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Fish hematology in Brazil: A review

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ABSTRACT

This article reviews data of Brazilian manuscripts on fish hematology in their natural environment (rivers, dams, lakes, and sea) and captivity, their relationship with parasitism, temperature, stress, nutrition, and water quality. A total of 205 articles were researched, of which 17 were published before the year 2000. Fish hematology has always been less studied than other classes of vertebrates, but blood analysis can provide information about disease processes in these animals. Although the robust interpretation of fish blood parameters is often hampered by the lack of reference values, this knowledge deficit represents an opportunity to expand clinical hematology studies across fish species. Fish hematologists are encouraged to obtain samples whenever possible as they may benefit individual animals or populations, contributing to the establishment of baseline data for many species not yet studied. Efforts are necessary to advance in this field of study clarifying some important points such as the function of each leukocyte and relating plasma changes to the species physiology.

Keywords: Hematology; Natural environment; Aquaculture; Nutrition; Parasitism; Pollution.

Hematologia de peixes no Brasil: Uma revisão

RESUMO

Este artigo revisa dados de estudos brasileiros sobre hematologia de peixes em seu ambiente natural (rios, represas, lagos e mar) e em cativeiro, sua relação com parasitismo, temperatura, estresse, nutrição e qualidade da água. São discutidos trabalhos publicados desde o início dos estudos de pioneiros nessa área e artigos publicados de 2000 a 2023. Foi pesquisado o total de 205 artigos. Destes, 17 foram publicados antes do ano 2000. A hematologia de peixes sempre foi menos estudada que de outras classes de vertebrados, mas as análises sanguíneas podem fornecer informações sobre os processos de doenças nesses animais. Embora a interpretação dos parâmetros sanguíneos de peixes seja muitas vezes dificultada pela falta de valores de referência, esse déficit de conhecimento representa uma oportunidade para a expansão de estudos de hematologia clínica entre espécies de peixes. Essa é uma discussão de alguns problemas mais comuns, anormalidades documentadas em peixes e percepções sobre algumas das causas de variações e anormalidades hematológicas que justificam as investigação e documentação adicionais sobre a hematologia de peixes. Os hematologistas de peixes são incentivados a obter amostras sempre que possível, pois assim podem beneficiar animais ou populações individuais, contribuindo para o estabelecimento de dados basais para muitas espécies ainda não estudadas. Esforços são necessários para o avanço dessa área da aquicultura, esclarecendo alguns pontos importantes, como a função de cada leucócito e a relação das alterações plasmáticas com as alterações fisiológicas das espécies.

Palavras-chave: Hematologia; Ambiente natural; Aquicultura; Nutrição; Parasitismo; Poluição.

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INTRODUCTION

Studies on fish blood in Brazil began with Oria (1932), who described the morphometric characteristics of erythrocytes from 24 freshwater species, belonging to five different families. The pioneering works by Pitombeira (1972), Pitombeira and Martins (1970), Pitombeira et al. (1968), Pitombeira et al. (1969), Pitombeira et al. (1975), and Ribeiro (1978) described the composition and characteristics of fish blood cells. Later, other authors endeavored to study fish blood in more depth and morphologically characterized the blood cells of different fish species: *Prochilodus scrofa* (Ranzani-Paiva & Godinho, 1983, 1985, 1986; Ranzani-Paiva et al., 1998/1999); *Rhamdia hilarii* (Kavamoto et al., 1983; Ranzani-Paiva, 1991), *Mugil platanus* (Ranzani-Paiva, 1995), *Oreochromis niloticus* (Ranzani-Paiva & Ishikawa, 1996; Tavares-Dias & Faustino, 1998; Ueda et al., 1997, 2001), *Colossoma macropomum* (Tavares-Dias & Sandrim, 1998; Tavares-Dias et al., 1998), *Piaractus mesopotamicus* (Tavares Dias et al., 1999), hybrid *Colossoma macropomum* × *Piaractus mesopotamicus* (Tavares-Dias et al., 2000b), *Leporinus macrocephalus* (Tavares-Dias et al., 2000a), *Schizodon borellii* (Ranzani-Paiva et al., 2000b), and *Tilapia rendalli* (Tavares-Dias & Moraes, 2003).

The erythrocyte number, hematocrit, and hemoglobin concentration are used to diagnose anemia in fish populations, thus indicating their health status. For example, these parameters decrease if the gills are affected. Hence, oxygen transport decreases, and oxygen absorption may also decrease due to inflammation of gill lamellae. Therefore, they may be indicators of the oxygen transport capacity in fish, thus relating to the concentration of oxygen available in the animal's environment. Changes in hematocrit caused by stress can lead to hemoconcentration or hemodilution; hemoconcentration can be due to the loss of fluids by the body or the release of erythrocytes from the spleen, increasing the hematocrit. Hemodilution can be due to some pathologies and a reduction in the number of erythrocytes and hematocrit. The total number of leukocytes and their subpopulations may indicate an immune response of fish to environmental challenges, while changes in the number of thrombocytes may indicate the conditions of hemostasis in fish.

Recent studies have shown that in Brazil the number of papers published on fish hematology increased in 2008 (43%) and 2012 (10.2%), and descreased again in 2020 (4.3%). Among native species, *Piaractus mesopotamicus* (pacu) and *Colossoma macropomum* (tambaqui) showed high frequency in these studies, as these species together with their hybrids are widely used in national fish farming (Lizama et al., 2020).

Initially, these articles were directed mainly to fish in their natural environments (Ranzani-Paiva, 1981), as it was necessary to know the species in their environment and then check their performance in captivity. Studies have been also focused on the biochemical constituents of fish blood plasma and serum (Castro et al., 2021; Labarrère et al., 2013; Tavares-Dias & Moraes, 2010; Tavares-Dias et al., 2004, 2008b), as these analyses of blood also help in assessing the general health status of these animals.

Blood parameters can be important diagnostic and prognostic tools in fish, with well-established laboratory protocols and reference values. Erythrogram is of great value in identifying fish anemic processes and respiratory capacity, while the leukogram can be used as a diagnostic aid in infectious processes, as well as in understanding the immune system (Tavares-Dias & Mataqueiro, 2004) and homeostatic imbalances in fish. Therefore, hematology, in addition to showing important information about the well-being of fish, contributes to the understanding of physiology, phylogenetic relationships, dietary conditions, and other ecological parameters in natural populations of fish, and in cultured populations.

Studies on the origin, morphology, and function of blood cells have been carried out to clarify the function of each one and their involvement in the protection against diseases. Cytochemistry studies have proven effective in differentiating specific cell lineages and elucidating their functional properties. In this sense, studies to characterize the structure and the morphocytochemical, immunohistochemical, and ultrastructural aspects of the fat snook *Centropomus parallelus* head kidney and peripheral blood were carried out by Santos et al. (2011), Silva et al. (2011), and Tavares-Dias and Moraes (2006, 2007). The head kidney is characteristically hematopoietic and presents in its parenchyma aggregates of lymphoid cells containing populations of immunopositive T lymphocytes in a nodular arrangement and around blood vessels, and melanomacrophage centers. Among the cells constituting and surrounding these aggregates, there were macrophages and monocytes, and their precursors. Among these, there are granulocytic lineage cells in various phases of maturation and positive for lysozyme and periodic acid Schiff (PAS). This organ is responsible for the erythrocytic, granulocytic, lymphocytic, monocytic, and thrombocytic series production.

A review of the fish blood clotting system was carried out by Tavares-Dias and Oliveira (2009), comparing and discussing the different aspects of hemostasis mechanisms in teleost fish. Calcium, in high concentrations, accelerates intrinsic coagulation factors (Doolittle & Surgenor, 1962; Doolittle et al., 1976). This study also discusses the implications of thrombocytes and differences in blood clotting time among fish species.

Eosinophil and basophil functions in fish are still uncertain. There is evidence of eosinophil participation in defense against parasites, as observed in other vertebrates, and basophil's involvement in the phagocytosis process, especially in the removal of cell debris. Perhaps for this reason, one can find basophils exhibiting cytoplasmic vacuolization in some fish (Ranzani-Paiva et al., 2023).

Blood cells play an important role in protecting organisms and the characterization of each, as well as the determination of their number in peripheral blood and immunocytochemical properties, merited studies in several species of Brazilian fish. Santos et al. (2011) and Veiga et al. (2000, 2002) described thrombocyte and leukocyte morphology and ultrastructure in *Salminus maxillosus* and *Centropomus parallelus*; in addition, other studies have also reported the blood count to add knowledge to the biology of species (Ranzani-Paiva et al., 2001, 2003).

Another important contribution of these studies concerns the phagocytosis some blood cells perform. Dias et al. (2011) carried out a study to determine the migration time of monocytes/macrophages to the peritoneal cavity in matrinxã *Brycon amazonicus*, through the technique of inoculation of *Saccharomyces cerevisiae*, and to check for possible alterations in hematological parameters after the stimulus. With Dias et al. (2011) studies, the phagocytic index and phagocytic activity were determined by quantifying macrophages and how many particles they phagocytosed.

Also, Tavares-Dias et al. (2007c) discussed that the phagocytic activity of thrombocytes, which are cells similar to mammalian platelets, possibly have this property of organism's "protection". In this study, thrombocytes were observed phagocytosing cell debris. This same function of thrombocytes was discussed by Meseguer et al. (2002), as these cells, like platelets beyond their primary function in hemostasis, seem to play an active role in inflammation. As regards their phagocytic ability, the results to date are confusing, incomplete, and somewhat contradictory.

All common routine procedures in fish farming can result in stress for fish, with physical damage, changes in homeostasis, immunosuppression, and eventually death. To minimize these stress factors and damage to the fish's physical integrity, the use of anesthetics has become routine in fish farms. The use of anesthetics is a necessary practice and, in accordance with ethical standards (Normative Resolution no. 49 of the Conselho Nacional de Controle de Experimentação Animal, 2021), it is essential. Weinert et al. (2015) evaluated the hematological parameters of Nile tilapia (*Oreochromis niloticus*) subjected to two different anesthetics, eugenol and benzocaine. Most of the observed changes in the erythrogram were higher for the benzocaine group in comparison to the control group. These results suggest that the anesthetic under investigation effectively minimizes the effects of stress caused by handling and invasive procedures. Thus, the hematological variations attributed to different anesthetic protocols should be considered for the different fish species.

Freshwater fish hematology

The blood composition of fish is subjected to physiological and ecological factors such as sex, stage of gonadal development, stress, parasitism, age, seasonal cycle, pollutants, and stress, generally related to the type of environment the fish inhabits. It is necessary to know the blood patterns of fish in the natural environment to later compare them with the values in captivity.

Studies were carried out to standardize the values of hematological parameters between sexes in *Gymnotus inaequilabiatus* from the Pantanal of Mato Grosso do Sul, Brazil (Rodrigues et al., 2018), and indicated that females had a higher percentage of immature leukocytes than males. *Osteoglossum bicirrhosum* (Tostes et al., 2019), *Mylossoma duriventre* (Chamy et al., 2015), *Cichla monoculus*, *Cichla temensis*, and *Cichla vazzoleri* (Castro et al., 2021), tropical species from lakes and rivers from the north of Brazil, have been studied. The results are pioneers and can be used to compare to other future ones.

Carvalho et al. (2009) examined the hematology of three species of fish native to the Tocantins River and found some differences in the number of erythrocytes among the species. *Squaliforma emarginata* presented a greater number of blast cells (orthochromatic and polychromatophilic erythrocytes), which were attributed to respiratory adaptations of a behavioral and/or physiological nature, related to the environment. These immature red cells are common in the peripheral blood of fish and rarely indicate any pathogenesis (Ranzani-Paiva et al., 2023). Immature leukocytes are commonly found in the circulation of healthy fish, and the final maturation of these cells can occur in the bloodstream. This physiological capacity for differentiation does not occur in mammals. For this reason, when immature leukocytes are present in fish blood, a false leukemic state is produced (Ranzani-Paiva et al., 2023). The leucocyte number is also different among species, possibly related to the niche occupied, favored by exposure to different microorganisms and environments.

Ranzani-Paiva et al. (2001, 2003) studied the blood of dourado, *S. maxillosus*, from the Mogi-Guaçu River, characterizing the cells and hematological parameters during different stages of the life cycle. These studies revealed that the blood undergoes changes in composition during the reproductive cycle and growth, and in the spawning period, and the blood red series tend to decrease in value, when the fish deplete their energy during reproduction. Another interesting finding was that younger fish tend to have higher erythrocyte number values than adults.

Studies compared the blood parameters of *Hoplias malabaricus* and *Geophagus brasiliensis* living in an urban and two rural areas and indicated that hematological indices among individuals collected from the rural area were lower than those from the urban area. This variation in hematological indices was related to poor water quality in the urban environment, which demands higher physiological and adaptive mechanisms. Eosinophil and basophil granulocytes were not found in both fish species (Romão et al., 2006). The same was described by Ranzani-Paiva et al. (2005) for parasitized Nile tilapia from the Guarapiranga Reservoir, a place with the existence of pathogenic enterobacteria in quantities greater than acceptable.

The results of blood cell parameters were established for *B. amazonicus* and other Bryconidae species with variations among species. However, the presence of the blood granulocytes, neutrophils, and heterophils in *B. amazonicus* suggests that the last leukocytes can be a characteristic of the family Bryconidae (Tavares-Dias et al., 2008a). The heterophil cells are involved in the acute inflammatory response, and work to control bacterial, viral, and parasitic infections. Heterophils are round cells when stained with Romanowsky stains, their fusiform granules appear in both colors similar to neutrophils and eosinophils (Maxwell & Robertson, 2007).

Marine fish hematology

Marine fish represent an important economic resource for many countries. They are often considered one of the most economical promising sources of animal protein for human consumption. Hematology and blood biochemistry are essential tools for the diagnosis of diseases caused by environmental changes, nutritional imbalance, or even the presence of pathogens. The erythrogram, in addition to being used to diagnose anemia, can characterize different populations and physiological strategies related to environmental variations in fish populations. The main difference between fresh and marine water fish blood is the erythrocyte volume. The total number of erythrocytes for marine fish is higher than for freshwater ones. This was shown by Guerra-Santos et al. (2012) in a study with *Rachycentron canadum* parasitized with *Amyloodinium ocellatum*. Even in the parasitized specimens, the mean erythrocyte number was $4.3 \times 10^6 \cdot \mu L^{-1}$, considered high compared to other marine fish species.

During their life, fish go through the entire process of gonad maturation and other stages until spawning, processes that require energy, which is accumulated during the resting phase and consumed during gonadal maturation and spawning. During the maturation period, there is an increase in the secretion of estradiol, a sex hormone which inhibits the release of thyroid hormones, which regulate the body's metabolism. In this way, the energy available in the fish, which would be destined for body growth, can be mostly destined for gonadal development (Lima et al., 2013).

In Brazil, a few studies have been conducted with marine fish comparing the gender and maturation stages. Parasitized males, females, and immatures of *Mugil liza* were studied, comparing the erythrogram, protein levels, and the hepato and splenosomatic ratios (Ranzani-Paiva & Tavares-Dias, 2002). Gueretz et al. (2020) revealed that hematocrit and total proteins were lower in immatures than in females of *Mugil curema*, while the number of lymphocytes was lower in males. Splenomegaly was recorded suggesting the development of leukocyte responses to infections or the production of erythrocytes for blood replacement in an anemic process. The increase in spleen volume occurs due to biochemical, physiological, and immunological changes necessary to maintain organic homeostasis in response to infections (Lowe-Jinde, 1980).

Fish live in very close contact with the environment and are, therefore, very susceptible to changes in the environment that can be reflected in their blood components. Comparison of the marine fish blood living close to large coastal cities are instruments that help environmental monitoring. Some changes were reported by Abujamara et al. (2011) in the croaker *M. furnieri* erythrogram, and by Seriani et al. (2013) in the leukogram and thrombogram of *C. parallelus* from the marine coast. In the last study, the frequency of micronuclei, nuclear anomalies, and erythroblasts was determined, in addition to the absolute number of leukocytes and thrombocytes. Micronucleus is the result of chromosome fragmentation, which produces a small nucleus next to the cell's original nucleus. As a genotoxicity marker, these variations in the erythrocyte nucleus are used for water quality monitoring. These studies results were attributed to natural variations of the species under study in the winter period, due to the great difficulty of isolating a single factor to correlate the changes. Similar results were observed by Cicero et al. (2014) and Dotta et al. (2015) for *Lutjanus analis*.

Macrophages represent a body defense cell belonging to the innate immune system acting by phagocytizing damaged and aged cells, cellular debris, foreign agents, and inert particles. Measurements *in vivo* of macrophage phagocytosis are used to evaluate the effect of different stimuli on the fish's health condition. One way to check is to inoculate *S. cerevisiae* solution intraperitoneally in fish and collect and examine the liquid a few hours later, counting the macrophages and phagocytosing macrophages (Jensch-Junior et al., 2006). Ranzani-Paiva et al. (2008) used this technique in *Centropomus parallelus* and noticed a significant decrease in almost all hematological parameters 8 hours after peritoneal inoculation of *S. cerevisiae*. Among the leukocytes, lymphocyte and neutrophil values were higher in fish inoculated with *S. cerevisiae*.

In fish, seasonal variation challenges the mechanisms of survival or acclimation mainly in the winter, when the specific immune response of these animals is suppressed, consequently making them widely susceptible to opportunistic pathogens (Jerônimo et al., 2011). Fat snook *C. parallelus* hematological parameters and the phagocytic capacity of peritoneal macrophages were analyzed related to sex, stage of gonadal maturation, and seasonal cycle (Ranzani-Paiva et al., 2008; Rôxo et al., 2018; Santos et al., 2009) verifying during female gonadal maturation stage, and in spawning period, the fish presented lower erythrocyte values than those of other stages. In addition to these results, the phagocytic capacity and index were higher in the summer and lower in the fall in females. The results showed that spring and summer correspond to seasons of the year for better hematological and phagocytic responses for the survival of the fat snook in its natural habitat. These results support the production of these animals in captivity, determining the best time to handle the animals.

Blood studies in farmed fish

Damage caused to fish is related, among other factors, to diet and supplementation, parasitism, and inadequate and excessive use of therapeutic products. With this increase, health problems become more common, requiring periodic monitoring of the fish health condition in the culture environment. Blood parameter evaluations help in understanding the homeostasis of fish under severe pathophysiological changes caused by water quality or diseases, to establish the baseline reference values of different species and to determine systematic relationships between them (Chagas et al., 2009).

Several farmed fish were studied for blood parameters determination used to increase the knowledge and for the treatment of diseases. Reference values for blood parameters, plasma levels of glucose and total proteins, alkaline phosphatase, total protein, urea, calcium, cholesterol, alanine aminotransferase, and aspartate aminotransferase, as well as the morphology of leukocytes in cultured species, were determined by Tavares-Dias et al. (2000a, 2000b) for hybrid tambacu, Tavares-Dias and Moraes (2003) for *Coptodon rendalli*, Tavares-Dias et al. (2002a) for *Rhamdia quelen*, Tavares-Dias al. (2003) for hybrids of *P. mesopotamicus*, *C. macropomum*, *B. amazonicus*, *Brycon hilarii*, *M. macrocephalus*, and *Cyprinus carpio*, Tavares-Dias et al. (2004) for *C. carpio*, Azevedo et al. (2016) and Bittencourt et al. (2003) for *O. niloticus*, and Barcellos et al. (2003) and Borges et al. (2004) for *R. quelen*.

Studies on macrophage migration have been also carried out in captive freshwater fish reporting blood parameters and plasma glucose levels for *Pseudoplatystoma* spp. inoculated intracoelomatically with 1.5 mL of *S. cerevisiae* solution, showing that the concentration of eosinophils was lower in incubated fish and there was no significant difference in plasma glucose between incubation times. Nunes et al. (2014) concluded that the 2-hour incubation time proved sufficient to promote the migration and maximum activation of macrophages in surubim.

Bittencourt et al. (2003) determined the reference ranges for hematological and biochemical values for *O. niloticus* cultivated in a semi-intensive system. Azevedo et al. (2006b), from pay-fishing and intercropped with pigs, observed a positive correlation among weight, length, mean corpuscular volume (MCV), and higher concentration hemoglobin (MCHC). Among blood cells, they did not observe basophils or eosinophils, nor their precursors.

For *P. mesopotamicus* juvenile (Tavares-Dias & Mataqueiro, 2004), *Cichla temensis* (Tavares-Dias et al., 2011), adult *P. mesopotamicus* (Bittencourt et al., 2010), *Gymnotus* spp. (Ventura et al., 2018), *P. mesopotamicus* breeders after spawning induction (Ventura et al. 2020), and tambacu hybrids (Oba-Yoshioka, 2017), the hemogram and plasma levels of cortisol, glucose, and total protein were characterized, and similarities among the hematological parameters were found.

The blood cell count of *S. brasiliensis* was done by Pádua et al. (2009), Ranzani-Paiva et al. (2002, 2003), and Satake et al. (2009) comparing the results among captive specimens. These results serve as a reference for future studies with this species, both free-living and captive. *Prochilodus lineatus* had higher erythrocyte counts, hematocrit, and hemoglobin, reflecting a considerable adaptation to survive in an environment with low oxygen levels (Tavares-Dias et al., 2008b).

The use of anticoagulants is necessary in studies with fish blood to prevent clotting, which is rapid in this group of animals and can compromise the analysis. The effects of heparin (100 IU) and sodium ethylene diamine tetracetic acid (EDTA) (3, 5, and 10%) on blood clotting and hematological parameters of surubim hybrids (*Pseudoplatystoma reticulatum* × *Pseudoplatystoma corruscans*) were described by Ishikawa et al. (2010). Coagulation was efficiently inhibited when using a_2EDTA, and samples with heparin were clotted 10 hours after the collection. There was an increase in the osmotic fragility of erythrocytes, mainly when 10 and 5% Na_2EDTA were used, but no difference between the control and heparin. The authors concluded that 3% Na_2EDTA is safe and efficient as an anticoagulant for hybrid surubim, preventing clotting for more than 10 hours and causing a slight effect on the osmotic fragility of erythrocytes.

The effect of using sodium heparin (5,000 IU) and tripotassium EDTA on hematological parameters of *C. macropomum* showed that heparin was the more appropriate anticoagulant for this fish since it was effective in preventing coagulation for more than 10 hours, without causing hemolysis, changes in hematological parameters or osmotic fragility of erythrocytes (Pádua et al., 2012). Most authors use heparin as an anticoagulant since this is a natural substance and easily accessible. However, heparin must be diluted in saline solution so that there is no waterproofing of the cell membrane of leukocytes, preventing the staining of blood smears (Ranzani-Paiva et al., 2023).

Jerônimo et al. (2015) and Labarrère et al. (2013) described total protein plasma levels and blood parameters of hybrid surubins (*P. reticulatum* × *P. corruscans*) in intensive and semi-intensive cultivation. These authors described the effects of stocking density on glucose, total cholesterol, triglycerides, urea, creatinine, alkaline phosphatase, aspartate aminotransferase, lactic dehydrogenase, calcium, phosphorus, potassium, and magnesium serum levels, but no on erythrocyte number.

Tavares-Dias et al. (2007a) described the hemogram and leukogram of *Arapaima gigas* grown in net-cages in the state of Amazon, Brazil. Red blood cell indices indicated a high demand for oxygen in this species. Another study with this same species was conducted by Drumond et al. (2010), who compared erythrocyte, leukocyte, and thrombocyte counts between fingerlings and juveniles in semi-intensive cultivation. Fingerlings had lower mean corpuscular volume (MCV), and higher mean corpuscular hemoglobin concentration (MCHC), erythrocyte number, hematocrit, and MCHC than juveniles. However, juveniles had lower lymphocyte numbers and higher monocyte, neutrophil, and eosinophil number, while both had similar thrombocyte and total leukocyte numbers.

Studies with hybrid tambacu and patinga (*P. mesopotamicus* × *P. brachypomum*) emphasized the influence of seasons and demonstrated that, in the hot season, tambacu presented higher hemoglobin concentration, MCV, MCHC values, and eosinophil numbers. In the cold season, erythrocyte, thrombocyte, total leukocyte, lymphocyte, and neutrophil numbers were higher. In patinga, the concentrations of hemoglobin, erythrocytes, MCHC, total leukocytes, and basophils were higher in the cold season (Brum et al., 2019a).

Fukushima et al. (2012) compared erythrocyte number and morphometry in blood smears of triploid *R. quelen*, and showed that triploidy increased the size and volume of erythrocytes, and, as expected, in triploid fish, circulating erythrocyte, leukocyte, and thrombocyte numbers are lower. Lymphocytes were the most predominant cells in the differential leukocyte count of diploid fish (62.5%), while monocytes were predominant in triploid fish (49.6%).

Brum et al. (2019b) evaluated the effect of a polyculture system with Pacific white shrimp (*Litopenaeus vannamei*) in brackish water on the blood parameters and condition factor of *O. niloticus* in the nursery and fattening phases. There was a significant increase in the hematocrit, MCV, MCHC, total leukocyte, and thrombocyte values, in the fattening phase compared to the nursery.

In recent years, a new form of fish farming has been added to hydroponics, which is the production of plants without soil, with roots in water, and fish in the same cultivation system, named aquapony. Only one study was carried out about fish hematology in this system. Machado et al. (2021) evaluated the hematological levels and husbandry performance of *P. mesopotamicus* in a hydroponic, biofloc systems and with water renewal, and there was no influence on erythrocytes, hematocrit, hemoglobin, MCV, MCHC, and total leukocyte number.

Some studies also evaluated the hematology of laboratory and aquarium fish. Fries et al. (2013) evaluated hematological parameters of *Carassius auratus*. Their results showed a variation in hematocrit, hemoglobin, MCV, MCHC, erythrocyte, leukocyte, and thrombocyte numbers. Maciel et al. (2016) described the morphology of blood cells of *Astronotus ocellatus* from commercial breeding and acclimatized to laboratory conditions identifying erythrocytes, three forms of thrombocytes, and four populations of leukocytes: neutrophilic and eosinophilic granulocytes, and lymphocyte and monocyte agranulocytes.

Fish hematology and nutrition

A recent study has reported that, in Brazil, 29% of published studies on fish hematology refer to nutrition (Lizama et al., 2020), thus demonstrating the use of this tool to assess the performance of animals on experimental diets. Fish health is predominantly dependent on intake, quantity, and quality of food consumed. Therefore, adequate nutrition is one of the main requirements for maintaining good quality fish in culture systems, as well as physiology. Dietary imbalances or deficiencies may lead to disease outbreaks and economic losses in fish farms. Blood parameters evaluation may help to understand fish homeostasis and biomonitoring of serious pathophysiological changes caused by inadequate nutrition in farmed fish.

Hematological changes also occur depending on the nutrients and/or supplements added to the feed of fish. Several of these substances were tested for different species, such as those by Levy-Pereira et al. (2018) and Sado et al. (2014), who fed *P. mesopotamicus* and tilapia, respectively, with Mannan-oligosaccharide (MOS), a functional oligosaccharide refined from the cell wall of *S. cerevisiae*. This yeast can activate and proliferate a large number of bifidobacteria and lactobacilli, regulate microecological balance, and it is also a high-quality soluble dietary fiber prebiotic with action on biochemical and hematological parameters. The MOS feeding increased the number of total leukocytes, monocytes, and lymphocytes in fish, but decreased the neutrophil number.

Vitamins are organic substances essential for animal health. Vitamin C assists in cellular metabolism by promoting chemical reactions that allow the absorption of nutrients. This increases the production of leukocytes and antibody levels when the body fights against pathogens. In fish, some experiments were carried out with vitamin C, for example, with *B. amazonicus*, which presented changes in hematological parameters such as in hematocrit, hemoglobin, and total numbers of erythrocytes and leukocytes (Affonso et al., 2007).

Vitamin E has as its main function the ability to act as an antioxidant, that is, this vitamin protects other molecules from oxidation reactions. In *A. gigas* (Andrade et al., 2007) and *P. mesopotamicus* (Garcia et al., 2011; Sado et al., 2013) supplemented with vitamin C or E, there was an increase in erythrocyte and leukocyte cellular numbers, and a decrease in MVC with an increase in hemoglobin and a decrease in total leukocytes, lymphocytes, neutrophils, monocytes, eosinophils, and special granulocytic cells. These studies demonstrated that vitamin E is essential for erythrocyte protection and maintenance of erythropoiesis since the highest level of this vitamin determines a smaller number of erythroblasts. In 2007, Garcia et al. tested the supplementation of vitamin C and/or E in *P. mesopotamicus* diet challenged with *A. hydrophila* and concluded that in this species these vitamins are essential for erythrocyte protection. Vitamin C alone induced an increase in the number of circulating thrombocytes in a dose-response relation, but did not protect the fish against infection with *A. hidrophila*. However, just like vitamin deficiency should be avoided, vitamins in excess can also cause damage to fish, as observed in the hematocrit and hemoglobin values.

Assays with vitamin A (vitA), an essential nutrient that acts as an endocrine regulator of several metabolic pathways, modulating normal growth and health status of animals, were carried out by Guimarães et al. (2016) in *O. niloticus*. Although the importance of vitamin A for baseline hematology and immune response is well documented for higher vertebrates, there is limited information on the physiological effects of vitA for fish. In their experiments, the animals were fed with a ration containing vitA, and hematology, immune function, and resistance to experimental infection with *A. hydrophila* and cold-induced stress were evaluated. The authors concluded that there were no clear protective effects of vitA supplementation on disease and cold stress resistance, and vitA does not seem to have a pronounced effect on leukocyte differentiation, but it plays an important role in maintaining normal erythropoiesis. According to these authors, the deficiency of vitA caused neutropenia, reduction in erythrocyte count, hematocrit, and hemoglobin, and high mortality rates in a short period.

To determine dietary levels of β-glucan and vitamin C that would allow fish to cope with different stresses, a Nile tilapia group fed these supplements was subjected to cold-induced stress and another to *A. hydrophila* infection. Red blood cells, hematocrit, MCV, total plasma protein, albumin:globulin ratio, and leukocyte, lymphocyte, neutrophil, and monocyte numbers were affected by stress and/or diets. In total, 0.1–0.2% β-glucan and 600 mg/kg vitamin C increased fish resistance to stress and 0.8% β-glucan resulted in reduced immune responses regardless of the vitamin C supplementation level (Barros et al., 2015a).

In *R. quelen* males and females fed different levels of energy and proteins. The total number of erythrocytes, hematocrit, hemoglobin, glucose, total proteins, and albumin were not altered (Higuchi et al., 2011), males had a higher erythrocyte total number, hematocrit and hemoglobin, and females had a higher thrombocyte number (Lazzari et al., 2011). These papers only has this information.

Other additives have been tested, as *Manihot esculenta* (cassava) in *C. macropomum* (Aride et al., 2016; Pereira-Junior et al., 2013), fish oil, linseed oil, soybean oil, olive oil (Araújo et al., 2011), and lysine (Liebl et al., 2022), showing no changes in the erythrogram and plasma levels of cortisol, glucose, total proteins, and triglycerides.

The use of essential oils in fish nutrition has been also a common practice in fish farming. These additives act in the intestine, modulate the innate and adaptive immune response, increasing resistance to stress and decrease the ability of pathogens to colonize the digestive tract, consequently, aiding digestion, and absorption of nutrients (Campagnolo et al., 2013).

Fish on a diet supplemented with essential oil showed a reduction in serum cholesterol and triglyceride levels after 35 days of supplementation, while the levels of serum glucose total protein, and immunoglobulins did not show differences (Brum et al., 2018). Valladão et al. (2019) used thyme (*Thymus vulgaris*) essential oil (TVEO) and medicinal plant in the diet for Nile tilapia. The total leukocyte and lymphocyte, neutrophil, monocyte, and basophil counts were increased in the TVEO group compared to the control.

The combination of essential oils and other additives is also common in experiments with tilapia. Addam et al. (2019) evaluated the effects of dietary supplementation of organic acids blend (OAB) alone or in combination with the essential oil of *Lippia origanoides* (OAE) for Nile tilapia. Increased glucose in fish fed OAB and a high number of circulating monocytes in fish fed OAE diet were observed. After 30 days of supplementation, there was an increase in the number of blood monocytes and glucose concentration. However, there were no changes in erythrocyte number, hemoglobin, hematocrit, leukocyte, lymphocyte, monocyte, and neutrophil numbers between treatments.

Oreochromis niloticus fed diets containing soybean oil (Costa et al., 2014; Martins et al., 2017), choline (Fernandes Júnior et al., 2010; Garcia et al., 2012), *Agaricus blazei* mushroom (Schalch et al., 2015), *Ocimum gratissimum* or *Zingiber officinale* (Brum et al., 2018) showed alterations in the erythrogram as such an increase in the percentage of immature leukocytes and hematocrit, and a decrease in the percentage of thrombocytes and leucogram, total proteins, total serum cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels.

The combination of oils and other additives was also tested in experiments with fish. Jesus et al. (2019) evaluated the effect of pure and protected sodium butyrate with palm oil and buffered solution in the diet for Nile tilapia on hematological parameters. An increase in the erythrocyte and monocyte numbers was observed in fish fed $Buffer_{0.5\%}$ and $Oil_{0.25\%}$, and the lowest monocyte number in different concentrations of buffer and oil.

Not only the quality of the feed but also the frequency of feeding and management are factors that interfere with the health of farmed fish. Hilbig et al. (2012) evaluated the feeding management in juvenile *P. mesopotamicus* under different feeding rates such as a period of feeding above the level used for maintenance and its hematological characteristics. The decrease in the feeding rate to 70% over satiation apparently improves the apparent feed conversion without harming the biochemical and hematological parameters of fish reared in net cages.

In *H. severus* fingerlings, Abe et al. (2022) evaluated the effects of feeding rate and frequency on blood parameters and plasma levels of glucose, total proteins, total cholesterol, and triglycerides, to improve knowledge of the adequate feeding rate and frequency for this ornamental fish species. Lower feeding rate caused blood alterations such as reduced levels of glucose, triglycerides, and erythrocyte, thrombocyte, and leukocyte numbers. The authors concluded that *H. severus* fingerlings can be reared using a 6% feeding rate with two meals a day for better utilization of the feed without affecting its hematological parameters.

Nutritional additives such as prebiotics, probiotics or symbiotics supplemented in fish feed are some of the practices most used nowadays in fish farms, because, in addition to growth promoters, they tend to increase the protection of fish against pathogens. Probiotics are generally live microorganisms that, when administered in adequate amounts, confer health benefits to supplemented fish (Rodrigues et al., 2021; Tachibana et al., 2011). The method of processing the diet and how the probiotic is included in the diet can interfere with the hematological, immunological, and microbiological parameters of the fish (Nakandakare et al., 2013).

Probiotics have a variety of beneficial effects, but their mechanisms of action have not been completely elucidated. By competitively excluding other microbiota and competing for adhesion sites in the digestive tract (Balcázar et al., 2006; Vanderpool et al., 2008), probiotics act to stimulate immunity (Coppola & Gil-Turnes, 2004) and greater production of lactic acid (Fuller, 1977; Verschuere et al., 2000), decreasing the production of toxic amines and increasing the availability of amino acids at absorption sites (Kozasa, 1989), and also to improve energy saving and increase the availability of vitamins and enzymes (Fuller, 1989). Probiotics have been also shown to enhance the non-specific phagocytic activity of alveolar macrophages, suggesting a systemic action by secretion of mediators that stimulate the immune system (Biller-Takahashi & Urbinati, 2014).

Prebiotics are components not metabolized by the body that become food for probiotics, microorganisms that stimulate the proliferation of good bacteria. The beneficial bacteria of the microbiota are essential to keep the body healthy and protected. In 2019, International Scientific Association for Probiotics and Prebiotics defined symbiotics, the use of prebiotic plus probiotic, as “a mixture involving living microorganisms and substrate(s) used selectively by host microorganisms that confers a benefit to the health of the host". Dias et al. (2011, 2020) studied feed supplementation with *Bacillus subtilis* for *B. amazonicus* in the spawning period and reported that females fed a probiotics-supplemented diet exhibited an increase in the number of oocytes and, consequently, had higher rates of fertilization and hatching of larvae. Probiotic-treated fish also exhibited an increase in the phagocytic activity of macrophages, indicating an improvement in the immune system of breeders. Hematological parameters were different in comparison to the time-control, and plasma cortisol and glucose levels were higher in the females (Farias et al., 2016).

Iwashita et al. (2015) conducted a feeding trial to investigate the effects of dietary administration of *B. subtilis*, *Aspergillus oryzae*, and *S. cerevisiae* on growth, innate immune response, hemato-immunological parameters, and disease resistance of Nile tilapia. There were no differences between the total number of leukocytes, and fish fed experimental diets supplemented with probiotics displayed elevated lymphocyte count at week 4. Fish fed the experimental diet without probiotic displayed elevated thrombocyte numbers, and fish fed probiotics showed elevated monocyte and thrombocyte numbers.

Tachibana et al. (2020, 2021) and Telli et al. (2014) added *B. subtilis* and *Bacillus licheniformis* in diets for Nile tilapia and monitored the effects on hematology, which presented variations according to the feeding strategy. Santos et al. (2020) evaluated the inclusion of increasing levels of *Saccharomyces boulardii* in the diet supplemented with palm (*Opuntia ficus-indica*) on the hematological parameters of Nile tilapia. They concluded that the inclusion of lyophilized *S. boulardii* yeast in diets containing palm positively interfered with the percentages of thrombocytes and lymphocytes, and maintained the glucose levels according to the recommended range for this species.

The biofloc system is an alternative technology for high-density production, which maintains water quality and a minimum renewal rate, thus stimulating the growth of heterotrophic bacteria and nitrifying bacteria, responsible for the autotrophic process, which plays an important role in the maintenance of water quality in the system (Barbosa et al., 2017). Poli et al. (2021) investigated the effect on fish health indicators of different Nile tilapia stocking densities when reared in an integrated culture with Pacific white shrimp (*L. vannamei*) using biofloc technology. Fish reared under the highest stocking density exhibited higher values of hematocrit and total leukocytes compared to the two lowest densities.

Jesus et al. (2021) studied the effects of dietary supplementation with sodium butyrate and *Lippia origanoides*, combined and isolated, on the health and zootechnical performance of Nile tilapia juveniles. The fish supplemented with butyrate + *L. origanoides* showed a significant reduction in the MCV, and an increase in the erythrocyte number in fish fed *L. origanoides* and butyrate + *L. origanoides*.

Propolis is a natural product produced by *Apis mellifera* with positive effects on immunological and hematological parameters, which has many different biological and pharmacological properties, such as antibacterial, antiviral, antioxidant, anti-inflammatory, and immunostimulant, among others. The effect of crude propolis powder on the growth and hemato-immune parameters of Nile tilapia, as well as its effects on resistance to challenge with *A. hydrophila*, was investigated by Orsi et al. (2017). Differences in crude propolis levels affected fish hemoglobin and neutrophil numbers following the bacterial challenge. The authors demonstrated the effects of dehydrated ginger and crude propolis powder on hemato-immune parameters in tilapia, as well as its effects on resistance to challenge with *A. hydrophila*. Differences in the supplement levels affected fish hemoglobin, and neutrophil numbers following the bacterial challenge. However, the other parameters were not altered. Dehydrated ginger powder alone affected hematological parameters, before or after challenge with *A. hydrophila*, while hemoglobin, hematocrit and MCV decreased after infection. Also, there was an increase in total leukocyte, lymphocyte, neutrophil, and monocyte numbers (Naliato et al., 2021).

Nucleotides are low molecular biochemical compounds with numerous essential physiological and biochemical functions, such as building monomeric units of nucleic acids, chemical energy transference, biosynthetic pathways, biological regulators, and coenzyme components. Functional proprieties of dietary nucleotides may have particular importance on the modulation of the fish's immune status. Indeed, it has been reported to enhance macrophages, natural killer cells, serum complement, and lysozyme and phagocytosis activity.

In this sense, Barros et al. (2015b) performed an experiment with dietary supplementation of a commercial nucleotide mixture, successfully used in terrestrial animals, for tilapia nutrition. After a period of feeding, these fish were challenged with *A. hydrophila*. Hematological profile and innate immune response were not influenced by the dietary nucleotides, but the Nile tilapia resistance to *A. hydrophila* tended to be improved.

Periods of food deprivation are frequent in many species of fish in their natural environment such as those caused by climate changes, seasonal variations, competition for food, and breeding migrations, common processes that involve starvation in fish

(Sakyi et al., 2020). Starvation causes metabolic stress attributed to metabolic changes for higher energy production and activates the production of acute-phase proteins, which defend the fish from oxidative and cellular damage (Arjona et al., 2009).

Rios et al. (2005) analyzing *Hoplias malabaricus* subjected to long-term starvation and re-fed found immature erythrocytes in peripheral blood, and showed that erythropoiesis decreased during food deprivation. Their results suggest that a process of senescence takes place in the pre-existent erythrocytes and that the cells are not replaced during starvation. After starvation, *H. malabaricus* had erythrocytes reduced, causing changes in hematocrit, MCV, and MCHC. Furthermore, during this period, the fish presented lymphocytopenia and thrombocytopenia. After re-feeding, the number of leukocytes and thrombocytes recovered, but the erythrocytes number remained reduced, and there was an increase in abnormal red cell nuclei.

Hematology and stress in fish

Stress is often described as a state in which an organism's energetic stability, termed homeostasis, is impaired by any internal or extrinsic stimuli, generally termed stressors. The response of a fish species to a stressful condition varies from species to species and depends on its ability to adapt to the surrounding environment. Common laboratory and field management practices, and those used in fish farms can cause stress in fish. Plasma glucose, which plays an important role in the metabolism of fish, is one of the most used indicators, together with cortisol, to diagnose the occurrence of physiological stress. The leukocyte profile is also particularly useful under stress, as it can be influenced by stress hormones (Urbinati et al., 2020). Changes caused by stress can increase the number of neutrophils (neutrophilia) and decrease the number of lymphocytes (lymphopenia), both of which are affected in the opposite direction. During stress, cortisol secretion reduces the lifespan of lymphocytes by promoting their apoptosis, which causes changes in the number of these cells. Under such conditions, circulating lymphocytes generally decrease in number due to their distribution between organs and/or also due to a reduction in lymphopoietic activity. Therefore, the number of leukocytes is an important immunological parameter and can also indicate the health of the fish under any stressor agent.

Hypoxic stress was studied in *C. macropomum* (Affonso et al., 2002), *B. amazonicus* (Abreu & Urbinati, 2006), *Hoplerythrinus unitaeniatus* (Mariano et al., 2009), and *Gymnotus carapo* (Moraes et al., 2002) causing alterations in some hematological, plasmatic, and seric parameters. Hypoxic stress caused by the capture of *P. mesopotamicus* provoked an increase in plasma cortisol and glucose levels, as well as a reduction in serum sodium levels, and an increase in chloride levels. However, the values of potassium, calcium, osmolarity, total erythrocyte number, hematocrit, hemoglobin, and MCV were not altered (Abreu et al., 2009). In adults of *O. niloticus* subjected to hypoxia by exposure to atmospheric air, the blood parameters, plasma levels of glucose, total protein, total cholesterol, triglycerides, calcium, chloride, sodium, and potassium were not significantly different from the control fish (Silva et al., 2012). These results corroborate the observation that differences in hematological responses depend on species and habitats, making it difficult to determine patterns for each.

Brycon amazonicus is a freshwater Neotropical fish that shows social interaction and aggressive behavior, especially in crowded environments where each dominant fish competes with other individuals for conditioned territorialism. The subordinate fish showed a significant increase in cortisol, glucose, hematocrit, and hemoglobin, demonstrating the stress they were subjected to. The immune system also indicates modulation caused by cortisol, which results in an increase in neutrophil number and a decrease in thrombocyte number. However, the dominant fish show a significant increase in monocyte numbers and a decrease in lymphocyte levels (Ferraz & Gomes, 2009). In general, when individuals are exposed to long-term stress, cortisol increases, resulting in a decrease in lymphocyte numbers with a consequent increase in neutrophils, preventing the production of antibodies, and leading to a decrease in immunity.

Neves et al. (2018), studying the blood parameters and plasma glucose levels of *Peckoltia oligospila* subjected to transport stress, concluded that the stress did not compromise their physiology, indicating that this fish is more resistant to stress than other species. However, these authors recommended that no other stressful procedures be carried out for at least 24 hours after the fish are recovered and transported, to guarantee the health and survival of the animals.

The effects of capture stress also cause different responses in different fish species. This statement can be verified by the results obtained after capture and transport stress by Barcellos et al. (2003) and Tavares-Dias et al. (2001), who observed differences in *C. macropomum* blood parameters and plasma glucose and cortisol levels, reduction in erythrocyte number, hemoglobin and hematocrit, and increase in MCV plasma glucose and cortisol levels, total leukocyte number, and lymphocyte and neutrophil numbers in *R. quelen*. In *R. quelen*, increases in plasma levels of cortisol, glucose, and alkaline phosphatase, without altering the levels of total protein, urea, calcium, cholesterol, alanine aminotransferase, and aspartate aminotransferase, were reported. Chronic and acute stress caused an increase in neutrophil number, and reduction in the number of lymphocytes, monocytes, eosinophils, and granulocytic cells (Barcellos et al., 2004). Another type of stress commonly practiced by breeders is transport. For *A. gigas*, transport caused an increase in cortisol and glucose levels, and hematocrit, with reduction in lactate levels, depending on the post-transport time (Brandão et al., 2006).

Stress caused by exposure to formalin caused an increase in glucose levels in *C. macropomum*, without altering blood levels of chloride, sodium, potassium, and calcium (Araújo et al., 2004), and the water range pH from 4 to 8 caused reduction in the number of erythrocytes, hematocrit, MCHC, and total protein levels, with an increase in plasma levels of triglycerides, sodium and potassium, and no changes in plasma glucose levels and calcium (Aride et al., 2007).

Several studies have been conducted using tilapia as an experimental animal to test different additives and different stresses due to low and high water temperatures. The use of immunostimulants for fish not only promotes immunity against pathogens, but also prevents animals from different forms of stress, including the common changes in water temperature. Furthermore, dissolved oxygen is directly related to water temperature and has a direct influence on important physiological processes for fish development, such as breathing, digestion, growth, reproduction, and behavior. Some species require low water temperatures such as trout and other species, higher temperatures such as tilapia and tropical fish. Many authors carried out experiments subjecting fish to temperature variations associated with nutritional factors. With *O. niloticus*, Araújo et al. (2004, 2011) submitted fish to stress using low water temperature (17ºC), which led to leukopenia without any alteration in the erythrogram. Similar results were obtained by Martins et al. (2004a).

In Nile tilapia, there was no change in plasma levels of cortisol, leukocytes total number, and hematocrit, while plasma levels of glucose and total number of erythrocytes increased according to the effects of capture stress. Dietary supplementation of *O. niloticus* with different levels of choline and stress by low temperature caused no changes in fish blood parameters (Fernandes Júnior et al., 2016), but chronic stress due to increased temperature (26 to 33ºC) caused reduction in the number of erythrocytes, leukocytes, and erythroblasts, and an increase in MCV (Garcia et al., 2012).

For Nile tilapia supplemented with choline (Fernandes Júnior et al., 2010), folic acid (Barros et al., 2010) increases levels of digestible protein and digestible energy (Fernandes Júnior et al., 2016). Omega-6 and omega-3 polyunsaturated fatty acids (Araújo et al., 2011), lipids such as soybean oil, fish oil, beef tallow, and their mixtures (Araújo et al., 2015), and stress by low temperature did not influence the erythropoiesis or the production of erythrocytes and leukocytes, but caused hypochromic microcytic anemia, leucopoiesis, and neutrophilia. Araújo et al. (2017) evaluated the potential functional effect of spray-dried plasma on Nile tilapia's capacity to improve health under cold-induced stress and concluded that dietary supplementation improved growth performance, intestinal health, hematological profile, and resistance to cold.

Barton and Iwama (1991) emphasized that leukopenia resulting from stress is due to a decrease in lymphocytes in the circulating blood. This was found by Barros et al. (2014) in tilapia with an increase in the number of neutrophils and monocytes, and consequently a decrease in lymphocyte number as an effect of different administration periods of dietary β-glucan and vitamin C on the non-specific immune response, physiological parameters, disease resistance to cold, transport-induced stress, and challenge with *A. hydrophila*. After transport-induced stress, fish fed the test diet for seven days required more hours to return to the baseline cortisol levels and neutrophil number. Moreover, regardless the administration period, fish required 24 hours for the number of leukocytes and glucose levels to return to the initial values. Signor et al. (2010) evaluated the hematological parameters of Nile tilapia fed diets supplemented with different levels of autolyzed yeast and zinc, before and after cold stimulation. Adding autolyzed yeast and zinc to the diets influenced the erythrocyte number. After cold stimulation, there was damage to erythropoiesis in fish that received the diets. The absence of test nutrients determined a drop in hematocrit, leukocyte number, and total plasma protein levels.

One of the consequences of the increase in water temperature is the reduction in the amount of oxygen in the water. For this reason, cold and heat are highly stressful for fish, causing harmful environmental changes. Freitas et al. (2022) evaluated the effects of dietary digestible protein levels and different stressors (cold-induced stress; heat/dissolved oxygen-induced stress; transport-induced stress; and size-sorting-induced stress) on hemato-biochemical parameters of Nile tilapia. Lymphopenia and neutrophilia were the main cell-mediated immune response, and the diet formulated to contain 22% protein was not suitable for maintaining homeostasis under temperature stress. However, Vicente et al. (2019) found no change in the hematological profile of fish subjected to heat stress when using orange peel fragments as functional feedstuff.

Stress caused by high stocking density provokes increases in lactate, cortisol, glucose, and hematocrit levels, depending on the time after stocking. The high stocking density of farmed fish is one of the most critical factors in aquaculture and animal welfare. Inadequate densities can promote aggressiveness, and excess population increases competition and negatively influences water quality. A lack of swimming space is also harmful to many fish species (Schwedler & Johnson, 1999/2000). Furthermore, the high stocking density of fish in tanks increases the amount of ammonia in the water, which is very toxic to fish, with many species not supporting concentrations above 5 $mg{\cdot}L^{-1}$; and values above 0.01$mg{\cdot}L^{-1}$ are not toxic to fish. However, exposure to ammonia did not immediately change fish physiological parameters, with latency in stress responses (Brandão et al., 2006). The high density of stocking of *C. macropomum* caused an increase in the number of thrombocytes, leukocytes, lymphocytes, monocytes, and eosinophils, without affecting the erythrogram (Costa et al., 2019).

On the blood parameters of *O. niloticus* and *C. carpio* kept under different management and feeding conditions, Ghiraldelli et al. (2006) found differences in fish between different ways to feed the animals. Some properties fed their fish (tilapia) with fish entrails, cooked rice, leftover food from restaurants, and artisanal feed or intercropping with pigs as the main source of food or commercial feed. High hematocrit, erythrocyte and lymphocyte numbers, and total leukocyte count values were observed, according to the diet. The hematological values of carp showed no variations that could be related to the environment.

Lima et al. (2015) studied *Leporinus macrocephalus* fed a diet containing garlic, cinnamon, and yeast before and after subjecting to stress from capture. After the performance test, fish were subjected to stress from capture, and the effect of additives as a stress reducer was evaluated through hematological analysis. A reduction in the number of leukocytes in fish subjected to stress was verified in all treatments. The authors concluded that diets supplemented with garlic and cinnamon led to a better performance, while not influencing the hematological patterns after the stress of capture.

Effects of parasitism on fish hematology

Physical injuries and stress are among the main causative factors of various diseases in fish, often leading to mortality in the natural environment and fish farms. Parasitic diseases, in general, can cause damage to fish production, resulting in mortalities, low growth and weight gain, and loss in the quality of the final product. However, many parasites can infect host fish without causing apparent damage; when the balance of this host-parasite relationship is broken, varied consequences may affect fish homeostasis, reflected in the blood. Thus, constant monitoring of the farmed fish health status is necessary because, with the expansion of fish farms to increase and intensify production and productivity, the increase in disease outbreaks is common.

As a consequence of parasitic infections, expressive blood parameter alterations may occur in host fish populations. Changes caused by parasites in blood parameters are related to parasite attachment sites in hosts. Parasites can compromise the gills, intestines, skin, or other organs of fish, which can harbor diverse species of parasites, affecting the endocrine, immune, and hematological systems (Bosi et al., 2022). Changes caused by parasites in the erythrogram may indicate possible anemia and in the leukogram an allergic, or inflammatory reaction.

When several species of parasites occur in various organs, changes in fish blood are difficult to interpret. Several articles were written to elucidate this situation, but the results were diverse and contradictory. For *Schizodon borellii* and *Prochilodus lineatus* infested with Dactylogyridae in the gills and captured in the floodplain of the upper Paraná River, Paraná, Brazil, and *Cucullanus pinnai* in the intestine, no significant change in leukocyte differential counts or the erythrocytic parameters was found. Those infected with Dactylogyridae, Ergasilidae in the gills, and *Neoechinorhynchus curemai* in the intestine had differences in hemoglobin rate, lymphocyte, neutrophil, and monocyte numbers. The specimen infected with *N. curemai* had a low percentage of lymphocytes and a high percentage of neutrophils and monocytes (Ranzani-Paiva et al., 2000a).

Steindachneridion parahybae infected with *Ichthyophthirius multifiliis* in gills and tegument presented an anemic process due to low hemoglobin values and total number of erythrocytes (Corrêa et al., 2019). However, in the gills of *O. niloticus*, *Cichlidogyrus sclerosus*, *Cichlidogyrus* sp., *Trichodina* sp., and *Lamproglena* sp., they caused alterations in the total number of erythrocytes, thrombocytes, and leukocytes, glucose levels, hematocrit, and percentage of lymphocytes, monocytes, and neutrophils (Azevedo et al., 2006a).

A reduction in the total number of erythrocytes and thrombocytes, and MCHC were verified in *Hoplias malabaricus* parasitized with monogeneans Dactylogyridae, *Anacanthorus* sp., and *Urocleidoides emeritus*, as well as *Contracaecum* larvae (Corrêa et al., 2013, 2015).

Arapaima gigas parasitized with *Dawestrema cycloancistrium*, *Dawestrema cycloancistrioides*, and *Polyacanthorhynchus* *macrorhynchus* showed lower values of hematocrit, MCV, and glucose levels, and higher concentrations of hemoglobin, CHCM, number of erythrocytes, monocytes, neutrophils, and eosinophils than fish infected only with *Ichthyophthirius multifiliis* (Marinho et al., 2015). In *P. mesopotamicus* with the gills infected with *Anacanthorus penilabiatus*, high parasitism caused a decrease in hematocrit, total number of erythrocytes, MCHC, and number of basophils, with an increase in the number of leukocytes and lymphocytes, while MCV and total number of thrombocytes, monocytes, eosinophils, neutrophils, and special granulocytic cells showed no differences between levels of parasitism (Jerônimo et al., 2014). However, feeding 1,000 and 2,000 $mg \cdot kg^{-1}$ of garlic in *P. mesopotamicus* infected with *A. penilabiatus* caused an increase in the erythrocyte number and thrombocyte percentage, and a decrease in lymphocyte percentage (Martins et al., 2002).

In *O. niloticus*, infection of gills with *I. multifiliis* and *Saprolegnia* sp. caused a reduction in the number of total erythrocytes, percentage of lymphocytes, hemoglobin, hematocrit, and MCHC, and an increase in MCV and percentage of neutrophils and monocytes (Tavares-Dias et al., 2002a). Studies have evaluated the effects of parasitic infections on hemoglobin, hematocrit, MCHC, and leukocytes and thrombocytes in *P. mesopotamicus*, *M. macrocephalus*, tambacu hybrids, and *B. amazonicus*. In *P. mesopotamicus*, infection with *A. penilabiatus* and *Piscinoodinium pillulare* caused an increase in the percentage of monocytes and reduction in the percentage of thrombocytes. In *M. macrocephalus* parasitized with monogenean and *P. pillulare*, there were a decrease in the percentage of lymphocytes and an increase in the percentage of neutrophils. In *B. amazonicus* parasitized with *I. multifiliis*, *P. pillulare*, and monogeneans, there was an increase in the percentage of neutrophils (Tavares-Dias et al., 2008c).

Infections with *Dawestrema cycloancistrioides* in gills of *A. gigas* caused reduction in plasma levels of total proteins and chloride, and an increase in levels of glucose, hemoglobin, number of erythrocytes, leukocytes, and lymphocytes (Araújo et al., 2009). In gills of *Epinephelus marginatus* parasitized with *Pseudorhabdosynochus beverleyburtonae* and *Neobenedenia melleni* captured from the coast of the state of São Paulo, Brazil, blood parameters were compared between the hot and cold seasons and natural and captivity environments. Similar differences were observed between the environments with higher values of hematocrit in the wild group than in cultured fish during the cold season, and no significant differences between the origins of fish were observed during the hot season (Roumbedakis et al., 2015).

A study evaluated for two years, during hot and cold seasons, hematological parameters and the occurrence of gill parasites in *R. quelen* farmed in the state of Santa Catarina, Brazil, simultaneous with the evaluation of the water quality parameters in the pond to analyze the relationship between environmental conditions, hematology, and parasitism. Monogeneans *Aphanoblastella mastigatus* and *Scleroductus* sp. were found in the gills of the host. During the hot season, there were an increase in the parasites mean intensity caused by these monogeneans, as well as in total number of leukocytes, thrombocytes, and lymphocytes, and a reduction in monocyte numbers. The changes might be caused by parasitism and/or environmental variations between seasons (Figueredo et al., 2014).

Prochilodus lineatus infected in the intestine with *Neoechinorhynchus curemai* showed an increase in MCV, and number of monocytes, and a reduction in the number of thrombocytes (Belo et al., 2013). In *M. macrocephalus* infected in the intestine with *Goezia leporini*, there were variations in the size (anisocytosis) and shape (poikilocytosis) of erythrocytes, as well as dividing erythrocytes, and a reduction in hematocrit, MVC, and percentage of lymphocytes, with an increase in the percentage of neutrophils and eosinophils (Martins et al., 2004b).

Fujimoto et al. (2009) observed a decrease in the number of erythrocytes, lymphocytopenia, neutrophilia, eosinophilia, and a significantly higher number of thrombocytes in parasitized *Centropomus undecimalis* from the region of Bragança, state of Pará, Brazil, infected with *Rhabdosynochus* sp. (gills), *Bucephalus* sp. (intestine), *Cucullanus* sp. (intestine), and *Lernanthropus* gen. sp. (gills). Santos and Tavares-Dias (2010) studied the blood parameters of *Oxydoras niger* from Coari Lake, state of Amazonas, Brazil, parasitized with *I. multifiliis*, *Chilodonella* sp., *Cosmetocleithrum* spp., *Paracavisona impudica*, *Cucullanus grandistomis*, Digenea and Cestoda. This parasite's association caused an increase in the levels of hemoglobin, MCHC, and total number of neutrophils, and a reduction in the total number of lymphocytes.

Silva-Souza et al. (2000) and Furtado et al. (2019) studied the leukocytes of *Schizodon intermedius* and *R. quelen* parasitized with *Lernaea cyprinacea* and showed a high number of immature leukocytes, monocytes, lymphocytes, neutrophils, and special granulocytic cells, and lymphocytopenia, monocytosis, and neutrophilia, as well as an increase in the number of immature leukocytes in infected fish. In *Astyanax altiparanae* from the Mogi-Guaçu River, São Paulo, Brazil, infested with *L. cyprinacea*, there was a reduction in the total number of erythrocytes and thrombocytes (Corrêa et al., 2016b). In hybrid tambacu, natural infestation with *Dolops carvalhoi* caused reduction in hematocrit and serum magnesium levels, and increase in MCV, plasma glucose levels, serum levels of total protein, sodium, chloride, number of monocytes, and special granulocytic cells (Tavares-Dias et al., 2007b).

Some studies such as by Carraschi et al. (2015) related parasitism to some treatments used to minimize the effects of chemical drugs on fish that could potentiate the deleterious effects of parasites instead of combating them. These authors studied hematological parameters of *P. mesopotamicus* naturally infected with *I. multifiliis*, *Trichodina heterodentata*, *A. penilabiatus*, *Aeromonas* sp., and *Streptococcus* sp., which were treated with enrofloxacin and toltrazuril or with florfenicol and thiamethoxam. Following toltrazuril and enrofloxacin treatment, fish exhibited leukocytosis due to lymphocytosis. With thiamethoxam and florfenicol treatment, fish showed an increase in hematocrit, hemoglobin level, MCV, and MCHC and a decrease in erythrocyte number.

Later, Carraschi et al. (2017) carried out another hematological study with *P. mesopotamicus* testing the treatment of pathogens with enrofloxacin, toltrazuril, and enrofloxacin. Fish were treated against *Aeromonas* sp., *Streptococcus* sp., *I. multifiliis*, *T. heterodentata*, and *A. penilabiatus*, and then exposed to these chemicals. The pathogens were treated with toltrazuril or thiamethoxam. Following treatment, fish exhibited leukocytosis with lymphocytosis. Thiamethoxam and florfernicol treatment increased hematocrit, hemoglobin level, MCV, and MCHC, and decreased the erythrocyte number. The infection caused death in the control fish. Drugs used in the study stimulated the immune systems of fish or caused temporary electrolyte imbalances.

Among Kinetoplastida, *Trypanosoma* is the genus with the highest occurrence in populations of marine and freshwater fish in the world, with high levels of prevalence, influencing fish health and consequently causing economic losses, mainly for fish populations kept in stress. Corrêa et al. (2016a) investigated infections with *Trypanosoma* spp. in the blood of *Hypostomus* spp. and leeches, as well as blood parameters of this host from the Tapajós River, state of Pará, eastern Amazon, Brazil. Among the 47 hosts examined, 89.4% were parasitized with *Trypanosoma* spp., and 55.4% also had leeches attached around the mouth. The number of erythrocytes, hemoglobin, MCV, MCHC, and total number of leukocytes and thrombocytes showed variations, and a negative correlation with the intensity of *Trypanosoma* spp. in hosts was found. The results suggested that the leeches were vectors of *Trypanosoma* spp. in these hosts.

Toxicity and fish hematology

During aquaculture production cycles, fish species can be impacted by multiple pathogens, being exposed to treatment with chemicals. Damasceno et al. (2016) tested graded levels of dietary copper supplementation on the growth, hematological parameters, ceruloplasmin activity, and resistance to high-temperature stress in *O. niloticus*. The toxicity of dietary copper supplementation impaired the growth performance and influenced the hematological parameters after the feeding period. Therefore, considering the fish's health, levels of available copper close to the required amount were sufficient to ensure the health of Nile tilapia, even under stress conditions, whereas an excess of this mineral in the diet impaired the health of this species.

Comparisons of the blood composition of some fish species from different environments were carried out, and the studies presented a wide variety of results. Hematological parameters of *Astyanax fasciatus* (Alberto et al., 2005), *H. malabaricus*, *Geophagus brasiliensis* (Romão et al., 2006), *Pimelodus maculatus* (Jerônimo et al., 2009), and *O. niloticus* were analyzed (Seriani et al., 2011) in environments with different levels of contamination. There was no alteration in *A. fasciatus* hematological parameters and rare gill tissue anomalies and parasites in fish demonstrating the great capacity of this species for adaptation to the environment with a high ability to live in ion-poor and soft waters, being able to compensate for environmental changes caused by untreated domestic sewage discharges. In *A. fasciatus*, *H. malabaricus*, and *G. brasiliensis*, there were an increase in the hematocrit and a reduction in the number of lymphocytes and monocytes in fish from the place that received sewage discharge; however, the total number of erythrocytes, leukocytes, and thrombocytes were similar between the environments. *O. niloticus* collected in the control pond exhibited increased thrombocytes and immature erythrocytes number, as well as lymphocytes, and monocytes percentages. Total leukocytes and neutrophils differed between the pond and control. For *P. maculatus*, there were an increase in hematocrit and a reduction in the number of lymphocytes and monocytes in fish from the polluted site. The total number of erythrocytes, leukocytes, and thrombocytes did not vary between environments.

Selenium is described as an essential micronutrient that participates in different biological functions, such as the maintenance and regulation of the antioxidant defense system. However, when in high concentrations, selenium may cause toxic effects, as well as hematological changes in fish. Seriani et al. (2012) determined the toxicity of selenium in the form of sodium selenate in *O. niloticus* using the hematological parameters, after exposure to different concentrations of the substance. The acute response to exposure to sodium selenate included alterations in the hemoglobin, MCV, and MCHC and an increase in the total number of leukocytes, mainly due to the increased number of lymphocytes, monocytes, and neutrophils. Selenite at the tested concentrations was sublethal, and also caused hematological changes in Nile tilapia (Ranzani-Paiva et al., 2014).

Seriani et al. (2015b) assessed the antagonistic action of selenium against the toxicity of mercury (Hg) in *O. niloticus*. After seven days of exposure, cytogenotoxic effects and increased erythroblasts were caused by mercury, as well as leukocytosis triggered by Hg + sodium selenite, leukopenia associated with sodium selenate, and anemia triggered by Hg + sodium selenate. The results suggest that short-term exposure to chemical contaminants elicited changes in blood parameters and produced cytogenotoxic effects.

Fish biomonitoring using biomarkers is a useful tool for the assessment of aquatic pollution. Seriani et al. (2015a) collected water samples and *O. niloticus* from a pond in the Parque Ecológico do Tietê (PET), that lies along the Tietê River, São Paulo, Brazil, and from a control site (an experimental fish farm). The results of the water analysis indicated metal levels above the legal standards for Fe, Ni, Mn, and Pb. Compared to the controls, the results revealed high numbers of erythrocytes, leukocytes, lymphocytes, erythroblasts, and MCV; however, the hemoglobin content and MCHC values were low. The frequencies of nuclear abnormalities and micronuclei were higher in the PET fish. These results suggest that fish from the contaminated sites exhibit physiological responses, which probably indicate health disturbances. Furthermore, the results suggest that blood parameters and non-destructive targets can be used for pollution monitoring.

One of the techniques used to verify a product toxicity or genotoxicity is the counting of micronuclei (MN), which designates the cellular structures resulting from whole or fragmented chromosomes, during the process of cell division and, thus, are not included in the nucleus of the daughter cell, remaining in the interphase cells cytoplasm. For these studies, blood cells in smears are stained with Fast-Green as previously described by Stich et al. (1982), and the MNs are counted in a certain number of cells. In addition to MNs, nuclear erythrocytes anomalies also suggest genotoxicity and are included in the counts. Seriani et al. (2010) carried out a study counting these anomalies using usual dyes (May-Grünwald Giensa) in hematology. The results were satisfactory and allowed the standardization of classic hematological dyes for these studies in a short space of time and with less difficulty in staining.

Despite the known potassium permanganate efficacy for the treatment of fish diseases, information about their potential toxicity in non-target organisms is still very limited. França et al. (2013) evaluated the sublethal effects of this compound in *O. niloticus* using hematological analysis and oxidative stress (reduced glutathione concentration, glutathione *S*-transferase activity, catalase, and lipid peroxidation) as biomarkers. Fish exposed to a concentration of 1 $mg{\cdot}L^{-1}$ had no variation in blood parameters. At 4 $mg{\cdot}L^{-1}$, changes in hematological parameters of the exposed fish indicated hemolysis as a result of the oxidizing action of potassium permanganate. Regarding the oxidative stress analysis, only the reduced glutathione presented an increase in fish exposed to potassium permanganate, indicating adaptive and protection responses against oxidative stress. This study demonstrated that concentrations of potassium permanganate (1 to 4 $mg{\cdot}L^{-1}$) usually recommended for the treatment of fish diseases can be toxic to this fish species.

One of the biggest concerns in fish farms is water quality. The amount of chemicals frequently used in fish farms is large, and among these products there are some insecticides and other products. Their use affects in both direct and indirect ways the aquatic communities, causing acute, subacute, or chronic effects. Furthermore, the indiscriminate use of nonregistered chemicals as drugs can result in bacteria resistance and direct toxicity to non-target animals (Carraschi et al., 2015; Ikefuti et al., 2015). In fish farms, inadequate management combined with changes in water temperature and excess metabolic waste can cause stress, interfering with fish immunity and increasing the possibility of diseases caused by a wide variety of pathogens, which can generate economic losses. Studies have been carried out to test some drugs used as parasiticides in fish to minimize the effects of the pathogenic agents on animal health. One of the studies was carried out by Ikefuti et al. (2020), who used hematological parameters of *P. mesopotamicus* exposed to teflubenzuron. They observed an increase in the number of erythrocytes, leukocytes, thrombocytes, and lymphocytes and reduction in MCHC compared to the control, and this chemical did not cause changes in water quality.

FINAL CONSIDERATIONS

Fish hematological studies are useful in diagnosing diseases, as well as investigating the extent of blood damage caused to animals. Blood parameters act as efficient indices to examine health status, physiological changes, and metabolic disturbances in fish; they also act as biomarkers in the field of environmental toxicology, water quality, deficiencies, and diseases caused by stress and pathogens in natural fish populations and aquaculture.

This study also showed that, in recent years, there has been an increase in the use of fish hematology as a complementary tool for analyzing the effects of captivity, nutrition, parasitism, stress, and water quality in analysis of fish health; however, there has been a reduction in the number of studies describing baseline blood parameters in wild fish. It is evident the difficulty of establishing baseline standards for any species of fish, mainly due to the great variability of the natural environments in which they live or are raised. Furthermore, it was found that there are few studies with native Brazilian species and that most studies concern species in captivity (Table 1). Also, articles on 26 species from freshwater environments, 13 from marine environments, and 135 in captivity were found.

Table 1. Number of fish species studied in Brazil from 2000 to 2022.

Species	Number	Species	Number
Osteoglossidae	1	*Brycon amazonicus*	8
Serrasalmids	1	*Brycon orbignyanus*	1
Oreochromis niloticus	56	*Arapaima gigas*	5
Tilapia rendalli	1	*Salminus brasiliensis (S.maxilosus)*	6
Cichla spp.	1	*Leporinus macrocephalus*	3
Geophagus brasiliensis	2	*Prochilodus lineatus (P. scrofa)*	4
Cichla temensis	1	*Carassius auratus*	1
Astronotus ocellatus	1	*Cyprinus carpio*	1
Heros severus	1	*Mylossoma duriventre*	1
Lutjanus analis	1	*Psectrogaster amazonica*	1
Pseudoplatystoma spp.	1	*Astyanax altiparanae*	1
Peckoltia oligospila	1	*Mugil platanus*	1
Rhandia quelen	9	*Schizodon borellii*	1
Squaliforma emarginata	1	*Schizodon intermedius*	1
Pimelodus maculatus	1	*Gymnotus inaequilabiatus*	1
Oxydoras niger	2	*Gymnotus spp.*	1
Ictalurus punctatus	1	*Gymnotus carapo*	1
Auchenipterus nuchalis	1	*Mugil curema*	1
Peckoltia oligospila	1	*Centropomus parallelus*	1
Hypostomus spp.	1	*Hoplerythrinus unitaeniatus*	1
Pachycentron canadum	1	*Steindachneridium parahybae*	1
Hoplerythrinus unitaeniatus	1	**Hybrids**	
Hoplias malabaricus	4	*P. punctifer x Leiarius marmoratus*	1
Colossoma macropomum	11	*P. mesopotamicus x C.*	4
Piaractus mesopotamicus	16	*P. reticulatum x P. corruscans*	3
Freshwater	**Marine**	**Farmed fish**	**Others**
26	13	135	12

CONFLICT OF INTERESTS

Nothing to declare.

DATA AVAILABILITY STATEMENT

All data sets were generated or analyzed in the current study.

AUTHORS' CONTRIBUTION

Investigation: Ranzani-Paiva, M.J T. and Tavares-Dias, M; **Formal Analysis:** Ranzani-Paiva, M.J T. and Tavares-Dias, M; **Writing – original draft:** Ranzani-Paiva, M.J T. and Tavares-Dias, M; **Writing – review & editing:** Ranzani-Paiva, M.J T. and Tavares-Dias, M; **Final approval:** Ranzani-Paiva, M.J T. and Tavares-Dias, M.

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