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Ectoparasites Influence on the Haematological Parameters of Nile Tilapia and Carp Cultured in the State of Santa Catarina, South Brazil

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Abstract: The present study compared the haematological characteristics of Nile tilapia and carp parasitized and non-parasitized captured in fish farms from the cities of Blumenau, Joinville and Ituporanga, SC, South Brazil. In Blumenau the animals were fed not only with dry ration, but also with fish entrails and restaurant scraps, while Ituporanga consorted system with pig manure was common. From 146 examined tilapia, 79% were parasitized: 81% with *Trichodina magna* and *Trichodina truncata* (Protozoa: Ciliophora); 76% with *Cichlydogyrus* sp., *Cichlydogyrus sclerosus* (Monogenoidea: Dactylogyridae) and *Gyrodactylus* sp. (Gyrodactylidae); 9% with *Lamproglena* sp. (Crustacea: Lernaeidae) and 15% with crustacean copepodids. From 20 examined carp, 55% were parasitized: 18% with *Trichodina* spp., 90% with *Dactylogyrus* sp. (Dactylogyridae) and 36% with crustacean copepodids. The total mean of hematocrit percentage, number of thrombocytes and erythrocytes, as well as the number of neutrophils and monocytes was not related to parasitism. Parasitized tilapia from Blumenau and Joinville showed reduced number of erythrocytes, thrombocytes, leucocytes and circulating lymphocytes. Parasitized carp showed reduced hematocrit and increased erythrocyte number. Possibly, the handling and the source of feeding affected the haematological response of tilapia from Blumenau.

Key words: *Oreochromis niloticus*, *Cyprinus carpio*, ectoparasites, haematology

Introduction

Little is known on the haematological parameters comparing parasitized and nonparasitized freshwater cultured fish in Brazil. Nowadays, fish parasites constitute the main cause of economic losses in aquaculture (Paperna, 1991; Moraes and Martins, 2004).

The haematological parameters constitute an important tool that reveals the health state of fish (Blaxhall, 1972). Parasitism may induce lowered growth (Evans, 1974; Ranzani-Paiva and Silva Souza, 2004) and haematological alterations (Sopinska, 1985; Yokoyama *et al*., 1996; Ruane *et al*., 2000; Martins *et al*., 2004). In the intensive culture these alterations may affect the natural resistance of fish to parasites. In Brazil the ectoparasites belong to the most responsible for fish damage in intensive culture as related by Moraes and Martins (2004). In the States of São Paulo and Paraná the effects of parasitism on the blood variables have been studied by Ranzani-Paiva et al. (1997a, 2000), Tavares-Dias *et al*. (1999, 2002), Silva Souza *et al*. (2000) and Martins *et al*. (2004). However, little is known on the haematological parameters of cultured fish in the State of Santa Catarina. Azevedo *et al*. (2006) related no alteration in these variables in tilapia maintained in feefishing and with pig manure as source of feeding.

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The present study showed how ectoparasites may affect the haematology of tilapia and carp captured from fish farming located in three regions in the State of Santa Catarina, Brazil. The haematological parameters in parasitized and non-parasitized fish was related to the environment in which the fishes were maintained.

Materials and Methods

From October 2004 through June 2005, 137 *Oreochromis niloticus* Linnaeus, 1758 (Cichlidae) body weight 381.2±173.2 g, length 215.3±37.3 cm and 20 *Cyprinus carpio* Linnaeus, 1758 (Cyprinidae) body weight 662.7±194.6 g, length 350.2±37.1 cm were collected from the facilities located in the cities of Blumenau (26°55'10''S, 49°03'58''W), Joinville (26°18'16''S, 48°50'44''W) and Ituporanga (27°24'52''S, 49°36'09''W), Santa Catarina State, Brazil, for haematological and parasitological analysis.

Within each sampling the water quality was measured. In the Blumenau region the temperature was 26.2+3.3°C; dissolved oxygen 8.0±1.4 mg L^{-1}; pH 7.2±0.9; ammonia 1.2±0.9 mg L^{-1}; alkalinity 66.7±25.2 mg L^{-1} and transparency 27.5±3.3 cm. In Joinville the temperature was 30.2±15.8°C; dissolved oxygen 6.0±1.9 mg L^{-1}; pH 7.1±0.6; ammonia 1.1±1.3 mg L^{-1}; alkalinity 37.5±9.6 mg L^{-1} and transparency 30.0±15.8 cm. In Ituporanga the temperature was 24.5±1.4°C; dissolved oxygen 8.8±0.3 mg L^{-1}; pH 6.2±0.8; ammonia 1.2±0.3 mg L^{-1}; alkalinity 60.0±10.0 mg L^{-1} and transparency 20.0±5.0 cm.

Fish were anesthetized with benzocaine solution (50 mg L^{-1}) and the blood was withdrawn from the caudal vein into a syringe containing a drop of 10% EDTA solution (Ethic Committee n° 23080.027275/2004-85/CEUA/UFSC). The blood was utilized to measure hematocrit (Goldenfarb *et al*., 1971); erythrocyte number with haemocytometer, leucocyte and thrombocyte number by indirect method (Martins *et al*., 2002) and differential counting of leucocytes by using the combination of Giemsa/May-Grunwald (Rosenfeld, 1947) staining 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 containing water at 60°C for slackness of Monogenoidea, which was shaked and fixed in 5% formalin solution. After that, in the laboratory, the gills were observed for counting of the lernaeid crustaceans and Monogenoidea in the gill filaments. For identification of trichodinids skin mucus and gill smears containing parasites were prepared on slides, stained by Giemsa or impregnated with Klein's dry silver impregnation method for adhesive disc observation as suggested by Lom (1958). Parasites prevalence was calculated according to Bush *et al*. (1997). The data were analysed by variance analysis (ANOVA) with 5% significance. The arithmetic averages of the haematological data were compared between the parasitized and non-parasitized fish in each region.

Results

In the region of Blumenau not only commercial and homemade ration, but also fish entrails, cooked rice and restaurant scraps were the main sources of feeding to fish that were maintained at a stocking density of 1.0 to 2.0 fish m^{-2}. On the other hand, fish from Ituporanga were fed with pig manure and a stocking density of 3.2 to 4.0 fish m^{-2}. The region of Joinville was characterized by fish production using commercial dry ration as the main source of feeding at a stocking density of 1.2 to 1.6 fish m^{-2}. It was also observed that the majority of the farms were not used to control the water quality.

From a total of 137 tilapia examined, 79% were parasitized, 86% with two species of trichodinid, *Trichodina magna* Van As and Basson, 1989 and *Trichodina truncata* Ghiraldelli, 2005 (Protozoa:

Table 1: Prevalence (%) of ectoparasites from the different localities in the State of Santa Catarina, Brazil. (PF: parasitized fish, EF: examined fish)

	Oreochromis niloticus								
Localities	Trichodinids	Monogenoidea	Copepodids	*Lamproglena*	*Epystilis*	*P. pillulare*	*Argulus*	PF/EF	Total
Blumenau	69	56	17	19	4	0	2	35/48	73
Joinville	68	81	13	2	8	8	8	62/63	98
Ituporanga	37	26	3	0	0	3	0	17/35	49
	Cyprinus carpio								
	Trichodinids	Monogenoidea	Copepodids	*Lamproglena*	*Epystilis*	*P. pillulare*	*Argulus*	PF/EF	Total
Blumenau	0	100	67	0	0	0	33	3/3	100
Ituporanga	0	41	12	0	0	0	0	8/17	47

Table 2: Mean values of the haematological characteristics in *Oreochromis niloticus* Parasitized (P) and Non Parasitized (NP) from the different localities of the State of Santa Catarina, Brazil. EGC: especial granulocytic cell. Different letters indicate significant difference between parasitized and non parasitized fish in each ($p<0.05$)

Variables	Fish	Blumenau	Joinville	Ituporanga	Total mean
Hematocrit	P	25.5±9.1a	29.0±5.5a	26.9±6.9a	27.6±7.1a
(%)		(n = 36)	(n = 54)	(n= 18)	(n = 108)
	NP	27.8±7.0a	37.0b	25.6±7.4a	26.9±7.4a
		(n =12)	(n = 1)	(n = 16)	(n = 29)
Erythrocyte	P	1.3±0.5a	1.8±0.5a	1.7±0.03a	1.6±0.5a
($\times 10^6$ µL^{-1})	NP	1.7±0.05b	2.3b	1.4±0.03b	1.6±0.4a
Thrombocyte	P	22.3±12.2a	41.9±20.2a	74.5±31.7a	40.6±26.6a
($\times 10^3$ µL^{-1})	NP	43.8±17.5b	97.4 b	45.2±24.1b	46.6±23.1a
Leucocyte	P	33.5±16.8a	42.6±21.5a	62.2±16.5a	42.7±21.9a
($\times 10^3$ µL^{-1})	NP	53.2±18.9b	64.4b	61.2±14.3a	57.9±16.3b
Lymphocyte	P	24.4±13.2a	34.1±20.3a	58.0±17.3a	34.7±20.8a
($\times 10^3$ µL^{-1})	NP	44.1±17.8b	62.5b	56.5±12.7a	51.5±15.9b
Neutrophill	P	8.7±5.4a	8.2±14.3a	4.2±5.7a	7.7±10.9a
($\times 10^3$ µL^{-1})	NP	8.7±10.2a	1.9b	4.4±5.6a	6.1±8.0a
Monocyte	P	0.5±1.1a	0.2±0.4a	0a	0.3±0.7a
($\times 10^3$ µL^{-1})	NP	0.4±0.8a	0b	0.3±0.6b	0.3±0.6a
EGC	P	0.02±0.11a	0	0	0
($\times 10^3$ µL^{-1})	NP	0a	0	0	0

Ciliophora) on body surface and gills; 81% with *Cichlydogyrus* Paperna, 1960, *Cichlydogyrus sclerosus* Paperna and Thurston, 1969 (Monogenoidea: Dactylogyridae) and *Gyrodactylus* von Nordmann, 1832 (*Gyrodactylidae*) on body surface and gills; 9% with *Lamproglena* von Nordmann, 1832 (Crustacea: Lernaeidae) in the gill archs and 16% with crustacean copepodids on body surface and gills. From 20 carp analysed, 55% were parasitized: 18% with *Trichodina* Ehrenberg, 1830 (Protozoa: Ciliophora) on body surface; 90% with *Dactylogyrus* Diesing, 1850 (Monogenoidea: Dactylogyridae) in the gills and 36% with crustacean copepodids in the gills (Table 1).

When analysing the total mean of hematocrit, the numbers of erythrocyte and thrombocyte and the numbers of neutrophils and monocytes in the differential count in tilapia, no significant difference was related to parasitism. In each locality, hematocrit did not vary with the parasitism (Table 2). Total number of erythrocyte, thrombocyte and leucocyte decreased in parasitized fish from Blumenau and Joinville. Nevertheless, there was a decrease in the total number of leucocytes accompanied by a decrease in the lymphocytes number in the differential count. Especial granulocytic cell was observed only in parasitized tilapia from Blumenau.

In carp, hematocrit percentage showed a strong relation with the parasitism, characterized by a decrease in parasitized fish from Ituporanga. On the other hand, erythrocyte number was higher in parasitized than non-parasitized fish. The number of neutrophils in the differential counting was decreased in parasitized fish (Table 3). Despite the fact that low number (n = 3) of carp was collected in Blumenau, the comparison with non-parasitized fish was prejudiced.

Table 3: Mean values of the haematological characteristics in *Cyprinus carpio* Parasitized (P) and Non Parasitized (NP) from the different localities of the State of Santa Catarina, Brazil. Different letters indicate significant difference between parasitized and non parasitized fish in each ($p<0.05$)

Variable	Fish	Blumenau	Ituporanga	Total mean
Hematocrit	P	32.7+1.9 (n = 3)	30.9+7.4 a (n = 9)	31.3+6.5 a (n = 12)
(%)	NP	-	37.5+4.7 b (n = 8)	37.4+4.7 b (n = 8)
Erythrocyte	P	1.5±0.02	1.6±0.03a	1.6±0.2a
($\times 10^6$ μL^{-1})	NP	-	1.3±0.02b	1.3±0.02b
Thrombocyte	P	20.9±3.8	36.4±11.9a	32.2±12.4a
($\times 10^3$ μL^{-1})	NP	-	27.8±10.1a	27.8±10.1a
Leucocyte	P	37.9±9.0	46.9±13.0a	44.4±12.3a
($\times 10^3$ μL^{-1})	NP	-	44.6±8.6a	44.6±8.6a
Lymphocyte	P	26.8±5.1	41.5±11.4a	37.5±12.0a
($\times 10^3$ μL^{-1})	NP	-	35.3±6.9a	35.3±6.9a
Neutrophill	P	8.9±4.6	2.4±1.9a	4.5±4.0a
($\times 10^3$ μL^{-1})	NP	-	5.9±2.1b	5.9±2.1a
Monocyte	P	0	0.05±0.1a	0.04±0.01a
($\times 10^3$ μL^{-1})	NP	-	1.1±1.6b	1.1±1.6b

Discussion

Haematological parameters in fish may vary according to stressor stimulus, treatment or parasitism showing different responses in each fish species (Boon *et al*., 1989; Ranzani-Paiva *et al*., 1997b; Omoregie 1998; Martins *et al*., 2002, 2004). In the present study, decreased erythrocyte number, hematocrit and the highest number of neutrophills corroborated the findings of Sopinska (1985) in carp parasitized by cestodes. Similarly, Boon *et al*. (1989) and Ruane *et al*. (2000) did not observe alterations in the hematocrit of *Anguilla anguilla* Linnaeus, 1758 parasitized by nematodes and *Oncorhynchus mykiss* Walbaum, 1792 with caligid copepods, respectively.

The values of hematocrit from *O. niloticus* here studied were similar to that related by Ranzani-Paiva *et al*. (1987, 1997b) in carp infested by *Argulus* Müller, 1785 and *Prochilodus lineatus* Valenciennes, 1836 exposed to organophosphate, respectively; to Martins *et al*. (2001) in *Piaractus mesopotamicus* Holmberg, 1887 infected by Monogenoidea and to Tavares-Dias *et al*. (2002) in *O. niloticus* parasitized by *Ichthyophthiririus multifiliis* Fouquet, 1876 and fungus, but lower than that observed in *P. mesopotamicus* and *Leporinus macrocephalus* Garavello and Britski, 1988 parasitized by Monogenoidea, trichodinids and *Lernaea cyprinacea* Linnaeus, 1758 (Tavares-Dias *et al*., 1999), in *P. lineatus* parasitized by Monogenoidea, ergasilids and acanthocephalan (Ranzani-Paiva *et al*., 2000) and *L. macrocephalus* infected by anisakid nematodes (Martins *et al*., 2004). It can also be observed that hematocrit may present changed behaviour depending on the environment, fish handling and with the fish health status. Pilarczyk (1986) related in carp infected by "spring viremia of carp" hematocrit from 4 to 48%. It is difficult to affirm that this parameter decrease or increase with stressor stimulus as supported by Martins *et al*. (2002).

Erythrocyte number was higher than that observed by Ranzani Paiva *et al*. (1987, 1997a) but similar to that related by Tavares-Dias *et al*. (1999, 2002). On the other hand, decreased erythrocyte number in tilapia from Blumenau and Joinville was, in fact, affected by the parasites. There was no difference in the total mean erythrocyte number between parasitized and non-parasitized fish, as also related by Ranzani-Paiva and Tavares-Dias (2002) in *Mugil platanus* Günther, 1880 parasitized by the blood protozoan parasites, trichodinids, Monogenoidea and copepods. Martins *et al*. (2004) have also related reduced number of erythrocytes in the blood of fish infected by anisakids.

Decreased counting of leucocytes in the blood of tilapia was different to that verified by Sopinska (1985). Two hypotheses are thought to these observations. One of them is that a decrease was attributed to reduction of lymphocyte number in the circulating blood enhancing its migration. The other is that in the region of Blumenau fish were fed not only by ration, but also with fish entrails and

restaurant scraps. This fact might contribute to fish response in adverse environmental condition. In Joinville only one out of 61 examined fish was not parasitized. In spite of the fact that fish sampling was great, the reduced number of non-parasitized fish possibly did prejudice the analysis, but reflects directly the real situation in that facility. Following this view, an increase in the numbers of erythrocytes and thrombocytes in tilapia from Ituporanga may be due to the fact that the animals were fed with pig manure constituting the main source of feeding in that region. It is known that thrombocytes are involved in the fish defence response, as supported by Matushima and Mariano (1996) and Martins *et al*. (2000). It was clearly observed an increase in the neutrophill number in tilapia from Joinville. Once more, it can also be inferred that the parasitism affect directly the neutrophils population in the circulating blood, by inducing more production enhancing the host responses. Our results were in agreed with Sopinska (1985) and Silva Souza *et al*. (2000).

When comparing our data with those obtained by Azevedo *et al*. (2006) in tilapia from the other locality of Santa Catarina, similar number of erythrocytes but an increase in the number of thrombocytes, hematocrit percentage, total number of leucocytes and neutrophils were observed. According to Azevedo *et al*. (2006) fish were fed by ration and pig manure. In fact, these differences observed are related to fish handling, water conditions and feeding that vary from one owner to another.

Carp showed alteration neither in the thrombocyte and leucocyte number nor in the lymphocyte percentage. A decrease in the hematocrit percentage was similar to that found by Sopinska (1985) in parasitized carp. Nevertheless, Pilarczyk (1986) observed lower hematocrit percentage in diseased carp than that verified in this work. The erythrocyte number was higher than that observed in parasitized carp (Sopinska, 1985; Ranzani-Paiva *et al*., 1987). It must be remembered that Sopinska (1985) studied carp infected by cestodes, parasites that affect the nutrient absorption in the organism.

In conclusion, some haematological variables might vary according to locality of sampling, fish handling, water conditions and the source of feeding. Moreover, these alterations are directly related to the degree of infection and number of pathogens on/in the host (Martins *et al*., 2004). The results comproved the damage caused to the health of tilapia fed with pig manure, fish entrails and restaurant scraps. Further studies have to be carried out to investigate the effects of parasitism in the other tropical fish species maintained in sport fishing and fish production facilities and their seasonality of occurrence.

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