HAEMATOLOGICAL AND GILL RESPONSES IN PARASITIZED TILAPIA FROM VALLEY OF TIJUCAS RIVER, SC, BRAZIL

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ABSTRACT: In the State of Santa Catarina there is no information about haematological and parasitological characteristics of fish maintained in feefishing operations and manured ponds. This work compared the haematological characteristics of Nile tilapia parasitized or not, captured in a property situated in Nova Trento, SC, Brazil, between August 2003 and July 2004. During the studied period, the water temperature, pH, dissolved oxygen, alkalinity, ammonia and transparency were measured. Ten fish were collected monthly either in a feefishing or in a pond receiving pig manure, and anesthetized with benzocaine solution for haematological, parasitological and histopathological exams. From a total of 360 analyzed fish, 64 (17.8%) were parasitized with *Cichlidogyrus sclerosus* and *Cichlidogyrus* sp. (Monogenoidea: Ancyrocephalidae), 20 (5.5%) with *Trichodina* sp. (Protozoa: Ciliophora), and four (1.1%) with *Lamproglena* sp. (Crustacea: Lernaeidae). The total number of erythrocytes, thrombocytes and leucocytes, glucose rate and the percentages of hematocrit, lymphocytes, monocytes and neutrophils did not show relation with parasitism. Histopathological analysis showed moderate hyperplasia and absence of congestion. The low level of parasitism found in the animals was not responsible for haematological alterations.
Key words: *Oreochromis niloticus*, Santa Catarina, parasitism, haematology, histopathology

RESPOSTAS HEMATOLÓGICA E BRANQUIAL DE TILÁPIA PARASITADA DO VALE DO RIO TIJUCAS, SC, BRASIL

RESUMO: No Estado de Santa Catarina não existe informação sobre características hematológicas e parasitológicas que compare peixes mantidos em pesque-pague e com dejetos de suínos. Este trabalho estudou as características hematológicas da tilápia do Nilo parasitada ou não, capturada numa propriedade em Nova Trento, SC, Brasil, entre agosto de 2003 e julho de 2004. Durante o período a temperatura da água, pH, oxigênio, alcalinidade e transparência foram medidos. Dez peixes foram mensalmente coletados no pesque-pague e num viveiro que recebia dejetos de suínos, anestesiados com benzocaína para análise hematológica, parasitológica e histopatológica. De 360 animais analisados, 64 (17,8%) estavam parasitados com *Cichlidogyrus sclerosus* e *Cichlidogyrus* sp. (Monogenoidea: Ancyrocephalidae), 20 (5,5%) com *Trichodina* sp. (Protozoa: Ciliophora) e quatro (1,1%) com *Lamproglena* sp. (Crustacea: Lernaeidae). O número total de eritrócitos, , trombócitos e leucócitos, a taxa de glicose, os percentuais do hematócrito, linfócitos, monócitos e neutrófilos não alteraram com o parasitismo. A análise histopatológica mostrou moderada hiperplasia e ausência de congestão. O baixo nível de parasitismo não foi responsável por alterações hematológicas.
Palavras-chave: *Oreochromis niloticus*, Santa Catarina, parasitismo, hematologia, histopatologia

INTRODUCTION

Aquaculture in Brazil has attracted the attention of producers and owners to the activities of rearing, fry production, and sport fishing. Its direct relation with job generation and wide economic relation with other activities has generate income of U$ 200 million, and more than 300,000 workers directly involved (Kitamura et al., 2002). As a result of the development of Aquaculture in the last 10 years, infectious and parasitic diseases outbreaks have occurred (Martins et al., 2002).

Parasitized fishes may present significant changes in their haematological and physiological characteristics (Ruane et al., 2000), affecting their development (Singhal et al., 1990; Ranzani-Paiva & Silva-Souza, 2004a,b). In the United States alone, parasitic

diseases of farmed fish caused in 1989 an economic loss estimated at US$ 11.5 million (Klesius & Rogers, 1995). Damage caused on the host depends on parasite species, type of injury in the host tissue, number of parasites and the health status of the host (Tavares-Dias et al., 1999a).

In Brazilian species, haematological changes have been caused by *Argulus* Müller 1785 (Ranzani-Paiva et al., 1987; 1989; Tavares-Dias et al., 1999b); protozoan, Monogenoidea, copepods and Hirudinea (Ranzani-Paiva et al., 1997); Monogenoidea, *Ichthyophthirius multifilis* Fouquet 1876, *Trichodina* Ehrenberg 1830, *Piscinoodinium pillulare* (Schäperclaus 1954), *Lernaea cyprinacea* Linnaeus 1758 (Tavares-Dias et al., 1999a; Silva-Souza et al., 2000); Monogenoidea, nematodes, acanthocephalans and ergasilids (Ranzani-Paiva et al., 2000) and anisakids (Martins et al., 2004b).

This work compared the haematological characteristics of Nile tilapia, parasitized or not with ectoparasites, in a property situated in Nova Trento, SC, Brazil. Histopathological analysis of the gills from fish maintained with pig manure and in feefishing operation was performed.

MATERIAL AND METHODS

From August 2003 through July 2004, ten *O. niloticus* (28 ± 0.6 cm total length; 420 ± 35 g mean body weight) were captured monthly by net in two ponds, in a fish farm situated in Nova Trento (Lat. 27°17'09'' S, Long. 48°55'47'' W), SC, Brazil. The animals from feefishing ponds were fed with commercial diet and the others were maintained in ponds with pig manure as main feed source. During the period, the water temperature was measured with bulb termometer, dissolved oxygen with oxymeter "YSI-Mod.55", pH with electronic peagameter Thermo Orion, alkalinity by colorimetric method and transparency with Secchi disc. A sample of 250 mL of water from the ponds was frozen for ammonia analysis according to Koroleff (1976).

After sedation with benzocaine solution (1 g 10 L^{-1}), the blood was withdrawn from the caudal vein into a syringe containing a drop of 10% EDTA solution (approved by the Ethic Committee - CEUA nº 23080.027275/2004-85/UFSC). This blood was utilized to measure hematocrit (Goldenfarb et al., 1971); erythrocyte number in a Neubauer chamber; glucose (Accu-Chek Advantage 2 Roche); leucocyte and thrombocyte number by indirect method (Martins et al., 2004a), and differential counting of leucocytes by using the combination of Giemsa/May-Grunwald staining (Rosenfeld, 1947), in which a hundred cells were counted for the establishment of each cell contents.

After blood collection, parasitological exam consisted of scraping of body mucus and smears of organs for parasite evaluation. The gills were removed, placed in a flask with water at 55ºC for slackness of Monogenoidea for one hour, shaked and fixed in 5% formalin solution. After that, in the laboratory the gills were carefully observed for counting and presence of lernaeid crustaceans in the gill archs. Prevalence and mean intensity was calculated according to Bush et al. (1997).

For histopathological analysis, small pieces of tissue were fixed in 10% buffered formalin solution and embedded in paraffin. Cross sections (6 μm) stained with haematoxilin and eosin were observed under microscope. The results were analyzed by Spearman's (rs) test ($\alpha = 0.05$) (Siegel, 1975) and the ANOVA F statistic for analysis of variance. The haematological analysis was done from the total mean of the blood variables during the whole period in parasitized and non-parasitized fish.

RESULTS AND DISCUSSION

No difference between the aquatic parameters of the feefishing lakes and manured ponds was observed (Table 1). Water temperature was lower in August 2003, May, June and July 2004, and higher in December 2003, January and February 2004. The total mean values of dissolved oxygen concentration and the water transparency were slightly lower in the manured pond than in the feefishing lakes.

During the studied period, water quality was adequate for tilapia maintenance. The lowest dissolved oxygen concentration of the manured pond (3.1 to 3.8 mg L^{-1}) and slightly acid pH (4.8 to 6.5) probably resulted from high organic matter concentration and intermittent water flow, as already observed by Allabaster & Lloyd (1982). Lower transparency of water of the manured pond was evident when compared to that of feefishing lake, differing from the findings of Sipaúba-Tavares et al. (1994), but similar to that related by Eler et al. (2001). In this study the presence of high contents of algae was directly related to nutrient input. Despite the low dissolved oxygen observed in the early morning, no mortality was reported, probably because the region's low water temperature avoided toxic substances liberation from organic matter. The oxygen concentration in the feefishing lake was similar to that reported by Tavares-Dias et al. (2001) in São Paulo State. However, a slight variation of pH during the whole period and the oxygen concentration between August 2003 and March 2004 corroborated the findings of Schalch (2002) for feefishing lakes in São Paulo State.

From a total of 120 examined fish, 35 (29.2%) were parasitized. Two (1.6%) with *Trichodina* sp. (Protozoa: Ciliophora); 16 (13.3%) with *Cichlydogyrus* Paperna 1960 and *Cichlydogyrus sclerosus* Paperna and Thurston 1969 (Monogenoidea: Dactylogyridae) on body mucus and gills, with mean intensity 4.2 (2.0 to 14.6), being three (2.5%) with only one *C. sclerosus* in the gills and four (3.3%) were parasitized by *Lamproglena* sp. (Crustacea: Lernaeidae) in the gill archs with mean intensity 1.5 (1.0 to 2.0). Table 2 shows the number of parasitized fish in each month of sampling, as well as their respective prevalence rates of Monogenoidea and *Trichodina* sp. in fish from feefishing and manured pond.

No difference on the haematological characteristics between parasitized and non-parasitized fish (Table 3) was recorded. It was observed slight decreasing of the erythrocyte number and hematocrit percentage in parasitized animals. Glucose rate, in this case, was not influenced by parasitism. In the differential

Table 1 - Aquatic characteristics of feefishing lakes (FF) and manured ponds (PM) in a facility situated in Nova Trento, SC, Brazil.

Months	Temperature		Oxygen		pH		Ammonia		Alkalinity		Transparency	
	----- ºC -----		---- mg L^{-1} ----				---------- mg L^{-1} ----------				------ cm ------	
	FF	PM	FF	PM	FF	PM	FF	PM	FF	PM	FF	PM
Aug/03	19.3	18.2	9.3	5.7	7.1	7.0	0.5	0.5	20.0	40.0	25.0	4.0
Sep/03	19.0	20.0	8.3	6.8	7.0	6.0	0	0	25.0	20.0	22.0	7.0
Oct/03	22.5	22.7	8.5	5.8	6.5	6.5	0.108	0.098	30.0	20.0	14.0	16.0
Nov/03	24.4	25.3	4.0	3.8	6.5	6.0	0.103	0.107	30.0	30.0	18.0	12.0
Dec/03	28.1	28.8	5.8	3.8	6.5	6.5	0	0.072	30.0	30.0	17.0	22.0
Jan/04	26.3	26.9	6.4	4.1	5.1	4.8	0.032	0.048	30.0	30.0	15.0	18.0
Feb/04	25.4	25.1	7.8	5.9	7.4	7.8	0.135	0.194	30.0	30.0	10.0	18.0
Mar/04	32.0	27.6	6.5	5.5	5.6	5.4	0.136	0.151	20.0	25.0	22.0	9.0
Apr/04	24.0	24.5	3.8	3.1	6.0	5.5	0.313	0.073	50.0	80.0	20.0	12.0
May/04	19.2	19.0	3.5	3.1	5.1	5.1	0.043	0.199	40.0	30.0	20.0	10.0
Jun/04	17.3	18.0	3.8	3.2	6.2	6.3	0.160	0.348	30.0	30.0	10.0	10.0
Jul/04	18.3	18.7	4.3	3.8	6.5	6.7	0.131	0.143	30.0	30.0	10.0	10.0
Total mean	23.0	22.9	6.0	4.5	6.3	6.1	0.116	0.143	30.0	33.0	17.0	12.0

Table 2 - Prevalence rate of ectoparasites in *Oreochromis niloticus* maintained either in feefishing Lakes or manured pond in Nova Trento, SC, Brazil.

Months	Pond of feefishing				Pond with pig manure				
	PF/EF	Monogenoidea	*Trichodina*	*Lamproglena*	PF/EF	Monogenoidea	*Trichodina*	*Lamproglena*	Statistic
	---------- % ----------				---------- % ----------				
Aug/03	5/5	60	20	20	3/5	60	0	0	-
Sep/03	5/5	40	20	40	4/5	60	0	20	$P = 0.410$
Oct/03	0/5	0	0	0	0/5	0	0	0	-
Nov/03	2/5	40	0	0	1/5	20	0	0	$P = 0.610$
Dec/03	1/5	20	0	0	0/5	0	0	0	-
Jan/04	1/5	20	0	0	1/5	20	0	0	$P = 0.610$
Feb/04	0/5	0	0	0	0/5	0	0	0	$P = 1.800$
Mar/04	0/5	0	0	0	0/5	0	0	0	$P = 0.610$
Apr/04	0/5	0	0	0	0/5	0	0	0	-
Mai/04	1/5	20	0	0	0/5	0	0	0	$P = 0.200$
Jun/04	0/5	0	0	0	0/5	0	0	0	$P = 0.410$
Jul/04	0/5	0	0	0	0/5	0	0	0	-
Total	15/60	33.3	20.0	30.0	9/60	40.0	0	20.0	-

PF: parasitized fish; EF: examined fish.

counting of leucocytes, lymphocytes predominated followed by monocytes and neutrophils.

Haematological processes of anaemia have been observed in fish parasitized by cestodes (Sopinska, 1985). Omoregie (1998) and Omoregie & Oyebanji (2002) registered haematological changes in tilapia exposed to oil and oxytetracycline, respectively. This shows that not only the presence of parasites but also altered environmental conditions may alter the haematology parameters of fish.

The discrete decreasing in the erythrocyte number and hematocrit in this study corroborated data reported by Sakanari (1984) for fish infected by anisakid nematodes; by Sopinska (1985) for carp parasitized by cestodes; by Ranzani-Paiva et al. (1987) for carp with *Argulus* sp.; by Yokoyama et al. (1996) for carp infected by *Myxobolus artus* Akhmerov 1960; by Tavares-Dias et al. (2002) for tilapia with *I. multifiliis*, and by Martins et al. (2004b) for *Leporinus macrocephalus* infected by anisakids. Decreased hematocrit in fish parasitized with Monogenoidea was also reported by Montero et al. (2004). The lack of alteration in the haematological characteristics was also observed by Tavares-Dias et al. (1999a) for *Piaractus mesopotamicus* parasitized with *Argulus* sp., by Tavares-Dias et al. (1999b) for *P. mesopotamicus* and *L. macrocephalus* with ectoparasites, by Ranzani-Paiva et al. (2000) for *Schizodon borelli* and *Prochilodus lineatus* with Monogenoidea, nematodes and acanthocephalans, and by Ranzani-Paiva & Tavares-Dias (2002) for *Mugil platanus* with trichodinids, Monogenoidea, copepods and Hirudinea.

In contrast with our results, Ruane et al. (2000) observed increased number of erythrocytes and no alteration of hematocrit in parasitized trouts. The predominance of lymphocytes in the differential counting of leucocytes was similar to that observed by Silva-Souza et al. (2000) for fish parasitized with *L. cyprinacea*, but higher than the normally verified in tilapia (Ezzat et al., 1974; Lea Master et al., 1990; Alkahem, 1994; Ueda et al., 1997; Tavares-Dias & Faustino, 1998; Tavares-Dias & Moraes, 2003). Histopathological analysis of gills did differ from Martins & Romero (1996) who reported basal hyperplasia, necrosis and oedema in fish parasitized by Monogenoidea; from Longshaw et al. (2004) who reported moderate gill hyperplasia in fish parasitized by trichodinids, and Montero et al. (2004), who reported distortion of lamellar structure and lamellar fusion in fish parasitized by Monogenoidea. Hyperplasia observed in the gills of fish raised in the manured pond probably resulted from contact with suspended, particulate material (Figure 1).

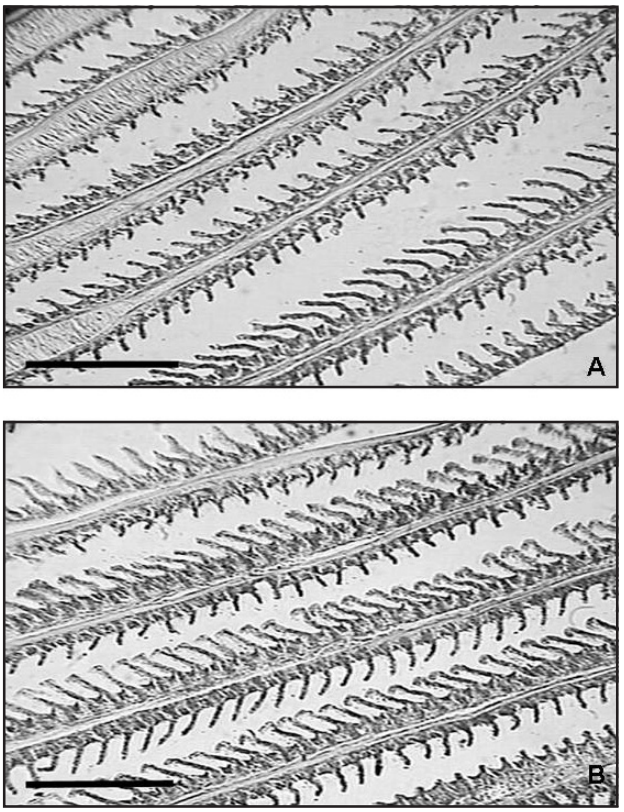

Figure 1 - Gill filaments of Nile tilapia from feefishing lakes (A) and manured ponds (B) in the State of Santa Catarina, Brazil. Scale bar = 100 µm.

Table 3 - Mean values (±SD) and range of the haematological characteristics of *Oreochromis niloticus* parasitized or not, in Nova Trento, SC, Brazil.

Variables	Non parasitized (n = 85)	Parasitized (n = 35)	Statistic
Erythrocytes (x 10^3 μL^{-1})	1.500 ± 340 (820-1.630)	1.070 ± 390 (880-1.580)	$P = 0.220$
Thrombocytes (x 10^3 μL^{-1})	31 ± 22 (18-33)	35 ± 23 (20-38)	$P = 0.950$
Hematocrit (%)	42 ± 15 (20-46)	33 ± 9 (22-44)	$P = 0.508$
Glucose (mg dL^{-1})	32 ± 8 (26-50)	29 ± 10 (24-45)	$P = 0.204$
Total leucocytes (x 10^3 μL^{-1})	8.2 ± 7.5 (2.9-8.8)	8.8 ± 5.7 (3.1-9.5)	$P = 0.341$
Lymphocytes (%)	96.6 ± 1.5 (95-97)	97.4 ± 1.1 (95-98)	$P = 0.947$
Monocytes (%)	1.9 ± 1.1 (1.1-1.9)	1.4 ± 1.2 (1.1-1.6)	$P = 0.692$
Neutrophils (%)	1.5 ± 1.6 (1.2-1.4)	1.2 ± 1.1 (1.1-1.3)	$P = 0.076$

The number of ectoparasites in tilapia was not sufficient to induce changes in the haematological parameters. The conditions in which the fish are maintained vary between fish farms. The low level of parasitism and the lack of the gill response confirmed the influence of environmental factors, especially water temperature in parasite reproduction. Consequently, the diversity of parasitic fauna could vary depending on the host-parasite-environment equilibrium. Good management practices (Queiroz et al., 2004) must be applied to improve fish ponds productivity and to avoid parasite reproduction.

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