1997 Asiatic Herpetological Research Vol. 7, pp. 139–146

Egg Components and Utilization During Incubation in the Turtle, *Chinemys reevesii*

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Abstract. -The energy content, water content and ratios of egg components in the turtle, *Chinemys reevesii*, were determined during 0-55 days of incubation. The total water content of the egg took 65% of egg mass on preincubation and increased 5.66% during incubation time. The total water content of the egg on preincubation was found 60.7% in the yolk, 35% in the albumen and 4.3% in the shell. The ratios of those varied with the embryonic age (days) growth, and the greater portion the water in the egg had transferred to the embryonic tissues on 55 days of incubation when the water content of the embryo was 50.4% of the egg total water content. The dried mass of shell, yolk and albumen on preincubation were 35.3%, 61.0% and 3.1% of the egg dry mass, respectively. The component materials of those, except for a major portion of the remained shell, were transferred into the embryonic tissues with the incubation days as the total transferred rate was 79.3%, and the cost rate for 20.7%. The lipid content in dried yolk averaged 20.4% of the yolk's dried mass during incubation. The energy content of the yolk, albumen and embryo were relatively stable through the incubation period and averaged 6462.5, 5087.7 and 6291.4 cal/g, respectively.

Key words: Turtles, Chinemys reevesii, egg components, energy content, Incubation.

Introduction

Oviparous reptiles characteristically allocate the majority of their total reproductive investment to their eggs (Fischer et al., 1991). The eggs of the turtle, *Chinemys reevesii*, reflect the total reproductive investment in the offspring by females. This post-hatching yolk is the source of energy for the survival and growth of the hatchlings in some reptiles during the first days of life (Kraemer and Bennett, 1981; Congdon et al., 1983a, 1983b; Troyer, 1987; Wang et al., 1990, 1995). The component materials and energy in the turtle egg are first utilized for embryonic development and, secondarily, for hatchling maintenance after it leaves the egg. However, the utilability rates of the component materials, such as the shell, albumen and yolk of the *Chinemys reevesii* egg for embryonic development at the different stages of incubation are not clear. How many component materials of the egg are allocated and which are necessary for the embryonic development and hatchling? So far, only limited data are available on changes of the shell, albumen and yolk rations in the egg during the different stages of the incubation for reptiles.

In this paper, we report some component (albumen, yolk and shell) ratios and energy values in freshly laid egg of the turtles, *Chinemys reevesii*, and also the changes of the component ratios during the different stages of incubation.

Materials and Methods

One hundred eggs of the common turtle, *Chinemys reevesii*, were collected in the morning after the oviposition of the females in June 1987 and 1988 at a turtle farm near East China Normal University in Shanghai, China. Each egg was removed from the nest on the day of laying, marked and weighed with a torsion balance (±0.01 mg). Ten fresh eggs were used for the standard (normal) level of the egg component materials and the energy content. The remaining 90 eggs were incubated in an environmental temperature of 28±0.05° on a water-saturated substrate (sand), and the inside of the incubation chamber was periodically misted with water. Those hatching eggs were used for some analyses of the ratios of the component materials and the energy content during the different stages of incubation (Tables 1-6).

When the eggs for assay were cracked, the embryo, yolk, albumen and shell were carefully separated and each one was separately placed in a pre-weighed glass container and then weighed. The embryo was separated from the yolk and both were freed from the surrounding choriallantoic and viteline tissue, and then blotted dry and weighed. The albumen was cleaned away from the shell membranes, and the shell was rinsed with distilled water and blotted dry and weighed. The samples were then dried to a constant mass in an oven at 60°C. All the weighings were accurate to the nearest milligram. All of the dried materials were stored in a desiccator until assayed.

Water content was calculated as the difference in the net wet and dry mass of the egg materials. The energy contents of the dry embryo, yolk and albumen were then measured. Triplicates of each individual sample were assayed by combustion to determine the energy content with the microbamb calorimeter (JR-2000 type made in China) and benzoic acid standards. The results were corrected for free ash energy content.

The lipid content of the yolk was removed with petroleum ether from an aliquot off the dry yolk mass in a soxhlet extractor; each length of the extractive time lasted 5 hours. The amount of lipid was determined by subtracting the mass of the sample after extraction from the sample mass before extraction. The sample weighings were accurate to the nearest milligram.

Results

The changes of the egg components of *Chinemys reevesii* at the different incubation times are shown in Tables 1, 2 and 3.

Eggshell. During day zero of incubation, the fresh mass of the eggshell was 14.6% of the fresh egg (Table 1) and the dried mass of the eggshell was 35.3% of the dried egg (Table 2). The values of the shell declined as the time of incubation increased, and exhibited an age phase of incubation (Tables 1 and 2).

Yolk Content and Incubation Time. During the first days of incubation, the yolk contents took the majority of the egg components (Tables 1 and 2). But, those contents declined as the incubation time increased. When the hatchlings left their eggshells, the remaining yolk was only about 6-9% of the fresh yolk mass on the zero day of incubation.

Albumen Contents. The fresh albumen contents (%) of the egg were higher after than before 20 days of incubation (Table 1). But, dried albumen (%) of the egg mass expressed relative stability (Table 2).

Embryonic Mass. From 0-10 days of incubation, the embryos had not been determined because of a little tissue of each embryo. We found that the embryonic wet and dry mass of *Chinemys reevesii* increased with the time (days) of incubation (Tables 1 and 2). The recently emerged hatchlings averaged 4.8 g (SD=0.78, N=10) or 79.31% (SD=2.4) of the total content in the egg mass compared with day zero of incubation, so that, the rate of the material transferred to the tissues of the embryos was 79.31% (SD=2.4) and the rate of cost was 20.69% (SD=2.39).

Water Content in Eggs. The water content in eggs was determined by subtracting the dried mass from the fresh mass of the egg, and the results are shown in Table 3.

During 0 day of incubation, the water content of each egg averaged 65% (SD=6.3, N=10). The rates of those after 20 days of incubation raised with the incubation days slightly increased (Table 3) as the mean growth rate my reach 9.2% until 55 days of incubation.

The water content of the shell averages 16.1% (SD=1.3, N=76) throughout the incubation period. The water in the yolk on 0 day of incubation was 65.5% (SD=7.8, N=10), but the value of that declined as the incubation time increased (Table 3). The water in the embryos also declined as the incubation time increased. But, the water in the albumen kept in a range of 95.3-97.3% on 0-55 days of incubation (Table 3).

Lipid Content in Yolk. Table 4 shows that the lipid content (%) in the dried yolk on the first periods of incubation reduced slightly, and then, increased with the incubation time did as raised to 26.51% on 55 days on incubation.

Energy Values of Egg Contents. The energy values (cal/g) of the yolk, albumen and embryo throughout incubation time are shown in Table 5.

The caloric values (free-ash) of the wet-yolk increased with the incubation days and those data are fitted into the following equation: for wet yolk, cal/g=2356.52±31.43 days, r=0.9745, $P<0.01$. But, the caloric values (free-ash) of the dried yolk stabilized relatively in the range of 6343.9-6609.8 cal/g.

The caloric values of the embryonic mass also increased with the incubation time (Table 5) as the relation between caloric values (Y) and days (X) of incubation expressed a positive linear regression correlation for $Y=1306.32+97.44X$, r=0.9820, $P<0.01$. The caloric values of dried embryonic mass were stabilized in the range of 6257.7-6337.7 cal/g (free-ash).

The albumen energy values are the lowest among components of the egg (Table 5). Such as the total mean energy values of wet albumen is 204.8 cal/g and dried albumen is 5087.7 cal/g throughout the period of incubation, but only equal to 5.95% of wet and 76.7% of dried yolk energy, respectively.

Discussion

Type and Mass of Eggshell

The types of eggshell in the turtles were classified to be both fundamental patterns of the rigid-shell (or brittle-shelled) and the flexible-shelled; in the former, the shell mass take above 30% of the whole egg dried mass and below 21% of one in the latter (Congdon and Gibbons, 1985). The dried mass of shells averaged 35.3% of the total dried mass of eggs in *Chinemys reevesii* and should be classified as a type of rigid eggshell, and the mass of that is approximate to 36.44% of *Gopherus polyphemus* eggshell mass and is lower by 4-8% than those of *Kinosternon subrubrum, K. coloratus* and *Clemmys marmorata* (Congdon and Gibbons, 1985).

The shell mass in the egg in *Chinemys reevesii* declined as the incubation days increased (Table 1 and 2), declining a total of 37.8% in fresh eggshell and 6.2% in the dried mass of eggshell with a part of calcium in the eggshell that was possibly transferred into the developing embryo. We have determined the contents of calcium and magnesium in whole egg (Table 6) for tested the above hypothesis. The results (Table 6) show that (1) the calcium and magnesium contents were less in posthatching eggshells than in preincubation ones; (2) the calcium and magnesium contents of the egg contents (yolk and albumen) only equaled 45.93% and 87.74% of those in hatchlings, respectively. Obviously calcium and magnesium in the egg contents were not enough to provide the embryonic development. The other 54.07% of calcium and 12.26% of magnesium in the newly hatchlings may come from reserves in the eggshell. Some previous papers also support our results: the sources of calcium in embryos of sea turtles came 60-80% from stores in the eggshell (Simkiss, 1967; Bustard et al., 1969) and the 56% of calcium in embryonic snapping turtles, *Chelydra serpetins*, obtained also from the development required (Packard et al., 1984b).

Growth of Embryos and Consumption of Yolk. We found that the yolk content declined as the incubation time increased, the embryo mass increased as the incubation time did. Both reflect a potential relationship between yolk consumption and embryonic growth. Because the turtle egg is a semi-closed system, all course of material transference, energy flow and embryonic development is carried out in the system throughout the incubation period. The yolk of the turtle is the main sources of energy and materials for the embryonic development, so the yolk of *Chinemys reevesii* declined as the embryo grew during the incubation times.

The just newly hatchlings of *Chinemys reevesii* averaged 4.8 g (SD=0.78, N=10) for 68.41% of the fresh egg content (albumen and yolk) mass at 28±0.5°C of the incubation temperature. If all of the egg contents may provide for the embryonic development, the transferred rate from the egg contents into embryonic tissues was 68.41%, and the rate of cost was 31.59% at 28±0.5°C of the incubation temperature in *Chinemys reevesii* eggs. The transferred rate of the *Trionyx triunguis* egg contents is 75.21% (Leshem et al., 1991). But, the transferred rate is influenced by the temperature of incubation, at 30°C for 65.54% and at 33°C for 62.61% in *Chinemys reevesii* egg contents (Wang et al., 1990).

Movement of Water Inside Eggs. The total water content of each *Chinemys reevesii* fresh eggs at preincubation averaged 65% that the percent water approximates to 66.9-70% water of the *Alligator mississippiensis* egg (Fischer et al., 1991). The water content of the reptile eggs have been defined as the hydric condition necessary for successful completion of the incubation (Packard and Packard, 1988). We found that the total lost water about 20% laid egg mass in the Grass lizard (*Takydromus septentrionalis*) had failed to hatch. In addition, the egg must absorb much more water from its surrounding substrata for the embryonic development completion, and the mass of eggs may be increased 342.2% of the initial egg mass over the course of incubation (Wang et al., 1989). In this study, the total water content of the *Chinemys reevesii* egg increased only 5.66% (SD=2.61, N=76) of the initial egg mass during incubation. But, eggs of some species hatch successfully even declining appreciably in mass. The eggs of the snapping turtles (*Chelydra serpentina*) frequently hatch after declining in mass by 18% over the course of incubation (Morris et al., 1983); the eggs of soft-shelled turtles (*Trionyx triunguis*) may lose approximately 21% water of the egg without ill effect during incubation (Leshem et al., 1991).

The total water content of the *Chinemys reevesii* egg on the preincubation reserved 60.7% in the yolk, 35% in the albumen and 4.3% in the shell (Table 3). The ratios of those varied with the incubation times (Table 3). The changes of the ratios may cause the yolk consumption and the embryonic growth: for example, on 55 days of incubation, the wet mass of yolk was only 7.5% of egg mass and the wet embryonic mass had grown to 45.9% of the egg mass (Table 1) when the water of the yolk took only 4.9% of the egg total water content and the water of embryo took 50.4% of the egg total water content (Table 3). Secondarily, yolk is also the approximate source of water used by embryos (Morris et al., 1983), so a quantity of water was transferred to the embryo. The albumen and shell water content were apparently not changed (Table 3).

The water content of the *C. reevesii* embryo declined significantly with the embryonic age (Table 3) that the changes of the relative hydration are similar to previous reports for the embryos of Green iguana (*Iguana iguana*) (Ricklefs and Cullen, 1973) and Colubrid snake (*Coluber constrictor*) (Packard et al., 1984). The water content of the embryo may be an index to the maturity of the embryo and can be used to estimate embryonic age.

Lipid of Yolk. The rate of the lipid content in the dry yolk of *Chinemys reevesii* averaged 20.4% during incubation. The value of that is close to 21% of the egg lipid in pained turtles, *Chrysemys picta* (Congdon et al., 1983). The lipid in the yolk may play an important role through embryonic development. It provides an embryo with greater amounts of usable metabolic energy and water (Gutzke and Packard, 1987), and the most part of one is transferred to the hatchling for it cost in the first days after it leaves the egg (Fischer et al., 1991).

Energy of Egg Contents. The changes of the energy content (cal/g) in the wet mass of albumen, yolk and embryo of the *Chinemys reevesii* are affected by their water content. However, the energy content in those of dried mass are relatively stable through incubation (Table 5). The caloric value, 6291.4 cal/g (SD=31.8), of the dried yolk in *Chinemys reevesii* is close to the value of those in the snapping turtles, *Chelydra serpentina* (6.6 cal/g), and the slider turtles, Pseudemys scripta (6.7 Kcal/g), at the time of laying (Slobodkin, 1962). The total energy content of the dry yolk in the *Chinemys reevesii* increased as the lipid content of the yolk did. Both show positive correlation, r=0.6768 ($P<0.01$), and the correlation is significant.

Acknowledgments

This research was supported by the Natural Scientific Fund of Educational Committee of China, Natural Scientific Fund of China, and East China Normal University. We would like to thank Dr. Allan Muth, Dr. Cary C. Packard, Dr. J. Whitfield Gibbons and Dr. Justin D. Congdon for providing some literature, and also special thanks to Prof. Ermi Zhao for his advice on my manuscript.

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Appendix I

Tables 1-6

Table 1. The changes of wet mass of egg components in *Chinemys reevesii* during incubation.

Day	N	Egg Mass in g	Component % of egg mass					Age phase of each component (%)			
		(M±SD)	Shell	Yolk	Albumen	Embryo	Other*	Shell	Yolk	Albumen	Embryo
0	10	5.89±1.43	14.6	54.4	23.9	-	7.1[a]	100	100	100	-
10	8	5.67±0.97	12.5	47.3	22.9	-	17.3[a]	85.7	86.9	95.7	-
20	10	6.15±0.98	12.2	35.3	43.1	3.2	6.2	83.2	64.9	179.9	100
30	9	6.72±1.05	12.0	27.6	40.1	15.5	4.8	82.3	50.7	166.8	493.0
40	10	7.36±0.79	11.3	16.9	32.1	35.7	4.0	77.5	30.8	134.1	1133.7
45	9	7.10±1.02	11.3	16.5	30.6	37.1	4.5	77.6	30.2	127.9	1175.6
50	10	7.28±0.43	9.9	10.9	30.5	42.5	6.2	67.5	20.0	127.4	1340.6
55	10	8.16±0.96	9.7	7.5	31.7	45.9	5.2	66.2	13.7	132.4	1455.2

*The values of those indicate the remains inside the shell after the embryos are out of the shell.

[a] shows the estimated values which includes those of the embryonic membranes and embryos etc.

Table 2. The changes of dry mass of egg components in *Chinemys reevesii* during incubation.

Day	N	Egg Mass in g	Component % of egg mass					Age phase of each component (%)			
		(M±SD)	Shell	Yolk	Albumen	Embryo	Other*	Shell	Yolk	Albumen	Embryo
0	10	2.09±0.22	35.3	61.0	3.1	-	0.6 [a]	100	100	100	-
10	8	2.12±0.42	35.0	60.7	3.5	-	0.8 [a]	99.2	99.5	112.9	-
20	10	1.89±0.39	34.9	60.2	3.8	0.7	0.4	98.9	98.7	122.6	100
30	9	2.02±0.30	34.6	55.7	3.6	5.5	0.6	98.0	90.3	116.1	785.7
40	10	2.02±0.32	34.0	40.4	3.4	21.7	0.5	96.3	66.2	109.7	3100.0
45	9	2.04±0.31	34.0	36.5	3.5	25.6	0.4	96.3	59.8	112.9	3657.1
50	10	1.93±0.28	33.6	27.9	3.4	34.1	1.1	95.2	45.7	109.7	4871.4
55	10	2.07±0.36	33.1	18.7	3.4	43.2	1.6	93.8	30.7	109.7	6171.4

*The values of those indicate the remains inside the shell after the embryos are out of the shell.

[a] shows the estimated values which includes those of the embryonic membranes and embryos etc.

Table 3. The changes of water content of eggs in *Chinemys reevesii* during incubation.

Day	N	Total water in egg		Ratios of water (%)				% of each component water			
		G		Shell	Yolk	Albumen	Embryo	Shell	Yolk	Albumen	Embryo
		M (SD)	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)
0	10	3.81	65.0	4.3	60.7	35.0	-	17.4	65.5	95.3	-
		(0.89)	(6.3)	(0.8)	(9.0)	(9.0)	-	(3.0)	(7.8)	(1.9)	-
10	8	3.57	63.1	3.6	55.2	41.2	-	14.9	59.6	95.5	-
		(0.86)	(5.4)	(0.5)	(8.4)	(8.7)	-	(2.5)	(6.4)	(1.1)	-
20	10	4.27	67.3	3.6	30.7	61.1	4.0	17.6	51.7	97.0	93.1
		(0.61)	(6.3)	(1.6)	(9.3)	(7.7)	(1.9)	(8.0)	(6.1)	(1.5)	(5.0)
30	9	4.69	70.6	2.6	18.7	57.8	20.7	15.7	45.2	97.3	90.0
		(0.95)	(2.7)	(0.4)	(2.7)	(4.3)	(5.6)	(1.5)	(2.0)	(0.4)	(0.7)
40	10	5.35	72.5	2.2	11.5	44.3	42.0	14.8	40.2	97.3	84.0
		(0.64)	(1.9)	(0.2)	(3.3)	(5.2)	(5.9)	(1.1)	(2.8)	(0.2)	(3.0)
45	9	5.06	71.3	2.4	9.4	43.2	44.7	14.6	38.9	96.5	82.4
		(0.72)	(1.1)	(0.3)	(1.7)	(5.2)	(6.3)	(1.9)	(0.9)	(0.5)	(2.2)
50	10	5.36	73.6	2.5	7.1	44.5	47.7	17.5	41.2	96.6	79.6
		(0.69)	(3.0)	(0.4)	(0.5)	(8.3)	(5.6)	(4.3)	(2.2)	(13)	(2.5)
55	10	6.06	74.2	2.4	4.0	43.8	50.4	15.9	40.8	96.6	77.1
		(0.76)	(2.1)	(0.6)	(0.3)	(4.9)	(4.2)	(1.4)	(2.1)	(0.6)	(1.3)

Table 4. The lipid contents in dry mass of yolk of *Chinemys reevesii* eggs during incubation.

Days	N (egg)	Lipid % of yolk (M±SD)
0	10	20.50±1.49
10	8	18.58±0.57
20	10	15.92±0.95
30	9	19.75±0.84
40	10	20.11±1.23
45	9	21.26±2.01
50	10	21.27±1.12
55	10	26.51±2.14

Table 5. The energy contents (cal/g) of albumen, yolk and embryo in the eggs of *Chinemys reevesii* during incubation.

Days	N (eggs)	Yolk		Albumen		Embryo	
		Wet	Dried	Wet	Dried	Wet	Dried
0	10	2331.4	6344.6	247.6	5313.5	-	-
10	8	2536.7	6372.7	240.3	5297.9	-	-
20	10	2967.2	6343.9	238.4	5281.2	199.6	6337.7
30	9	3549.8	6486.1	217.3	5188.3	956.1	6256.0
40	10	3674.6	6538.4	185.7	5144.8	2245.2	6287.4
45	9	3907.6	6397.5	173.8	4895.8	2331.2	6295.5
50	10	3831.7	6609.8	165.0	4853.5	2600.4	6257.7
55	10	3911.8	6606.7	170.2	4726.3	2894.3	6313.8
Mean		3338.9	6462.5	204.8	5087.7	1871.1	6291.4
SD		637.9	112.8	34.8	229.4	1054.7	31.8

Table 6. Calcium and magnesium contents (M±SD, in mg) of eggshell, egg content and hatchlings in *Chinemys reevesii*.

Contents	N	Eggshells		Egg contents	Hatchlings
		Preincubation	Posthatching		
Ca	5	227.10±19.20	183.30±29.05	15.67±4.67	34.12±1.04
Mg	5	1.63±0.14	0.99±0.15	2.02±0.14	2.30±0.55