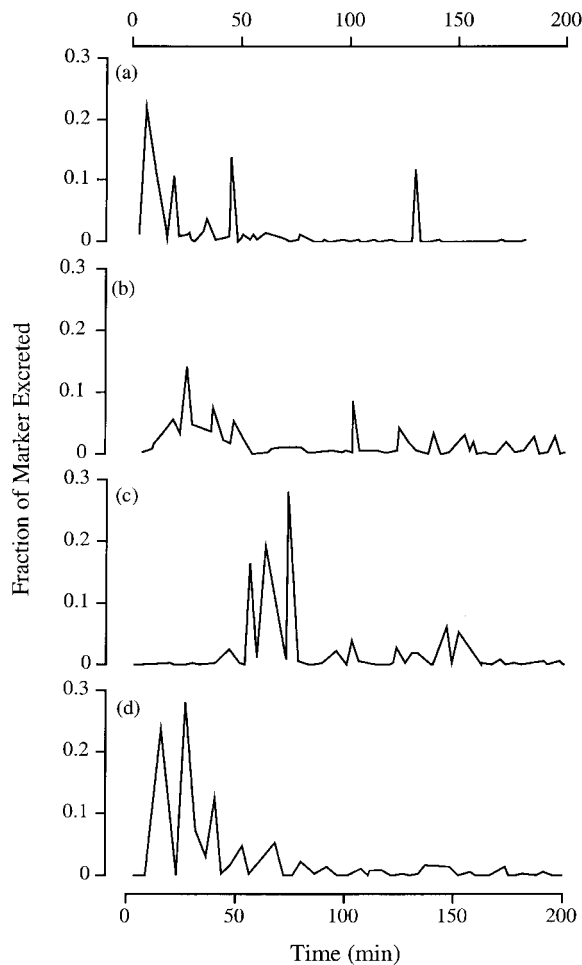


Figure 3. Retention time distributions in broad-tailed hummingbirds. The impermeant marker used was ^{14}C sodium ferrocyanide. Plots of the proportion of ^{14}C dpm excreted versus time were variable in shape, and most trials showed jagged multi-peaked distributions of marker retention. For illustration, we present four retention time distribution curves selected at random for birds feeding on each of our experimental concentrations. Differences in the qualitative shape of retention time distributions among sugar concentrations were not readily apparent by visual examination. The average MRT for *Selasphorus platycercus* was 74.6 ± 18.29 min ($N = 16$).

($67.12\% \pm 8.82\%$ of total sugars excreted), followed by glucose ($25.01\% \pm 7.84\%$ of total sugars excreted). These monosaccharides were about eight and three times more abundant than the disaccharide sucrose ($8.00\% \pm 3.86\%$ of total sugars excreted), respectively. All hummingbirds showed the same ranking in the proportion of the different sugars excreted.

Sucrase Activity Measurements

Sucrase activity standardized by intestinal length declined sharply (by a factor of about 20) from the most proximal to

the most distal section of the small intestine (Fig. 5). However, intestinal diameter declined from 1.6 to 0.6 mm from the duodenum to the intestinal junction with the cloaca. Therefore, sucrase activity standardized by the volume of intestinal contents decreased only modestly (by a factor of about 3) along the length of the small intestine (Fig. 5). We calculated total sucrase activity by summing the activity in each section and estimated maximal sucrase activity using the Michaelis constant for our preparation. Maximal total intestinal sucrase activity equaled $12.72 \pm 3.1 \mu\text{mol min}^{-1}$ (mean \pm SD).

Discussion

Many birds and mammals increase food ingestion rates when acclimated to cold temperatures (Hammond and Diamond 1997; McWilliams and Karasov 1998). Often, these increases in food intake are accompanied by changes in digestive function such as intestinal enlargement and increases in the expression of digestive enzymes and nutrient transporters (Konarzewski and Diamond 1994; McWilliams et al. 1998). The main objective of our study was to determine whether the intake response of hummingbirds to sugar concentration was the result of compensatory feeding or of a physiological constraint. Thus, to avoid the changes that follow chronic acclimation to the cold, we exposed broad-tailed hummingbirds to cold temperatures under acute conditions. When exposed to a relatively sudden drop in environmental temperature and, hence, to an acute increase in thermoregulatory energy expenditures, broad-tailed hummingbirds did not increase their rate of energy consumption and lost mass. In following paragraphs, we suggest that our results are contrary to the notion that the relationship between intake and sugar concentration in these birds is the

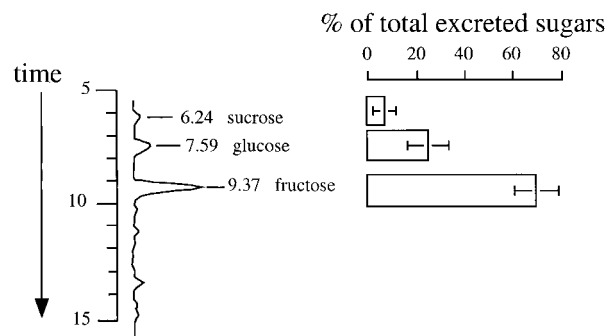


Figure 4. Differential abundance of sucrose and the products of its hydrolysis, glucose and fructose, in broad-tailed hummingbird excreta. The percentage data is for all trials ($N = 16$). Sucrose, glucose, and fructose appeared in excreta in significantly different proportions (repeated measures ANOVA, $P < 0.001$). The graph on the left is a representative HPLC trace. Time is retention time (min) in the chromatography column. The numbers labeling each peak are the retention times for each sugar.

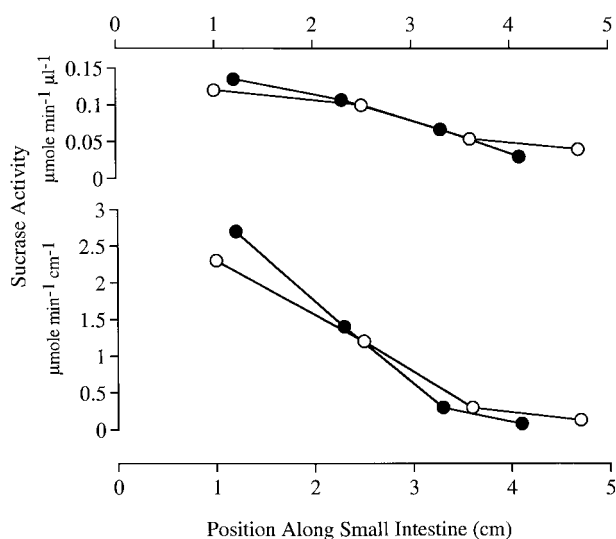


Figure 5. Distribution of sucrase activity along the length of the small intestine of two broad-tailed hummingbirds. Activity was standardized by intestinal length (*lower panel*) and volume of digesta (*upper panel*) in each intestinal section. Activity was measured at pH 6.5 and at a sucrose concentration equal to 28 mmol L⁻¹.

result of compensatory feeding. We also assert that the failure of broad-tailed hummingbirds to increase food intake points to the existence of physiological limitations to the rate at which they can ingest food. We present a mathematical model that suggests that intestinal sucrose hydrolysis rates in broad-tailed hummingbirds were operating at near-maximal levels and, thus, were probably imposing limits to the rate at which sucrose was ingested and assimilated. We then discuss the match between sucrose hydrolysis and the uptake of glucose and fructose. Finally, we consider the ecological consequences that the limitations imposed by digestive function can have on a high altitude small endotherm that faces unpredictable weather and the expense of fattening during migration.

Physiological Constraint or Compensatory Feeding?

Volumetric food intake decreased with increasing sugar concentration in broad-tailed hummingbirds. This pattern is exhibited by many nectar-feeding birds (Collins 1981; Downs 1997; López-Calleja et al. 1997) and is often attributed to compensatory feeding. To test the compensatory feeding hypothesis, we predicted that volumetric food (and thus energy) intake would increase when birds faced higher energetic demands. Contrary to our prediction, food intake did not increase significantly when the birds were exposed to higher energetic demands produced by exposure to low temperatures. We interpret this failure to increase feeding when challenged by cold temperatures as evidence for the existence of limits imposed by

the physiological capacity of broad-tailed hummingbirds to process energy.

Two lines of evidence support the notion that broad-tailed hummingbirds were unable to increase their feeding rate to match increased energy demands. First, although birds lost mass at both experimental temperatures, birds at 10°C lost mass at significantly higher rates. Second, behavioral observations provided further, albeit anecdotal, evidence for increased energy deficits in birds exposed to cold temperatures. At 10°C, hummingbirds were often observed emerging from torpor in the morning, whereas birds at 22°C did not appear to use torpor. Nocturnal torpor is used by hummingbirds to conserve energy when daily energy intake is low (reviewed by Calder 1994). In addition, at 10°C, birds exhibited behaviors commonly associated with energy conservation. They spent less time flying, exhibited ptiloerection while perching, and held their feet close to their body in flight (Gass and Montgomerie 1981; Udvardy 1983). The energy saved by these behavioral responses was presumably not sufficient to completely offset the increased demand. Independently of the energy conservation mechanisms involved, it appeared that birds at 10°C could not process energy fast enough to compensate for their higher energy demands. The observation of increased torpor in cold-exposed hummingbirds points to the subtle interrelation between digestive and metabolic traits in hummingbirds. In these animals, balancing a sometimes precarious energy budget may require the use of energy-conserving strategies such as nocturnal torpor when daily energy output is increased and energy acquisition is constrained.

Does Intestinal Hydrolytic Capacity Limit Food Intake?

The failure of broad-tailed hummingbirds to increase sugar intake in the cold led us to speculate about the existence of factors imposing an upper limit to food intake rate. The presence of characteristic intake-response curves in broad-tailed hummingbirds eliminated two possible limitations to food intake: food harvesting rate and water processing by the kidney (Beuchat et al. 1990). Here we use harvesting rate in the limited sense of ingesting food without processing it in the gut. Harvest rate is potentially limited by the rate at which food can be licked by hummingbirds (Gass and Roberts 1992 and references therein). Although volumetric intake rates did not differ significantly between experimental temperatures, they ranged about fourfold from the highest to the lowest sugar concentration. Clearly, at sucrose concentrations higher than 292 mmol L⁻¹, broad-tailed hummingbirds were not limited by food harvesting or water processing rates.

The vast majority of the energy ingested by hummingbirds comes from dietary sugar. Thus, the physiological processes that determine the rate at which ingested sugar is assimilated and metabolized are good candidates for factors limiting food intake. Physiological limits to sugar processing in humming-

birds can occur at several steps. Sugar ingestion can be limited by characteristics of the digestive tract, namely by the rates at which sucrose is hydrolyzed and at which the products of its hydrolysis are transported across the intestine into circulation (Karasov et al. 1986; Martínez del Rio 1990a). In addition, the rate at which sugar is processed can be limited by the rate at which absorbed glucose and fructose are catabolized and/or shunted into the synthesis of glycogen and lipid (Suarez et al. 1988; Suarez et al. 1990). It is likely that these steps are all matched to each other so that no step is more limiting than the other (Hammond and Diamond 1997). In the next two sections we focus on the potential role of digestive processes in limiting sugar processing.

Diamond and Hammond (1992) proposed a method to compare the capacity of the intestine to hydrolyze and absorb nutrients with the ingested loads of these nutrients. They suggested integrating the maximal reaction velocity (V_{\max}) of brush-border hydrolases or transporters along the length of the intestine to yield total hydrolytic or transport capacity (reviewed by O'Connor and Diamond 1999). This capacity can then be compared with the ingested load. They use the term "safety factor" for the ratio of capacity (i.e., V_{\max} integrated along the intestine) to load (i.e., the amount of nutrient ingested; Weiss et al. 1998). Using this method yields a maximal rate of sucrose hydrolysis of $0.26 \pm 0.06 \text{ g h}^{-1}$ (mean \pm SD) and safety factors that range from 1.3 to 4.4 (average \pm SD = 2.2 ± 0.8 , $N = 16$). These relatively high safety margins can be interpreted as evidence of hydrolytic "spare capacity" (sensu Diamond 1991) in hummingbirds and against the notion that sucrose hydrolysis rates can impose limits to food intake.

Using V_{\max} as an estimate of capacity assumes that nutrient concentrations in the intestinal lumen are saturating (i.e., higher than the Michaelis constant of the process in question) throughout the intestine. This assumption is probably false for both glucose transport and sucrose hydrolysis (see Ferraris et al. 1990). Moreover, it is likely that sucrose concentration decreases as sucrose is hydrolyzed as digesta flows along the length of the intestine. This reduction in sucrose concentration probably leads to reduced sucrose hydrolysis rates. Thus, the assumptions required to use V_{\max} to estimate the gut's digestive capacity probably lead to its overestimation for both sucrose hydrolysis and glucose uptake. Here we propose an alternative method to estimate the capacity of hummingbirds to hydrolyze sucrose.

A Model of Sucrose Hydrolysis in Hummingbird Guts

Our method relies on modeling the intestine of hummingbirds as a plug-flow chemical reactor (Penry and Jumars 1987). The model makes two assumptions. (1) Digesta flows unidirectionally (Jumars and Martínez del Rio 1999), and (2) the rate at which sucrose is hydrolyzed in the intestine ($-r_s$) follows simple Michaelis-Menten kinetics:

$$-r_s = S_{\max} C_s (K_m + C_s)^{-1}, \quad (1)$$

where S_{\max} equals the rate of hydrolysis along the intestine ($\mu\text{mol min}^{-1} \mu\text{L}^{-1}$), K_m is sucrose's Michaelis constant ($\mu\text{mol} \mu\text{L}^{-1}$), and C_s is the concentration of sucrose ($\mu\text{mol} \mu\text{L}^{-1}$) down the intestine or with time (Jumars and Martínez del Rio 1999). Equation (1) can be integrated to yield the throughput time (τ) required to reduce the initial sucrose concentration (C_{s0}) to a given final value (C_{sf}):

$$\tau = (S_{\max})^{-1} [K_m \ln(C_{s0} C_{sf}^{-1}) + (C_{s0} - C_{sf})]. \quad (2)$$

In plug-flow reactors if one knows τ and the volume of gut contents (G in μL), intake rate (\dot{V}_0 in $\mu\text{L min}^{-1}$) can be estimated as

$$\dot{V}_0 = G\tau^{-1}. \quad (3)$$

We used sucrose activity values measured in vitro and data on intestinal morphology to predict intake rates for our four experimental sucrose concentrations. The parameter values used in the model were S_{\max} averaged along the intestine's length ($0.22 \mu\text{mol min}^{-1} \mu\text{L}^{-1}$), K_m ($0.0524 \mu\text{mol} \mu\text{L}^{-1}$), and G ($46 \mu\text{L}$). Because we found that approximately 99.6% of sucrose was hydrolyzed, we assumed that C_{sf} was equal to $0.004 C_{s0}$.

The intake rates estimated from this analysis are shown in Figure 1. Estimated intake rates are described by a power function that overestimates observed intake by a margin that increases from 15% at the lowest concentration to 35% at the highest. Although the model overestimates intake, the qualitative resemblance between its output and the observed pattern is remarkable. The model predicts that the relationship between volumetric intake and sugar concentration should follow a power function with a slope lower than 1 (Fig. 1). The model also predicts an increase in sugar intake and assimilation with increased concentration in food. This result is also in accordance with our observations and the consequence of lower average hydrolysis rates at lower food concentrations (Fig. 1; Jumars and Martínez del Rio 1999). In compensatory feeding, the slope of the log-log relationship between volumetric intake and sugar concentration equals -1 , and, hence, there is no correlation between sugar intake and sugar concentration.

Why does the model overestimate intake? Here we propose a possible explanation and in the next section we suggest another one. Our model does not include the kinetics of glucose and fructose uptake. It is possible that the inclusion of hexose uptake in the model would yield lower predicted intakes. Unfortunately, available methods to estimate hexose intestinal uptake in vitro (i.e., the intestinal everted sleeve; Karasov and Diamond 1983) yield glucose uptake values that are too low to account for the glucose assimilation observed in vivo in birds (Caviedes-Vidal and Karasov 1996). For example, glucose uptake rates measured in vitro using everted sleeves in rufous

hummingbirds were approximately four times lower than glucose assimilation rates observed in vivo (see Karasov et al. 1986). Including hexose uptake in a model of hummingbird function requires developing methods that yield realistic estimates of uptake kinetics.

Our analysis suggests some observations regarding estimates of digestive capacity, and hence of safety factors, as well as comments about the use of chemical reactor approaches to model digestive processes. Using integrated V_{\max} to estimate hydrolytic and transport capacity appears to lead to overestimation of the gut's digestive capacity and hints at the existence of large safety margins. Although the more complex method described here is not free of assumptions (see below), it includes significantly more physiological detail and hence may lead to a less biased estimate of hydrolytic capacity. Recall that safety factors calculated using intestinal sucrose's V_{\max} to estimate capacity were much higher than one (see previous section). In contrast, safety factors estimated using the method proposed here are relatively small (capacity/load \pm SD = 1.2 ± 0.2 , $N = 16$), suggesting that there is a close match between the ability to hydrolyze sucrose and the amount of sucrose consumed. Because our analysis takes into account the decline in sucrose concentration along the gut that accompanies hydrolysis as well as the residence time of digesta in the gut, it leads to lower safety factors. These lower safety factors indicate that broad-tailed hummingbirds ingest as much sucrose as they have the capacity to hydrolyze. Consequently, when they were challenged with increased energy demands, they were unable to increase food consumption to match them.

Most previous analysis of guts as chemical reactors used models to predict throughput times that maximize the rate of nutrient absorption (Dade et al. 1990; Martínez del Río and Karasov 1990; Jumars and Martínez del Río 1999). Here we have used a different approach. Following Levey and Martínez del Río (1999), we assumed that hummingbirds must show high assimilation efficiencies to prevent osmotic imbalances in the lower gut and used this physiological detail as a constraint on the model to estimate intake rates. The model appeared to perform well, as predicted intake values closely matched observed values (Fig. 1). Our approach highlights the usefulness of chemical reactor models in understanding gut function even when these models are used outside of the context of optimality (Levey and Martínez del Río 1999).

Although our model seems to adequately capture several features of gut function in hummingbirds, it must be considered preliminary and its assumptions should be examined experimentally. Specifically, we assumed that the concentration of sucrose in the intestinal lumen changed simply as a result of hydrolysis. In reality, sucrose concentration changes with hydrolysis and the addition and removal of water by secretion and absorption into and from the intestinal lumen (see the next section and Chang and Rao 1994). A more realistic model of gut function in hummingbirds may require inclusion of these

processes. Data on the concentration of solutes in hummingbird intestinal contents can help to evaluate the validity of our model's assumptions (see Ferraris et al. 1990).

The Relationship between Intake Rate, Sugar Concentration, and Mean Retention Time

To estimate intake rates we relied on the notion that throughput time and intake rate are reciprocally related (see eq. [3]). Although our model predicted intake rate accurately, it failed to predict gut mean retention time. The model predicts that MRT should increase linearly with sugar concentration and that it would vary reciprocally with intake rate. MRT, however, was independent of both sugar concentration and volumetric intake. Here we discuss the mismatch between the model's predictions and our observations.

The reciprocal relationship between residence time in the gut and intake rate was established for chemical reactors on the basis of reasonable assumptions (see Penry and Jumars 1987). Given that the volume of digesta in the gut remains relatively constant, the time required to replace these contents should decrease as flow of materials into the gut increases (Prop and Vulink 1992; Levey and Martínez del Río 1999). However, despite a fourfold increase in volumetric intake from the highest to lowest sugar concentration, broad-tailed hummingbirds exhibited no significant change in MRT. Why did hummingbirds fail to show any relationship between MRT and volumetric intake?

McWhorter and Martínez del Río (1999) have shown that most of the water consumed in food by hummingbirds is absorbed in the gut. Thus, the volume of water ingested does not flow through the intestine but is absorbed and then excreted by the kidneys into the cloaca. In hummingbirds, it is likely that water absorption is a relatively fast process that takes place primarily in the intestine concurrently with glucose absorption (Loo et al. 1996; McWhorter and Martínez del Río 1999). The morphology of hummingbird gastrointestinal tracts supports the notion that ingested water does not flow through but, rather, is absorbed in the intestine. In *Selasphorus platycercus*, the diameter of the intestinal lumen decreases dramatically from the duodenum to the intestinal junction with the cloaca (see "Results"), indicating that digesta volume decreases distally. If ingested water does not flow through the intestine but is rapidly absorbed across its walls, there is no reason to expect a reciprocal relationship between intake rate and gut mean retention time. We hypothesize that intestinal water absorption uncouples MRT from the rate of volumetric food intake. A consequence of intestinal water absorption is that the concentration of nonabsorbable solutes, such as sucrose, along the intestinal length may not decrease as rapidly as our model predicts. Thus, hydrolysis rates may be higher than predicted. Intestinal water absorption may be one of the reasons why our model overestimates volumetric intake (Fig. 1).

Several authors have suggested the use of the ratio of intestinal volume to volumetric ingestion rate as an estimate of gut retention time (Martínez del Rio 1990a; Prop and Vulink 1992; López-Calleja et al. 1997; Witmer and Van Soest 1998). Although this ratio may be useful in animals that ingest food containing large amounts of solid indigestible material (e.g., geese; Prop and Vulink 1992), it is probably not a meaningful index of retention time in animals that ingest highly digestible foods with large water content. Here, it is necessary to point out the differences between MRT and intestinal throughput time (τ) predicted by our model. The former is the average time that a marker particle is retained in the whole gut, whereas the latter is the time that a particle is retained in the small intestine. Because the volume of the whole gut is larger than that of the intestine and is potentially variable, as it depends on meal volume (Hainsworth and Wolf 1972), MRT cannot be directly predicted from τ . Ideally, τ should be calculated independently of MRT (Martínez del Rio and Karasov 1990). Levey and Martínez del Rio (1999) point out the challenges of estimating intestinal throughput times for animals with complex guts and feeding behaviors.

Glucose and Fructose Uptake in Broad-Tailed Hummingbirds

Although near complete assimilation of nectar constituents seems to be characteristic of nectarivorous birds (Hainsworth 1974; Karasov et al. 1986; Martínez del Rio 1990b; Jackson et al. 1998), our results revealed subtle differences in the assimilation of sugars. In hummingbirds, fructose was present in excreta at approximately 2.5 times the level of glucose. These two monosaccharides were about eight and three times more abundant than the disaccharide sucrose, respectively. The differences in the uptake efficiency of glucose and fructose in broad-tailed hummingbirds may be the result of differences in their mechanisms of intestinal transport. Glucose is transported across the luminal membrane of enterocytes by a Na^+ -dependent active transporter (SGLT1; Pajor and Wright 1992), whereas fructose is transported by a distinct transporter (GLUT5; Rand et al. 1993). In humans, the rate of intestinal fructose uptake is roughly half that of glucose (Holdsworth and Dawson 1965; Gitzelmann et al. 1989). In hummingbirds, fructose uptake also seems to be slower than glucose uptake. Because fructose transport in birds has not been researched in detail, the difference in glucose and fructose transport rates in hummingbirds cannot be explained. Lower fructose uptake rates may be due to lower densities or lower turnover constants of GLUT5 relative to SGLT1.

Fructose and glucose were much more abundant than sucrose in broad-tailed hummingbird excreta. This result indicates that the ability of broad-tailed hummingbird intestines to absorb the glucose and fructose produced from the hydrolysis of sucrose is limited. It is high enough to absorb most, but not all, the monosaccharides produced by the action of intestinal

sucrase. Weiss et al. (1998) reported that glucose transport and sucrase activity remained approximately matched to each other in mice (*Mus musculus*). They concluded that neither sucrase nor the glucose transporter was the rate-limiting step for sucrose digestion but that both steps were equally limiting. The same conclusion would seem to apply to broad-tailed hummingbirds: our model suggests that the capacity of the intestine to hydrolyze sucrose is not much greater than the amount of sucrose ingested. The presence of small but significant amounts of glucose and fructose in excreta suggests that hexose uptake rates are slightly lower than those needed for complete absorption. Although glucose and fructose appeared in excreta at higher abundance than sucrose, their concentration in excreta was very low. Our conclusion about the match between sucrose hydrolysis and hexose uptake must be tempered by the fact that what is known about glucose and fructose transporter physiology is from studies of nonnectarivorous mammals (Holdsworth and Dawson 1965; Gitzelmann et al. 1989; Pajor and Wright 1992; Rand et al. 1993; Weiss et al. 1998).

Does Gut Function Limit Intake in Other Nectar-Feeding Birds?

Although the characteristic intake response exhibited by broad-tailed hummingbirds is probably the result of limitations to food intake imposed at least in part by digestive processes, it is unwise to assume that this response has the same underlying mechanisms in all nectar-feeding birds or that all nectar-feeding birds show the same slim digestive safety margins. Beuchat et al. (1979) compared the food intake rates of rufous (*Selasphorus rufus*) and Anna's hummingbirds (*Calypte anna*) at several temperatures. These two species exhibited different responses to temperature. Between 0° and 20°C, *S. rufus*, like *S. platycercus*, exhibited relatively constant energy intake rates. In contrast, *C. anna* showed the negative correlation between energy intake and environmental temperature that is expected in animals that compensate for increased expenditures by increasing energy intake. Beuchat et al. (1979) hypothesized the existence of a digestive limitation to intake rate in *S. rufus*. Our results echo this hypothesis. In a recent study, Lotz (1999) exposed lesser double-collared sunbirds (*Nectarina chalybea*) to variable temperatures. These birds, like *C. anna*, exhibited a linear decrease in sugar intake with increased temperature. It is interesting to note that, in contrast to the *Selasphorine* hummingbirds in question, both *C. anna* and *N. chalybea* inhabit relatively stable environments and are not considered to be long-distance migrants (Russell 1996; Lotz 1999). There are too few comparable studies of volumetric intake over large enough sugar concentration ranges and time scales (e.g., Tamm and Gass 1986; Lloyd 1991) to discern if the limitations to food intake displayed by broad-tailed hummingbirds are a general trait of nectar-feeding birds.

Ecological Significance of Gut Limitations to Food Intake in Broad-Tailed Hummingbirds

Although it is risky to extrapolate from laboratory results, we venture that hummingbirds in the field are also subject to limitations to food intake imposed in part by their digestive system. Broad-tailed hummingbirds are the smallest birds that breed in the central Rocky Mountains. Low ambient temperatures are common in their montane meadow habitats (Calder 1994). Adverse weather conditions may destroy food resources, disrupt foraging behavior, and impose high thermoregulatory costs (Gass and Lertzman 1980). Although the cold spells experienced by *S. platycercus* are probably sporadic and of short duration (Calder 1975, 1994), our results suggest that in the field their limited ability to assimilate food fast enough when challenged by low temperatures can lead to periods of mass loss. A small body, an energetically expensive foraging mode, and a harsh and unpredictable environment are challenges that make the lives of broad-tailed hummingbirds precarious. It is possible that a limiting gut must be added to this list of challenges.

Broad-tailed hummingbirds face occasional intervals of high energy demands due to adverse weather. In addition, they face the task of increasing fat reserves to fuel migration each year (Calder and Calder 1992). Broad-tailed and rufous hummingbirds gain from 1.2 to 2.3 g over a week during refueling stopovers (Carpenter et al. 1993; Calder 1994). During periods of premigratory fattening, hummingbirds must generate an energy budget with a surplus. Two nonexclusive mechanisms can be used to produce a positive energy budget: increasing the capacity of the gut to deliver energy or reducing energy expenditures. Carpenter and Hixon (1988) have documented torpor in a healthy migrant hummingbird under thermally favorable conditions. They interpret their observation as evidence of torpor as a mechanism that reduces nocturnal energy expenditure and thus hastens fat accumulation. It is important to point out, however, that their hypothesis is based on a single, very rare observation (Carpenter and Hixon 1988). Given the limited field data at hand, we speculate that one of the reasons that migrant hummingbirds might use torpor during premigratory fattening is the existence of a central digestive limitation to food assimilation. In the face of constrained energy intake rates, broad-tailed hummingbirds may reduce the expenditure side of their energy budget by using torpor to increase their rates of fat accumulation.

The speculations presented in this section presume that the digestive limitations documented for broad-tailed hummingbirds in the laboratory operate in the field. They would be idle if they were not testable. We present them because all the ingredients to test them are available: daily energy expenditures can be measured using standard methods (Powers and Nagy 1988; Tiebout and Nagy 1991) and digestive capacities can be estimated from sugar composition and concentration of floral nectars and the simple mathematical model presented in this

article. Hummingbirds present an unparalleled opportunity to test the notion that digestive constraints have ecological consequences for animals under natural conditions.

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