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Influence of anticoagulants and blood storage on hematological values in tambaqui, *Colossoma macropomum*

Marcos Tavares-Dias[1]* and Elziane F. Silva Sandrim[3]

*[1] Laboratório de Patologia de Organismos Aquáticos, CAUNESP, Rodovia Carlos Tonani, Km 05, 14870-000, Jaboticabal-São Paulo, Brazil. [2] Piscicultura Usina São Geraldo, Rodovia Armando Salles Oliveira, Km 8, 14160-00, Sertãozinho-São Paulo, Brazil. *Author for correspondence.*

ABSTRACT. Tambaqui *Colossoma macropomum*, Cuvier, 1818 (Osteichthyes: Characidae) were used in this study. The blood of the animals was obtained by punction on the tail vessel with a heparinized syringe (5000 IU) and with one containing EDTA 10%. After collection, the blood was immediately processed in order to measure hematocrit percentage and hemoglobin concentration. It was then left in room temperature where it was kept for a ten-hour period, after which hematocrit and hemoglobin concentrations were measured again. Results indicated that, in teleosts, anticoagulant selection can interfere significantly with the results of the studied hematological parameters. However, blood storage at room temperature did not have a significant effect (α=0.05) on hematocrit percentage, hemoglobin rate or mean corpuscular hemoglobin concentration (MCHC).

Key words: anticoagulants, *Colossoma macropomum*, fish, hematocrit, hemoglobin, storage.

RESUMO. Influência de anticoagulantes e estocagem de sangue em valores hematológicos de tambaquis, *Colossoma macropomum*. Foram empregados, neste estudo, tambaqui *Colossoma macropomum* Cuvier, 1818 (Osteichthyes: Characidae). O sangue de cada animal foi coletado através de punção da veia caudal utilizando-se seringas heparinizadas (5000 IU) ou contendo EDTA 10%. Após a coleta do sangue procedeu-se imediatamente à medida do hematócrito e da taxa de concentração da hemoglobina. Posteriormente, o sangue foi deixado em temperatura ambiente, onde permaneceu durante 10 horas, quando se realizou novamente a medida do percentual de hematócrito e da taxa de hemoglobina. Os resultados indicaram que em teleósteos a escolha do anticoagulante pode interferir significativamente (α=0,05) nos resultados dos parâmetros hematológicos estudados. Entretanto, a estocagem do sangue em temperatura ambiente (10 horas) não teve efeito significativo (α=0,05) sobre o percentual de hematócrito, taxa de hemoglobina e concentração da hemoglobina corpuscular média (CHCM).

Palavras-chave: anticoagulantes, *Colossoma macropomum*, hematócrito, hemoglobina, peixe, sangue.

Several anticoagulants are used in hematological studies with mammals. Heparin anticoagulants, sulfated glycosaminoglycans which are responsible for several pharmacological activities have been the most frequently used substances. For studies on hematological parameters normality in fish, the collected blood should not be altered by anticoagulants (Hattingh, 1975).

To determine the normal hematological parameters in fresh-water teleosts, heparin has been the most frequently anticoagulant used by researchers such as Martins and Pitombeira (1968), Ranzani-Paiva and Godinho (1983), Zuim *et al.* (1984), Ranzani-Paiva and Godinho (1985), Zuim *et al.* (1986), Satake *et al.* (1986, 1991), Ranzani-Paiva (1991), Borghi and Silva-Souza (1992), Wepener *et al*. (1992), Wilhelm-Filho *et al*. (1992), Shiau *et al*. (1993), Martínez *et al*. (1994), Martins *et al*. (1995) and Ranzani-Paiva and Ishikawa (1996). It has also been used in hematological variation studies in parasited fish (Ranzani-Paiva *et al.*, 1987). According to literature, few researchers have used EDTA as an anticoagulant (Kavamoto *et al.*, 1983a; Kavamoto *et al.*, 1983b; Lochmiller *et al*., 1989; Lea Master *et al*., 1990; Sun *et al*., 1994). However, in other hematological analysis studies, the use of anticoagulants has not been mentioned (Dabrowska and Wlasow, 1986; Cavalcante *et al., 1*995; Ranzani-Paiva *et al.*, 1996; Rodrigues *et al.*, 1996).

According to Allen (1993), whenever it is intended to determine hematological parameters in fish, the selection of anticoagulants as well as that of

anesthetic are very important so as not to interfere with the parameters. Under certain conditions, anticoagulants can interfere in the procedures of automated blood measurements; therefore, if they are made in that way, the minimum possible amount of anticoagulant must be used.

The present study aims at verifying the effects of EDTA anticoagulants and heparin on hematocrit percentage, hemoglobin rate, and the mean corpuscular hemoglobin concentration (MCHC) in teleosts *Colossoma macropomum* as well as the effect of blood storage time for this species at room temperature.

Material and methods

Twenty specimens of *Colossoma macropomum* (tambaqui) with mean weight 487.5 g and mean length 25.9cm were used. The blood was obtained from the twenty animals by a punction on the tail vessel using a heparinized syringe (heparin 5000 U.I.) or containing 10% EDTA without the use of anesthetics. The duration of each punction varied from 30 to 50 seconds. After collection, the blood was immediately processed (0 hour of storage) in order to measure hematocrit percentage and hemoglobin concentration. It was then left at room temperature and kept for a ten-hour period, after which hematocrit and hemoglobin concentrations were measured again.

Hematocrit measurement was carried out according to Goldenfarb *et al.* (1971) and the values found were expressed as a percentage of the total blood volume.

Hemoglobin rate was determined by the cyanometahemoglobin method according to Collier (1944), and its values were expressed in g/% ml of blood.

The mean corpuscular hemoglobin concentration (MCHC) was calculated according to Wintrobe (1934).

The results obtained were analyzed statistically by the "Statistical Analysis System/SAS where they were submitted to variance analysis (ANOVA). Tukey's test was used in order to analyze the differences between the hematological parameters values (α=0.05 was considered significant).

Results

The results indicated that the hemoglobin rate (0 and 10 hours of storage) in heparinized blood was significantly higher (α=0.05) when compared to samples with EDTA (Figure 1).

The mean percentage of hematocrit obtained with heparin (0 and 10 hours of storage) was considered significantly higher (α=0.05) than that obtained with EDTA (Figure 1). MCHC values obtained from heparinized blood and with EDTA did not show significant difference (α=0.05).

Statistical analysis did not show significant differences (α=0.05) between the results obtained from immediate blood processing and those obtained from blood stored for a 10-hour period at room temperature with regard to hematocrit percentage, hemoglobin concentration and MCHC (Figure 1).

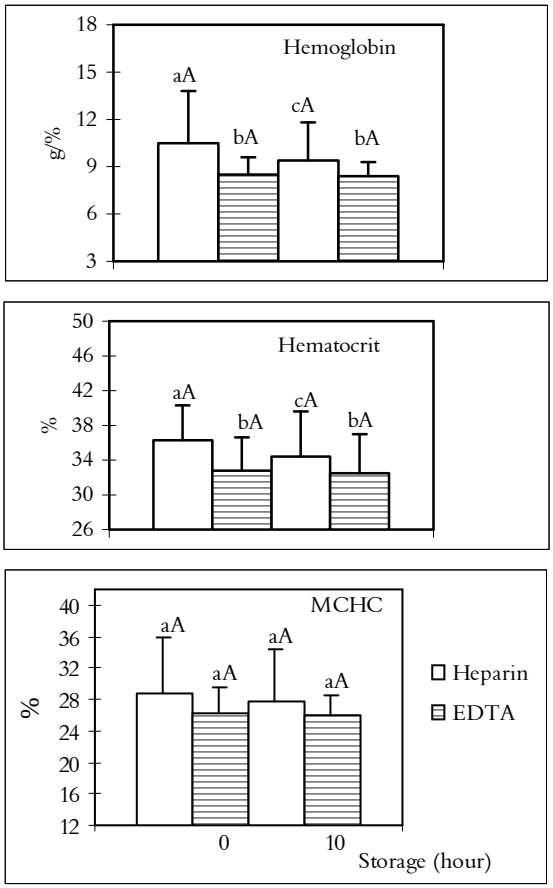

Figure 1. Effect of EDTA and heparin anticoagulants and of *Colossoma macropomum* blood storage time at room temperature on hematocrit percentage, hemoglobin rate, and the mean corpuscular hemoglobin concentration (MCHC). a, b, c - Mean values (effect of EDTA and heparin anticoagulants) followed by the same small letter do not differ statistically by Tukey's test (α=0.05). A, B - Mean values (blood storage time at room temperature) followed by the same capital letter do not differ statistically by Tukey's test (α=0.05)

Discussion

Results show that the selection of anticoagulants in teleosts can interfere significantly with hematocrit and hemoglobin values. However, it does not interfere with the mean corpuscular hemoglobin concentration (MCHC). Higher values for hematocrit and hemoglobin using heparin (5000 I.U) were also described in *Brycon orbignyanus* by Bazzoli *et al.* (1996).

Similarly, when comparing the effects of several heparin solutions on the hematological parameters of *Oreochronis aureus,* Allen (1993) verified an increase in hematocrit percentage in heparinized blood when compared to non-heparinized. He also showed that the blood collected with heparin 250 U.I./ml and diluted in a solution of 0.65% NaCl produces more stability to the hematocrit percentage than other dilutions. The use of NaCl solution seems to have a stabilizing effect reflected in blood and plasma osmolality. Heparin without NaCl depresses blood osmolality.

In spite of the above, studies by Smit and Hattingh (1980) show that heparin produces more stability in hematological profiles than EDTA and other anticoagulants. On the other hand, Clarke *et al.* (1979) relate that heparin does not prevent blood coagulation in *Micropterus salmonides* as effectively as EDTA. However, studies in mammals show a correlation between anticoagulant activity and the molecular weight of heparin. Significant anticoagulant activity (140 I.U) can be observed in heparin molecules with a molecular weight of at least 12 kDa. Heparin with molecular weight below 5kDa shows negligible anticoagulant activity (50 I.U) (Nader and Dietrich, 1994).

Heparin has also been shown to inhibit several enzymes, among which are myosin ATPase (Cruz and Dietrich, 1967; Tersariol *et al*., 1992), hyaluronidase, elastase and renin (Sealey *et al*., 1967). Furthermore, it has been recently shown that heparin binds itself to numerous growth factors such as the fibroblast growth factor (Lobb and Fett, 1984; Gambarini *et al.*, 1993) and the endothelial cell growth factor (Maciag *et al*., 1984). In addition, heparin can also bind itself to several adhesion proteins such as laminin (Sakashita *et al*., 1980), fibronectin (Yamada, 1983) and vitronectin (Hayman *et al*., 1983). Binding of heparin to endothelial cells has also been shown by *in vivo* and *in vitro* experiments (Jaques, 1980).

The present work also suggests that in the studied conditions the storage of *C. macropomum* blood at room temperature for ten hours did not have a significant effect on hemoglobin concentration or on hematocrit percentage as it was expected. EDTA seems to have a more stable conservative effect than heparin even when it is necessary to store blood samples for later measurement. However, this point needs to be better investigated. Railo *et al.* (1985) suggest that blood parameters must preferably be made without blood storage.

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