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Hematological parameters of cachara *Pseudoplatystoma reticulatum* during the reproductive period in fish farming

Parâmetros hematológicos de cachara durante o período reprodutivo em piscicultura

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Abstract: For several species such as fish, during the reproductive period, hematological variations may occur. Related to this, the objective of this study was to evaluate the hematological variables of males and females of cachara (*Pseudoplatystoma reticulatum)* during their reproductive period in fish farming. During six months, the animals (n =17) were submitted to blood collections once a month, by puncture of the caudal vessel for determination of hemoglobin, hematocrit, erythrocyte counts, and differential counting of defense cells. The experimental design used was entirely random, considering as a source of variation the sex factor. *P. reticulatum* females had significantly higher mean values of hemoglobin and immature leukocytes compared to males, however, males had higher mean values of neutrophils than females. The reproductive period of *P. reticulatum* may influence the hematological characteristics, with possible differences in the sex dependent response.

Key words: Carnivorous fish. Hematology. Sex diferences. Siluriformes.

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Resumo: Nos peixes, assim como para outras espécies de animais, durante o período reprodutivo podem ocorrer variações hematológicas. Com este estudo foram avaliadas variáveis hematológicas de machos e fêmeas de cachara (*Pseudoplatystoma reticulatum)* durante o seu período reprodutivo em piscicultura. Durante seis meses, os animais (n=17) foram submetidos a colheitas sanguíneas mensalmente, por punção do vaso caudal, para determinação de hemoglobina, hematócrito, contagem do número de eritrócitos, e contagem diferencial de células de defesa. O delineamento experimental utilizado foi inteiramente casualisado, considerando como fonte de variação o fator sexo. As fêmeas de *P. reticulatum* apresentaram médias de hemoglobina e de leucócitos imaturos significativamente maiores em relação aos machos, entretanto, os machos apresentaram valores médios de neutrófilos superiores aos das fêmeas. O período reprodutivo de *P. reticulatum* pode influenciar as características hematológicas, com possíveis diferenças na resposta dependente do sexo.

Palavras-chave: Peixe carnívoro. Hematologia. Diferença sexual. Siluriformes.

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Introduction

The surubim (cachara [*Pseudoplatystoma reticulatum*], pintado [*Pseudoplatystoma corruscans*], and hybrids) are species of great economic importance in South American countries. This is especially true in Brazil, where they are one of the most largely produced fish species. In 2017, Brazil produced 16 tons of this fish (IBGE, 2018).

P. reticulatum is a fish native to the Midwest region of Brazil and its rapid growth and high market value have led to increased interest on the part of fish farmers (Campos, 2005). It is a fish with positive organoleptic characteristics for the cultivation, with firm texture and good meat quality, good carcass yield and absence of intramuscular spines (Neto *et al.*, 2010). In addition, this specie is used to produce the “cachapinta” or “pintachara” hybrids when they are crossed with *Pseudoplatystoma corruscans*, and the jundiara, crossed with *Leiarius marmoratus* (Alves, Varela, Moro & Kirschnik, 2014; IBGE, 2017).

Hematological parameters are practical measures, which are susceptible to alterations according to species, age, sex, reproductive period, water quality, capture methods and environmental changes (Tavares-Dias *et al*., 2009; Ranzani-Paiva, Romagosa, & Ishikawa, 2005; Lazzari *et al.,* 2011). Understanding the adaptations of these individuals organisms in their environment, leads to obtaining reference values in hematology. Thus allowing, a good monitoring of the health status of the fish and can be a tool to improve reproductive rates under cultivation conditions (Oliveira *et al.,* 2009).

There are records in the literature of hematological parameters in cachara juveniles and their hybrids, (Campos *et al*., 2014; Sanchez *et al*., 2014; Rodrigues *et al.,* 2018) however, the reference values of the hematological profile in *P. reticulatum* in reproductive period are scarce. Thus, the objective of this work was to evaluate the hematological variables of the red and white series of males and females of *P. reticulatum*, throughout the reproductive period.

Material and Methods

For this study, broodstock of *P. reticulatum* (n = 17) with an average weight of 3,458 g and an average length of 82 cm were used, electronically marked with a chip and raised in a dug tank, with independent entrance and exit of water, in the fish farm sector, from the State University of Mato Grosso do Sul (UEMS), in Aquidauana - MS. This work was authorized by the UEMS Animal Ethics Committee, according to the CEUA/UEMS 0014/2013 protocol.

The fish were stocked in earthen ponds with 280 m^2 capacity to be monthly monitored from October 2010 to April 2011. These fish were not used for reproduction on this year. During six months, the fish were fed commercial diet with 40% crude protein (10-12 mm pellet) and forage fish *ad libidum.* Water quality was measured each 15 days with a multiparameter YSI. Water temperature varied from 24 to 32ºC and dissolved oxygen from 4 to 5 mgL^{-1} considered adequate for this species.

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A completely randomized design was adopted, with the sex factor as an experimental group. For the management and blood analysis procedure, the individuals were captured and anesthetized with 10% benzocaine, and physically contained with a moistened cloth for handling, being weighed with an electronic scale.

After biometrics, blood aliquots were collected from each individual, using blood vessel puncture, with the aid of needles and disposable syringes bathed in 3% EDTA. The following hematological variables of the red series were determined: hemoglobin by Collier's cyanomethaemoglobin method (1944); percentage of hematocrit (Ht) by the microhematocrit method of Goldenfarb et al. (1971) and counting of red blood cells (RBC) in a Neubauer chamber using a 1:200 dilution in formaldehyde-citrate solution under an optical microscope (400 x).

Blood extensions were made in duplicate of each fish, air-dried and stained May Grünwald-Giemsa-Wright (Tavares-Dias & Moraes, 2004) for the differential leukocyte count, total thrombocyte and leukocyte count (Knowles, Hrubec, Smith, & Bakal, 2006) .

For statistical analysis of the data, the results of the hematological variables were subjected to analysis of variance (ANOVA) with a significance level of 5%. When there were significant differences, the means were compared using the Tukey test ($p<0.05$).

Results

In this study, no significant difference ($p<0.05$) was found in the percentage of hematocrit and in the number of erythrocytes between the specimens of *P. reticulatum* in the reproductive period. However, a difference ($p<0.05$) was observed in relation to hemoglobin (HB) values with females presenting a higher value than males (Table 1).

Table 1. Means (± standard deviation) of variables from the red male and female series of *Pseudoplatystoma reticulatum* during reproductive period.

	Sex						
	Female value			Male value			
Parameters	Means	Maximum	Minimum	Means	Maximum	Minimum	P
HTC (%)	29.45±6.34	41.00	19.00	27.47±6.34	37.00	17.00	0.31
HB (g dL-1)	5.88±3.41a	12.60	1.86	3.66±3.42b	11.06	1.42	0.02
RBC (106 µL-1)	1.96±0.47	2.85	1.03	2.32±0.47	4.28	1.48	0.07

HTC = hematocrit, HB = hemoglobin, RBC = red blood cells. Different letters indicate significant difference by Tukey's test ($p<0.05$) between sex.

In the leukogram of the fish there was no difference ($p<0.05$), between genders, for monocytes, lymphocytes, basophils, eosinophils and PAS^+ leukocyte. However, the quantification of neutrophils was different ($p<0.05$), with male individuals presenting higher values than females. Regarding leukocytes, the values were higher ($p<0.05$) in females (Table 2).

Table 2. Means (± standard deviation) of the hematological variables of the white series of male and female breeding *Pseudoplatystoma reticulatum* during the reproductive period.

Parameters	Relative Values (%)			Absolute Values (μL^{-1})		
	Male	Female	P	Male	Female	P
Monocytes	0.95±0.77	1.31±0.76	0.12	4.044±499	587±499	0.08
Lymphocytes	74.21±10.95	76.19±10.95	0.55	6.017±2.449	4.835±2.449	0.13
Basophils	3.67±2.62	3.86±2.62	0.82	2.411±1.684	1.988±1.684	0.44
Eosinophils	3.52±2.50	2.77±2.50	0.33	2.448±1.956	1.945±1.956	0.36
Neutrophils	14.64±8.28	11.81±8.27	0.26	1.092±501[a]	707±501[b]	0.0001
PAS^+	0.73±0.37	0.81±0.374	0.51	316±470	515±470	0.12
Leuko I	1.41±1.72[a]	2.79±1.721[b]	0.007	1.184±1.19	1.711±1196	0.15

PAS^+ = leukocyte PAS positive, Leuko I = immature leukocyte. Different letters indicate significant difference by Tukey's test ($p<0.05$) between sex.

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Discussion

During the reproductive period, a positive relationship between the level of hemoglobin and the increase in muscle activity of fish has already been proven (Ranzani-Paiva *et al*., 2005). Still, a difference in hemoglobin concentration may be associated with different erythrocyte maturation degrees, since immature erythrocytes contain less hemoglobin compared to mature ones (Lusková, 1997). According to Pereira *et al*. (2011), hemoglobin is a blood parameter that can act as an indicator of the response to acute stress in fish, a situation that requires greater oxygen transport. The higher hemoglobin level in females differs from the results obtained by Ranzani-Paiva *et al*. (2005) in a study to evaluate the hematological parameters of cachara, *Pseudoplatystoma fasciatum*, bred in captivity, where male individuals had higher hemoglobin averages than females. Among the biological manifestations, spawning appears to be the most important event changing the animal homeostasis, which was reflected in the hematological parameters (Lusková, 1997). Sex could be associated with differences in blood cell constituents that could be caused by higher metabolic activity of male versus female fish (Collazos, Ortega, Barriga, & Rodriguez, 1998).

For teleost fish such as cachara, the mean leukocyte values may vary intraspecific and interspecific. Interspecific variations can occur due to factors such as reproduction, seasonality, eating habits and ecophysiological conditions (Tavares-Dias & Matiqueiro, 2004; Tavares-Dias & Moraes, 2004). Leukocyte variations can also be caused by stress such as changes in temperature, osmotic concentration, nutritional deficiency and pathologies (Andrade, 2016). During the period of blood sampling, the presence of white parasites was found on the external surface, especially in the fish's operculum. According to Pereira *et al*. (2011), when the animal is subjected to stressful situations, like parasitic infections, the action of cortisol intensifies, which will act on hematopoietic tissues resulting in blockage in the production of lymphocytes, monocytopenia and neutrophilia, as an immunosuppressive response.

Considering that a high number of neutrophils and a high hemoglobin can be indicative of stress, both males and females of cachara could be more stressed during the studied reproductive period, however, the data are insufficient for this conclusion. In addition, it must be taken into account that during the period in which the breeders were sampled for blood analysis, there was no event that would impair the quality of the water in the nursery and / or intensive management that would lead them to stressful situations. One must take into account the fact that fish have parasitism, which can explain stress conditions.

Conclusions

In the reproductive period, *P. reticulatum* has a higher concentration of hemoglobin in females and neutrophilia in males.

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