HEMATOLOGICAL CHARACTERISTICS OF BRAZILIAN TELEOSTS. VII. PARAMETERS OF SEVEN SPECIES COLLECTED IN GUARIBA, SÃO PAULO STATE, BRAZIL

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ABSTRACT

The intensive aquaculture needs accurate information on the identification and control of stress situations and/or diseases in order to ensure healthy fishes. The evaluation of blood parameters is the fastest way to detect stress symptoms. In this paper the hematocrit, hemoglobin concentration, mean corpuscular hemoglobin concentration (MCHC), differential count of defense cells (leucocytes and thrombocytes) of *Piaractus mesopotamicus* Holmberg, 1887; *Colossoma macropomum* Cuvier, 1818; tambacu hybrid (male of *P. mesopotamicus* x female of *C. macropomum*); *Brycon cephalus* Günther, 1869; *Brycon hilarii* Cuvier & Valenciennes, 1849; *Leporinus macrocephalus* Garavello & Britski, 1988 and *Cyprinus carpio* Linnaeus, 1758, collected in a fee-fishing farm were evaluated. The hemoglobin concentration, hematocrit and MCHC were similar in all species studied. However, inter-specific variation in the percentage of thrombocytes and neutrophils and special granulocytic cells was observed. Further studies on hematological parameters for fish species, mainly in culture environments are needed for the establishment of normal values, providing standards for physiological or pathological investigation.

Key Words: blood, freshwater fish, hematocrit, hemoglobin, leucocytes

CARACTERÍSTICAS HEMATOLÓGICAS DE TELEÓSTEOS BRASILEIROS. VII. PARÂMETROS DE SETE ESPÉCIES COLETADAS EM GUARIBA, ESTADO DE SÃO PAULO, BRASIL

RESUMO

A aqüicultura intensiva necessita de informações precisas sobre a identificação e controle de estresse e/ou doenças a fim de assegurar a saúde dos peixes. A avaliação das características sangüíneas é uma das maneiras de avaliar os sintomas de estresse. Neste estudo foram analisados o hematócrito, a concentração de hemoglobina, a concentração da hemoglobina corpuscular média (CHCM) e a contagem diferencial de células sangüíneas de defesa orgânica (leucócitos e trombócitos) de *Piaractus mesopotamicus* Holmberg, 1887; *Colossoma macropomum* Cuvier, 1818, híbrido tambacu (macho de *P. mesopotamicus* x fêmea de *C. macropomum*); *Brycon cephalus* Günther, 1869; *Brycon hilarii* Cuvier & Valenciennes, 1849; *Leporinus macrocephalus* Garavello & Britski, 1988 e *Cyprinus carpio* Linnaeus, 1758 capturados em um pesque-pague. A concentração de hemoglobina, o hematócrito e CHCM foram similares em todas as espécies. Porém, ocorreu variação interespecífica no percentual de trombócitos, neutrófilos e células granulocíticas especiais. Futuros estudos sobre características hematológicas de peixes, principalmente em condições de cultivo, são necessários para estabelecer valores basais provendo, assim, padrões para investigações fisiológicas ou patológicas.

Palavras-Chave: hematócrito, hemoglobina, leucócitos, peixe dulciaqüícola, sangue

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INTRODUCTION

The blood of fishes has been studied in order to determine the hematological picture of species in their natural environment, so that values of each species can be standardized and factors that alter them can be verified. It has also been undertaken in fish living in captivity so that abnormalities occurring in temperature and dissolved oxygen variations, diseases and others factors can be detected (RANZANI-PAIVA *et al.*, 2000). Moreover, blood analysis has also been used to determine the effects of stress caused by confinement, capture and handling (TORRES *et al.*, 1986; MARTINS *et al.*, 2000; TAVARES-DIAS *et al.*, 2001). Thus, intensive aquaculture needs accurate information on the identification and control of stress situations and/or diseases in order to ensure healthy fishes (TAVARES-DIAS *et al.*, 2000a). The evaluation of blood parameters is the fastest way to detect stress symptoms (TORRES *et al.*, 1986).

In fact, the hematological characteristics of confined Brazilian teleosts have been studied under several aspects: determination of normal values (ZUIM *et al.*, 1986; TAVARES-DIAS and FAUSTINO, 1998; TAVARES-DIAS *et al.*, 1998; TAVARES-DIAS *et al.*, 1999; TAVARES-DIAS *et al.*, 1999a,b;c; TAVARES-DIAS *et al.*, 2000b,c,d; RANZANI-PAIVA *et al.*, 1986; RANZANI-PAIVA, 1991,1996; RANZANI-PAIVA *et al.*,1998/1999), effects of capture and handling stress (MARTINS *et al.,* 2000;TAVARES-DIAS *et al.*, 2001), pesticides (RANZANI-PAIVA *et al.*, 1997; TAVARES-DIAS *et al.*, 1999d) and parasites (TAVARES-DIAS *et al.,* 1999c,d; SILVA-SOUZA *et al.*, 2000).

In the present study, the mean values of hematocrit, hemoglobin, mean corpuscular hemoglobin concentration (MCHC), differential count of defense cells (leucocytes and thrombocytes) of seven teleosts collected in a fee-fishing farm in Guariba, São Paulo State, Brazil, were evaluated.

MATERIAL AND METHODS

Fish and culture characteristics

From April 1997 to March 1999, 157 specimens of the hybrid tambacu (170.0 to 3,100.0 g and 20.0 to 59.0 cm), 44 pacus *Piaractus mesopotamicus* Holmberg, 1887 (125.0 to 1,700.0 g and 18.0 a 43.5 cm), 31 tambaqui *Colossoma macropomum* Cuvier, 1818 (225.0 to 3,900.0 g and 23.0 to 62.0 cm), 23 common carp *Cyprinus carpio* Linnaeus, 1758 (100.0 to 3,700.0 g and 19.5 to 82.0 cm), 23 matrinxã *Brycon cephalus* Günther, 1869 (500.0 to 1,045.0 g and 34.5 to 43.0 cm), 13 piraputanga *Brycon hilarii* Cuvier & Valenciennes, 1849 (300.0 to 800.0 g and 24.0 to 37.0 cm) and 26 piauçu *Leporinus macrocephalus* Garavelo & Britski, 1988 (500.0 to 1,200.0 g and 31.0 to 42.0 cm), kept in earth ponds of a fee-fishing farm situated in Guariba, São Paulo State, Brazil (21° 19" 17' S - 48° 12" 37,8' W) were captured with a hook for subsequent analyses:

Hematological analysis

The blood was withdrawn (0.5 ml) from the caudal vessel into a syringe containing a drop of EDTA (10%) solution. This blood was used to determine the hemoglobin concentration, hematocrit, mean corpuscular hemoglobin concentration (MCHC) (for details see TAVARES-DIAS *et al.*, 2000a;b;c). In the differential count of leucocytes and thrombocytes, in this paper called differential count of organic defense blood cells (for details see TAVARES-DIAS *et al,*2000a;b;c), blood smears were prepared and stained with May Grünwald-Giemsa. Two hundred cells were counted to establish the amount of each cell content. Immature leucocytes were observed but not quantified.

Parasitological analysis

Body mucus and pieces of gills, kidney, liver, spleen and heart were compressed between a glass and a coverslip with a drop of 0.65% saline solution for microscope observation. Intestines were also observed in a Petri dish containing saline solution.

RESULTS AND DISCUSSION

The fishes showed neither external or internal structural changes nor the presence of parasites.

In this essay, the hemoglobin concentration, hematocrit and MCHC were similar in the four species studied (Table 1). On the other hand, *P. mesopotamicus* and *B. hilarii* (Characidae) showed higher hemoglobin mean values and lower hematocrit mean values, when compared to other Characidae, which are also kept in captivity and distinct environments (RANZANI-PAIVA *et al.*, 1998/1999; TAVARES-DIAS *et al.*, 1998; TAVARES-DIAS *et al.*, 1999b; TAVARES-DIAS *et al.*,1999a) but similar *C. macropomum* (TAVARES-DIAS and SANDRIN, 1998a). *C. carpio* (Cyprinidae) showed higher mean values of hemoglobin and hematocrit than the ones described by VUREN and HATTINHG (1978) and FERREIRA *et al.* (1981) for the same carp. However, those fishes were maintained in distinct environments. Inter-specific variations in hematological values have been reported in fishes in their habitat (VUREN and HATTINHG, 1978;

RANZANI-PAIVA *et al*., 1998/1999) and attributed to different factors, such as genetic variation, stress - caused by capture and handling (KORI-SIAKPERE, 1985; TAVARES-DIAS *et al*., 2001) - and also blood sampling procedures (LUSKOVÁ, 1998; TAVARES-DIAS and SANDRIN, 1998a).

In previous studies with freshwater teleosts, the role of thrombocytes as organic defense blood cells like the leucocytes was broadly discussed, mainly under a pathological approach (for details see TAVARES-DIAS *et al*., 2000a;b;c). Leucocytes have been widely used in clinical diagnosis of diseases in humans and domestic animals. In the count of organic defense blood cells of tambacu hybrid, *P. mesopotamicus*, *C. macropomum*, *B. cephalus, B. hilarii* (Characidae) and *L. macrocephalus* (Anostomidae), blood smears displayed higher percentage of thrombocytes, followed by lymphocytes or neutrophils, monocytes, special granulocytic cells and eosinophils (Table 2). Previous studies also reported higher frequency of thrombocytes in marine (SAUNDERS, 1966) and freshwater teleosts such as *Clarias gariepinus* (BOOMKER, 1981), *Oreochromis niloticus* (TAVARES-DIAS and FAUSTINO, 1998; TAVARES-DIAS *et al*., 2000c), *P. mesopotamicus* (TAVARES-DIAS *et al*.,1999a;c;d), *B. cephalus* (TAVARES-DIAS *et al*., 1999b), *L. macrocephalus* (TAVARES-DIAS *et al*.,1999c; TAVARES-DIAS *et al*., 2000d), hybrid Florida red tilapia (TAVARES-DIAS *et al*., 2000a) and hybrid tambacu (TAVARES-DIAS *et al*., 2000b). On the other hand, the percentage of lymphocytes was higher than the one of thrombocytes for *C. carpio* in this work. Similar results had been found by HINES and YASHOUV (1970).

Table 1. Mean values ± standard deviation and variation range (values in parentheses) of hematological parameters of teleosts species collected in a fee-fishing farm from Guariba, SP, Brazil, April 1997 to March 1999. MCHC) = mean corpuscular hemoglobin concentration

Species	N	Hemoglobin (g/dL)	Hematocrit (%)	MCHC (g/dL)
Tambacu hybrid	157	12.8 ± 3.7	40.2 ± 7.8	31.5 ± 6.4
	---	(5.1 – 18.9)	(20.0 – 57.0)	(19.7 – 45.8)
P. mesopotamicus	44	13.6 ± 2.3	40.2 ± 4.4	34.3 ± 8.0
	---	(8.9 – 15.8)	(31.0 – 45.0)	(23.4 – 50.6)
B. hilarii	13	12.2 ± 3.7	35.5 ± 6.4	33.9 ± 4.3
	---	(9.6 – 14.8)	(31.0 – 40.0)	(30.9 – 37.0)
C. carpio	23	14.0 ± 1.7	44.0 ± 8.4	32.0 ± 2.3
	---	(12.8 – 15.2)	(38.0 – 50.0)	(30.4 – 33.7)

This paper revealed similar percentages of lymphocytes in all species of Characidae family (*P. mesopotamicus*, *C. macropomum*, *B. hilarii* and *B. cephalus*) and hybrid tambacu. However, this family showed inter-specific variation in the thrombocyte and granulocyte percentage, which was also observed in other families. The thrombocyte percentage in *P. mesopotamicus* and *C. macropomum* (Myleinae subfamily), and in its hybrid tambacu, was higher than in *B. hilarii* and *B. cephalus* (Bryconinae subfamily) and *C. carpio*. Eosinophils were absent in *B. cephalus*. Moreover, special granulocytic cells were not present in *B. cephalus* and *B. hilarii* (Characidae family) and *L. macrocephalus* (Anostomidae family). BARBER and WESTERMANN (1978) reported that in certain taxonomic groups the presence of special granulocytic cells may be consistent. These authors also found that eosinophils are rare in the peripheral blood of freshwater and marine teleosts.

Fishes leucocytes, mainly the granulocytes, have been subject of some controversy. According to MESEGUER *et al*. (1994), the available morphological data indicate a wide structural heterogeneity and diversity of cell types. In the present study, basophils were observed only in the peripheral blood of *L. macrocephalus*. BARBER and WESTERMANN (1975) suggest that the absence of basophils may be due to inadequate staining procedure. These cells are characterized by metachromatic granules and their solubility. Thus, there may be failure to preserve the granules, which makes their identification more difficult. The presence of basophils in peripheral blood of *Sparus aurata* was only confirmed by transmission electron microscopy (LÓPEZ-RUIZ *et al*., 1992).

Table 2. Mean values ± standard deviation and variation ranges (values in parentheses) of percentage of organic defense blood cells of teleosts species collected in a fee-fishing farm from Guariba, SP, Brazil, April 1997 to March 1999. SGC= Special granulocytic cells

Species	Thrombocytes	Lymphocytes	Neutrophils	Monocytes	SGC	Eosinophils	Basophils
Tambacu hybrid	67.7 ± 12.8	19.2 ± 11.3	7.8 ± 8.4	3.2 ± 2.9	1.1 ± 1.7	1.0 ± 2.0	0.0 ± 0.0
	(38.0 − 93.0)	(0.0 − 48.0)	(0.0 − 27.0)	(0.0 − 12.0)	(0.0 − 11.0)	(0.0 − 13.0)	(0.0 − 0.0)
P. mesopotamicus	68.7 ± 10.8	20.0 ± 9.1	4.0 ± 6.6	2.5 ± 1.9	3.7 ± 5.4	1.1 ± 3.0	0.0 ± 0.0
	(54.0 − 87.0)	(4.0 − 43.0)	(0.0 − 29.0)	(0.0 − 7.0)	(0.0 − 17.0)	(0.0 − 14.0)	(0.0 − 0.0)
C. macropomum	72.6 ± 9.7	16.4 ± 10.3	6.4 ± 6.2	3.6 ± 2.4	0.6 ± 1.6	0.4 ± 0.8	0.0 ± 0.0
	(48.0 − 89.0)	(4.0 − 43.0)	(1.0 − 22.0)	(0.0 − 8.0)	(0.0 − 5.0)	(0.0 − 3.0)	(0.0 − 0.0)
B. cephalus	47.5 ± 12.4	22.6 ± 9.1	22.2 ± 10.6	7.7 ± 8.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	(29.0 − 75.0)	(11.0 − 34.0)	(7.0 − 40.0)	(1.0 − 27.0)	(0.0 − 0.0)	(0.0 − 0.0)	(0.0 − 0.0)
B. hilarii	54.4 ± 19.2	25.8 ± 19.6	15.4 ± 13.2	4.2 ± 3.7	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0
	(32.0 − 71.0)	(11.0 − 60.0)	(4.0 − 38.0)	(1.0 − 10.0)	(0.0 − 0.0)	(0.0 − 1.0)	(0.0 − 0.0)
L. macrocephalus	64.7 ± 18.5	10.0 ± 10.5	23.0 ± 26.4	2.0 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.6
	(46.0 − 85.0)	(0.0 − 21.0)	(3.0 − 53.0)	(1.0 − 2.0)	(0.0 − 0.0)	(0.0 − 0.0)	(0.0 − 1.0)
C. carpio	37.0 ± 10.9	50.8 ± 12.8	10.0 ± 7.3	1.0 ± 1.4	1.0 ± 1.4	0.2 ± 0.5	0.0 ± 0.0
	(21.0 − 59.0)	(29.0 − 71.0)	(2.0 − 25.0)	(0.0 − 3.0)	(0,0 − 4,0)	(0.0 − 0.5)	(0.0 − 0.0)

In fact, a granulocyte with an eccentric, large, round or oval nucleus, and cytoplasmic granules strongly stained with the periodic acid-Schiff (PAS) method was described in the peripheral blood, hemopoietic tissue of the kidney, and in connective tissues of a Cypriniform of North America, white sucker *Catostomus commersoni* (BARBER and WESTERMANN, 1975). This granulocyte was also observed in marine and freshwater fishes (BARBER and WESTERMANN, 1978). These authors named this granulocyte "PAS-positive granular leucocyte" (PAS-GL), and suggested that when it was detectable in the kidney tissue, it indicated the presence of histamine. As basophils/mast cells were absent in the peripheral blood of the *C. commersoni*, it seems that PAS-GL is an evolutionary precursor of these cells. Subsequently, Barber and Westermann's PAS-GL was characterized cytochemically in blood and hemopoietic tissues of catfish *Pimelodus maculatus* (Siluriformes of South America) and called "special granulocytic cell", recognized as a distinct granulocyte (RIBEIRO, 1978). The PAS-GL differ from the basophils and eosinophils. PAS-GL was described as type I neutrophil in the peripheral blood of dourado *Salminus maxillosus* (Characiformes of South America) (VEIGA *et al*., 2000) and also as special granulocytic cell in the peripheral blood of surubin barred *Pseudoplatystoma fasciatum,* a Siluriform of South America (CARVALHO, 2002). However, the granules of that granulocyte, which strongly stain with PAS, are composed of neutral glycoprotein in *P. maculatus* (RIBEIRO, 1978) and *C. commersoni* (BARBER and WESTERMANN, 1975) and glycogen in *S. maxilosus* (VEIGA *et al*., 2000) and *P. fasciatum* (CARVALHO, 2002). Therefore, it is necessary to clarify if the granules of that granulocyte contain glycoproteins or glycogen. We have been working on this subject in our laboratory.

The literature suggests that granulocyte precursors have fewer carbohydrates than mature cells, and the amount of these substances increases with cell maturation (LORENZI, 1999). Thus, a strong positive reaction to the PAS suggests a great amount of carbohydrates in the cell. This is an indicative that the Barber and Westermann's PAS-GL cannot be a precursor cell, given its cytochemical characteristics.

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