



Effect of heparin and EDTA as anticoagulants on hematological values in farmed juvenile of *Arapaima gigas*

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Received: 14 May 2021 / Accepted: 25 October 2021
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Abstract

The rapid coagulation of fish blood makes the use of anticoagulants in hematological analysis necessary and means that choosing the most appropriate product is important. The aim of the present study was to evaluate the effect of two different anticoagulants (heparin sodium and K₂EDTA), storage time, and temperature on the hematological values of *Arapaima gigas* juveniles. Twelve juveniles (905.79 ± 111.2 g and 49 ± 1.74 cm) kept in captivity were used for the tests. Blood was collected from the caudal vein and divided for the analysis of the effects of K₂EDTA 10% and heparin sodium 5000 IU, storage time (0, 5, 10, and 24 h), and temperature (4 and 28 °C). The hematocrit, erythrocyte count, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), and differential leukocyte count were evaluated. The results revealed significant differences in the hematocrit, lymphocytes, and neutrophils when the anticoagulants were compared at 0 h of storage. In the hematocrit, heparin sodium produced variations, while K₂EDTA maintained the values constant at 5 h, the erythrocyte count constant for up to 24 h, with the exception of at 4 °C, while hemoglobin produced variations in all the groups at 5 h. The blood indices MCV, MCHC, and MCH were best preserved, without variations, using K₂EDTA at 28 °C for up to 5 h. In conclusion, K₂EDTA 10% at 28 °C maintained constant hematological values of *Arapaima gigas* for 5 h, with the exception of hemoglobin, which should be analyzed as soon as possible.

Keywords Aquaculture · Fish farming · Pirarucu · Paiche · Peru

Handling Editor: Dr. Gavin Burnell

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Introduction

The evaluation of hematological values is a useful method for knowledge the physiological state and health of fish which face different conditions during culture (Blaxhall 1972; Tavares-Dias and De Moraes 2004; Ekanem et al. 2012; Ranzani-Paiva et al. 2013; Sánchez et al. 2017). It is essential to establish the reference hematological parameters for each species to determine changes caused by external and internal factors (Knowles et al. 2006; Tavares-Dias and Sandrim 1998). The use of anticoagulants is important in hematological analyses, as fish blood coagulates rapidly, so it is necessary to determine the most appropriate anticoagulant to avoid alterations in the values of hematological parameters (Korcock et al. 1988; Faggio et al. 2014; Ciepliński et al. 2019). Hematological parameters in aquaculture are indicators of fish health status that can identify alterations caused by stress, diseases, and culture conditions affecting production (Yousefi et al. 2012; Sánchez et al. 2017; Fazio 2019).

Anticoagulants, through their chemical nature, act at different stages of the coagulation cascade (Harr et al. 2005), preserving blood fluidity and making the blood count feasible (Ranzani-Paiva et al. 2013). However, it appears that anticoagulants can cause additional changes in the blood, according to their composition (Oliveira et al. 2015). When the concentrations used are not ideal, they can cause hemolysis, coagulation, an increase in the number of erythrocytes, and changes in the morphology of blood cells (Hattingh 1975; Mafuvadze and Erlwanger 2007). The anticoagulants ethylenediaminetetraacetic acid (EDTA) and heparin sodium, at different concentrations, have been used most frequently in routine hematological analyses of cultured fish (Fazio 2019). According to literature, disadvantages have been reported for both anticoagulants in some fish species; heparin interferes with the staining of leukocytes when subjected to Romanowsky-based staining methods (Ranzani-Paiva et al. 2013), and EDTA has been reported to cause changes erythrocyte morphology (Walencik and Witeska 2007).

Arapaima gigas is considered the most emblematic fish of the Amazon basin, due to its high growth potential (10 kg/year) in captivity (Imbiriba 2001; Nuñez 2009), rapid acceptance of balanced diets in its early stages, and high density growth (Cavero et al. 2003; Oliveira et al. 2012; Gonzales et al. 2016; Fernandez-Méndez et al. 2017). Due to the increased cultivation of this species, there is now greater interest in the use of hematological values to assess the health of fish under different culture conditions (Andrade et al. 2007; Sanchez et al. 2017; Luz et al. 2019). In different studies involving *A. gigas*, the most used anticoagulants were EDTA 10% and heparin 5000 IU (Andrade-Porto et al. 2017; Dias et al. 2019), with lower doses of heparin 100 IU causing alteration of parameters, and EDTA 3% resulting in hemolysis from the beginning of preservation (Dantas et al. 2012). The objective of the present study was to determine the effects of the anticoagulants heparin sodium 5000 IU and K₂EDTA 10%, time, and storage temperature on the hematological values of cultured juvenile *A. gigas*.

Materials and methods

Fish farming conditions

Juvenile *A. gigas* were cultured in 1000 L concrete tanks with water recirculation and mechanical filtration. The fish were fed a commercial extruded diet (Aquatech, Naltech, Lima, Peru: 42% protein, 10% lipids, 12% ash; 1.5% calcium; 1% phosphorus and 4 mm pellets), three times a day until apparent satiety and were maintained at a density of one fish per 100 L. Measurements of temperature (28.9 ± 0.6 °C), dissolved oxygen (5.7 ± 1.5 mg L⁻¹), using an oximeter (YSI Model 55, USA), and pH (6.8 ± 0.5), using a pH meter (Oakton pH 110 Series, USA), were taken twice a day (08:00 and 16:00 h). Ammonium (3.0 ± 0.2 mg L⁻¹) and nitrite (0.03 ± 0.01 mg L⁻¹) were evaluated once a week using a colorimetric photometer (HI 83203, USA).

Blood parameters

A total of 12 fish with an average weight of 905.79 ± 111.2 g and a total length of 49 ± 1.74 cm were used for the experiment. The fish were extracted using wet towels for immobilization and eye bandages, while their bodies were constantly hydrated with water. A 2.5 mL blood sample was drawn from the caudal vein for each fish using 3-ml disposable syringes. Each sample was then divided into two parts: one part to be treated with K₂EDTA 10% anticoagulant (50 µL mL⁻¹ blood), and the other part to be treated with heparin sodium 5000 IU (50 µL mL⁻¹ blood). Each subsample was then divided into three groups, each containing 0.4 ml of blood. The first of these groups was used in the evaluation of the effect of the anticoagulant (K₂EDTA and sodium heparin) on the hematological parameters at 0 h, at room temperature (28 °C). The other two groups were used to evaluate each storage temperature (4 and 28 °C), with four treatments obtained (Hep 28 °C, Hep 4 °C, EDTA 28 °C, and EDTA 4 °C), which were then subjected to the respective storage temperatures, with the 4 °C samples kept refrigerated and the 28 °C samples kept in a room with constant temperature. The hematological parameters of each treatment were analyzed at 5, 10, and 24 h and were compared with the initial results at 0 h for each anticoagulant from the first group, to evaluate variations. In all analyses, the 12 samples were evaluated for each treatment and storage time. No anesthetics were used, to prevent possible interference in the results (Korcock et al. 1988; Tavares-Dias and Sandrim 1998).

The sample hematocrit (%) was determined using the micro hematocrit method (Goldenfarb et al. 1971). Sample hemoglobin concentration (g dL⁻¹) was measured using the cyanmethemoglobin method (Biolabtest Laboratories, Lima, Peru) at 540 nm with a UV visible spectrophotometer (Cary 60, USA). Erythrocyte counts (10⁶ cells µL⁻¹) were performed using Natt-Herrick solution (Natt and Herrick 1952) and a Neubauer chamber. Mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) were determined using the Wintrobe (1934) method. To perform differential leukocyte counts, individual blood smears from each fish were prepared, and anticoagulant samples were taken on glass slides. The smear was allowed to air dry for 24 h before staining. The staining procedure consisted of the use of Wright solution for 10 min (Hawkey and Dennett 1989), followed by rinsing with water. Once dried, the stained slides were examined under a compound light microscope (Leica DM750, Germany) at 1000 × magnification. Leukocytes were counted using a zig zag

approach, from head to tail on the slide (Ranzani-Paiva et al. 2013), with the identification of a minimum of 200 cells per slide for differential leukocyte counting (lymphocytes, monocytes, neutrophils, and eosinophils). The results were expressed as a percentage of each leukocyte identified.

Analysis of data

The results of the anticoagulants at 0 h were evaluated by the Student's t-test. One-way analysis of variance (ANOVA), followed by Dunnett's test ($p \leq 0.05$), was used to determine differences in storage time and temperature from the baseline (0 h) for each combination of temperature and anticoagulant. All analyses were performed after testing for normality (Shapiro-Wilk test) and homoscedasticity (Bartlett's test), and the Sigma Plot 11 statistical software package was used.

Results

The results showed that the use of heparin sodium 5000 IU and K₂EDTA 10% as anticoagulants for the blood of *Arapaima gigas* effectively prevented blood clotting at both temperatures for 24 h of storage. The hematological parameter values preserved with heparin sodium 5000 IU and K₂EDTA 10% at 0 h at room temperature (28 °C) are shown in Table 1. The percentage of hematocrit had an increase ($p < 0.05$) in the samples with K₂EDTA 10%, and in the differential leukocyte count, there was an increase of lymphocytes and reduction of neutrophils with heparin 5000 IU. Erythrocytes, hemoglobin, MCV, MCHC, MCH, monocytes, and eosinophils showed no differences ($p > 0.05$) by type of anticoagulant.

The results of anticoagulant storage time and temperature on hematological parameters are shown in Fig. 1. The hematocrit percentage (Fig. 1a) when heparin sodium was used showed changes ($p < 0.05$) at all preservation times compared to 0 h. However, with K₂EDTA, there were increased values ($p < 0.05$) at 10 h at both temperatures (4 and 28 °C) of preservation. In the erythrocyte count (Fig. 1b), only variations of the values ($p < 0.05$) were observed with K₂EDTA at 4 °C, which decreased at 10 and 24 h. Hemoglobin values (Fig. 1c) with both anticoagulants (heparin sodium and

Table 1 Effect of heparin sodium 5000 IU and K₂EDTA 10% at 0 h and 28 °C on hematological parameters of juvenile *Arapaima gigas* ($n = 12$)

Parameters	Heparin 5000 IU	K ₂ EDTA 10%
Hematocrit (%)	31.44 ± 0.78*	33.21 ± 1.40*
Erythrocytes (10 ⁶ µL ⁻¹)	1.27 ± 0.43	1.38 ± 0.41
Hemoglobin (g dL ⁻¹)	10.11 ± 0.94	10.36 ± 0.42
MCV (fL)	282.13 ± 123.10	277.95 ± 147.8
MCHC (g dL ⁻¹)	32.18 ± 3.21	31.23 ± 1.03
MCH (pg)	90.11 ± 39.30	87.37 ± 49.22
Lymphocytes (%)	95.83 ± 4.92*	86.5 ± 8.14*
Monocytes (%)	2.0 ± 2.28	3.33 ± 3.68
Eosinophils (%)	1.33 ± 1.63	2.5 ± 2.74
Neutrophils (%)	0.83 ± 1.32*	7.67 ± 2.58*

Asterisks (*) in the same row indicates a significant difference

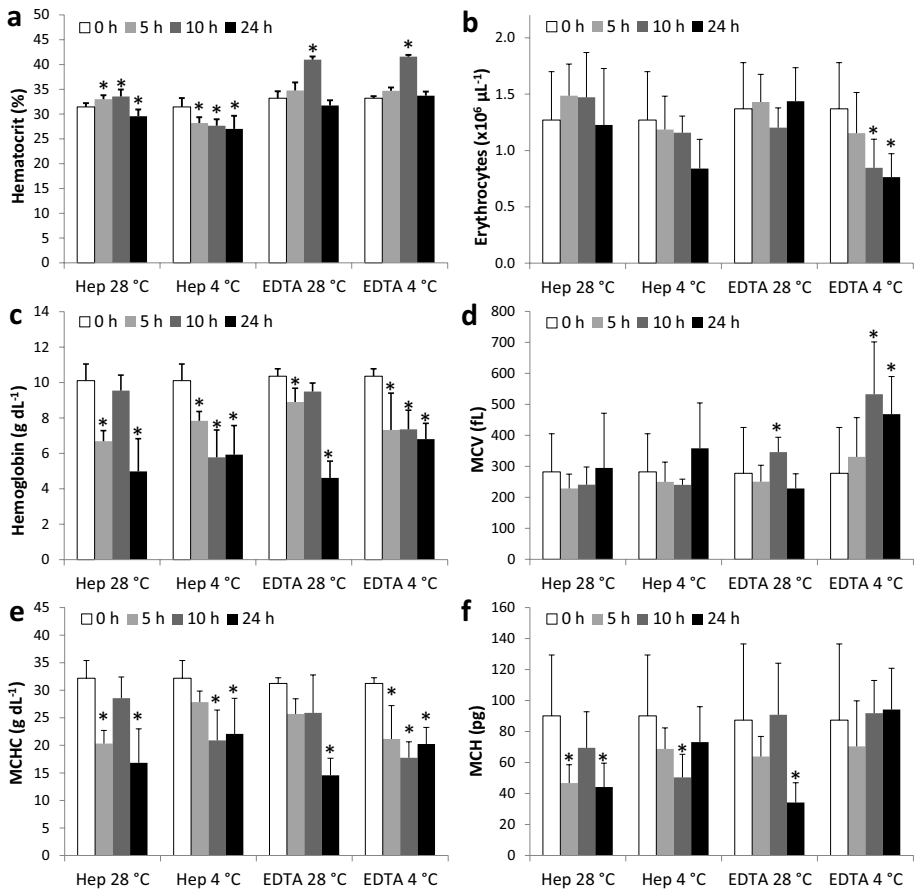


Fig. 1 Effect of storage time (0, 5, 10, 24 h), anticoagulant (heparin sodium 5000 IU and K_2EDTA 10%), and storage temperature (4 and 28 °C) on hematological parameters of juvenile *Arapaima gigas*. Asterisks (*) indicate significant differences ($p < 0.05$) between hours of storage and 0 h for each treatment ($n = 12$)

K_2EDTA) at the temperature of 4 °C showed reduction ($p < 0.05$) at all storage times, whereas at 28 °C, temperature with heparin sodium and K_2EDTA decreased ($p < 0.05$) at 5 and 24 h of storage. MCV (Fig. 1d) with the use of heparin sodium remained unchanged ($p > 0.05$) during the storage time at the two temperatures, however, with K_2EDTA increased the value ($p < 0.05$) at 5 h at 28 °C and at 10 and 24 h at 4 °C. MCHC (Fig. 1e) with heparin sodium decreased ($p < 0.05$) at the temperature of 28 °C at 5 and 24 h, as well as at 4 °C at 10 and 24 h. However, with K_2EDTA , MCHC at the temperature of 28 °C decreased ($p < 0.05$) at 24 h and at 4 °C decreased ($p < 0.05$) for all storage times. MCH (Fig. 1f) with heparin sodium decreased ($p < 0.05$) at the 28 °C temperature at 5 and 24 h, while at 4 °C decreased ($p < 0.05$) at 10 h. With K_2EDTA , the MCH decreased ($p < 0.05$) at 28 °C, only at 24 h, and at 4 °C there was no alteration of the values ($p > 0.05$) at any of the storage times.

Discussion

The use of anticoagulants during hematological analyses in fish has been shown to have different effects on the blood cells of teleosts (Tavares-Dias and De Moraes 2004). However, it is important to determine the most appropriate anticoagulant for each species to establish reference values (Tavares-Dias and Sandrim 1998; Witeska et al. 2017). In the present study, the use of the anticoagulants succeeded in preventing coagulation in the blood of juvenile *A. gigas* for up to 24 h. Other studies in species such as *Mugil cephalus* also prevented the coagulation up to 24 h (Faggio et al. 2014), in *Colossoma macropomum* for more than 10 h (Padua et al. 2012) and in *A. gigas* only EDTA 10% compared to lower concentrations and heparin sodium was effective for more than 10 h (Dantas et al. 2012). However, although the blood remained uncoagulated, they presented alterations in hematological values due to hemolysis.

The increase in hematocrit percentage with EDTA was associated with the anticoagulant effect to trap Ca^{2+} ions to prevent the coagulation (Harr et al. 2005), which produces decalcification of the cell membrane and alteration of the osmotic gradient that increased the cell volume (Smit et al. 1977; Kaestner 2013; Bogdanova et al. 2013). Similar results were found in teleosts such as *Cyprinus carpio* L., *Oncorhynchus mykiss*, and *Piaractus mesopotamicus* (Witeska and Wargocka 2011; Maqbool et al. 2013; Farias et al. 2016). In *Arapaima gigas*, the increase was also reported although this was non-significant (Dantas et al. 2012). The increase in the percentage of lymphocytes and the reduction of neutrophils with heparin compared to EDTA could be related to the differentiation of leukocytes in the count (Campbell 2004); high concentrations of heparin can cause interference in Romanowsky-based staining methods, by impeding the entry of the colorant into the cells (Ranzani-Paiva et al. 2013) making leukocyte counting difficult. These alternations have also been evidenced in other studies with *Cyprinus carpio* L. and *Clarias gariepinus* (Witeska et al. 2017; Adeyemo et al. 2009). Some authors have highlighted the superior quality of blood smears with EDTA in comparison with those performed with heparin (Campbell 2015; Bain et al. 2006).

The results in the time and temperature of preservation in hematocrit showed that K_2EDTA maintained stable values for a longer time versus sodium heparin. The increase in values (EDTA, 10 h) is associated with increased erythrocyte volume and reductions to hemolysis of cells over time (Walencik and Witeska 2007). Similar results were obtained in *Clarias gariepinus* and *Cyprinus carpio* however at lower concentrations of both anticoagulants the hematocrit remained constant longer (Hattingh 1975). In contrast, *Mugil cephalus* and *C. carpio* showed no variation in hematocrit results with the use of both anticoagulants for 24 h (Faggio et al. 2014; Witeska et al. 2017). In the present study, erythrocyte counts reported stable preservation for 24 h, except for EDTA at 4 °C, which had a reductive effect and in related to the increase of MCV at the same temperature at 10 and 24 h. This behavior was also reported for *Piaractus mesopotamicus* with EDTA 10% (Farias et al. 2016) and was associated with an increase in erythrocyte volume (MCV) until hemolysis, which caused a reduction in the number of erythrocytes (Kaestner 2013; Bogdanova et al. 2013), which was intensified at a refrigerated temperature (4 °C). In some treatments where changes were associated with hemolysis, there were no significant changes in erythrocytes up to 24 h, as reported in *M. cephalus* with EDTA and heparin (Faggio et al. 2014), while in *C. carpio*, changes were reported with heparin at 2 h of preservation (Witeska et al. 2017). Hemoglobin proved to be a highly variable parameter during blood preservation in *A. gigas*; the decrease which occurred in less than 5 h would be related to

the degeneration of erythrocytes during the storage period (Pádua et al. 2012). In refrigerated samples (4 °C), the changes were slow; however, at 28 °C, they caused rapid changes and interference in the cyanmethemoglobin method due to the release of substances during hemolysis (Mirghaed et al. 2017) and a decrease hours later by degradation (24 h). In other species such as *C. carpio* (heparin) and *M. cephalus* (EDTA and heparin), the values remained constant for 24 h (Faggio et al. 2014; Witeska et al. 2017). In the blood indices, MCV showed a similar behavior with the erythrocyte count, while MCHC and MCH were highly variable as an effect of the influence of hemoglobin on the values. This relationship of variables was confirmed with *M. cephalus*, where MCV, MCHC, and MCH values showed no changes with EDTA and heparin, because there were also no changes in the hematocrit, erythrocyte, and hemoglobin (Faggio et al. 2014). When evaluating the blood indices as a whole, only with EDTA 10% at 28 °C, the values remained constant up to 5 h. Different studies on anticoagulants in teleosts have shown that each species reacts differently to the dose, concentration, and chemical nature of the anticoagulant (Ranzani-Paiva et al. 2013).

The results of this study showed that blood samples from juvenile *A. gigas* with anticoagulant K₂EDTA 10% preserved at room temperature (28 °C) maintained constant hematocrit, erythrocyte, MCV, MCHC, and MCH values for 5 h and also showed less interference in differential leukocyte count. Furthermore, hemoglobin is a parameter that should be determined for both anticoagulants before 5 h to avoid alterations in the results.

Data availability All data generated or analyzed during this study are included in this published article.

Code availability Not applicable.

Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by APGF, FIMP, KAHQ, IYSC, and JLV.R. The first draft of the manuscript was written by CFM, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding The present study was funded by the project “Reproductive and nutritional study to increase the yield of aquaculture production in Loreto” (41-2016) of the Instituto de Investigaciones de la Amazonia Peruana (IIAP).

Declarations

Ethics approval Experimental procedures with animals were carried out in accordance with Peruvian legislation for animal protection and welfare in compliance with the protocol of the ethics committee for the use of animals in acts of experimentation, research, and teaching (30407/2016).

Conflict of interest The authors declare no competing interests.

References

- Adeyemo OK, Okwilagwe OO, Ajani F (2009) Comparative assessment of sodium EDTA and heparin as anticoagulants for the evaluation of haematological parameters in cultured and feral African catfish (*Clarias gariepinus*). Braz J Aquat Sci Tech 13:19–24
- Andrade JIA, Ono EA, Menezes GC, Brasil EM, Roubach R, Urbinati EC, Tavares-Dias M, Marcon JL, Affonso EG (2007) Influence of diets supplemented with vitamin C and E on pirarucu (*Arapaima gigas*) blood parameters. Comp Biochem Physiol Part A Mol Integr Physiol 146:576–580

- Andrade-Porto SM, Affonso EG, Kochhann D, Malta JCO, Roque R, Ono EA, Araujo CSO, Tavares-Dias M (2017) Antiparasitic efficacy and blood effects of formalin on *Arapaima gigas* (Pisces: Arapaimidae). *Aquaculture* 479:38–44
- Bain BJ, Lewis SM, Bates I (2006) Basic haematological techniques. In: Lewis SM, Bain BJ, Bates I (eds) *Dacie and Lewis Practical Hematology*, 10th edn. Churchill living stone Elsevier, Germany, pp 25–54
- Bogdanova A, Makhro A, Wang J, Lipp P, Kaestner L (2013) Calcium in red blood cells: a perilous balance. *Int J Mol Sci* 4:9848–9872
- Blaxhall PC (1972) The hematological assessment of the health of freshwater fish: a review of selected literature. *J Fish Biol* 4:593–604
- Campbell TW (2015) *Exotic animal hematology and cytology*, 4th edn. Wiley-Blackwell, Ames
- Campbell TW (2004) Hematology of Birds. In: Thrall MA (ed) *Veterinary hematology and clinical chemistry*. Lippincott Williams and Wilkins, New York, pp 225–258
- Cavero BAS, Ituassú DR, Pereira-Filho M, Roubach R, Bordinhon AM, da Fonseca FAL, Ono EA (2003) Uso de alimento vivo como dieta inicial no treinamento alimentar de juvenis de pirarucu. *Pesq Agropec Bras* 38:1011–1015
- Ciepliński M, Kasprzak M, Grandtke M, Steliga A, Kamiński P, Jerzak L (2019) The effect of dipotassium EDTA and lithium heparin on hematologic values of farmed brown trout *Salmo trutta* (L.) spawners. *Aquac Int* 27:79–87
- Dantas PDS, Chagas E, Dairiki J, Boijink CDL (2012) Heparina e EDTA como anticoagulantes para pirarucu (*Arapaima gigas*). In: Moraes RR, Boijink CDL, Da Silva KE, Quisen RC (eds) *Anais da IX Jornada de iniciação científica da Embrapa Amazônia Ocidental*. Embrapa Amazônia Ocidental, Manaus, pp 203–209
- Dias MKR, Yoshioka ETO, Rodriguez AFR, Ribeiro RA, Faria FSEDV, Ozório ROA, Tavares-Dias M (2019) Growth, physiological and immune responses of *Arapaima gigas* (Arapaimidae) to *Aeromonas hydrophila* challenge and handling stress following feeding with immunostimulant supplemented diets. *Fish Shellfish Immunol* 84:843–847
- Ekanem AP, Udoh AJ, Inyang-Etoh AP (2012) Effect of different anticoagulants on hematological parameters of *Oreochromis niloticus*. *Int J Sci Adv Technol* 2:17–20
- Faggio C, Arfuso F, Piccione G, Zumbo A, Fazio F (2014) Effect of three different anticoagulants and storage time on haematological parameters of *Mugil cephalus* (Linnaeus, 1758). *Turkish J Fish Aquat Sci* 14:615–621
- Farias THV, Pereira NL, Pádua SBD, Alves LDO, Sakabe R, Belo MADA, Pilarski F (2016) Na₂EDTA anticoagulant impaired blood samples from the teleost *Piaractus mesopotamicus*. *Pesq Vet Bras* 36:431–435
- Fazio F (2019) Fish hematology analysis as an important tool of aquaculture: a review. *Aquaculture* 500:237–242
- Fernandez-Méndez C, Troncoso AG, Green SB, Gonzales AF, Ruiz JM, Chirinos CR (2017) Artemia in la adaptación al consumo de alimento balanceado de paiche *Arapaima gigas*. *Folia Amazon* 26:187–194
- Goldenfarb PB, Bowyer FP, Hall E, Brosious E (1971) Reproducibility in the hematology laboratory: the microhematocrit determination. *Am J Clin Pathol* 56:35–39
- Gonzales A, Mejía F, Huanuiri K, Sánchez I, Vásquez J, Fernández-Méndez C (2016) Valores hematológicos y bioquímicos de juveniles de paiche *Arapaima gigas* en cultivo intensivo. *Folia Amazon* 25:137–144
- Harr KE, Raskin RE, Heard DJ (2005) Temporal effects of 3 commonly used anticoagulants on hematologic and biochemical variables in blood samples from macaws and Burmese pythons. *Vet Clin Pathol* 34:383–388
- Hattingh J (1975) Heparin and ethylenediamine tetra-acetate as anticoagulants for fish blood. *Pflügers Archiv* 355:347–352
- Hawkey CM, Dennett TB (1989) Color atlas of comparative veterinary hematology. *Vet Clin Pathol* 18:108–108
- Imbiriba EP (2001) Production potential of pirarucu, *Arapaima gigas*, in captivity. *Acta Amazon* 31:299–299
- Kaestner L (2013) Cation channels in erythrocytes - historical and future perspective. In: *Calcium signalling*. Springer, Berlin
- Korcock DE, Houston AH, Gray JD (1988) Effects of sampling conditions on selected blood variables of rainbow trout, *Salmo gairdneri* Richardson. *J Fish Biol* 33:319–330
- Knowles S, Hrubec TC, Smith SA, Bakal RS (2006) Hematology and plasma chemistry reference intervals for cultured shortnose sturgeon (*Acipenser brevirostrum*). *Vet Clin Pathol* 35:434–440

- Luz JR, Ramos APS, Melo JFB, Braga LGT (2019) Use of sodium butyrate in the feeding of *Arapaima gigas* (Schinz, 1822) juvenile. *Aquaculture* 510:248–255
- Mafuvadze B, Erlwanger KH (2007) The effect of EDTA, heparin and storage on the erythrocyte osmotic fragility, plasma osmolality and haematocrit of adult ostriches (*Struthio camelus*). *Vet Arh* 77:427–434
- Maqbool A, Ahmed I, Sheikh ZA (2013) Effects of two commonly used anticoagulants on haematology and erythrocyte morphology of rainbow trout (*Oncorhynchus mykiss*). *Int J Fish Aquat Stud* 2:239–243
- Mirghaed AT, Ghelichpour M, Hoseini SM, Amini K (2017) Hemolysis interference in measuring fish plasma biochemical indicators. *Fish Physiol Biochem* 43:1143–1151
- Natt P, Herrick A (1952) A new blood diluent for counting the erythrocytes and leucocytes of the chicken. *Poult Sci* 31:735–738
- Nuñez J (2009) Domestication de nouvelles espèces d'intérêt piscicole en Amazonie. *Cah Agri* 18:136–143
- Oliveira EG, Pinheiro AB, de Oliveira VQ, da Silva-Júnior ARM, de Moraes MG, Rocha ÍRCB, Costa FHF (2012) Effects of stocking density on the performance of juvenile pirarucu (*Arapaima gigas*) in cages. *Aquaculture* 370:96–101
- Oliveira AT, Pantoja-Lima J, Aride PHR, Tavares-Dias M, Marcon JL (2015) In: Tavares-Dias M, Mariano WS (eds) *Fisiologia de arraías de água doce: subsídios para aplicabilidade na aquicultura*. Aquicultura no Brasil: novas perspectivas, São Carlos, pp 45–74
- Pádua SBD, Pilarski F, Sakabe R, Dias-Neto J, Chagas EC, Ishikawa MM (2012) Heparin and K₃EDTA as anticoagulants for tambaqui (*Colossoma macropomum*, Cuvier, 1816). *Acta Amazon* 42:293–298
- Ranzani-Paiva MJ, Pádua SB, Tavares-Dias M, Egami M (2013) Métodos para análise hematológica em peixes. Eduem, Maringá
- Sánchez I, Mejía F, Huanuiri K, Vásquez J, Gonzales A, Fernández-Méndez C (2017) Respuesta hematológica y bioquímica en juveniles de paiche *Arapaima gigas* sometidos a diferentes concentraciones de amonio. *Folia Amazon* 26:51–58
- Smit GL, Hattingh J, Schoonbee HJ (1977) Observations on some effects of disodium ethylenediamine tetraacetate and heparin on fish blood. *Comp Biochem Physiol C Toxicol Pharmacol* 57:35–38
- Tavares-Dias M, Sandrim EFS (1998) Influence of anticoagulants and blood storage on hematological values in tambaqui, *Colossoma macropomum*. *Acta Sci Biol Sci* 20:151–155
- Tavares-Dias M, De Moraes FR (2004) *Hematologia de peixes teleósteos*, 2nd edn. Ribeirão Preto, São Paulo
- Tavares Dias M, Moraes FR (2007) Haematological and biochemical reference intervals for farmed channel catfish. *J Fish Biol* 71:383–388
- Walencik J, Witeska M (2007) The effects of anticoagulants on hematological indices and blood cell morphology of common carp (*Cyprinus carpio* L.). *Comp Biochem Physiol C Toxicol Pharmacol* 146:331–335
- Wintrobe M (1934) Variations on the size and haemoglobin content of erythrocytes in the blood various vertebrates. *Folia Haematol* 51:32–49
- Witeska M, Wargocka W (2011) Disodium EDTA used as anticoagulant causes hemolysis in common carp blood. *Turkish J Vet Anim Sci* 35:99–104
- Witeska M, Biardzka J, Kniaz J (2017) The effects of heparin concentration, storage time, and temperature on the values of hematological parameters in *Cyprinus carpio*. *Turkish J Vet Anim Sci* 41:351–356
- Yousefi M, Abtahi B, Kenari AA (2012) Hematological, serum biochemical parameters, and physiological responses to acute stress of Beluga sturgeon (*Huso huso*, Linnaeus 1785) juveniles fed dietary nucleotide. *Comp Clin Path* 21:1043–1048