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The hematological profile of farmed *Sorubim lima*: reference intervals, cell morphology and cytochemistry

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ABSTRACT

Hematology implies rapid and practical analysis to assist the diagnosis of fish homeostatic imbalance. This study determined the blood reference intervals in an important native South American catfish farmed in the Brazilian Pantanal wetland, *Sorubim lima* (Siluriformes, Pimelodidae), and describes the morphological and cytochemical characteristics of the cells. A total of 92 fish reared in a fish farm were examined for reference intervals 25 - 75% of total plasmatic protein, hemoglobin, red blood cell count (RBC), hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and total thrombocyte and leukocyte count (WBC). Periodic acid Schiff (PAS) with salivary amylase, bromophenol blue, Sudan black B and toluidine blue (metacromasy) were the cytochemical stains employed to signal glycogen, proteins, lipids and nuclei in thrombocytes and leucocytes. In the blood smears, monocytes, lymphocytes, neutrophils, basophils, eosinophils, PAS - positive granular lymphocytes (LG-PAS) and thrombocytes were observed. Siluriforme similar results were observed in neutrophils and eosinophils morphology. Compared to other pimelodidae RBC, hematocrit and MCV showed the lowest index variation. In contrast to other siluriformes fish, *S. lima* showed the concomitant presence of circulating eosinophils and basophils.

Key words: freshwater fish, jurupensém, health, hematology, farming

Introduction

The species *Sorubim lima* Bloch and Schneider, 1801, commonly known as "jurupensém", a long-distance migrating South American catfish, does not reproduce in

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a lentic environment and has external fertilization. A carnivorous fish, it feeds mainly on invertebrates and other fish (ROSSI, 2001) and can reach 4 kg and 60 cm, being appreciated for its meat with few bones (SHIBATTA et al., 2011). This fish is an important species of the fish fauna of the Pantanal, Mato Grosso do Sul, Brazil, also being highlighted as an ornamental feature (PÁDUA et al., 2012). Currently, its artificial breeding is common (SHIBATTA et al., 2011) allowing intensive rearing. Recent studies have reported the presence of etiologic agents that compromise its production (PÁDUA et al., 2013).

Hematological studies are of biological, ecological and veterinary interest and can help the diagnosis of stress and fish diseases. Changes in blood parameters indicate the occurrence of hemo-concentration or hemo-dilution due to osmoregulatory dysfunction (TAVARES-DIAS and MORAES, 2004). Containment, transport, capture and mismanagement cause stress and increase susceptibility to infections. Thus, stressed fish may have increased levels of circulating cortisol and hematological changes that can endanger the health of animals and cause economic losses (MORAES and MARTINS, 2004).

The reference intervals are defined by upper and lower limits, 25 - 75 percentiles, and cover most of the values obtained for each hematological variable of the individuals analyzed (TAVARES-DIAS and MORAES, 2007a). In turn, the reference population is defined by the set of individuals that meet certain criteria, especially the absence of disease. Different fish species have variations in the number, size and volume of erythrocytes, hemoglobin concentration and hematocrit percentage, that may be related to their different characteristics, such as eating habits (CAMARGO et al., 2005), fish size, stress level (MARTINS et al., 2011a), stage of gonadal development, sex, photoperiod, dissolved oxygen, pH, temperature (MARTINS et al., 2011b) and infections (SILVA et al., 2012a). The cytochemical methods, besides allowing the identification of cell lineages, suggest the immune function of each leukocyte component (TAVARES-DIAS, 2006a).

In neo-tropical pimelodids, blood parameters have been studied in mandi *Pimelodus maculatus* (JERÔNIMO et al., 2009), channel catfish *Ictalurus punctatus* (TAVARES-DIAS and MORAES, 2007b), "jundiá" *Rhamdia quelen* (BARCELLOS et al., 2003; BORGES et al., 2004; FUKUSHIMA et al., 2012), cachara *Pseudoplatystoma fasciatum* (RANZANI-PAIVA et al., 2005), "pintado" *Pseudoplatystoma corruscans* (BEELEN et al., 1998) and hybrid of *P. corruscans* x *P. fasciatum* (BEELEN et al., 2003; ISHIKAWA et al., 2010; PÁDUA and ISHIKAWA, 2011; SILVA et al., 2012a). However, there are no studies on the hematology of *S. lima.* This study investigated the blood characteristics of *S. lima* from intensive farming and described the reference ranges, morphology and cytochemical properties of leukocytes and thrombocytes.

Materials and methods

Fish and rearing conditions. A total of 92 juveniles were obtained from commercial fish farming in the municipality of Terenos (20º 25' 57.7" S, 55º 17' 08.9" W), Mato Grosso do Sul, Brazil. The animals were maintained at the Laboratory of Aquaculture Embrapa, Dourados, Mato Grosso do Sul, Brazil, for eight months in an intensive system. The fish were kept in fiberglass tanks, with 1,000 L capacity, with continuous water flow, and commercial feed for carnivorous fish (45% crude protein) was offered three times daily.

The tanks were cleaned daily and the water quality parameters monitored: dissolved oxygen 5.2 ± 0.7 mg/L (± standard deviation), temperature 25.0 ± 2.7 °C, pH 7.4 ± 0.1 measured with a multiparameter Hanna, and total ammonia 0.33 ± 0.10 mg/L analyzed by the colorimetric method. At the end of the laboratory period, the fish were 22.53 ± 1.65 cm in average total length, 54.06 ± 15.10 g average weight and did not exhibit gonadal development, which explains the lack of standardization for sex and hematological and cytochemical analyses.

Hematological analysis. After anesthesia with clove oil, 75 $mg.l^{-1}$ (SILVA et al., 2012b), the fish blood was collected from the caudal vessel using 3-mL syringes containing 3% EDTA to hematocrit percentage by the microhematocrit method (GOLDENFARB et al., 1971), hemoglobin concentration by the cianometahemoglobin method (RANZANI-PAIVA et al., 2013) and the total number of erythrocytes (RBC) in a Neubauer chamber. From these data, the RBC indices (RANZANI-PAIVA et al., 2013) were calculated: mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). The total protein concentration in the plasma was determined with a portable refractometer. The total counts of leukocytes (WBC) and thrombocytes were calculated by an indirect method from blood smears stained with May-Grünwald Giemsa-Wright (MGGW) (TAVARES-DIAS and MORAES, 2006).

Cytochemical analysis. Ten fish were used for cytochemical study. After blood collection, the blood smears were subjected to cytochemical reactions to demonstrate glycogen by periodic acid Schiff (PAS) (McMANUS, 1946) using the control of specificity by salivary amylase (LISON, 1960), total proteins by bromophenol blue (MAZIA et al., 1953), nuclei (metachromasia) by toluidine blue (TAVARES-DIAS, 2006b) and lipids by 0.3% Sudan black B (LISON, 1960). The results were expressed in terms of the intensity of the cytochemical reactions: negative reaction (-), weakly positive reaction (+), positive reaction (++) and highly positive (+++).

Statistical analysis. The CBC data were tested for normality by the Kolmogorov-Smirnov test and since most had non-Gaussian distributions, the reference intervals were determined using the 25 - 75 percentiles. The relative condition factor (Kn) (LE CREN,

1951) was calculated from the ratio between the weight (g) and total length (cm) of 92 fish.

Results

The relative condition factor (Kn) corresponded to 1.004 ± 0.046 (mean ± standard deviation) and correlation (R^2) between weight and length was equal to 0.597. Fig. 1 shows the growth of farmed *Sorubim lima*, where $y = 0.005x^{2.956}$ (y = weight, x = length).

The values for hemoglobin, hematocrit, MCV, MCHC, thrombocytes, monocytes, basophils, neutrophils, eosinophils and LG-PAS showed non-normal distribution, while the normal distribution of values was observed in RBC, total plasma proteins, WBC and lymphocytes. For each blood parameter the reference ranges 25 - 75%, means ± standard deviations (SD) were determined, followed by the median in brackets, and values of minimum - maximum, considering the lower (Kn<1.004) and higher (Kn>1.004) indices (fish with the best zootechnical indexes) to the relative condition factor found (Tables 1 and 2).

Table 1. Reference ranges 25-75%, means ± standard deviations (SD) followed by the median in brackets and values of minimum - maximum for the red series of farmed *Sorubim lima* (n = 92) from the Pantanal of Mato Grosso do Sul, Brazil

	Reference intervals		Means ± SD (median)		Minimum - maximum	
Parameters	Kn<1.004	Kn>1.004	Kn<1.004	Kn>1.004	Kn<1.004	Kn>1.004
Total plasmatic protein ($g \times dL^{-1}$)	4.20 - 5.00	4.20 - 5.00	4.71 ± 0.54 (4.60)	4.64 ± 0.53 (4.80)	3.80 - 6.00	3.00 - 5.60
Hematocrit (%)	27.00 - 31.00	26.75 - 30.00	28.50 ± 4.06 (30.00)	28.33 ± 3.24 (29.00)	13.00 - 34.00	17.00 - 33.00
RBC ($\times 10^6\ \mu L^{-1}$)	2.13 - 2.64	2.14 - 2.50	2.37 ± 0.36 (2.38)	2.31 ± 0.39 (2.25)	1.69 - 3.17	1.45 - 3.54
Hemoglobin ($g \times dL^{-1}$)	4.90 - 9.32	4.96 - 7.21	7.02 ± 2.79 (5.78)	6.33 ± 1.97 (5.94)	3.50 - 13.82	2.14 - 12.14
MCV (fL)	112.76 - 135.29	116.86 - 134.53	122.28 ± 22.61 (116.68)	125.51 ± 19.77 (126.18)	45.30 - 171.60	76.92 - 178.57
MCHC ($g \times dL^{-1}$)	18.54 - 31.14	17.37 - 25.46	24.54 ± 8.49 (20.58)	22.45 ± 7.46 (20.76)	12.88 - 44.58	11.61 - 48.56

RBC: total number of erythrocytes, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration

Table 2. Reference ranges 25-75%, means ± standard deviations (SD) followed by the median in brackets and values of minimum - maximum for thrombocytes and white series of farmed *Sorubim lima* (n = 92) from the Pantanal of Mato Grosso do Sul, Brazil.

	Reference intervals		Means ± SD (median)		Minimum - maximum	
Parameters	Kn<1.004	Kn>1.004	Kn<1.004	Kn>1.004	Kn<1.004	Kn>1.004
Thrombocytes ($\times 10^3\ \mu L^{-1}$)	9.02 - 19.15	11.29 - 19.95	14.18 ± 7.22 (12.09)	18.18 ± 13.76 (15.20)	3.48 - 31.46	47.5 - 90.04
WBC ($\times 10^3\ \mu L^{-1}$)	36.24 - 56.77	30.21 - 52.47	47.74 ± 15.80 (45.51)	43.31 ± 19.16 (41.42)	24.60 - 101.46	17.25 - 95.20
Monocytes ($\times 10^3\ \mu L^{-1}$)	0.00 - 1.49	0.00 - 0.96	0.93 ± 1.05 (0.67)	0.66 ± 0.74 (0.50)	0.00 - 5.02	0.00 - 3.11
Lymphocytes ($\times 10^3\ \mu L^{-1}$)	29.62 - 45.70	24.05 - 42.12	38.64 ± 11.96 (38.92)	36.22 ± 15.75 (34.13)	16.72 - 68.93	14.36 - 82.82
Basophils ($\times 10^3\ \mu L^{-1}$)	0.00 - 1.13	0.17 - 1.01	0.85 ± 1.06 (0.56)	0.69 ± 0.69 (0.53)	0.00 - 5.92	0.00 - 3.11
Eosinophils ($\times 10^3\ \mu L^{-1}$)	0.00 - 70.00	0.00 - 0.31	0.81 ± 4.91 (0.00)	0.35 ± 0.95 (0.00)	0.00 - 35.51	0.00 - 5.71
Neutrophils ($\times 10^3\ \mu L^{-1}$)	1.52 - 6.39	1.02 - 5.23	5.13 ± 5.20 (3.08)	3.68 ± 3.85 (2.46)	0.64 - 23.62	0.00 - 18.65
LG-PAS ($\times 10^3\ \mu L^{-1}$)	0.46 - 1.86	0.47 - 2.13	1.37 ± 1.79 (0.80)	1.69 ± 1.51 (1.33)	0.00 - 11.16	0.00 - 6.53

WBC: total number of leukocytes, LG-PAS: PAS-positive granular leucocyte

Table 3. Cytochemical staining of blood cells of *Sorubim lima* (n = 10). Bromophenol blue staining (BB), periodic acid-Schiff staining (PAS), sudan black B staining (SBB) and toluidine blue staining (TB)

Cells	BB	PAS	SBB	TB
Thrombocytes	-	++	-	-
Monocytes	-	-	-	-
Lymphocytes	-	-	-	-
Basophils	++	-	++	+++
Eosinophils	++	-	+++	-
Neutrophils	++	++	++	-
LG-PAS	-	+++	-	-

Negative (-), weakly positive (+), positive (++), highly positive (+++)

The cytochemistry showed thrombocytes stained by PAS, basophils stained with bromophenol blue, Sudan black B and toluidine blue, eosinophils stained with bromophenol blue and Sudan black B, neutrophils stained by bromophenol blue, PAS

and Sudan black B and PAS-LG only stained by PAS. Monocytes and lymphocytes (non-granular mononuclear cells) were not stained (Table 3).

The blood smears stained by MGGW showed the presence of erythrocytes, thrombocytes, monocytes, lymphocytes, neutrophils, PAS-positive granular leukocytes (LG-PAS), eosinophils and basophils (Fig. 2).

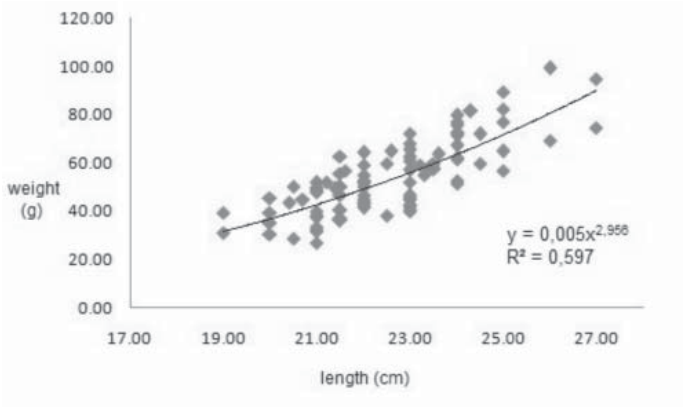

Fig. 1. Weight-lenght relationship in *Sorubim lima* (n = 92) demonstrated by relative condition factor (Kn = 1.004)

Neutrophils (Fig. 2bc) are circular cells with cytoplasmic neutrophil staining that occasionally exhibit smaller vacuoles, the nucleus has various shapes, and sometimes fewer segmentations are verified, usually located at the periphery of the cell. This granulocyte showed diffuse positive reaction for PAS (Fig. 3), sudanophil cells (Fig. 3d) and positive reaction to bromophenol blue (Fig. 3g). The PAS-positive granular leukocyte (Fig. 2d) has a circular shape, eccentric nuclei and neutral cytoplasmic granulation without affinity for acid or basic dye, but it showed an intensely positive reaction to PAS (Fig. 3b), persisting after treatment with salivary amylase, revealing the presence of neutral glycoproteins.

Eosinophils (Fig. 2e) have cytoplasm and circular granulation of eosinophilic staining, with intense sudanophil cells (Fig. 3e) and positive reaction to bromophenol blue (Fig. 3h). Basophils (Fig. 2f) were the smallest granular leukocytes, exhibiting fine granulation and occasionally small cytoplasmic vacuoles. Their granules showed sudanophil cells (Fig. 3f), a positive reaction to bromophenol blue (Fig. 3i) and a positive reaction to toluidine blue, with metachromasia.

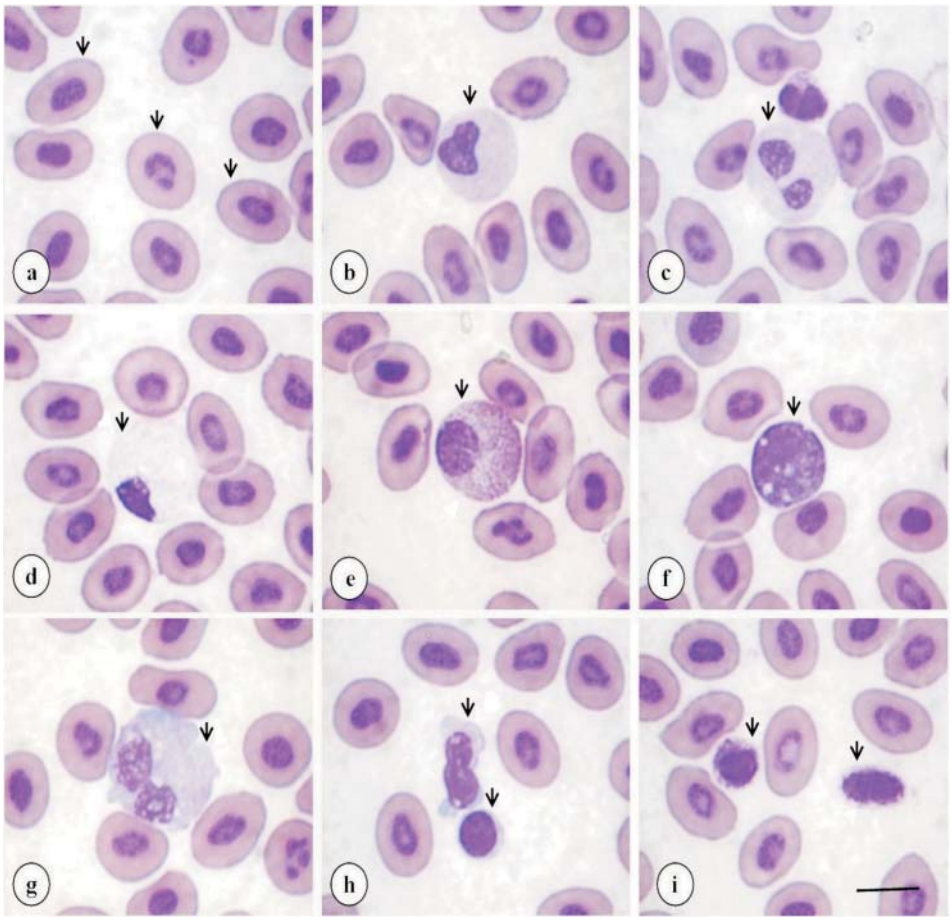

Fig. 2. Blood cells of *Sorubim lima*. Erythrocytes (a), neutrophils (bc), LG-PAS (d) eosinophil (e), basophil (f), monocyte (g), lymphocytes (h) and thrombocyte (i). MGGW staining (May Grunwald Giemsa Wright). Scale bar = 5 μm

Monocytes (Fig. 2g) are pleomorphic cells showing cytoplasmic projections and few vacuoles. Lymphocytes (Fig. 2h) are the smallest non-granular leukocytes with a high nucleus/cytoplasm ratio and can display small cytoplasmic projections. Monocytes and lymphocytes showed no positive reaction to any of the cytochemical methods used. Thrombocyte cells are predominantly elliptical and occasionally circular (Fig. 2i), with fine acidophilic granulation in their cytoplasm, and small vacuoles, showing a positive

reaction for PAS (Fig. 3c); glycogen granules located predominantly at the poles or in the center of the cell with diffuse reaction were observed in some cells.

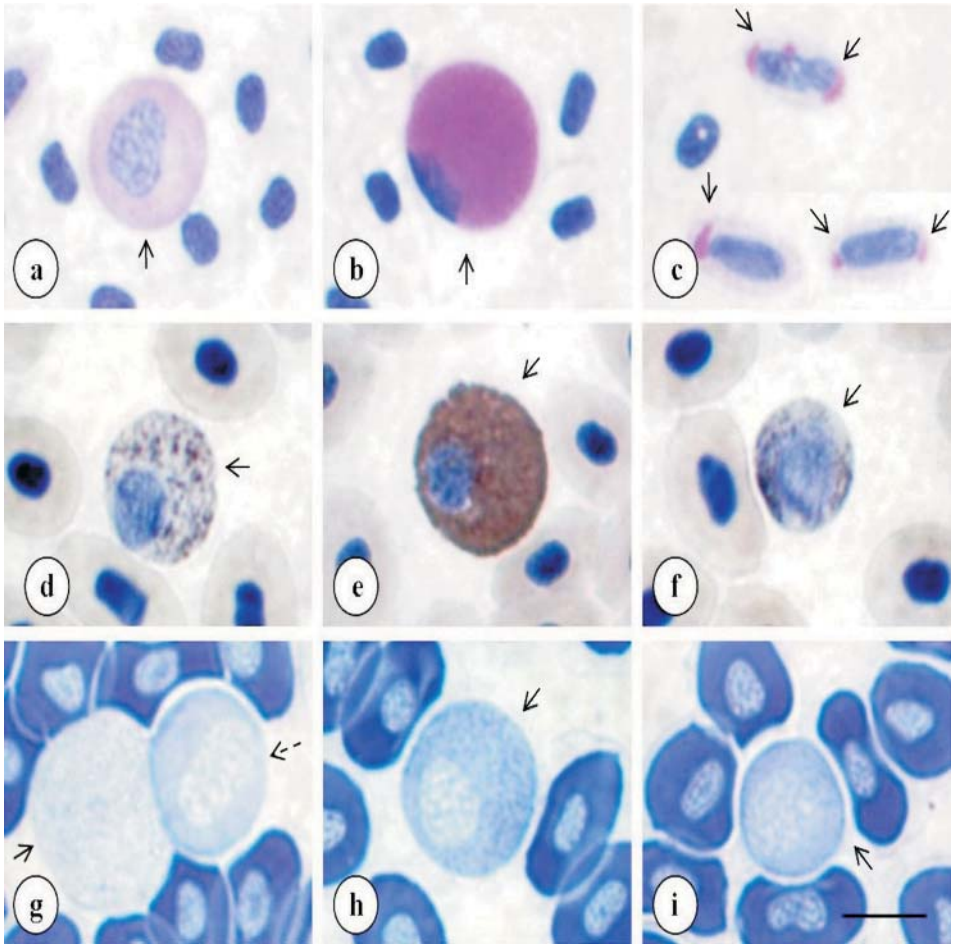

Fig. 3. Cytochemical staining of blood cells of *Sorubim lima*. Positive PAS staining in neutrophil granules (a), LG-PAS (b) and thrombocytes (c). Sudanophillic reaction in a neutrophil (d), eosinophil (e) and basophil (f). Positive staining with bromophenol blue in neutrophil granules (g - dotted arrow) with a negative reaction in LG-PAS (g - solid arrow), positive for eosinophils (h) and basophils (i). Scale bar = 5 μm.

Discussion

This study quantified the cells of white and red series, and determined the blood concentration of total protein in *S. lima* by morphological and cytochemical means, besides providing reference intervals for circulating cells. In contrast to the findings of this study, only neutrophils and eosinophils were recorded in the hybrid catfish *Pseudoplatystoma corruscans* x *P. reticulatum* (BEELEN et al., 2003), neutrophils and basophils in channel catfish (TAVARES-DIAS and MORAES, 2007a) and neutrophils, eosinophils and heterophils in Atipa *Hoplosternum littorale* (Callichthyidae) (TAVARES-DIAS and BARCELLOS, 2005). Moreover, the morphological characteristics of neutrophils and eosinophils were similar to previous studies.

Sudanophillic staining observed in granules of eosinophils, basophils and neutrophils of *S. lima* was also reported in *Salminus brasiliensis* (VEIGA et al., 2000) hybrid catfish (BEELEN et al., 2003), blue tilapia *Oreochromis aureus* (UEDA et al., 2001), Nile tilapia *Oreochromis niloticus* (UEDA et al., 2001), tuna *Thunnus maccoyii* (ROUGH et al., 2005) and *Araipama gigas* (ARAÚJO et al., 2009), indicating the presence of lipids. These lipid components act as energy reserves of the cells where they are degraded by lysosomal enzymes (ARAÚJO et al., 2009).

Neutrophils, eosinophils and basophils of *S. lima* showed positive staining for bromophenol blue, like the eosinophils of "dourado" (VEIGA et al., 2000) and Nile tilapia (UEDA et al., 2001), as well as monocytes, neutrophils and eosinophils of pirarucu (ARAÚJO et al., 2009) . This cytochemical method marks proteins in granular leukocytes, which are organic molecules involved in the killing of microorganisms (ARAÚJO et al., 2009). The morphological and cytochemical features of LG-PAS granulocytes in *S. lima* were similar to those described for the common carp *Cyprinus carpio* (TAVARES-DIAS and MORAES, 2004) and bighead carp *Aristichthys nobilis* (TAVARES-DIAS, 2006b). These granulocytes showed a strong positive reaction to PAS after digestion by salivary amylase, indicating the presence of glycoproteins (TAVARES-DIAS, 2006b), supporting the LG-PAS nomenclature given by BARBER and WESTERMANN (1975), and indicating the PAS method as a marker for such a cell.

The glycogen, an energy source for phagocytic cells, accumulates with cell maturation (VEIGA et al., 2000; UEDA et al., 2001). Thrombocytes and neutrophils of *S. lima* showed a positive reaction to PAS, similar to that described for other species of freshwater fish (VEIGA et al., 2000; UEDA et al., 2001; TAVARES-DIAS, 2006b; TAVARES-DIAS and MORAES, 2006) and marine fish (ROUGH et al., 2005). On the other hand, a PAS-positive reaction has been described for monocytes and lymphocytes of Nile tilapia (UEDA et al., 2001), eosinophils of tuna (ROUGH et al., 2005), monocytes and eosinophils of arapaima (ARAÚJO et al., 2009), heterophils of *Brycon orbignyanus* (TAVARES-

DIAS and MORAES, 2006) and heterophils, basophils, lymphocytes and thrombocytes of "Murray cod" *Maccullochella peelii peelii* (SHIGDAR et al., 2009).

The hematological profile of fish is directly related to gender, gonadal development, nutritional status, seasonality, environment and dietary supplementation (TAVARES-DIAS and MORAES, 2004; BABALOLA et al., 2009). Among the cytoplasmic proteins, enzymes are the most important for participating in phagocytosis (TAVARES-DIAS, 2006a; TAVARES-DIAS et al., 2007a). The values of total protein for *S. lima* were similar to those found in channel catfish (TAVARES-DIAS and MORAES, 2007a) and "Günther's catfish" *Horabagrus brachysoma* (PRASAD and CHARLES, 2010) and were lower than those observed in "pintado" (BEELEN et al., 1998). The plasma protein concentration may vary according to the immune status and should be considered the interspecific difference in fish (TAVARES-DIAS and MORAES, 2004).

Changes in RBC of fish may indicate responses to dissolved oxygen levels in water tanks, ponds and the natural environment. Therefore, hemoglobin, MCV and MCHC are useful to identify and classify the type of anemia occurring in fish. For example, the MCHC reduction may indicate an anemic framework, while its increase may be related to hemolysis in the blood sample (LABARRÈRE et al., 2012).

Erytrogram values for *S. lima* were relatively close to those described for the pimelodid catfish *R. quelen* (BARCELLOS et al., 2003; BORGES et al., 2004), channel catfish (TAVARES-DIAS and MORAES, 2007a), cachara (RANZANI-PAIVA et al., 2005) and *Synodontes membranacea* (OWOLABI, 2011). In contrast, the MCV of *S. lima* was greater than that found in *R. quelen* (BORGES et al., 2004) and *S. membranacea* (OWOLABI, 2011). The larger size indicates a larger erythrocyte membrane area for gas exchange, thus optimizing breathing (CARVALHO et al., 2009). Therefore, fish with larger erythrocytes may have fewer red blood cells to adduce hemoglobin (TAVARES-DIAS and MORAES, 2004) with efficient gas exchange (CARVALHO et al., 2009). The circulating blood of *S. lima* is composed of a large variety of cell types, including monocytes, lymphocytes (predominantly), basophils, eosinophils, neutrophils and LG-PAS cells, besides thrombocytes, which have the functions of protection and blood clotting (TAVARES-DIAS et al., 2007a; TAVARES-DIAS et al., 2007b). This study showed larger reference intervals in thrombocytes, monocytes, basophils and neutrophils, and smaller 25 to 75% ranges in lymphocytes when compared to those of *I. punctatus* (TAVARES-DIAS and MORAES, 2007a). Among the granulocytes, neutrophils were the most abundant cells and showed positive staining for proteins, glycogen and lipid.

Differences in reference intervals among fish are related to interspecific and environmental variations. Variables related to the red series are of great value in identifying anemic processes, while the WBC may be useful in understanding infectious processes and other states of homeostatic imbalance. The hematological profile of *S. lima* under

appropriate conditions, such as those studied here, is useful for diagnosis in fish farming and allows comparison between fish of the same species, family and/or order.

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References

ARAÚJO, C. S. O., M. TAVARES-DIAS, A. L. S. GOMES, S. M. S. ANDRADE, J. R. G. LEMOS, A. T. OLIVEIRA, W. R. CRUZ (2009): Infecções parasitárias e parâmetros sanguíneos em *Arapaima gigas* Schinz, 1822 (Arapaimidae) cultivados no estado do Amazonas, Brasil. In: Manejo e sanidade de peixes em cultivo. (Tavares-Dias, M., Org.), Embrapa Amapá, Brazil, 724 pp.

BABALOLA, T. O. O., M. A. ADEBAYO, D. F. APATA, J. S. OMOTOSHO (2009): Effect of dietary alternative lipid sources on haematological parameters and serum constituents of *Heterobranchus longifilis* fingerlings. Trop. Anim. Health and Production 41, 371-377.

BARBER, D. L., J. E. M. WESTERMANN (1975): Morphological and histochemical studies on a PAS-positive granular leucocyte in blood and connective tissues of *Catostomus commersoni* Lacêpède (Teleostei: Pisces). Am. J. Anat. 142, 205-220.

BARCELLOS, L. J. G., L. C. KREUTZ, L. B. RODRIGUES, I. FIOREZE, R. M. QUEVEDO, L. CERICATO, J. CONRAD, A. B. SOSO, M. FAGUNDES, L. A. LACERDA, S. TERRA (2003): Haematological and biochemical characteristics of male jundiá (*Rhamdia quelen* Quoy e Gaimard, Pimelodidae): changes after acute stress. Aquac. Res. 34, 1465-1469.

BEELEN, R., T. HEIJDEN, R. BOOMS, M. VERDEGEM, G. C. PAVANELLI (1998): Blood values of young brazilian catfish *Pseudoplatystoma corruscans* (Agassiz, 1829). Acta Sci. Biol. Sci. 20, 147-150.

BEELEN, R., B. BOYD, J. C. GARAVELLO, G. C. PAVANELLI, A. J. AINSWORTH (2003): A cytochemical, light and electron microscopic study of the peripheral blood leucocytes of hybrid surubim catfish, *Pseudoplatystoma corruscans* x *Pseudoplatystoma fasciatum*. Comp. Clin. Pathol. 12, 61-68.

BORGES, A., L. V. SCOTTI, D. R. SIQUEIRA, D. F. JURINITZ, G. F. WASSERMANN (2004): Hematologic and serum biochemical values for jundiá *Rhamdia quelen*. Fish Physiol. Biochem. 30, 21-25.

CAMARGO, S. O., J. L. POUEY, C. MARTINS (2005): Erythrocytic parameters of silver catfish (*Rhamdia quelen*) as affected by different protein level diets. Ciênc. Rural 35, 1406-1411.

CARVALHO, E. G., C. S. SEIBERT, M. S. COELHO, E. E. MARQUES (2009): Hematological parameters of native fish species of the Tocantins river, *Auchenipterus nuchalis*, *Psectrogaster amazonica* and *Squaliforma emarginata* (Teleostei, Ostariophysi). Acta Sci. Biol. Sci. 31, 173-177.

FUKUSHIMA, H., R. L. BAILONE, L. A. WEISS, M. L. MARTINS, E. ZANIBONI-FILHO (2012): Triploidy in the hematology of jundia juveniles (Siluriformes: Heptapteridae). Braz. J. Biol. 72, 147-151.

GOLDENFARB, P. B., F. P. BOWYER, E. HALL, E. BROSIUS (1971): Reproductibility in the hematology laboratory: the microhematocrit determinations. Am. J. Clin. Pathol. 56, 35-39.

ISHIKAWA, M. M., S. B. PÁDUA, F. SATAKE, H. HISANO, G. T. JERÔNIMO, M. L. MARTINS (2010): Heparin and Na2EDTA as anticoagulants for hybrid surubim catfish *Pseudoplatystoma reticulatum* x *P. corruscans*: efficacy and hematological changes. Ciênc. Rural 40, 1557-1561.

JERÔNIMO, G. T., M. L. MARTINS, F. BACKMANN, J. A. GREINERT-GOULART, A. A. SCHMITT-JÚNIOR, L. GHIRALDELLI (2009): Hematological parameters of *Pimelodus maculatus* (Osteichthyes: Pimelodidae) from polluted and non-polluted sites in the Itajaí-Açú river, Santa Catarina state, Brazil. Acta Sci. Biol. Sci. 31, 179-183.

LABARRÈRE, C. R., P. M. C. FARIA, E. A. TEIXEIRA, M. M. MELO (2012): Erythrogram of hybrids surubins (*Pseudoplatystoma corruscans* X *P. reticulatum*) kept at different stocking densities. Arq. Bras. Med. Vet. Zoot. 64, 510-514.

LE CREN, E. D. (1951): The length-weight relationship and seasonal cycle in gonadal weight and condition in the perch *Perca fluviatilis*. J. Anim. Ecol. 20, 201-219.

LISON, L. (1960): Lipides et lipoproteines. In: Lison, L. Histochemie et cytochimie animales. Principes et methods. Paris, Gauthier Villars 2, 449-530.

MARTINS, M. L., C. A. SHOEMAKER, D. XU, P. H. KLESIUS (2011a): Effect of parasitism on vaccine efficacy against *Streptococcus iniae* in Nile tilapia. Aquaculture 314, 18-23.

MARTINS, M. L., D. XU, C. A. SHOEMAKER, P. H. KLESIUS (2011b): Temperature effects on immune response and hematological parameters of channel catfish *Ictalurus punctatus* vaccinated with live theronts of *Ichthyophthirius multifiliis*. Fish and Shellfish Immunol. 31, 774-780.

MAZIA, D., P. A. BREWER, M. ALFERT (1953): The cytochemical staining and measurement of protein with mercuric bromophenol blue. Biol. Bull. 104, 57-67.

McMANUS, J. F. A. (1946): Histological demonstration of mucin after periodic acid. Nature 158, 202.

MORAES, F. R., M. L. MARTINS (2004): Predisposing conditions and principal diseases of intensive fish farming teleosts. In: Especial Topics in Intensive Freshwater Fish Culture in the Tropics. (Cyrino, J. E. P., E. C. Urbinatti, D. M. Fracalossi, N. Castagnolli, Eds.). São Paulo/SP, Brazil, TecArt 343-383.

OWOLABI, O. D. (2011): Haematological and serum biochemical profile of the upside-down catfish *Synodontis membranacea* Geoffroy Saint Hilaire from Jebba Lake, Nigeria. Comp. Clin. Pathol. 20, 163-172.

PÁDUA, S. B., M. M. ISHIKAWA (2011): Metacromasia para identificação de basófilos sanguíneos em surubim híbrido: contribuição metodológica. Rev. Bras. Med. Vet. 33, 147-150.

PÁDUA, S. B., M. M. ISHIKAWA, R. Y. D. KASAI, G. T. JERÔNIMO, J. R. CARRIJO-MAUAD (2012): Parasitic infestations in hybrid surubim catfish fry (*Pseudoplatystoma reticulatum* x *P. corruscans*). Rev. Bras. Med. Vet. 34, 235-240.

PÁDUA, S. B., M. M. ISHIKAWA, A. S. VENTURA, G. T. JERÔNIMO, M. L. MARTINS, L. E. R. TAVARES (2013): Brazilian catfish parasitized by *Epistylis* sp. (Ciliophora, Epistylididae), with description of parasite intensity score. Parasitol. Res. 112, 443-446.

PRASAD, G., S. CHARLES (2010): Haematology and leucocyte enzyme cytochemistry of a threatened yellow catfish *Horabagrus brachysoma* (Gunther 1864). Fish Physiol. Biochem. 36, 435-443.

RANZANI-PAIVA, M. J. T., E. ROMAGOSA, C. M. ISHIKAWA (2005): Hematological parameters of "cachara", *Pseudoplatystoma fasciatum* Linnaeus, 1766 (Osteichthyes, Pimelodidae), reared in captivity. Bol. Inst. Pesca 31, 47-53.

RANZANI-PAIVA, M. J. T., S. B. PÁDUA, M. TAVARES-DIAS, M. I. EGAMI (2013): Métodos para análise hematológica em peixes. EDUEM, Brazil, 135pp.

ROSSI, L. M. (2001): Ontogenetic diet shifts in a neotropical catfish, *Sorubim lima* (Schneider) from the river paraná system. Fish. Manag. Ecol. 8, 141-152.

ROUGH, K. M., B. F. NOWAK, R. E. REUTER (2005): Haematology and leukocyte morphology of wild caught, *Thunnus maccoyii*. J. Fish Biol. 66, 1649-1659.

SHIBATTA, O. A., J. L. NOVELLI, J. H. P. DIAS, S. G. C. BRITTO, M. C. FILHO (2011): Reprodução em cativeiro do Jurupecê, *Sorubim lima* (Siluriformes, Pimelodidae), por meio de indução hormonal. Semina: Ciênc. Agr. 32, 363-372.

SHIGDAR S., A. HARFORD, A. C. WARD (2009): Cytochemical characterization of the leucocytes and thrombocytes from Murray cod (*Maccullochella peelii peelii*, Mitchell). Fish Shellfish Immunol. 26, 731-736.

SILVA, B. C., J. L. P. MOURINO, F. N. VIEIRA, A. JATOBA, W. Q. SEIFFERT, M. L. MARTINS (2012a): Haemorrhagic septicaemia in the hybrid surubim (*Pseudoplatystoma corruscans* x *Pseudoplatystoma fasciatum*) caused by *Aeromonas hydrophila*. Aquac. Res. 43, 908-916.

SILVA, L. L., T. V. PARODI, P. RECKZIEGEL, V. O. GARCIA, M. E. BÜRGER, B. BALDISSEROTTO, C. A. MALMANN, A. M. S. PEREIRA, B. M. HEINZMANN (2012b): Essential oil of *Ocimum gratissimum* L.: Anesthetic effects, mechanism of action and tolerance in silver catfish, *Rhamdia quelen*. Aquaculture 350-353, 91-97.

TAVARES-DIAS, M. (2006a): A morphological and cytochemical study of erythrocytes, thrombocytes and leukocytes in four freshwater teleosts. J. Fish Biol. 68, 1822-1833.

TAVARES-DIAS, M. (2006b): Cytochemical method for staining fish basophils. J. Fish Biol. 69, 312-317.

TAVARES-DIAS, M., F. R. MORAES (2004): Hematologia de peixes teleósteos. Vilimpress, Brazil, pp. 144.

TAVARES-DIAS, M., J. F. M. BARCELLOS (2005): Peripheral blood cells of the armored catfish, *Hoplosternum littorale* Hancock, 1828: a morphological and cytochemical study. Braz. J. Morphol. Sci. 22, 215-220.

TAVARES-DIAS, M., F. R. MORAES (2006): Morphological, cytochemical, and ultrastructural study of thrombocytes and leukocytes in Neotropical fish, *Brycon orbignyanus* Valenciannes, 1850 (Characidae, Bryconinae). J. Submicrosc. Cytol. Pathol. 38, 209-215.

TAVARES-DIAS, M., F. R. MORAES (2007a): Haematological and biochemical reference intervals for farmed channel catfish. J. Fish Biol. 71, 383-388.

TAVARES-DIAS, M., F. R. MORAES (2007b): Leukocyte and thrombocyte reference values for channel catfish, *Ictalurus punctatus* Raf, with an assessment of morphologic, cytochemical, and ultrastructural features. Vet. Clin. Pathol. 36, 49-54.

TAVARES-DIAS, M., E. A. ONO, F. PILARSKI, F. R. MORAES (2007a): Can thrombocytes participate in the removal of cellular debris in the blood circulation of teleost fish? A cytochemical study and ultrastructural analysis. J. Applied Ichthyol. 23, 709-712.

TAVARES-DIAS, M., F. R. MORAES, E. M. ONAKA, P. C. B. REZENDE (2007b): Changes in blood parameters of hybrid tambacu fish parasitized by *Dolops carvalhoi* (Crustacea, Branchiura), a fish louse. Vet. arhiv 77, 355-363.

UEDA, I. K., M. I. EGAMI, W. S. SASSO, E. R. MATUSHIMA (2001): Cytochemical aspects of the peripheral blood cells of *Oreochromis niloticus*, tilápia, Linnaeus, 1758 (Cichlidae, Teleostei) - Part II. Braz. J. Vet. Res. Anim. Sci. 38, 273-277.

VEIGA, M. L., M. I. EGAMI, M. J. P. RANZANI-PAIZA, E. L. RODRIGUES (2000): Aspectos morfológicos y citoquímicos de las células sanguíneas de *Salminus maxillosus* Valenciennes, 1840 (Characiformes, Characidae). Rev. Chilena Anat. 18, 245-250.

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SAŽETAK

Hematologija podrazumijeva brze i praktične analize koje pomažu dijagnostici poremećene homeostatske ravnoteže u riba. Ovim istraživanjem utvrđeni su referentne vrijednosti morfoloških i citokemijskih obilježja krvnih stanica južnoameričkog soma *Sorubim lima* (Siluriformes, Pimelodidae), uzgajanog u močvarnim područjima Brazila. Kod ukupno 92 farmski uzgojene ribe istraženi su referentne vrijednosti za ukupne bjelačevine plazme, hemoglobin, broj eritrocita, hematokrit, srednji volumen eritrocita, prosječnu koncentraciju hemoglobina te ukupni broj trombocita i leukocita. Za citokemijsko određivanje glikogena, bjelančevina, masti te jezgri u trombocitima i leukocitima upotrijebljen je Schiffov perjodni reagens (PAS) s amilazom sline, bromfenolnim plavilom, sudanskim crnilom B i toluidinskim plavilom (metakromatska zrnca). U krvnim razmascima utvrđeni su monociti, limfociti, neutrofili, bazofili, eozinofili, PAS pozitivni granulirani limfociti i trombociti. Ustanovljeno je da je morfologija neutrofila slična onoj u riba somovki (Siluriformes). U usporedbi s ostalim dugobrkim ribama (Pimelodidae), broj eritrocita, hematokrit i srednji volumen eritrocita pokazali su najniži indeks varijacija. Za razliku od ostalih somovki, *S. lima* je pokazala istodobnu prisutnost cirkulirajućih eozinofila i bazofila.

Ključne riječi: slatkovodna riba, *Sorubim lima*, som, zdravlje, hematologija, farmski uzgoj