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Biochemical and hematological reference ranges for Amazon freshwater turtle, *Podocnemis expansa* (Reptilia: Pelomedusidae), with morphologic assessment of blood cells

A.A. Oliveira-Júnior [a], M. Tavares-Dias [b,*], J.L. Marcon [a]

[a] *Department of Physiological Sciences, Institute of Biological Sciences, Federal University of Amazonas, Av. Gal. Rodrigo Octávio Jordão Ramos, 3000, 69.077-000 Manaus, Amazonas State, Brazil*

[b] *Health and Biotechnology Institute, Federal University of Amazonas, Estrada Coari-Mamiá, 305, 69.460-000 Coari, Amazonas State, Brazil*

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Abstract

Blood parameters are useful to measure physiological disturbances in chelonians. Thus they can provide important information for the diagnosis and prognosis of diseases. The aim of this paper was to determine the reference range of plasma glucose, total protein, triglycerides, cholesterol and urea levels, as well as hematocrit, hemoglobin, red blood cell, thrombocyte and white blood cell counts for healthy *Podocnemis expansa* (Schweigger, 1812), bred in a turtle farm in the Amazonas State, Brazil. Plasma glucose, hemoglobin, mean corpuscular volume (MCV), lymphocytes and heterophils were the parameters with the smallest variations. Significant ($p < 0.001$) correlations between red blood cells count and hematocrit and hemoglobin concentration were found. Turtle *P. expansa* had lymphocytes, azurophils, heterophils, eosinophils and basophils with morphologic features similar to the ones of others turtles species. No investigated blood parameter was influenced by sex. In further studies, the established reference ranges might be useful for the health assessment of this turtle species.

Keywords: Blood; Physiology; Hematology; Leukocytes; Chelonian; Turtles

1. Introduction

The giant turtle from Amazon, *Podocnemis expansa* (Schweigger, 1812), is the largest freshwater chelonian of South America, measuring from 75.0 to 107.0 cm in length. This turtle species has a wide geographic distribution in Amazon, inhabiting rivers, lagoons, swamps and flood plains of Brazil, Bolivia, Colombia, Ecuador, Guyana, Peru and Venezuela (Viana et al., 2004). In Brazil, *P. expansa* turtles have been used for human feeding since the beginning of colonization (Mundim et al., 1999), because its meat and eggs are very appreciated by the Amazon people who live near the forests and rivers. The Amazon turtle is at risk of extinction (Diniz and Santos, 1997), and therefore it is protected by federal law. As a consequence, the culture of the Amazon turtle was introduced in the Amazonas State by environmental authorities in 1999, in an attempt to preserve the natural populations of this species. However, despite all these facts, there is still little information on the physiology of *P. expansa*, especially regarding their hematological profile.

For wild *P. expansa* population from Araguaia River (GO, Brazil) some serum biochemical parameters (Mundim et al., 1999) and leukocytes percentage (Oliveira et al., 2000) have been reported, whereas for captive turtles only serum biochemical values have been reported (Santos et al., 2005). For wild *P. expansa* from Amazon, biochemical plasma and red blood cells parameters have been studied in a population from Purus River (Marcon et al., 2004).

* Corresponding author. Tel./fax: +55 9735612363.
E-mail address: mtavaresdias@ufam.edu.br (M. Tavares-Dias).

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Therefore, no reference range for such hematological parameters has yet been established for this important turtle species, and data regarding leukocytes are still extremely modest.

In chelonian species, intraspecific variation has been reported in different turtle species, because variability may be expected among different individuals (Anderson et al., 1997; Troiano and Silva, 1998; Pires et al., 2006), due to some internal and external factors such as the quality of the aquatic environment, genetic variation, nutritional status, sex and age. Within chelonian species, normal values may also vary with geographic localization of the turtle, age or size, sex, season, diet and activity (Taylor and Jacobson, 1982; Bolten and Bjorndal, 1992; Anderson et al., 1997; Peterson, 2002; Pires et al., 2006; Casal and Orós, 2007). In addition, some turtles are capable of great adaptations (Peterson, 2002), which might make difficult a comparison among chelonian species with very different lifestyles.

To help assess the causes of disease (Munro et al., 1998; Knotková et al., 2005, Casal and Orós, 2007) and to evaluate populations that are potentially at risk, the establishment of reference ranges are needed. In addition, assessing the physiological status of endangered species is a high priority because it helps to develop an appropriate management for wild as well as for captivity individuals (Bolten and Bjorndal, 1992; Pires et al., 2006). Therefore, the present study determined the reference ranges of twenty-two blood parameters for farmed *P. expansa* turtles. Thus, this is the most complete report regarding hematological parameters for this important chelonian species from Amazon.

2. Materials and methods

2.1. Turtles and culture conditions

Twenty-eight specimens of healthy *P. expansa* (9 males and 19 females) collected from a commercial farm in Manaus (Amazon State, Brazil) were used for the determination of blood parameters. The turtles were maintained in ponds of 470 m^2. They were feed daily with aquatic macrophites (*Pistia stratiotes* and *Azolla filiculoides*) and pelleted fish meal with 34.0% of crude protein. When the blood samples were collected, the water temperature was 28.2 °C and oxygen concentration was 3.55 mg/L.

2.2. Blood parameters determination

Each turtle was quickly caught and while it was physically restrained, a blood sample was collected from the caudal vessel with syringes coated with sodium heparin (2.500 UI/mL). These samples were used for determining the hematological parameters. Red blood cell counts (RBC) were determined optically with a Neubauer chamber using a stain solution (38.0 g sodium citrate, 20.0 mL 37–40% formalin, 0.2 g toluidine blue, distilled water in q.s.p 1000.0 mL). Hematocrit (Hct) was determined by microhematocrit method, and hemoglobin concentration (Hb) by cyanomethaemoglobin method. From these primary indices resulted the secondary Wintrobe indices, the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC).

In order to control as many variables as possible and to produce good-quality blood smears, they were made immediately after blood collection and actively air-dried to avoid thrombocyte clumping and morphologic changes. In addition, the blood smears were soon stained with a combination of May Grünwald–Giemsa–Wright (Tavares-Dias and Moraes, 2003). Blood smears were used for total thrombocyte (TT), total white blood cell (WBC) counts (Tavares-Dias et al., 2008), and WBC differential relative and absolute counts. Two hundred leukocytes were counted for each blood smear for differential relative and absolute counts. They were classified as lymphocytes, azurophils, heterophils, eosinophils and basophils. The blood was centrifuged and the plasma was used for determining glucose, total protein, urea, triglycerides and cholesterol, which were measured by commercial kits (Doles, Brazil).

2.3. Statistical analysis

The normality of the data was evaluated by applying the Kolmogorov–Smirnov test. The outliers were identified and discarded, thus the remaining data yielded a normal distribution (Horn et al., 2001). For each normally distributed parameter analyzed the mean, the standard deviation and reference ranges (defined as 95% confidence intervals) were calculated. In addition, linear regression was performed between RBC, hematocrit and hemoglobin concentration, and significance testing was set at 0.05.

3. Results

In order to establish reference ranges, no turtle with lesions on the plastron or carapace was used. The turtles ranged from 0.5 to 12.8 Kg (4.3 ± 3.3 Kg) in weight, straight-line carapace length ranged from 19.0 to 53.0 cm (33.6 ± 10.3 cm) and plastron length ranged from 16.0 to 45.5 cm (28.0 ± 9.0).

Reference ranges established for biochemical and red blood cells parameters of *P. expansa* are given in Table 1. No hematological parameters were influenced by sex. The number of samples tested for each blood parameter is indicated at the table. Triglycerides and cholesterol were the parameters with the highest variation, while glucose, hemoglobin and MCV the variables with the smallest variation. Positive correlations ($p < 0.001$) of red cells count with hematocrit [$\text{Hct} = 3.319 + (79.052) \times (\text{RBC})$] and hemoglobin [$\text{Hb} = 0.623 + (20.607) \times (\text{RBC})$] (Fig. 1), as well as between hematocrit and hemoglobin [$\text{Hb} = 1.066 + (0.209) \times (\text{Hct})$] (Fig. 2) were found.

In blood smears, mature erythrocytes were homogeneous in size, shape and color. They were typically oval,