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Original Article

Changes in hematological and biochemical parameters of tambaqui (*Colossoma macropomum*) parasitized by metazoan species

Alterações nos parâmetros hematológicos e bioquímicos de tambaqui (*Colossoma macropomum*) parasitado por espécies de metazoários

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Abstract

The aim of this study was to evaluate the impacts of metazoan parasites on hematological and biochemical parameters and relative condition factor of tambaqui (*Colossoma macropomum*) farmed in northern Brazil. A total of 32 juvenile fish were captured from a commercial fish farm located in the municipality of Rio Preto da Eva, Amazonas state, Brazil. Parasite prevalence was 100% for *Anacanthorus spathulatus*, *Mymarothecium boegeri* and *Notozothecium janauachensis*, 100% for *Neoechinorhynchus buttnerae* and 53.13% for *Dolops geayi*. The greatest mean parasite intensity was found in acantocephalans followed by monogeneans and branchiuran crustaceans. A negative correlation was observed between abundance of *N. buttnerae* and hematocrit percentage, hemoglobin concentration, total thrombocyte count and glucose and between abundance of the monogenean and glucose concentration. Parasitic infections caused damage in tambaqui in terms of the observed hematological parameters that were characterized by hypochromic anemia and thrombocytopenia, which are important parameters to be used in parasitic diagnosis. This study is the first record of the occurrence of *Dolops geayi* in farmed tambaqui in the Amazon.

Keywords: Fish parasites, fish farming, Monogenea, Acanthocephala, Branchiura, hematology.

Resumo

O objetivo deste estudo foi avaliar o impacto do parasitismo por metazoários sobre os parâmetros hematológicos, bioquímicos e fator de condição relativo de tambaquis (*Colossoma macropomum*) cultivados na região Norte do Brasil. Foram coletados 32 tambaquis em fase de engorda numa piscicultura comercial no município de Rio Preto da Eva, estado do Amazonas, Brasil. A prevalência de parasitos foi de 100% para *Anacanthorus spathulatus*, *Mymarothecium boegeri* e *Notozothecium janauachensis*, 100% para *Neoechinorhynchus buttnerae* e 53,13% para *Dolops geayi*. A maior intensidade média foi de acantocéfalos, seguida por monogenéticos e branquiúros. Correlação negativa significativa foi observada entre abundância de *N. buttnerae* e os parâmetros de hematócrito, concentração de hemoglobina, trombócitos totais e glicose, bem como entre a abundância de monogenéticos e glicose. A infecção por parasitos metazoários foi capaz de debilitar os tambaquis como observado por meio das alterações hematológicas; sendo este quadro de anemia hipocrômica e trombocitopenia importante para ser utilizado no diagnóstico destas parasitoses. Este estudo foi o primeiro registro da ocorrência de *Dolops geayi* em tambaquis cultivados na Amazônia.

Palavras-chave: Parasitos de peixes, piscicultura, Monogenea, Acanthocephala, Branchiura, hematologia.

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Introduction

Colossoma macropomum, commonly known as tambaqui, is the most cultured native freshwater fish in Brazil at 137 thousand tons (27.0% of total production) (IBGE, 2016) due to its good zootechnical performance, rusticity, diet acceptance and number of fingerlings throughout the year (GOMES et al., 2010). In intensive culture of *C. macropomum* that uses aerators, production can lead to 18 tons per hectare and approximately 2.5 kg weight in 10 months of culture (IZEL et al., 2013).

In addition to production intensification and inadequate animal care, the occurrence fish diseases can compromise part or all of the production of fish (VALLADÃO et al., 2018). Economic impacts from diseases in Brazilian fish farms has totaled 25 thousand tons of production with U$ 5 million in economic losses (TAVARES-DIAS & MARTINS, 2017). Among the main parasites that affect tambaqui culture in northern Brazil, acanthocephalans must be highlighted because they cause significant damage to fish production due to their high parasitic loads (CHAGAS et al., 2015; GOMES et al., 2017; JERÔNIMO et al., 2017; PEREIRA & MOREY, 2018). However, these studies also emphasized losses due to infection by the protozoans *Ichthyophthirius multifiliis* and *Piscinoodinium pillulare* and monogenean helminths.

Such obstacles are directly related to parasite damage of host tissue that can vary according to the parasitic species and the health status of the host (TAVARES-DIAS, 2006). Consequently, parasitism causes immune suppression and reduces fish resistance to diseases (FAST, 2014). Regular fish and facility assessments including hematological and biochemical parameters as stress indicators constitute a rapid assessment tool in fish diagnosis (TAVARES-DIAS, 2015).

Elevated levels of parasitism by the monogenean *Anacanthorus penilabiatus* caused a significant decrease in the hematocrit percentage, red blood cells count, mean corpuscular hemoglobin concentration and number of circulating basophils in the characid fish pacu *Piaractus mesopotamicus* (JERÔNIMO et al., 2014). The hybrid tambacu (*Colossoma macropomum* × *Piaractus mesopotamicus*) parasitized by the branchiuran *Dolops* sp. showed reduced hematocrit percentage and increased mean corpuscular hemoglobin concentration, glucose, total protein, sodium and chlorides indicating osmoregulatory damage to the host (TAVARES-DIAS et al., 2007a). Nevertheless, little is known regarding the physiological alterations from parasitic infection in tambaqui (TAVARES-DIAS et al., 2011).

The aim of this study was to evaluate the impact of parasitic metazoans on the hematological and biochemical parameters and relative condition factor of tambaqui cultured in northern Brazil.

Materials and Methods

Study site and fish collection

A total of 32 specimens of tambaqui (436.41 ± 177.7 g weight and 29.17 ± 3.8 cm long) were collected in December 2016 with nets in a fish farm in the municipality of Rio Preto da Eva, Amazonas State, Brazil. The fish were reared in ponds that were 8,000 m^2 and an intensive system of culture, and they were fed twice a day with a commercial diet for omnivorous fish with 28% crude protein.

Water quality was measured at three different points in the ponds at the sampling time and were as follows: water temperature 30.40 ± 0.10 °C and dissolved oxygen 5.78 ± 0.67 mg L^{-1} measured with an oximeter YSI Pro20 (Ohio, USA), pH 6.30 ± 0.12 measured with a YSI Environmental pH100 (Ohio, USA) and ammonia 0.36 ± 0.17 mg L^{-1} measured by the indophenol method (APHA, 1998).

After capture, the fish were anesthetized in a benzocaine solution (100 mg L^{-1}) for blood collection and biometry and were euthanized by a transversal section of the spinal cord for necropsy, and organs were collected for parasitological examination. All animal procedures were approved by the Animal Ethics Committee of Embrapa Pesca e Aquicultura, in Palmas, Tocantins state, Brazil (certificate no. 24, protocol 10/2016).

Hematological and biochemical analysis

Blood was withdrawn from the caudal vein with syringes containing a drop of EDTA 10% for determination of the hematocrit percentage by the microhematocrit method, the total erythrocyte count (RBC) in a Neubauer chamber after dilution 1:200 in a Natt & Herrick (1952) solution, and the hemoglobin concentration by the cyanmethemoglobin method (COLLIER, 1944). The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Ranzani-Paiva et al. (2013). Blood smears were stained with May Grünwald-Giemsa-Wright for a total count of leukocytes (WBC) and thrombocytes (RANZANI-PAIVA et al., 2013). A blood aliquot was used to obtain the plasma and centrifugation at 75 *G*, and the plasma was kept at -18 °C until the biochemical analysis. Glucose was determined by the glucose oxidase method, and total protein was determined by the biuret method with a commercial kit (Labtest, MG, Brazil).

Parasite collection and identification

Parasites were collected and fixed according to Eiras et al. (2006). The gills and intestines were removed and fixed in a 5% formalin solution, and the parasites were preserved in 70% alcohol for posterior identification and quantification under a stereomicroscope. The identification was based on Golvan (1956), Kritsky et al. (1979), Malta (1982, 1984), Cohen & Kohn (2005) and Belmont-Jégu et al. (2004). Prevalence, mean intensity and mean abundance were calculated according to Bush et al. (1997).

Relative condition factor (Kn)

Weight and length data were used to calculate the relative condition factor (Kn). Standard length (Ls) in cm and total weight (Wt) in g of each host were adjusted to a weight-length curve by the formula: $Wt = a.Ls^{B}$. With the coefficients *a* and *b* of the equation, the estimated weight values (We) were calculated with the relative condition factor (Kn) corresponding to a quotient between the observed and expected weight to determined length (Kn = Wt/We) (LE CREN, 1951).

Statistical analysis

The results obtained were expressed as the mean ± standard error. Spearman (rs) correlation coefficient was used to determine the possible correlations among the parasites, hematological and biochemical parameters and condition factor (Kn). The significance level was $p<0.05$ (ZAR, 1999).

Results

Parasite prevalence was 100% for monogeneans, identified as *Anacanthorus spathulatus* Kritsky et al. (1979), *Mymarothecium boegeri* Cohen & Kohn (2005) and *Notozothecium janauachensis* Belmont-Jégu, Domingues and Martins (2004), 100% for acanthocephalans *Neoechinorhynchus buttnerae* Golvan (1956) and 53.13% for branchiura crustaceans identified as *Dolops geayi* Bouvier (1897) (Table 1). The fish were parasitized by two (46.88%) or three (53.13%) parasite groups (Monogenea, Acanthocephala, and Branchiura). A total of 1,762 monogenean parasites were collected in the gills, and from these, 296 monogeneans were identified as 282 *A. spathulatus* (95.27% prevalence), 3 *M. boegeri* (1.01%) and 11 *N. janauachensis* (3.72%). A total of 2,770 *N. buttnerae* were found in the intestine and 29 *D. geayi* in the gill cavity of *C. macropomum*. Also, 1,005 free living adult copepods non-parasite (Class Maxillopoda, Subclass Copepoda, Order Cyclopoida) were found in gill filaments.

Table 2 shows the condition factor values and hematological and biochemical parameters as well as the reference values for tambaqui according to Tavares-Dias (2015), study in which non parasitized fish are similar in size and weight and maintained under reared conditions as registered in this study. Negative significant correlations among abundance of acanthocephalans, hematocrit, hemoglobin, thrombocytes and glucose as well as between abundance of monogeneans and glucose were found (Table 3). Nevertheless, no correlation among the parasites was verified (Table 4).

Table 1. Parasitological indices of tambaqui (*Colossoma macropomum*).

Indices	Parasites		
	Monogenea	**Acanthocephala**	**Branchiura**
Examined fish	32	32	32
Parasitized fish	32	32	17
Prevalence (%)	100	100	53.13
Mean intensity	55.06 ± 38.59	86.56 ± 76.09	1.71 ± 1.05
Mean abundance	55.06 ± 38.59	86.56 ± 76.09	0.91 ± 1.15
Range	12 - 187	5 - 386	0 - 4
Site of infection	Gills	Intestine	Gills

Discussion

The municipality of Rio Preto da Eva is the greatest tambaqui producer in Brazil as a result of intensive management practices, the use of aeration and high stocking densities (IZEL et al., 2013; IBGE, 2016). However, the lack of implementation of best management practices in fish farming has favored outbreaks of mortality and disease dissemination (MACIEL et al., 2016). Fish cohabit with pathogens naturally in the environment, and increased parasite populations are attributed to high stocking densities and the lack of prophylactic measures. Protozoans, helminths, myxozoans and crustaceans are the main parasites found to cause fish diseases that compromise fish physiology (JERÔNIMO et al., 2015; MACIEL et al., 2018).

Neoechinorhynchus buttnerae is a unique acantocephalan species parasitizing tambaqui (MALTA et al., 2001; THATCHER, 2006; DIAS et al., 2015; PEREIRA & MOREY, 2018). In the present study, the mean intensity of infection by *N. buttnerae* was lower than that found by Malta et al. (2001), Lourenço et al. (2017) and Jerônimo et al. (2017) in fish farms in the Amazon State. In contrast, no fish mortality was found in the present study, but other studies have related fish mortality (MALTA et al., 2001) and cachexia in cultured fish in Rondônia and the Amazon (JERÔNIMO et al., 2017). Mechanical damage in intestinal tissues is associated with proboscis attachment into the mucosa

Table 2. Mean values ± standard deviation of condition factor, hematological and biochemical parameters of tambaqui (*Colossoma macropomum*), from Rio Preto da Eva, Amazonas state, Brazil.

Variables	Mean ± SD	Ranges	Reference values*
Hematocrit (%)	28.94 ± 4.26	21.00 - 38.00	26.0 - 47.0
Hemoglobin (g dL^{-1})	9.40 ± 1.85	5.93 - 12.85	6.3 - 12.4
RBC (10^6 μL^{-1})	2.11 ± 0.86	1.08 - 5.07	2.44 - 5.19
MCV (μm^3)	155.12 ± 57.82	69.03 - 311.48	70.8 - 142.9
MCH (pg)	50.33 ± 19.95	22.00 - 105.31	-
MCHC (%)	32.41 ± 3.68	26.38 - 41.40	20.2 - 36.5
WBC (10^3 μL^{-1})	110.03 ± 54.38	50.46 - 303.3	22.1 - 170.3
Thrombocytes (10^3 μL^{-1})	19.04 ± 13.57	0.00 - 67.76	2.8 - 59.2
Glucose (mg dL^{-1})	83.84 ± 28.71	43.12 - 151.47	31.7 - 102.9
Total protein (g dL^{-1})	3.21 ± 0.97	1.99 - 5.86	2.9 - 4.1
Condition factor (Kn)	1.02 ± 0.22	0.42 - 1.64	-

RBC: red blood cells, WBC: white blood cells, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration; SD: standard deviation; *Source: Tavares-Dias (2015).

Table 3. Spearman correlation coefficient values among parasites abundance, condition factor, hematological and biochemical parameters of tambaqui (*Colossoma macropomum*).

Variable	Monogenea	Acanthocephala	Branchiura
Hematocrito	-0.04^{NS}	-0.59*	-0.01^{NS}
Hemoglobin	-0.13^{NS}	-0.55*	0.04^{NS}
RBC	-0.09^{NS}	-0.19^{NS}	-0.01^{NS}
MCV	0.16^{NS}	-0.04^{NS}	-0.00^{NS}
MCH	0.09^{NS}	-0.12^{NS}	0.01^{NS}
MCHC	-0.20^{NS}	-0.2^{NS}	0.09^{NS}
WBC	-0.07^{NS}	-0.17^{NS}	-0.02^{NS}
Thrombocytes	-0.0^{NS}	-0.39*	0.13^{NS}
Glucose	-0.55*	-0.35*	-0.24^{NS}
Total protein	-0.03^{NS}	-0.19 ^{NS}	-0.10^{NS}
Condition factor	-0.14^{NS}	-0.14^{NS}	0.20^{NS}

RBC: red blood cells, WBC: white blood cells, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration; *significant correlation ($p<0.05$); ^{NS}no significant correlation ($p\geq0.05$).

Table 4. Spearman correlation coefficient values among parasites groups of tambaqui (*Colossoma macropomum*).

Parasites	Monogenea	Acanthocephala	Branchiura
Monogenea	1.00	0.30^{NS}	0.32^{NS}
Acanthocephala		1.00	0.25^{NS}
Branchiura			1.00

^{NS}no significant correlation ($p\geq0.05$).

that leads to an inflammatory reaction by macrophages, Langerhans cells and lymphocytes that result in thickening the intestinal wall (JERÔNIMO et al., 2017). Histopathological findings showed a disappearance of the intestinal villi, hypertrophy of the goblet cells and leukocyte inflammatory infiltrate in the submucosa layer (MATOS et al., 2017).

In this study, the negative correlation observed in acantocephalan parasitized fish with hematocrit and hemoglobin concentrations could be associated with a hypochromic anemia possibly explained by hemorrhages at the attachment site in the intestine, causing lower number of circulating erythrocytes in the parasitic tambaquis when compared to the reference values (TAVARES-DIAS, 2015). Similar results were found in *Seriola dumerili* parasitized by the monogenean *Zeuxapta seriolae* in the gills (MONTERO et al., 2004). On the other hand, the increased mean corpuscular volume observed in *P. lineatus* parasitized by acantocephalans (BELO et al., 2013) suggests an advanced anemic response due to immature erythrocytes release in the blood stream.

The number of thrombocytes described for tambaqui in this study is within of the reference values described for the species by Tavares-Dias (2015), however the negative correlation of *N. buttnerae* abundance with total thrombocyte count suggests that these cells were being recruited by the parasitized animals. Similar alterations were reported in *P. lineatus* parasitized by acantocephalans (BELO et al., 2013), in *P. mesopotamicus* parasitized by the monogenean *A. penilabiatus,* the dinoflagellate *P. pillulare* (TAVARES-DIAS et al., 2008) and *Argulus* sp. (TAVARES-DIAS et al., 1999). Fish thrombocytes are cells involved in not only coagulation (TAVARES-DIAS & OLIVEIRA, 2009) but also phagocytosis (TAVARES-DIAS et al., 2007b; DOTTA et al., 2014). Coagulation can be influenced by parasitic infections, and thrombocytopenia in fish is related to the migration of thrombocytes to hemorrhagic lesions (TAVARES-DIAS & OLIVEIRA, 2009; BELO et al., 2013).

Monogeneans are known as one of the most important ectoparasites in fish (SANTOS et al., 2013) provoking gills lesions, necrosis, edema, displacement of the gill epithelium and pillar cell rupture (MARTINS & ROMERO, 1996). Such alterations compromise physiological processes such as breathing, nitrogenate excretion, acid-base equilibrium and osmoregulation (BECKER & BALDISSEROTTO, 2014; FERNANDES & MORON, 2014). Monogeneans (flatworms) are among the most host-specific of parasites in general and may be the most host-specific of all fish parasites (WHITTINGTON et al., 2000). The species found in this work were the same as those observed by Godoi et al. (2012) in *C. macropomum* collected from fish farms in the Western Amazon, Brazil. The authors further point out that the specificity of monogeneans in a particular host can be influenced by numerous factors, as different fixing strategies related to mechanical and chemical factors that stimulate the permanence on their hosts. In the present study, no significant correlation was observed between abundance of monogeneans and hematological parameters in contrast to the results found by Montero et al. (2004) and Tavares-Dias et al. (2008), possibly due to a low parasite load.

The fish louse *Dolops geayi* is widely distributed in South America, with reports of occurrences in Argentina, Brazil, Paraguay and Venezuela. The present study provides the first record of *C. macropomum* as a host for *D. geayi*. However, it should be emphasized that there are no records of tambaquis in natural environments as hosts for this species of Branchiura. Because fish farming consists of a confined environment, it allows these two species (parasite and host) to coexist, which possibly favors parasitism by *D. geayi* in tambaquis. According to some studies, the parasitic specificity of this species of Branchiura is low when they are in confined environments such as fish farms and aquariums (RINGUELET, 1943; MALTA, 1982, 1984). All the 29 specimens of *D. geayi* examined in this study were young and parasitizing the gill cavity, which was the same infestation site that Malta (1982) found in four other species of fish (*Megalodoras* sp., *Crenicichla* sp., *Hoplias malabaricus* and *Astronotus ocellatus*), which were natural hosts for *D. geayi* in the Janauacá Lake. We did not find any correlations between parasitism by *D. geayi* and the hematological and biochemical parameters of tambaquis, which suggests that the mean parasite intensity of 1.7 that we found was insufficient to cause physiological changes. On the other hand, regarding the tambacu hybrid parasitized by *Dolops* sp., there have been reports of diminished hematocrit and increased concentrations of mean corpuscular hemoglobin, plasma glucose, serum protein, sodium and chlorides, thus resulting in osmoregulatory disorders in the host (TAVARES-DIAS et al., 2007a).

The condition factor can also be used to detect parasitic species that affect host health (LIZAMA et al., 2007). However, in the present study there was no correlation between the condition factor and the hematological and biochemical parameters or between the condition factor and the parasite groups in tambaquis.

The variation amplitude of glucose levels in this study was higher than the reference values described for tambaqui by Tavares-Dias (2015). However, the plasma glucose levels had negative correlation with Monogenoidea and Acanthocephala, suggesting that this index is altered in the parasitized fish. This result can be explained by the fact that high levels of parasite infestation promote a stress reaction in the host, with the occurrence of a series of physiological and behavioral responses (URBINATI et al., 2015), including secondary responses as well as changes in glucose and hematological parameters, as demonstrated in the current study. In pirarucus (*Arapaima gigas*) with polyparasitism, the same pattern was observed, with changes in glucose levels and no changes in total plasma protein levels (MARINHO et al., 2015). In tambacus parasitized by *Dolops* sp. (Branchiura), there were increases in plasma glucose and serum protein levels (TAVARES-DIAS et al., 2007a). In the present study, the range of glucose and total protein levels in tambaquis corroborated those that had previously been reported for tambaquis, tambacus and tambatingas (TAVARES-DIAS, 2015; OBA-YOSHIOKA et al., 2017). It can also be highlighted that glucose and total protein levels may vary according to species, sex, state of gonad development, seasonality, diet and management (MARINHO et al., 2015; OBA-YOSHIOKA et al., 2017).

In the present study, the tambaquis farmed in intensive system presented moderate infestation by Monogenoidea, Acanthocephala and Branchiura, and the correlations between parasitism and blood variables suggests worsening of fish health condition. The application of information on occurrences of hypochromic anemia, thrombocytopenia can be used to guide the diagnosis of these parasitosis. Assessing fish-farming facilities on a regular basis is strongly recommended as a prophylactic measure to avoid diminished fish performance and productivity indices among farmed tambaqui. The present study also provides the first record of *D. geayi* parasitizing tambaquis in captivity.

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