

Haematological Parameters of the Hybrid Surubim (*Pseudoplatystoma reticulatum* x *P. corruscans*) farmed in Brazil

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ABSTRACT

This study evaluated the haematological parameters of the hybrid surubim (*Pseudoplatystoma corruscans* x *P. reticulatum*) farmed in intensive and semi intensive system. A total of 240 fish were examined, 120 from fish farm and 120 from a semi-intensive for comparison between the hot and cold season. The water quality was weekly measured to evaluate the influence of environmental conditions on the haematology. Fish from intensive system showed hematocrit and white blood cell count (WBC) higher in hot season while the total plasmatic protein, hemoglobin concentration, mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were higher in cold season. In fish from semi intensive system, the red blood cells count (RBC) and total thrombocyte number was higher in hot season while the MCHC and MCV were higher in cold season. There was no alterations in the numbers of lymphocytes, neutrophils, basophils, eosinophils and PAS – positive granular leukocyte (PAS-LG). The results showed the influence of seasonality on the haematological parameters of the hybrid surubim kept under normal farming conditions. Regarding water quality, hot season was directly related to increased water temperature and decreased dissolved oxygen while the opposite was found in cold season.

Key words: culture system, hematology, seasonality, Siluriformes

INTRODUCTION

Brazil currently ranks among the top countries with great growth potential in continental aquaculture. The intense capture of species of economic importance has resulted in decrease of inventory and consequent reduction of natural fish production, leading to increased interest in its cultivation (Crepaldi et al. 2006). Fish of the genus *Pseudoplatystoma* (Siluriformes) are of great importance, both in fishing and in aquaculture, with significant contribution in the fishing

economy of central Brazil (Campos 2005). In 2011, about 1.747 tons of *Pseudoplatystoma* spp. were grown in fish farms in Brazil, generating revenues of US\$ 3.825.140 (FAO 2013). *Pseudoplatystoma corruscans* Spix and Agassiz, 1829 (popularly known in Brazil as "pintado" or "surubim") and *P. reticulatum* Eigenmann and Eigenmann, 1889 (known as "cachara" or "surubim") are migratory carnivorous species, which in adulthood can reach up to 100 kg and 20 kg, respectively (Campos 2005). In farms, surubins reaches about 2 kg in just one year

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(Zaniboni Filho 2004). The strategy of crossing *P. corruscans* with *P. reticulatum* to produce hybrid fingerlings increased the survival in the hatchery over the cultivation of *P. corruscans* (Naldoni et al. 2009), favoring management practices (Ishikawa et al. 2012). The hybrid surubim shows excellent husbandry for cultivation, high market value, international market potential, good carcass yield, flavor and enjoyed the absence of spines (Labarrère 2012). However, to promote good management practices and optimize production, it is necessary to know their biological peculiarities (Ishikawa et al. 2012).

The intensive fish farming is responsible for constant pressure in the fish due to the stress caused by high stocking densities, transport, fluctuations in water quality parameters, inadequate nutrition and pressure by pathogens. Thus, the hematological parameters could be interesting tools to show the health condition of the fish (Labarrère et al. 2012). Studies on the hematology of surubim hybrids are still scarce. The morphological description and cytochemistry of leukocytes were reported by Beelen et al. (2003). Later, other studies reported high erythrocyte and total plasma proteins values of surubins hybrids cultured in the state of Mato Grosso do Sul (Tavares-Dias et al. 2009) and under different stocking densities (Labarrère et al. 2012). Some others studies reported hematological changes caused by the infection with *Pseudomonas* sp. (Tavares-Dias et al. 2009), dietary supplementation symbiotic (Mouriño et al. 2012), experimental infection with *Aeromonas*

hydrophila (Silva et al. 2012), and testing of various blood anticoagulants (Ishikawa et al. 2010). However, little is known about the blood profile of these animals on fish farms, especially in relation to leukocyte number, which is one of the main ways of defense of the immune system (Tavares-Dias et al. 2009). Considering the growing interest, in hybrid surubim farming, in Brazil and the lack of information that could serve as a reference to assess their health status, the objective of this study was to compare the hematological parameters of fish raised in feedlot and fishing ponds, in two seasons (hot and cold).

MATERIAL AND METHODS

A total of 240 surubins hybrids were captured by dragnet from an intensive system (n=120) and from a semi intensive pond (n=120) from June 2010 to May 2011 in the warm seasons (October to March) and cold (April to September) in State of Mato Grosso do Sul, Brazil. During both the seasons the physico-chemical characteristics of water from fish farms was monitored weekly from 08:00 to 09:00 am. The following parameters were measured: dissolved oxygen, temperature, pH and electrical conductivity with multiparameter HANNA®, transparency (with the aid of Secchi disk) and alkalinity (by titulation method). Samples of output pond water were collected for the determination of orthophosphate, total ammonia, nitrite and nitrate by colorimetric kit (Alfakit®, São Paulo, Brazil). The results are shown in Table 1.

Table 1 - Water quality of ponds measured during of seasons of intensive and semi-intensive fish farms.

Parameters	Intensive Farm		Semi Intensive Farm	
	Cold Season	Hot Season	Cold Season	Hot Season
Alcalinity (mg.L ⁻¹)	61.55 ± 5.09	63.29 ± 10.41	38.56 ± 4.12	39.67 ± 8.87
pH	7.25 ± 0.30	7.36 ± 0.50	7.13 ± 0.42	7.35 ± 0.54
Electrical conductivity (µS.cm ⁻¹)	72.36 ± 5.64	77.33 ± 3.58	25.91 ± 1.74	26.70 ± 3.80
Dissolved Oxygen (mg.L ⁻¹)	6.41 ± 1.04	3.12 ± 1.32	6.78 ± 0.81	4.35 ± 1.29
Temperature (°C)	18.90 ± 2.33	28.75 ± 0.80	18.97 ± 3.17	28.29 ± 0.89
Transparency (cm)	40.29 ± 18.31	25.08 ± 6.93	25.98 ± 4.10	19.79 ± 3.56
Ammonia (mg.L ⁻¹ NH ₃)	0.20 ± 0.10	0.32 ± 0.20	0.27 ± 0.07	0.13 ± 0.05
Total Iron (mg.L ⁻¹ Fe)	0.04 ± 0.09	0.13 ± 0.17	0.17 ± 0.17	0.14 ± 0.27
Nitrate (mg.L ⁻¹ NO ₃)	0.19 ± 0.19	0.15 ± 0.07	0.19 ± 0.13	0.29 ± 0.16
Nitrite (mg.L ⁻¹ NO ₂)	0.03 ± 0.02	0.02 ± 0.04	0.08 ± 0.13	0.03 ± 0.08
Orthophosphate (mg.L ⁻¹ PO ₄)	0.00 ± 0.00	0.49 ± 0.41	0.23 ± 0.36	0.09 ± 0.26

After capture, the animals were anesthetized with clove oil (75 mg L⁻¹) for blood collection and its

analysis (Ishikawa et al. 2011) (Ethics Committee 29979/2009-05/CEUA/UFSC 23080.0). Blood was

collected by puncture of the caudal vessel with syringes containing EDTA 3%, packed, cooled and transported to the laboratory to measure the percentage of hematocrit by the microhematocrit method (Goldenfarb et al. 1971); concentration hemoglobin by the cyanometahemoglobin (Collier 1944), erythrocyte count (RBC) in a Neubauer chamber after dilution 1:200 in formalin-citrate, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) (Wintrobe 1934) and total plasma proteins with portable refractometer. The total numbers of leukocytes (WBC) and thrombocytes were obtained by indirect method from extensions stained with May-Grünwald-Giemsa-Wright (MGGW) (Ishikawa et al. 2008).

Biometric and haematological parameters data were checked for normality, using Kolmogorov-Smirnov test, and homoscedasticity, using Levene's test and were subjected to the analysis of variance (ANOVA), comparing between the seasons and properties upon satisfying the requirements. Data transformations were used according to pertinence and Tukey's test was used

to compare the means at a significance level of 5%. The water quality data were subjected to principal components analysis (PCA) in order to verify the relationship of the variables with the different seasons.

RESULTS AND DISCUSSION

In principal component analysis conducted with the data from the fishing ponds of semi-intensive system (Fig. 1), the joint representation of the two main factors accounted for 62% of the variance. A higher correlation between the cold season and high levels of transparency, orthophosphate and dissolved oxygen was observed, whereas the hot season was more strongly correlated with higher levels of nitrites, nitrates and temperature. These relationships could be due to lower primary productivity in the ponds during the cold season, associated with higher values of ammonia due to lower capacity of nutrient cycling, followed by increased nitrification as productivity increased in the hot season.

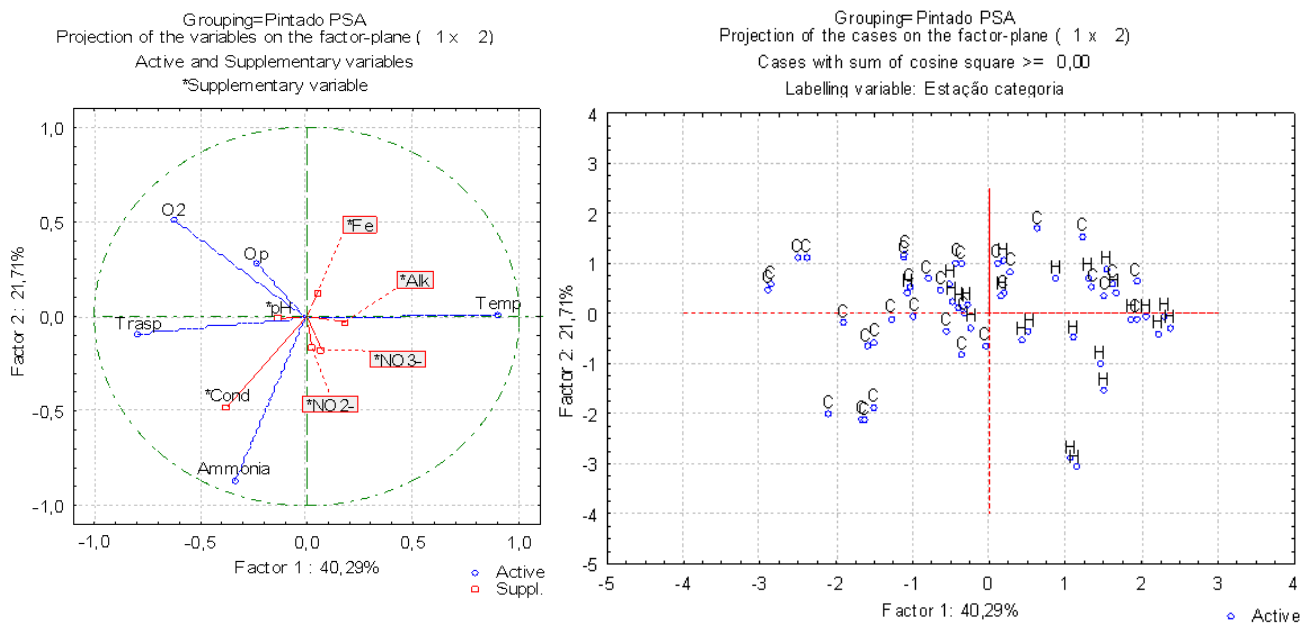


Figure 1 - Principal Components Analysis (PCA) of water quality parameters from semi-intensive culture of hybrid surubim. O₂= Dissolved Oxygen; Op= Orthophosphate; Trasp= Transparency; Cond= Electrical Conductivity; Temp= Temperature; Alk= Alkalinity; Fe= Iron; C = Cold season; H = Hot season.

In the PCA from the intensive system, the joint representation of the two main factors accounted for approximately 68% of the variance. There was diffuse relationships between the seasons and

water quality parameters. However, higher conductivity values were more related with the cold season (Figure 2).

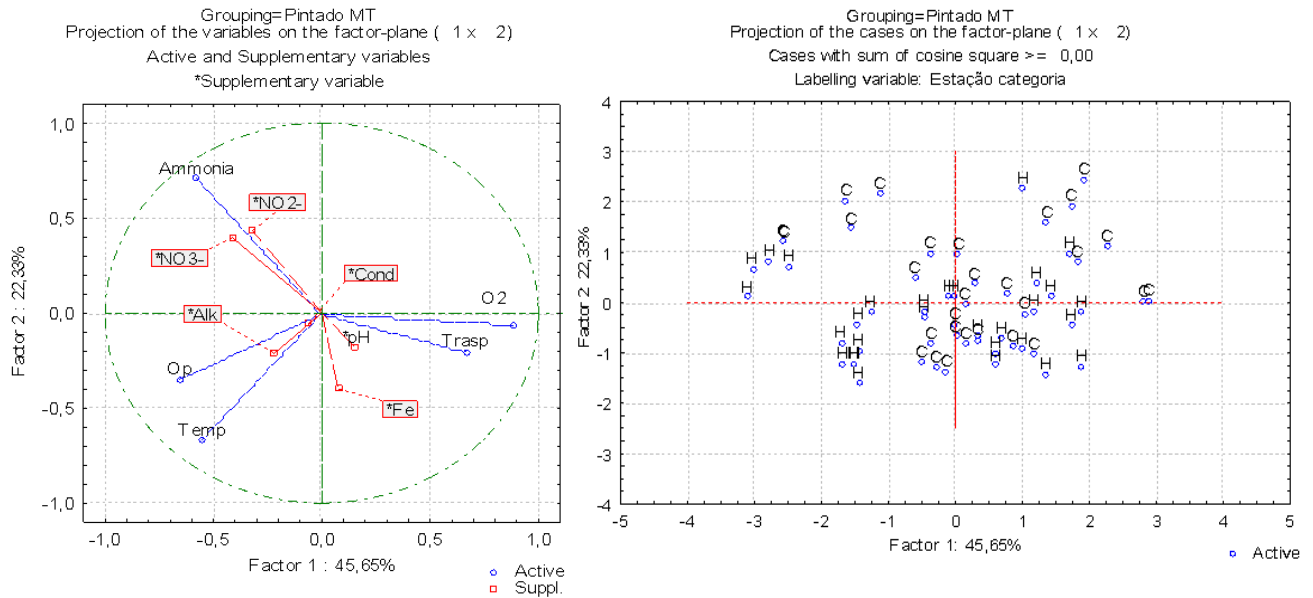


Figure 2 - Principal Components Analysis (PCA) of water quality parameters from intensive culture of hybrid surubim. O₂= Dissolved Oxygen; Op= Orthophosphate; Trasp= Transparency; Cond= Eletrical Conductivity; Temp= Temperature; Alk= Alkalinity; Fe = Iron; C = Cold season; H = Hot season.

Hematology has been widely used as an important tool for monitoring the health status of fish farming, allowing inferences about their healthiness conditions (Ishikawa et al. 2010). Hematological parameters are likely to change according to the changes of the aquatic environment; they can help to understand the process of adaptation of animals to their environment (Ranzani-Paiva et al. 2005) and provide references to identify any changes in the condition fish health (Ranzani-Paiva et al. 1997). This type of monitoring is important in aquaculture, given that the conditions imposed by the cultivation affect the basic physiological functions of farmed fish due to submission to stressors (Tavares-Dias and Moraes 2007).

The body weight of the animals showed a significant difference ($p < 0.05$) between the seasons and was higher in the cold season than in warm, depending on the actual fattening period. However, there was no significant change in length (Table 2). Fish from the intensive system had higher mean hematocrit in the cold season, however, total plasma protein, hemoglobin, MCV and MCHC. WBC values were higher in the hot season, but there were no significant differences in the numbers of RBC, thrombocytes, lymphocytes, neutrophils, monocytes, eosinophils, basophils and

PAS-LG between the seasons. In the animals from the semi-intensive pond, the cold season was also followed by a significant increase in hemoglobin, MCHC and MCV ($p < 0.05$).

Hematocrit values in this study were within the range of values reported by Tavares-Dias et al. (2009); however, these were higher when compared to other studies of hybrid surubim (Beelen et al. 2003; Ishikawa et al. 2010; Mouriño et al. 2012). There was also an increase during the cold season in fish intensive system, with values significantly higher than those animals from the semi-intensive ponds in the same season as shown by significantly higher amounts of erythrocytes. Since the values of erythrocytes were similar to those reported in the literature for this hybrid, the hematocrit was high due to the high MCV of erythrocytes.

In the hot season, the numbers of RBC and thrombocytes were higher, but no significant seasonal influence was observed on hematocrit, total plasma protein, WBC, lymphocytes, neutrophils, monocytes, eosinophils, basophils and PAS-LG (Table 2).

Comparing the farming systems within the same season, the total plasma protein values were higher in the semi-intensive culture in comparison to intensive fish farming, both in cold as in hot

seasons. In the cold season, animals from the semi-intensive culture also had higher levels of hemoglobin, MCHC and PAS-LG and lower hematocrit, erythrocytes, leucocytes and thrombocytes. In the hot season, the total numbers of leukocytes and thrombocytes were higher in the animals of intensive fish farming. In the hot

season, there were no differences in hematocrit, hemoglobin, erythrocyte, MCHC, MCV, lymphocytes, neutrophils, monocytes, eosinophils, basophils and PAS-LG, while in cold season, the animals of both the systems showed similar values of MCV, lymphocytes, neutrophils, monocytes, eosinophils and basophils (Table 2).

Table 2 - Biometry and hematological parameters of hybrid surubim farmed in Brazil. PPT: total plasma proteins, MCHC: mean corpuscular hemoglobin concentration, MCV: mean corpuscular mean, PAS-LG: granular leukocyte PAS-positive.

Variables	Intensive Farm		Semi Intensive Farm	
	Hot Season	Cold Season	Hot Season	Cold Season
Weight (g)	565.83±158.50 ^b	637.77± 114.80 ^a	939.38±383.48 ^b	1203.73±409.20 ^a
Lenght (cm)	42.23 ± 3.34	42.35 ± 6.12	48.93 ± 5.35	49.97 ± 5.35
PPT (g.dL ⁻¹)	4.68 ± 0.39 ^{ab}	5.41 ± 0.64 ^{ab}	6.19 ± 1.30 ^A	6.35 ± 0.65 ^A
Hematocrit (%)	34.83 ± 3.00 ^b	37.09 ± 4.19 ^{aA}	33.05 ± 7.30	32.93 ± 6.82 ^B
Hemoglobin (g.dL ⁻¹)	3.14 ± 0.72 ^b	7.35 ± 3.65 ^{ab}	3.74 ± 0.93 ^b	8.65 ± 3.31 ^{aA}
Erythrocyte (x10 ⁶ .µL ⁻¹)	2.23 ± 2.04	1.96 ± 0.51 ^B	1.92 ± 0.36 ^a	1.57 ± 0.26 ^{bA}
MCHC (g.dL ⁻¹)	9.04 ± 2.06 ^b	20.03 ± 10.00 ^{aA}	1.72 ± 3.46 ^b	28.97 ± 17.26 ^{ab}
MCV (fL)	179.84 ± 41.61 ^b	202.76 ± 59.68 ^a	178.57 ± 53.24 ^b	215.89 ± 65.00 ^a
Total Leukocyte (x10 ³ .µL ⁻¹)	71.83 ± 31.85 ^{aA}	61.43 ± 18.69 ^{bA}	52.59 ± 25.46 ^B	46.39 ± 16.83 ^B
Thrombocyte (x10 ³ .µL ⁻¹)	43.10 ± 26.61 ^A	42.99 ± 20.47 ^A	29.81 ± 21.33 ^{ab}	21.90 ± 15.86 ^{bb}
Lymphocyte (x10 ³ .µL ⁻¹)	64.17 ± 27.65	54.20 ± 17.16	46.58 ± 23.74	40.68 ± 15.93
Neutrophil (x10 ³ .µL ⁻¹)	4.58 ± 3.86	4.86 ± 3.12	3.41 ± 2.26	3.21 ± 3.00
Monocyte (x10 ³ .µL ⁻¹)	0.78 ± 0.88	0.84 ± 1.00	0.30 ± 0.51	0.32 ± 0.46
Eosinophil (x10 ³ .µL ⁻¹)	0.80 ± 1.00	0.42 ± 0.54	0.55 ± 0.61	0.30 ± 0.38
Basophil (x10 ³ .µL ⁻¹)	0.86 ± 1.38	0.76 ± 0.85	0.70 ± 0.74	0.41 ± 0.64
PAS-LG (x10 ³ .µL ⁻¹)	0.64 ± 0.98	0.39 ± 0.61 ^A	0.97 ± 1.58	0.76 ± 0.77 ^B

*Lowercase letters indicate significant differences (p <0.05) between stations. capital letters indicate significant differences between sites within the same season.

In the cold season, the average hemoglobin concentrations were similar to those reported by Labarrère et al. (2012) for the surubim hybrid in different densities of stock. Fish from both the systems showed a reduction in hemoglobin concentration in the hot season. Santos et al. (2012) observed an increase in hemoglobin in the summer. During this season, the availability of dissolved oxygen in the water was lower and the metabolic activity of the fish increases. The MCHC showed hemoglobin values similar to Labarrère et al. (2012) and Dias-Tavares et al. (2009) in the cold season; during the warm season, there was a significant reduction in both local cultivation. Lower CHCM values are indicative of anemia (Labarrère 2012), which may also indicate a higher proportion of young erythrocytes, since they do not possess adequate amount of hemoglobin (Tavares-Dias et al. 2009). This may be the case due to concomitant low MCV and elevated number of erythrocytes. During the cold season, animals from the semi-intensive ponds showed higher values of hemoglobin and MCHC

compared to fish from the intensive system, which indicated reduced dissolved oxygen in the water. The red blood cell MCV was higher than the values reported by Mourinho et al. (2012), but was within the recorded by Tavares et al. (2009) for hybrid surubim. MCV was reduced in the warm season in both the places. In the case of semi-intensive culture, this change was accompanied by an increase in the number of erythrocytes. Therefore, the lower values of MCV were due to the higher amount of immature red blood cells (Affonso et al. 2002), recruited to optimize the oxygen transport, as the hot season was usually related to lower concentrations of dissolved oxygen in the water. The erythrocyte volume is also related to activity level (Glazova 1976, Wintrobe 1934), being higher when the animal is more sedentary. Thus, the increase in MCV in the cold season could be strongly associated with a decrease in metabolic activity and locomotion of animals, which reduced the oxygen demand. The total plasma protein concentrations were similar to those observed by Ishikawa et al. (2010)

and Dias-Tavares et al. (2009) in hybrid surubim. Plasma proteins are responsible for vital functions such as carrying of metabolites, humoral defense and coagulation (Tavares-Dias et al. 2009). In the animals from the intensive system, a significant increase of this parameter occurred in the cold season, while in the semi-intensive ponds, it remained unchanged. Similar to that observed in fish from the semi intensive ponds, De Pedro et al. (2005) and Swain et al. (2007) observed no seasonal change in this parameter. The increase that occurred during the cold season was still within the variation described by Tavares et al. (2009). Total protein was altered primarily by the changes in plasma volume, with increases normally related to loss of plasma fluid into the intracellular compartment. This occurs by osmotic imbalance between the intracellular and extracellular compartments, thus any stress that induces such an imbalance can lead to increased plasma protein (Milligan and McDonald 1992). Previous studies have shown that stress increases the amount of total serum proteins (Milligan and Wood 1986; Milligan and McDonald 1992; Melo et al. 2009). In this study, the cold season was associated with increased concentrations of ammonia, which could be an additional stress factor, associated with the high density and temperature reduction. Regarding the system of cultivation, animals from semi-intensive systems ponds showed higher total plasma protein in both seasons. Hrubec et al. (2000) observed higher values in fish from intensive cultivation compared with fish raised at low density.

The values of erythrocytes were similar to those reported in the literature for this hybrid (Tavares-Dias et al. 2009; Mouriño et al. 2012). In semi-intensive ponds, higher values were observed during the warm season, as also reported by Jerônimo et al. (2011), De Pedro et al. (2005) and Guijarro et al. (2003). Given that this season was related to lower concentrations of dissolved oxygen in the water, it could be stated that the fish increased red blood cell production to improve oxygen transport capacity, compensating for the reduced availability (Jerônimo et al. 2011). During the cold season, the animals of the intensive system ponds had higher erythrocyte, therefore, it was possible that in this system the concentrations of dissolved oxygen were lower, also demonstrating a compensatory effect.

The WBC count and thrombocytes number in blood may be indicative of the health status of fish

(Tavares-Dias and Moraes 2007). In the fishes from semi-intensive ponds, it was similar to that reported by Mouriño et al. (2012) and Silva et al. (2012) for hybrid surubim, while in the intensive system, there was a tendency to higher values in both the seasons. This increase could be related to stress (Martins et al. 2004) due to higher densities and more intense management. The animals in intensive system showed an increase in the number of leukocytes in the hot season, supporting the studies of Morgan et al. (2008), De Pedro et al. (2005) and Collazos et al. (1998).

The number of thrombocytes in fish from the semi-intensive ponds was similar to that reported for the hybrid surubim by Mouriño et al. (2012) and Silva et al. (2012), but in the intensive system, higher values were similar to those reported by Tavares-Dias et al. (2009), which showed no seasonal variation, while fish from the semi-intensive ponds had more thrombocytes in the hot season. Thrombocytes participate in blood coagulation and may also possess defense function (Martins et al. 2008). Seasonal variation found in the WBC and thrombocytes number supported Schreck and Slater (1998) hypothesis that the immunological behavior usually consisted of increase in the summer and decrease in winter. According to Engelsma et al. (2003), lower temperature affects both the quantity and function of immune cells.

Regarding the differential leukocyte count, none of the mentioned parameters showed a significant difference between seasons, a result also reported by Swain et al. (2007). The numbers of lymphocytes were similar to those reported by Mouriño et al. (2012) in hybrid surubins that received dietary supplementation with prebiotic and/or probiotic, and higher than those observed by Tavares-Dias et al. (2009) in hybrid surubins cultured in Mato Grosso do Sul. The numbers of neutrophils were higher than those reported by Mouriño et al. (2012), slightly higher than those found by Tavares-Dias et al. (2009) and similar to the findings of Mouriño et al. (2012), where hybrid surubins were supplemented with probiotics and challenged with or without *Aeromonas hydrophila*. The values of monocytes were below those reported by Tavares-Dias et al. (2009), and in the intensive system, were similar to those observed by Mouriño et al. (2012), but fish from the semi-intensive ponds showed lower monocytes values than those observed by Tavares-Dias et al. (2009) and Mouriño et al. (2012). The

higher number of monocytes found in fish from the intensive system could be associated with higher stocking density.

In relation to eosinophils, the average values were similar to those reported by Tavares-Dias et al. (2009) and Mouriño et al. (2012) for this hybrid. Basophils appeared similar in the quantity to those observed by Mouriño et al. (2012), but dramatically higher than those reported by Tavares-Dias et al. (2009). Regarding PAS-LG, quantities were similar to those reported by Mouriño et al. (2012), being higher in the free-fishing ponds than in intensive system during the cold season. According to Schalch et al. (2005), changes in the amounts of PAS-LG are related to stress and may be caused by parasitism. Similar was observed by Ranzani-Paiva et al. (1987) on the high-frequency of this cell in carps parasitized by *Argulus* sp. It was possible that the reduction in the efficiency of the immune system was associated with the cold season, which made the fish more susceptible to parasitism.

CONCLUSION

The present results demonstrated the influence of the seasons on the hematological values of hybrid surubim kept in intensive and semi-intensive ponds of fish farm. The reduction in the number of WBC along with the increase in MCHC, MCV, hematocrit and total plasma protein in intensive culture system during the cold season could be related to the need to improve the body's defenses during this season. The increase in hemoglobin concentration, MCHC, MCV and a reduction in the number of thrombocytes animals from semi intensive ponds, could be related to system maintenance.

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