Digestive performance in neonatal Southern Pacific Rattlesnakes (Crotalus oreganus helleri)

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Abstract: Despite significant research on the metabolic characteristics of digestion in adult snakes, the digestive performance of neonatal snakes is poorly characterized. We examined the energetic costs associated with digestion and the energetic profit derived from the first meal eaten by neonatal Southern Pacific Rattlesnakes (Crotalus oreganus helleri Meek, 1905). Composition of venom of C. o. helleri changes through ontogeny, becoming richer in proteolytic components that are hypothesized by some to enhance the rate of digestion. Therefore, we also investigated whether venom type (proteolytic-component-rich “adult” versus neurotoxin-rich “neonate” venom) affected digestive performance. We examined specific dynamic action (SDA), apparent assimilation efficiency, rate of digestion, and gut passage time in snakes fed prey killed with either “adult” or “neonate” venom at 22 and 30 °C. Although digestion progressed more quickly at 30 °C compared with 22 °C, there were no significant differences in digestion rate or assimilation efficiency owing to venom type; however, our statistical power was limited by small sample size. Despite the lack of “digestive experience”, the apparent assimilation efficiency was remarkably high (mean of 92%) and greater than published values for Crotalinae species. Based on these results, we hypothesize that neonatal C. o. helleri make a big energetic profit from their first meal by digesting efficiently and economically.

Résumé : Malgré d’importantes recherches sur les caractéristiques métaboliques de la digestion chez les serpents adultes, la performance des nouveau-nés est mal décrite. Nous examinons les coûts énergétiques associés à la digestion ainsi que l’avantage énergétique obtenu du premier repas ingéré chez des crotales de l’ouest (Crotalus oreganus helleri Meek, 1905). La composition du venin de C. o. helleri change au cours de l’ontogénèse, s’enrichissant en composantes protéolytiques qui, pense-t-on, accélèrent la vitesse de digestion. C’est pourquoi, nous avons aussi déterminé si le type de venin (type « adulte » riche en composantes protéolytiques ou type « néonate » riche en neurotoxines) affecte la performance de la digestion. Nous avons examiné l’action dynamique spécifique (SDA), l’efficacité apparente de l’assimilation, le taux de digestion et la durée du passage dans le tube digestif chez des serpents nourris de proies tuées avec du venin « adulte » ou « néonate » à 22 et à 30 °C. Bien que la digestion progresse plus rapidement à 30 °C qu’à 22 °C, il n’y a pas de différence significative dans le taux de digestion ni dans l’efficacité de l’assimilation en fonction du type de venin; le faible échantillon réduit cependant la puissance des statistiques. Malgré l’absence d’« expérience digestive », l’efficacité apparente de l’assimilation est remarquablement élevée (moindre de 92 %) et supérieure à toutes les valeurs publiées pour les espèces de Crotalinae. D’après ces résultats, nous émettons l’hypothèse selon laquelle les C. o. helleri nouveau-nés reçoivent un profit énergétique considérable de leur premier repas à cause d’une digestion efficace et économique.

[Traduit par la Rédaction]

Introduction

Newborn rattlesnakes (genus Crotalus L. 1758), like other animals with little or no parental care after birth, are under significant pressure to successfully forage to avoid starvation. Like neonates of many other species, they must derive materials and energy from body stores and maternal yolk for initial growth and maintenance (Clutton-Brock 1991). These metabolic fuel stores must also meet the demands of initial foraging activities, as well as supply the energy requirements of ingestion and digestion of prey. Secor (1995) showed that newborn Burmese Pythons (Python molurus (L., 1758)) have enough abdominal fat stores to supply approximately 52 days of energy at resting metabolic rates. Given the additional energy required for foraging activities and digestion, even less time than this is available to find, capture, ingest, and digest a prey item before starvation.

For infrequently foraging snakes (e.g., genus Python Daudin, 1803 and Crotalus spp.), specific dynamic action (SDA; the energy expended during digestion and assimilation of prey) can be dramatically greater than basal energy expenditure (Secor and Diamond 1995, 1997, 1998), typically on the order of 5–10 times standard metabolic rate (SMR) (Secor et al. 1994; Andrade et al. 1997; McCue and Lillywhite 2002;
Dietary enzymes and membrane transporter activity (Secor and Diamond 1995, 1998; McCue 2006b; Secor 2008b); and hypertrophy of organs supportive of digestive function including the pancreas, liver, kidneys, and heart (Secor and Diamond 1998; Andersen et al. 2005; Cox and Secor 2008; Secor 2008b). However, the degrees to which hypertrophy of digestive organs (Overgaard et al. 2002) and acid production (Andrade et al. 2005; Cox and Secor 2008; Secor 2008b) contribute to SDA in snakes may not be as great as previously thought.

The energetic costs associated with ingestion and digestion, as well as the energetic profit derived by neonates with naïve digestive systems, remains poorly characterized. It is possible that neonate snakes with no prior digestive experience possess limited capacities for hypertrophy of digestive tissues, as well as for digestion and absorption of nutrients from the first meal. Secor (1995) found that juvenile P. molurus increase their metabolic rates while digesting their first meals to a similar extent as adult snakes. However, few data exist concerning digestive performance in the neonates of other species of snakes.

In this study, we ask how the energetic costs associated with SDA compare with the energy derived from ingestion of the first meal by neonatal rattlesnakes. Southern Pacific Rattlesnakes (Crotalus oreganus helleri Meek, 1905) shift from “neonate” venom that consists primarily of neurotoxic components to “adult” venom characterized by proteolytic components (Mackessy 1988). These proteolytic components may be responsible for enhancement of digestive performance in adult rattlesnakes (Thomas and Pough 1979; Mackessy 1988). Thus, as part of this study, we conducted experiments using prey injected with either juvenile or adult venom to compare SDA, the rate of digestion, occurrence of prey regurgitation, and digestive efficiency of neonatal snakes given meals injected with different venom types. Because temperature is well known to affect digestion in snakes (Skoczylas 1970; Greenwald and Kanter 1979; Naulleau 1983; Beaura and Zaidan 2001; Wang et al. 2002; Toledo et al. 2003; Zaidan and Beaura 2003), we determined the effects of two different temperature treatments on these digestive parameters.

**Materials and methods**

**Study animals and treatment groups**

All animals in this study were used in accordance with the guidelines of the Institutional Animal Care and Use Committee of the University of California, Santa Barbara. Adult female C. o. helleri were collected from Santa Barbara County between May and September of 2004 and 2005 and assessed for reproductive condition. Eleven female snakes with embryos were transported to the Animal Resource Center at the University of California, Santa Barbara, where they were housed in captivity until parturition. Forty-six neonatal C. o. helleri from 11 clutches were used in this study (mass 12.4 ± 2.1 g (mean ± SE); snout–vent length 250 ± 13 mm (mean ± SE)).

Snakes were randomly assigned to one of six groups: four treatment groups of nine snakes per group and two neonate venom donor groups of five snakes per group. Three snakes from each treatment group were euthanized during digestion to measure gut passage rates, leaving six snakes per treatment for measurement of metabolic rates. In addition to the gravid female snakes, 10 adult snakes were captured and held for adult venom samples.

The oxygen consumption rates during digestion, rate of digestion, and occurrence of putrefaction in four test groups were assessed. Each snake was fed a small laboratory mouse (Mus musculus, L., 1758) weighing 35% ± 3% (mean ± SE) of the body mass of that snake. Live mice were injected with either adult venom or neonate venom. Treatment groups were maintained and tested at 30 or 22 °C, thus making four treatments of nine snakes each: adult venom at 30 °C; adult venom at 22 °C; neonate venom at 30 °C; neonate venom at 22 °C. Because rattlesnakes are ectothermic, varying temperature allowed us to determine whether digestive performance and the effects of venom type are temperature-dependent. The temperatures selected are similar to the minimum and maximum values that variation in weather may impose on wild neonates during their first autumn.

**Venom samples and venom injection**

To obtain venom samples, 10 randomly selected neonates and 10 wild-caught adults were reserved for extracting venom samples. Constituents of rattlesnake venom can vary both with age and among individual snakes (Mackessy 1988; Hayes 1991). To minimize potential effects of individual variation and to create a venom sample representing the general neonate or adult constituents, pooled venom samples were created before injecting mice. For each treatment, either five neonates or five adults from the venom donor groups were anesthetized. The venom glands of each snake were gently palpated to release venom. Venom was collected in 1 µL capillary tubes and combined in a sterilized test tube. Neonatal snakes used for venom samples were excluded from the treatment groups and were only used to obtain one venom sample per snake. Venom was obtained once from each of 10 adult rattlesnakes and combined to produce pooled adult venom samples for 22 °C (five adults) and 30 °C (remaining five adults) treatments. Snakes in this study were collected from similar localities within the range of the snakes examined by Mackessy (1988). We assume that the snakes used for venom samples in this study have a similar shift in venom components as observed by Mackessy (1988).

Once venom had been extracted from all five venom donors for a given treatment and the venom combined to create a pooled sample, 10 µL of venom was drawn from the test tube and injected into a living laboratory mouse. This amount was based on studies reporting venom expenditure during predatory strikes by neonatal rattlesnakes (Mackessy 1988; Hayes 1991; Mackessy et al. 2003; W.K. Hayes, personal communication) and is approximately one-third of the mean maximum amount of venom that could be expressed from venom extraction of 20 neonates obtained during a companion study (0.028 ± 0.002 mL (mean ± SE), n = 20; J.P. LaBonte, unpublished data).

To mimic the natural characteristics of envenomation, prey items were injected in vivo. This allowed the circulatory sys-
tem of live prey to help disperse the venom from the site of injection to other parts of the body (Kardong 1986; Macknessy 1988). Venom was injected with a syringe and a 25-gauge needle into each mouse’s abdominal cavity at approximately 10 mm anterior to the hip and 5 mm below mid-dorsal at a depth of 3 mm. Envenomation depth was a conservative estimate based on fang length of 4.0 ± 0.5 mm (mean ± SE) measured in 15 neonates obtained in previous years (J.P. LaBonte, unpublished data).

Feeding protocol

After each mouse had been injected with venom and reached a state of incapacitation, they were force-fed to neonates anesthetized with Isoflurane. Force feeding under anesthesia was conducted for three critical reasons. First, neonatal snakes are often reluctant to willingly feed on prey offered in captivity; neonatal Western Rattlesnakes (Crotalus oreganus Holbrook, 1840) generally refuse rodent prey, including laboratory mice (J.P. LaBonte, personal observation). Second, to eliminate variation in the amount of time that venom is acting on the prey and the time that the prey is exposed to digestive enzymes, it was important to standardize the time between injecting venom into the prey and feeding it to the snake. Third, to maintain the integrity of the treatments, it was important to guarantee that the snakes did not introduce additional venom into the prey item.

With the aid of blunt forceps, each mouse was gently guided through the oral cavity and into the esophagus of the snake. Through gentle palpation and additional aid of the forceps, the prey was then moved into the stomach of the snake. After feeding, each snake was placed into a cage and allowed to recover. Snakes appeared to be alert and behaving normally between 15 and 60 min after this procedure. Snakes force-fed in this way showed no qualitative differences in metabolic response when compared with a subset of two similar snakes allowed to feed willingly, without anesthesia. McCue (2006a) found no significant difference in mean hourly metabolic rate between a group of snakes (Western Diamondback Rattlesnake, Crotalus atrox Baird and Girard, 1853, and Copperhead, Agkistrodon contortrix (L., 1766)) that were anesthetized with Isoflurane vapor and a control group, not anesthetized, for the first 72 h after recovery from anesthesia.

Metabolic response

Oxygen consumption rates (\( \dot{V}_{O_2} \)) were recorded to measure metabolic response during digestion. Prior to each set of measurements, snakes were placed into a 300 mL jar sealed tightly with a rubber stopper with one input and one output 1/8 inch (1 inch = 25.4 mm) diameter plastic tube extending through the stopper. Once in place, snakes were allowed to acclimate to the chamber for a minimum of 30 min prior to recording measurements. Data were not collected until routine checks showed that each study animal was inactive and remained so throughout the measurements. Gas-tight Tygon™ tubing attached the output tube to a FOXBOX Field O₂ Analysis System (Sable Systems International, Inc., Las Vegas, Nevada, USA). A flow pump, built into the oxygen analyzer, maintained constant gas flow of 250 mL·min⁻¹ between the jar containing the snake and the analyzer and thus provided continuous delivery of room air to the snakes. Ex-

current air was scrubbed of water and carbon dioxide by passage through a column of drierite–ascarite–drierite (drierite: W.A. Hammond Drierite Co., Ltd., Xenia, Ohio, USA; ascarite II: Arthur H. Thomas Company, Philadelphia, Pennsylvania, USA) before delivery to the oxygen analyzer. The oxygen content of the gas exiting the jar was recorded for approximately 25 min for each snake. Immediately prior to and immediately following these measurements, oxygen content was recorded for approximately 10 min from an equal-sized empty reference jar in the same manner.

STPD-corrected (standard temperature pressure dry) oxygen levels were recorded by PC and analyzed with the Expedata version 1.0.1 (Sable Systems International, Inc.). The rate of oxygen consumption was determined by subtracting baseline values (determined as the linear extrapolation of traces from the reference jar directly before and after the trial in question). Standard equations were then used to convert raw oxygen traces into rates of oxygen consumption (Withers 1977; Lighton 2008). The oxygen consumption rates were taken as the mean value of approximately 10 min of recording from the lowest, relatively stable section of the recording.

Snakes were maintained in individual cages in a temperature controlled room for 7 days at the appropriate treatment temperature prior to commencing respirometry measurements. One group was maintained at the ambient temperature of the laboratory (22.0 ± 0.7 °C; mean ± SE) and respirometry measurements were performed at the same temperature. Another group was acclimated to 30.0 ± 0.3 °C and respirometry was performed in jars 75% immersed in a water bath maintained at 30.0 ± 0.5 °C. A thermocouple inserted into the jar was used to monitor temperature.

All snakes used in this experiment were similar in mass (12.4 ± 2.1 g, \( n = 36 \)); mass specific \( \dot{V}_{O_2} \) (mL O₂·g⁻¹·h⁻¹) was calculated for each measurement and used in all analyses. \( \dot{V}_{O_2} \) was measured daily between 0800 and 1200. During measurements made 3–6 days prior to feeding, minimum metabolic rate was determined each day as the mean \( \dot{V}_{O_2} \) value over approximately 10 min when this value was lowest and relatively stable. SMR was determined by taking the mean of minimum metabolic rate values across the 3–6 days over which data were obtained for each snake. After feeding, measurements of \( \dot{V}_{O_2} \) were continued daily between 0800 and 1200 until \( \dot{V}_{O_2} \) measurements had returned to a stable level not significantly different from SMR (8–12 days).

Assimilation efficiency

The apparent assimilation efficiency was estimated in the 22 °C treatments. Because nondigestible contents and nitrogenous components of egesta were included, apparent assimilation efficiency is reported, although it is referred to as “assimilation efficiency” henceforth. Assimilation efficiencies could not be calculated for the 30 °C treatments because of inadequate feces samples. Assimilation efficiency was calculated as \( (\text{Cal}_{\text{ingested}} - \text{Cal}_{\text{acs}}) / \text{Cal}_{\text{ingested}} \) where \( \text{Cal}_{\text{ingested}} \) is the number of the calories contained in each mouse, calculated by conducting bomb calorimetry on a representative sample of mice \( (n = 3) \) that were of similar size and age as those used in the 22 °C treatments to obtain a mean calories per gram mouse (5863 ± 31 cal·(g dry mass)⁻¹). Mice used for this estimate were humanely euthanized via CO₂ inhalation, promptly lyophilized, and sent to the Central Analytical...
Laboratory at the Department of Poultry Science, University of Arkansas, for bomb calorimetry analysis. The calories per gram for scat, Cal_{scat}, was calculated by conducting bomb calorimetry on egesta obtained from six of the snakes in the 22 °C treatments, three from the adult venom group, and three from the neonate venom group. Snakes were checked four times daily for egesta following feeding. Excrement was not observed 11 days after feeding, although snakes were checked once daily for an additional 14 days after the last excrement was collected. All egesta, including nondigestible contents, for each snake were combined and dried in an oven at 80 °C for 72 h, weighed, then ground to a fine powder with mortar and pestle (Paine 1971) and sent to the Central Analytical Laboratory at the Department of Poultry Science Laboratory, University of Arkansas, for bomb calorimetry analysis.

**Gut passage**

To assess the rate of gut passage, three snakes from each treatment group were initially selected at random to be euthanized and dissected at 1.5, 3.5, and 5.5 days following feeding. The stomach, small intestine, and large intestine were then removed. Each section was then weighed, cut open and flushed of contents, and reweighed. The difference in mass was taken as the wet mass of remaining prey in each section of the intestinal tract. This was expressed as a percentage of the original mass of the prey item (cf. Secor and Diamond 2000).

**Statistical analysis of data**

Differences in oxygen consumption rates among and within experimental treatments were assessed by utilizing Student’s t-test. Throughout the text, Student’s t values are presented with their associated P values. The effects of treatments were assessed by two-way ANOVA. Data were verified to meet assumptions of normality and equal variance. Differences were considered statistically significant at α = 0.05. All values are presented as mean ± SE. All statistical analyses were performed utilizing the statistical software package S-Plus version 7.0 (Insightful Inc., Seattle, Washington, USA).

**Results**

**Metabolic response**

SMR at 30 °C was 0.099 ± 0.015 and 0.091 ± 0.010 mL O₂·g⁻¹·h⁻¹ in snakes prior to feeding on mice injected with adult and neonate venom, respectively (Table 1). Snakes at 22 °C displayed SMR of 0.075 ± 0.012 and 0.074 ± 0.007 mL O₂·g⁻¹·h⁻¹ prior to feeding on mice injected with adult and neonate venom, respectively (Table 1). SMR did not differ significantly between snakes in the adult and neonate venom treatments at 30 °C (t_{10} = -0.621, P = 0.550) or at 22 °C (t_{10} = -0.979, P = 0.325).

In the 30 °C treatment, the VO₂ of snakes fed mice injected with adult venom peaked at 0.362 ± 0.017 mL O₂·g⁻¹·h⁻¹ approximately 24 h after feeding (Table 1, Fig. 1). The metabolic scope, defined as the maximum postprandial VO₂ divided by SMR, was 2.73 ± 0.57. Snakes at 30 °C fed mice injected with neonate venom also reached peak VO₂ at approximately 24 h after feeding at 0.267 ± 0.043 mL O₂·g⁻¹·h⁻¹ with a scope of 2.58 ± 0.52 (Table 1, Fig. 1). At this temperature, peak VO₂ in the adult venom treatment was significantly greater than the peak VO₂ in the neonate venom treatment (t_{10} = 2.647, P = 0.027), although the metabolic scopes were not significantly different (t_{10} = 0.433, P = 0.675).

VO₂ values of snakes fed mice injected with adult venom at 30 °C remained significantly greater than SMR until day 5 when postfeeding VO₂ returned to prefeeding SMR levels (t_{10} = -0.850, P = 0.420). At 5 days, postfeeding VO₂ values of snakes fed mice injected with neonate venom at 30 °C remained significantly greater than SMR (t_{10} = -2.739, P = 0.021) and significantly greater than VO₂ in the adult venom treatment on day 5 (t_{10} = -2.405, P = 0.040). VO₂ in the 30 °C neonate venom treatment was not significantly different from SMR at 6 days (t_{10} = -1.322, P = 0.216).

SMR at 22 °C was 0.075 ± 0.012 and 0.074 ± 0.007 mL O₂·g⁻¹·h⁻¹ in snakes fed mice injected with adult and neonate venom, respectively (Table 1). Peak VO₂ of snakes in the adult venom treatment was observed at 3 days at 0.196 ± 0.008 mL O₂·g⁻¹·h⁻¹, exhibiting a scope of 3.87 ± 0.88 (Table 1, Fig. 1). Snakes in the neonate venom treatment reached peak VO₂ at approximately 4 days at 0.153 ± 0.002 mL O₂·g⁻¹·h⁻¹ with a scope of 3.28 ± 0.67 (Table 1, Fig. 1). Peak VO₂ of snakes fed mice injected with adult venom treatment was significantly greater than the peak VO₂ of snakes in the neonate venom treatment (t_{10} = 2.561, P = 0.028). However, the metabolic scopes were not significantly different (t_{10} = 1.60, P = 0.144).

VO₂ in the adult venom treatment remained significantly elevated above SMR until day 7 after feeding (t_{10} = -2.011, P = 0.072). VO₂ in the neonate venom treatment was significantly greater than SMR at 8 days after feeding, (t_{10} = -2.797, P = 0.023), although it was not significantly greater than VO₂ in the adult venom treatment at 8 days (t_{10} = -0.309, P = 0.764). At 9 days after feeding, VO₂ in the 22 °C neonate venom treatment was not significantly greater than SMR (t_{10} = -1.939, P = 0.089).

**Gut passage**

Because of the limited number of animals available for these studies, we sacrificed only a few to study gut passage. Despite the consequent lack of statistical power, a consistent pattern emerged, suggesting that higher temperature resulted in higher rates of digestion, as did injection of adult venom into the mice, irrespective of temperature. At 36 h (1.5 days) after feeding, the wet mass of the stomach contents in a snake fed a mouse injected with adult venom at 30 °C was 85% of the initial mass of the mouse, whereas the wet mass of stomach contents remaining at 36 h in a snake from the 30 °C neonate venom treatment was 91% of the original mouse mass (Fig. 2). At 84 h (3.5 days), wet mass of stomach contents were 14% of the original prey mass in the adult venom treatment and 30% of the original prey mass in the neonate venom treatment. At 132 h (5.5 days), stomach contents were 1% of prey mass in the adult venom treatment and 22% in the neonate venom treatment.

Digestion of mice injected with adult venom progressed more slowly at 22 °C than at 30 °C (Fig. 2). At 36 h (1.5 days), the wet mass of the stomach contents in the adult...
Table 1. Comparisons of metabolic variables of Southern Pacific Rattlesnakes (*Crotalus oreganus helleri*) between venom and temperature treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SMR (mL O₂·g⁻¹·h⁻¹) ± SE</th>
<th>Peak $\dot{V}O_2$ (mL O₂·g⁻¹·h⁻¹) ± SE</th>
<th>Scope (peak $\dot{V}O_2$/SMR)</th>
<th>Time to peak (h)</th>
<th>Time to return to SMR (h)</th>
<th>SDA (total mL O₂) ± SE</th>
<th>Assimilation efficiency (%) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult venom at 30 °C (n = 6)</td>
<td>0.099±0.015</td>
<td>0.362±0.017</td>
<td>2.73±0.57</td>
<td>24</td>
<td>120</td>
<td>19.7±4.09</td>
<td>na</td>
</tr>
<tr>
<td>Neonate venom at 30 °C (n = 6)</td>
<td>0.091±0.010</td>
<td>0.267±0.043</td>
<td>2.58±0.52</td>
<td>24</td>
<td>144</td>
<td>15.9±4.80</td>
<td>na</td>
</tr>
<tr>
<td>Adult venom at 22 °C (n = 6)</td>
<td>0.075±0.012</td>
<td>0.196±0.008</td>
<td>3.87±0.88</td>
<td>72</td>
<td>168</td>
<td>13.5±3.08</td>
<td>92.4±1.8 (n = 3)</td>
</tr>
<tr>
<td>Neonate venom at 22 °C (n = 6)</td>
<td>0.074±0.007</td>
<td>0.153±0.002</td>
<td>3.28±0.67</td>
<td>96</td>
<td>216</td>
<td>13.4±4.3</td>
<td>92.1±0.8 (n = 3)</td>
</tr>
</tbody>
</table>

Note: Values are presented as mean ± SE. Time to peak is the number of hours from consumption of meal to peak $\dot{V}O_2$. Time to return to standard metabolic rate (SMR) is the number of hours from consumption of meal to time when $\dot{V}O_2$ was not significantly different from SMR. Specific dynamic action (SDA) is the total O₂ consumed when $\dot{V}O_2$ was significantly greater than SMR. Assimilation efficiency is the apparent assimilation efficiency calculated as (estimated calories ingested – calories excreted) / estimated calories ingested. na indicates that the assimilation efficiency was not available because it was not calculated for the 30 °C treatments.

Fig. 1. Mass-specific O₂ consumption rates at 22 and 30 °C of Southern Pacific Rattlesnakes (*Crotalus oreganus helleri*) fed laboratory mice (*Mus musculus*) injected with adult venom versus neonate venom.

venom treatment at 22 °C was 91% of the original mass of the mouse, whereas the wet mass of stomach contents in the 22 °C neonate venom treatment was 93% (Fig. 2). At 84 h (3.5 days), the wet mass of stomach contents was 86% of the original prey mass in the adult venom treatment and 91% in the neonate venom treatment. At 132 h (5.5 days), the wet mass of stomach contents was 73% in the adult venom treatment and 81% in the neonate venom treatment. However, because it was possible to euthanize and dissect only one snake per interval for each treatment, these data must be considered preliminary.

Specific dynamic action

SDA (an estimate of the total energetic costs incurred resulting from digestion and assimilation) was quantified for each treatment as total oxygen consumption above SMR during the period of digestion when $\dot{V}O_2$ was significantly greater than SMR (calculated as the area under curve for the period during which significantly elevated metabolic rates were observed in Fig. 1). A two-way ANOVA was performed to test for differences in SDA as a result of treatment (adult venom versus neonate venom) and temperature (22 versus 30 °C) effects. Temperature had a significant effect on SDA.
Assimilation efficiency

Mice contained, on average, 5863 ± 31 calories·(g dry mass)⁻¹ (n = 3), resulting in an estimated 8445 ± 766 total calories per mouse used in the 22 °C treatments. Scat samples contained, on average, 3588 ± 679 calories·(g dry mass)⁻¹ (n = 6) for 651 ± 88 total calories in the egesta materials collected. Snakes in the adult venom treatment at 22 °C had an estimated assimilation efficiency of 92.4% ± 1.8% (Table 1). Snakes in the neonate venom treatment at 22 °C had an estimated assimilation efficiency of 92.1% ± 0.8% (Table 1). There was no significant difference in SDA as a result of venom treatment (F₁,20 = 1.282, P = 0.271).

Discussion

Assimilation efficiency

The lack of “digestive experience” does not appear to adversely affect the assimilation efficiencies of neonatal C. o. helleri. Snakes in the adult venom treatment at 22 °C had an estimated assimilation efficiency of 92.4%, whereas those in the neonate venom treatment at 22 °C had an estimated assimilation efficiency of 92.1% (Table 1). These values are substantially greater than those reported by McCue (2007), who found an apparent assimilation efficiency of 79.1% in adult C. atrox, and Chu et al. (2009), who observed a mean value of 85.5% in adult Taiwan Mountain Pitviper (Trimeresurus gracilis) and 80.0% in adult Bamboo viper (Trimeresurus stejnegeri stejnegeri) Schmidt, 1925 (Table 2). McCue (2007) and Chu et al. (2009) used smaller prey items (10% of snake mass) and maintained the study animals at different temperatures (30 and 14 °C, respectively); however, meal size has not been shown to affect digestive efficiency in snakes (Smith 1976; Bedford and Christian 2000), whereas higher ambient temperature has been shown to have either no effect on (Bedford and Christian 2000) or to slightly increase (Greenwald and Kanter 1979) assimilation efficiency in snakes. Our results are within the range of assimilation efficiencies of 89%–98% measured in various species of Australian pythons (Bedford and Christian 2000). However, an additional consideration in our study is the potential influence of indigestible components (i.e., hair). Our snakes were fed young mice that had substantially less hair than adult mice used in other studies; this may have resulted in greater assimilation efficiencies.

SDA and scope

The scope of the peak SDA response (defined as peak VO₂ during digestion divided by mass-specific SMR) seen in neonate C. o. helleri at 30 °C was generally lower than that seen in other rattlesnakes under similar conditions ingesting similarly large meals relative to body mass (Table 3). The scopes we measured are similar to values reported in adult snakes fed meals less than one-third as large relative to body mass. This is contrary to what might be expected, given that the scope of peak SDA generally increases with increasing relative meal size (Andrade et al. 1997; McCue 2006; Secor 2008a), and given that Secor (1995) found that juvenile P. malarus ingesting their first meal exhibit similar metabolic responses during digestion as adults of the same species. It is possible that our data collection frequency (one period of measurement per snake per day) was too low to capture the peak SDA response for most individuals. This would have resulted in systematic underestimation of the scope of peak SDA and absolute SDA values. However, the low scope may simply be due to higher SMR values than those measured in other studies that typically involve adult snakes. The significant cost of growth and development in neonatal reptiles might be expected to result in metabolic rates significantly above those predicted by scaling equations based largely on data from adults (reviewed in Nagy 2000). Beaupre and Zaidan (2001) reported rates of CO₂ production in neonatal Timber Rattlesnakes (Crotalus horridus L., 1758) that were 2–4 times greater than values predicted by scaling equations derived from data obtained from adults of the same species. Equations relating metabolic rate (as VO₂ or VC O₂) to body mass and temperature have been derived for a handful of rattlesnake species (Rock Rattlesnake, Crotalus lepidus; Kennicott, 1861, and Black-tailed Rattlesnake, Crotalus molossus; Baird and Girard, 1853; Beaupre 1993; C. atrox: Beaupre and Duvall 1998; C. horridus: Beaupre and Zaidan 2001; Sidewinder, Crotalus cerastes Hallowell, 1854; Secor et al. 1994; Secor and Diamond 2000). VO₂ values recorded in resting juvenile C. o. helleri in this study are not significantly greater than the those predicted by each of these scaling equations. On the balance of these data, we cannot conclude that SMR in the snakes in our study were unusually elevated.
with the proteolytic enzyme-rich adult venom. Nonetheless, this slightly more rapid digestive progression did not result in significant energetic benefit to the snakes at either temperature in the form of either lower SDA or greater assimilation efficiency. McCue (2007) suggested that the lack of any observed effect on assimilation efficiency in C. atrox may have been due to the relatively small prey mass (10%) used in his experiments or the methodology of injecting venom into dead, rather than live, mice which may limit venom dispersal (Mackessy 1988). Likewise, Chu et al. (2009) used relatively small prey (10%) to test for differences associated with venom types. In our study, mice were 35% of the mass of each snake and were injected with venom while alive; thus, snakes received a relatively large meal that contained venom in circulation. Despite this, there was no difference in assimilation efficiency between venom treatments. Although further work is certainly warranted, our results do not support the hypothesis that proteolytic components of venom enhance the digestion of ingested prey. Although digestion of prey injected with adult venom containing proteolytic components

Effects of venom type

The finding that venom type had no effect on SDA is consistent with other studies that examined the effects of venom on SDA in Crotalinae (Mendes and Abe 1999; McCue 2007; Chu et al. 2009). These results are interesting, given the partial “predigestion” of ingested prey by proteolytic components of venom (Thomas and Pough 1979; Nicholson et al. 2006). In agreement with hypotheses regarding the benefits of partial predigestion (Thomas and Pough 1979; Mackessy 1988; Nicholson et al. 2006), we found that digestion progressed slightly faster in snakes that ingested mice injected

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<th>Table 3. Comparisons of published studies of metabolism during digestion in Crotalinae species conducted at 30 °C.</th>
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<td><strong>Treatment</strong></td>
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<tr>
<td><strong>Western Diamondback Rattlesnake (Crotalus atrox)</strong></td>
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<td><strong>Sidewinder (Crotalus cerastes)</strong></td>
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<td><strong>South American Rattlesnake (Crotalus durissus L., 1758)</strong></td>
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Note: All results reported here are from metabolic studies conducted at 30 °C. Values are presented as mean ± SE, or when SE was not reported, with value ranges in parentheses.
seems to have progressed slightly faster than digestion of prey injected with neonate venom, the putrefaction of gut contents did not appear to be a significant danger for our animals, as not one snake regurgitated its meal.

Factors other than enhancement of digestion may play more significant roles in shaping the evolution and ontogeny of neonatal venom (Daltry et al. 1996; Andrade and Abe 1999; Barlow et al. 2009). For instance, venom components that rapidly immobilize prey may be more important to the survival of neonates than those that accelerate digestion. Neonatal C. o. helleri from Santa Barbara County show high levels of the neurotoxin phospholipase A₂ that decline dramatically as snakes increase in size (Mackessy 1985, 1988). Along with this ontogenetic shift in venom components is an apparent shift in preferred prey. Neonatal and juvenile C. o. helleri less than 500 mm in total length from Santa Barbara County prefer lizard prey, as evidenced by gut content analysis in museum specimens (Mackessy 1988) and behavioral experiments showing innate prey preference (LaBonte 2008). Above this size, and concurrent with the shift in venom makeup, mammals become the exclusive prey type (Mackessy 1988). Neurotoxin-rich neonatal venom immobilizes lizard prey (Sagebrush Lizard, Sceloporus gracilus Baird and Girard, 1852) more quickly than adult venom (Mackessy 1985, 1988), thus limiting the distance that the prey can move after envenomation and increasing the likelihood of successful prey location and ingestion. Successful foraging soon after birth may be extremely important to survival of neonates during their first year. Birth in C. o. helleri occurs in the late summer and early fall, which provides little time to acquire a meal before the onset of winter conditions when thermal conditions preclude foraging.

In adult Ball Pythons (Python regius (Shaw, 1802)), the use of stable carbon isotope tracking techniques revealed a progressive increase in reliance on exogenous (prey-derived) fuels beginning as soon as 4 h after feeding (Waas et al. 2010). Thus, prey-derived metabolic fuels can rapidly be used to substitute for endogenous fuel reserves. The application of techniques like those used by Waas et al. (2010) would shed light on possible interspecific differences in ontogenetic shifts in exogenous fuel use in infrequently foraging snakes.

Acknowledgements

S. Secor, W. Hayes, and S. Beaupre provided helpful comments and suggestions during the early stages of this research. We thank M. Ball and K. LaBonte for assistance with fieldwork and S. Sweet for logistical and bureaucratic support.

References


