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PLASMA BIOCHEMISTRY AND CONDITION OF CONFISCATED HATCHLING PIG-NOSED TURTLES (*CARETTOCHELYS INSCULPTA*)

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***Abstract.*—Plasma biochemical analysis is an important tool for health assessment in many species. Reference intervals for certain chelonian species, particularly sea turtles, have been previously established. However, baseline biochemical values for freshwater turtles, particularly species in the superfamily Trionychia, are lacking. We evaluated plasma biochemical parameters in 20 confiscated hatchling Pig-nosed Turtles (*Carettochelys insculpta*) to establish baseline parameters for health assessment. The majority of turtles exhibited various degrees of dermal lesions and two turtles had suspected yolk peritonitis based on the presence of cloudy intracoelomic fluid; the turtles were treated accordingly for these problems. Plasma values showed decreased levels for packed cell volume (PCV), total protein, blood urea nitrogen (BUN), and uric acid compared to previous chelonian studies, which may be attributed either to hatchling age, previous malnutrition, and/or disease. Additionally, alkaline phosphatase (ALP) was increased while aspartate aminotransferase (AST) was decreased compared to previous studies. Three biochemical parameters (albumin, creatinine, and gamma-glutamyltransferase GGT) were below the analyzer measurement threshold for all turtles.**

Key Words.—biochemistry; *Carettochelys insculpta*; chelonians; confiscation; health; Pig-nosed Turtle; reptiles

INTRODUCTION

The Pig-nosed Turtle (*Carettochelys insculpta*), also known as the Fly River Turtle or Pitted-Shell Turtle, is a freshwater chelonian inhabiting rivers and billabongs in the wet-dry tropics of northern Australia and southern New Guinea (Georges 1992; Rhodin and Genorupa 2000). The Pig-nosed Turtle is the only surviving species of the once-widespread family Carettochelyidae and is widely studied for several unique aspects of its reproductive biology (Elsemberg et al. 2011).

The Pig-nosed Turtle is currently listed as Vulnerable on the IUCN Red List, and its export and use is restricted under CITES Appendix II. Major threats to Pig-nosed Turtle populations include habitat destruction and consumption of meat and eggs by local populations (Rhodin and Genorupa 2000). Additionally, despite legal restrictions, Pig-nosed Turtles are increasingly exported illegally for the exotic pet trade abroad (Rhodin and Genorupa 2000). Health assessments of Pig-nosed Turtles may, therefore, have implications for wildlife biology and conservation, as well as the exotic pet trade. Although a large amount of natural history research has been performed on this species, health parameters of the Pig-nosed Turtle have not been previously reported in the literature.

Biochemical parameters, as measured in peripheral blood, are a useful diagnostic tool in animal health management. To determine the significance of alterations in biochemical values, it is essential to establish species-specific (or at least taxon-specific) normal values for the parameters of interest. Baseline biochemical values for certain North American (Brenner et al. 2002; Innis et al. 2007; Chaffin et al. 2008; Müller and Brunnberg 2009) South American (Frair et al., 1978) and Asian (Chung et al. 2009; Oliveira-Júnior et al. 2009; Chansue et al. 2011) freshwater turtles have been reported . However, to our knowledge, there are no published data for complete biochemistry values in a turtle species of the superfamily Trionychia (including softshell turtles, flapshell turtles, and the Pig-nosed Turtle), although one study investigated select biochemistry parameters in the context of studying oxygenation during hibernation (Reese et al. 2003). We evaluated plasma biochemical parameters from 20 hatchling Pig-nosed Turtles to establish baseline values for this species.

MATERIALS AND METHODS

***Turtle procurement and husbandry.*—**The United States Fish and Wildlife Service confiscated over 100 hatchling Pig-nosed Turtles after illegal importation to the United States in January 2003. The North Carolina State University College of Veterinary Medicine (NCSU-CVM) temporarily housed 25 of the confiscated turtles from 17 January 2003 until 22 October 2003. Turtles were group-housed (two to four turtles per tank) in 75 L glass aquariums, half filled with dechlorinated water. Hang-on-back power filters preserved water

quality, and water temperature remained at 25.5–26.5° C with a salinity of five parts per thousand. Caretakers cleaned the tanks every 1–2 d and performed water changes as necessary. Turtles received two small pieces of assorted fruits/vegetables, one small piece of lettuce, and one aquatic turtle pellet every 1–2 d. Turtles varied in health status on presentation and received medical treatment accordingly. All animals were weighed at least weekly. Because sexual dimorphism is not apparent in Pig-nosed Turtles until maturity, we were unable to determine sex of the turtles noninvasively.

Plasma sample collection.—We collected blood from the 20 largest turtles on a single day (21 October 2003) prior to shipment out of NCSU-CVM (9 mo of captivity). All turtles appeared healthy based on general physical examination and absence of gross shell or skin lesions. We manually restrained turtles in ventral recumbency and collected blood from the subcarapacial sinus using a heparinized 28 gauge, 1.3 cm needle attached to a 0.5 mL syringe. We collected between 0.3–0.5 mL of blood from each turtle and immediately transferred the sample to a plain 1.5 mL microcentrifuge tube.

Plasma biochemistry parameters.—We performed plasma biochemistry analysis on the day of blood collection at the NCSU-CVM Clinical Pathology Laboratory. We centrifuged the samples at 10,000 rpm for 15 min and separated the plasma component. We analyzed plasma samples using a Roche Hitachi 912 biochemistry analyzer (F. Hoffmann-La Roche, Basel, Switzerland). The automated analyzer measured the following analytes: total protein, albumin, glucose, blood urea nitrogen (BUN), creatinine, uric acid, alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), total bilirubin (BILI), creatine kinase (CK), sodium, potassium, chloride, calcium, phosphorus, magnesium, and bicarbonate. Measured values allowed calculation of the following values: globulins, albumin/globulin ratio, anion gap, sodium/potassium ratio, and plasma osmolality. We measured packed cell volume (PCV) manually using hematocrit tubes.

Values for certain chemistry parameters (creatinine, albumin, GGT) were consistently below the measurable threshold of the analyzer; thus, these values were not included. Because albumin levels were too low to be measured, values for globulins and albumin/globulin ratio could not be calculated. Blood volume collected from some animals was insufficient to perform all biochemistry tests.

Statistical parameters.—We calculated mean, median, range, and standard deviation (SD) for each of the biochemical parameters For purposes of calculating the mean and SD, we counted values below the measurable threshold of the instrument as one-half of the lowest measurable value. We omitted such values from the calculation of the range. We defined outliers as values more than two times the standard deviation above or below the mean and excluded these.

RESULTS

Turtle demographics and health status.—On presentation, all 25 turtles were emaciated with a mean body weight of 27 g (23–32 g). Although no information was available regarding hatch date of these turtles, size and weight were consistent with hatchlings under one year of age (Cann 1998); necropsy of one animal revealed a yolk sac still present in the coelomic cavity, further supporting this age assessment. On presentation, the majority of turtles exhibited various degrees of dermal lesions (raised white/yellow plaques on carapace, plastron, and/or skin), and two turtles had suspected yolk peritonitis based on the presence of cloudy intracoelomic fluid. Prior to presentation at NCSU-CVM, all turtles had received amikacin (2.5 mg/kg intracoelomically) and enrofloxacin (5 mg/kg intracoelomically) every 72 h for four doses. During the first 2 mo of temporary housing at NCSU-CVM, animals with visible lesions received ceftazidime (20 mg/kg intracoelomically every 72 h for 4–5 doses), and lesions were debrided and cleaned with povidone iodine or silver sulfadiazine. After 2 mo, all turtles were treated with 1 h dips in dilute Acriflavine Plus (Kordon LLC, Hayward, California, USA) daily for 7–14 d. Diagnostics performed on skin lesions revealed heavy growth of *Chrysosporium* sp. on bacterial culture. There was no evidence of iridovirus on virus isolation. Three turtles from the original confiscation group died during holding at NCSU-CVM. The first animal died after 1 mo at NCSU-CVM and necropsy revealed emaciation, septicemia, and fungal dermatitis. The second animal died after 5 mo at NCSU-CVM and necropsy revealed emaciation and systemic fungal infection consistent with aspergillosis. The third animal died after 7 mo at NCSU-CVM; necropsy revealed poor body condition and fungal dermatitis, but no evidence of systemic disease. At the time of blood collection (following 9 mo of rehabilitation, consistent nutrition, and medical care), mean weight of the turtles had increased to 59 g (43–88 g).

Plasma biochemical analysis.—The number of samples for various measures of plasma biochemistry from hatchling Pig-nosed Turtles, including the packed cell volume (PCV), ranged from 15 to 20 (Table 1). Parameter analysis revealed single outlier values for the following analytes: BUN, AST, potassium, phosphorus, and bicarbonate. Outliers for BUN, AST, and phosphorus were from the same individual; outliers

TABLE 1. Mean, median, range, and SD for the plasma biochemical values of confiscated hatchling Pig-nosed Turtles. Values reported as "<" (less than) indicate values below measurable threshold of the analyzer.

Analyte	Units (SI)	*N*	Mean	Median	Range	SD
PCV	%	19	12	11	5–22	5.0
Total protein	g/L	20	12	12	8–18	2.8
Glucose	mmol/L	20	2.9	2.8	2.0–4.0	0.53
BUN	mmol/L	16	1	1	0.4-2	0.5
Uric acid	mmol/L	20	8	< 12	< 12-18	4
ALP	U/L	16	353	302	149–792	193
AST	U/L	17	33	34	13–77	18
LDH	U/L	16	250	269	112–400	96
Total bilirubin	µmol/L	16	1	< 2	< 2-2	0.437
CK	U/L	16	378	323	99–897	252
Sodium	mmol/L	16	138	137	134–142	2.40
Potassium	mmol/L	15	3.6	3.6	2.9–4.3	0.36
Na/K ratio		16	38	38	30–47	4.4
Chloride	mmol/L	16	112	112	107–117	2.54
Calcium	mmol/L	20	1.9	1.9	1. 7–2.2	0.13
Phosphorus	mmol/L	15	0.55	0.58	0.34–0.77	0.090
Magnesium	mmol/L	16	0.99	1.1	0.95–1.4	0.12
Bicarbonate	mmol/L	15	25	25	21–29	2.4
Anion gap		16	5.0	5.1	2.3–6.6	1.2
Osmolality	mmol/kg	16	267	266	259–276	4.50

for potassium and bicarbonate were from different specimens.

DISCUSSION

There appear to be very few publications examining blood parameters of confiscated turtles. One study reporting both hematology and plasma chemistry values from nine Giant Asian River Turtles (*Orlitia borneensis*) suffering from shell necrosis found that despite marked improvement in the shell condition of the seven surviving animals, anemia and hypoproteinemia persisted for nearly 3 y (Knotkova et al. 2005). Another study (McCallum et al. 2011) evaluated body condition and reported on metabolic bone disease of confiscated hatchling Alligator Snapping Turtles (*Macrochelys temminckii*). This work did not include any blood parameters.

Other studies have reported baseline hematology and biochemical parameters for a number of chelonian species, including sea turtles (Kakizoe et al. 2007; Flint et al. 2010), freshwater turtles (Frair et al. 1978; Brenner et al. 2002; Innis et al. 2007; Chaffin et al. 2008; Müller and Brunnberg 2009), and tortoises (Dickinson et al. 2002). However, clinicians require species-specific (or at least taxon-specific) baseline values for the parameters commonly measured by commercial chemistry analyzers. When working with reptiles, species-specific health data are even more important due to the wide variety in environment and habitats within a taxon. This study represents the first published values for a plasma biochemistry panel in a turtle of the family Trionychidae. While complete blood cell count hematology would have enriched this study, funds and personnel resources were limited, so a decision to focus on plasma biochemistry values was made.

A number of parameters can influence biochemistry data in chelonians, including sex, age, and environmental conditions. Studies have found that females have consistently higher calcium, phosphorus, albumin, cholesterol, and triglyceride values compared to males, generally assumed to represent the metabolic consequences of egg production and vitellogenesis (Taylor and Jacobson 1982; Christopher et al. 1999; Brenner et al. 2002; Metin et al. 2008; Yilmaz and Tosunoglu, 2010). Other studies have shown increased enzyme activity in males, including alanine aminotransferase (ALT; Metin et al. 2006; Chung et al. 2009) and AST (Dickinson et al. 2002; Chung et al. 2009), attributed to either increased metabolic rate or aggression and subsequent tissue damage.

Published literature also shows that age can impact certain biochemical parameters in sea turtles. Kakizoe et al. (2007) tracked juvenile Loggerhead Sea Turtles (*Caretta caretta*) as they matured from 1 mo to 3 y of age, and noted the following: (1) progressively decreasing ALP (presumably due to gradual cessation of bone growth); (2) gradually increasing total protein, BUN, and creatinine; and (3) relatively low cholesterol and triglyceride values compared to adult animals. Casal et al. (2009) found that juvenile Loggerhead Sea Turtles had lower values of packed cell volume, total protein, albumin, globulins, cholesterol, triglycerides, and calcium compared with adult animals, presumably related to egg production in adult animals. Labrada-Martagón et al. (2010) found that total protein, albumin, and triglyceride levels increased with age in Green Turtles (*Chelonia mydas*). In contrast, other studies of Loggerhead Sea Turtles (Flint et al. 2010) and Alligator Snapping Turtles (Chaffin et al. 2008) found no significant differences in biochemical values between juvenile and adult turtles. It is important to recognize that the aforementioned species are not closely related to Pig-nosed Turtles and the juvenile versus adult data in this species could differ markedly.

Previous studies also demonstrate that environmental conditions can affect biochemical parameters in turtles. Seasonal changes may reflect reproductive events, food and water availability, or metabolic changes (hibernation/aestivation). For example, Dickinson et al. (2002) noted increases in cholesterol and triglycerides in female Desert Tortoises (*Gopherus agassizii*) in May and September, corresponding to egg production in this species. Several studies have found associations between water availability and biochemical evidence of dehydration, including elevated PCV and increased electrolyte and uric acid values (Taylor and Jacobson 1982; Christopher et al. 1999; Zaias et al. 2006). Additionally, several studies have correlated increased food availability with biochemical evidence of increased protein consumption and metabolism, such as elevated BUN and CK (Dickinson et al. 2002; Zaias et al. 2006; Chaffin et al. 2008). Metabolic demand also affects biochemical profiles, as demonstrated by hibernating Desert Tortoises, who experienced decreased glucose, total protein, albumin, cholesterol, calcium, phosphorus, and enzyme activity (AST, ALT, ALP), as well as increased BUN and plasma osmolality (Christopher et al. 1999). It should be noted that data obtained from unrelated chelonian species, especially terrestrial forms, cannot be accurately aligned with data for the Pig-nosed Turtle.

Our study population consisted of a group of hatchling turtles for which we could not determine sex who experienced consistent environmental conditions during the course of rehabilitation, and who were all sampled at a single point in time. Therefore, the structure of this study did not allow comparison among sexes, age groups, seasons, or environmental conditions. However, it is important to consider two major factors when comparing the results from Pig-nosed Turtles to the results of other studies: first, the young age of the turtles sampled and second, the health status of the study population. The sample population in this study was hatchling Pig-nosed Turtles, all presumed to be under one year old. The confiscated Pig-nosed Turtles were malnourished on arrival at NCSU-CVM and a number of the turtles suffered from presumed or confirmed bacterial and fungal infections during rehabilitation. Although the turtles gained nearly 100% of their body weight during 9 mo of rehabilitation and appeared overtly healthy at the time of blood sample collection, it is likely that these turtles displayed effects of chronic infection/inflammation, malnutrition, and stress.

Data from the Pig-nosed Turtles in our study differ in significant ways from previously published biochemical parameters in other turtle species. First, values for total plasma protein reported in our study were lower than previously established reference values for a number of land, sea, and freshwater turtle species (Marks et al. 1990; Raphael et al. 1994; Anderson et al. 1997; Hidalgo-Vila et al. 2007; Santoroand Meneses 2007). Furthermore, albumin was undetectable in the plasma samples from Pig-nosed Turtles in our study, while albumin was detectable in all studies previously cited. In addition, PCV values from our Pig-nosed Turtles were below reported values in other species (Raphael et al. 1994; Brenner et al. 2002; Hidalgo-Vila et al. 2007; Innis et al. 2007, 2009).

Values for BUN and uric acid in our Pig-nosed Turtles were lower than those previously reported in other species (Pagés and Peinado 1992; Anderson et al. 1997; Brenner et al. 2002; Chung et al. 2009; Flint et al. 2010). Furthermore, values for creatinine were below the measurable threshold for the analyzer for all turtles, while creatinine was detected in a number of previous studies (Anderson et al. 1997; Hidalgo-Vila et al. 2007; Gelli et al. 2008; Innis et al. 2009; Flint et al. 2010). In reptiles, BUN and creatinine are generally considered to be poor markers of renal function when compared to uric acid (Divers et al. 1996). However, BUN is a marker for protein consumption and metabolism (Chaffin et al. 2008; Dickinson et al. 2002; Zaias et al. 2006). It is plausible that despite appropriate diet during rehabilitation, the Pig-nosed Turtles in this study still suffered from protein deficiency as a result of prior malnutrition. In addition, low BUN and creatinine values may again be related to the hatchling age of the population (Kakizoe et al. 2007).

When considering hepatic enzymes, ALP values in our Pig-nosed Turtles were generally higher than those reported in other species, while AST values in our turtles were lower (Dickinson et al. 2002; Hidalgo-Vila et al.

2007; Santoro and Meneses 2007; Gelli et al. 2008; Labrada-Martagón et al. 2010). Alkaline phosphatase is found in hepatocytes, bone, and other tissues, while AST is found in hepatocytes and muscle tissue. Both enzymes can increase in liver disease in reptiles, although AST appears to have more clinical relevance (Divers et al. 1996). The high values for ALP in this study likely reflect increased bone growth in these young turtles (Kakizoe et al. 2007). Increased ALP could also suggest liver disease, although a concurrent decrease in AST would not be expected.

Values for glucose, sodium, potassium, chloride, and calcium in our Pig-nosed Turtles were generally consistent with levels previously reported in other chelonian species (Taylor and Jacobson 1982; Brenner et al. 2002; Reese et al. 2003; Innis et al. 2009; Flint et al. 2010). Values for phosphorus in our Pig-nosed Turtles were lower than some previously reported results (Chaffin et al. 2008; Gelli et al. 2008; Innis et al. 2009; Labrada-Martagón et al. 2010) but were more consistent with those reported in other studies (Brenner et al. 2002; Innis et al. 2007; Santoro and Meneses 2007; Flint et al. 2010). This discrepancy could either represent a relevant physiologic difference across taxa, a result of nutritional imbalance, or simply the young age and lack of reproductively active females in our study population (Taylor and Jacobson 1982; Christopher et al. 1999).

Albumin, creatinine, and GGT were not detected in the plasma of any turtle in our study. Undetectable serum albumin and creatinine in the present population may be the result of health status or age of the confiscated turtles, as previously discussed. Undetectable levels of GGT are consistent with the findings of Flint et al. (2010), who also found no measurable GGT in a population of Loggerhead Sea Turtles. However, other studies (Chaffin et al. 2008; Deem et al. 2009; Innis et al. 2009) have detected GGT in plasma of various turtle species. This discrepancy may reflect variations in laboratory equipment or technique, or may suggest differences in enzymology or metabolism among chelonian species.

The present study had several limitations. First, the small sample size (n = 20) precluded the calculation of formal reference intervals, which would require a minimum of 120 individuals (Geffre et al. 2009). However, given the limited sample population available, and the lack of previously published data regarding biochemical parameters in Trionychid turtles, we feel these results still provide a useful baseline for clinicians and researchers. Second, it is possible that the results of this study may have been affected by lymph contamination of blood samples. Previous studies have documented that lymph contamination in chelonians can result from certain sampling sites, including the dorsal coccygeal vein (López-Olvera et al. 2003; Chaffin et al. 2008). Lymph contamination may affect the results of multiple biochemical parameters, including PCV, total protein, uric acid, ALP, AST, LDH, calcium, and phosphorus (López-Olvera et al. 2003). Although no gross lymph contamination was witnessed during sampling for this study, and no specific studies have investigated lymph contamination from the subcarapacial sinus, it is possible that minor lymph contamination affected our results. Finally, our study population consisted of hatchling turtles that experienced varying degrees of malnutrition, stress, and disease. Given this single population, it is therefore difficult to tease out the effects of age, malnutrition, and disease when comparing plasma biochemical values to those obtained by other studies.

Data reported in this study represent a baseline against which future plasma biochemistry results in Pig-nosed Turtles may be compared and also demonstrate associations between certain biochemical parameters, age, and health status. Such assessments are important for health monitoring and disease diagnostics in this species. Because Pig-nosed Turtles are a vulnerable species with importance in the wildlife biology research community, pet trade, and aquarium/zoo industry, assessments of health and disease are important from the standpoint of sustainable conservation and management. These biochemistry data add to a growing database of knowledge about health management in wild chelonian species. Future research should continue to establish reference values in other turtle species, as well as compare biochemical values across age groups and disease states.

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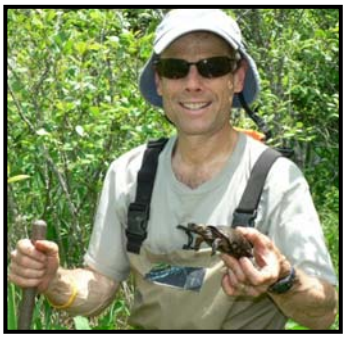

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