International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(2): 416-420
www.biochemjournal.com
Received: 08-11-2023
Accepted: 18-12-2023

Sagar G Satkar
Dept. of Aquatic Animal Health Management, Faculty of Fisheries, Kerala University of Fisheries and Ocean Studies, Panangad, Kochi, Kerala

Saiprasad Bhusare
Fish Nutrition, Biochemistry and Physiology Division, ICAR-Central Institute of Fisheries Education, Mumbai, India

Ashish Sahu
Dept. of Fisheries Resources Management, Faculty of Fisheries, Kerala University of Fisheries and Ocean studies, Pannagad, Kochi, Kerala

Vaijnath S Aitwar
Dept. of Aquatic Environment Management, College of Fisheries, Mangaluru, Karnataka, India

Santosh N Kunjir
Dept. of Fisheries Extension, Economics and Statistics, College of Fishery Science, Udgir, Maharashtra, India

Milind M Girkar
Dept. of Aquatic Environment Management, College of Fishery Science, Udgir, Maharashtra, India

Corresponding Author:
Saiprasad Bhusare
Fish Nutrition, Biochemistry and Physiology Division, ICAR-Central Institute of Fisheries Education, Mumbai, India

Stress-induced hematological and serum alterations as biomarkers in fish health assessment

Sagar G Satkar, Saiprasad Bhusare, Ashish Sahu, Vaijnath S Aitwar, Santosh N Kunjir and Milind M Girkar

DOI: https://doi.org/10.33545/26174693.2024.v8.i2Sf.614

Abstract
Hematological parameters are particularly sensitive to a wide range of environmental factors, including stress, infections, and water quality. Peripheral blood tests examine several red and white blood cell indices and frequently include biochemical assays as well. Despite the fact that employing haematological procedures is challenging and requires knowledge, there are several advantages, including the wealth of information and relatively low cost. Today, 50% of the fish consumed worldwide comes from aquaculture, and by 2030, it's expected to be the main source of fish. Due to the growth of aquaculture, importance of fish health monitoring systems have come to light more and more.

Keywords: Hematology, Fish, Serum protein, Health

Introduction
Hematological parameters are helpful tools for detecting physiological and pathological changes in fish, and they are used by researchers all over the world (Gabriel, Akinrotimi and Eseimokumo, 2011) [19]. Parameters like total Red Blood Cell (RBC) count, hematocrit (PCV), Hemoglobin concentration (Hb), erythrocyte indexes Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), total White Blood Cell (WBC) count, and thrombocytes count are all routinely assessed in fish (Campbell, 2004) [7]. Evaluation of these parameters, together with routine analysis of serum biomarkers such as glucose, triglyceride, total protein, and serum enzymes, will continue to improve fish culture by allowing for early detection of infectious disease and determining sub-lethal conditions that affect production performance (Öner, Atli and Canli, 2008; Fazio, 2019) [32, 14]. Analysis of hematological parameters can also help in the early detection of infectious diseases and identification of sub-lethal conditions that affect production (Fazio, 2019) [14]. Contaminants can be ingested by fish from water, food, sediments, and suspended particulate matter, having harmful effects on their health (Hardersen and Wratten, 1998) [21]. The pollutants in aquatic environments can cause an increase in the intracellular production of Reactive Oxygen Species (ROS), which can cause oxidative damage to the functioning of biological systems (Di Giulio *et al.*, 1989) [10].

Hematological profile
The hematology profile consists of various blood parameters that indicate the health status of the organism. Establishing hematological reference values in fish poses greater challenges compared to higher vertebrates, primarily due to their poikilothermic nature and significant reliance on physiological processes and metabolic rates influenced by external factors, with water temperature likely playing the most crucial role. Interpreting blood parameters is complex due to fluctuations caused by both internal and external factors, including sex, size, stock (density), and environmental conditions (Witeska *et al.*, 2016) [48].
Fish live in close interaction with their environment which makes them more vulnerable to physical and chemical changes that could be reflected in their blood components. There also exists a close interaction between the circulatory system and the external environment, so hematology is a useful indicator of physiological dysfunctions (Elahee & Bhagwant, 2007)

[12]. In recent years, fish hematology has been used as one of the ways for early disease detection. Comparative investigations on blood samples collected from live or anesthetized fish have shown that the hematological values in fish alter with gender, reproductive period, sexual maturity, water quality parameters, diseases, and other environmental factors (Fazio, 2019) [14], thus making it an area of great concern for the fish hematologists.

Effect of stress on hematology

When fish experience stress, blood parameters are altered. It is reported that salinity stress modifies the standard hematological characteristic of teleosts (Yada *et al.*, 2002) [49]. The leucocytes, erythrocytes, hematocrit, hemoglobin, and mean corpuscular volume of the fish decreased significantly when were kept in contact with crude oil (Omoregie, 1998) [31]. Four teleost fish species (*Gobius niger, Mugil cephalus, Sparus aurata,* and *Dicentrarchus labrax*) were analyzed to compare their hematological profiles in the Tyrrhenian Sea. The study suggested that the differences found may be associated with the feeding habits, lifestyle, and adaptability of different fish species to their habitats (Fazio *et al.*, 2013 and Malathi *et al.*, 2012) [15, 27]. Hematological parameters were determined and compared between *Channa punctata* and *C. striatus* from freshwater bodies in the Cauvery delta, Thanjavur, India. They claim there is a slight distinction between the two species. *C. punctata* had increased red blood cell count, hemoglobin, packed cell volume, mean corpuscular volume, and mean corpuscular hemoglobin concentration compared to *C. striatus. C. striatus* exhibited greater WBC count and MCH compared to *C. punctata.*

Water quality effects on hematology

Water quality characteristics including temperature, Dissolved Oxygen (DO) level, pH, calcium, magnesium, and nitrate values were proposed to affect the blood parameter values of *Silurus glanis*. When analyzing blood parameters in fish, it is crucial to take into account factors such as the fish's age, size, breeding periods, gender, seasons, physiology, habitat, and environmental variables (Küçükgül *et al.*, 2019) [25]. In 2013, Gaber *et al.* conducted a study on the impact of water quality in the El-Rahawy drain on African catfish in El-Rahawy village, Egypt. The study revealed significant positive relationships between hematological and biochemical indicators, various physico-chemical factors, and heavy metal levels in the drain.

Temperature

Various fish species exhibit distinct alterations in their blood composition when subjected to elevated water temperatures. Eels demonstrated a significant increase in plasma glucose concentrations after 6 hours of exposure to high water temperatures. Eels' hematocrit and hemoglobin levels grew considerably after six hours of exposure to severe temperatures (Gollock *et al.*, 2005) [20]. In *B. balcanicus* exposed to thermal stress produced by a temperature rise of 10 ºC for 60 minutes, the mean PCV value rose significantly, and the erythrocyte volume increased (Radoslav *et al.*, 2013) [34]. Tilapia glucose concentration rises (150-200%) in response to heat stress, indicating that the organisms are attempting to maintain a physiologically stable state. The metabolism and activity of an organism increase as the temperature rises (Zaragoza *et al.*, 2008) [51].

Ammonia

Fromm PO and Gillette JR, (1968) [17] demonstrated that environmental ammonia levels and plasma levels were correlated in rainbow trout after the exposure of 2 hr. When there is a higher concentration of ammonia in the environment, the hemoglobin has a stronger propensity to bind ammonia than it does oxygen. It was discovered by Tomasso *et al*. (1981) [42] that some freshwater fishes can experience an increase in plasma cortisol when they are subjected to acute exposure to ammonia. The morphological changes that occur in the blood are a reflection of the physiological effects that stressors have on fish. (Das *et al*., 2004 and Jeney *et al.*, 1992) [9, 23]. The Total Leucocyte Count (TLC) has raised with low total ammonia nitrogen (TAN) and declined with high TAN indicates the increase in TLC as a defence means against the stress at lower TAN. Furthermore, the increase in neutrophils and macrophages that occurs in conjunction with an increase in the concentration of TAN serves as further evidence that fish have a defensive mechanism against stress. On the other hand, this results in the opposite happening at greater TAN levels due to the enormous stress. As the amounts of ammonia in the blood grow, the glucose level in the blood will also rise. The increase in glucose levels is a consequence of the increased conversion of glycogen in the liver to glucose in order to satisfy the energy requirements brought about by the stress-induced increase in the metabolism of the cells. (Das *et al*., 2004) [9]. Ammonia stress affected the serum protein level in fingerlings of *Cirrhinus mrigala* (Das *et al*., 2004) [9]

Zhang *et al.* (2019) [50] a significant reduction in the levels of hemoglobin and white blood cells was seen at ammonia concentrations of 15 and 20 mg/l, as well as a drop in the values of red blood cells and hematocrit at 20 mg/l of ammonia. There is a possibility that the decreased total erythrocyte count is the result of decreased erythropoietic activity in the kidney (Santhakumar *et al*., 1999) [38] or hemodilution due to impaired osmoregulation across the gill epithelium (Wedemeyer *et al*., 1984) [46].

Salinity

The oxygen transport in the blood and across the gills can be affected by the difference in the decline or rise in some blood parameters. The drop in RBC is linked to osmoregulatory dysfunction brought on by excessive salinity (Soltanian *et al*., 2016 and Usha, 2011) [39, 44]. According to Elarabany *et al*., (2017) [13] There was a statistically significant increase in HCT, Hb concentration, and platelet count in (4gNaCl\L, 8gNaCl\L, and 12gNaCl\L at the corresponding times). When compared to the control group, the levels of superoxide dismutase activity (SOD), total protein, red blood cells (RBCs), and catalase activity were shown to be considerably lower at the concentrations of 4gNaCl\L, 8gNaCl\L, and 12gNaCl\L, respectively. Other parameters such as WBCs, hematometric indices (MCV, MCH, MCHC), malondialdehyde (MDA) level, glutathione reductase (GR) activity, as well as glucose, cortisol and IgM did not show any significant differences in the estimated salinity concentrations.

Effect of pollutants on hematology

According to Soni *et al.* (2006) [40], the permeability of erythrocyte membranes to pollutants not only caused the membranes to change in size and form by affecting the structure and function of the cell membranes, but it also caused the membranes to damage and even destroy them. When compared with the controls, all of the species that were found in contaminated waters had considerably reduced erythrocyte numbers, hematocrit, hemoglobin, and thrombocyte percentages, as well as significantly larger mean cell volume (MCV), leucocyte numbers, and lymphocyte percentages.

Hematology and sex

From the Musa Creek in the north-west Persian Gulf, researchers investigated the effect of sex on the hematological parameters of the yellowfin seabream, *Acanthopagrus latus*. They discovered that the red blood cell count was higher in male fish than in female fish. However, other parameters such as white blood cell count, platelet count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin differential count, and leukocyte differential count did not show a significant difference between male and female fish (Karimi *et al.*, 2013) [26]. According to the research carried out by Motlagh *et al.* (2012) [28], females had considerably larger white blood cells (WBC) and lymphocytes than men did. However, females had a significantly lower density of heterophils compared to males. Other hematological parameters did not change significantly between the sexes.

Disease

A number of blood cell abnormalities, including anemia, leukopenia, leukocytosis, thrombocytopenia, and others, can be brought on by diseases that affect fish. The examination of morphology, the determination of the packed cell volume (PCV), and the acquisition of total erythrocyte counts and RBC indices, such as mean cell volume, mean corpuscular hemoglobin concentration, and mean corpuscular hemoglobin, are all methods that can be helpful in the diagnosis of illness. In the most prominent examples, packed cell volumes were around one-third of normal, which is sixteen percent, compared to the typical range of forty-eight to fifty percent. Variable alterations were seen in the blood smears; however, in general, there was a rise in the number of immature erythrocytes, which is indicative of a response anemia (Ferguson *et al.*, 2014) [16]. Amend and Smith (1975) [1] show that hemoglobin, hematocrit and RBC count levels were significantly decreased in fishes affected by Infectious Hematopoietic Necrosis Virus (IHNV), as a result of which anemia was considered as the typical sign of IHN.

Parasitic effect on hematology

The blood-sucking behavior of young *Alitropus typus* leads to changes in the blood composition of the host fish. The fish afflicted with parasites become anemic due to blood loss, as shown by a reduction in total red blood cell count, hemoglobin levels, and hematocrit. Anemia can be classified as macrocytic or hypochromic depending on certain blood parameters such as mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and volume index (Nair & Nair, 1983) [29]. The fish had a significant drop in total protein and albumin levels due to a parasite illness. Parasitic illness also produces reactive oxygen species (ROS). The hematological and biochemical study revealed a notable decrease in red blood cells, hemoglobin, packed cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total protein, albumin, globulin, and A/G ratio. Conversely, total white blood cells, mean corpuscular volume, aspartate aminotransferase, alanine aminotransferase, urea, creatinine, uric acid, and glucose levels were considerably elevated in the catfish that were infested (Radwan *et al.*, 2021) [35].

Immuno-Biochemical Status

Immuno-biochemical profiles offer insights into the health of fish, water quality, and the relationship between immune-biochemical factors and an organism's susceptibility to changes in environmental conditions. According to reports, the increase in plasma corticosteroids due to salt stress is a feature of teleost fish (Barton & Iwama, 1991) [5]. Serum biochemical analysis, together with hematological markers, plays a crucial role in diagnosing diseases in both human and veterinary medicine. Pathological changes can be detected in the serum long in advance of clinical illness manifestation, making it a valuable supplemental diagnostic tool (Tripathi, 2003) [43].

Serum protein

According to Oluwalola *et al.*, (2020) [30], total serum protein is the component of the blood that is composed of proteins, and it rises along with fasting or any other form of stress. According to Riche (2007) [36], the concentration of this substance is an essential clinical indication of the health, stress relief, humoral defense system, and welfare of aquatic creatures. According to De Lisle (1971) [11], in order to keep the blood pH and osmotic pressure stable, blood proteins are absolutely necessary. The decline in protein levels can be attributed to a number of factors, including the fish's lower hunger, diminished capacity for synthesis and absorption, or increased protein loss owing to hemodilution (Patriche *et al.*, 2011) [33].

Globulin

According to Wiegertjes *et al.* (1996) [47], a rise in serum protein, albumin, and globulin levels is associated with a higher innate immune response in fish. This is the hypothesis expressed by the researchers. Increases in globulin levels are a consequence of stress. Low levels of globulin in fishes confer immunological tolerance, which enables them to survive in contaminated environments for longer periods of time (Javed & Usmani, 2014) [22].

Albumin

Albumin helps in the transportation of lipids in fishes and also helps in the general metabolism of fishes. It is osmotically active, transports and plastic proteins as well as changes in its blood content during fish prolonged starvation (Andreeva, 2010) [2].

Glucose

Blood glucose is a vital energy source for multiple cells. Normal blood glucose levels are regulated by the breakdown of dietary carbohydrates and a complex mechanism of internal production. There was no significant difference in glucose levels between fish that were fasted for three days and those fasted for seven days (Wang *et al.*, 2019) [45]. There was a positive correlation between blood glucose level and weight and length, and a positive correlation between total protein and hemoglobin (Bittencourt *et al.*, 2003) [6]. The stress reaction triggered by the alarm cue in Nile tilapia may vary depending on the species under investigation or the characteristics of the stimulus, as the observed pattern does not align with findings from other research. Elevated cortisol levels were noted, with no impact on glucose levels (Sanches *et al.*, 2015) [37].

Conclusion

This paper thoroughly examines the importance of hematological parameters in evaluating fish health and how they are influenced by different environmental and physiological conditions. Hematological characteristics are essential for identifying both normal and abnormal changes in fish, allowing researchers globally to track their health condition.

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