Hematological parameters for the *Brycon orbignyanus* Valenciennes, 1850 (Osteichthyes: Characidae) intensively bred

Parámetros hematógicos para la piracanjuba *Brycon orbignyanus* Valenciennes, 1850 (Osteichthyes: Characidae) en cultivo intensivo

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Abstract. This paper determined red blood cell number, total thrombocyte count, and total leukocyte, and differential counts for young *Brycon orbignyanus* Valenciennes 1849 (Characidae) maintained in fish farming ponds. Mean values and range obtained were as follows: red blood cell count 3.283 (2.110-4.235) x 10^6/µl, hemoglobin 10.7 (8.4-13.1) g/dl, hematocrit 40.2 (35.0-47.0) %, mean corpuscular volume 125.0 (91.5-199.1) fl, mean corpuscular hemoglobin concentration 26.9 (19.5-35.6) g/dl, total thrombocyte count 25,743.0 (11,228.0-54,152.0) µl, total leukocyte count 23,290.0 (9,601.0-37,520.0) µl, lymphocytes 3,642.0 (709.0-9,005.0) µl, neutrophils and heterophils 18,178.0 (7,488.0-29,783.0) µl and monocytes 1,471.0 (147.0-4,976.0) µl. These results found should be useful for evaluation of health status of this fish, when in intensive breeding or other cultivation forms, in addition to serving for comparison of this species in a natural environment.

Key Words: *Brycon orbignyanus*, erythrocytes, hematology, leukocytes, thrombocytes.

Resumen. Este trabajo determina los índices eritrocítários, el número de trombocitos y de leucocitos en especimenes jóvenes de piracanjuba, *Brycon orbignyanus* Valenciennes, 1849 (Characidae), cultivados intensivamente en viveros. Los valores medios y las variaciones son descritas a continuación: eritrocitos totales 3,283 (2,110-4,235) x 10^6/µl, hemoglobina, 10,7 (8,4-13,1) g/dl, hematocrito 40,2 (35,0-47,0), volumen corpuscular medio 125,0 (91,5-199,1), concentración de hemoglobina corpuscular media 26,9 (19,5-35,6) g/dl, trombocitos totales 25.743,0 (11.228,0-54.152,0) µl, leucocitos totales 23.290,0 (9.601,0-37.520,0) µl, linfocitos 3.642,0 (709,0-9.005,0) µl, neutrofilos e heterofilos 18.178,0 (7.488,0-29.783,0) µl e monocitos 1.471,0 (147,0-4.976,0) µl. Estos resultados encontrados podrán ser útiles en la evaluación de la salud de este pez cultivado intensivamente o en otra forma de cultivo, además de servir para la comparación de la especie en ambiente natural.

Palabras clave: *Brycon orbignyanus*, eritrocitos, hematologia, leucocitos, trombocitos.

The fish of the Characidae family belong to the Bryconinae sub-family, which includes species of the *Brycon* genus, and are thoroughly distributed in Central and South America (Lima, 2003). In this sub-family there is an important species for the Brazilian aquaculture, *Brycon orbignyanus*, popularly known as piracanjuba, which is originally from the La Plata River basin (Argentina, Brazil and Uruguay). As this fish respond very well to hormonal induction for reproduction, its use in Brazilian fish farming began in the decade of 1930, with the studies of Rodolpho von Ihering. However, only in 1994 the studies for its viability in captivity were intensified, with the purpose of its reintroduction into areas threa-

tened with extinction, and data concerning blood variables in this species are still incomplete.

Hematological data are used routinely in health care of humans and domestic mammals. Hematological evaluations are also gradually becoming a routine practice for determining health status in fish (Tavares-Dias *et al*., 2003; Ranzaini-Paiva *et al*., 2003; Cazenave *et al*., 2005). As the aquaculture industry expands, tools to monitor the health status of fish using standardized non-lethal and expressive methods will be needed (Hrubec *et al*., 2000). Evaluation of hematologic analysis will enhance fish cultivation by facilitating early detection of situations of stress and/or diseases that could affect production performance (Tavares-Dias *op.cit*.; Tavares-Dias & Moraes, 2004; Rehulka *et al*., 2004). This, in turn, will contribute to more specific, timely and effective disease treatments in the future. Thus, in previous studies we determined the glucose levels, protein total, sodium potassium, calcium, magnesium and chloride for *B. orbignyanus* and *B. amazonicus* (Tavares-Dias, 2004) in intensive culture. In continuity, we proposed in the present study to determine red blood cell numbers, thrombogram, and leukogram of *B. orbignyanus* in fish farming ponds.

Forty clinically healthy young *B. orbignyanus*, 272 days of age, were obtained from a commercial fish farm (21º 07'45"S, 48º03'57"W), and transported to the Aquaculture Center/Caunesp, Jaboticabal, São Paulo, Brazil. They were maintained in three cement tanks (500 l) and acclimatized for 14 days, with a controlled flow-through water system. They received pelleted fish meal (35% of crude protein) diets once a day. During the acclimatization, and study periods the fish were observed daily for any clinical signs of disease, including lack of appetite, increased opercular movements, or visible lesions of the skin, tail, and fins. Signs of disease were not apparent during any period of observation. At the fish farm, during their culture, the fish received pelleted and extruded fish diets with 42% and 28% of crude protein, respectively. After that, they were stocked in ponds of 1000 m^2 and received extruded ration with 28% of protein. During this period, the water temperature was 24.5 to 30.2ºC; pH 6.7 to 6.9; dissolved oxygen 5.4 to 6.2 mg/l; electric conductivity 115.6 to 129.8 µS/cm and pluviometric index 0.0 to 342.0 mm^3.

From each specimen 0.5 ml of blood was collected by puncture of the caudal vein using a syringe containing EDTA (10%) for red blood cell number analysis. Then, as anesthetic substances may cause stress in fish (Tavares-Dias & Moraes, 2004), none of these products were used. After a rapid capture of the fish from the tanks by hand nets, they were immobilized and placed on a level surface, where the caudal puncture was performed rapidly.

The blood samples were used for determination of red blood cell (RBC) counts using an automatic blood cell counter (Celm, Model CC510). Hematocrit was determined by microhematocrit method, and hemoglobin concentration by cyanomethemoglobin method, being both methods usually well known. From these primary indicators resulted the secondary Wintrobe indices, such as mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC).

The blood samples were used for the confection of blood smears for morphological observation, which were stained with May Grünwald-Giemsa-Wright (MGGW) according to the Tavares-Dias & Moraes (2003) method, and for thrombocyte and total leukocyte count (WBC), and differential leukocyte counts. Total leukocytes, and thrombocytes counts were performed in relation to the erythrocytes in randomly selected fields of stained blood smear, and recalculated per unit volume: leukocytes (µl) = number of leukocytes in blood smear x RBC (µl)/2,000 erythrocytes counted in smear, and thrombocytes (µl)= number of thrombocytes in blood smear x RBC (µl)/2,000 erythrocytes counted in blood smear.

The red blood cell indices for *B. orbignyanus* are summarized in Table 1. Erythrocyte count showed a higher range while hemoglobin a smaller range, influencing thereby MVC, and MCHC.

Main values for thrombocyte counts were 25743.0 µl. Lymphocytes, neutrophils/heterophils, and monocytes constituted the leukocyte populations observed (Table 2). As heterophils, and neutrophils are granulocytes that possess equivalent functions, these were quantified together.

Changes in blood values are particularly important for diagnosis of disease and stress (Tavares-Dias *et al*., 2003; Ranzani-Paiva *et al*., 2003; Rehulka *et al*., 2004; Cazenave *et al*., 2005). Evaluation of hematologic parameters will enhance fish cultivation by facilitating early detection of infective diseases (Rehulka *et al*., *op. cit*) and identification of subletal conditions (Cazenave *et al*., *op. cit*.) that affect production performance. However, data for *B. orbignyanus* were fragmented until the present moment

Table 1. Mean values ± standard deviation (SD) and range for red blood cells indices in *B. orbignyanus* intensively bred

Parameter	Mean ± SD	Range
Weight (g)	466.9 ± 75.8	360.0-628.5
Total length (cm)	32.4 ± 1.7	29.5-36.0
Erythrocytes (x 10^6/µl)	3.283 ± 0.48	2.110–4.235
Hemoglobin (g/dl)	10.7 ± 1.3	8.4–13.1
Hematocrit (%)	40.2 ± 3.1	35.0–47.0
MVC (fl)	125.0 ± 22.0	91.5–199.1
MCHC (g/dl)	26.9 ± 3.9	19.5–35.6

Table 2. Mean values ± standard deviation (SD) and range for thrombocyte and leukocyte counts in *B. orbignyanus* intensively bred

Parameters	Mean ± SD	Range
Thrombocytes (µl)	25,743.0 ± 12,519.0	11,228.0–54,152.0
Leukocytes (µl)	23,290.0 ± 7,041.0	9,601.0–37,520.0
Lymphocytes (µl)	36,42.0 ± 2,039.0	709.0–9,005.0
Neutrophils/Heterophils (µl)	18,178.0 ± 5,657.0	7,488.0–29,783.0
Monocytes (µl)	1,471.0 ± 1,144.0	147.0–4,976.0

since only glucose levels (47.7-114.2 mg/dl), total protein (2.5-6.2 g/dl), sodium (135.0-153.5 mmol/l), potassium (2.0-5.2 mmol/l), calcium (2.3-3.1 mmol/l), magnesium (0.7-2.0 mmol/l) and chloride levels (103.0-127.0 mmol/l) were studied (Tavares-Dias, 2004).

Our complemental studies in *B. orbignyanus* show that erythrocyte numbers varied from 2.110–4.235 x 10^6/µl, hematocrit 35.0–47.0%, hemoglobin 8.4–13.1 g/dl, MCV 91.5–199.1 fl and MCHC 19.5–35.6 g/dl. These values are similar to those of young *B. amazonicus* (Tavares-Dias *et al*., 1999) and fry of *B. cepahus* (Arévalo & Castellanos, 2003) also in artificial cultivation systems. On the other hand, when compared to the young *B. hilarii* (Tavares-Dias *et al*., *op. cit*), the hematocrit, hemoglobin and MCHC values were relatively alike, while that for young and adult *B. cepahus* the hemoglobin; MCV and MCHC values were different (Arévalo & Castellanos, *op.cit.*), as were the erythrocyte count and the MCV values for adult *Brycon* sp. (Ranzani-Paiva, 1991). Inter-specific variations in red blood cell parameters have been reported in different teleosts and been attributed to factors such as sex, genetic variation, stress caused by capture, and handling (Tavares-Dias *et al*., 2003; Rehulka *et al*., 2004; Cazenave *et al*., 2005), species, water temperature, and environment. Therefore, hematologic values frequently can be affected by various intrinsic, and extrinsic factors (Tavares-Dias & Moraes, 2004; Cazenave *et al*., *op. cit*) when comparing these values among different species, with results appearing differently than expected.

The aim of stringent health control values is to provide a fish pathologist with a choice of sensitive methods to enable early detection of physiological deviations from standard form, which may signal the on come of disease. The numbers, and proportions of leukocytes, and thrombocytes (organic defense cells) in circulation provide an important representation of defense cell distribution throughout the body. In *B. orbignyanus* there was intraspecific variation in total thrombocyte count, and the mean values were similar to those in *Cyprinus carpio* (Tavares-Dias *et al*., 2004), however were lesser than those in the also characid *Piaractus mesopotamicus* (Tavares-Dias & Mataqueiro, 2004), and, as the counting method used was the same, these differences appear to be due to the species in question.

It was observed that the total leukocyte count in *B. orbignyanus* presented intraspecific variation, besides the fact that the mean values were much higher than in *B. cepahus* (Arévalo & Castellanos, 2003). However, this difference could be due to the counting method applied since the low values for *B. cepahus* suggest that this counting was probably done by direct method. Low leukocyte values obtained in these counts resulted from inefficient staining of these cells in the hemocytometer. Nevertheless, due to this factor, indirect methods can be considered more accurate that the direct methods (for details see Tavares-Dias *et al*., 2002) in spite of being a estimated count. This demonstrates the urgent need to develop a method that would allow more precision in leukocyte counting in fishes, preferably an automated method, as those already used for other vertebrates with anucleated erythrocytes.

Hine *et al*., (1990) hypothesized that neutrophils, and heterophils represent cellular types of two different lines that evoluted, and they appear today as alternative types in lower vertebrates of the zoological scale, thus heterophils developed eosinophilic proprieties while neutrophils basophilic proprieties. Nevertheless, neutrophils, and heterophils were found in *B. orbignyanus,* and they exhibited both proprieties, eosinophilic, and basophilic. In addition, blood lymphocytes, and monocytes were observed in *B. orbignyanus*. Arévalo and Castellanos, *op. cit*, also reported blood neutrophils, and heterophils in *B. cepahus* alevins, although in young, and adult fishes they found eosinophils, a granulocyte that was not seen here in peripheral blood of *B. orbignyanus*. The occurrence of granulocytes in fish has been subject of some controversy, including Bryconinae. Therefore, the granulocytes that have been described, as special granulocytic cells in *Brycon* sp. (Ranzani-Paiva, 1996) and *B. amazonicus* (Tavares-Dias *et al*., 1999), are in fact heterophils. Such identification problems can be caused by inadequate Romanowsky staining (e.g. May Grünwal-Giemsa), when used for staining blood smears of these species. These findings seem indicate that presence of both heterophil, and neutrophil seem be a characteristic of species from Bryconinae sub-family.

In *B. orbignyanus* the neutrophil/heterophil number was found to be higher than that described for the characid *P. mesopotamicus* (Tavares-Dias & Mataqueiro, 2004), while lymphocyte numbers were smaller, and monocyte count very similar. However, when compared to *C. carpio* (Tavares-Dias *et al*., 2004), the number of these leukocytes was different. The methods used for counting were alike, but these are different species and from different environments, and other factors should be considered since the leukocytes are immune cells traveling between and through tissues to the bloodstream (Tavares-Dias & Mataqueiro, *op. cit*). Moreover, the response to different stimuli from the environment may vary among individuals of a same species, as well as among species. The presence of leukocytes

relates to important characteristics of health status in fish and in many cases they are also helpful in immune system evaluation. Therefore, variations in the proportions of these defense cells in the blood are usually expected.

To conclude, as hematology assessment is gradually becoming routine practice for intensively bred fish, since intensive aquaculture needs accurate information for identification and control of stress situations and/or diseases in order to ensure healthy fish, the evaluation of blood parameters may be the quickest way to detect these symptoms. Therefore, there is an urgent need of reliable normal databases to be available for species of economic importance, as is the *B. orbignyanus*. Thus, we studied the hematological values for this species, and concluded that they are similar to those of other characids reported in literature. Moreover, other complemental studies will be done in this species, such as cytochemistry and ultrastructure, for concluding compilation of reference data for this Brazilian bryconin.

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