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ZOOLOGÍA

HEMATOLOGYCAL AND BLOOD BIOCHEMISTRY PARAMETERS OF CAPTIVE BIG-HEADED AMAZON RIVER TURTLES, *Peltocephalus dumerilianus* (TESTUDINES: PODOCNEMIDIDAE)

Parámetros hematológicos y bioquímica sanguínea de tortugas amazónicas cautivas de cabeza grande, *Peltocephalus dumerilianus* (Testudinas: Podocnemididae)

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ABSTRACT

The determination of hematological values is used to obtain knowledge about the health conditions of animal species. The big-headed Amazon River turtles, (*Peltocephalus dumerilianus*) are considered one of the least known testudine species concerning their biology and health status. Herein, we determined the hematological and plasma biochemical parameters of 17 (eight males and nine females) adult *P. dumerilianus* to provide reference interval values for clinically healthy individuals. We collected the blood samples by puncturing the femoral vein using long heparinized hypodermic syringes. Sexual dimorphism for individuals was determined by external observation of the shape of the plastron. The average values obtained for the ten hematological and biochemical parameters analyzed were red blood cell count = 0.32 million μL^{-1}; hematocrit = 20.6 %; hemoglobin = 8.5 g dL^{-1}; mean corpuscular volume = 681.6 fL; mean corpuscular hemoglobin = 267.8 pg; mean corpuscular hemoglobin concentration = 41.9 g dL^{-1}; glucose = 80.6 mg dL^{-1}, total protein = 4.1 g dL^{-1}, triglycerides = 388.9 mg dL^{-1}, and total cholesterol = 79.3 mg dL^{-1}. Despite the sexual dimorphism evidenced for the species, there was no significant statistical difference between males and females for both hematological and biochemical parameters analyzed herein. Based on these results, the population is considered healthy, with parameter values coinciding with previously reported reference ranges for testudines species in the region. The results obtained in this study can be used for assessing the health status of other Amazonian turtle populations, especially in actions aimed at cultivation strategies, management, and species conservation.

Keywords: Amazon, blood, hematology, physiology, reptiles.

RESUMEN

La determinación de valores hematológicos se ha utilizado para conocer las condiciones sanitarias de algunas especies animales. La tortuga cabezona del río Amazonas, *Peltocephalus dumerilianus*, se considera una de las especies de testudines menos conocidas en relación a su biología y estado de salud. Aquí, determinamos los parámetros bioquímicos hematológicos y plasmáticos de 17 adultos (ocho machos y nueve hembras) de *P. dumerilianus* con el fin de proporcionar valores de intervalo de referencia sobre los

individuos clínicamente sanos. Recolectamos las muestras de sangre perforando la vena femoral con jeringas hipodérmicas largas heparinizadas. El dimorfismo sexual de los individuos se determinó mediante la observación externa de la forma del plastrón. Los valores medios obtenidos para los diez parámetros hematológicos y bioquímicos analizados fueron: recuento de glóbulos rojos = 0,32 millones μL^{-1}; hematocrito = 20,6 %; hemoglobina = 8,5 g dL^{-1}; volumen corpuscular medio = 681,6 fL; hemoglobina corpuscular media = 267,8 pg; concentración media de hemoglobina corpuscular = 41,9 g dL^{-1}; glucosa = 80,6 mg dL^{-1}, proteína total = 4,1 g dL^{-1}, triglicéridos = 388,9 mg dL^{-1} y colesterol total = 79,3 mg dL-1. A pesar del dimorfismo sexual evidente para la especie, no hubo diferencia estadística significativa entre machos y hembras para los parámetros hematológicos y bioquímicos analizados aquí. Con base en estos resultados, la población se considera saludable y los valores de los parámetros coinciden con los rangos de referencia reportados previamente de las especies de testudines en la región. Los resultados obtenidos en este estudio pueden utilizarse en la evaluación del estado de salud de otras poblaciones de tortugas amazónicas, considerando especialmente aquellas acciones dirigidas al manejo, conservación y estrategias de cultivo de la especie.

Palabras clave: amazónica, fisiología, hematología, reptiles, sangre.

INTRODUCTION

The determination of both hematology and biochemistry reference values is used for obtaining knowledge regarding the health status of wild populations (Aride *et al*., 2015; Oliveira *et al.,* 2016; Nascimento *et al.,* 2020). According to Stevenson *et al.* (2005), this practice has led to the strengthening of a research field known as conservation physiology. This is an important concept since it aims to understand in detail the mechanisms that cause conservation problems based on the hematological, metabolic, endocrine, and immunological parameters of animals (Stevenson *et al*., 2005).

These evaluations are important to characterize distinctive physiological parameters and determine specific values for different types of disease (Aguirre and Balazs, 2000; Kakizoe *et al*., 2007; Oliveira *et al.,* 2017). Therefore, this type of clinical study has often been applied to research involving endangered species, such as frequently is the case for the species of sea turtle (Prieto-Torres *et al.*, 2012; 2013; Montilla *et al.*, 2014), or even in species that have potential for commercial use (Stevenson *et al*., 2005; López-Martínez *et al.*, 2020). Maceda-Veiga *et al.* (2015) affirm that research on blood components contributes to the monitoring of ecological conditions of vertebrate species and can be used in the preservation of animal health and environmental health.

In the Amazon region, the Testudines (Animalia: Vertebrate: Reptilia) have great economic value, since they are commonly exploited for their meat, eggs, paws, and the carapace, as well as for use as adornments (Oliveira *et al*., 2011; Tavares-Dias *et al*., 2012). Due to this scenario, and because they are very easy to capture in the natural environment, several species have virtually disappeared in some regions. Nevertheless, the Brazilian government has created laws aimed at protecting these species, as well as encouraging their rearing in a captive environment (Andrade, 2008). However, currently, few studies assess the physiological state and clinical health of individuals from both natural and captive environments.

The big-headed Amazon River turtle (*Peltocephalus dumerilianus* Schweigger, 1812) is considered to be one of the least known Testudine species regarding its biology and health status. Recent information suggests that big-headed Amazon River turtles are being widely used as a food source in locations where populations of larger species, such as *Podocnemis unifilis* (Troschel, 1848) and *Podocnemis expansa* (Schweigger, 1812), have become reduced. Because of its economic and social relevance, the conservation status of *P. dumerilianus* has become ever more critical, and it is now classified by the IUCN (2015) as a species that is vulnerable to extinction. Moreover, due to a variety of reasons, baseline health data are not available for many populations throughout the Amazon region. Therefore, it is very important to perform local studies to establish reference values for the conditions of animals and their environment (Aguirre and Balazs, 2000; Montilla *et al.*, 2006; 2014; Stevenson *et al*., 2005; Kakizoe *et al*., 2007; Prieto-Torres *et al*., 2012; 2013; Lara Resendiz, 2020).

The aims of this study were as follows: 1) obtain the hematological and blood biochemistry reference values for the *P. dumerilianus* population from the Amazon region; 2) compare the blood chemistry values with data previously reported for Testudine populations; 3) determine hematological and blood biochemistry differences within the population, according to sex and size of the animals.

MATERIALS AND METHODS

Sample size, blood collection, and physical examination

All procedures developed in this study were performed according to the license (number 41350) provided by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) and approved by the CEUA (Animal Ethics Committee) at the Universidade Federal do Amazonas (UFAM) under protocol number. 005/2016. From January to March 2018, we collected blood samples from the seventeen adult *P. dumerilianus* (eight male and nine female) from rearing systems located in the municipality of Manaus, Amazonas, Brazil. The animals were kept in a

20 000 liter tank of water and subsequently captured with the aid of a trawl net. All individuals appeared to be clinically healthy and were feeding normally on vegetables and fish. Immediately after capture, animals were disinfected with iodized alcohol to obtain blood samples by puncture of the femoral vein using 25 gauge, long, heparinized hypodermic syringes (5000 IU, SR brand, Brazil) (Oliveira-Júnior *et al*., 2009; Oliveira *et al*., 2011).

After blood collection, some pressure was applied and the affected area was treated to avoid the formation of hematomas (Aguirre *et al*., 1995). The tubes with the samples were kept on ice in a cooler (for no more than one hour) before being processed at the Laboratory of Animal Morpho-Physiology at the Instituto Federal de Educação, Ciência e Tecnologia (IFAM), Manaus Central Campus (MCC).

We performed a physical examination on each big-headed Amazon River turtle to assess the behavior, movement, body condition, absence of ectoparasites, tumors, and lesions on skin and carapace (Harris *et al*., 2011). The sexual determination was made by external observation of the shape of the end of the plastron. Likewise, for all turtles, the straight carapace length (SCL), straight width of the carapace (SCW), straight length of the plastron (SLP), and straight width of the plastron (SWP) were measured using a flexible measuring tape. Bodyweight (kg) for each individual was obtained using a portable set of scales. Sexual dimorphism for individuals was determined by external observation of the shape of the plastron.

Hematological parameters and biochemical analysis

Erythrocyte counts (RBC) were conducted in a Neubauer chamber, after dilution of the samples in a formalin-citrate solution (Oliveira-Júnior *et al*., 2009); hematocrit (Ht) was determined using the microhematocrit method; and the hemoglobin (Hb) concentration was obtained using the cyanmethemoglobin method (Oliveira-Júnior *et al*., 2009). Via these data, the following red cell indexes were calculated: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) (Wintrobe, 1934).

Blood smears were prepared and stained following the recommendations of Oliveira *et al*. (2011). Subsequently, these were used for morphological identification of leukocyte and total thrombocyte counts (Oliveira-Júnior *et al*., 2011), and for leukocyte differential counts, which were based on the counts of 200 leukocyte types of interest.

After blood centrifugation, plasma was obtained to determine glucose (mg dL^{-1}), total protein (g dL^{-1}), triglycerides (mg dL^{-1}), and total cholesterol (mg dL^{-1}) concentrations with the aid of specific commercial kits (Labtest Diagnóstica®, Brazil).

Statistical analyses

Data were expressed as mean, standard deviation (SD), and reference intervals for each hematological and biochemical parameter. To evaluate data normality, we used the Shapiro-Wilk test. Because our sample size was low, reference interval values were only calculated for the whole population. We calculated these values in two ways (Prieto-Torres *et al*., 2012;2013): 1) all values between the mean and two SDs were included for normally distributed variables, and 2) data found between the 2.5^{th} and 97.5^{th} percentiles were selected for variables that were not normally distributed. All analyses were performed using a significance level of 95 % (p = 0.05) using R software, v. 3.5.3.

RESULTS

All the big-headed Amazon River turtle individuals were alert and active during capture and were considered clinically healthy upon physical examination (Thomson *et al*., 2009; Ferrando Gaibisso, 2010; Harris *et al*., 2011). Captured animals were classified as adults based on their morphological measurements. We observed statistically significant differences (p <0.05) among mostly morphological measurements among individuals according to their sex; the males were those with the higher values for SCL, SCW, SLP, and weight (Table 1).

Regarding the leukocyte and thrombocyte morphology in *P. dumerilianus*, the staining technique used did not

Table 1. Values obtained for morphological variables of big-headed Amazon River turtles (*Peltocephalus dumerilianus* Schweigger, 1812), sampled in a captive breeding system located in the city of Manaus, Amazonas state, Brazil.

Parameters	Male (n= 8)	Female (n= 9)	*P*
Straight carapace length (cm)	43.0 ± 8.5 (27.0 – 52.0)	33.0 ± 6,3 (23.0 – 42.0)	0.025*
Straight carapace width (cm)	38.7 ± 8.9 (22.0 – 48.0)	30.2 ± 4.9 (22.0 – 37.0)	0.029*
Straight plastron length (cm)	31.0 ± 6.7 (18.0 – 37.0)	23.9 ± 4.4 (17.0 – 30.0)	0.023*
Straight plastron width (cm)	28.9 ± 5.8 (18.0 – 35.0)	24.5 ± 4.2 (18.0 – 31.0)	0.096
Weight (kg)	18.2 ± 8.2 (3.2 – 26.2)	9.2 ± 5.5 (2.4 – 17.8)	0.020*

* denotes a significant difference between males and females: Student's t-test (p < 0.05).

present satisfactory results, making the morphological and quantitative analysis of leukocytes and thrombogram impossible. This difficulty may have been caused by a specific biological agent in the blood of *P. dumerilianus*, demonstrating that the blood extension staining technique used in other species of Amazonian turtles (Oliveira-Júnior *et al.,* 2009; Oliveira *et al.*, 2011; Tavares-Dias *et al.*, 2012) is not effective for all species of testudinids in the Amazon region.

Despite the sexual dimorphism evidenced for the species, there was no significant statistical difference in the hematological parameters analyzed between males and females (Table 2). No statistically significant differences in the results of the erythrogram were found between adult *P. dumerilianus* males and females (Table 3).

DISCUSSION

Sexual dimorphism has been previously observed in studies conducted with *P. dumerilianus* individuals in natural environments. Males are generally larger than females (De La Ossa-V *et al.*, 2011). The study by Pritchard and Trebbau (1984) recorded male specimens with SCL ranging from 37.4 to 42.2 cm, while females showed values between 27.1 and 27.6 cm, both of which are lower than the average values obtained in this study. Similar results to those found in our study were also recorded in studies by Pezzutti (2003) and Iverson and Vogt (2002). On the other hand, we observed a maximum bodyweight of 26.2 kg for males and 17.8 kg for females, which is higher than the weight recorded for wild individuals (15 kg) by Iverson and Vogt (2002) and Pezzutti (2003).

Differences observed for the morphological variables among individuals from different populations (including captive populations) may be explained by several factors, such as aquatic environment quality, geographical location, genetic variability, gender, age, and nutritional status (Oliveira-Júnior *et al*., 2009). For the present study, we considered that nutritional status was the most probable factor for such differences regarding previous studies.

Table 2. Hematological and biochemical parameters of the big-headed Amazon River turtles (*Peltocephalus dumerilianus* Schweigger, 1812) compared with *Podocnemis* species. Animals from a captive breeding system located in the city of Manaus (Brazil).

Species	*P. dumerilianus*			*P. erythrocephala*		*P. expansa*	*P. expansa*	*P. unifilis*	*P. sextuberculata*
Parameters	**Adult male (n= 8)**	**Adult female (n= 9)**	***p****	**Adult male (n= 68)**	**Adult female (n= 35)**	**Adult male and female (n= 28)**	**Adult male and female (n= 28)**	**Adult male and female (n= 28)**	**Adult male and female (n= 28)**
Hematological parameters									
Ht (%)	20.6. ± 3.7	20.5 ± 3.6	0.945	21.5 ± 0,4	20.8 ± 0.4	25.1 ± 6.9	21.8 ± 6.6	23.2 ± 4.7	17.6 ± 5.4
Hb (g dL^{-1})	7.9 ± 2.4	9.0 ± 2.7	0.384	6.3 ± 1.3	5.7 ± 1.1	6.5 ± 1.21,3	5.1 ± 2.3	7.0 ± 1.6	4.7 ± 1.2
RBC (million μL^{-1})	0.34 ± 0.08	0.29 ± 0.05	0.171	0.44 ± 0.08	0.41 ± 0.08	0.28 ± 0.07	0.28 ± 0.08	0.18 ± 0.08	0.16 ± 0.05
MCV (fL)	637.2 ± 71.2	716.2 ± 173.1	0.278	485.5 ± 90.3	477.2 ± 117.8	922.3 ± 150.2	851.4 ± 282.1	1425.1 ± 448.3	1,291.3 ± 652,3
MCH (pg)	247.8 ± 37.1	285.3 ± 69.6	0.226	29.11 ± 3.4	27.6 ± 3.6	26.2 ± 5.4	-	-	-
MCHC (g dL^{-1})	39.0 ± 4.9	44.2 ± 12.1	0.306	142.1 ± 26.6	130.5 ± 32.6	-	22.3 ± 5.5	30.1 ± 3.3	27.3 ± 4.2
Biochemical parameters									
Glucose (mg dL^{-1})	76.4 ± 8.1	83.4 ± 8.0	0.138	116.4	116.4	91.3 ± 17.7	92.7 ± 22.2	149.9 ± 66.5	127.1 ± 42.2
Total proteins (g dL^{-1})	3.6 ± 1.2	4.4 ± 2.1	0.471	20.8 ± 3.4	20.8 ± 4.3	3.5 ± 1.3	2.4 ± 0.7	3.8 ± 0.7	1.6 ± 0.3
Triglycerides (mg dL^{-1})	379.1 ± 46.5	397.5 ± 31.2	0.377	12.72 ± 3.6	14.5 ± 7.3	35.4 ± 19.7	18.9 ± 7.0	27.8 ± 8.3	37.7 ± 9.9
Total cholesterol (mg dL^{-1})	86.7 ± 10.9	73.8 ± 27.6	0.192	32.7 ± 9.1	38.2 ± 12.7	58.1 ± 18.3	38.3 ± 26.5	125.0 ± 55.2	117.3 ± 51.4
Locality	Farm in Manaus, Amazonas, Brazil			Mariuá Archipelago, Middle Rio Negro, Amazonas, Brazil		Farm in Manaus, Amazonas, Brazil	Abufari Biological Reserve, downstream Purus River, Amazonas, Brazil		
References	Present study			Santos, 2011		Oliveira-Júnior *et al.,* 2009	Tavares-Dias *et al.,* 2012		

* Student's t test.

Table 3. Mean and reference values for hematological and biochemical parameters of healthy big-headed Amazon River turtles (*Peltocephalus dumerilianus* Schweigger, 1812) from a captive breeding system, located in the city of Manaus, Amazonas state, Brazil.

Parameters	Mean ± standard deviation	Confidence interval 95 %	Median	25° - 75° percentile
Hematological parameters				
Ht (%)	20.6 ± 3.5	18.7 – 22.4	21.0	18.0 – 23.0
Hb (g dL^{-1})	8.5 ± 2.5	7.2 – 9.8	8.7	7.1 – 10.2
RBC (million μL^{-1})	0.32 ± 0.07	0.28 – 0.35	0.31	0.26 – 0.37
MCV (fL)	681.6 ± 140.1	606.9 – 756.3	673.2	557.6 – 733.7
MCH (pg)	267.8 ± 58.2	235.6 – 300.1	276.4	234.9 – 290.5
MCHC (g dL^{-1})	41.9 ± 9.8	36.7 – 47.1	39.8	34.6 – 47.8
Biochemical parameters				
Glucose (mg dL^{-1})	80.6. ± 8.5	75.9 – 85.3	80.2	72.6 – 86.0
Total protein (g dL^{-1})	4.1 ± 1.7	2.9 – 5.2	4.2	2.0 – 5.4
Triglycerides (mg dL^{-1})	388.9 ± 38.8	367.4 – 410.4	394.4	354.9 – 422.5
Total cholesterol (mg dL^{-1})	79.3 ± 22.3	66.4 – 92.2	78.6	55.3 – 97.4

According to De La Ossa-V *et al*. (2011), the big-headed turtle is classified as an omnivorous species, and carnivorous and omnivorous individuals tend to have plasma biochemistry values superior to herbivorous species.

The results of the erythrogram (Ht, Hb, RBC, MCV, MCH, and MCHC) help to determine the type of anemia, volume, dehydration, and responses associated with parasite levels in chelonian individuals reared in both natural and cultivated environments (Marcon *et al.*, 2008; Oliveira-Junior *et al.*, 2009; Oliveira *et al.*, 2011; Morselli *et al.*, 2016). The study by Tavares-Dias *et al*. (2012) establishes erythrogram values for the Amazonian freshwater turtles *P. expansa*, *P. unifilis* (Troschel, 1848), and *Podocnemis sextuberculata* (Cornalia, 1849) considering ontogeny, fishing tools, and forced diving. The Ht, RBC, and MCV values for *P. dumerilianus* are similar to the values of captive *P. expansa* individuals (Oliveira-Júnior *et al*., 2009; Tavares-Dias *et al*., 2012), which indicates an oxygen uptake similar to the described Testudine species. For Hb, MCH, and MCHC, the values were closer to those related to the *P. unifilis*. However, it is noteworthy that these studies did not investigate issues associated with sexual dimorphism. Thus, according to the erythrogram, *P. dumerilianus* has intermediate biological characteristics that fall between *P. expansa*, and *P. unifilis*.

In the biochemical analysis, no statistically significant differences were found between males and females about plasma glucose, total protein, triglyceride, and total cholesterol levels, which is similar to the results described for *P. expansa, P. unifilis* and *P. sextuberculata* (Tavares-Dias *et al.*, 2012). In the present study, we observed that the glucose values were similar to those described by Oliveira-Júnior *et al*. (2009) and Tavares-Dias *et al*. (2012) for *P. expansa*. These indices indicate normoglycemia in the *P. dumerilianus* when compared to other species sampled from the natural environment (Tavares-Dias *et al*., 2012). Total protein levels for captive animals are generally lower than those in wild animals (Norton, 1990; Christopher *et al.*, 2003), and this variable is associated with food management. Thus, the total protein levels of *P. dumerilianus* were similar to those described for *P. expansa* (Marcon *et al*., 2008; Oliveira-Junior *et al.*, 2009; Tavares-Dias *et al.*, 2012), but were lower than those described for *P. unifilis* and *P. sextuberculata* (Tavares-Dias *et al*., 2012).

For triglyceride and total cholesterol levels, the values found were higher than those described for *P. expansa* (Marcon *et al.*, 2008; Oliveira-Junior *et al.*, 2009; Tavares-Dias *et al.*, 2012). Cholesterol levels were lower than those shown for *P. unifilis* and *P. sextuberculata* (Tavares-Dias *et al.*, 2012). Thus, we can infer that, for the big-headed turtle, there is possibly a case of a lipid metabolism disorder. This can be measured by changes in cholesterol, which is secreted from the liver as bile acids (Swimmer, 2000). These changes may occur due to the diet provided, which presents high-fat values and promotes an increase of triglycerides and cholesterol. Also, factors such as lack of exercise in a confined condition promote increased blood fat levels.

CONCLUSIONS

Based on the erythrogram data, we can infer that the big-headed turtle is a species with intermediate characteristics that fall between the species *P. expansa* and *P. unifilis*. In the plasma biochemistry, glucose and total protein values are similar to *P. expansa*. Total cholesterol and triglyceride levels were high,

certainly resulting from inadequate nutrition management, as well as the size of the tanks to promote greater swimming activity for this Amazonian Testudine species.

The application of reference hematological intervals is fundamental for the monitoring of species in both natural and farm environments since they serve as a basis for actions related to the sustainable use of these resources. The hematological parameters for the *P. dumerilianus* determined in the present study can be used in actions aimed at species management, conservation, and captive breeding strategies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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