

# HEMATOLOGICAL PARAMETERS OF THE HYBRID SERRASALMIDS FARMED IN CENTRAL-WESTERN BRAZIL\*

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## ABSTRACT

This study evaluated the hematological parameters of tambacu and patinga, two the hybrid serrasalmid fishes farmed in the state of Mato Grosso do Sul, Brazil, with emphasis on the influence of seasons on them. Two-hundred forty hybrid fishes, 120 tambacus and 120 patingas, were collected over 12 months to assess their hematological parameters and compare them between the warm (October-March) and cold seasons (April-September). Water quality parameters were also measured weekly to test for the influence of environmental conditions on the results. The dissolved oxygen content in the water decreased in the warm season, and increased in the cold season. In the warm season, tambacus presented significantly higher values ( $p < 0.05$ ) of the total plasmatic protein (TPP), hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and eosinophil count parameters. In the cold season, counts of erythrocytes, thrombocytes, total leukocytes, lymphocytes, and neutrophils were higher in tambacus ( $p < 0.05$ ). For patinga, MCV was higher in the warm season, while hemoglobin, erythrocytes, MCHC, total leukocytes, and basophil counts were higher ( $p < 0.05$ ) in the cold season. These results demonstrated the influences of seasonality on the hematological parameters of the hybrids tambacu and patinga when held under normal farming conditions in the region.

**Key words:** tambacu; patinga; season; water quality; *Colossoma*; *Piaractus*.

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## PARÂMETROS HEMATOLÓGICOS DE SERRASALMÍDEOS HÍBRIDOS CULTIVADOS NA REGIÃO CENTRO-OESTE DO BRASIL

### RESUMO

Este estudo avaliou os parâmetros hematológicos de híbridos tambacu e patinga cultivados no Mato Grosso do Sul, Brasil, com ênfase na influência das épocas do ano. Duzentos e quarenta peixes híbridos, 120 tambacus e 120 patingas, foram coletados ao longo de 12 meses para análises hematológicas, comparando a estação quente (Outubro-Março) com a estação fria (Abril-Setembro). Os parâmetros de qualidade de água foram medidos semanalmente, para verificar sua influência sobre os parâmetros hematológicos. A estação quente mostrou tendência ao declínio nos níveis de oxigênio dissolvido, enquanto na estação fria ocorreu o inverso. Na estação quente, tambacus apresentaram valores mais altos ( $p < 0,05$ ) de proteína plasmática total (TPP), concentração de hemoglobina, volume corpuscular médio (MCV), concentração de hemoglobina corpuscular média (MCHC) e número de eosinófilos. Na estação fria, as contagens de eritrócitos, trombócitos, leucócitos totais, linfócitos e neutrófilos foram mais altas ( $p < 0,05$ ). Em patingas, valores mais altos de MCV foram observados na estação quente, enquanto a concentração de hemoglobina, eritrócitos, MCHC, leucócitos totais e basófilos foram mais altos ( $p < 0,05$ ) na estação fria. Os resultados mostram influência da sazonalidade sobre os parâmetros hematológicos dos híbridos tambacu e patinga mantidos em condições típicas de cultivo na região.

**Palavras-chave:** tambacu; patinga; estação; qualidade de água; *Colossoma*; *Piaractus*.

### INTRODUCTION

The production of farmed fish in Brazil has been expanding in recent years due to the availability of water resources, vast territorial extent, favorable weather, and wide variety of native fish species suitable for farming there. In 2015, the total production of farmed fish in Brazil was 483,241 tons, accounting for 69.9% of the country's aquaculture production and corresponding to an income of about ~942,982 million US dollars (IBGE, 2015). There has also been a considerable increase in the production of hybrids from native freshwater fishes for use in farm production, with the main emphasis

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being on hybrids of serrasalmid species (family Serrasalminidae), such as “tambacu” and “patinga” (Jerônimo et al., 2015a, 2015b).

In 2015, the Brazilian production of the hybrids tambacu and tambatinga combined was 37,443 tons, corresponding to approximately 81,043 US dollars (IBGE, 2015). The patinga is another hybrid that was recently introduced into fish farming, so there is still no official data on its separate production, but the joint production of patinga and pacu (not a hybrid) in 2015 accounted for 3,480 tons and approximately 7,779 US dollars (IBGE, 2015). The statistical data available to date have not yet demonstrated an increase in hybrid fish production alone in Brazil (Jerônimo et al., 2015a, 2015b).

The tambacu and patinga hybrids belong to the family Serrasalminidae, also known as “roundfishes”, and comprise the majority of the production of native species in continental fish farming in Brazil (IBGE, 2015). Fishes of the genera *Colossoma* and *Piaractus* are considered high-quality food items (Jégu, 2003). Among these are the “tambaqui” (*Colossoma macropomum* Cuvier, 1816) and the “pirapitinga” (*Piaractus brachypomus* Cuvier, 1818), which is considered the largest scaled fish in the Amazon (Resende et al., 1998), as well as the pacu (*Piaractus mesopotamicus* Holmberg, 1887), the species exploited the most by commercial and sport fishing in Brazil (Resende, 2003).

Tambaqui (*C. macropomum*) is cultivated throughout Brazil, and can reach a mass of 1.8 kg in one year, but does not tolerate low temperatures, which impairs its performance in production in the Southern and Southeastern regions of the country, which are characterized by having harsher winters (Souza, 1998). The pirapitinga (*P. brachypomus*) is widely used for hybridization, either with the tambaqui, generating the “tambatinga” and the “piqui”, or with the pacu, generating the “patinga” (De Paula, 2009). The pacu (*Piaractus mesopotamicus* Holmberg, 1887) is cultivated throughout the Brazilian territory, and is valued for its precocity, rusticity, easy adaptation to commercial feeds, and the high economic value of its meat due to its organoleptic characteristics (Castagnolli and Cyrino, 1986; Bittencourt, 2012). In some places in Brazil, hybrid serrasalmid fishes are cultivated more often than pure species, as is the case in the Central-Western region, especially in the State of Mato Grosso do Sul (IBGE, 2015).

Hematological analysis is a relevant tool for these purposes, which allows for the early detection of conditions that affect the performance of the production system (Labarrère et al., 2012; Bosisio et al., 2017). The assessment of hematological characteristics in farmed fish is important for the prevention of diseases and mortality because the blood parameter values of fish reflect their general physiological status, and also allow morbid conditions to be diagnosed. Further, in addition to infectious diseases and parasitism, these hematological parameters may be also influenced by stress and changes in water quality (Martins et al., 2000; Ranzani-Paiva et al., 2000). These changes, in turn, may naturally occur due to seasonality, causing differences among hematological values in different times of the year (Jerônimo et al., 2011).

For tambacu, reference values for hemoglobin, hematocrit, mean corpuscular hemoglobin concentration (MCHC), and counts of total and different types of blood cells were previously published (Tavares-Dias et al., 2000, 2003). These parameters were also

described for tambacus with parasites (Tavares-Dias et al., 2007, 2008; Varandas et al., 2013) and subjected to capture stress in a “catch-and-release” fishing establishment (Varandas et al., 2013). Other studies reported the parameters of this species related to its red blood cells under the influence of exposure to nitrite (Moraes et al., 2006). The biochemical parameters of its blood, including quantification of total serum protein (Tavares-Dias and Moraes, 2010), were also previously assessed, along with the characterization of the acute inflammatory response in this species based on the counts of defensive (i.e. immunity-related) blood cells in inflammatory exudate (Martins et al., 2009).

For patinga, there are still no hematological studies in the literature. Due to the increased production of these hybrids in Brazilian farms and the scarcity of information about their hematology, the aim of this study was to verify the hematological parameters of tambacu and patinga farmed in an intensive system in the State of Mato Grosso do Sul, Central-West region of Brazil. This was done while also checking the influence of the season of the year (warm or cold) on these parameters, and considering the influences of variations in water quality parameters.

## MATERIAL AND METHODS

A total of 240 hybrid fish, 120 tambacus (female *C. macropomum* × male *P. mesopotamicus*) and 120 patingas (female *P. mesopotamicus* × male *P. brachypomus*), were collected by dragnet from two fish farms in the region of Grande Dourados, state of Mato Grosso do Sul, Central-Western Brazil, from June 2010 to May 2011. This period was divided into a “cold season” from April to September and a “warm season” from October to March, between which there is an average variation of approximately 5 °C.

The physicochemical characteristics of the water in the fish ponds were measured once a week. These included: dissolved oxygen, temperature, pH, and electrical conductivity, which were measured with a multiparameter HANNA® device (Hanna instruments, Inc., USA); transparency, measured with the Secchi disk method; and alkalinity, measured by the titration method. Samples of farm output water were also collected for the determination of orthophosphate, total ammonia, nitrite, and nitrate content using a colorimetric kit (Alfakit®, Brazil).

After capture, the animals were anesthetized with clove oil (75 mg L<sup>-1</sup>) for blood collection (Ishikawa et al., 2011) (Ethics Committee 29979/2009-05/CEUA/UFSC 23080.0). During collection, fish were macroscopically analyzed to ensure the absence of signs of infection or parasitism. Blood was collected by puncture of the caudal blood vessel with syringes containing 3% ethylenediaminetetraacetic acid (EDTA), and samples were then packed, cooled, and transported to the laboratory. Hematocrit was determined by the microhematocrit method (Goldenfarb et al., 1971), and hemoglobin concentration was assessed by the cyanmethemoglobin method (Ranzani-Paiva et al., 2013). Erythrocyte counts (RBC) were performed in a Neubauer chamber after dilution of the blood in a 1:200 formalin-citrate solution, while mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated

according to the methods of Ranzani-Paiva et al. (2013), and total plasma protein (TPP) content was determined with a portable refractometer. In extensions stained with May-Grünwald-Giemsa-Wright (MGGW) staining (Ranzani-Paiva et al., 2013) were obtained the counts of the total numbers of leukocytes (WBC) and thrombocytes by the indirect method (Ishikawa et al., 2008) and the differential count of leukocytes. In the differential count, the numbers of lymphocytes, neutrophils, monocytes, basophils, eosinophils and PAS-positive granular leukocytes (PAS-GL) were registered.

Biometric (length and weight) and hematological parameters data were checked for normality using the Kolmogorov-Smirnov test, and for homoscedasticity using Levene's test. If they passed these tests, they were then subjected to an analysis of variance (ANOVA) comparing them between seasons. Data transformations were used as needed, and Tukey's HSD test was used to compare means among groups at a significance level of 5% ( $p < 0.05$ ). The water quality data were also subjected to principal component analysis (PCA) to verify whether the variables differed between the different seasons.

RESULTS

Regarding water quality parameters (Table 1), the principal components analysis (PCA) of the data from the ponds where the patinga were collected showed that the two main principal components accounted for 68% of the variance in the data (Figure 1). Higher dissolved oxygen and transparency values were found here in the cold season, while higher values of ammonia, orthophosphate, nitrate, and nitrite content, as well as temperature (as would be expected), were found in the warm season.

In the pond from which the tambacu were collected, the two main principal components accounted for 59% of the variance in the data (Figure 2). Higher values of dissolved oxygen, transparency, and pH occurred here in the cold season, whereas in the warm

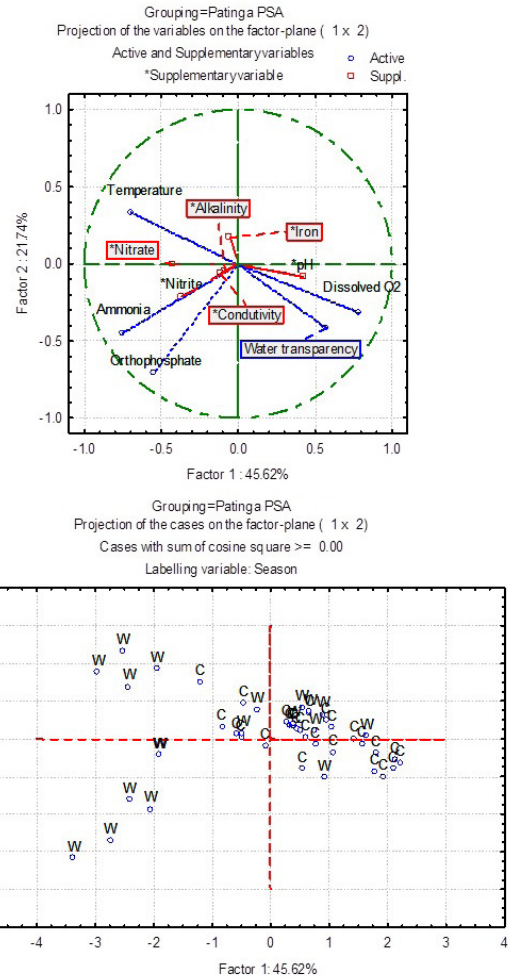
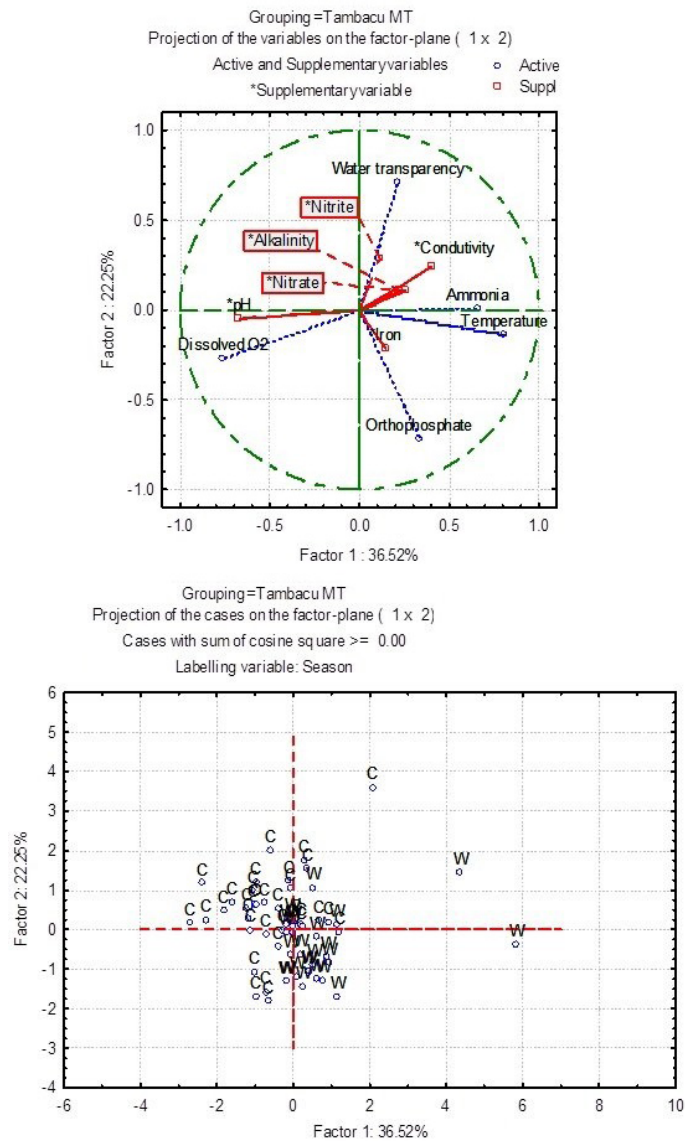


Figure 1. Principal component analysis (PCA) of the water quality parameters measured in the pond where the patingas (*Piaractus mesopotamicus* x *Piaractus brachypomum*) studied were collected. C = cold season; W = warm season.

Table 1. Water quality parameters in ponds where the hybrids tambacu (*Colossoma macropomum* x *Piaractus mesopotamicus*) and patinga (*P. mesopotamicus* x *Piaractus brachypomum*) were farmed in the state of Mato Grosso do Sul, Central-Western Brazil, in the warm and cold seasons. Lowercase letters indicate significant differences between seasons ( $p < 0.05$ ).

Parameters	Tambacu (n=120)		Patinga (n=120)	
	Warm Season	Cold Season	Warm Season	Cold Season
Total Alkalinity (mg.L <sup>-1</sup> )	48.57±11.65	50.68±7.43	40.69±6.77	40.74±5.28
pH	7.07±0.59	7.27±0.30	7.91±1.37	7.09±0.42
Conductivity (µS.cm <sup>-1</sup> )	62.21±12.01	63.56±8.21	31.00±4.55	35.95±9.27
Dissolved Oxygen (mg.L <sup>-1</sup> )	5.39±1.56	6.76±1.06	6.33±1.07	7.41±1.55
Temperature (°C)	25.10±3.44	19.87±3.01	24.93±3.27	20.44±3.23
Transparency (cm)	16.54±2.92	18.38±5.52	21.32±5.11	25.70±9.75
Ammonia (mg.L <sup>-1</sup> )	0.19±0.12	0.12±0.06	0.25±0.22	0.14±0.12
Total iron (mg.L <sup>-1</sup> )	0.26±0.41	0.37±0.60	0.29±0.28	0.20±0.41
Nitrate (mg.L <sup>-1</sup> )	0.42±0.46	0.30±0.45	0.24±0.99	0.29±1.17
Nitrite (mg.L <sup>-1</sup> )	0.02±0.03 <sup>b</sup>	0.04±0.08 <sup>b</sup>	0.20±0.07 <sup>a</sup>	0.03±0.03 <sup>b</sup>
Orthophosphate (mg.L <sup>-1</sup> )	0.66±0.36	0.33±0.36	0.16±0.34	0.03±0.14





**Figure 2.** Principal component analysis (PCA) of the water quality parameters measured in the pond where the tambacus (*Colossoma macropomum* x *Piaractus mesopotamicus*) studied were collected. C = cold season; W = warm season.

season higher values of temperature, ammonia, orthophosphate, and electrical conductivity occurred.

Tambacus showed significant increases in length and weight in the warm season compared to those in the cold season ( $p < 0.05$ ). There was no significant difference in the length and weight of the patinga hybrids between the seasons ( $p > 0.05$ ) (Table 2). Regarding hematological parameters, the tambacus presented higher values of hematocrit, TPP, hemoglobin, MCHC, MCV, and eosinophil counts in the warm season. In the cold season, higher numbers of erythrocytes, total leukocytes, lymphocytes, neutrophils, and thrombocytes were observed in them.

Patingas showed greater MCV in the warm season and higher values of hemoglobin, erythrocytes, MCHC, total leukocytes, and basophil counts in the cold season. The other parameters did not show significant differences in them between the seasons (Table 2).

## DISCUSSION

The hematological values of fish may be influenced by intrinsic biological factors, such as age, developmental phase, and size (Ranzani-Paiva et al., 2013). Thus, the significant increase in the body length and weight of tambacus from the cold to the warm season could explain some of the differences found between the seasons for this hybrid. For patingas, the values of length and weight were similar in both seasons, so the majority of hematological parameters in this hybrid also did not show differences between the seasons.

In both seasons, tambacu showed percent hematocrit values within the range reported previously for this hybrid (Tavares-Dias et al., 2000, 2003). The hematocrit was significantly higher in the warm season due to the significant increase in the mean corpuscular volume (MCV) of the erythrocytes in this season. Regarding patinga, the percent hematocrit observed herein was similar to those previously reported in pacu (*Piaractus mesopotamicus*) (Belo et al., 2014; Klein et al., 2014; Sado et al., 2014; Dieterich et al., 2015). The patinga hybrid did not show significant differences in hematocrit between the seasons, corroborating a previous study's results for the hybrid surubim (female *Pseudoplatystoma reticulatum* x male *P. corruscans*) farmed in the same region (Jerônimo et al., 2015a).

Regarding the concentrations of hemoglobin and mean corpuscular hemoglobin concentration (MCHC) in the blood of tambacu, the values found herein were lower than those found in previous studies of this hybrid (Martins et al., 2002; Tavares-Dias et al., 2003), particularly in the cold season. This difference may have been due to the size of the fish used, as these were larger in the present study, and/or differences in the availability of oxygen in the water between studies. Both parameters were significantly higher in the warm season, which agreed with the previously reported increase in hemoglobin in fat snook (*Centropomus parallelus*) during the warm season (Santos et al., 2012). This may be explained by the fact that at elevated temperatures there is an increase in the metabolic activity of fish, which diminishes the oxygen concentration in the water during this time; thus, fish tend to show an increase in hemoglobin as a compensatory mechanism to improve respiratory efficiency under such conditions.

The hemoglobin and MCHC of patinga presented low values when compared with those previously reported for pacu (*Piaractus mesopotamicus*) in normal, healthy conditions (Tavares-Dias et al., 2003). Contrarily to tambacu, the values of hemoglobin of patinga were lower in the warm season, which could be explained by the lower number of erythrocytes. However, this does not explain the reduction in MCHC, therefore, it is possible to infer that reduction in these parameters is an indicative of anemia (Labarrère et al., 2012).

**Table 2.** Biometric and hematological data for the hybrids tambacu (*Colossoma macropomum* x *Piaractus mesopotamicus*) and patinga (*P. mesopotamicus* x *Piaractus brachypomum*) farmed in the state of Mato Grosso do Sul, Central-Western Brazil, in the warm and cold seasons.

Parameters	Tambacu (n=120)		Patinga (n=120)	
	Warm Season	Cold Season	Warm Season	Cold Season
Weight (g)	1,356.07±313.85 <sup>a</sup>	950.80±173.95 <sup>b</sup>	667.28±147.09	832.75±175.61
Length (cm)	38.44±3.69 <sup>a</sup>	37.08±2.63 <sup>b</sup>	32.74±2.75	33.22±2.80
Hematocrit (%)	36.47±3.67 <sup>a</sup>	32.40±3.87 <sup>b</sup>	36.94±4.65	36.19±4.38
TPP (g.dL <sup>-1</sup> )	5.73±0.99 <sup>a</sup>	5.17±0.36 <sup>b</sup>	6.50±0.71	5.83±0.93
Hemoglobin (g.dL <sup>-1</sup> )	7.18±3.27 <sup>a</sup>	4.50±1.09 <sup>b</sup>	6.22±1.77 <sup>b</sup>	7.66±3.79 <sup>a</sup>
Erythrocytes (x10 <sup>6</sup> .µL <sup>-1</sup> )	1.57±0.31 <sup>b</sup>	1.75±0.31 <sup>a</sup>	1.51±0.29 <sup>b</sup>	1.89±0.41 <sup>a</sup>
MCHC (g.dL <sup>-1</sup> )	20.36±10.18 <sup>a</sup>	14.04±3.84 <sup>b</sup>	17.38±6.19 <sup>b</sup>	20.87±9.33 <sup>a</sup>
MCV (fL)	238.88±40.58 <sup>a</sup>	190.64±41.66 <sup>b</sup>	251.60±44.43 <sup>a</sup>	200.74±54.44 <sup>b</sup>
Total leukocytes (x10 <sup>3</sup> .µL <sup>-1</sup> )	24.18±5.71 <sup>b</sup>	33.56±9.14 <sup>a</sup>	21.60±6.34 <sup>b</sup>	32.06±8.75 <sup>a</sup>
Thrombocytes (x10 <sup>3</sup> .µL <sup>-1</sup> )	17.68±4.34 <sup>b</sup>	21.58±6.94 <sup>a</sup>	16.10±6.04	19.20±9.93
Lymphocytes (x10 <sup>3</sup> .µL <sup>-1</sup> )	20.86±5.02 <sup>b</sup>	28.19±7.96 <sup>a</sup>	19.26±6.28	28.01±7.21
Neutrophils (x10 <sup>3</sup> .µL <sup>-1</sup> )	1.03±0.83 <sup>b</sup>	1.93±1.33 <sup>a</sup>	0.27±0.25	0.62±0.72
Monocytes (x10 <sup>3</sup> .µL <sup>-1</sup> )	1.19±0.79	2.13±1.96	0.63±0.62	1.22±1.35
Eosinophils (x10 <sup>3</sup> .µL <sup>-1</sup> )	0.30±0.57 <sup>a</sup>	0.11±0.23 <sup>b</sup>	0.23±0.37	0.46±0.62
Basophils (x10 <sup>3</sup> .µL <sup>-1</sup> )	0.10±0.18	0.10±0.21	0.01±0.05 <sup>b</sup>	0.22±0.49 <sup>a</sup>
PAS-GL (x10 <sup>3</sup> .µL <sup>-1</sup> )	0.71±0.88	0.55±0.52	1.18±0.62	1.52±1.30

Lowercase letters indicate significant differences between seasons ( $p < 0.05$ ). TPP = total plasmatic protein; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; PAS-GL = PAS-positive granular leukocytes.

For tambacu, in both seasons the mean corpuscular volume of erythrocytes was higher than the values previously reported for this hybrid (Tavares-Dias et al., 2007). This may be due to the size of the fish (Ranzani-Paiva et al. 2013), because those used in the present study were larger. Ranzani-Paiva et al. (2013) state that there may be quantitative and morphological changes in fish blood elements due to endogenous factors, such as length and weight. However, MCV also depends on level of activity, being higher in more sedentary animals (Glazova, 1976). Variations in hematological parameters, such as erythrocyte size and MCV, can be verified not only for different species but also for the same species in different environmental conditions, revealing physiological adaptation (Campbell and Murru, 1990). For patinga, in both seasons the MCV was within the range of values previously reported for pacu (*P. mesopotamicus*) of similar weight (Jerônimo et al., 2014). Regarding seasonality, the values of MCV were lower in the cold season for both hybrids, coinciding with the time when there were higher numbers of erythrocytes in tambacu. The number of erythrocytes in tambacu was similar to that in a previous study of this hybrid in the absence of a stress stimulus (Martins et al., 2002). It is possible to infer that, in the cold season, there was an increase in the production of these cells that resulted in there being a higher proportion of young erythrocytes, which tend to have lower volumes. Some studies state that fish erythrocytes usually show a progressive increase in the size with maturation (Härdig, 1978; Glomski et al., 1992; Passantino et al., 2004). This could also explain the lower values of MCHC recorded in the cold season for tambacu, because young

erythrocytes contain less hemoglobin than older cells (Kumar et al., 1999). On the other hand, contradicting this hypothesis, other studies show that younger erythrocytes are larger than mature ones (Esteban et al., 1989). In patinga, the amount of erythrocytes was within the range previously reported for its parental species (Tocidowski et al., 1997; Jerônimo et al., 2014). In the hybrid surubim, when farmed in an intensive system, there was also no difference in erythrocyte counts between warm and cold seasons (Jerônimo et al., 2015a).

Tambacu and patinga presented higher total plasmatic protein (TPP) concentrations compared to those of pacu (*P. mesopotamicus*) (Sado et al., 2013, 2014; Bicudo et al., 2014; Jerônimo et al., 2014) and pirapitinga (*P. brachypomus*) (Vásquez-Torres et al., 2012). In tambacu, higher values were observed in the warm season. Changes in TPP are mainly caused by alterations in plasmatic volume due to osmotic disequilibrium between intracellular and extracellular compartments, so any stress that induces this situation may alter the total protein values (Melo et al., 2009). A decrease in TPP may also occur as a consequence of alimentary restriction (Chagas et al., 2005), which could have been the cause of the lower values observed in the cold season, given that at lower temperatures fish reduce their feeding. The patinga hybrid did not show differences in TPP between the seasons, corroborating a previous study's results for the hybrid surubim farmed in the same region (Jerônimo et al., 2015a). Despite being a different species, the fact of being raised in a nearby location illustrates the possibility of this response under similar environmental conditions.

The number of thrombocytes in the blood of tambacu in this study was low in comparison to that reported in other studies of this hybrid (Martins et al., 2002; Tavares-Dias et al., 2007; Varandas et al., 2013), with lower numbers occurring during the warm season. Fish thrombocytes have the potential to be powerful immune cells due to their phagocytic activity (Nagasawa et al., 2015). Higher water temperatures result in higher numbers of phagocytic cells being present in the tissues to face potential parasitic and bacterial infections, which may reduce the number of these cells circulating in the blood (Buchtíková et al. 2011). The thrombocytes may also migrate to perform coagulation in injured sites, which could also explain the lower amount of these cells observed circulating in the blood during the warm season. In pacu (*P. mesopotamicus*), infestation by *Argulus* sp. (Crustacea) induced decreases in thrombocyte counts, supporting this hypothesis (Tavares-Dias et al., 1999). However, considering that thrombocytes perform immune responses, their numbers may have increased in the cold season to prevent infections. Patinga also presented lower thrombocyte counts compared to those reported for pacu (*P. mesopotamicus*) in previous studies (Sado et al., 2013, 2014; Belo et al., 2014; Jerônimo et al., 2014), but there was no difference between the seasons in this case. A lack of seasonal differences in thrombocyte counts was also reported for the hybrid surubim farmed in an intensive system (Jerônimo et al., 2015a).

The numbers of total leukocytes in tambacu was similar to previous findings for this hybrid (Tavares-Dias et al., 2007). In patinga, total leukocyte counts were similar to the ranges reported for its parental species, pirapitinga (*P. brachyponus*) and pacu (*P. mesopotamicus*) (Tocidowski et al., 1997; Belo et al., 2014; Jerônimo et al., 2014). For both hybrids, increased total leukocyte counts were found during the cold season. Lower temperatures may trigger susceptibility to certain pathogens, so the production of defense cells may be increased to prevent eventual infections. In tambacu, the increase in total leukocyte number was due to the significant increases in the numbers of lymphocytes and neutrophils in the blood during the cold season.

The average counts of lymphocytes in the blood of tambacu were much lower than those previously reported for this hybrid (Tavares-Dias et al., 2007), and there was an increase in these in the cold season. Lymphocytes are directly involved in the specific immunological responses of fishes, and higher numbers of lymphocytes in the colder months suggest that more are needed to prevent infections then and maintain stability in these organisms (Jerônimo et al., 2011). For patinga, the amounts of lymphocytes were similar to those reported in a previous study with pacu (Jerônimo et al., 2014), and higher than those in other studies of pacu and pirapitinga (Tocidowski et al., 1997; Sado et al., 2013, 2014; Belo et al., 2014), without there being any significant differences between the seasons.

In both seasons, the number of neutrophils in the blood of tambacu was much lower than that reported previously for this hybrid in normal conditions (Tavares-Dias et al., 2007). The values were higher in the cold season, corroborating the findings of a study that reported neutrophilia in Nile tilapia during the colder months of the year (Jerônimo et al., 2011). Neutrophils are

responsible for innate immune responses, so an increase in the abundance of these cells during the cold season may be a means of preparing for possible infections during the colder periods of the year. In patinga, the number of neutrophils in the warm season was slightly lower than was previously reported for pacu (Sado et al., 2013, 2014; Jerônimo et al., 2014), but in the cold season it was similar to these findings. However, there was no significant difference between the seasons.

In tambacu, the number of monocytes was similar to that observed in this hybrid when parasitized by *Dolops carvalhoi* (Crustacea) and higher than that in non-parasitized fish in a previous study (Tavares-Dias et al., 2007). In patinga, the number of monocytes was within the range reported for pacu of similar weight (Jerônimo et al., 2014). For both hybrids, there was no significant difference in monocyte counts between the seasons, corroborating previous findings for the hybrid surubim (Jerônimo et al., 2015a).

The number of eosinophils in tambacu was lower than that previously reported for this hybrid (Tavares-Dias et al., 2007). Eosinophils are rare in the peripheral blood of freshwater and marine teleosts, being more abundant in the intestinal submucosa, peritoneal liquid, mesentery, and gills (Tavares-Dias et al., 2000). The tambacus studied presented increased numbers of eosinophils during the warm season, which could be explained by increased parasitism because these cells are known to perform innate immune responses against parasites (Reite and Evensen, 2006). The warm season was associated with lower water transparency, which indicates increases in the amount of organic matter in the water. In addition to higher temperatures, the proliferation of bacteria and parasites is favored in these conditions. This hypothesis of parasitism would coincide with the decreased numbers of erythrocytes and thrombocytes in tambacus during the warm season. The patingas presented values of eosinophil counts within the ranges previously reported for pacu and pirapitinga (Tocidowski et al., 1997; Sado et al., 2013, 2014; Belo et al., 2014). No significant difference in eosinophil counts between the seasons was observed, corroborating previous results for the hybrid surubim (Jerônimo et al., 2015a).

This was the first study to quantify the numbers of basophils in the blood of tambacu and patinga. In both hybrids, the counts found agreed with those reported for pacu of similar weight (Jerônimo et al., 2014). In tambacus, there was no significant difference in basophil numbers between the seasons, corroborating a previous study's result for the hybrid surubim farmed in the same region (Jerônimo et al., 2015a). The blood from patinga presented higher numbers of basophils in the cold season, coinciding with the increase in total leukocyte numbers at this time. The proliferation of phagocytic cells in the cold season may prevent eventual infections, although little is known about the mechanism of action of basophils in fish (Ranzani-Paiva et al., 2013).

The numbers of PAS-positive granular leukocytes (PAS-GL) in the blood of tambacu were lower than those previously reported for this hybrid (Tavares-Dias et al., 2007). The hybrid patinga presented values of PAS-LG within the range reported for pacu of similar weight (Jerônimo et al., 2014). For both hybrids, there was no difference in this parameter between the seasons. Changes



in the amounts of PAS-LG are related to stress, and may be caused by parasitism (Schalch et al., 2005). Therefore, the lack of seasonal differences in PAS-GL may indicate that there was a certain degree of stability in the health conditions of these fish throughout the year.

## CONCLUSIONS

This study was the first hematological assessment of the patinga hybrid, which was found to present similar values to its parental species, pacu (*P. mesopotamicus*) and pirapitinga (*P. brachypomus*). The present study's results demonstrated the influence of the season on the hematological parameters of the tambacu and patinga hybrids. Understanding the effects of seasonality and environmental factors on the health status of farmed fish is necessary to develop better strategies for controlling disease outbreaks.

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