HEMATOLOGICAL ASSESSMENT IN FOUR BRAZILIAN TELEOST FISH WITH PARASITIC INFECTIONS, COLLECTED IN FEEFISHING FROM FRANCA, SÃO PAULO, BRAZIL

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ABSTRACT

The effects of parasitic infections in condition factor, hematocrit, hemoglobin, mean corpuscular hemoglobin concentration (MCHC), and leucocytes and thrombocytes distribution in *Piaractus mesopotamicus*, *Leporinus macrocephalus*, hybrid tambacu (*P. mesopotamicus* x *Colossoma macropomum* and *Brycon amazonicus* collected in feefishing from Franca, São Paulo, Brazil were evaluated. Parasitized tambacu and *L. macrocephalus* had higher ($p<0.05$) condition factor than unparasitized fish. However, the contrary occurred in *P. mesopotamicus* and *B. amazonicus*. Changes in the hematocrit, hemoglobin and MCHC were not related to parasitism. Parasitic infections did not cause effect on leucocytes and thrombocytes percentage ($p>0.05$) of tambacu. In *P. mesopotamicus* parasitized by Monogenea *Anacanthorus penilabiatus* and dinoflagellate *Piscinoodinium pillulare,* increase in monocytes and decrease in thrombocytes percentage ($p<0.05$) were found. However, the same parasitic association in *L. macrocephalus* caused a decrease in lymphocytes percentage accompanied by increase in neutrophils percentage ($p<0.05$). In *B. amazonicus*, infection by *Ichthyophthirius multifiliis*, *P. pillulare* and monogeneans caused increase in neutrophils percentage.

Key words: blood, condition factor, freshwater fish, leucocytes, hematology, parasites

AVALIAÇÃO DA HEMATOLOGIA DE QUATRO PEIXES TELEÓSTEOS BRASILEIROS COM INFECÇÕES PARASITÁRIAS, COLETADOS EM PESQUE-PAGUES DE FRANCA, SÃO PAULO, BRASIL

RESUMO

O presente trabalho avaliou os efeitos de infecções parasitárias no fator de condição, na hemoglobina, no hematócrito, na concentração da hemoglobina corpuscular média (CHCM) e na distribuição de leucócitos e trombócitos em *Piaractus mesopotamicus*, *Leporinus macrocephalus*, híbrido tambacu (*P. mesopotamicus* x *C. macropomum*) e *Brycon amazonicus* coletados de pesque-pagues de Franca, São Paulo, Brasil. O fator de condição em tambacu e *L. macrocephalus* parasitados foi significativamente ($p<0,05$) maior que nos peixes controles. O inverso ocorreu em *P. mesopotamicus* e *B. amazonicus*. Em nenhuma das espécies houve alteração significativa ($p>0,05$) no hematócrito, hemoglobina e CHCM que pudesse ser atribuída ao parasitismo. As infecções parasitárias também não exerceram efeito significativo ($p>0,05$) sobre o percentual de leucócitos e trombócitos em tambacus. Entretanto, em *P. mesopotamicus* a associação de Monogenea *Anacanthorus penilabiatus* com o protozoário *Piscinoodinium pillulare* provocou significativo ($p<0,05$) aumento no percentual de monócitos e redu-ção no percentual de trombócitos nos peixes parasitados. Em *L. macrocephalus* parasitados ocorreu diminuição no percentual de linfócitos ($p<0,05$) e quando houve associação entre monogenea e *P. pillulare* a redução do percentual de linfócitos foi acompanhada por aumento do percentual de neutrófilos ($p<0,05$). Em *B. amazonicus* parasitados por *Ichthyophthirius multifiliis*, *P. pillulare* e monogenean ocorreu significativo ($p<0,05$) aumento no percentual de neutrófilos sangüíneos.

Palavras chave: Fator de condição, Sangue, Parasitas, Peixes de água doce, Hematologia, Leucócitos

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INTRODUCTION

Aquaculture in Brazil has attracted the attention of producers and owners to the activities of rearing, fry production and sport fishing. Due to development of aquaculture, the infectious and parasitic diseases outbreaks have also increased (TAVARES-DIAS *et al.*, 2001a,b; TAVARES-DIAS *et al.*, 2002;MARTINS *et al.*, 2004; AZEVEDO *et al.*, 2006). Fish serve as hosts to a range of parasites that are taxonomically diverse and that exhibit a wide variety of life cycle strategies (BARBER *et al.,* 2000). Some parasites not change to their host health when under stable existence, but if this existence is disturbed, the epizootic events can occur. Damages caused on the host depends on parasite species, type of injury in the host tissue, number of parasite and the health status of the host (TAVARES-DIAS *et al*., 1999a), as well as of the physiologic disturbances.

Blood parameters have been important tools to diagnosis and prognosis of fish diseases. In fish parasitized, several hematological studies have been reported anemia (RAUTHAN *et al*. 1995; TAVARES-DIAS *et al*., 2002; HORTON and OKAMURA, 2003; MARTINS *et al*., 2004; CHAVES *et al*., 2006) and leukocyte counts changes (SOPINSKA, 1985; RAHKONEN & PASTERNACK, 1998; TAVARES-DIAS *et al*., 1999b; SILVA-SOUZA *et al*., 2000; HORTON and OKAMURA, 2003; MARTINS *et al*., 2004; CHAVES *et al*., 2006) due to a response of the major hematopoietic organs (CHAVES *et al*., 2006). Therefore, the aim of this paper was to evaluate the effects of parasitic infections on the hematological parameters in *Piaractus mesopotamicus*, *Brycon amazonicu*s (Characidae) (Characidae), *Leporinus macrocephalus* (Anostomidae) and hybrid tambacu (*P. mesopotamicus* x *Colossoma macropomum*).

MATERIAL AND METHODS

In feefishing from Franca, São Paulo State, Brazil (20º 35′ 27″- 47º 26′ 33″ and 20º 34′ 50″ - 47º 21′ 29″) 92 specimens of *Piaractus mesopotamicus* Holmberg, 1887 (0.08-2.00 Kg and 15.0 to 47.0 cm length), 111 *Leporinus macrocephalus* Garavello and Britsky, 1988 (0.011-1.36 Kg and 21.5 to 46.0 cm length), 76 hybrid tambacu (0.01-2.27 Kg and 17.0 to 50.0 cm length) and 26 *Brycon amazonicus* Spix & Agassiz, 1829 (0.61-1.98 Kg and 33.5 to 48.0 cm length) were collected. The fish of both feefishing were juveniles and young specimens, and has been acquired from a same fish farm. For the analyses, the fish were captured with hook as followed.

During the studied period (April of 1997 to March of 1999) the water temperature ranged 17.8 to 29.3 ºC measured with a bulb termometer; pH 6.9 to 8.7 measured with an electronic "Corning" pHmeter; electric conductivity 27.0 to 119.4 μS/cm measured with a "Corning" conductivimeter and dissolved oxygen 5.2 to 7.0 mg/l measured with an "YSY-Mod.50" oxymeter.

Body mucous and pieces of gills, kidney, liver, spleen and heart were compressed between a glass and a coverslip with a drop of 0.65% saline solution for microscope observation. Intestines were also observed in Petri dish containing saline solution. The collection, fixation, quantification and identification of parasites were performed according to methods reported previously (TAVARES-DIAS *et al.,* 2001a,b).

The blood was withdrawn from the caudal vessel into a syringe containing 0.01 mg/mL of EDTA (10%). This blood was utilized to determine the hematocrit by microhematocrit method and hemoglobin by cyanomethaemoglobin technique. From these primary indices resulted the secondary Wintrobe indices, mean corpuscular hemoglobin concentration (MCHC). For differential count of leukocytes and thrombocytes, blood smears were stained using a combination of May Grünwald-Giemsa-Wrigth (TAVARES-DIAS and MORAES, 2003) and two hundred cells were then counted for the establishment of each cell percentage.

After blood collection, body weight (g) and length (cm) of the fish were measured for the calculation of allometric condition factor using $K_{a} = W/L^{b}$; where W= weight, L= total length and b= angular coefficient of weight/length relation.

For each species of fish, analysis of variance of the data from body weight and length was made with the aim of checking whether there was significant difference between the treatments of both biometric variables. When there was significant effect of body weight and/or length, the hematological variables and the factor of condition were studied for covariance as a function of body weight and/or length (in accordance with the significance as found). The percentage of thrombocytes and leukocytes were transformed in senus arch (square root of % + 0.5) and the averages were compared the by the Tukey test, at 5% probability (STEEL and TORREY, 1980).

RESULTS

The prevalence and mean intensity of parasites in the fish from two feefishing are demonstrated in the Table 1. *Ichthyophthirius multifiliis* Fouquet, 1876; *Piscinoodinium pillulare* Schäperclaus, 1954, Lom, 1981; *Trichodina* sp.; *Myxobolus colossomatis* Molnar and Békési, 1993 and *Henneguya piaractus* Martins and Souza, 1997; copepodids and adults of *Lernaea cyprinacea* Linnaeus, 1758, *Argulus* sp. and monogeneans were the observed parasites. Monogeneans from the gills of *P. mesopotamicus* were identified as *Anacanthorus penilabiatus* Boeger, Husack and Martins, 1995. The larger prevalence of *A. penilabiatus* and intensity of *Trichodina* sp. was observed in *P. mesopotamicus*, while *B. amazonicus* presented the smaller prevalence and intensity of parasites.

Parasitized *P. mesopotamicus* showed significant decrease ($p<0.05$) in the condition factor and thrombocytes percentage compared to unparasitized fish. Parasitized fish with *A. penilabiatus* and *P. pillulare* showed increase ($p<0.05$) in the monocytes percentage and decrease ($p<0.05$) in the thrombocytes percentage (Table 2). Under these conditions, the blood smears revealed the presence of vacuolated monocytes, generally accumulated and of different sizes (Figure 1).

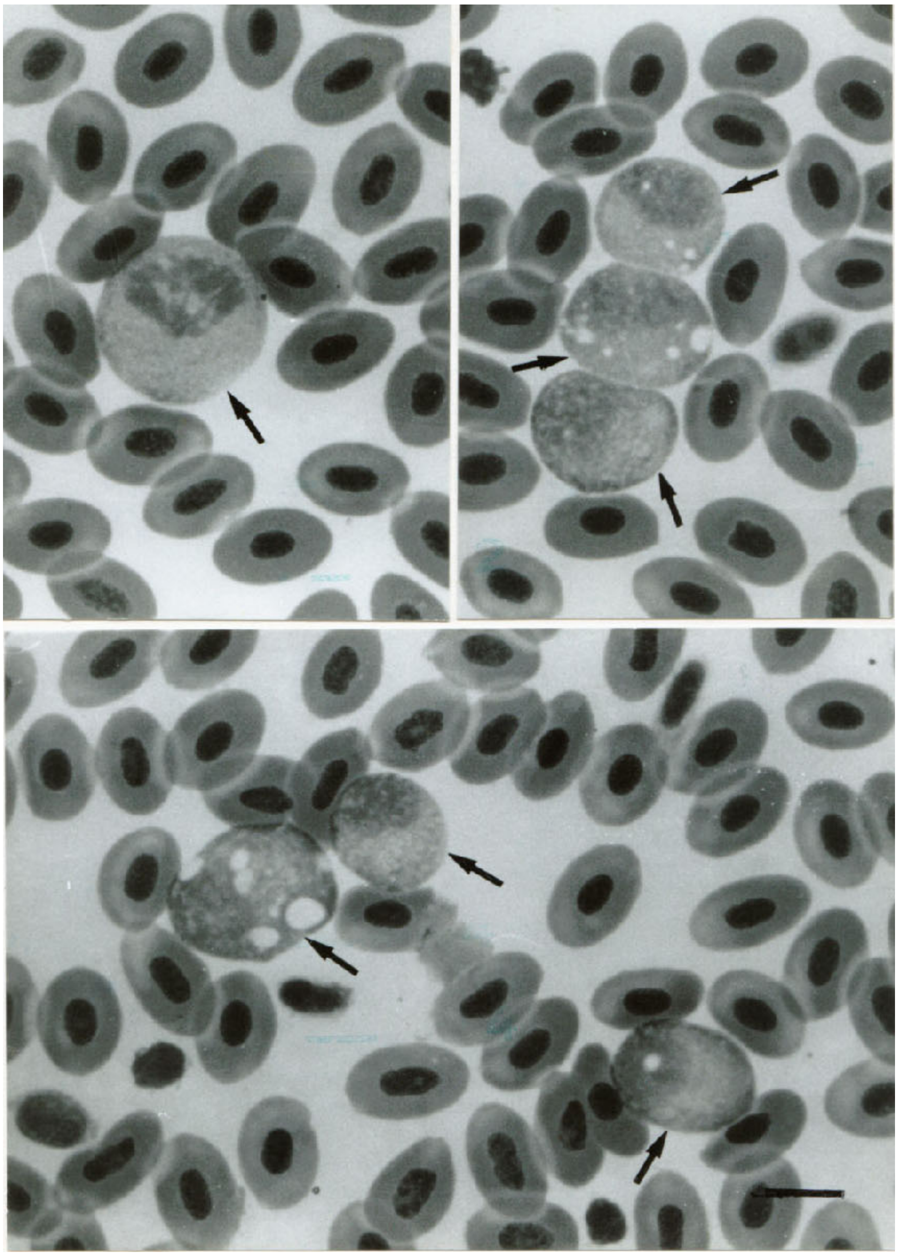
Table 1. Mean intensity and prevalence (P %) of parasites from 305 examined fish in feefishing from farm Franca, São Paulo, Brazil during April/1997 to March/1999. *Copepodids of *L. cyprinacea*

Hosts	*P. mesopotamicus*		*L. macrocepahlus*		*Hybrid tambacu*		*B. amazonicus*	
Parasites	Mean intensity	P (%)	Mean intensity	P (%)	Mean intensity	P (%)	Mean intensity	P (%)
I. multifiliis	8315.4 ±11287.0	9.0	442100.3 ±856446.5	6.0	11747.7 ±19847.9	3.5	222.0 ±0.0	6.2
Trichodina sp	105519.8 ±359374.9	13.0	598.4 ±1450.4	17.0	248.0±0.0	1.2	_	_
P. pillulare	4558959.0 ±1491097.3	24.0	5050224.0 ±1198839.0	42.0	6658437.0 ±14062126.0	20.0	360.0 ±0.0	6.2
Myxosporean	_	6.0	_	_	_	_	_	_
Monogenean	2287.6 ±6630.4	76.0	251.5 ±292.4	58.0	433.8 ±6040.0	11.7	1720.0 ±125.4	18.7
Copepodids*	9.6 ±8.6	7.0	8.5 ±7.8	11.0	13.4 ±11.4	5.9	_	_
L. cyprinacea	2.0 ±0.0	2.1	_	_	2.6 ±2.4	15.3	_	_
Argulus sp	5.0 ±2.0	1.0	4.0±3.0	0.9	_	_	_	_

Table 2. Mean values ± standard deviation of condition factor and hematological parameters of unparasitized (N=15) and parasitized *P. mesopotamicus,* independently of the parasite species (General values) (N=42), parasitized by *A. penilabiatus* (N=18), by *A. penilabiatus* and *P. pillulare* (N=15). In each line, values followed by the same letter are not significantly different ($p>0.05$) by Tukey test; PAS-GL= PAS-positive granular leukocytes

Parameters	Unparasitized Control	Parasitized		
		General Values	*A. penilabiatus*	*A. penilabiatus* and *P. pillulare*
Condition factor	13.9±0.27Aa	10.6±0.12B	10.6±0.17b	10.5±0.10b
Hemoglobin (g/dL)	10.3±3.4A,a	9.7±3.4A	10.5±3.5a	9.3±3.7a
Hematocrit (%)	37.0±10.2A,a	34.1±9.3A	35.8±10.1a	34.5±7.8a
MCHC (g/dL)	28.0±4.6A,a	29.7±11.0A	31.3±10.5a	27.7±9.3a
Thrombocytes (%)	61.7±12.4A,a	53.5±16.2B	56.7±10.7b	50.4±17.9b
Lymphocytes (%)	17.4±7.6A,a	22.3±11.0A	21.5±9.5a	20.7±10.6a
Neutrophils (%)	14.7±8.5A,a	13.5±11.5A	13.9±7.1a	17.2±18.0a
Monocytes (%)	8.7±4.4A,b	14.5±15.2A	10.8±7.4b	12.2±9.7a
PAS-LG (%)	3.9±3.3A,a	5.8±2.2A	4.9±1.5a	6.0±3.4a
Eosinophils (%)	1,4±0.6A,a	0.9±1.1A	1.0±1.4a	0.6±0.3a

Figure 1. Blood cells of *P. mesopotamicus* parasitized with *A. penilabiatus* and *P. pillulare*. Note monocytes (arrow) with agglomeration and vacuoles of different size, after staining with MayGrünwald-Giemsa-Wrigth. Bars= 8 µm



In parasitized *L. macrocephalus* there was a significant increase ($p<0.05$) in the condition factor and decrease of the lymphocytes percentage. Hematocrit, hemoglobin concentration and MCHC did not alter when compared to unparasitized fish. In this specie of fish, the association between monogenean and *P. pillulare* infection caused decrease in lymphocytes percentage accompanied by increase in neutrophils percentage (Table 3).

In parasitized hybrid tambacu was found high condition factor ($p<0.05$) than in controls. However, the parasitism did not provoke significant changes ($p>0.05$) in the hematological variables and in the leukocytes and thrombocytes percentage (Table 4).

No difference ($p>0.05$) in the hematocrit, hemoglobin and MCHC was observed in parasitized *B. amazonicus*. However, parasitized fish showed significant decrease ($p<0.05$) in the condition factor and increase in neutrophils percentage compared to unparasitized fish. A reduced value in the lymphocytes percentage was observed in parasitized fish but without significant difference (Table 5).

Table 3 – Mean values ± standard deviation of condition factor and haematological parameters of unparasitized (N=17) and parasitized *L. macrocephalus*, independently of parasite species (General values) (N=38), parasitized by monogenean (N=21), by monogenean and *P. pillulare* (N=18). In each line, values followed by the same letter are not significantly different (p>0.05) by Tukey test

Parameters	Unparasitized Control	Parasitized		
		General values	Monogenean	Monogenean and *P. pillulare*
Condition factor	10.2±0.20B,b	20.8±0.43A	20.6±0.50a	20.1± 0.24a
Hemoglobin (g/dL)	9.5±2.6A,a	8.9±2.0A	10.1±1.4a	8.9±3.1a
Hematocrit (%)	34.6±6.6A,a	35.6±7.9A	35.6±1.1a	33.5±5.8a
MCHC (g/dL)	27.7±6.6A,a	25.6±5.6A	27.5±4.8a	26.9±6.3a
Thrombocytes (%)	51.3±16.6A,a	53.0±15.6A	55.6±12.0a	50.2±18.9a
Lymphocytes (%)	30.7±14.5A,a	19.5±11.9B	25.1±12.3a	13.9±10.0b
Neutrophils (%)	14.6±5.3A,b	19.8±15.3A	17.0±7.5b	24.9±20.6a
Monocytes (%)	8.7±3.8A,a	12.6±11.8A	9.0±4.3a	14.8±13.5a
Basophils (%)	4.2±1.6A,a	5.4±2.4A	3.6±2.3a	6.3±2.8a

Table 4 – Mean values ± standard deviation of condition factor and haematological parameters in unparasitized (N=14) and parasitized hybrid tambacu, independently of the parasite species (general values) (N=18), parasitized by monogenean (N=09), by *L. cyprinacea* (N=08), by monogenean and *P. pillulare* (N=10). In each line, values followed by the same letter are not significantly different (p>0.05) by Tukey test; PAS-GL= PAS-positive granular leukocytes

Parameters	Unparasitized Control	Parasitized			
		General values	Monogenean	*L. cyprinacea*	Monogenean and *P. pillulare*
Condition factor	10.1±0.16B,b	13.4±1.30A	13.1±1.33a	13.4±2.16a	13.2±0.86a
Hemoglobin (g/dL)	13.1±2.9A,a	14.1±3.8A	16.9±1.3a	13.1±3.8a	14.9±3.0a
Hematocrit (%)	38.8±10.0A,a	39.9±9.5A	41.5±2.6a	39.8±11.9a	37.4±6.9a
MCHC (g/dL)	35.4±11.1A,a	35.5±7.5A	33.0±6.9a	33.2±2.9a	40.4±7.2a
Thrombocytes (%)	52.8±17.5A,a	56.3±10.4A	62.4±12.2a	52.7±7.8a	57.1±9.7a
Lymphocytes (%)	26.0±19.1A,a	23.5±8.6A	22.1±7.6a	23.3±13.7a	22.8±6.8a
Neutrophils (%)	15.1±6.0A,a	15.5±7.9A	9.7±3.0a	17.1±13.9a	16.6±4.8a
Monocytes (%)	11.1±6.8A,a	10.6±3.1A	7.8±2.5a	11.8±4.3a	12.0±2.5a
PAS-LG (%)	4.3±1.7A,a	4.7±1.4A	4.3±1.3a	4.6±1.6a	2.4±0.5a
Eosinophils (%)	0.8±0.4A,a	0.7±0.5A	0.9±0.4a	0.9±0.4a	0.7±0.6a

Table 5 – Mean values ± standard deviation of condition factor and hematological parameters in unparasitized (N=21) and parasitized *B. amazonicus* by monogenean, *I. multifiliis* and *P. pillulare*. In each line, values followed by the same letter are not significantly different (p>0.05) by *t* test

Parameters	Unparasitized	Parasitized
Condition factor	11.7±0.02A	9.8±0.4B
Hemoglobin (g/dL)	11.4±1.4A	10.1±3.6A
Hematocrit (%)	42.7±8.0A	43.5±4.9A
MCHC (g/dL)	27.7±7.7A	25.2±9.9A
Thrombocytes (%)	48.0±18.4A	47.1±14.8A
Lymphocytes (%)	19.4±14.4A	9.8±9.6A
Neutrophils (%)	20.5±5.1B	31.3±5.2A
Monocytes (%)	12.1±3.3A	11.8±6.2A

DISCUSSION

Parasites have been responsible for delay in fish growth and gain of weight by affecting the food ingestion (BARBER *et al*., 2000; BARKER *et al*., 2005). The relationship between weight and length has been used as a tool to estimate body conditions of healthy fish (TAVARES-DIAS *et al*., 2000a) and parasitized fish (LAIDLEY *et al*., 1988; TAVARES-DIAS *et al*., 1999a; RANZANI-PAIVA *et al*., 1997; TAVARES-DIAS *et al*., 2002; LIZAMA *et al*., 2006).

In the present work, parasitized *P. mesopotamicus* and *B. amazonicus* had low condition factor. Similar finding has been reported for *Onchorhynchus keta* (URAWA, 1996) and for *Heterrobranchus longifilis* (EKANEM and OBIKEZIE, 1996). On the other hand, parasitized tambacu and *L. macrocephalus* had higher condition factor than unparasitized fish, corroborating the results described in trout experimentally infected with *Cryptobia salmositica* (LAIDLEY *et al*., 1988) and in *Prochilodus lineatus* parasitized by Monogenea, Digenea, Cestoda, Nematoda, Copepoda and Branchiura (LIZAMA *et al*, 2006). This increase in condition factor of tambacu and *L. macrocephalus* can be a consequence of liquid in the visceral cavity, which has also been reported in parasitized trout (LAIDLEY *et al.,* 1988). However, in *Prochilodus lineatus* has been reported that largest fish with highest condition factor can tolerate higher levels of parasitic infection (LIZAMA *et al*., 2006).

In present study, a great number of parasites in hosts did vary; hence there was no change in the hematocrit, hemoglobin concentration and MCHC in parasitized *P. mesopotamicus*, tambacu, *B. amazonicus* and *L. macrocephalus*. Similar finding have been in *Mugil platanus* with parasites monogenean or copepod (RANZANI-PAIVA *et al*. 1997), in *P. mesopotamicus* with *Argulus* sp (TAVARES-DIAS *et al*., 1999b) and *Oreochromis niloticus* with monogenean, *Trichodina* sp. and *Lamproglena* sp. (AZEVEDO *et al*., 2006). Therefore, these results suggest that the parasite and host are adapted to survival in these conditions, without significant losses to the host health. On the other hand, decrease in primary red blood cell indices has been reported in *O. niloticus* parasitized by *Ichthyophthirius multifiliis* and *Saprolegnia* sp. (TAVARES-DIAS *et al*., 2002), in *Dicentrarchus labrax* parasitized by *Ceratothoa oestroides* (HORTON and OKAMURA, 2003), in *L. macrocephalus* parasitized by *Goezia leporini* (MARTINS *et al*., 2004) and in *Trachinotus marginatus* parasitized by *Bicotylophora trachinoti* (CHAVES *et al.,* 2006).

It is difficult to comment on changes in number of blood immune cells in parasitized fish, because the exact function of each cell is still little known. Moreover, these cells can confound the interpretation of results when attempting to make conclusions about the degree of stress experienced by parasitized fish. Nevertheless, when fish were parasitized by different parasites in unlike intensity levels, has been reported lymphocytosis accompanied by neutrophilia and monocytosis (TAVARES-DIAS *et al*., 2002), lymphocytopenia accompanied by monocytosis and neutrophilia (SOPINSKA, 1985; SILVA-SOUZA *et al*., 2000) and lymphocytopenia accompanied by neutrophilia and basophilia (Martins *et al*., 2004), as well as lymphocytosis and neutrophilia (RAHKONEN and PASTERNACK, 1998). Blood leukocytes, especially granulocytes and monocytes, could destroy pathogenic organism. In parasitized fish, increase in levels circulating of monocytes has been attributed to an improvement of cell defense system (SOPINSKA, 1985). Neutrophils possessing phagocytic capability are often the first leukocytes to migrate to the site of infecting parasite (RAHKONEN and PASTERNACK, 1998; SILVA-SOUZA *et al*., 2000), therefore increase or decrease of these granulocytes may be found, on the dependence of the infection stage.

In present study, *L. macrocepalus* and *P. pillulare* parasitized with monogenean, had decrease in lymphocytes percentage and increase in neutrophils percentage, while *B. amazonicus* parasitized with monogenean, *I. multifiliis* and *P. pillulare* had decrease neutrophils percentage. On the other hand, tambacu parasitized with monogenean, *L. cyprinacea* or *P. pillulare* did not showed alteration in the leukocytes percentage. Similarly, has been reported no changes in leukocytes distribution in *M. platanus* parasitized by monogenean, copepods, trypanosomes and *Trichodina* sp. (RANZANI-PAIVA *et al*., 1997) and in *O. niloticus* parasitized by monogenean, *Trichodina* sp. and *Lamproglena* sp. (AZEVEDO *et al*., 2006).

Mechanisms of specific immunity in fish are significantly less developed and play marked less important role than in birds or mammals (STOSIK *et al*., 2001). In contrast, fish have non-specific resistance system, which plays the basic role in defense of the organism against pathogenic (STOSIK *et al*., 2001; PASSANTINO *et al*., 2005). Then, piscine thrombocytes represent a link between innate and adaptive immunity (PASSANTINO *et al*., 2005). In *P. mesopotamicus*, independently of parasite species, was found decrease in thrombocytes percentage. Similar change has been reported for *P. mesopotamicus* with *Argulus* sp. (TAVARES-DIAS *et al*., 1999b), for *B. bendelisis* with trypanossomiasis (RAUTHAN *et al*., 1995), *Salmo trutta* with *Diphyllobotrium dentriticum* (RAHKONEN and PASTERNACK, 1998) and for *O. niloticus* with *I. multifiliis* and *Saprolegnia* sp. (TAVARES-DIAS *et al*., 2002). Possibly, the thrombocytes were being mobilized to contribute in the organic defense mechanisms, because these cells together with the leukocytes seem to represent an important defense line in these hosts. However, other factors should be also considered. RAHKONEN and PASTERNACK (1998) suggested that this thrombocytopenic response might serve to release clotting factors in preparation for tissue damage.

To summary, the results demonstrated different leukocytes responses to the parasites, which may be due to different degree of susceptibility of each host. Fish were captured with hook and line, and had food in their stomach, then probably were not captured sick fish, because fish in an advanced disease stage have not appetite. Therefore, this fact was responsible for non-change in the hemoglobin concentration, hematocrit and MCHC of fish studied.

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