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Blood values of young Brazilian catfish *Pseudoplatystoma corruscans* (Agassiz, 1829)

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ABSTRACT. The ranges and mean values of haematocrit, leucocrit, red cell, white cell and differential cell counts and serum protein concentration of young growing *Pseudoplatystoma corruscans* (n=6) were measured, and serum electrophoretic patterns on cellulose acetate described. Blood was obtained by puncture of the caudal vessels. Using routine staining methods, five different cell types could be distinguished by light microscopy. Mean total cell count was 1.29 x 10^6 cells.ml^{-1}, while mean plasma protein content was 11.82g.l^{-1} consisting of five major protein fractions. These haematological characteristics were established as a "working document" upon which to base more extensive studies.

Key words: Blood, electrophoresis, fish, plasma proteins, *Pseudoplatystoma corruscans.*

RESUMO. Valores sangüíneos de pintados juvenis, *Pseudoplatystoma corruscans* (Agassiz, 1829). Foram analisadas amostras sangüíneas de seis exemplares de *Pseudoplatystoma corruscans*. As amostras foram coletadas por punção caudal e foram determinados os valores e as médias de hematócrito, leucócrito, eritrócitos e leucócitos e das concentrações protéicas plasmáticas. O plasma sangüíneo foi submetido a eletroforese, para determinação das diferentes frações protéicas. A partir de extensões sangüíneas coradas com métodos rotineiros, cinco tipos celulares puderam ser distinguidos sob microscopia ótica. A média das células sangüíneas totais foi de 1.29 x 10^6 células.ml^{-1}, enquanto que a concentração protéica média do plasma sangüíneo foi 11.82g.l^{-1}, constituindo-se de cinco frações principais. Essas características hematológicas foram estabelecidas no intuito de se obter um primeiro quadro hematológico da espécie, no qual serão baseados estudos mais aprofundados.

Palavras-chave: eletroforese, peixe, proteínas plasmáticas, *Pseudoplatystoma corruscans,* sangue.

There are different health monitoring programmes in domestic farm animals using blood parameters to assess animal health. Sometimes it is a key for diseases diagnosis, but mostly the results of haematological investigations allows for acceptance or rejection of a possible diagnosis. In fish health research the need to use such parameters has been underlined in a review by Blaxhall (1972), but in fish health management the relevance of haematology is being challenged mainly by the scarce availability of data on physiological ranges of the blood parameters of cultured fish species. Nevertheless, it is reasonable to assume that haematology will play an even more eminent role in fish health, as opposed to farm animal health, because of the impracticability of physically examining respiratory, circulatory, digestive or nervous systems of live fish. Moreover, in contrast to post-mortem studies, the fish do not have to be sacrificed (Peutz *et al*., 1996). Therefore the objectives of the present study are to describe the normal morphology of the cellular blood components and to establish values of a selected number of cellular and humoral blood parameters of the pintado, *Pseudoplatystoma corruscans* (Agassiz, 1829), which are lacking. The reason of our choice for this species is that among the various species of Siluriformes found in South America, the pintado, a catfish with wide geographical distribution in South American rivers (Amazonas, Paraguay, São Francisco and Paraná), may be considered as one of the most promising species for the Brazilian fish culture. At present several studies are undertaken to generate information about this animal to provide a sound basis for establishing the Pintado as a «cultivable species».

Material and methods

Fish. Two-month old *Pseudoplatystoma corruscans* (12.4 ± 1.5 g) were obtained from a commercial farm (Peixe Vivo-Campo Grande-MS) in south western Brazil. Upon arrival at the laboratory (Dept. Fish Culture & Fisheries, Wageningen Agricultural University, The Netherlands), 6 animals (randomly chosen) were placed into 140-l aquarium, with recirculating, aerated, filtered and ultraviolet-sterilised water at 27 ± 1°C. Animals were fed above satiation with frozen artemia, frozen mosquito larvae and catfish dry pellets (Trouvit, Trouw & Co BV), and were allowed to acclimatise for 4 weeks before blood sampling. Water quality parameters were measured weekly. Temperature and acidity were measured using a pH meter (HANNA Instruments, Belgium), conductivity with a conductivity meter (HANNA Instruments, Belgium). Nitrate, nitrite and ammonium were estimated using test kits (Merk, Germany). The photoperiod was fixed at 12L/12D.

Sampling procedure. After sedation in an immersion bath of 0.02% tricaine methane sulfonate (TMS) (Crescent Research Chemicals, Phoenix, AZ, USA) and 0.06% sodium bicarbonate, blood samples of ± 0.5ml were collected from each fish by puncturing the caudal vessels using a 1cm^3 sterile plastic syringe coated with potassium salt of ethylenediamine tetra-acetic acid (EDTA). All fish were weighed and the standard length measured.

Blood cells. *In duplo* measurements were made for haematocrit (Hct) and leucocrit (Lct) determination immediately after blood sampling (Schippers *et al.*, 1994). Blood smears were stained with a modified May-Grünwald-Giemsa method in order to allow observation of white blood cell morphology and the relative abundance of cell types. Next, the same smears were stained with the periodic-acid-Schiff (PAS) method to identify granulocytes with and without polysaccharides (PAS$^+$ and PAS$^-$). The relative abundance of each cell type was determined by counting a total of 100 granulocytes at a magnification of 1000X (Schippers *et al.*, 1994). Total cell count was determined using a coulter counter Model ZM (Coulter Electronics Ltd, UK).

Plasma proteins. Plasma fraction was separated immediately after blood sampling by centrifugation at 1500g for ten minutes and plasma was used to determine the total plasma proteins and plasma protein fractions. The total plasma protein (TPP) concentration was measured with the modified Lowry method (Lowry *et al.*, 1951), (Sigma Chemical Company 1989). The different fractions of the plasma proteins (PPF) were determined by electrophoresis of plasma samples using cellulose acetate paper in a Boskamp electrophoresis chamber powered with Boskamp Pherostat 273 (Schippers *et al.*, 1994). Electrophoresis was allowed to proceed for 60 min at a constant voltage of 300Volts. Protein bands were scanned by Ultroscan XL scanning laser densitometer. Results were evaluated by Gelscan XL 2.1.

Results

The water quality parameters were maintained constant. Temperature varied between 27°C and 28°C, pH remained between 8.04 and 8.19, and conductivity between 38.6 and 53.0mS. Nitrate was lower than 50mg.l^{-1}, and nitrite and ammonium were negligible. The mean values and range of the most relevant haematological parameters are given in Table 1, together with the standard deviation of each mean.

Table 1. Summary of the haematological parameters

Parameter	Range	Mean	Std
Hematocrit (%)	16.58-23.46	20.43	3.06
Leucocrit (%)	0.29-2.23	1.26	0.70
Cell counts (million/ml)	0.90-1.58	1.29	0.22
Young lymphocytes (% of total white cells)	6-15	8.17	3.49
Old lymphocytes (% of total white cells)	45-66	58.50	7.64
PAS neg. granulocytes (% of total white cells)	21-48	31.83	9.91
PAS pos. granulocytes (% of total white cells)	0-6	1.50	2.26
Total plasma protein (g. l^{-1})	7.40-15.02	11.82	2.68
PPF I (%)	12.9-17.3	15.3	1.49
PPF II (%)	31.8-38.1	35.78	2.26
PPF III (%)	13.4-15.2	13.93	0.68
PPF IV (%)	20.5-28.2	25.45	2.68
PPF V (%)	8.2-11.9	9.67	1.49

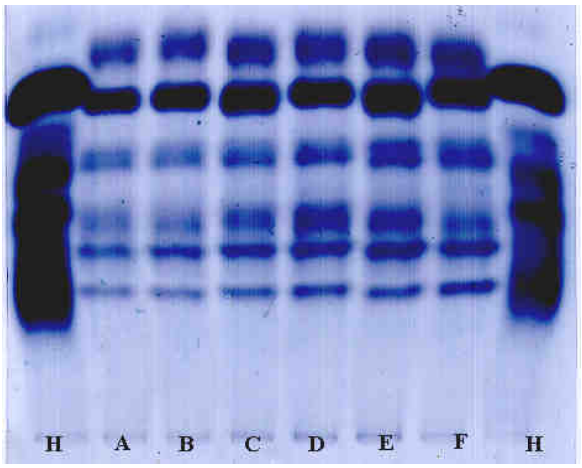

Figure 1. Electrophoretic separation of *Pseudoplatystoma corruscans* plasma proteins on cellulose acetate paper. (H) Human serum albumin as a standard, (A, B, C, D, E, F) normal fish plasma

The electrophoretic pattern of the plasma proteins showed five fractions (Figures 1 and 2) which were numbered I to V in function of decreasing electrophoretic mobility.

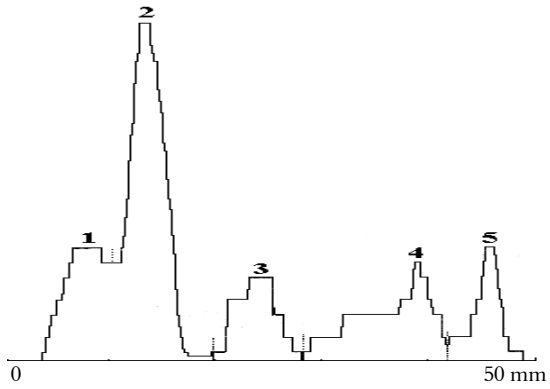

Figure 2. Cellular acetate electropherogram of plasma proteins of Pintado, *Pseudoplatystoma corruscans*. Arabic numerals indicate five different protein fractions

According to form, shape and colour, five blood cell types could be identified. The cells (Figure 3) were identified, as described by Blaxhall and Daisley (1973):

Erythrocytes. The cells were oval and had a homogenous light red cytoplasm and an oval, centrally located nucleus. The nucleus colour was purple.

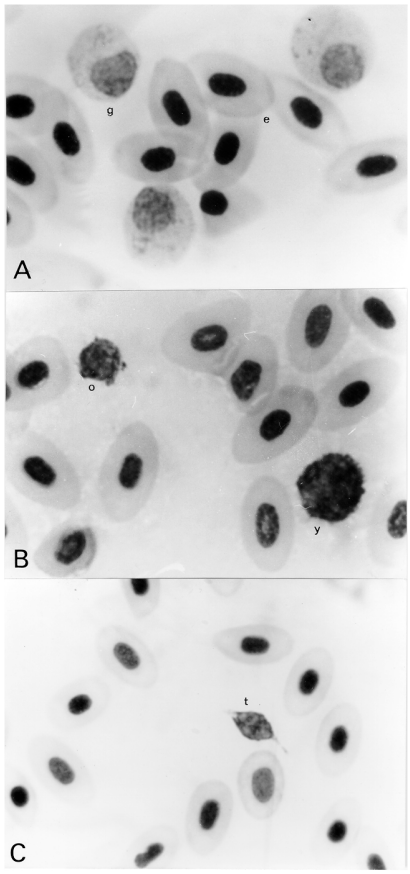

Figure 3. Light micrographs of the peripheral blood cells of *Pseudoplatystoma corruscans*, stained according to a modified May-Grünwald-Giemsa (1000X). (A) Erytrocyte (e), Granulocyte (g); (B) Young lymphocyte (y), Old lymphocyte (o); (C) Thrombocyte (t)

Young (large) lymphocytes. These cells had dark blue and fairly homogenous nucleus. The cytoplasm was nongranular, often with pseudopod-like projections on the surface and stained light blue.

Old (small) lymphocytes. These cells were small and round, the nucleus occupied most of the cell, leaving a small border of light blue cytoplasm. The nucleus was a compact darker mass.

Granulocytes. These cells had segmented nucleus separated by thin filaments. In young granulocytes the nucleus was round or horse-shoe shaped. The nucleus was purple red and the cytoplasm light pink with small granules (violet droplets).

Thrombocytes. These cells were variable in shape, sometimes oval, elongated or flask-shaped. The nucleus was purple and the cytoplasm blue. The cell gives the general appearance of a badly distorted lymphocyte. The thrombocytes were not included in the counting.

Discussion

The Hct and Lct values of this study agree with those found in other research on fish blood (Hattingh, 1972, McCarthy *et al*., 1973 and 1975, Schippers *et al*., 1994). The relative abundance of the different peripheral blood cells showed a predominance of old lymphocytes which is consistent with that reported for other species (Boon *et al*., 1990b, Peutz *et al*., 1996, Schippers *et al*., 1994). The high percentage level of lymphocytes suggests that the fish were not stressed (Pickering, 1986). There were more PAS^- than PAS^+ granulocytes which is also in accord with other studies. The literature concerning fish granulocytes is confusing and contradictory, the nomenclature is usually based on morphologic description of mammalian cells. Presumptive identification of granulocytes is based on morphologic criteria as cell size, cytoplasmic staining, nuclear shape, and histochemical reactions. As the peripheral blood leukocytes (PBL) are of key importance in the defence mechanisms, it seems very important to obtain in subsequent reports a more accurate classification of the PBL population of *Pseudoplatystoma corruscans* (electron microscope & functional criteria) than it was possible with the conventional staining techniques used in this study.

The TPP values obtained were lower than those found in other studies (Hille, 1985, Schippers *et al*., 1994). In the electrophoretic pattern, five major protein fractions (PPF I, II, III, IV and V) could be recognised. This agrees with the findings of Boon *et al*. (1990a) for European eel, *Anguilla anguilla* L., but disagrees with those of other species, in which four major protein fractions were identified (Boon *et al*., 1986, Boon *et al*., 1987). A number of authors have assigned these fractions in analogy with mammalian plasma proteins

as, albumin(s) and α, β and γ globulin. In our study the percentage of PPF I is low and the percentage of PPF II is quite high. In the TPP, the relative content of PPF II is much smaller in other species and the biggest fraction is PPF I. In this study the latter moved closely to PPF II and suggests they might be complementary proteins. As refereed by Kirsipuu (1979) a pure albumin-like protein fraction could be included in the two or three fastest-running fractions of the electrophoretic pattern. So, when the two are considered as albumin, relative amounts are above 50%. This is the percentage expected with juvenile fish, because PPF I includes carrying proteins which are very useful in young growing fish. The amount of fraction IV-V is also at a higher level. Normally, under the experimental conditions the fish were not in contact with pathogens, and in consequence the immune system was not stimulated; this should result in low level of immunoglobulins which are considered part of the PPF IV (Hille, 1985). It is known that depending on the type of electrophoresis, different fractions could be demonstrated (Mulcahy, 1970), but the differences obtained in this study are still unclear and remains to be investigated in further studies. The main purpose of this study was to obtain a basis for comparison with standardised techniques to enable future studies. Much work has still to be done on pintado's haematological investigations, especially the establishment of normal values together with the identification of those environmental and disease conditions that may affect these values.

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