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HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS FOR THE PIRARUCU *ARAPAIMA GIGAS* SCHINZ, 1822 (OSTEOGLOSSIFORMES, ARAPAIMATIDAE) IN NET CAGE CULTURE

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Abstract: In the present work fish blood parameters such as erythrocytes, thrombocytes, leukocytes, electrolytes (Cl^-, Na^+, K^+ and Ca^{+2}) and metabolic products (total protein, glucose, urea, triglycerides and cholesterol) were measured in healthy *Arapaima gigas*, when kept in culture in Central Amazon. The mean ± standard deviation and range were established. Red blood cell indices of *A. gigas* indicate a high demand for oxygen with high carrying oxygen capacity. Hematological values reported in this work provide basic information on a species of increasing economic interest, and therefore these values may be a useful tool for the control of their state of health and nutritional conditions. Furthermore, comparisons of these results with those of wild *A. gigas* could help to better understand these parameters in natural populations.

Key Words: freshwater fish, haematology, *Arapaima gigas*, leukocytes, plasma chemistry

Introduction

The *Arapaima gigas* (pirarucu) is the unique representative fish from the Arapaimatidae family and is an obligatory air breathing found only in South America. It is one of the most important species to improve the intensive aquaculture development in the Amazonian area (Ono et al. 2003; 2004; Andrade et al. 2006). Despite its carnivorous diet, it exhibits a high growth rate (Ono et al. 2003; 2004). It can be easily trained to accept artificial feeding (Ono et al. 2003; 2004, Andrade et al. 2006) and great roughness in handling (Andrade et al. 2006).

Knowledge of the pirarucu's physiology is sometimes obscure, especially concerning blood characteristics. In animals, the blood evaluation parameters provide a routine important tool in clinical veterinary medical practice (Tavares-Dias et al. 2004; Tavares-Dias and Mataqueiro 2004; Tavares-Dias and Moraes 2006). In such matters, the same principle can be used for fish. The study of the fish blood parameters are also important for determining factors related to its physiological capacity (Affonso 2001; Wells et al. 2005). Hematocrit, haemoglobin and the erythrocytic haemoglobin concentration values indicate the oxygen-carrying capacity in teleosts. Such parameters are highly variable among the species interfering in the oxygen-carrying capacity (Affonso 2001; Tavares-Dias and Moraes 2004; Wells et al. 2005).

The precise blood oxygen affinity observed within the species may represent and adaptation to its particular oxygen requirements. The ability of fish haemoglobin to reversibly bind oxygen represents a compromise between the affinity required to extract oxygen from water and the necessity of delivering the oxygen to the tissues (Affonso 2001; Wells et al. 2005). For this reason, fish differ considerably in their activity patterns and habitats. Therefore, the primary hematological variables associated with oxygen transport may serve as indices of the aerobic capabilities of the fish (Affonso 2001; Wells et al. 2005; Tavares-Dias and Moraes 2004). Leukocytes and thrombocytes are considered important parameters to evaluate both the fish's state of health and their immune system (Tavares-Dias et al. 2004; Tavares-Dias and Mataqueiro 2004; Tavares-Dias and Moraes 2006).

Intensive aquaculture conditions put increasing demands on the fish, with different stress factors affecting their basic physiological functions (Tavares-Dias and Moraes 2003; Tavares-Dias and Mataqueiro 2004; Borges et al. 2004; Tavares-Dias and Moraes 2006). Therefore, fish farming requires an efficient health monitoring system. The objective of stringent health control is to provide the fish pathologist with sensitive physiological methods that may signal a disease (Hrubec et al. 2001; Tavares-Dias and Moraes 2003; Tavares-Dias and Mataqueiro 2004; Tavares-Dias and Moraes 2006). Thus, in the present work, we propose to determine the red blood cells indices, metabolic products (total protein, glucose, urea, triglycerides and cholesterol), total thrombocytes count and white blood cell counts of *Arapaima gigas,* in net cage culture.

Material and Methods

Fish and rearing characteristics

Arapaima gigas' alevins obtained from a fish farm (Coari – AM) were transfer to the tanks Aquiculture Department (CPAQ/ INPA, Manaus, Amazonas, Brazil) and fed by zooplankton for 30 days. At this time, the juveniles were around 7cm in length and transferred to net-cages with 1m^3 each during another 25 days for feeding training as described by Ono et al. (2003, 2004). After the training period, they were maintained with commercial fishmeal (40% crude protein - Nutron, Brazil) up to 14 months old; when the blood of 20 fish were collected for analysis.

Blood analyses proceedings

Blood samples from each animal were collected by puncturing the caudal vein using heparinzed syringes (5.000 UI) and divided in two aliquots. The first aliquot was used to count the red blood cells (RBC) number and white blood cells (WBC) in Neubauer chamber using a Natt & Herrick (1952) solution. Haemoglobin concentration was determined by the cyanonmethaemoglobin method and hematocrit by microhematocrit method. These data were used to calculate the mean corpuscular volume (MCV) and the mean corpuscular haemoglobin concentration (MCHC). Blood smears were stained with May Grünwald-Giemsa-Wright (Tavares-Dias and Moraes, 2003) and used to estimate total thrombocytes (Tavares-Dias and Moraes 2006) and differential leukocyte counts.

The second aliquot was used to determine the plasma glucose by enzymatic methods, plasma urea, triglycerides and cholesterol using a commercial kit (Doles, GO, Brazil). Total plasma protein was determined by the biuret reaction. All analysis was determined by using an automated chemical system regularly monitored for accuracy and precision in accordance to "good labora-tory practices".

Results

During the fish cultivating period, the water temperature ranged from 27.6 to 28.9ºC, the pH 5.5 to 7.4, oxygen 6.4 to 7.6 mg/L, electric conductivity 18.8 to 41.3 µS/cm, nitrites 0.01 to 0.02 mg/L and ammonia 0.49 to 0.52 mg/L. These values are considered normal for tropical fish.

The mean values and the variation of the metabolites and the erythrogram of *A. gigas* are shown in Table 1. The metabolites' concentration, haemoglobin and MCHC are the parameters with higher variation in this specie.

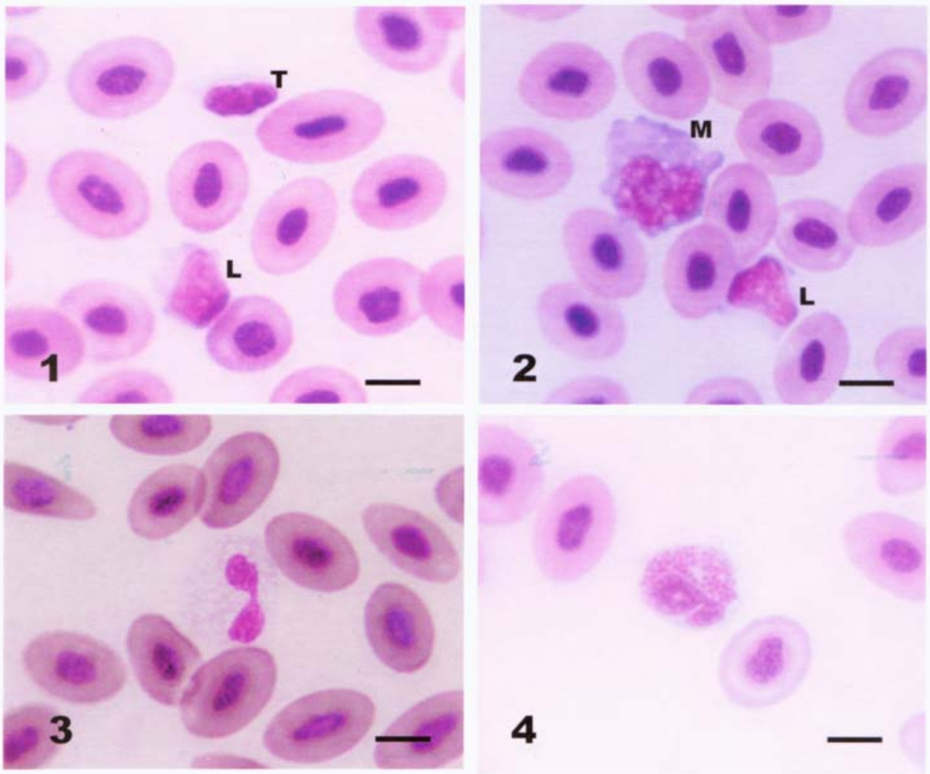
Thrombocytes and white blood cell counts (WBC) are shown in Table 2. In pirarucu blood smears, thrombocytes, lymphocytes, monocytes, neutrophils and eosinophils were identified and characterized. In general, thrombocytes are fusiform and round. The nuclei correspond to the cell shape and the cytoplasm is hyaline with no visible granules. Lymphocytes are round cells with variable size. The nucleus is also round, and with high relationship to its basophilic cytoplasm. The nucleus exhibits a dense chromatin and the cytoplasm has no visible granules (Figure 1). Monocytes are larger rounded cells with basophilic cytoplasm (Figure 2). The nucleus is generally eccentric and occasionally horseshoe-shaped. Neutrophils are round, with the cytoplasm containing fine neutrophilic granules. The nucleus is eccentric, rod-shaped and occasionally segmented with a compact chromatin (Figure 3). Eosinophils are round granulocytes, which are slightly smaller than neutrophils with a cytoplasm rich in eosinophilic granules and an eccentric nuclei (Figure 4).

Table 1 – Mean values ± standard deviation (SD) for biochemical values and red blood cells parameters of *A. gigas* in net cage culture

Parameters	Range	Mean ± SD
Weight (g)	701.8-892.4	793.4 ± 28,4
Total length (cm)	44.5-54.0	49.7 ± 3.1
Total protein (g/dL)	4.8-7.7	6.5 ± 0.6
Glucose (mg/dL)	112.4-198.5	152.4 ± 27.3
Triglycerides (mg/dL)	154.6-622.7	308.1 ± 140.2
Cholesterol (mg/dL)	150.6-263.7	204.1 ± 33.0
Urea (mg/dL)	7.4-14.5	9.3 ± 1.8
RBC (x 10^6/µL)	3.720-4.620	4.123 ± 0.280
Haemoglobin (g/dL)	7.6-12.4	10.4 ± 1.0
Hematocrit (%)	31.0-38.5	34.4 ± 2.3
MCV (fL)	82.1-83.3	83.3 ± 0.3
MCHC (g/dL)	23.8-34.4	30.2 ± 2.2

Table 2 – Mean values ± standard deviation (SD) for thrombocytes and leukocytes count of *A. gigas* in net cage culture

Parameters	Range	Mean ± SD
Thrombocytes (µL)	3,960-122,880	46,097 ± 42,799
WBC (µL)	14,000-18,000	15,833 ± 1,150
Lymphocytes (µL)	9,000-15,130	12,669 ± 1460
Lymphocytes (%)	9.0-89.0	80.2 ± 9.0
Monocytes (µL)	140.0-1,760	748.9 ± 449.1
Monocytes (%)	1.0-12.0	5.3 ± 3.1
Neutrophils (µL)	980.0-4,960	2,419 ± 1,261
Neutrophils (%)	1.0-31.0	14.3 ± 7.9
Eosinophils (µL)	0.0-1,500	116.0 ± 344.8
Eosinophils (%)	0.0-2.0	0.2 ± 0.5



Figures 1-4. *Arapaima gigas* peripheral blood cells stained with May Grünwald-Giemsa-Wright. (1) Fusiform thrombocyte (T), and lymphocyte (L), (2) monocyte (M) and lymphocyte (L), (3) segmented neutrophil and (4) eosinophil. Bar = 5.0 µm.

Discussion

The study of blood characteristics may corroborate important subsidies of diagnoses and prognoses of morbid conditions in fish populations (Tavares-Dias and Moraes 2004) and therefore, contribute to better comprehending comparative physiology, phylogenetic relations, feeding conditions and other ecological parameters.

Very little is still known about the metabolites' values in tropical fishes, especially Brazilian teleost. In *A. gigas* the glucose levels are higher than those described for *Oncorhynchus mykiss* (Rehulka 2000), *Piaractus mesopotamicus* (Tavares-Dias and Mataqueiro 2004), *Brycon amazonicus* and *B. orbignyanus* (Tavares-Dias 2004), *Rhamdia quelen* (Borges et al. 2004) and *Tinca tinca* (Pedro et al. 2005). However, they are similar to the hybrid striped bass (Hrubec et al. 2001). Previous studies demonstrated that glucose plasma basal levels varied in ecological distinct species, in part, influenced by environmental and non-environmental factors such as feeding habits and life mode of the fish, particularly related to its locomotivity (Tavares-Dias and Mataqueiro 2004; Tavares-Dias 2004). The higher values occur in active fishes whose feeding habits are in water column or in predators. Lower values are reported for sluggish fish, a bottom feeder (Larson et al. 1976; Ryzhova 1981; Lésel et al. 1986; Hepher 1988). The former pattern was observed in *A. gigas*. Although the specie does not migrate during the reproductive cycle, it is considered active habit specie, such as a carnivore predator its prey and thus displaying high plasma glucose levels, as expected. Hence, these variations might be related with the mobilization of the energetic supplement in response to adverse situations that each individual or species are exposed to in the environment. Therefore, an attempt to supply additional energy would be required. In addition, there are differences in carbohydrate metabolism among species.

Previous works suggest that in tropical fishes the total serum protein levels range from 2.8 to 8.2 g/dL (Tavares-Dias et al. 2004). In the present study, the juvenile *A. gigas* presented a total protein plasma level of 6.5 g/dL, similar to those related to young hybrid striped bass (Hrubec et al. 2001), but slightly higher than those described in adults *T. tinca* (Pedro et al. 2005). Such results are to be expected when both species are carnivorous. However, the values found in *A. gigas* are higher than those in the omnivorous feeding habits species such as *Brycon amazonicus*, *B. orbignyanus* (Tavares-Dias 2004), *O. mykiss* (Rehulka 2000) and *R. quelen* (Borges et al. 2004). In teleosts, total serum protein levels are influenced by specie (Ryzhova 1981; Tavares-Dias 2004), and by age (Ryzhova 1981; Hrubec et al. 2001), since adult fishes need more protein to form their gametes.

The lipids are the economical usual form used by fishes to stock energy and can be stored in many different organs (Guijarro et al. 2003). The cholesterol and triglycerides plasma levels in juvenile *A. gigas* are higher than those in males and females of *T. tinca* (Guijarro et al. 2003; Pedro et al. 2005). However, the triglyceride levels are lower than those present in *R. quelem* males but the cholesterol is still the same (Borges et al. 2004). In adults, large amounts of lipids are necessary during the reproductive process. Thus, the cholesterol and triglyceride plasma levels differ according to age, general health conditions and fish feeding diet.

In the present study, *A. gigas* red blood cell counts, haemoglobin and MVC were higher than values reported by Andrade et al.(2006), for this same specie when supplemented with high vitamin C and E levels in diet, yet, hematocrit and MCHC were similar. Nevertheless, we analyzed farmed fish in net cage while Andrade et al.(2006) studied farmed fish in laboratory. On the other hand, such values are higher in pirarucu than those described in *Hoplosternum littorale* (Affonso 2001), except for the MVC, which is higher in the latter specie. The differences can be explained by the pirarucu's erythrocyte volume, which is smaller than those in *H. littorale*. The *A. gigas* is obligatorily air-breathing specie with nearly 75% of its oxygen captured directly from the air (Stevens and Holeton 1978). However, *H. littorale* is a bimodal air-breathing fish, which in well oxygenated water uses its gills to obtain O_2, but during hypoxia uses its posterior gut as an accessory air-breathing organ to obtain almost 30% of the oxygen from the air (Val 1995; Affonso 2001).

The thrombocytes in teleost fish are multifunctional cells, once they are involved in eicosanoids releasing (Hill et al. 1999), blood coagulation process (Tavares-Dias and Mataqueiro 2004; Witeska 2005) and immune defence (Tavares-Dias and Mataqueiro 2004; Tavares-Dias and Moraes 2004). Therefore, as they are in constant movement in haematopoietic organs and systemic circulation, this might cause a variant change in its regular number (Tavares-Dias and Mataqueiro 2004; Witeska 2005). A recent review demonstrated that in freshwater teleost the thrombocyte numbers range from 870.0 to 100, 800.0 µL (Tavares-Dias and Moraes 2004). In *A. gigas*, the range goes from 3,960.0 to 122,880.0 µL, with mean values of 46,097 µL. Such values are similar to those found in the hybrid striped bass (Hrubec et al. 2001) and the *P. mesopotamicus* (Tavares-Dias and Mataqueiro 2004). Therefore, these baseline thrombocytes values in *A. gigas* are, as well as intraspecific variations can be expected.

The white blood cell numbers range widely among teleosts (Tavares-Dias and Moraes 2004) due to the phylogenetic position, sex, season, environmental conditions and age (Hrubec et al. 2001, Tavares-Dias and Moraes 2004), as well as may be influenced by the count methodology (Tavares-Dias et al. 2003, Tavares-Dias and Mataqueiro 2004). However, in *A. gigas* the total leukocyte number was similar to those reported to the hybrid striped bass (Hrubec et al. 2001), *P. mesopotamicus* (Tavares-Dias and Mataqueiro 2004) e lower than in *C. carpio* (Tripathi et al. 2004) and *R. quelen* (Borges et al. 2004). By the other hand, the values were higher than those reported to *A. gigas* feed by high levels of vitamins C and E, performed by hemocytometer, while in the present work the count was performed by estimation method on staining blood smears.

In the blood smears of *A. gigas* are found, thrombocytes, monocytes, lymphocytes, neutrophils and eosinophils with similar morphologic features to reported in *C. carpio* (Tavares-Dias et al. 2004; Tripathi et al. 2004) and *P. mesopotamicus* (Tavares-Dias and Mataqueiro 2004). The most frequent white blood cells in pirarucu are the lymphocytes, while eosinophils are rarely observed. Similar result has been stated for different species in the families: Anostomidae, Pimelodidae, Erythrinidae, Ictaluride, Characidae, Cichlidae, Mugilidae and Cyprinidae (Tavares-Dias and Moraes 2004).

To summarize, there is an urgent need to make reliable normal databases available for this species of economic importance. We established hematological values for the pirarucu *A. gigas*, which can be used as interpretative data obtained from this specie kept in similar environmental conditions. However, other studies will have to be lead for knowledge of the hematological values of this species when in other different modalities of culture and in natural environment.

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