Haematological and biochemical values for Nile tilapia *Oreochromis niloticus* cultured in semi-intensive system

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ABSTRACT. The aim of the present study was to investigate the reference ranges for haematological and biochemical values for Nile tilapia, *Oreochromis niloticus* (Cichlidae), cultivated in a semi-intensive system. Erythrocytes number was 6.93 ± 8.28 x 10^6/mm^3, hemoglobin 10.52 ± 3.09g/dL of blood and hematocrit 31.85 ± 8.45%, mean corpuscular volume (MVC) 148.80 ±. 153.19μ^3, mean corpuscular hemoglobin (MCH) 40.74 ± 34.19 pg and mean corpuscular hemoglobin concentration (MCHC) 35.24 ± 14.92%. Total plasma protein and glucose values obtained were 3.06 ± 0.65g/dl and 60.32 ± 20.22mg/dl, respectively. A correlation matrix was established to compare de degree of association among biometric data and hematological parameters, and among each of them and biochemical values. A positive correlation was observed among weight, length, MCV and MHC, as well as between length and hematocrit. The blood glucose level was positively correlated with weight and length, whereas total protein was positively correlated with hemoglobin. The erythrocytes count was positively correlated with hemoglobin and negatively correlated with MCV and MHC. Microscopy examination of blood smears revealed the presence of erythrocytes, neutrophils, lymphocytes, and monocytes. Under the conditions employed here, no basophils or eosinophils, nor their precursors, could be found in blood smears of Nile tilapia.

Key words: Nile tilapia, *Oreochromis niloticus*, haematology, biochemistry.

RESUMO. Valores hematológicos e bioquímicos em tilápia do Nilo *Oreochromis niloticus* cultivada em sistema semi-intensivo. O objetivo do presente estudo foi investigar os valores de referências hematológicos e bioquímicos para a tilápia do Nilo, *Oreochromis niloticus* (Cichlidae), cultivada em sistema semi-intensivo. A contagem de eritrócitos foi 6,93 ± 8,28 x 10^6/mm^3, hemoglobina 10,52 ± 3,09g/dL de sangue, hematócrito 31,85 ± 8,45%, volume corpuscular médio (MVC) 148,80 ±.153,19μ^3, hemoglobina corpuscular média (MCH) 40,74 ± 34,19pg e concentração de hemoglobina corpuscular média 35,24 ± 14,92%. Os valores de proteína plasmática total e da glicose obtidos foram de 3,06 ± 0,65g/dl e 60,32 ± 20,22mg/dl, respectivamente. Uma matriz de correlação foi estabelecida para comparar o grau de associação entre os dados biométricos e parâmetros hematológicos, e entre estes e os valores bioquímicos. Uma correlação positiva foi observada entre peso, comprimento, MCV e MHC, bem como entre comprimento e hematócrito. O nível de glicose sanguínea foi positivamente correlacionado com o peso e o comprimento, enquanto proteína total foi positivamente correlacionada com a hemoglobina. A contagem de eritrócito foi positivamente correlacionada com a hemoglobina e negativamente correlacionada com MCH e MHC. A observação microscópica do esfregaço sangüíneo revelou a presença de eritrócitos, neutrófilos, linfócitos e monócitos. Sob as condições empregadas no estudo não foram encontrados basófilos, ensinófilos ou seus precursores no esfregaço sangüíneo de tilápia do Nilo.

Palavras-chave: tilápia do Nilo, *Oreochromis niloticus*, hematologia, bioquímica.

Introduction

Tilapia (*Oreochromis niloticus*) culture is widely practiced in many tropical and subtropical regions of the world and constitutes the third largest group of farmed finfish, right after carp and salmonids, with an annual growth rate around 11.5%. Much of the rapid increase in aquaculture production in recent years has come from the increasing of existing systems.

Increase in stocking densities can make fish more susceptible to stress and disease, in turn, may affect or cause severe stock loss (Schreck, 1996). The physiological stress response, although initiated as an adaptive response to destabilizing factors, can have damaging effects if prolonged. It is well established that continuous stress affects the behavior and normal development, with growth reduction (Jobling and Reinsnes, 1986), suppression of reproduction (Gerking, 1980), and an increase in susceptibility to infections, through immunodepression (Schreck and Bradford, 1990), which may cause mortality. Therefore, there has been a greater understanding of the need to establish reference haematological and biochemical values in fish in order to assess health status and the subsequent diagnosis of disease.

Prompted by these reports, the present study aims to establish reference ranges for haematological values for healthy Nile tilapia *O. niloticus* cultivated in a semi-intensive system. This level of semi-intensive management is common for small-scale tilapia operations with limited capital, or in situations where high-quality food is not available.

Material and methods

Fish. Nile tilapia, *O. niloticus,* semi-intensively cultured, came from the Estação Experimental de Piscicultura, Universidade Estadual de Maringá. The fish (females and males) used had 100-900g in average weight, 16-37cm in lenght and were 6 to 9 months old. The weather at *Maringá* region, in northwestern State of *Paraná*, Brazil, belongs to type CW 'h (Köppen classification) and is called "tropical", with two wet seasons (fall, late spring and early summer) and two dry seasons (winter and early spring). The annual rainfall is about 1582.2mm. The annual mean temperature is approximately 22ºC (Deffune *et al.,* 1994).

Sample Preparation. Blood was taken from fish on four occasions between March and April, when the water temperature varied from 16 to 28.3ºC. Fish (randomly chosen) were weighted and anesthetized with 1:15 benzocaine solution after being captured. Approximately 1ml of blood was extracted from the caudal vein, using a heparinized syringe.

Haematological techniques. One drop of whole blood was placed in a microscope slide, two slides for each fish; smeared and allowed to dry for later staining with Leishman's reagent and used for differential counting of leukocytes (Vallada, 1988). Haematocrit and Hemoglobin were determined according to Vallada (1988). Erythrocyte number was determined by using Neubauer chamber. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were analyzed according to the method proposed by Wintrobe (1934). Total plasma protein and glucose determination followed Henry *et al*. (1974) and Bergmeyer (1986), respectively.

Statistical analysis. Results were analyzed by linear regression and *t*-test, at 5% of confidence level.

Results and discussion

Summary for haematological and biochemical parameters are presented in Table 1. Erythrocytes amount was 6.93 ± 8.28 x 10^6/mm^3, hemoglobin 10.52 ± 3.09g/dL of blood and hematocrit 31.85 ± 8.45%, mean corpuscular volume (MVC) 148.80 ± 153.19μ^3, mean corpuscular hemoglobin (MCH) 40.74 ± 34.19 pg and mean corpuscular hemoglobin concentration 35.24 ± 14.92%. Total plasma protein and glucose obtained were 3.06 ± 0.65g/dl and 60.32 ± 20.22mg/dl, respectively. A correlation matrix was established to compare the degree of association among the biometric data and hematological parameters and among each of them and biochemical values (Table 2). A positive correlation was observed among weight, length, MCV and MHC, as well as between length and hematocrit. The blood glucose level was positively correlated with weight and length, whereas total protein was positively correlated with hemoglobin. The erythrocytes count was positively correlated with the hemoglobin and negatively correlated with MCV and MHC.

Table 1. Summary of the haematological and biochemical parameters of Nile tilapia *Oreochromis niloticus* cultured in semi-intensive system.

Parameters	Mean	Range
Erythrocytes (10^6/mm^3)	6.93 ± 8.28	0.7-28
Hemoglobin (g/dl)	10.52 ± 3.09	6.58-15.98
Hematocrit (%)	31.85 ± 8.45	15-45
MCV (μ^3)	148.80 ± 153.19	12.36-528.57
MHC (pg)	40.74 ± 34.19	5.07-120.86
MCHC (%)	35.24 ± 14.92	19.84-87.73
Glucose (mg/dl)	60.32 ± 20.22	22.7-107
Total protein (g/dl)	3.06 ± 0.65	1.81-3.98

Table 2. Correlation coefficients among the haematological, biochemical and biometric parameters of Nile tilapia *Oreochromis niloticus* cultured in semi-intensive system.

Parameters	Correlation coefficients								
Length	0.95*								
Erythrocytes	-0.33	-0.26							
Hemoglobin	-0.07	0.04	0.54*						
Hematocrit	0.39	0.50*	-0.11	0.31					
MCV	0.56*	0.54*	-0.60*	-0.35	0.47*				
MHC	0.50*	0.49*	-0.66*	-0.33	0.40	0.98*			
MCHC	-0.10	-0.12	0.42	0.49*	-0.59*	-0.54*	-0.49*		
Glucose	0.66*	0.60*	-0.28	-0.09	0.18	0.44	0.43	-0.03	
Total Protein	-0.08	-0.02	0.28	0.49*	0.37	-0.13	-0.17	0.11	-0.17
	Weight	Length	Erythrocytes	Hemoglobin	Hematocrit	MCV	MHC	MCHC	Glucose

Significance level: *P < 0,05.

Microscopic examination of blood smears revealed the presence of erythrocytes, neutrophils, lymphocytes, and monocytes (Figure 1). Under the conditions employed here, no basophils or eosinophils, nor their precursors, could be found in blood smears of Nile tilapia.

An attempt was made to compare the reference range for haematological values for healthy Nile tilapia and the range of the corresponding values for Brazilian fish specimens reported by other investigators. A direct comparison was not possible because of the different experimental conditions; however, some points were evident.

Erythrocytes count at 6.93 x 10^6/mm^3 in Nile tilápia are within the range described for "piava" *Schizodon borellii*, 2.09 x 10^6/mm^3 and "curimbatá" *Prochilodus lineatus*, 2.33 x 10^6/mm^3 (Ranzani-Paiva *et al*., 2000). On the other hand, it is significantly lower than those described for Florida red tilapia *Oreochromis urolepis hornorun x O mossambicus*, 256.7 x10^6/mm^3 (Tavares-Dias *et al*., 2000a), and "pacu" *Piaractus mesopotamicus*, 249.4 x 10^6/mm^3 (Tavares-Dias *et al*., 1999).

The mean value for hematocrit in Nile tilápia obtained in this study is within the range of the corresponding values described for specimens of "piauçu" *Leporinus macrocephalus*, *P. mesopotamicus* (Tavares-Dias *et al*., 1999), Florida red tilapia (Tavares-Dias *et al*., 2000a), and *S. borellii* (Ranzani-Paiva *et al*., 2000). This value is lower than those reported for hybrid "tambacu" (*Piaractus mesopotamicus* Holmberg, 1887, male x *Colossoma macropomum* Cuvier, 1818 female) (Tavares-Dias *et al*., 2000b) and *P. lineatus* (Ranzani-Paiva *et al*., 2000).

The hemoglobin concentration (10.52g/dl) in Nile tilapia is similar to those reported for "pacu" (10.40g/dl), "curimbatá" (9.70g/dl), and hybrid "tambacu" (12.7g/dl), but higher than those for Florida red tilapia (7.3g/dl) and "piava" (7.7g/dl).

Higher value of MVC (148.8μ^3) than those referred for "pacu" (117.6μ^3) and Florida red tilápia (113.6μ^3) were found in the present work. However, this value is significantly lower than the values obtained for "piava" (166.6dμ^3) and "curimbatá" (169.3μ^3).

As pointed by Teixeira *et al*. (2000) reference values determined for hemogram elements and biochemical tests may not represent precisely those of a certain population or animal species and should, therefore, be carefully interpreted, once there is a wide range of physiological variation. According to these authors, these variations are influenced by environmental conditions, gender, age, origin, breeding system, feeding and lineage, which may also interfere with the results obtained in tests.

The blood glucose level (60.32mg/dl) in Nile tilápia was similar to those reported by Tavares-Dias *et al*. (1999) for "pacu" (76.4mg/dl). However, this value is significantly higher than the value obtained for channel catfish *Ictalurus melas* (39.7mg/dl). In the present work, blood was taken on four occasions between March and April, when water temperature varied from 16 to 28.3°C. Seasonal temperature changes may affect blood sugar levels, although the data refer to fishes from tropical regions, where temperature is more or less stable throughout the year. The seasonal difference in blood glucose level may also be due to the different sugar metabolism in the various seasons. As pointed by Chavin and Young (1970), temperature affects the blood sugar levels, but the pattern is inconsistent. According to these authors, at low temperatures, *Ictalurus melas* and *C. carpio* become hyperglycemic, *Pomoxix annularis* becomes hypoglycemic, and *Lepomis macrochirus* remains unchanged. Other environmental and physiological factors may contribute to the reported variations. The total protein values obtained were lower than those found in two-month old *Pseudoplatystoma corruscans* (Beelen *et al*., 1998).

According to form, shape and color, erythrocytes, neutrophils type I and II, lymphocytes and monocytes could be identified. Under the conditions employed in this experiment, no basophils or eosinophils, nor their precursors, could be found in blood smears of Nile tilápia. Basophils and eosinophils are generally lacking in fish blood, but studies by Pitombeira and Martins

(1970), Ezzat *et al.* (1974) indicate that they are present in at least some fish species. According to Boomker (1981), although it is generally accepted that most helminths elicit an eosinophilic reaction, no eosinophils could be found in catfish, around either living or dead nematodes. In studies by Ranzani-Paiva *et al*. (2000) the highest percentage of eosihophils was found in non-parasitized *S. borelli.* Erythrocytes in circulating blood were oval with a homogenous light red cytoplasm. Their nucleus color was purple and centrally located (Figure 1A). Blood smears revealed the presence of two types of cells of the neutrophilic series: Neutrophils type I (Figure 1A) and type II (Figure 1C). Neutrophils type I were round and had a cytoplasm stained light blue. Their nucleus was fairly large, round, oval or roughly triangular, and was always eccentrically situated. Neutrophil type II was usually round and the cytoplasm contains numerous neutrophilic granules. Their nucleus was either ring-shaped or polymorphic and contains 1 or 2 lobes. Lymphocytes were smaller than erythrocytes, usually spherical and containing a large nucleus surrounded by a thin rim of cytoplasm (Figure 1B). Their nucleus stained intensely purplish-blue and the cytoplasm stained pale blue. Monocytes were round, although cytoplasmic projections and pseudopodia were often seen. The nucleus of the monocytes was round or triangular in shape and usually eccentrically situated (Figure 1D).

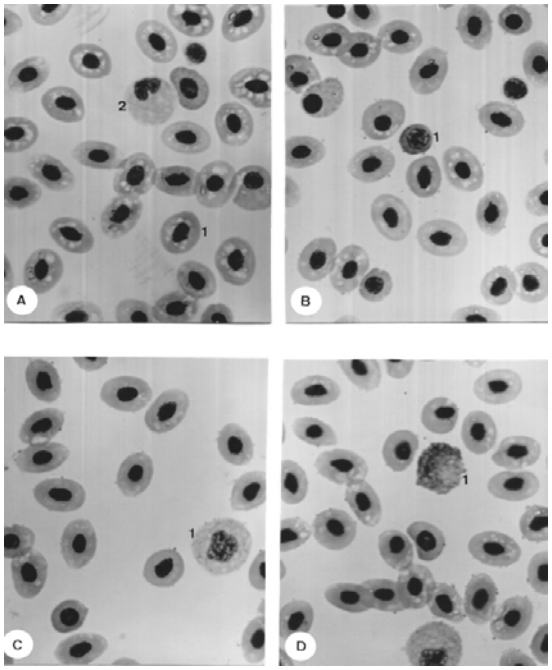

Figure 1. Microscopy examination of blood smears of *Oreochromis niloticus* shown the presence of (A) erythrocyte (A1), neutrophil type I (A2); lymphocytes(B1); Neutrophil type II (C1), monocytes(D1).

The large variation in hematological and biochemical parameters obtained emphasizes the need of more extensive study with a large number of animals to confirm whether age, sex, environmental and disease conditions modify these hematological and biochemical profiles, before the establishment of normal values for healthy Nile tilápia (*Oreochromis niloticus)*.

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References

BEELEN, R. *et al*. Blood values of young Brazilian catfish *Pseudoplatystoma corruscans* (Agassiz, 1829). *Acta Scientiarum,* Maringá, v. 20, n. 2, p. 47-150, 1998.

BERGMEYER, H.U. *Methods of enzimatic analysis.* Deerfield Beach: VCH, 1986.

BOOMKER, J. The haemocytology and histology of the haemopoietic organs of South African freshwater fish. III. The leucocytes, plasma cells and macrophages of *Clarias gariepinus* and *Sarotherodon mossambicus. Onderstepoort J. Vet. Res*., Onderstepoort, v. 48, p. 185-193, 1981.

CHAVIN, W.; YOUNG, J.E. Factors in the determination of normal serum glucose of goldfish *Carassius auratus* L. *Comp. Biochem. Physiol*., London, n. 33, p. 629-653, 1970.

DEFFUNE, G. *et al***.** Classificação climática e índice de aridez para Maringá-PR, de 1976-1992. *Boletim de Geografia*, Maringá, Suppl. 12, 1994.

EZZAT, A.A. *et al.* Studies on the blood characteristics of *Tilapia zillii* (Gervais). I. Blood cells. *J. Fish Biol.,* London, n. 6, p. 1-12, 1974.

GERKING, S.D. Fish reproduction and stress. *In: Environmental physiology of fishes*. New York: Plenum Press, 1980.

HENRY, R.J. *et al. Clinical Chemistry, principles and technics.* New York: Harper & Row, 1974.

JOBLING, M.; REINSNESW, T.C. Physiological and social constraints on growth of Arctic charr. *Salvelinus alpinus* L.: an investigation of factors leading to stunting. *J. Fish Biol*., London, n. 28, p. 379-384, 1986.

PITOMBEIRA, M.S.; MARTINS, J. M. Haematology of the Spanish mackerel, *Scomberomorus maculatus. Copeia,* Charleston, n. 1, p. 182-186, 1970.

RANZANI-PAIVA, M.J. *et al*. Haematological characteristics and relative conditions factor (Kn) associated with parasitism in *Schizodon borellii* (Osteichthyes Anostomidae) abd *Prochilodus lineatus* (Osteichthyes Prochilodontidae) from Paraná River, Porto

Rico region, Paraná, Brasil. *Acta Scientiarum*, Maringá, n. 22, v. 2, p. 515-521, 2000.

SCHRECK, C.B. *The fish immune system, organism and environmental*. London: Academic Press, 1996.

SCHRECK, C.B.; BRADFORD, C.S. Internal corticosteroid production: potential regulation by immune system in the salmonids. *In: Progress in Comparetive Endocrinology*. New York: Wiley & Liss, 1990.

TAVARES-DIAS, M. *et al*. Características hematológicas de teleósteos brasileiros. II. Parâmetros sangüíneos do *Piaractus mesopotamicus* Holmberg (Osteichthyes, Characidae) em policultivo intensivo. *Rev. Bras. Zool.,* Curitiba, n. 16, v. 2, p. 423-431, 1999.

TAVARES-DIAS, M. *et al*. Haematological characteristics of hybrid Florida red tilapia *Oreochromis niloticus* x *O. mossambicus* under intensive rearing. *Proceeding from The Fifth International Symposium on Tilapia Aquaculture,* v. 2, p. 533-541, 2000a.

TAVARES-DIAS, M. *et al*. Haematological characteristics of Brazilian teleosts. III Parameter of the hydrib tambacu (*Piaractus mesopotamicus* Holmberg x *Colossoma macroponum* Cuvier) (Osteichthydes, Characidae. *Rev. Bras. Zool*., Curitiba, n. 17, p. 899-906, 2000b.

TEIXEIRA, M. A. *et al*. Hematological and biochemical profiles of rat (*Rattus norvegicu)* kept under microenvinmental ventilation system. *Braz. J. Vet. Res. Anim. Sci*., São Paulo, n. 37, v. 5, 2000.

VALLADA, K. *Manual de técnicas hematológicas*. Rio de Janeiro: Livraria Atheneu, 1988.

WINTROBE, M.M. Variations of the size and haemoglobin content of erythrocytes in the blood various vertebrates. *Folia Hematol.*, Leipzig, v. 51, p. 32-49, 1934.

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