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Effects of Starvation on Urinary Nitrogen Composition of Juvenile Chinese Three-keeled Pond Turtles (*Chinemys reevesii*)

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Abstract.- We investigated the effect of starvation on urinary nitrogen composition in the juvenile Chinese three-keeled pond turtle (*Chinemys reevesii*). Under normal conditions, ammonia, urea, and uric acid constitute 2.3, 95.8 and 1.9% of total urinary nitrogen, respectively. During starvation periods of one to four weeks, the concentration of urea changed little, while that of ammonia rose sharply and that of uric acid fell significantly. After feeding was resumed for four weeks, the levels of ammonia and uric acid returned to control levels. Changes in urinary nitrogen composition during starvation may be related to the anti-oxidative function of uric acid during periods of stress.

Keywords.- Pond turtle, *Chinemys reevesii*, bladder urine content, stress, uric acid.

Introduction

The nature of an animal's nitrogenous waste, including ammonia, urea and uric acid, is dependent on the environment in which it lives (Delaunay, 1931). Furthermore, the spatial distribution of nitrogenous end products in the body provides important data on how that animal responds physiologically to that environment. The highly diverse excretory function of the Reptilia has been well-documented (Campbell, 1995), and although aquatic and semiaquatic turtles are primarily ureotelic, a number of exceptions are known where the predominant form of nitrogenous waste is ammonia (Lee et al., 2007).

The first objective of this study was to determine the composition of excretory nitrogen in the bladder of the Chinese Three-keeled pond turtle *Chinemys reevesii*. This research will add to the body of literature on nitrogen excretion in freshwater turtles, which is currently very limited (Lee et al., 2007; Singer, 2003).

Nitrogenous end-products have diverse physiological functions in different animal groups, including acid-base regulation, osmoregulation, etc. (Wright, 1995). In turtles, two of the major factors affecting nitrogen excretion are availability of water and amount of dietary nitrogen ingested (Singer, 2003), which are of course highly influenced by starvation and dehydration, which turtles have developed a magnificent physiological capability to resist. The aquatic Sonoran mud turtle (*Kinosternon sonoriense*), for example, can aestivate for 11 weeks without food or water; during the initial period of deprivation, urine in the bladder is apparently used for osmoregulation (Peterson and Stone, 2000). While ammonia is quite toxic, more inert forms of excretory nitrogen (e.g. urea and uric acid) are more costly to synthesize (in ATP equivalents; Baze, 1970), making ammonia the most frequently produced form of waste in aquatic reptiles because they can lose this waste rapidly to the environment (Cragg et al., 1961). Uric acid is considered to be an endogenous antioxidant, and as such, might play a role in the clearance of free radicals that are produced during starvation (Zhu et al., 2005). The second objective of the present work is to investigate how *Chinemys reevesii* changes its nitrogen metabolism during starvation.

Materials and methods

Experimental animals and diet.- Juvenile turtles were obtined from a turtle farm in Guangzhou, China and acclimated to a fixed photoperiod of 12L:12D at a temperature of 29±°C for three weeks in December 2004. Each of the three turtles were placed in a glass tank with the dimension of 19 × 23 × 27 cm. Turtles were fed to apparent satiation twice a day on a commercial formulated feed powder (composition as percentage of dry matter: crude protein 41.66%, crude lipid 5.84%, ash 18.24%, and water 8.62% of total weight), which was added to water and extruded to strip pellets before usage. Each turtle was marked by sawing one to three notches at the edge of its carapace.

Experimental procedure.- Before the experiment, 108 healthy turtles with a mean body mass of 13.58±1.84 g (mean±*SD*) were chosen, randomly divided into five groups (C [control], S1, S2, S3 and S4), and placed into a total of 36 tanks. This experiment lasted eight weeks, with a period of starvation interrupting periods when the

Table 1. Percentage of urine in body weight, nitrogen concentration (mmol•L^{-1}) and partition (percent) formed as ammonia, urea and uric acid in the bladder of fresh-water turtles (*Chinemys reevesii*) at the end of fasting and after feeding for four weeks. Data expressed as Mean ± SE. Different letters denote significant differences within the same column, p <0.05.
Group C was the control group. Group S1 was starved during the fourth week; group S2 was starved during the third and fourth weeks; group S3 was starved from the second to the fourth week; group S4 was starved for the first four weeks.

Group	N	Percentage of urine in body weight	Concentration: mmol•L^{-1}				Partition of total Nitrogen		
			Urea	Ammonia	Uric acid	Total N	Urea	Ammonia	Uric acid
At the end of fasting									
C	5	2.1±0.6	172.5±33.0	9.21±0.44 ^{b}	2.19±0.20 ^{a}	363.0±66.5	94.5±0.8	2.9±0.6 ^{b}	2.6±0.3 ^{a}
S1	2	2.1±0.5	166.3±38.5	17.45±3.64 ^{ab}	0.44±0.42 ^{b}	352.0±82.0	94.6±0.3	5.0±0.1 ^{ab}	0.4±0.4^{ab}
S2	5	4.0±1.2	136.1±45.6	9.37±3.21 ^{b}	0.71±0.14 ^{b}	284.5±94.2	94.7±0.9	3.4±0.3 ^{ab}	1.9±0.8 ^{ab}
S3	6	3.2±0.6	139.4±41.9	13.24±5.44 ^{ab}	0.36±0.13 ^{b}	267.4±104.1	95.6±0.4	3.6±0.5 ^{ab}	0.9±0.3 ^{b}
S4	6	3.1±0.5	235.7±32.3	24.25±2.61^{a}	0.22±0.11 ^{b}	496.6±66.8	94.8±0.3	5.0±0.2 ^{a}	0.2±0.1 ^{b}
F		0.915	1.178	3.103	25.559	1.311	0.417	5.015	4.789
p		0.475	0.352	**0.040**	**0.000**	0.304	0.794	**0.007**	**0.008**
After feeding for four weeks									
C	6	2.4±0.2	165.7±24.9 ^{ab}	6.10±0.56	0.96±0.17	341.3±49.7 ^{ab}	96.8±0.5	1.9±0.2	1.3±0.3
S1	5	2.1±0.5	242.4±40.5 ^{a}	7.43±1.1	1.08±0.32	496.6±80.9 ^{a}	97.5±0.5	1.6±0.3	1.0±0.4
S2	6	2.1±0.3	150.0±26.7 ^{ab}	7.40±1.58	1.37±0.33	312.8±53.8 ^{ab}	95.1±0.9	2.6±0.5	2.3±0.7
S3	7	2.8±0.3	119.8±10.1 ^{b}	5.59±0.88	1.45±0.19	251.1±21.0 ^{b}	95.4±0.5	2.2±0.3	2.3±0.3
S4	7	2.7±0.2	107.3±21.6 ^{b}	4.81±0.83	1.21±0.17	224.1±43.8 ^{b}	94.7±1.2	2.6±0.6	2.7±0.6
F		1.261	4.077	1.138	0.776	4.064	1.947	1.010	2.010
p		0.313	**0.012**	0.362	0.552	**0.012**	0.135	0.422	0.125

turtles were fully fed. The C group included eight tanks where the turtles were always fed to satiation. The S1, S2, S3 and S4 groups included seven tanks each. The S4 group was starved in the first four weeks of the experiment. The S3 group was fed in the first week and then starved for three weeks. The S2 group was fed for two weeks and then starved for two weeks. The S1 group was fed for three weeks and then starved for one week. All groups were fed to satiation from the fifth to eighth weeks.

Analytical methods.- One turtle was sampled randomly from every tank at the end of the fourth and eighth weeks, starved for 24 hours to empty the gut, toweled off, weighed to within 0.01 g with an electronic balance, placed in a plastic bag and euthanized at a temperature of -80°C.

The turtles were dissected and the urinary bladders, which contained frozen urine, were extracted and weighed. Urine samples were centrifuged and diluted twenty times with 0.9% NaCl. Urea and uric acid concentrations were determined using a Roche Diagnostics Cobas INTEGRA 400. The concentration of ammonia was measured using Roche Modular-P and Integra systems.

Data processing.- All statistical analyses were performed using the SPSS13.0 software package. The Kolmogorov-Smirnov test revealed that the data (including percentages) followed a normal distribution. A one-way ANOVA was employed to assess the effects of starvation. The Tukey HSD or Games-Howell test was used for making multiple comparisons between the means of different groups; $p < 0.05$ was taken as the level of significance.

Results

Table 1 shows the changes in percentage of urine, total excretory nitrogen concentration, and the proportion of ammonia, urea, and uric acid among the different groups at the end of the fasting and refeeding periods.

After fasting, significant differences between the control and deprived groups were found in the concentrations of ammonia ($F_{4,19} = 3.103$, $p = 0.040$)and uric acid ($F_{4,18} = 25.559$, $p = 0.000$), and in the composition of total excretory nitrogen ($F_{4,18} = 5.015$, $p = 0.007$; $F_{4,18} = 4.789$, $p = 0.008$). Ammonia concentration and its relative concentration showed a positive relationship with increading periods of starvation, while those of uric acid showed the reverse trend. Excretory urea did not appear to be significantly affected by starvation ($p > 0.05$).

After feeding for four weeks, urea concentration and total excretory nitrogen of S1 were found to be significantly higher in groups S3 and S4, while there were no clear differences between the other groups for these two parameters. Furthermore, all groups did not differ clearly in other parameters measured ($p > 0.05$). Urinary nitrogen composition was approximately 2.3% ammonia, 95.8% urea, and 1.9% uric acid in the control group.

Discussion

In our study, *Chinemys reevesii* was found to be primarily ureogenic like other freshwater turtles, but it exhibited a relatively higher proportion of urea (about 95.8%) in excretory nitrogen compared to that of other freshwater turtles such as *Trachemys scripta* (about 70%, urine in ureter; Dantzler and Schmidt-Nielson, 1966) and *Pelodiscus sinensis* (54%, water samples; Lee et al., 2007). Schmidt-Nielsen and Skadhauge (1967) reported that ureotelic fresh water turtles excreted 45–95% of their waste nitrogen in the form of urea, comparable to the results found here.

During food deprivation, the concentration of uric acid in the turtle's bladder fell significantly ($p = 0.000$) while that of ammonia clearly rose ($p = 0.040$), even though they constitute only a small proportion of total nitrogenous end products. Rapatz and Musacchia (1957) found that the fasted fresh water turtle *Chrysemys picta* (fasted for 6–8 weeks at 22°C) showed characteristic biochemical properties in decreased liver total fatty acids, decreased blood glucose and increased urine uric acid levels. Meanwhile, specimens in cold torpor (4–8 weeks at 4°C) had increased liver total fatty acids, a significant increase in liver glycogenolysis and increased urine uric acid levels. Zhu et al. (2005) found that *Chinemys reevesii* retained higher uric acid in its bladder during cold torpor, but these contents rapidly decreased when exposed to air because of oxidation. They presumed that retention of uric acid in the bladder during cold torpor (they induced hibernation for about one year) might have a benificial function during long periods of food deprivation, as uric acid or urate is known to have antioxidative properties similar to those of vitamin C and E. This may be the cause of the observed decrease of uric acid in the bladder during starvation in the present study. Conflicting results observed between this and previous studies may be due to differences in rearing conditions, the use of a different species (*Chinemys reevesii* vs. *Chrysemys picta*) or ontogenetic differences (juvenile vs. adult). The increase of ammonia in the bladder of the turtles may be the result of increased activity in innate protein catabolism during starvation. Further research on nitrogen metabolism in stressful

environments should be conducted.

The concentration of total urinary nitrogen and urea in our experiment did not significantly change with starvation, conflicting with prior experiments (Zhu et al., 2005). This conflict may be due to differences in nitrogen metabolism when hibernation is not induced.

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