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ORIGINAL ARTICLE

Hematological alterations in fish exposed at agricultural fair

Emerson Giuliani Durigon[1] · Fernanda Picoli[1] · Suélen Serafini[1] · Géssica Perin[1] · Tais Inês Zuffo[1] · Monique Berticelli Morselli[1] · Sidinei Follmann[2] · Diogo de Alcântara Lopes[1] · Aleksandro Schafer Da Silva[1,3]

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Abstract

The objective of this study was to evaluate if there were alterations in hematological variables of fish after transportation and exhibition at an agricultural fair. We first collected blood samples from 12 fish of the species *Prochilodus lineatus* (curimbatá) at the Goio-En research institute; then, these animals were transported (60 km) to the agricultural fair, where they stayed for 3 days in glass aquariums exposed to the visitors of the event. On the return of the fish to the institute, we collected blood again. We performed hematological analyses, measuring hematocrit, hemoglobin concentration, and total erythrocyte count in the harvested material. We also determined mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. Total leukocyte counts and leukocyte differential performed on blood smears. There were no differences in hematocrit, hemoglobin, mean corpuscular hemoglobin, leukocyte, lymphocyte, neutrophil, and monocyte concentration ($P > 0.05$) before and after the fair. However, the number of erythrocytes and thrombocytes were lower after the fish show ($P < 0.05$). Mean values of mean corpuscular volume and mean corpuscular hemoglobin was higher after the fair ($P < 0.05$). Stress at the fair may have reduced the number of erythrocytes and thrombotic agents, as a result of which there may have been a compensatory effect in the fish that increased the mean corpuscular volume and mean corpuscular hemoglobin concentrations, in order to maintain the transport of oxygen in the cells and homeostasis. Based on these lines of evidence, it appears that exhibition of curimbatá in fairs caused alterations in the hematological parameters, damaging homeostasis and metabolism, thus negatively affecting their development.

Keywords Stress · Hematology · Hemostasis · *Prochilodus lineatus*

Introduction

The curimbatá (*Prochilodus lineatus*) is a species native to Brazil that has been progressively increasing its reach in the national fish culture. In Brazil, it is one of the most produced native species, with around 2747 tons produced in 2016, an increase of more than 7% compared to the previous year (IBGE 2015). Nevertheless, despite this significant production, there are more than 4000 tons of *Prochilodus* spp. produced in other countries costing approximately US$5 million, according to Baptista et al. (2018).

Fish are commonly exhibited at agricultural fairs, either for attraction, a distraction to visitors, or commercialization of fresh fish. These exposures may be responsible for stressful situations (Iwama et al. 2004) that begin with transportation, aggravated by the days of exposure, usually in high densities and in environments that are not in harmony with their natural habitat, and ending with their return (Mendes et al. 2015). Generally, in such a scenario, the immunological conditions and the physiological homeostasis of the animals are affected, damaging their physiological homeostasis and predisposing them to diseases and death (Conte 2004).

Measurement of hematological variables is essential for evaluation of health as well as stress in animals (Ribeiro et al. 2000). These parameters are altered by several factors, including temperature, oxygen, pH, stress, nutritional status, and diseases (Da Silva et al. 2012). Because we consider agricultural fairs a stressful situation, the aim of this study was to

✉ Aleksandro Schafer Da Silva
aleksandro_ss@yahoo.com.br

[1] Programa de Pós-graduação em Zootecnia, Universidade do Estado de Santa Catarina, Chapecó, Santa Catarina, Brazil

[2] Instituto Goio-En, Águas de Chapecó, Santa Catarina, Brazil

[3] Department of Animal Science, Universidade do Estado de Santa Catarina, Rua Zanin Beloni Trombeta 680 D, Chapecó/, SC 89815-630, Brazil

evaluate whether there were alterations in the hematological variables of curimbatás after transportation and exhibition in an agricultural fair.

Materials and methods

Fish, transport, and installations at the agricultural fair

The Curimbata (*Prochilodus lineatus*) used in this study came from the Goio-En Institute, located in the municipality of Águas de Chapecó, Santa Catarina, Brazil. Twelve fish were used, with a mean weight of 209 ± 55 g and mean length of 38 ± 12 cm. The fish were transferred an approximate distance of 60 km (1 h 20 min) to an agricultural fair held in the city of Chapecó, Santa Catarina, Brazil, in a transport box with controlled temperature, oxygen, and pH.

At the fair, the fish were housed over 3days in two 2.5-m^3 glass aquariums, and six fish were allocated to each aquarium. Water quality parameters such as temperature, pH, oxygen, and ammonia were monitored daily, and the results were 21.73 ± 0.48 °C, 5.99 ± 0.58, 6.03 ± 1.45 mg L^{-1}, and 1.19 ± 0.63 mg L^{-1}, respectively. Approximately 30% of the water in the aquariums was replaced daily, in order to maintain the quality and to control nitrogen compounds. After the 3 days of the fair, the animals returned to the Goio-En Institute, under the same conditions as in outward transport.

Blood collection and hematological analysis

Blood collections were performed before and after the fair at the Goio-em Institute at 3-day intervals. Prior to collection, the fish were anesthetized with eugenol (1 mg/L^{-1}) and with the aid of syringes containing EDTA 10%, blood samples were collected by puncturing the caudal vessel. The samples were placed in microtubes for complete blood count.

From the blood samples, we determined hematocrit (%) (Goldenfarb et al. 1971), hemoglobin concentration (g/dl) (Collier 1944), and total erythrocyte count (μL), performed after 1:200 dilution in sodium chloride solution (0.65%), according to Ghiraidelli et al. (2006). Total leukocyte and leukocyte differential counts (lymphocytes, neutrophils, thrombocytes, and monocytes) were evaluated by blood smear and May-Grunwald/Giemsa-stained slides (Rosenfeld 1947) and were read by the indirect method (Ishikawa et al. 2008). The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated according to the methodology described by Ranzani-Paiva et al. 2013).

Statistical analysis

After verification of normality (Shapiro-Wilk) and homoscedasticity (Levene), the abnormal data were transformed. Subsequently data were subjected to analysis of variance (ANOVA) to evaluate possible significant differences between the two transport moments (before/after the fair). Differences were considered significant when P was < 0.05.

Results and discussion

There were no differences in the hematocrit, hemoglobin concentration, MCHC, total leukocyte and lymphocyte counts, neutrophils, and monocytes ($P > 0.05$). However, erythrocyte and thrombocyte values were lower after the fair than prior ($P < 0.05$). On the other hand, the values of MCV and MCH were higher after the agricultural fair ($P < 0.05$) (Table 1).

Table 1 Hematological variables before and after the transportation of curimbata fish exposed to the agricultural fair for a period of 3 days

Variables	Before				After				
	Mean	±	SD	CV	Mean	±	SD	CV	*P*
Hematocrit (%)	35.25	±	1.28	3.64	35.64	±	8.54	23.95	NS
Erythrocytes (× 10^6/μl)	2.38	±	0.43	17.93	1.32	±	0.42	31.00	< 0.001
Hemoglobin (g/dL)	7.95	±	1.52	19.16	7.74	±	1.85	23.86	NS
MCH (g/dL)	34.82	±	11.71	33.64	60.01	±	24.33	37.08	0.004
MCV (fL)	155.99	±	43.76	26.48	279.95	±	91.35	33.07	0.001
MCHC (g/dL)	22.56	±	4.37	19.37	22.05	±	4.85	22.01	NS
Leukocytes (× 10^3/μl)	29.37	±	13.37	45.52	25.91	±	6.37	24.59	NS
Lymphocytes (× 10^3/μl)	85.71	±	5.56	6.48	78.41	±	12.60	16.08	NS
Neutrophils (× 10^3/μl)	7.57	±	3.55	46.91	9.50	±	8.47	89.26	NS
Monocytes (× 10^3/μl)	7.12	±	5.91	83.01	9.27	±	8.01	86.42	NS
Thrombocytes (× 10^3/μl)	36.00	±	11.24	31.23	9.15	±	4.22	46.28	< 0.001

SD, standard deviation; *CV*, coefficient of variation; *MCH*, mean corpuscular hemoglobin; *MCV*, mean corpuscular volume; *MCHC*, mean corpuscular hemoglobin concentration

The values found in this study for hematocrit were within the range observed by Gonçalves et al. (2010). Those authors found variations between 25.1 and 55.8% when subjecting curimbatás to various storage densities in transportation. In another study evaluating hematological parameters of curimbata infected or not with *Neoechinorhynchus curemai*, researchers found MCV values between 139.64 and 152.18 fl (Belo et al. 2013), similar to those found in this study.

According to the literature, in stress situations, plasma cortisol levels increase considerably, inhibiting production of leukocytes. Cortisol evokes immunosuppressive responses hematopoietic tissues, causing a decrease in the production of lymphocytes, monocytes, and neutrophils, all of which are forms of white blood cells, also called leukocytes (Cardoso Pereira et al. 2005). In our study, there was no significant decrease in leukocyte production after the fair, nor in any of the abovementioned components. This finding suggests that the animals were not stressed enough to cease production of leukocytes.

Cardoso Pereira et al. (2005) reported that hematocrit, hemoglobin, and the relative number of lymphocytes may be a response to acute stress. They also comment that, in stressful situations with the elevation of cortisol levels, there is an increase in the demand for oxygen and energy in the tissues and, consequently, rapid production and differentiation of red blood cells. During this hemo-concentration (common in stress situations), there is an increase in hemoglobin and hematocrit levels. These variables did not differ in our study, reinforcing the hypothesis that the fish were not in severe stress.

It is important to emphasize that the function of erythrocytes is the transport of oxygen through hemoglobin and carbon dioxide exchange with tissues (Tavares-Dias and Moraes 2004; da Silva et al. 2012). For fish, the value of erythrocytes may vary according to the physiological state of the animal, remaining between 1 and $3 \times 10^6/\mu L$ (Andrade 2016). In addition to red blood cells and hemoglobin, hematocrit also indicates the oxygen transport capacity in fish closely related to the concentration of oxygen available in the culture environment (Tavares-Dias et al. 2002). When there is a decrease in red blood cell count, there is a decrease in oxygen transport in the body of the animals and consequent damage to their health and consequently their growth (Da Silva et al. 2012). Despite the difference between collection times, the number of erythrocytes remained within normal range as reported in the literature (Andrade 2016).

We observed higher MCV in the fish after return from the fair, suggesting that stress interfered with the animals' homeostasis. From this interference, electrolytic changes that cause water to enter the cell, increasing its volume. This is in agreement with a study by Gonçalves et al. (2010), in which MCV increased to 167.3 after the transport of curimbatá for 96 h. In addition to MCV, there was elevation of MCH, possibly an adaptive response to minimize the damage caused by the decrease in red cell numbers. From this adaptation, it is possible to increase O_2 transport to desirable levels. Stress causes biochemical and physiological changes in animals as an adaptive measure, and support for stressful conditions results in reaching a degree of balance (Wendelaar Bonga 1997). The fish analyzed in this study presented with hypochromic anemia, confirmed by the presence of large red blood cells and not fully hemoglobinized juvenile forms, as reported by Belo et al. (2013).

The primary function of thrombocytes in fish is hemostatic regulation (Tavares-Dias et al. 2008). Some studies have demonstrated that these cells act as a defense system through phagocytic and bactericidal activity, and their activity may vary according to the physiological state (Tavares-Dias et al. 2008). Similar results were found by Belo et al. (2013) with *P. lineatus*, in which there was a decrease in the number of thrombocytes after the animals were infected with *Neoechinorhynchus curemai*. As a result, researchers suggested that the reduction in the number of these cells may indicate a decline in the immune system function in fish (Tavares-Dias et al. 2008).

According to the literature, it is known that after exposure to stressful management, fish tend to change physiological responses, including decreased weight gain, increased susceptibility to diseases and ionic imbalances, resulting in immediate or future mortality (Urbinati et al. 2004). In this study, there was no mortality; however, necrotic dermal lesions were observed as the absence of scaling caused by friction and possible cannibalism linked to animal stress.

We conclude that the curimbata fish that were exhibited in agricultural fairs did not present acute stress. Nevertheless, changes in critical hematological variables related to tissue oxygenation and hemostasis occurred in compensatory situations, in order to minimize the adverse effects caused by transport stress and exposure, as well as small consumption of food during the 3 days of the agricultural fair.

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Compliance with ethical standards

This experiment was approved by the Animal Welfare Committee of the State University of Santa Catarina (UDESC), protocol number 1977230318.

Conflict of interest The authors declare that they have no conflict of interest.

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