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Meal size effects on the postprandial metabolic response of *Bothrops alternatus* (Serpentes: Viperidae)

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ABSTRACT. We examined the effects of meal size on the postprandial metabolic response of the lancehead *Bothrops alternatus* (Duméril, Bibron & Duméril, 1894), fed mice equaling to 5, 10, 20, and 40% of the snake’s body mass. The maximum O_2 consumption rates measured during digestion increased with meal size, reaching levels up to 2.8-7.8-fold higher than the metabolic rate measured during fasting. Specific Dynamic Action (SDA) duration also increased with meal size, lasting from 54 to 212 hours to complete. Under our experimental conditions, 30°C, the majority of our snakes failed to completely digest prey with a relative size of 40%. The SDA coefficient ranged from 17 to 27% of the energy content of the meal and was not affected by meal size.

KEY WORDS. Metabolism; postprandial; prey size; snakes.

Food intake is followed by an increase in metabolic rate, commonly referred to as specific dynamic action, SDA (Kleiber 1961), which have been documented in all major animal clades (see Secor 2009). This postprandial metabolic response depends primarily on the size and nature of the meal (McCue *et al.* 2002, Toledo *et al.* 2003) associated with factors such as body size and environmental temperature (Luo & Xie 2008). Among these factors, meal size is known to influence the maximum rates of metabolism attained during digestion, the time to reach these peak values, the duration, and the energetics of the digestive process (Jobling & Davies 1980, Andrade *et al.* 1997, Secor & Diamond 1997a, Secor & Faulkner 2002). Despite its universal distribution, the magnitude of the SDA response can vary widely among different animal groups. For instance, in sit-and-wait snake species that experience long periods of fasting occasionally interrupted by the ingestion of large prey which, in extreme cases, may even exceed their own body mass (Greene 1992, 1997, Secor & Diamond 1997a), the SDA response is especially remarkable (Tattersall *et al.* 2004, McCue 2006).

According to Greene (1997), since its origin in the Cretaceous Period, the snakes have experienced a high degree of adaptive radiation. Changes in feeding behavior have been treated as key elements in the evolutionary success of this group and some studies suggest adaptive advantages associated with capture of relatively large prey (Greene 1983). One of these advantages could be expected to involve energetic considerations since the cost of meal digestion can be quite substantial in snakes accounting for up to 33% of the caloric content of the meal (Secor 2009), and over to 43% of the total energy budget under natural conditions (Secor & Nagy 1994). However, the relative cost of meal digestion (i.e.,% of meal energy allocated for digestion) seems to be unaffected by its size (Andrade *et al.* 1997, Toledo *et al.* 2003) or, in some cases, to be greater for larger meals (Secor & Diamond 1997a, b, McCue & Lillywhite 2002). Whether this pattern is affected by differences in feeding habits still deserves further examination, since the effects of prey size on SDA have been quantified only in a limited number of snake species. Hence, herein we extended such an investigation to the crossed pitviper *Bothrops alternatus* (Duméril, Bibron & Duméril, 1894), a Neotropical Viperid snake known for its ability to feed upon a broad range of prey sizes. More particularly, we were interested in verify whether an energetic advantage (or of any other nature) could be associated to the digestion of larger prey items. On the other hand, we sought to identify whether the digestion of larger prey would impose functional constraints, specially associated to the transport of respiratory gases, since the SDA response on snakes is almost entirely aerobically supported (Wang *et al.* 2001).

MATERIAL AND METHODS

We used six juveniles (both sexes) of *B. alternatus*, born in captivity from pregnant females collected at different localities in the State of São Paulo, Brazil. *Bothrops alternatus* has a terrestrial habit, occupying mainly humid open fields from center-west of Brazil to central Argentina (Melgarejo 2003, Campbell & Lamar 2004). This mammal specialist species is considered a sit-and-wait forager, whose feeding behavior is characterized by an infrequent ingestion of ambushed prey with a wide range of sizes (Greene 1992, 1997, Martins *et al.* 2002).

Animals were kept individually in wooden boxes with glass front and side holes for ventilation (30 x 29 x 27 cm), lined with corrugated cardboard, kept in a temperature-controlled room at approximately 25°C (±5°C). Snakes were fed on mice, *Mus musculus* Linnaeus, 1758, and water was offered *ad libitum*. The experiments were conducted under permission of the "Animal Use Ethic Committee", CEUA (protocol number 4587, July 22th 2009), from the Instituto de Biociências, Universidade Estadual Paulista.

To investigate the meal size effect on the specific dynamic action, the metabolic rate of *B. alternatus* was analyzed before (fasting) and after the snakes being fed with a single mouse with relative mass equivalent to 5, 10, 20, or 40% of the snake's own body mass, hereafter referred as G5%, G10%, G20%, and G40%, respectively. Each snake was measured once for each and every meal size treatment in a randomized order. Metabolic rates were measured by using closed system respirometry as detailed below.

Resting metabolic rate (RMR) of fasting animals was analyzed over a period of 24 hours. After that, the snakes were removed from the respirometer and returned to the maintenance box where a live mouse of appropriate mass was offered. After spontaneous capture and ingestion of prey, snakes were immediately brought back to the respirometer. From this moment, $\dot{V}O_2$ was monitored continuously until values returned to levels of RMR, thereby indicating the end of digestion.

To quantify the oxygen consumption rates an automated respirometry was used. Initially, snakes were placed in hermetically sealed respirometric chambers with a volume appropriate to the size of snakes (~700 ml). By using the program DATACAN V (Sable Systems), we programmed a multiple flow controller (Multiplexer TR-RM8, Sable Systems) to acquire one measurement of $\dot{V}O_2$ for each snake every 70 minutes. The system was set for ventilating the animal chambers with outside air for 60 minutes periods, in order to renew the air contained in the respirometers (open phase). Open phase periods were interspaced by 10 minutes periods in which the external ventilation was interrupted and the air contained in the respirometer was recirculated (closed phase) through an oxygen analyzer (Sable Systems, PA-1B), connected to a data acquisition system (DAC, Sable Systems). The slopes of the decrement in oxygen fractional concentration, recorded during the closed phase periods, were used for $\dot{V}O_2$ calculations. To control the experimental temperature, snakes were kept in a climatic chamber BOD (Fanem, 347CD, and Eletrolab, 122FC) with temperature set to 30°C, being periodically checked with a maximum and minimum thermometer, accepting a variation of ±1°C.

RMR was estimated as the average value of $\dot{V}O_2$ recorded during the fasted period. The maximal oxygen consumption during digestion was defined as $\dot{V}O_{2peak}$, while T_{peak} was taken as the time required to reach this level. SDA duration was determined as the time required for the lower limit of the 95% confidence interval of the postprandial period to overlap with the upper limit of the 95% confidence interval calculated during the RMR (see Andrade *et al.* 1997). $\dot{V}O_{2peak}$, T_{peak}, and SDA duration were all extracted from a fourth-order polynomial curve fitted to the temporal variation of $\dot{V}O_2$ using TableCurve 2D (Systat Software). The factorial metabolic increment was calculated as the ratio between $\dot{V}O_{2peak}$ and RMR. Meal energy content (ME) was calculated in kJ based on Cox & Secor (2007). Energetics cost of digestion (E_{SDA}) was estimated by removing the maintenance cost, estimated on the basis of the RMR, from the total amount of oxygen consumed by snakes during SDA, and considering that 0.0198 kJ is expended for each 1 mL of oxygen used in aerobic metabolism (Gessman & Nagy 1988). The SDA coefficient was calculated as the percentage of energy spent on digestion (E_{SDA}) in relation to the total meal energy ingested by the snakes (ME).

To analyze the effects of prey size on the different parameters associated with the SDA we used ANOVA test for Repeated Measures (Oneway) followed, whenever necessary, by a pairwise multiple comparison procedure (Holm-Sidak method). The premises of normality and/or homoscedasticity of variances of the data were checked before running the statistical tests. All data are presented as mean ± standard error. The level of significance was set at $p < 0.05$.

RESULTS

The mass of the snakes ($F_{3,15} = 0.45$, $p = 0.722$), as well as the RMR ($F_{3,15} = 1.27$, $p < 0.321$), did not differ among experimental groups presenting an average of 153.33 ± 3.58 g (Table I).

The postprandial metabolic increase of *B. alternatus*, regardless of meal size, was characterized by rapid initial increase in oxygen uptake after ingestion to reach a peak ($\dot{V}O_{2peak}$), followed by a gradual return to the levels recorded during the pre-feeding period (RMR) (Fig. 1). However, even thought all snakes in G40% were able to digest their prey for long enough to permit the identification of $\dot{V}O_{2peak}$, only one individual was able to fully complete the process. All the other snakes in this group regurgitated their meal within 62.02 ± 6.16 hours post feeding (see Fig. 1). For this reason, we were unable to perform any statistical analyzes for SDA duration, E_{SDA}, and SDA coefficient for the G40% group.

The values for maximum oxygen uptake during SDA ($F_{3,15} = 121.21$, $p < 0.001$) and the time taken to reach $\dot{V}O_{2peak}$ ($F_{3,15} = 45.82$, $p < 0.001$) were significantly higher as the meal mass increased (see Table I). The factorial increment of metabolic rate ($F_{3,15} = 33.39$, $p < 0.001$) and the duration of the digestion ($F_{2,10} = 103.86$, $p < 0.001$) also increased with prey size (Table I). The single individual of *B. alternatus* that was able to fully digest its prey equaling 40% of its body mass spent 212.33 hours (ca. 9 days) to complete digestion (Fig. 1, Table I). Both meal energy ($F_{3,15} = 580593.70$, $p < 0.001$) and E_{SDA} ($F_{2,10} = 138.04$, $p < 0.001$) were significantly higher with the increase in prey size (Table I), while the SDA coefficient did not differ statistically among treatments (($F_{2,10} = 2.05$, $p = 0.179$).

Table I. Metabolic and energetic parameters associated with the SDA of *Bothrops alternatus* fed on meals with different relative sizes (G5% to G40%) at 30°C (mean values ± SE). RMR, resting metabolic rate; $\dot{V}O_{2peak}$, maximum rate of oxygen uptake during digestion; T_{peak}, time taken to reach $\dot{V}O_{2peak}$; E_{SDA}, energetic cost of digestion; ME, energy content of the meal; SDA coefficient, percentage of the energy expended in SDA (E_{SDA}) in relation to meal energy (ME). N = 6 in all cases except for Duration, E_{SDA}, and SDA coefficient in the G40% (n = 1, see text for details). Groups identified with an asterisk are statistically different to each other.

Relative prey size	G5%	G10%	G20%	G40%
Body mass (g)	153.50 ± 7.64	151.18 ± 6.43	153.39 ± 8.23	155.25 ± 8.20
RMR (mL O_2 kg^{-1} h^{-1})	51.98 ± 3.78	47.59 ± 3.23	48.12 ± 8.10	54.00 ± 3.00
$\dot{V}O_{2peak}$ (mL O_2 kg^{-1} h^{-1})	143.13 ± 5.43*	213.96 ± 13.27*	297.22 ± 12.37*	410.35 ± 13.48*
T_{peak} (h)	14.68 ± 1.01*	18.24 ± 1.20*	25.30 ± 0.98*	38.10 ± 2.33*
Factorial increase (VO_{2peak}/RMR)	2.80 ± 0.15*	4.56 ± 0.31*	6.25 ± 0.27*	7.78 ± 0.68*
Duration (h)	54.09 ± 1.76*	70.19 ± 4.38*	106.60 ± 4.03*	212.33
E_{SDA} (kJ kg^{-1})	58.04 ± 3.45*	153.02 ± 15.61*	332.75 ± 18.34*	892.66
ME (kJ kg^{-1})	336.87 ± 3.51*	756.19 ± 3.18*	1,589.66 ± 4.05*	326.51 ± 4.73*
SDA coefficient (%)	17.22 ± 0.99	20.24 ± 2.09	20.93 ± 1.16	27.33

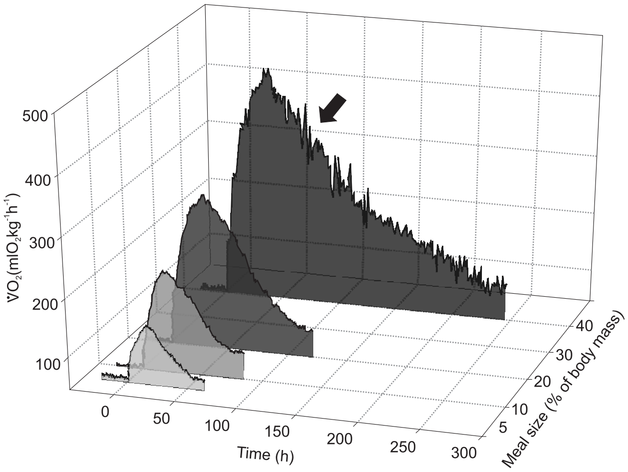

Figure 1. Time course variation in mean oxygen consumption rates ($\dot{V}O_2$) during the digestion of *Bothrops alternatus* fed on meals with different relative sizes at 30°C. Time zero indicates the moment of meal ingestion. The arrow in G40% indicates the averaged time in which all but one snakes in this group regurgitated their prey. N = 6 in all cases, except for G40% after the arrow, n = 1. SE not shown for clarity.

DISCUSSION

The postprandial metabolic response of *B. alternatus* was characterized by a rapid increase in the rates of O_2 consumption after meal ingestion reaching maximum values between 14 and 38 hours post feeding, with no plateau formation. Then, $\dot{V}O_2$ decreased slowly over several days before returning to the metabolic levels recorded before feeding. Such a pattern was not affected by meal size and is congruent with the general response commonly reported for snakes (see Andrade *et al.* 2005).

Meal size affected virtually all SDA parameters in *B. alternatus*. The maximum rates of oxygen consumption during digestion increased with prey size, which resulted in a proportional increase of factorial increment. This scope reached maximum values up to almost eightfold above the resting metabolic rate, which is consistent with the figure found for other snake species studied under similar conditions (Wang *et al.* 2001, Andrade *et al.* 2005, McCue 2006). For *B. alternatus*, the factorial increase in metabolic rate as a function of meal size rose almost linearly from 5 to 20% but, from 20 to 40%, this increment appeared asymptotic (see Fig. 2). In other words, as relative meal size was increased from 20% to 40%, a less than proportional increase in the maximum rates of metabolism followed. Therefore, we suspect that *B. alternatus* digesting prey with relative size above 20%, at 30°C, experiences rates of metabolism probably approaching the maximum capacity of the snakes' cardiorespiratory system. Since the lowering of body temperature is accompanied by a decrease in the maximum

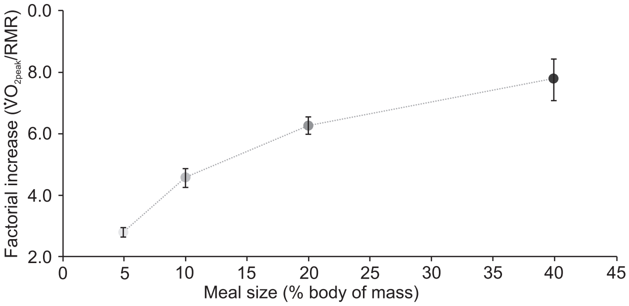

Figure 2. Factorial increment of the postprandial metabolism in relation to the rates measured during fasting (RMR) for *Bothrops alternatus* (mean values ± SE, n = 6) fed with rodent prey of different relative sizes.

metabolic rates measured during digestion (Hailey & Davies 1987, Wang *et al.* 2003, Zaidan & Beaupre 2003), the reaching of this meal-size metabolic ceiling during digestion is probably modulated by temperature (Toledo *et al.* 2003).

One of the most impressive features of Macrostomata snakes is the ability to feed on large prey (Gans 1983, Greene 1983, Pough 1983), often exceeding 30% of their own body mass (see Secor 2009). The ingestion of large meals, however, imposes important physiological challenges. For example, the concurrent decrease in the surface-to-volume ratio following the increase in meal size, may impair the efficiency of digestive enzymes (Pough & Grooves 1983) and lead to a longer SDA duration (Andrade *et al.* 1997). Even more importantly is the fact that once swallowed, the prey must be processed before the intestinal flora causes it to putrefy (Pough *et al.* 2003), which may also explain why most *B. alternatus* failed in completing the digestion of prey with relative mass of 40%. As discussed above, snakes fed on such large prey were unable to increase their maximum postprandial metabolic rates proportionally (Fig. 2) which, perhaps, compromised the early stages of digestion. As a consequence, meal putrefaction may have proceeded faster than enzymatic digestion, ultimately leading to the regurgitation of the meal, since that have occurred almost 24 hours after snakes reaching $\dot{V}O_{2peak}$ (Fig. 1). Indeed, all regurgitated mice were extremely bloated, indicating that fermentation caused by bacterial action was occurring within the meal, inside the snakes' stomach (Anderson & VanLaerhoven 1996).

As the cost of meal digestion varied proportionally with the energy content of the meal, the relative cost of meal digestion (i.e., SDA coefficient) was not affected by meal size. Thus, the ingestion of a large or small prey accounted for the same relative energetic investment, which seems to be in conflict with the evolutionary scenario proposed by Greene (1983) for the evolution of feeding habits in some snake groups. However, in absolute terms, larger prey still provides an energetic return substantially greater than smaller prey. In fact, whereas the difference in the absolute cost of digestion between G20% compared with G5% represents an additional investment of only 95 $kJ.kg^{-1}$, the absolute energy return obtained from a 20% prey is 1,257 $kJ.kg^{-1}$ greater than that provided by the ingestion of a 5% prey (see Table I). Therefore, for a sit-and-wait snake species, the acquisition of a single large meal may still result in important energetic advantage compared to a small meal item, even if they have the same relative cost. Furthermore, the successful capture of a large prey item may result in important ecological advantages related to survival, growth, and allocation of time and energy to different activities (Greene 1983).

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