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Gastric function and its contribution to the postprandial metabolic response of the Burmese python *Python molurus*

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Summary

The large intact prey ingested by Burmese pythons require considerable processing by the stomach before passage into the small intestine. To investigate the function and cost of gastric digestion and its contribution to postprandial metabolic response for the Burmese python, I examined the rate of gastric digestion, the postprandial profile of gastric pH and the effects of decreasing gastric workload on the metabolic cost of digestion, referred to as specific dynamic action (SDA). Ingested meal mass (equivalent to 25% of snake body mass) was reduced by 18% within 1 day postfeeding, by which time intragastric pH had decreased from 7.5 to 2. Gastric pH was maintained at 1.5 for the next 5–7 days, after which it returned to 7.5. The SDA generated by digesting an intact rat meal was reduced by 9.1%, 26.0%, 56.5% and 66.8%, respectively, when pythons were fed steak, ground rat, liquid diet or ground rat directly infused into the small intestine. The production of HCl and enzymes and other gastric functions represent an estimated 55% of the python's SDA generated from the digestion of an intact rodent meal. Additional contributors to SDA include protein synthesis (estimated 26%), gastrointestinal upregulation (estimated 5%) and the activities of the pancreas, gallbladder, liver, kidneys and intestines during digestion (estimated 14%). Operating on a 'pay before pumping' principle, pythons must expend endogenous energy in order to initiate acid production and other digestive processes before ingested nutrients can be absorbed and channeled into metabolic pathways.

Key words: reptile, snake, *Python molurus*, digestion, stomach, gastric pH, specific dynamic action.

Introduction

A physiological response to feeding is an increase in metabolic rate, a phenomenon commonly referred to as specific dynamic action (SDA). SDA represents the summed energy expended on the ingestion, digestion, absorption and assimilation of a meal (Brody, 1945; Kleiber, 1975). The magnitude of SDA varies dramatically among organisms, largely due to the individual or combined effects of meal size (Tandler and Beamish, 1981; Janes and Chappell, 1995; Secor and Diamond, 1997a), meal composition (Tandler and Beamish, 1980; Hailey, 1998; Secor and Faulkner, 2002), body size (Tandler and Beamish, 1981; Secor and Diamond, 1997a; Secor and Faulkner, 2002), body temperature (Soofiani and Hawkins, 1982; Secor and Faulkner, 2002; Wang et al., 2003) and feeding habits (Secor, 2001). Several of these factors contribute to the large SDA response (5–44-fold increases in metabolism) characteristic of the Burmese python *Python molurus* (Benedict, 1932; Secor and Diamond, 1997a; Overgaard et al., 1999). Burmese pythons feed in the wild on large intact prey at relatively infrequent intervals and upregulate and downregulate gastrointestinal (GI) performance, respectively, with the initiation and completion of each digestive bout (Pope, 1961; Secor and Diamond, 1995, 1997b).

It has therefore been suggested that the python's large postprandial metabolic response is attributed to the digestion of large intact meals (up to 100% of body mass), their relatively low standard metabolic rate (SMR) above which SDA is quantified, and the added cost of upregulating their quiescent guts immediately following feeding (Secor and Diamond, 1995). Understandably, the digestion of large intact meals would significantly contribute to the python's large SDA response. The digesta exiting the stomach consists of a soup-like chyme for practically all vertebrates, whereas the physical state of food entering the stomach differs dramatically among species. Humans swallow small macerated pieces of highly digestible food, most carnivores swallow small intact prey that have been crushed or pieces of meat torn from larger prey, and many reptiles (lizards, turtles, and crocodilians) commonly ingest crushed or fragmented animal or plant material. In stark contrast, snakes swallow only intact prey and must delegate to the stomach the whole job of breaking down that prey before its passage into the small intestine. Therefore, for snakes, the digestion of a meal requires a relatively larger effort by their stomachs. Given that gastric function (i.e. acid and enzyme secretion) is energetically demanding (Reenstra and Forte,

1981; Helander and Keeling, 1993), the gastric breakdown of the python's meal may occur at a relatively high cost (compared with that of other carnivores) and thus explain their comparatively larger SDA.

Studies that have attempted to elucidate the relative contribution of the various components of digestion to SDA have divided SDA into 'mechanical SDA' and 'biochemical SDA' (Tandler and Beamish, 1979, 1980; Jobling and Davies, 1980; Carefoot, 1990). Mechanical SDA represents the cost of physically processing the food (i.e. chewing, swallowing and peristalsis), whereas biochemical SDA is the postabsorptive cost of assimilation (including nutrient transport and protein and tissue synthesis). Finding that meal passage represents only 8–12% of SDA, whereas protein synthesis contributes as much as 44% to SDA, researchers have concluded that postabsorptive costs (biochemical SDA) dominate SDA (Jobling, 1981; Brown and Cameron, 1991; Lyndon et al., 1992). Contrary to this conclusion, python SDA may be largely dominated by preabsorptive costs.

The goal of the present study was to ascertain information on gastric digestion and its energetic cost for the Burmese python. I hypothesized that gastric breakdown of the large intact meals consumed by Burmese pythons would incur a substantial energetic expense and thus be an important contributor to their SDA. The aims of this study were to: (1) document the gastric breakdown of an intact meal; (2) profile the postprandial pattern of intragastric pH; (3) assess changes in the postprandial metabolic response to decreasing gastric workloads and (4) estimate the relative contributions of gastric performance, protein synthesis and gastrointestinal upregulation to the python's SDA. As shall be shown, pythons maintain a highly acidic environment within their stomach during digestion, the cost of which may dominate their SDA.

Materials and methods

Animals and their maintenance

The 62 Burmese pythons (*Python molurus* L.) used in this project were purchased as hatchlings (Bob Clark Captive Bred Reptiles, Oklahoma City, OK, USA) and maintained on a bi-weekly diet of rodents and chickens. Prior to study, snakes were fasted for one month to ensure that they were postabsorptive (Secor and Diamond, 1995). Snakes ranged in mass from 340 g to 6280 g (mean ± 1 S.E.M., 1510±180 g) and in age from 0.5 years to 6 years (2.1±0.3 years). Python care and study were conducted under UCLA Animal Research Committee protocol number 93-204 and the University of Alabama Institutional Animal Care and Use Committee protocol number 168.

Rate of gastric digestion

I used two methods to examine the rate at which ingested rat meals were broken down within the python's stomach and passed into the small intestine. First, four pythons (449±60 g) were fed one or two intact rats equaling 28.6±0.7% of their body mass. These snakes were maintained at 27–30°C and were x-rayed each day for 6 days following feeding in order to visualize the breakdown of the skeleton of the ingested rat(s) within the python's stomach. Second, 36 pythons (882±37 g) were fed rat meals (1–3 rats) equaling 25.7±0.6% of their body mass. Following feeding, snakes were maintained at 27–30°C and sacrificed at 12 h and at 1, 2, 3, 4, 6 and 14 days postfeeding (3–8 snakes per time). For each snake, stomach contents were weighed and compared with the original wet mass of the ingested meal in order to quantify the percentage of the ingested meal still remaining within the stomach.

Gastric pH

I monitored gastric pH of seven pythons (1250±130 g) following their ingestion of a single intact rat equaling 24.7±1.7% of the snake's body mass. Stomach pH was measured using an infant gastric pH electrode (model 91-0011; Synectics Medical Inc., Irving, TX, USA) sutured to the head of the ingested rat. The electrode cable extended from the snake's mouth and was sutured to the side of the snake immediately posterior to its head. A reference electrode (model 4011; Synectics Medical Inc.) was attached to the dorsum of each snake, and both electrodes were connected to a pH monitor (Digitrapper Mk II, Synectics Medical Inc.). Soon after feeding and for the remainder of the measurements, snakes exhibited no observable discomfort to the cable extending from their mouth or to the electrode attached to their back. Position of each pH electrode was checked by x-ray. Prior to use, each pH electrode was calibrated using commercial pH buffers of pH 1.07 and 7.01 (Synectics Medical Inc.). Following feeding, pH was recorded sporadically at 2–4 h intervals for up to 14 days while snakes were maintained at 23–28°C. Measurements ended when either the snake was able to dislodge the probe from its mouth (one case) or when stomach pH returned to its initial level (approximately 7.5). Data from all snakes were grouped into 3 h intervals (5.6±0.2 snakes per interval) beginning at the time of feeding.

Feeding treatments to assess gastric workload

I assessed the effects of different gastric workloads on SDA by feeding pythons one of five meal treatments (4–6 snakes per treatment) and measuring their postprandial metabolic responses. Meal treatments included normal intact rats and the following four meals designed to reduce the workload of the stomach while still inducing full postgastric responses: steak, ground rat, liquid meal and ground rat infused directly into the proximal small intestine. Five snakes (2390±340 g) consumed 2–3 intact rats whose combined mass equaled 25.0±0.1% of their body mass. Steak meals (25.3±0.2% of body mass) were made from slabs of lean sirloin steak (3.8% fat) rolled into a tube with either a rat or chicken head attached to one end to entice pythons (2320±430 g; N=6) to consume them. Six snakes (2430±190 g) were fed meals (25% of body mass) of finely ground pre-killed rats by forcing the meal through a tube inserted into the snake's esophagus. The liquid meal (25% of body mass) was formulated to match the nutrient composition of an intact rat and consisted (by mass) of 70% mammalian Ringer's solution (composition of mammalian Ringer's

solution in mmol l^{-1}: NaCl 128, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 20, pH 7.4, 290 mosmol l^{-1}), 15% casein (C-7078; Sigma, St Louis, MO, USA), 12% homogenized chicken fat and 3% D-glucose (G-8270; Sigma). The liquid meal was similarly gavaged into the esophagus of four pythons (5240±510 g).

Ground rat was infused through rubber catheters surgically inserted into the proximal end of the small intestine of four pythons (1670±280 g). To implant catheters, snakes were anesthetized with halothane (Halocarbon Laboratories, River Edge, NJ, USA), their ventral midsection scrubbed with a topical antiseptic (Betadine solution; Purdue Frederick Co., Norwalk, CT, USA), and a 6-cm incision was made between the ventral scales and the first set of lateral scales at a site approximately 65% of the distance from the snout to the cloaca. The incision was retracted open and a small hole was made in the proximal end of the small intestine just distal to the pyloric sphincter and the junction with the pancreaticobiliary duct. A 10-cm rubber catheter (8 mm diameter) was inserted through the hole, extended 2 cm downstream into the intestinal lumen and attached to the intestinal wall by a series of 'purse-string' 4-0 silk sutures. The other end of the catheter was exteriorized through a small incision in the snake's body wall and sutured to lateral scales. The incision through the body wall was closed with an inner (muscular layer) and outer (scales) set of interrupted sutures (3-0 Vicryl; Ethicon Inc., Somerville, NJ, USA), followed by an application of New-Skin® (Medtech Laboratories Inc., Jackson, WY, USA). Immediately following surgery, each snake was given a single injection of antibiotic (1 ml kg^{-1} enrofloxacin; Baytril, Bayer Co., Shawnee Mission, KS, USA) and analgesic (0.5 mg kg^{-1} flunixin meglamine; Phoenix Pharmaceutical Inc., St Joseph, MO, USA). Snakes recovered from anesthesia within 1 h and were allowed one month of recovery before the start of the experiment. The ground rat meal was infused at 6-h intervals over a period of 5 days, such that the combined mass of the infusate was equivalent to 25% of the snake's body mass.

Measurements of oxygen consumption and quantification of SDA

I measured rates of oxygen consumption ($\dot{V}_{O_2}$) of pythons using closed-system respirometry as described by Vleck (1987) and Secor and Diamond (1997a). Snakes were placed into individual respirometry chambers (9–39 liters) and maintained within an environmental chamber at 30°C. Each respirometry chamber was constructed with an incurrent and excurrent air port, each attached to a three-way stopcock. For each metabolic trial, a 50-ml gas sample was withdrawn from each chamber, the chambers were then sealed (closing the incurrent and excurrent stopcocks), and a second gas sample withdrawn 0.5–1 h later from the reopened excurrent air port. Gas samples were pumped through a column of H_2O absorbent (Drierite; W. A. Hammond Drierite Co., Xenia, OH, USA) and CO_2 absorbent (Ascarite II; Thomas Scientific, Swedesboro, NJ, USA) into an O_2 analyzer (S-3A/II; AEI Technologies, Pittsburgh, PA, USA). I calculated whole-animal (ml h^{-1}) and mass-specific (ml g^{-1} h^{-1}) rates of O_2 consumption corrected for standard temperature and pressure.

Each SDA trial began by measuring the $\dot{V}_{O_2}$ of fasted snakes once or twice a day for 3 days. For each snake, I assigned the lowest measure of its $\dot{V}_{O_2}$ during those days as its standard metabolic rate (SMR). In this and previous studies (Secor and Diamond, 1997a), the lowest measures of $\dot{V}_{O_2}$ were commonly recorded during the morning (06.00–08.00 h), a time when pythons were least active. Following SMR measurements, snakes were fed (or infused) and metabolic measurements were continued at 12-h intervals for 2 days and thereafter at 1-day intervals for up to 7 days. For each trial, I quantified the following six variables as described and illustrated by Secor and Diamond (1997a): 'SMR', as described above; 'peak $\dot{V}_{O_2}$', the highest recorded $\dot{V}_{O_2}$ during digestion; 'factorial scope of peak $\dot{V}_{O_2}$', calculated as peak $\dot{V}_{O_2}$ divided by SMR; 'duration', time from feeding when $\dot{V}_{O_2}$ was no longer significantly greater than SMR; 'SDA', the total energy expended above SMR during the duration of significantly elevated $\dot{V}_{O_2}$, quantified as kJ and kJ kg^{-1}; and 'SDA coefficient', SDA quantified as a percentage of the energy content of the meal. I calculated SDA (kJ) from the extra oxygen consumed above SMR over the duration of significantly elevated $\dot{V}_{O_2}$, assuming that 19.8 J are expended per ml of O_2 consumed (Gessaman and Nagy, 1988). Energy content of each meal was calculated as the product of meal wet mass and the energy equivalent of that meal (kJ g^{-1} wet mass). Energy equivalent of the intact rat, steak and ground rat meals were 8.0 kJ g^{-1} wet mass, 6.2 kJ g^{-1} wet mass and 8.0 kJ g^{-1} wet mass, respectively, as determined by bomb calorimetry, and 8.0 kJ g^{-1} wet mass for the liquid diet, assuming 17.6 kJ g^{-1} of casein (protein), 39.3 kJ g^{-1} of fat and 17.6 kJ g^{-1} of glucose (Schmidt-Nielsen, 1997).

Statistical analyses

Postfeeding changes in stomach content (analyzed as calculated percentages and actual mass of stomach content) were evaluated using analysis of covariance (ANCOVA) with body mass as a covariate. A repeated-design analysis of variance (ANOVA) was applied to test for significant effects of sampling time on gastric pH and $\dot{V}_{O_2}$ for each meal treatment. To test the effects of meal treatment on metabolic variables, I used ANCOVA (body mass as a covariate) for whole-animal measures and ANOVA for mass-specific measures. Because of the significant variation in body mass among the five meal treatments, I recalculated whole-animal measures of SMR, peak $\dot{V}_{O_2}$ and SDA of each snake assuming a body mass of 2400 g. Adjusted values were calculated from allometric equations presented in table 2 from Secor and Diamond (1997a), assuming mass exponents of 0.7, 0.9 and 1.01, respectively, for SMR, peak $\dot{V}_{O_2}$ and SDA. In conjunction with ANOVA and ANCOVA, *post-hoc* pairwise mean comparisons (Tukey–Kramer procedure) were used to compare treatments (sampling times or meal type). I present the *P* value results of ANOVA and ANCOVA and significant pairwise mean comparisons. The level of statistical significance is

designated as $P<0.05$ and mean values are reported as means ± 1 S.E.M.

Results

Rate of gastric digestion

At 12 h after feeding, ingested rat meals had not experienced any significant loss in mass (Fig. 1). Rat meals had been significantly ($P<0.01$) reduced to 81.6±3.4% of their original mass within stomachs by 24 h. As all rats were swallowed head first, degradation began at the head and proceeded distally (Fig. 2). Within the first day, the head and shoulders of ingested rats were in varying states of digestion, ranging from being partly digested with the skull remaining intact (as illustrated in Fig. 2) to being completely digested and absent from the stomach. Up to this time, the ingested rats were decomposing and becoming bloated with gases (CO_2 and H_2) produced by the still-living resident gut microflora. This was clearly evident for snakes that had consumed several rats, as their girth had increased an additional 30–40%. By day 2, most of the anterior half of the rat had passed into the small intestine, leaving 56.7±1.8% of the original meal within the stomach. If two rats had been consumed, the head of the second rat was now showing signs of digestion. By day 3, gastric distention was significantly reduced as 29.6±4.5% of the original meal mass remained in the stomach. In cases where two rats were consumed, the first rat and the anterior half of the second rat had completely passed, and only the distal half of the second rat was present within the stomach. By day 4, only 23.0±2.7% of the meal remained within the stomach and was largely composed of portions of distal vertebrae and musculature, along with the hindlimbs and tail. Only parts of the hindlimbs and mats of hair (10.5±2.8% of ingested mass) were present in the stomach by day 6; by day 14, the stomachs were empty.

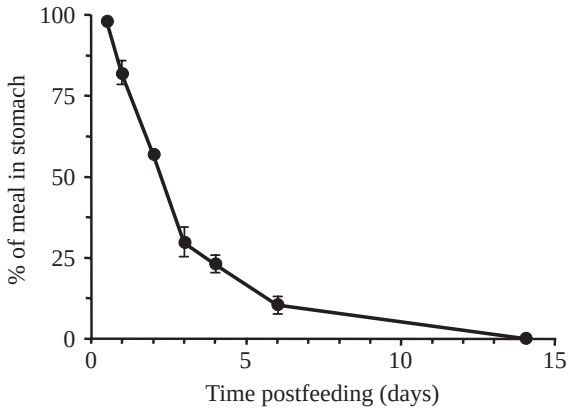

Fig. 1. Percentage of ingested meal remaining in the stomach as a function of time postfeeding (days) for *Python molurus*. All pythons had consumed meals equaling approximately 25% of their body mass. Mass of stomach contents was measured from 3–8 pythons per sampling period. In this and all following figures, error bars represent ±1 S.E.M. and are omitted if the S.E.M. is smaller than the width of the symbol for mean value.

Gastric pH

Feeding triggered a rapid decrease in gastric pH (Fig. 3). During the first 12 h after feeding, gastric pH dropped ($P<0.0001$) from 7.5 to 2.9, representing, on average, more than a 10-fold increase in intragastric $[H^+]$ every 3 h. By 24 h postfeeding, gastric pH had declined to approximately 2, and for the next 5–7 days held steady between 1.1 and 1.8 (mean pH during that time was 1.52±0.05). After the meal had passed from the stomach (usually 6–8 days after feeding), gastric pH increased at a rate that mirrored the rapid postfeeding decrease, such that over a span of only 18–24 h gastric pH had returned to 7.5. The individual variation in the time it took gastric pH to return to initial levels (7–12 days postfeeding) is explained by the differences among snakes in relative meal size (19.7–32.9% of body mass), as larger meals took more time to digest and thus induced longer episodes of acid production (Fig. 4).

Varying gastric workload and SDA

For each meal type, $\dot{V}_{O_2}$ (ml g^{-1} h^{-1} or ml h^{-1}) varied significantly (all $P<0.0001$) among pre- and postfeeding samples (Fig. 5). Whereas mass-specific SMR (ml g^{-1} h^{-1}) varied significantly ($P=0.008$) among meals, whole-animal measures of SMR (ml h^{-1}; recorded or adjusted to a body mass of 2400 g) did not differ (Table 1). Pythons reached peaks in $\dot{V}_{O_2}$ at 12 h (infused ground rat), 36 h (intact rat, steak and ground rat) or 48 h (liquid meal) postfeeding. Peak $\dot{V}_{O_2}$ varied

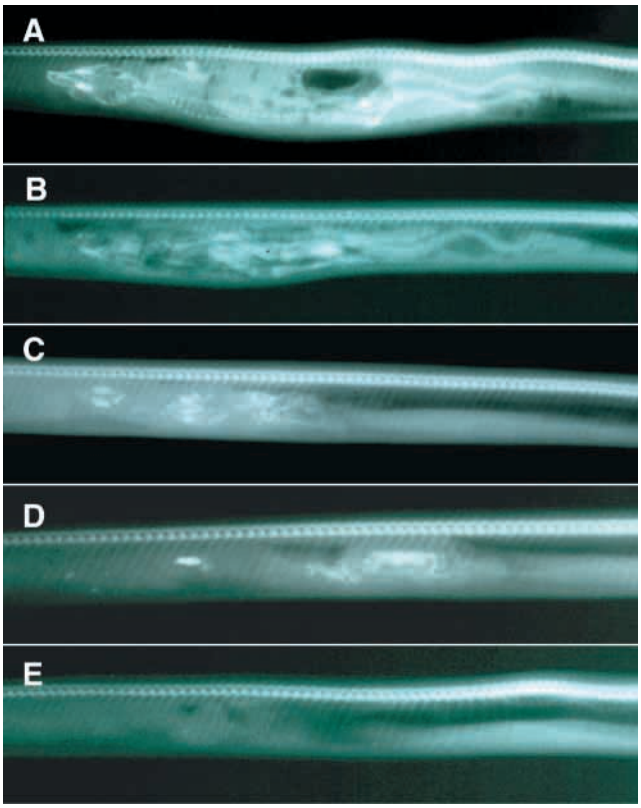

Fig. 2. X-rays of the midsection of a *Python molurus* taken at (A) 1 day, (B) 2 days, (C) 3 days, (D) 4 days and (E) 6 days following consumption of a rat weighing 25% of the snake's body mass.

significantly (all $P<0.0001$) among meal treatments as peak values during the digestion of intact rats were significantly (all $P<0.007$) greater than during the digestion of ground rat, which was greater (all $P<0.004$) than that during the digestion of the infused ground rat (Table 1). The factorial scope of peak $\dot{V}_{O_2}$ (peak $\dot{V}_{O_2}$/SMR) was highest for the digestion of intact rats, significantly ($P=0.005$) lower for the digestion of the ground rat and even lower (both $P<0.05$) for the digestion of the liquid meal and intestinal-infused ground rat (Table 1). The duration of significantly elevated $\dot{V}_{O_2}$ was 8 days for the digestion of the intact rat meals and 6 days for the other four meal treatments (Table 1).

SDA differed significantly (all $P<0.0001$) among meal treatments (Table 1). Although not differing between the intact rat and steak meals, SDA (adjusted to 2400 g) decreased significantly ($P<0.013$) with the digestion of ground rat and decreased even further (both $P<0.003$) during the digestion of the liquid meal and intestinal-infused ground rat. By infusing ground rat directly into the small intestine, thereby bypassing the workload of the stomach, SDA was reduced to one-third of

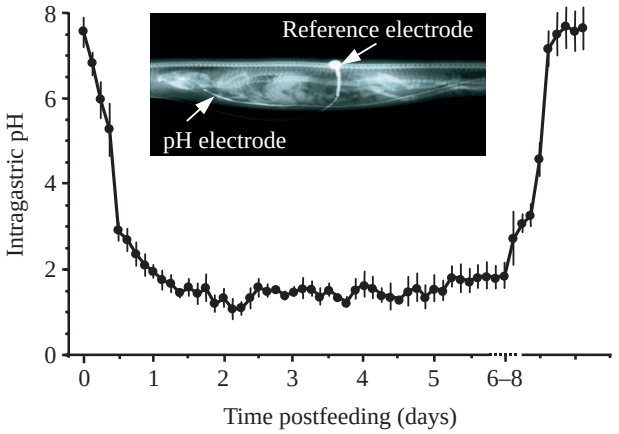

Fig. 3. Intragastric pH of *Python molurus* ($N=7$) as a function of time postfeeding (days). The insert illustrates the position of the pH electrode (attached to the rat's head) within the python's stomach. Note the decline in gastric pH following feeding and subsequent return after 7–9 days of digestion. The dotted portion of the *x*-axis signifies that the initial increase in pH upon completion of gastric digestion began 6–8 days postfeeding.

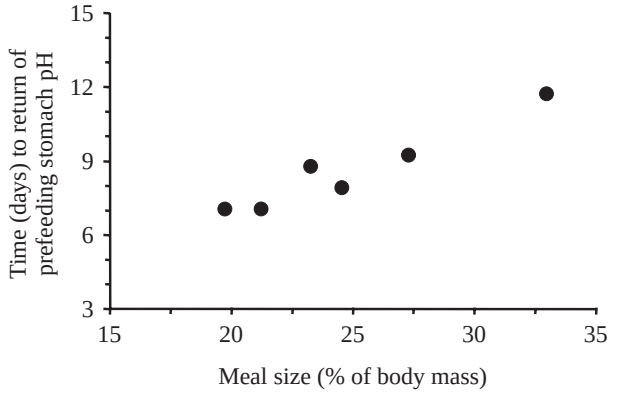

Fig. 4. The time (days) it took after feeding for stomach pH of *Python molurus* ($N=6$) to return to prefeeding levels plotted as a function of meal size (% of snake body mass). Note that as relative meal size increases so does the duration of maintaining an acidic stomach.

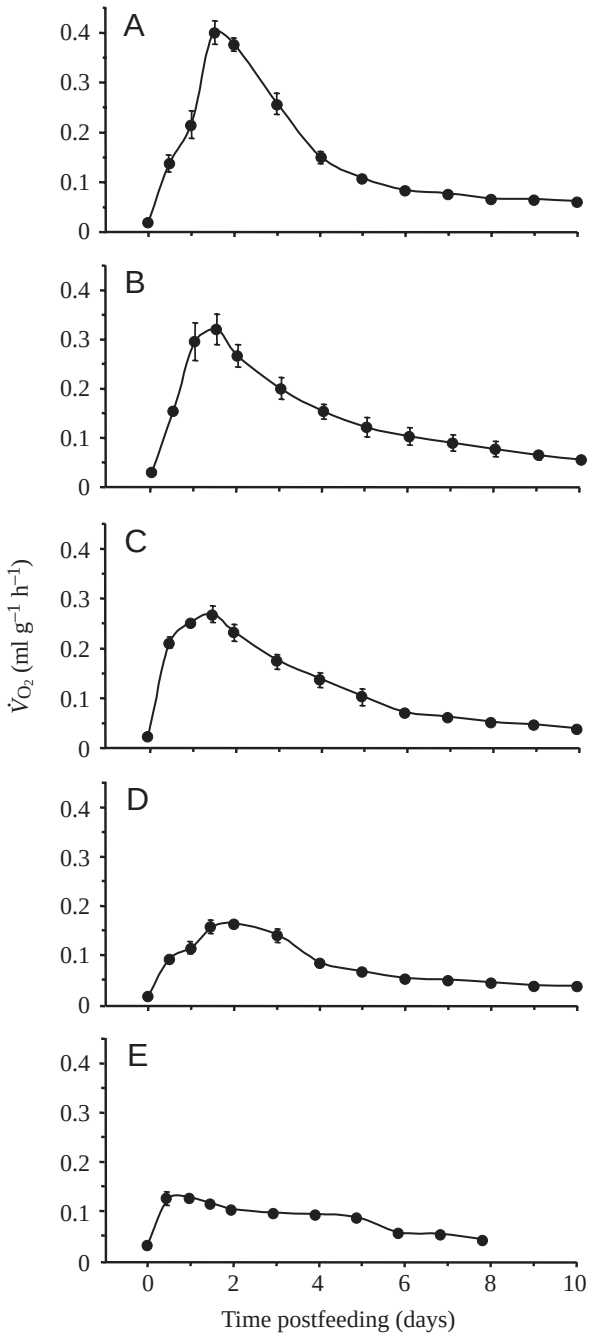

Fig. 5. Mean $\dot{V}_{O_2}$ at 30°C of *Python molurus* prior to (day 0) and up to 10 days following the ingestion of (A) intact rats, (B) steak, (C), ground rat, (D) liquid diet and (E) ground rat directly infused into the proximal small intestine. All meals were equal in mass to approximately 25% of snake body mass. Note the decrease in the magnitude of the postfeeding metabolic response as the workload on the stomach is reduced from digesting intact rat to intestinally infused ground rat meals.

Table 1. *Body mass, meal size, standard metabolic rate (SMR), and postfeeding metabolic measures of peak oxygen consumption ($\dot{V}_{O_2}$), scope of peak $\dot{V}_{O_2}$, duration, specific dynamic action (SDA) and SDA coefficient of Burmese pythons (*Python molurus*) in response to five meal treatments*

	Meal treatment					
Variable	Intact rat	Steak	Ground rat	Liquid meal	Infused ground rat	*P*
Body mass (g)	2394±341[a]	2316±434[a]	2429±192[a]	5240±509[b]	1673±275[a]	<0.0001
N	5	6	6	4	4	
Meal size (% of body mass)	25.0±0.1	25.3±0.02	25.0±0.0	25.0±0.0	25.0±0.0	0.41
SMR (ml h^{-1})	56.6±7.8	56.8±8.5	57.5± 4.4	111.6±7.1	49.4±4.5	0.53
SMR (ml h^{-1}) adjusted to 2400 g	56.6±7.8	58.4±1.8	57.2±2.5	66.0±1.4	64.2±2.6	0.052
SMR (ml g^{-1} h^{-1})	0.024±0.001[a]	0.025±0.001[a,b]	0.024±0.001[a]	0.022±0.001[a]	0.031±0.003[b]	0.008
Peak $\dot{V}_{O_2}$ (ml h^{-1})	963±145[a]	781±160[a,b]	681±68[b]	867±68[a]	229±24[c]	<0.0001
Peak $\dot{V}_{O_2}$ (ml h^{-1}) adjusted to 2400 g	965±48[a]	797±77[a,b]	672±34[b]	433±25[c]	328±29[c]	<0.0001
Peak $\dot{V}_{O_2}$ (ml g^{-1} h^{-1})	0.402±0.021[a]	0.330±0.031[a,b]	0.278±0.013[b]	0.166±0.010[c]	0.139±0.011[c]	<0.0001
Scope of peak $\dot{V}_{O_2}$	16.8±0.6[a]	13.2±1.5[b]	11.7±0.7[b]	7.7±0.58[c]	4.5±0.2[c]	<0.0001
Duration (days)	8	6	6	6	6	
SDA (kJ)	1259±174[a]	1111±203[a,b]	943±87[b]	1196±92[a]	289±38[c]	<0.0001
SDA (kJ) adjusted to 2400 g	1269±47[a]	1154±48[a]	938±54[b]	549±29[c]	422±18[c]	<0.0001
SDA (kJ kg^{-1})	528±19[a]	480±20[a]	391±22[b]	231±12[c]	175±7[c]	<0.0001
SDA coefficient	26.5±1.0[a]	30.5±1.2[a]	19.5±1.1[b]	14.4±0.8[c]	8.8±1.2[d]	<0.0001

Variables are defined in the text. Values are presented as means ± 1 S.E.M.

For each metabolic measure, superscript letters that differ denote significant (P<0.05) differences between means as determined from *post-hoc* pairwise comparisons.

that generated by the intact rat meals. Whereas SDA coefficients (SDA expressed as a percentage of the ingested meal energy) likewise did not differ between the intact rat and steak meals, values were significantly (both P<0.001) lower with the ground rat meal, decreasing again (P=0.022) with the liquid meal and declining even further (P=0.021) with the intestinal-infused ground rat meal (Table 1).

Discussion

Digestion of large and intact meals is an expensive endeavor for the Burmese python (Benedict, 1932; Secor and Diamond, 1997a; Overgaard et al., 1999). Mandatory for this process is the gastric breakdown of the ingested intact meal to a soup-like chyme that can then be passed to the small intestine. The highly acidic environment within the stomach when food is present indicates that gastric digestion by pythons is accomplished, in part, by a high production of hydrochloric acid (HCl). The reduction in the magnitude of the python's postprandial metabolic response when food bypasses the stomach provides evidence that the cost of HCl production and overall gastric performance is a substantial component of their SDA. In the following discussion, I shall address the breakdown of the rodent meal within the python's stomach, the postprandial profile of intragastric pH, the cost of gastric performance and the components of SDA and shall comment on the outlook for further studies of python gastric physiology.

Gastric breakdown

Following gastric upregulation during the first 12 h after feeding, secreted HCl and the protease pepsin begin to digest away the rat's head and anterior thorax. Concurrently, gas begins to build up within the body cavity of the ingested rats, a phenomenon that was also observed by Blain and Campbell (1942) in digesting boa constrictor (*Boa constrictor*) and indigo snake (*Drymarchon corais couperi*). Once HCl and pepsin have breached the rat's body cavity, the gas is released and gastric distention is relaxed. Breakdown of each rat proceeded from its head to its tail (rats were all swallowed head first), as it was continuously pushed towards the more distal portion of the stomach. The last materials to exit the stomachs were mats of hair, suggesting that the indigestible hair is either selectively held back, giving priority to the passage of more nutritional and digestible material, or is simply more difficult to pass through the pylorus.

In an x-ray study evaluating body temperature effects on gastric digestion, Skoczylas (1970a) observed rapid decomposition of ingested frogs within the stomachs of grass snakes (*Natrix natrix*) maintained at 25°C. Following their consumption of frog meals equaling 20% of body mass, *N. natrix* had cleared their stomachs within 3 days, which is considerably faster than the 5–7 days it took pythons to empty their stomachs. Plausible explanations for these differences are that *N. natrix* had consumed relatively smaller meals, their frog meals were digested more rapidly compared with rat meals due to a thinner integument, they had consumed a single frog whereas two-thirds of the pythons had consumed at least two rats (a single large prey item may pass faster than multiple smaller prey items) and, as a frequent feeder, *N. natrix* are able to initiate gastric digestion faster than the infrequently feeding

P. molurus, which must first upregulate gastric function before digestion can commence. In support of this last point, 24 h following consumption of a common meal size (25% of body mass), frequently feeding snake species pass twice the percentage of ingested prey (30%) from their stomachs than do infrequently feeding species (15%; Secor and Diamond, 2000).

Gastric pH

During fasting, the quiescent stomach of pythons apparently does not secrete acid, as is evident by the slight alkalinty of the gastric lumen immediately after swallowing the rat meal. The presence of the meal within the stomach (if not sooner) triggers the secretion of H^+, Cl^- and enzymes from the oxyntic cells of the gastric epithelium. This causes a rapid decrease in luminal pH to a stable level of approximately 1.5. Once the meal passes from the stomach, acid production ceases and luminal pH returns to around 7.5. Similarly, following ingestion of a frog meal, gastric pH of *N. natrix* decreases from 7.2 to 3.3 within 4 h, a response noticeably faster than that of *P. molurus* (Skoczylas, 1970b). For turtles, lizards, alligators and other snake species, gastric pH of fasting individuals ranges between 7 and 8 and declines to a range of 1.5–4 during digestion (Blain and Campbell, 1942; Coulson et al., 1950; Wright et al., 1957; Fox and Musacchia, 1959).

The pre- and postprandial profile of gastric pH of pythons (and other reptiles) is markedly different from that of mammals. In contrast to the slight alkalinity of the fasting python's stomach, mammals maintain a highly acidic environment (pH 1.1–3) within their stomachs between bouts of digestion (Youngberg et al., 1985; Evans et al., 1988; Cilluffo et al., 1990; Viani et al., 2002). Whereas pythons experience a dramatic postprandial decrease in gastric pH, the luminal pH of mammal stomachs increases rapidly after feeding to range between 3 and 6, presumably as the ingested meal buffers the gastric acid (Savarino et al., 1988; McLauchlan et al., 1989; Cilluffo et al., 1990). Within a few hours after feeding, intragastric pH of mammals drops as acid production, which has increased 20-fold, overwhelms the buffering capacity of the food, which is then being passed through the pyloric sphincter into the small intestine (Fordtran and Walsh, 1973).

The duration of gastric acid and enzyme production is a function of meal size, meal composition and body temperature. For pythons, increasing meal size by 65% (from 19.7% to 32.9% of body mass) resulted in a 50% increase in the duration that gastric pH was maintained at 1.5 (Fig. 4). Decreasing the structural composition of the python's meal results in an apparent decrease in gastric workload and acid and enzyme secretion (Fig. 5; Table 1). Intuitively, intact meals possessing a hard exo- or endoskeleton would require more time and effort to digest and pass than fragmented and/or soft-bodied food items. The turtle *Kinixys spekii* and the toad *Bufo marinus* required more time and energy, respectively, to digest millipedes and superworms (*Zophobas* larva), both possessing a chitinous exoskeleton, than to digest soft fungi and earthworms (Hailey, 1998; Secor and Faulkner, 2002). There is a direct relationship between body temperature and rates of chemical reactions; therefore, decreasing body temperature decreases rates of acid and enzyme secretions and thus increases the duration of digestion (Skoczylas, 1970a; Stevenson et al., 1985). The snakes used to monitor gastric pH were maintained at a lower body temperature (23–28°C) than those used to evaluate gastric digestion (27–30°C) and consequently experienced longer bouts of digestion.

Cost of gastric digestion

The results of this study suggest that as the workload of the python's stomach is reduced, so is the cost of digestion (Fig. 5; Table 1). One means to reduce gastric workload is to reduce meal size; for pythons, smaller meals are digested faster and incur a lower SDA and SDA coefficient (Secor and Diamond, 1997a). Another means (employed in this study) is to reduce the structural integrity of the meal while maintaining a constant meal size, thereby accelerating its passage into the small intestine. The rolled steaks lacked the outer integument and bones of an intact rat. The ground rat was finely pureed, thereby greatly increasing its surface area for acid and enzymatic degradation, and the liquid diet was already equivalent to or beyond the state of particle reduction characteristic of the chyme exiting the stomach. Whereas the steak meal passed from the stomach faster than intact rats but did not produce a lower SDA, both the ground rat and liquid diet reduced gastric resident time and SDA. In bypassing the stomach and infusing ground rat directly into the small intestine, the generated SDA was 33% of that resulting from the digestion of intact rat meals.

The large reduction in SDA when the functions of the stomach are bypassed suggests that gastric digestion is apparently an expensive component of the python's SDA. Contributing to this cost are three well-cited activities of the stomach; motility, enzyme production and acid secretion. Contractions of the stomach's smooth muscles serve to churn and grind the ingested meal, thereby facilitating contact of the food with enzymes and HCl, and to drive chyme through the pyloric sphincter. For non-mammalian species, a single cell type, the oxyntic cell, is responsible for the secretion of pepsinogen and HCl (handled by the chief and parietal cells, respectively, in mammals; Helander and Keeling, 1993). Secreted pepsinogen, when exposed to a luminal pH of 2–3.5, is cleaved to the active proteolytic enzyme pepsin. Pepsin begins the process of protein digestion by acting on collagen and hydrolyzing proteins. HCl is formed from Cl^- and H^+; Cl^- is passively released from oxyntic cells whereas H^+ is actively pumped from cells by the ATP-driven H^+/K^+-exchanger (H^+/K^+-ATPase or proton pump; Forte et al., 1980).

While motility and pepsinogen production undoubtedly both contribute to the cost of gastric digestion, at least five lines of evidence emphasize the cost of acid production: (1) pythons maintain an intragastric pH of 1.5 in spite of the large buffering capacity of the rat meals for 5–7 days; (2) the production of such a quantity of HCl requires the proton pumps of the oxyntic cells to move H^+ from the cytosol into the gastric lumen against

a concentration gradient in excess of a million-fold (Helander and Keeling, 1993); (3) the proton pumps operate *via* the hydrolysis of ATP with a stoichiometry of one H^+ pumped per ATP hydrolyzed (Reenstra and Forte, 1981; Norberg and Mårdh, 1990); (4) the gastric parietal cells of mammals contain the highest concentration of mitochondria (34–44% by volume) compared with any other mammalian cell type (Helander and Hirschowitz, 1972; Helander et al., 1986), and, in a preliminary study, I found python oxyntic cells to be 40% mitochondria by volume; and (5) acid secretion is absolutely dependent upon oxygen delivery (Forte et al., 1975; Berglindh, 1984). Collectively, these findings indicate that pythons expend considerable amounts of cellular energy *via* aerobic metabolic pathways to generate the vast quantity of HCl necessary to digest their large intact meals.

Components of SDA

One goal of this project was to ascertain the relative contribution of pre- and postabsorptive activities to the python's SDA. To begin, I calculated that a 1 kg python digesting a 250 g rat (25% of snake body mass) would experience an SDA of 600 kJ based on the published regression equation: log SDA = log body mass × 1.01 – 0.25 (table 2 in Secor and Diamond, 1997a). I next estimated the cost of gastric performance as 330 kJ (55% of SDA) based on the differences between the SDA resulting from digesting intact rat meals (528 kJ kg^{-1}) and that generated by the intestinally infused ground rat meals (175 kJ kg^{-1}) and considering that the infused ground rat meals may not have fully generated all postgastric activities (therefore decreasing the assumed cost of gastric function). Lacking, at least from the infused ground rat response, is the production and secretion of bicarbonate solution by the pancreas and small intestine in response to the introduction of the acidic chyme from an ingested meal.

Following feeding, pythons upregulate the performance of their dormant guts in order to digest their meals (Secor and Diamond, 1995, 1997b). To quantify the cost of gastrointestinal (GI) upregulation, I first estimated that the difference in SMR over a 24-h period (time taken to upregulate the gut) between a 1 kg python with a quiescent gut (from table 2 in Secor and Diamond, 1997a) and a 1 kg python with an upregulated gut (calculated from the SMR of frequently feeding snakes that maintain an upregulated gut; Secor and Diamond, 2000) was equivalent to 12 kJ. Next, I calculated that the cost of the postprandial increase in stomach and intestinal mass for a 1 kg python was 17.5 kJ. I assumed that these organs gained 2.5 g in protein with an energetic value of 44 kJ (17.5 kJ g^{-1} protein) and that the cost of protein synthesis is 0.4 kJ expended per kJ of protein synthesized (Aoyagi et al., 1988). Combining metabolic and growth costs, the estimated cost of GI upregulation is 29.5 kJ, which is 4.9% of SDA (Fig. 6).

To calculate the cost of post-absorptive protein synthesis, I estimated that a 1 kg python would gain 103 g in body mass from the digestion of a 250 g rat, assuming a growth efficiency

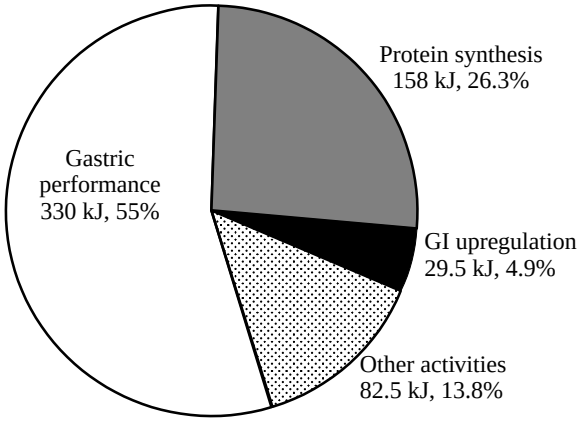

Fig. 6. Total specific dynamic action (SDA; 600 kJ) of a 1 kg *Python molurus* resulting from the digestion of a rat meal equal in mass to 25% of the snake's body mass, partitioned among the components of gastric performance, protein synthesis, gastrointestinal (GI) upregulation and other activities. Note in this example that the energy expended on gastric performance is the largest component of SDA.

(mass gained/mass consumed) of 41.2% (S. M. Secor, personal observations; based on 40 pythons each consuming 10 meals, each equaling 25% of body mass). Assuming that 22.5 g of the mass gained is protein (the rest being water, bone and fat), with an energy value of 394 kJ (17.5 kJ g^{-1} protein), the cost of protein synthesis is therefore 158 kJ (0.4 kJ g^{-1} protein), equivalent to 26.3% of SDA (Fig. 6). The combined cost of gastric performance, GI upregulation and protein synthesis is 517.5 kJ, 86.2% of the projected SDA. The remaining component, 82.5 kJ, 13.8% of SDA, would include the activity costs of the pancreas, gallbladder, liver, kidneys and small and large intestines (Fig. 6). Also included are the costs stemming from the postprandial increase in pulmonary and cardiovascular performance (Secor et al., 2000).

A universal phenomenon following feeding is activation of tissues of the GI tract to propel food through the oesophagus, stomach and intestines, to secrete H^+, enzymes, bicarbonate solution and bile, and to hydrolyze and transport nutrients. Given that these activities are energy consuming and are initiated prior to assimilating the meal, all digesting organisms must first expend energy before harvesting and metabolizing any of the ingested nutrients. Secor and Diamond (1995) in discussing this physiological phenomenon used the analogy 'pay before pumping' in reference to self-service fuel stations. Burmese pythons, like other organisms, must expend energy (pay) to generate the HCl and pepsin necessary to initiate gastric digestion, propel food into the small intestine, produce and release bile, enzymes and bicarbonate, upregulate and operate intestinal hydrolases and nutrient transporters, and lengthen intestinal microvilli before they can use any of the ingested nutrients in metabolic pathways (pump). Assuming that none of the ingested nutrients has crossed the intestinal wall by 18 h after consuming an intact rat (25% of body mass; Fig. 5), the minimal cost that is paid upfront is 62 kJ, which is

5.5% of SDA. This start-up cost must be met by endogenous energy stores, most likely by lipids mobilized from fat bodies. Support for this response is the rapid postprandial 50-fold increase in plasma triglycerides observed for Burmese pythons (Secor and Nagy, 2000).

The Burmese python's impressive upregulation of their GI tract following feeding was earlier suggested as one of several important contributors to their relatively large SDA (Secor and Diamond, 1995). The combination of 5- to 20-fold increases in small intestinal nutrient transport rates, up to a 3-fold increase in pancreatic and intestinal enzyme activities, a doubling of small intestinal mass, and a 5-fold increase in microvillus length was reasoned to have an impact on their SDA (Secor and Diamond, 1995, 1998). As previously calculated, the cost of GI upregulation may represent approximately 5% of the python's SDA. Similarly it was concluded from metabolic measurements taken during overlapping digestive bouts (thereby the gut was not allowed to down- and upregulate performance) that the cost of postprandial GI upregulation for the turtle *Kinixys spekii* and Burmese python is not large (Hailey, 1998; Overgaard et al., 2002). Granted that the postprandial upregulation of the python's GI tract occurs at some cost, it apparently does not dominate SDA.

Outlook on python gastric physiology

The findings of this project raise several interesting points warranting future investigation of the python's gastric physiology. First, the python's ability to activate and deactivate gastric function, well beyond that of mammals, avails them as an excellent model to investigate the underlying mechanisms involved in the regulation of gastric performance. For the python, the intragastric presence of the meal undoubtedly triggers neural and endocrine signals that stimulate the production of HCl and pepsin, and induces gastric hypertrophy and motility. It is well known that the hormone gastrin stimulates HCl production and gastric hypertrophy in mammals (Walsh, 1994), although recent attempts to assay gastrin in pythons using mammalian probes have been unsuccessful (Secor et al., 2001). Of interest is determining whether pythons possess a gastrin structurally distinct from that of mammals or employ a different regulatory peptide to control gastric function.

Second, mammals characteristically maintain an acidic environment within their stomachs between digestive bouts, whereas for pythons intragastric pH is kept slightly alkaline between meals. It has been suggested that mammals maintain an acidic gastric lumen as a protective means against ingested bacteria and other pathogens. Therefore, are pythons susceptible to being colonized by pathogenic microorganisms or do they possess some alternative protective mechanism against them?

And third, given that their large postprandial metabolic response is dominated by gastric function, do pythons possess an oxidative capacity of their stomach and oxyntic cells of unprecedented magnitude for an ectothermic vertebrate? This is suggested from the effort they expend to generate the HCl that can maintain an intragastric pH of 1.5 for a week against the constant buffering actions of their large meal, to constantly produce pepsin and perhaps other enzymes during that interval, to propel a large amount of material through the pyloric sphincter, and to produce new mucosal cells. Thus, the stomach's capacity for high aerobic performance may be necessary for pythons to digest their large intact meals.

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References

Aoyagi, Y., Tasaki, I., Okumura, J.-I. and Muramatsu, T. (1988). Energy cost of whole-body protein synthesis measured *in vivo* in chicks. *Comp. Biochem. Physiol. A* **91**, 765-768.

Benedict, F. G. (1932). *The Physiology of Large Reptiles with Special Reference to the Heat Production of Snakes, Tortoises, Lizards, and Alligators. Carnegie Inst. Wash. Publ. No. 425*. Washington, DC: Carnegie Institution of Washington.

Berglindh, T. (1984). The mammalian gastric parietal cell *in vitro*. *Annu. Rev. Physiol*. **46**, 377-392.

Blain, A. W. and Campbell, K. N. (1942). A study of digestive phenomena in snakes with the aid of the roentgen ray. *Am. J. Roentgenol. Radium Ther*. **48**, 229-239.

Brody, S. (1945). *Bioenergetics and Growth*. New York: Reinhold Publishing Company.

Brown, C. R. and Cameron, J. N. (1991). The relationship between specific dynamic action (SDA) and protein synthesis rates in the channel catfish. *Physiol. Zool*. **64**, 298-309.

Carefoot, T. H. (1990). Specific dynamic action (SDA) in the supralittoral isopod, *Ligia pallasii*: identification of components of apparent SDA and effects of dietary amino acid quality and content on SDA. *Comp. Biochem. Physiol. A* **95**, 309-316.

Cilluffo, T., Armstrong, D., Castiglione, F., Emde, C., Galeazzi, R., Gonvers, J.-J. and Blum, A. L. (1990). Reproducibility of ambulatory gastric pH recordings in the corpus and antrum. *Scand. J. Gastroenterol.* **25**, 1076-1083.

Coulson, R. A., Hernandez, T. and Dessauer, H. C. (1950). Alkaline tide in the alligator. *Soc. Exp. Biol. Med*. **74**, 866-869.

Evans, D. F., Pye, G., Bramley, R., Clark, A. G., Dyson, T. J. and Hardcastle, J. D. (1988). Measurement of gastrointestinal pH profiles in normal ambulant human subjects. *Gut* **29**, 1035-1041.

Fordtran, J. S. and Walsh, J. H. (1973). Gastric acid secretion rate and buffer content of the stomach after eating. *J. Clin. Invest*. **52**, 645-657.

Forte, J. G., Machen, T. E. and Öbrink, K. J. (1980). Mechanisms of gastric H^+ and Cl^- transport. *Annu. Rev. Physiol*. **42**, 111-126.

Forte, T. M., Mache, T. E. and Forte, J. G. (1975). Ultrastructural and physiological changes in piglet oxyntic cells during histamine stimulation and metabolic inhibition. *Gastroenterology* **69**, 1208-1222.

Fox, A. M. and Musacchia, X. J. (1959). Notes on the pH of the digestive tract of *Chrysemys picta*. *Copeia* **1959**, 337-339.

Geesaman, J. A. and Nagy, K. A. (1988). Energy metabolism: errors in gas-exchange conversion factors. *Physiol. Zool*. **61**, 507-513.

Hailey, A. (1998). The specific dynamic action of the omnivorous tortoise *Kinixys spekii* in relation to diet, feeding patterns, and gut passage. *Physiol. Zool*. **71**, 57-66.

Helander, H. F. and Hirschowitz, B. I. (1972). Quantitative ultrastructure studies on gastric parietal cells. *Gastroenterology* **63**, 951-961.

Helander, H. F. and Keeling, D. J. (1993). Cell biology of gastric acid secretion. *Baillière's Clin. Gastroenterol.* **7**, 1-21.

Helander, H. F., Leth, R. and Olbe, L. (1986). Stereological investigations on human gastric mucosa: I. normal oxyntic mucosa. *Anat. Rec*. **216**, 373-380.

Janes, D. N. and Chappell, M. A. (1995). The effect of ration size and body size on specific dynamic action in Adélie penguin chicks, *Pygoscelis adeliae*. *Physiol. Zool*. **68**, 1029-1044.

Jobling, M. (1981). The influences of feeding on the metabolic rate of fishes: a short review. *J. Fish Biol.* **18**, 385-400.

Jobling, M. and Davies, P. S. (1980). Effects of feeding on metabolic rate, and the specific dynamic action in plaice, *Pleuronectes platessa* L. *J. Fish Biol*. **16**, 629-638.

Kleiber, M. (1975). *The Fire of Life*. New York: Krieger.

Lyndon, A. R., Houlihan, D. F. and Hall, S. J. (1992). The effect of short-term fasting and a single meal on protein synthesis and oxygen consumption in cod, *Gadus morhua*. *J. Comp. Physiol. B* **162**, 209-215.

McLauchlan, C., Fullarton, G. M., Crean, G. P. and McColl, K. E. L. (1989). Comparison of gastric body and antral pH: a 24 hour ambulatory study in healthy volunteers. *Gut* **30**, 573-578.

Norberg, L. and Mårdh, S. (1990). A continuous technique for analysis of stoichiometry and transport kinetics of gastric H, K-ATPase. *Acta Physiol. Scand.* **140**, 567-573.

Overgaard, J., Busk, M., Hicks, J. W., Jensen, F. B. and Wang, T. (1999). Respiratory consequences of feeding in the snake *Python molurus*. *Comp. Biochem. Physiol. A* **124**, 359-365.

Overgaard, J., Andersen, J. B. and Wang, T. (2002). The effects of fasting duration on the metabolic response to feeding in *Python molurus*: an evaluation of the energetic costs associated with gastrointestinal growth and upregulation. *Physiol. Zool*. **75**, 360-368.

Pope, C. H. (1961). *The Giant Snakes*. New York: Knopf.

Reenstra, W. W. and Forte, J. G. (1981). H^+/ATP stoichiometry for the gastric (K^++H^+)-ATPase. *J. Membr. Biol*. **61**, 55-60.

Savarino, V., Mela, G. S., Scalabrini, P., Sumberaz, A., Fera, G. and Celle, G. (1988). 24-hour study of intragastric acidity in duodenal ulcer patients and normal subjects using continuous intraluminal pH-metry. *Dig. Dis. Sci.* **33**, 1077-1080.

Schmidt-Nielsen, K. (1997). *Animal Physiology, Adaptation and Environment*. Cambridge: Cambridge University Press.

Secor, S. M. (2001). Regulation of digestive performance: a proposed adaptive response. *Comp. Biochem. Physiol. A* **128**, 565-577.

Secor, S. M. and Diamond, J. (1995). Adaptive responses to feeding in Burmese pythons: pay before pumping. *J. Exp. Biol.* **198**, 1313-1325.

Secor, S. M. and Diamond, J. (1997a). Determinants of post-feeding metabolic response in Burmese pythons, *Python molurus*. *Physiol. Zool*. **70**, 202-212.

Secor, S. M. and Diamond J. (1997b). Effects of meal size on the postprandial responses in juvenile Burmese python (*Python molurus*). *Am. J. Physiol*. **272**, R902-R912.

Secor, S. M. and Diamond J. (1998). A vertebrate model of extreme physiological regulation. *Nature* **395**, 659-662.

Secor, S. M. and Diamond, J. (2000). Evolution of regulatory responses to feeding in snakes. *Physiol. Biochem. Zool.* **73**, 123-141.

Secor, S. M. and Faulkner, A. C. (2002). Effects of meal size, meal type, body temperature, and body size on the specific dynamic action of the marine toad, *Bufo marinus*. *Physiol. Biochem. Zool.* **75**, 557-571.

Secor, S. M. and Nagy, T. (2000). Postprandial response of plasma lipids and the hormone leptin in pythons. *Am. Zool.* **40**, 1205.

Secor, S. M., Hicks, J. W. and Bennett, A. F. (2000). Ventilatory and cardiovascular responses of a python (*Python molurus*) to exercise and digestion. *J. Exp. Biol.* **203**, 2447-2454.

Secor, S. M., Fehsenfeld, D., Diamond, J. and Adrian, T. E. (2001). Responses of python gastrointestinal regulatory peptides to feeding. *Proc Natl. Acad Sci. USA* **98**, 13637-13642.

Skoczylas, R. (1970a). Influence of temperature on gastric digestion in the grass snake, *Natrix natrix* L. *Comp. Biochem Physiol*. **33**, 793-804.

Skoczylas, R. (1970b). Salivary and gastric juice secretion in the grass snake, *Natrix natrix*, L. *Comp. Biochem. Physiol.* **35**, 885-903.

Soofiani, N. M. and Hawkins, A. D. (1982). Energetic costs at different levels of feeding in juvenile cod, *Gadus morhua* L. *J. Fish Biol.* **21**, 577-592.

Stevenson, R. D., Peterson, C. R. and Tsuji, J. S. (1985). The thermal dependence of locomotion, tongue flicking, digestion, and oxygen consumption in the wandering garter snake. *Physiol. Zool.* **58**, 46-57.

Tandler, A. and Beamish, F. W. H. (1979). Mechanical and biochemical components of apparent specific dynamic action in largemouth bass, *Micropterus salmoides* Lacépède. *J. Fish Biol.* **14**, 343-350.

Tandler, A. and Beamish, F. W. H. (1980). Specific dynamic action and diet in largemouth bass, *Micropterus salmoides* (Lacépède). *J. Nutrit.* **110**, 750-764.

Tandler, A. and Beamish, F. W. H. (1981). Apparent specific dynamic action (SDA), fish weight and level of caloric intake in largemouth bass, *Micropterus salmoides* Lacepede. *Aquaculture* **23**, 231-242.

Viani, F., Verdú, E. F., Idström, J. P., Cederberg, C., Fraser, R., Fried, M., Blum, A. L. and Armstrong, D. (2002). Effect of omeprazole on regional and temporal variations in intragastric acidity. *Digestion* **65**, 2-10.

Vleck, D. (1987). Measurement of O_2 consumption, CO_2 production, and water vapor production in a closed system. *J. Appl. Physiol.* **62**, 2103-2106.

Walsh, J. H. (1994). Gastrointestinal hormones. In *Physiology of the Gastrointestinal Tract* (ed. L. R. Johnson), pp. 1-128. New York: Raven.

Wang, T., Morten, Z., Arvedsen, S., Vedel-Smith, C. and Overgaard, J. (2003). Effects of temperature on the metabolic response to feeding in *Python molurus*. *Comp. Biochem. Physiol. A* **133**, 519-527.

Wright, R. D., Florey, H. W. and Sanders, A. G. (1957). Observations on the gastric mucosa of reptilia. *Quart. J. Exp. Physiol.* **42**, 1-14.

Youngberg, C. A., Wlodyga, J., Schmaltz, S. and Dressman, J. B. (1985). Radiotelemetric determination of gastrointestinal pH in four healthy beagles. *Am. J. Vet. Res*. **46**, 1516-1521.