

ORIGINAL ARTICLE

Influence of seasonality on the haematological and biochemical parameters of native species *Rhamdia quelen*

Influência da sazonalidade sobre os parâmetros hematológicos e bioquímicos da espécie nativa Rhamdia quelen

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Abstract

The objective of this study was to evaluate if there is any influence of the seasons on the homeostasis of silver catfish (*Rhamdia quelen*) through haematological and serum biochemistry evaluations. During one year, blood samples were collected from groups of 40 silver catfish in each season (spring, summer, autumn, and winter), totaling 160 animals. Blood samples were collected individually and used for haematological and serum biochemistry evaluations. In general, the main haematological findings were observed in the summer and in the spring, with hemoglobin levels ($6.32 \text{ g.dL}^{-1} \pm 0.20$), total erythrocyte counts ($1.62 \times 10^6 \cdot \mu\text{L}^{-1} \pm 0.05$) and neutrophil counts ($14.21 \times 10^3 \cdot \mu\text{L}^{-1} \pm 0.60$) higher in the summer than in the other seasons ($p < 0.05$), whereas in the spring the total leukocyte count ($25.89 \times 10^3 \cdot \mu\text{L}^{-1} \pm 1.02$) and the number of eosinophils ($9.08 \times 10^3 \cdot \mu\text{L}^{-1} \pm 0.11$) were higher when compared to the other seasons ($p < 0.05$). The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyltransferase (GGT), and albumin

levels were significantly altered between all seasons, but remained within the normal intervals for the species. Seasonality significantly influences haematological parameters and biochemical parameters of *Rhamdia quelen*, but without a clear determination of the types of change in each season.

Keywords: Fish. Hematology. Homeostasis. Catfish. Metabolism.

Resumo

O objetivo deste estudo foi avaliar se há alguma influência das estações do ano sobre a homeostasia do jundiá (Rhamdia quelen) através de avaliações hematológicas e de bioquímica sérica. Durante o período de um ano foram realizadas coletas de sangue de grupos de 40 jundiás em cada uma das estações do ano (primavera, verão, outono e inverno), totalizando 160 animais. As amostras de sangue

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foram coletadas de forma individual e utilizadas para avaliações hematológicas e de bioquímica sérica. De forma geral, os principais achados nas análises hematológicas ocorreram no verão e na primavera, sendo que no verão os níveis de hemoglobina ($6,32 \text{ g.dL}^{-1} \pm 0,20$), a contagem total de eritrócitos ($1,62 \times 10^6 \cdot \mu\text{L}^{-1} \pm 0,05$) e o número de neutrófilos ($14,21 \times 10^3 \cdot \mu\text{L}^{-1} \pm 0,60$) foram maiores em relação às outras estações ($p < 0,05$), enquanto que na primavera a contagem total de leucócitos ($25,89 \times 10^3 \cdot \mu\text{L}^{-1} \pm 1,02$) e o número de eosinófilos ($9,08 \times 10^3 \cdot \mu\text{L}^{-1} \pm 0,11$) foram maiores quando comparados às demais estações ($p < 0,05$). Em relação às análises bioquímicas, os níveis de alanina aminotransferase (ALT), aspartato aminotransferase (AST), fosfatase alcalina (FA), gama glutamiltransferase (GGT) e de albumina se alteraram significativamente entre todas as estações do ano, porém permaneceram dentro dos intervalos normais para a espécie. A sazonalidade influencia significativamente parâmetros hematológicos e parâmetros bioquímicos do *Rhamdia quelen*, porém sem uma determinação clara dos tipos de mudança em cada estação.

Palavras-chave: Peixes. Hematologia. Homeostase. Jundiá. Metabolismo.

Introduction

Due to the lack of haematological and biochemical reference values for the different species of fish, and consequent difficulty in interpreting the data, haematological analysis are often not used in the practice of commercial aquaculture (Clauss et al., 2008). This practice could be reversed if there were more reference values, since they are useful analyses to evaluate the animals' health.

However, even with the facts highlighted above, haematological evaluations may be useful when monitoring the health status of fish, whereas the factors that may influence cell counts are taken into consideration. Because of that, caution should be exercised when comparing results obtained with values found in scientific papers, since the factors influencing the results may or may not be present in these cases (Clauss et al., 2008).

The different biochemical and cellular constituents of the blood are important in the physiological and pathophysiological evaluation

of the animals due to the various alterations induced by different stressors, like physiological or environmental factors (Ranzani-Paiva, 2007). These external influences may occur for example through variations of temperature, salinity, pH, dissolved oxygen and luminosity (Tavares-Dias and Moares, 2004; Valenzuela et al., 2007; Bowden, 2008).

Water temperature, for example, has an extreme importance, since fish are ectothermic and their biological functions are directly affected by variations in ambient temperature (Jerônimo et al., 2011). Temperature oscillation also influences dissolved oxygen levels, that can act as a limiting factor in life along with other factors inherent to the environment (Bowden, 2008).

Another characteristic of the ectothermic organisms is that the physiological parameters can be adjusted to compensate some changes in temperature. In fish, temperature acclimatization is determined by changes in the metabolic rate (Baldisserotto, 2002; Souza et al., 2005). The increase in water temperature leads to a rise in biological and metabolic activities of the fish, increasing heart and respiratory rates as a result of the oxygen demand (Baldisserotto, 2002).

The species of fish utilized in this study was Silver catfish (*Rhamdia quelen*), a native fish of the Siluriformes order endemic from the south of Mexico to the center of Argentina, widely distributed in all Brazilian regions (Baldisserotto and Neto, 2004). This species has good rusticity, mainly to large fluctuations in temperature and dissolved oxygen (Gomes et al., 2000).

Since fish are ectothermic, the temperature variations that occur between the seasons and influence the physiology of the animals may represent a determinant factor in the possible oscillations of the haematological and biochemical parameters over a year, thus demonstrating the importance and significance of studies that improve the understanding of these physiological changes in order to facilitate laboratory evaluations in both commercial and research creations.

The silver catfish was chosen because there are few references that demonstrate the normal biochemical and haematological values for the species. Besides, the species has a moderate importance in the south of Brazil.

The aim of this study was to evaluate if the seasons of the year could influence the haematological and serum biochemical parameters of *Rhamdia quelen* cultivated in earthen ponds from the Fisheries Research Laboratory.

Material and methods

This project was approved by the Animal Ethics Committee (AEC) of the Pontificia Universidade Católica do Paraná (PUCPR) under number 641 - 2nd version. The experimental design was completely randomized, longitudinal and prospective.

Animals

The fish were captured from the same culture pond (20 m width x 80 m length x 1.50 m depth), with population density of 4 fish.m³ of water sheet, located at the Fisheries Research Laboratory (LAPEP, Laboratório de Pesquisa em Piscicultura) of PUCPR. It lies between the geographical coordinates: latitude 25°35'02.8" S and longitude 49°13'18.2" W, where the fish were raised.

In each season of the year 2016, 40 juveniles of silver catfish (*Rhamdia quelen*) were used for all the measurements (weight, total length, haematological and serum biochemical parameters), amounting a total of 160 animals from the same lot, with 150 ± 35 days of age at the beginning of the experiment. The samples were collected in the middle of each season with mean interval of 91 days (summer: 01.27.2016; autumn: 04.27.2016; winter: 07.27.2016; spring: 10.26.2016), starting on summer.

During the experimental period, fish were fed twice a day, (9 am and 5 pm) until complete satiety, with a commercial feed (Supra[®] Acqua Line), according to Table 1.

At the time of capture, these parameters were measured of the rearing pond: dissolved oxygen (DO - mg.L⁻¹), salinity (ppm), temperature (°C), water pH, toxic ammonia (NH₃ - ppm) and nitrite (NO₂ - ppm), with the aid of an oximeter (YSI[®], model 85) and a digital pH meter (YSI[®], model pH 100). Toxic ammonia and nitrite were monitored with the aid of a colorimetric kit (Alcon[®] - LabconTest). Initially, the value of total ammonia was measured, then with

the combination of pH and temperature of the water, the value of toxic ammonia was found in a reference table that accompanied the colorimetric kit.

These variables were conducted once during each season (spring, summer, autumn and winter).

Table 1 - Composition of the commercial feed

Nutritional levels	% Dry matter
Crude protein	28.00
Crude fat	5.00
Ash	12.00
Crude fiber	12.00
Calcium (min)	1.20
Calcium (max)	2.50
Phosphorus	0.90
Digestible energy, kcal.kg ⁻¹	3,100.00

Haematological evaluation

After biometry and anesthesia with benzocaine solution 2% (Sigma Chemical Co., USA), blood was collected by puncturing of the caudal vein using syringes containing 3% EDTA. After the blood collection, the animals underwent euthanasia.

The analyses procedures were performed immediately after the blood sample collections. Total counts of erythrocytes, leukocytes and thrombocytes were performed manually in Neubauer's chamber after 1:200 dilution of blood in Natt/Herrick's (1952) dye. The differential count leukocyte was performed by stained blood smears through the Rosenfeld method (1947). The hematocrit level (%) was determined using capillary tubes in a micro-hematocrit centrifuge (Sislab[®]/MH) operated at 11.000 rpm for 5 minutes.

The hemoglobin level (g.dL⁻¹) was determined by spectrophotometry using the cyanomethemoglobin reaction after sample centrifugation at 3.500 rpm for 5 minutes (Fanem[®] - Excelsa Baby II - mod. 206-R) (Larsen and Snieszko, 1961). An aliquot of the sample was centrifuged at 4.000 rpm for 10 minutes to be used for total plasmatic protein (g.dL⁻¹), and evaluated through a refractometer (Kernco[®] - OS1270).

Serum biochemical evaluation

For the serum biochemical analysis, an aliquot of blood from each animal was packed in Eppendorf® tubes without anticoagulant for precipitation of the serum. The levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) and albumin were obtained using commercial kits (Labtest®; Lagoa Santa, Brasil) by spectrophotometry (Drake - mod. Quick lab/Siel-mod. EPECTROMATIC 710®).

Statistical analysis

Statistical analysis of the parametric data was performed using one-way ANOVA followed by Bonferroni test. The non-parametric data was analyzed using Kruskal-Wallis test followed by Dunn's test. The normality test was Kolmogorov-Smirnov. The significance level considered was 5% (Petrie and Watson, 2009). All data were described in mean and

standard error and all calculations were performed using GraphPad Prism Statistical software, version 5.00 for Windows, San Diego, CA, USA.

Results

The results of water quality parameters such as temperature, DO, pH, salinity, toxic ammonia and nitrite measured in the culture ponds are described in Table 2. All these variables were measured only at the time of collection, in each season of the year; therefore the data will not be presented in averages and standard deviations.

Mean weight (g) and total length (cm) ± standard error for the silver catfish during the seasons were, respectively: summer - 86.3 ± 1.1 g and 20.9 ± 0.1 cm; autumn - 200 ± 5.4 g and 25.3 ± 0.1 cm; winter - 60.8 ± 1.3 g and 16.8 ± 0.1 cm; spring - 116 ± 3.2 g and 21.6 ± 0.1 cm. There was no significant difference ($p > 0.05$) in mean weight and total length in the different seasons of the year.

Table 2 - Values of water quality parameters in each season of 2016

Parameters	Seasons			
	Summer	Autumn	Winter	Spring
Temperature (°C)	23.40	19.40	14.00	22.40
Dissolved oxygen (mg.L ⁻¹)	6.39	6.06	11.87	9.83
pH	8.70	8.70	8.70	8.60
Salinity (ppm)	0.12	0.10	0.09	0.19
Toxic ammonia (ppm)	0.00	0.00	0.00	0.00
Nitrite (ppm)	0.18	0.18	0.19	0.19

Hematological parameters

The highest red blood count (RBC) occurred in the summer and the lowest in spring. The level of hemoglobin (Hb) was significantly lower ($p < 0.05$) in winter and in autumn.

The highest ($p < 0.05$) hematocrit level (Ht) occurred during autumn in contrast to spring, summer and winter. Mean corpuscular volume (MCV) was significantly lower ($p < 0.05$) in summer compared to the other seasons.

The mean corpuscular hemoglobin (MCH) was significantly higher ($p < 0.05$) in spring compared to all the other seasons of the year. The mean corpuscular hemoglobin concentration (MCHC) was significantly higher ($p < 0.05$) in spring and summer.

The total plasmatic protein level was significantly lower ($p < 0.05$) in winter. The erythrogram results are described in Table 3.

The highest mean value for the white blood count (WBC) occurred in spring and the lowest in autumn. The number of neutrophils was significantly higher

($p < 0.05$) in summer and spring compared to autumn and winter. Eosinophils were significantly higher in the spring, and significantly lower ($p < 0.05$) in autumn compared to winter.

Lymphocytes were significantly higher ($p < 0.05$) in the winter and spring, and the lowest count was

in autumn. Monocytes were significantly lower ($p < 0.05$) in the autumn compared to other seasons. The total thrombocyte count (TTC) in summer and winter were higher ($p < 0.05$) compared to spring and autumn. The leukogram results are described in Table 4.

Table 3 - Values (mean \pm standard error) of erythrograms of silver catfish (*Rhamdia quelen*) at different seasons of the year

Parameters	Seasons				Reference values ¹
	Summer	Autumn	Winter	Spring	
RBC ($\times 10^6 \cdot \mu\text{L}^{-1}$)	1.62 \pm 0.05 ^a	1.38 \pm 0.05 ^b	1.26 \pm 0.04 ^b	1.21 \pm 0.04 ^b	2.20 - 4.10
Hb (g.dL ⁻¹)	6.32 \pm 0.20 ^a	5.47 \pm 0.15 ^{bc}	4.96 \pm 0.15 ^c	5.85 \pm 0.13 ^{ab}	7.50 - 9.10
Ht (%)	24.54 \pm 1.62 ^b	35.33 \pm 1.12 ^a	29.13 \pm 0.82 ^b	25.05 \pm 1.71 ^b	37.00 - 51.00
MCV (fL)	15.15 \pm 1.00 ^b	26.30 \pm 1.06 ^a	24.53 \pm 1.31 ^a	21.58 \pm 1.72 ^a	104.30 - 156.80
MCH (pg)	3.95 \pm 0.11 ^b	4.07 \pm 0.13 ^b	4.14 \pm 0.21 ^b	5.05 \pm 0.22 ^a	22.60 - 33.50
MCHC (%)	33.45 \pm 3.93 ^a	16.13 \pm 0.80 ^b	17.77 \pm 0.94 ^b	34.04 \pm 4.92 ^a	16.90 - 23.60
TPP (g.dL ⁻¹)	6.05 \pm 0.37 ^a	6.50 \pm 0.14 ^a	4.96 \pm 0.11 ^b	6.36 \pm 0.33 ^a	-

Note: Different letters in the same line indicate significant difference between the means ($p < 0.05$) and same letters indicate no significant difference ($p > 0.05$). ¹ Reference values extracted from Borges et al. (2004). RBC: red blood cells; Hb: hemoglobin; Ht: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; TPP: total plasmatic protein.

Table 4 - Values (mean \pm standard error) of leukograms of silver catfish (*Rhamdia quelen*) at different seasons of the year

Parameters	Seasons				Reference values ¹
	Summer	Autumn	Winter	Spring	
WBC ($\times 10^3 \cdot \mu\text{L}^{-1}$)	20.33 \pm 0.88 ^b	10.64 \pm 0.66 ^c	21.51 \pm 1.01 ^b	25.89 \pm 1.02 ^a	70.10 - 111.80
Neutrophils ($\times 10^3 \cdot \mu\text{L}^{-1}$)	14.21 \pm 0.60 ^a	7.47 \pm 0.49 ^c	10.76 \pm 0.60 ^b	15.26 \pm 0.68 ^a	0.00 - 8.40
Eosinophils ($\times 10^3 \cdot \mu\text{L}^{-1}$)	5.56 \pm 0.09 ^b	1.65 \pm 0.02 ^c	4.38 \pm 0.05 ^b	9.08 \pm 0.11 ^a	-
Lymphocytes ($\times 10^3 \cdot \mu\text{L}^{-1}$)	3.23 \pm 0.25 ^b	1.65 \pm 0.15 ^c	7.48 \pm 0.49 ^a	5.81 \pm 0.36 ^a	18.30 - 115.90
Monocytes ($\times 10^3 \cdot \mu\text{L}^{-1}$)	2.59 \pm 0.33 ^b	1.37 \pm 0.13 ^c	2.83 \pm 1.73 ^{ab}	3.98 \pm 0.30 ^a	5.80 - 27.50
Thrombocytes ($\times 10^3 \cdot \mu\text{L}^{-1}$)	14.71 \pm 0.75 ^a	8.21 \pm 0.47 ^b	15.30 \pm 0.86 ^a	10.56 \pm 0.95 ^b	1.30 - 64.10

Note: Different letters in the same line indicate significant difference between the means ($p < 0.05$) and same letters indicate no significant difference ($p > 0.05$). ¹ Reference values extracted from Borges et al. (2004). WBC: white blood cells.

Serum biochemical parameters

ALT was significantly increased in the summer and autumn. The highest mean value of AST was observed in the winter and a significant decrease in ALP and albumin levels was observed in winter

when compared to the other seasons. GGT levels in autumn were significantly lower in contrast to other seasons.

The results of the biochemical parameters are available in Table 5.

Table 5 - Values (mean \pm standard error) of the biochemical parameters of silver catfish (*Rhamdia quelen*) at different seasons of the year

Parameters	Seasons				Reference values ¹
	Summer	Autumn	Winter	Spring	
ALT (U.L. ⁻¹)	32.26 \pm 5.05 ^a	16.89 \pm 1.21 ^{ab}	16.31 \pm 2.43 ^b	15.42 \pm 1.60 ^b	21.00 - 68.00
AST (U.L. ⁻¹)	104.20 \pm 18.16 ^b	85.70 \pm 6.99 ^b	136.70 \pm 10.98 ^a	112.90 \pm 14.66 ^{ab}	64.00 - 215.00
ALP (U.L. ⁻¹)	27.03 \pm 2.80 ^a	24.47 \pm 2.31 ^a	5.60 \pm 0.73 ^b	24.30 \pm 1.71 ^a	61.00 - 155.00
GGT (U.L. ⁻¹)	10.97 \pm 0.89 ^a	1.58 \pm 0.14 ^c	3.04 \pm 0.17 ^b	6.89 \pm 0.65 ^a	-
Albumin (g.dL ⁻¹)	0.94 \pm 0.03 ^a	0.85 \pm 0.02 ^a	0.75 \pm 0.01 ^b	0.87 \pm 0.02 ^a	1.60 - 2.20

Note: Different letters in the same line indicate significant difference between the means ($p < 0.05$) and same letters indicate no significant difference ($p > 0.05$). ¹Reference values extracted from Borges et al. (2004). ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase.

Discussion

The greater number of RBC and Hb level in summer compared with the colder seasons may be explained by the higher temperature of water. Similar results were described in another species like tench (*Tinca tinca*) (Collazos et al., 1998; De Pedro et al., 2005), Nile tilapia (*Oreochromis niloticus*) (Azevedo et al., 2006; Jerônimo et al., 2011), and rainbow trout (*Onchorhynchus mykiss*) (Morgan et al., 2008).

Besides the temperature, the luminosity may be another important factor that influenced in the counting of RBC and Hb, since in the summer there is a higher incidence of direct sunlight on the external culture ponds. Moreover, studies that maintained the photoperiod unchanged for a certain period of time and had controlled water temperature did not find differences in RBC or in Hb values (Meling et al., 2002; Leonardi and Klempau, 2003; Valenzuela et al., 2007; Srivastava and Choudhari, 2010).

These finds can be explained by the higher activity of fish in warmer temperatures (Azevedo et al., 2006), and consequently with higher luminosity when the culture ponds are external. A status of most intense activity causes a release of red cells from spleen in vertebrates (Gallaughan and Farrel, 1998), and this could be associated with the higher values of RBC. The decrease in erythropoiesis in the colder seasons (Hofer et al., 2000) also helps to explain the decreased level of these parameters in autumn and winter.

Other causes like hypophagia, which is characteristic in the colder seasons, can diminish the values of RBC and WBC (Ríos et al., 2002). Even though the feed consumption has not been measured, the hypophagia may have been an important factor in the responses found.

In the present study, Hb levels were significantly higher in the summer compared to winter. This is directly related to tissue oxygenation, and the increase may be associated with the same reasons for the increase in RBC, since aquatic environments under higher temperatures have a lower rate of dissolved oxygen (Ranzani-Paiva, 2007). Valenzuela et al. (2007), in an experiment with rainbow trout (*Onchorhynchus mykiss*), did not observe changes in these parameters; this finding may be due to the constant temperature along the trial. The oscillations of these parameters found in the present study can be justified by the changes in temperature through the different seasons.

Possible changes in Ht may be related to a mechanism called respiratory compensation (Jerônimo et al., 2011). This mechanism occurs because the fish needs to keep tissue oxygenation in adequate levels, despite low levels of oxygen found in the water. In the present study an increase in Ht was found in the season with the lowest level of oxygen dissolved (autumn), resembling with a study in Brazil (Jerônimo et al., 2011). Studies in the central region of Spain (Collazos et al., 1998; Guijarro et al., 2003; De Pedro et al., 2005) found an increase in Ht in the season with the highest

temperature (summer), differing from the results of the present study, where autumn's temperature was not the highest. This suggests that dissolved oxygen has a greater influence on Ht than the water temperature.

Regarding defense cells, the highest WBC value in *Rhamdia quelen* was obtained in the spring. The same result was found by De Pedro et al. (2005) working with tench (*Tinca tinca*). Other studies observed this result in the autumn (Collazos et al., 1998; Guijarro et al., 2003; Jerônimo et al., 2011). A factor that could be responsible for the increase of the WBC in the spring is that in this season the immune system of vertebrates is more active (Zapata et al., 1992).

In a study with rainbow trout (*Onchorhynchus mykiss*), Valenzuela et al. (2007) observed a decrease in WBC under larger artificial photoperiods. This result is different of that found in the present study, which had the lowest WBC values in the autumn, season that does not have the larger photoperiod. There are others studies that report changes in leukocytes due to variations in the photoperiod (Meling et al., 2002; Leonardi and Klempau, 2003; Biswas et al., 2004), but the relationship between these variables is unclear.

The highest number of neutrophils in *Rhamdia quelen* was observed in the spring. Studies with Nile tilapia (*Oreochromis niloticus*) (Jerônimo et al., 2011) and tench (*Tinca tinca*) (De Pedro et al., 2005) found this increase in the autumn. Neutrophilia was observed in walking catfish (*Clarias batrachus*) (Srivastava and Choudhari, 2010) and Atlantic salmon (*Salmo salar*) (Meling et al., 2002) with the increase of the photoperiod to which the fish were submitted. Another factor that interferes with neutrophil counts is the stress, since neutrophilia in vertebrates is usually related with increased glucocorticoids (Davis et al., 2008), which may occur during capture (Ghiraldelli et al., 2006).

Probably, larger photoperiods could be related to increases in leukocytes counts due to higher level of stress, since as previously mentioned, whenever there is an increase in light hours, there is also an increase in these counts. A possible parameter to define if this effect exists in the different seasons of the year is the measurement of blood glucocorticoids related to stress, like cortisol and aldosterone.

An increase in the mean value of eosinophils was observed in spring, and in every season the value for this parameter was higher than those found in *Rhamdia quelen* by Tavares-Dias et al. (2002) and Borges et al. (2004). However, Guijarro et al. (2003) did not observe a relationship between number of granulocytes and the seasons of the year in tench (*Tinca tinca*).

The highest mean value of lymphocytes was observed during winter, a result that is in line with those of Jerônimo et al. (2011) with Nile tilapia (*Oreochromis niloticus*); the same author reports lymphocytosis also in the autumn, as does Hofer et al., (2000) in arctic char (*Salvelinus alpinus*). In contrast, Ranzani-Paiva et al. (2005) reported lymphocytosis in Nile tilapia (*Oreochromis niloticus*) in the summer. Lymphocytosis in the coldest seasons may be related to the stability of the fish organism (Jerônimo et al., 2011), since this kind of response is intimately involved with the immune system, and probably demonstrates its competence.

Because seasonal variations challenge the survival or acclimatization mechanisms of fish, it can be said that at lower temperatures, the specific immune response of these animals is suppressed and they become susceptible to pathogens (Santos et al., 2009).

TTC was higher in winter and summer. Conversely, Jerônimo et al. (2011) reported a decrease in this parameter in summer and an increase in autumn. According to Jerônimo et al. (2011), thrombocytes can play an important role in the immune response when the rest of defense cells are diminished, situation that did not occur in the present study, since the smallest WBC counts was found in the autumn.

Total plasmatic protein levels were significantly higher in the spring, summer and autumn. Nayak et al. (2004) report that total plasmatic protein reduction may indicate immunosuppression in Indian major carp (*Labeo rohita*), but Swenson (1996) indicates that this reduction may be related to the decrease in protein feed intake, that is a normal occurrence in colder seasons due to diminution of the metabolic rate (Santos, et al., 2009). In the case of the present study, the values found can indicate a satisfactory protein intake and an adequate metabolic rate in these seasons, but not in the winter.

In relation to the levels of hepatic enzymes from *Rhamdia quelen*, there were some significant variations between the seasons. In general, these results may indicate lower liver activity in winter, since ALT, AST, ALP and GGT are enzymes related mainly to hepatic tissue but not only to it, and albumin indicates the liver function and status of protein synthesis (Thrall et al., 2007). In addition, despite the differences between the seasons, all values remained within the range considered normal for the species (Table 5) according to Borges et al. (2004), and do not suggest possible hepatic lesions in any of the seasons.

The higher concentration of serum AST in the winter, combined with a lower concentration of albumin in the same season, may indicate a possible lower feed intake and a consequent muscular catabolism. Since there is AST also in the muscular tissue, it is recognized as an evaluator of disease and lesions in these cells (Thrall et al., 2007), and, furthermore, AST can be related to protein catabolism in liver (Begum, 2005).

These findings are in agreement with the results of RBC, Hb and TTP, all indicating a relative hypophagia in the winter, but that seems to be normal since there are reports of hypophagia at colder temperatures without major problems to animals (Tavares-Dias et al., 2002).

Conclusion

As expected, there is a seasonal influence on the haematological and biochemical parameters of silver catfish (*Rhamdia quelen*). These influences seem to be related to different factors, such as the reduction of feed consumption in the colder seasons, and the greater activity and stress of the animals in the hotter seasons.

The results should be evaluated according to the current season, and behaviors different from those expected for that season should be further investigated in order to ensure that good sanitary and/or productive conditions are maintained.

The results found help to understand the physiology of the species since there are few studies that accompany these variables over a year.

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