白条草蜥(*Takydromus wolteri*)胚胎生长及物质和能量的动用

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摘要:研究白条草蜥卵在温、湿度分别为 30℃、-12kPa 的条件下,孵化过程中胚胎生长以及对物质和能量的动用。孵化过程中,每隔 5d 测定卵重。孵化第 10、15、20 天,分别解剖来自不同窝的卵 15、15、12 枚,分离为胚胎、卵黄和卵壳。初生幼体测量、称重后冰冻处死,随后解剖分离为躯干、剩余卵黄和腹脂肪体。所有材料 65℃恒温干燥,用索氏脂肪提取仪测定脂肪含量,氧弹式热量计测定能量含量,马福炉测定无机物含量。白条草蜥卵的平均孵化期为 24.7d。卵孵化时从基质中吸水导致重量增加。卵孵化 0~15d、15~20d、20~24.7d,胚胎分别利用新生卵能量的 11%、14%和 75%。0~20d,胚胎生长缓慢;20d 后生长迅速。卵孵化过程中,干重、非极性脂肪和能量的转化率分别为 50.3%、24.9%和 51.9%。初生幼体的能量组分为:躯干 95.2%,脂肪 2.2%,剩余卵黄 2.6%。胚胎发育所需要的无机物来自卵黄和卵壳。结果显示,白条草蜥从卵到孵出幼体物质和能量较低的转化率主要与较高胚胎发育投资和较小的剩余卵黄有关。

关键词:白条草蜥;卵;孵化;胚胎生长;物质和能量动用
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Embryonic growth and mobilization of material and energy in oviposited eggs of the white-striped grass lizards *Takydromus wolteri*

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Abstract: We incubated *Takydromus wolteri* eggs at 30℃ using wet vermiculite as the incubation substrate of which the moisture was kept at -12kPa, we paid particular attention to the embryonic growth and embryonic use of material and energy during incubation. Eggs were weighed at 5-day intervals to test for temporal changes in egg mass. From the tenth, fifteenth, twentieth day of incubation, we randomly selected 15, 15, 12 eggs from different clutches respectively, opened and separated them into shell, embryo and yolk. The three egg components were oven dried to constant mass at 65℃, weighed and preserved frozen for later determination of composition. Upon emergence, size and mass were measured on each hatchling. Hatchlings ($n = 29$) were then killed by freezing to -15℃ for later study. Upon thawing, we separated each hatchling into weighed and preserved frozen for later determination of composition. We extracted non-polar lipids from dried carcass, residual yolk and fat bodies. The three hatchling components were oven dried to constant mass at 65℃, samples in a Soxhlet apparatus for a minimum of 5.5 h using absolute ether as solvent. The amount of lipids in a sample was

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determined by subtracting the lipid-free dry mass from the total sample dry mass. The total lipid in each hatchling was calculated as the sum of the lipids in its carcass, residual yolk and fat bodies. We determined energy content of dried samples using an adiabatic bomb calorimeter and ash content in each sample using a muffle furnace at 700℃ for a minimum of 8 h and then weighing the remaining ash. The duration of incubation averaged 24.7 days in this study. Eggs increased in wet mass during incubation due to the absorption of water from the substrate. At the stage of 0－15, 15－20, 20－24.7 days of incubation, the developing embryos mobilized approximately 11%, 14% and 75% of the total egg energy in the yolk of the freshly laid egg, respectively. Embryos grew slowly during the first 20 days, the maximal embryonic growth occurred at the stage of 20－24.7 days of incubation. During incubation, approximately 50.3% dry material, 24.9% non-polar lipids and 51.9% energy in egg contents of the freshly laid egg were transferred to the hatchling. Of all energy in the newly emerged hatchling, 95.2% was in the carcass, 2.2% in fat bodies and 2.6 % in the residual yolk. Embryos used both yolk and eggshell as the sources of inorganic material for development. Our data show that the lower conversion efficiencies of energy and material from egg to hatchling in *T. wolteri* can be mainly attributed to their greater energetic costs of embryonic development and lower residual yolk sizes.

Key Words: *Takydromus wolteri*; egg; incubation; embryonic growth; mobilization of material and energy

爬行动物的繁殖投入主要以在卵黄沉积期向卵内分配物质和能量的形式体现。卵生爬行动物卵内储存的物质和能量一部分用于形成一个完整的幼体,另一部分以剩余卵黄和脂肪体的形式存在于初生幼体体内,用于早期的活动、维持和生长[1~5]。大多数卵生有鳞类爬行动物胚胎发育包括两个时期:第 1 时期是从排受精卵到产卵前;尽管这一时期的胚胎发育情况存在种间差异,但相对于卵内卵黄的能量而言,胚胎所包含的能量可以忽略[6~10];第 2 时期是从产卵到幼体孵出;由于卵产出时胚胎分化程度低、对卵内物质和能量的利用少,第 2 时期卵内物质和能量的转化几乎相当于整个胚胎发育过程物质和能量的转化[3,5,10,11]。

已有研究表明:在卵孵化过程中,胚胎发育的前半段时期胚胎生长十分缓慢,胚胎对卵内物质和能量的利用主要集中在后半段时期[5,11~13]。爬行动物卵内物质和能量的转化与孵化环境、胚胎发育能耗、幼体内剩余卵黄和脂肪体大小密切相关[2~4,10,14]。因而,在同一条件下研究胚胎发育过程中卵内物质和能量利用的动态,并与已有研究结果进行比较分析,将有助于解释爬行动物卵内繁殖投入以及胚胎物质和能量利用的一般模式。

白条草蜥(*Takydromus wolteri*)是年产多窝卵的小型昼行性蜥蜴,分布于江苏、安徽、江西,向西分布至湖北和四川东部,向北分布至东北诸省及朝鲜和俄罗斯(Southern Primorskiy Territory)[15],安徽境内仅分布于滁州琅琊山和当涂[16]。由于该种卵产出时胚胎分化程度低,根据 Zehr[17] 的经典文献,白条草蜥产出时的胚胎在 25 ~26 期,因此是研究卵孵化过程胚胎物质和能量利用动态较为理想的动物。本文通过研究白条草蜥胚胎发育过程中物质和能量利用动态,旨在为爬行动物卵内繁殖投入以及胚胎物质和能量利用理论提供新的证据。

1　材料和方法

研究用白条草蜥亲体于 2005 年 4 月上旬捕自安徽滁州琅琊山,补充材料于 2007 年 4 月捕自同一地理种群。捕捉的蜥蜴带回滁州学院实验室,经测量、称重和鉴定性别后被关养在蜥蜴专用玻璃缸(长 × 宽 × 高 = 1000mm ×800mm ×500mm)内,动物能在缸内自由取食黄粉虫幼虫(larvae of *Tenebrio molitor*),接受自然光照。预先在玻璃缸的顶部安装 100W 灯泡,光照周期为 14L:10D,0060h 灯泡自动开启,使缸内形成从室温到 55℃的温度梯度,动物自由选择最适活动温度。定期在蜥蜴饮水中添加爬行动物专用复合维生素和儿童钙粉,确保动物全面的营养需求。定期触摸判断雌体的怀卵状态,将怀输卵管卵的雌体单个关养在有潮湿沙质基质的产卵缸(长 × 宽 × 高 =300mm ×150mm ×250mm)内。每隔两小时检查蜥蜴的产卵情况,确保所有卵在产后被及时收集。对所有卵进行测量、称重和编号。随机从 15 窝新生卵随机取 1 枚,分离成卵壳和卵内容物,剩余一部分新生卵被移入内含孵化基质的塑料盒中,孵化基质的湿度设置为 －12kPa,另一部分新生卵被移入预先

设置好不同温湿度的塑料盒中(温度:27、30、33℃;湿度:-220、0kPa),用于研究水热环境对孵出幼体特征的影响。孵化盒用穿孔的塑料薄膜覆盖,放置在温度设置为(30±0.5)℃的生化培养箱内。卵的1/3埋于基质中。定期向孵化盒内加水,以保持基质湿度的恒定;每日调整孵化盒的位置以减少相互间的温差。当卵孵化到第10、15、20天时,随机从不同窝卵中分别取15、15、12枚,将其分离为胚胎、卵黄和卵壳。卵壳用清水冲洗、纸巾吸干后,称出湿重。卵内容物、卵黄和胚胎分别移入已知重量的玻璃皿内,称出重量。然后,在65℃烘箱中干燥至恒重,称出干重。称出每隔5d孵化卵的重量,直至幼体孵出。共孵出29条幼体,幼体出壳后的1h内被冷冻。

冷冻幼体以后经解冻,解剖分离成躯干、剩余卵黄和脂肪体,分别在65℃烘箱中干燥至恒重,称出干重。卵内容物、胚胎、卵黄和幼体中的非极性脂肪用索氏脂肪提取器在55℃条件下抽提脂肪,分析纯乙醚作抽提溶剂。上述物质的能量用HR-15型弹式氧弹仪(长沙高教仪器厂)测定。灰分含量用马福炉在700℃条件下焚烧8h测得。

卵孵化过程中,死亡胚胎对应的数据不用于统计分析。所有数据在作参数统计分析前,分别检验其正态性(kolmogorov-smirnov test)和方差同质性(f-max test)。经检验,部分原始数据须经ln转化后能用于参数统计分析。用线性回归、单因素方差分析(one-factor ANVOA)以及单因素协方差分析(one-factor ANCVOA)处理和比较相应的数据。描述性统计值用平均值±标准误表示,显著性水平设置为$\alpha = 0.05$。

2 结果

在30℃、-12kPa条件下,白条草蜥卵的平均孵化期为(24.7±0.1)d(23.7~26.1,$n = 29$)。卵孵化时从基质中吸水导致重量增加($F_{4,140} = 652.74, P < 0.0001$),孵化第20、15、10、5天的卵重分别是新生卵的4.7、3.7、2.6、1.6倍。

图1显示,白条草蜥卵孵化过程中,卵黄重量和能量随孵化时间增加而减少(重量:$F_{4,80} = 161.53, P < 0.0001$;能量:$F_{4,80} = 167.51, P < 0.0001$),胚胎重量和能量随时间增加而增加(重量:$F_{3,66} = 1544.77, P < 0.0001$;能量:$F_{3,66} = 1462.65, P < 0.0001$)。孵化最初15d,胚胎仅动用卵内约11%的能量;孵化第15~20天,胚胎动用卵内约14%的能量;孵化第20天到幼体孵出时,胚胎动用卵内约75%的能量。卵黄非极性脂肪随孵化时间的增加而减少($F_{4,80} = 140.62, P < 0.0001$),胚胎非极性脂肪随孵化时间的增加而增加($F_{3,66} = 592.28, P < 0.0001$)。卵黄灰分随孵化时间的增加而减少($F_{4,80} = 112.86, P < 0.0001$),胚胎灰分随孵化时间的增加而增加($F_{3,66} = 3201.14, P < 0.0001$)。卵孵化第10、15、20天,胚胎干重分别为初生幼体的10.9%、24.8%、60.3%。

比较本研究白条草蜥新生卵和初生幼体的成分发现:初生幼体的干重、非极性脂肪含量、能量均低于新生卵内容物;幼体灰分含量则高于新生卵内容物;孵化后卵壳的干重和灰分含量明显低于新生卵卵壳(表1)。卵的干重、非极性脂肪和能量转化到初生幼体内的比率分别为50.3%、24.8%、51.9%;非极性脂肪在幼体内的分配为躯干83.4%、脂肪体4.5%、剩余卵黄12.1%;能量在幼体内的分配为:躯干95.2%、脂肪体2.2%、剩余卵黄2.6%。初生幼体剩余卵黄干重占新生卵内容物干重的2.9%,占幼体干重的3.2%。

表1 白条草蜥新生卵和已孵卵成分比较

Table 1 Composition of freshly laid and hatched eggs of *T. wolteri*

项目 Item	新生卵 (n=15) Freshly laid egg		已孵卵 (n=29) Hatched egg		$F_{1,41}$	
	卵内容物 Egg contents	卵壳 Egg shell	初生幼体 Hatchling	卵壳 Egg shell		
湿重 Wet mass(mg)	187.90±1.0		267.5±3.4		262.33****	
干重 Dry mass(mg)	97.10±2.3	12.6±0.3	46.8±0.6	11.7±0.2	752.80****	5.25*
非极性脂肪 Non-polar lipid mass (mg)	28.90±0.8		7.2±0.1		541.55****	
能量 Energy content(kJ)	2.62±0.06		1.36±0.01		60.07****	
灰分 Ash mass(mg)	3.30±0.1	1.7±0.1	3.9±0.1	1.3±0.1	54.07****	4.58*

数据用矫正平均值±标准误表示,新生卵为协变量(210mg) Date are expressed as adjusted mean ± *SE*, with egg mass at oviposition (set at 210mg) as the covariate; * $P<0.05$; **** $P<0.0001$

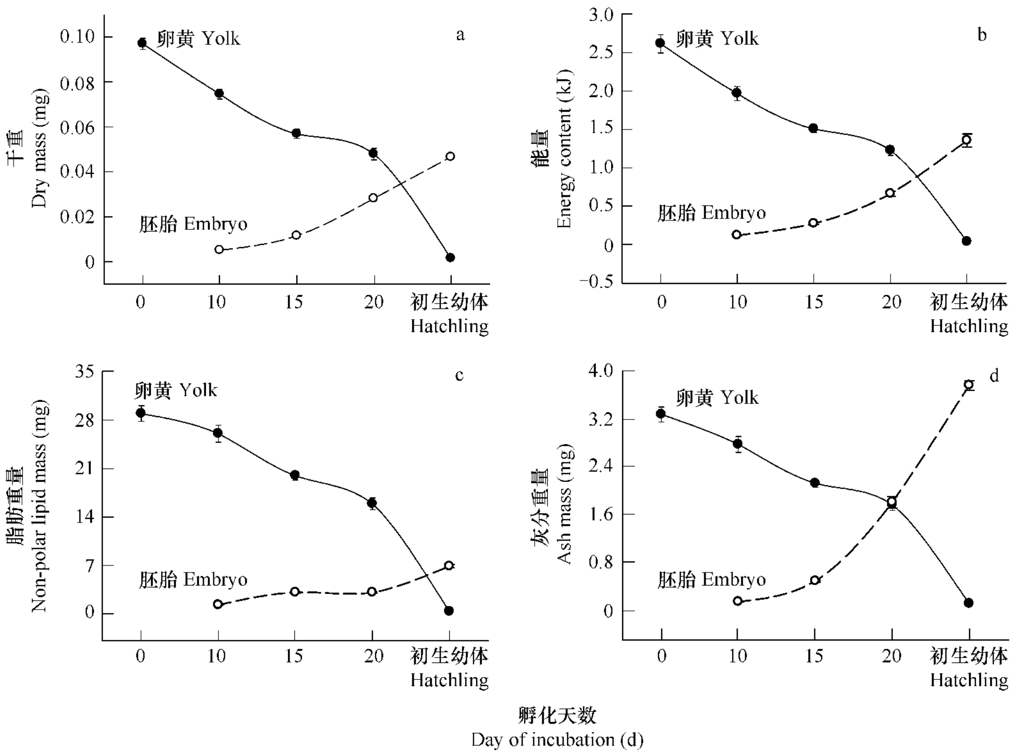

图 1　白条草蜥胚胎发育过程中物质和能量的动态(数据用平均值 ± 标准误表示)

Fig. 1　Dynamics of material and energy during embryonic development in the *T. wolteri* (Data are expressed as mean ± SE)

3　讨论

与龟鳖类、鳄类和有鳞类壁虎科等极端卵生类不同,不少卵生有鳞类处在卵生-卵胎生连续谱的中点附近[18]。新生卵胚胎发育程度,以及产后胚胎发育过程很大程度上与受精卵在母体输卵管内滞留时间有关,致使胚胎发育过程中物质和能量的转化存在显著的种间差异[5,7,19,20]。胚胎发育过程中物质和能量的转化还受到卵内外环境因素的影响[7,8,14]。用相似条件的实验设计,比较研究爬行动物卵孵化过程中物质和能量的利用可以得到有意义的结果。

白条草蜥卵孵化过程中,卵内容物中的卵黄干重、脂肪含量和能值随卵孵化时间的增加而不断减少,这意味着胚胎发育过程中能量消耗日益增加(图 1)。孵出幼体的灰份含量比新生卵内容物的灰份含量要高,而孵化卵卵壳的灰份含量却比新生卵卵壳的灰份含量要低(表 1)。这些结果表明白条草蜥利用卵壳作为胚胎发育过程中的第二矿物质来源,与以往对山地麻蜥(*Eremias brenchleyi*)[12]、北草蜥(*Takydromus septentrionalis*)[5]、五线石龙子(*Eumeces fasciatus*)[6]和虎斑颈槽蛇(*Rhabdophis tigrinus lateralis*)[11]的研究结果相类似。卵壳作为胚胎所需无机物的另一来源在卵生羊膜动物比较普遍[3,10,21,22]。

白条草蜥卵孵化过程中其胚胎发育可大体分为两个主要阶段(图 1),第 1 阶段即卵孵化的 0 ~ 15d,卵黄转移到胚胎的物质和能量很少,胚胎生长缓慢;第 2 阶段即卵孵化的 20 ~ 24. 7d,卵黄转移到胚胎的物质和能量很多,胚胎生长迅速,这一结果与山地麻蜥(*E. brenchleyi*)[8]、北草蜥(*T. septentrionalis*)[10]、五线石龙子(*E. fasciatus*)[12]类似,而不同于虎斑颈槽蛇(*R. tigrinus lateralis*)[7]。

已研究的 16 种卵生有鳞类新生卵内容物转移到孵出幼体的干物质、非极性脂肪和能量的转化率表明,胚胎发育过程中物质和能量的转化存在显著的种间差异,尤其是蜥蜴的物质和能量转化率明显低于蛇类[11]。本研究白条草蜥的干物质、非极性脂肪和能量的转化率明显小于蛇类而与蜥蜴类接近。这些结果可能提示,相对于大型有鳞类爬行动物,小型有鳞类物质和能量的转化率较低。

特定卵生有鳞类幼体干重不是取决于胚胎发育过程中物质和能量的转化率,而是直接地与亲体投入到单

个后代的繁殖投资有关。在缺乏亲代抚育的爬行动物中,剩余卵黄是亲体投入到卵内的一部分超出胚胎发育所需要的物质和能量,用于幼体的早期维持和组织生长[3, 10, 23 ~25]。本研究白条草蜥的剩余卵黄与蜥蜴类接近而明显小于蛇类。蛇类卵大,胚胎发育投资的成本小,蜥蜴类卵较小,胚胎发育投资的成本大,蛇类孵出幼体比蜥蜴孵出幼体获得更多的剩余卵黄[7],从而使胚胎发育过程中物质和能量有较高的转化率。

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