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A seasonal hematology study in major carps cultured in Ahmednagar district, and effects of probiotic feed on fingerlings growth

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Abstract :

A fish's hematological indicators are vital diagnostic tools for determining its health. A variety of studies have shown variations in hematological parameters, such as white blood cells, red blood cells, hematocrit (Hct), hemoglobin (Hb) content, mean corpuscular hemoglobin concentration, and mean corpuscular volume in fish, can provide insight into the fish's health status. To supplement or perhaps replace the use of antibacterial medicines as a disease control strategy, aquaculture has turned to probiotics, which are microorganisms or their products that benefit the host. Research on farmed fish has shown that using probiotics, whether they are monospecific or multispecific, makes fish more resistant to disease and other environmental changes like physiological stressors. This is because probiotics boost both specific and nonspecific immune parameters, such as lysozyme activities and phagocytic expression of different cytokines. Hematological indices in fish may still be useful diagnostic tools, and researchers are trying to find a safe range of values for blood parameters in different fish species. Several studies have shown that aquaculture-based probiotics may improve fish blood profiles. This review focused on hematological profile of fish, probiotics and their effect on fish hematology.

Key words : Aquaculture; Blood cells; fish health; Hematology; Probiotics.

1. Introduction :

Due to rising consumer demand and the depletion of wild-catch fisheries, aquaculture production of finfish is expected to become the world's primary source of fish by 2030, with aquaculture accounting for 50 percent of all fish eaten worldwide today. To feed the world's growing population, aquaculture will have to expand and develop sustainably, adapt to climate-driven changes in ecosystem productivity, and reduce its dependence on wild fish for aquafeed [1-3]. When it comes to protein sources that can be readily digested and offer a wide range of therapeutic effects against various health concerns, fish has long been regarded as one of the finest options. Indian aquaculture had its start in the Northeast, where fish was a staple of the cuisine. West Bengal was the most progressed in using wild-caught carp seed in the Northeastern states by the early 1950s. An early attempt at aquaculture had promising results, although farmers' lack of technical expertise hampered their performance. Freshwater fish productivity in southern India is heavily dependent on *Labeo rohita* [4-8].

Fish illness is a major concern in aquaculture because it is extensively dispersed over the globe. A large variety of aquatic species, both for human sustenance and as attractive specimens, have been produced via aquaculture in recent years. Disease concerns in Indian aquaculture are exacerbated by bacterial infections, particularly in the production of catfish. It's easy for *Aeromonas hydrophila* to spread via inadvertent abrasions. *Staphylococcus xylosus*, *Aeromonas hydrophila*, and *Streptococcus agalactiae* have all been shown to be deadly infections in humans. In aquaculture, the use of probiotics is a great way to prevent infections and take the place of antibiotics and chemotherapy drugs. Beneficial microorganisms or their products that help the host have been used in aquaculture to prevent disease, boost growth, and in some cases replace antimicrobial compounds. Most likely, Vergio was the first person to use the term "probiotic." In his manuscript "Anti-undprobiotika," he compared the harmful effects of antibiotics and other antimicrobial substances on the gut microbial population with "Probiotika" factors that help the gut microflora. Lilly and Stillwell defined probiotics as "microorganisms that help other microorganisms grow." As feed supplements, probiotics help the host by increasing the value of the feed, helping digestion with enzymes, stopping pathogenic microorganisms, preventing mutations and cancer, boosting growth, and boosting the immune response [9-11].

Probiotics have long been recognized as essential for human, poultry, and cow health. Probiotics are often thought to be helpful bacteria that can survive in the digestive system due to their ability to tolerate acidity and bile salts. Buttermilk and yogurt, among other fermented milk products, may contain these bacteria. In addition, poultry and livestock are fed probiotics. Probiotics have only been used in aquaculture for a short time, but their importance in preventing disease is quickly becoming clear. In this review we will discussed about hematology and effects of probiotic on fish.

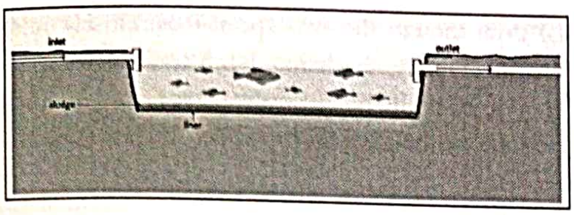

Figure 1. Diagrammatic view of aquaculture.

2. Hematology parameters :

Fish is a valuable economic resource for a country. They are typically regarded as one of the most cost-effective and promising sources of animal protein for humans to ingest. High protein and omega fatty acid levels made fish a significant source of nourishment for many people, particularly in the developing world. Das (2012) [12] evaluated that fish have a close relationship with their environment in terms of their growth, body processes, and development. Because of this, it is important to know how environmental factors affect fish health. Lack of information regarding fish hematology, particularly reference values, makes it difficult to conclude research on fish hematology [13,14].

Using blood parameters to assess the health of various farmed fish species is an effective method. A fish's hematological health status may be determined by knowing the physiological changes in hematological parameters, as well as the link between the changes in the fish's external and internal environments and changes in the fish's hematological health status. During the routine hematological evaluation of fish, erythrocyte indices such as the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) are measured, as well as the total white blood cell count (WBC), which are all diagnostic tools. Fish are very sensitive to physical and chemical changes in their blood cell components because they dwell according to their environment. Biometric indicators such as fish body weight and length, as well as other blood parameters, have been extensively studied in recent years [15-19].

Species	Anesthesia	Sampling	Anticoagulant	Diluent	n	Parameters	Reference
Acanthopagrus latus	2-phenoxyethanol	Caudal puncture	Heparin	Hayem, Turk	9	Ht, Hb, RBC, MCV, MCH, MCHC, WBC, DLC	20
T. tinca	Stunned	Cardiac puncture	EDTA	Hayem, Turk	8–16	Ht, Hb, RBC, MCV, MCH, MCHC, Lt, WBC	21
Oreochromis mossambicus	Caudal puncture	Heparin, EDTA	Automated		5	Ht, Hb, RBC, MCV, MCHC, WBC, DLC, TC	22
Oreochromis niloticus	Eugenol	Caudal puncture	EDTA	Dacie	25	Ht, RBC, MCV, MCHC, WBC, DLC	23
O. niloticus	MS-222	Cardiac puncture	EDTA	Growers	10	Ht, Hb, RBC, MCV, MCH, MCHC	24
Sander lucioperca	MS-222	Caudal puncture		Heparin	Semiautomated	Ht, RBC, MCV, MCH,	25

						MCHC, WBC, TC	
O. mykiss	Eugenol	Caudal puncture	Heparin	Dacie	6	Ht, Hb, RBC, MCV, MCH, MCHC	26

Table 1. Analytical factors and evaluated parameters in fish hematological studies.

➢ RBC :

Fange (1994) [27] says that most of the blood cells in fish are erythrocytes. Usually, they make up 98–99% of all the blood cells in these animals. Erythrocyte count (RBC), which is an important diagnostic parameter, is affected by things like the temperature of the water. It can also be changed by many biological factors, such as the fish's activity, age, sex, nutritional state, and reproductive status. It can also be different in different populations of the same species. RBC is usually between 0.5 and 1.5*106 cells per L in less active species and between 3.0 and 4.2*106 cells per L in more active species. Fazio et al. (2019) [28] found that the RBC in different fish species ranged from 0.81*106/L to 3.73*106 /L. Witeska (2016) [29] found that the RBC count in the blood of Cyprunis carpio was between 0.33 and 2.95*106/L. It's also important to note that the number of erythrocytes can change with the seasons, even if the fish are kept in a lab [30-32].

➢ Haemoglobin :

Fishery red blood cells possess tetrameric hemoglobin, like in other vertebrates. For regular measurements of fish blood hemoglobin content, scientists use the spectrophotometric cyanmethemoglobin technique (SPC). Blood hemoglobin is converted to stable methemoglobin using the Drabkin reagent, then the extinction at 540 nm wavelength is measured. The link between standard hemoglobin solutions and their extinction may be used to quantify hemoglobin concentration. Faggio (2013) found no statistical difference between the manual spectrophotometric approach and the automated method when it came to the data produced. Automated Hb measurements in fish species ranged from 4.70 to 16.6 g/dL, according to Fazio and colleagues (2019). Blood Hb levels in healthy Cyprunis carpio, aged 5–8 months, were shown to range from 62.4–69.6 g/L in research by Witeska and colleagues (2016). Hb concentrations in C. carpio vary from 34.1 to 114.3 g/L, according to data re-evaluated by Witeska (2016). According to Suljevic et al. (2016), the hemoglobin concentration of C. carpio was much lower than that of C. carassius (60.42–97.66 g/L) even though the two species are closely related. More specifically, the hemoglobin concentration in the blood of juvenile Solea senegalensis was 26–63 g/L (Peres and colleagues, 2015) under intense aquaculture conditions [33-39].

➢ WBC :

The immunological state of invertebrates may be gauged by looking at their leukocyte count, or white blood cell count (WBC). There is a frequent practice of counting white blood cells manually in fish. Because all fish blood cells are nucleated, leukocytes may be mistaken for thrombocytes or even erythroblasts when calculating WBCs. A hemocytometer and microscope are used to count fish white blood cells in diluted blood, much like erythrocytes. WBC numbers were most often assessed using Natt-Herricks, Prochazka- Skrobake's, or Dacies solutions by different authors according to Witeska (2016). Using the RBC value and the leukocyte-to-erythrocyte ratio in the blood smear, an indirect technique of calculating WBC may be used sometimes. While a colored smear may make it simpler to identify leukocytes than a hemocytometer, lymphocytes may occasionally resemble round thrombocytes [40-46].

The WBC levels obtained by Lugowska (2017) [47] were not affected by the kind of solution used. WBC levels acquired using the indirect approach were greater than those obtained using direct hemocytometer counting, indicating that the rater (the person who carried out the operation) and method type (direct vs. indirect) had a substantial impact on the leukocyte count. No significant difference was seen in WBC collected manually and mechanically, according to Faggio (2013). For fish leukocyte determination, however, automated analyzers are seldom employed. Even within the same species, the WBC levels of fish exhibit considerable variations. In invertebrates, the white blood cell count (WBC) is a critical metric in determining the state of the immune system. The amount of white blood cells in fish is often counted by hand. Because all fish blood cells are nucleated, it's important to proceed with care and expertise while calculating WBCs, as leukocytes might be mistaken for thrombocytes or even erythroblasts.

A hemocytometer and microscope are used to count fish white blood cells in diluted blood, much like erythrocytes. Natt-Herrick, Prochazka-Skrobak, and Dacie [48] solutions were most often used by different writers to estimate the amount of WBC, according to Witeska (2016) [49]. Using the RBC value and the leukocyte-to-erythrocyte ratio in the blood smear, an indirect technique of calculating WBC may be used sometimes. While a colored smear may make it simpler to identify leukocytes than a hemocytometer, lymphocytes may occasionally resemble round thrombocytes. The WBC levels obtained by Lugowska (2017) [50] were not affected by the kind of solution used. No significant difference was seen in WBC collected manually and mechanically, according to Faggio (2013) [51]. For fish leukocyte determination, however, automated analyzers are seldom employed. Even within the same species, the WBC levels of fish exhibit considerable variations.

3. Hematological Analyses :

Aquaculture, veterinary medicine, and scientific research all employ hematological analyses to assess fish health and wellbeing. Several environmental variables, including diet, water quality, stress, and infections, were shown to have a significant effect on hematological parameters. Biochemical testing may be used in conjunction with peripheral blood tests to further strengthen the evaluation of various indicators of red and white blood cells. Using hematological techniques is time-consuming and requires special training, however, the enormous amount of data and low cost make up for these drawbacks. Most fish hematology operations are performed manually, however, automated analyzers that have been adapted to deal with fish blood are also often used.

There are three basic hematological indices to be evaluated - the erythrocyte count, hematocrit, and hemoglobin concentration, which are used to produce the secondary or derived Win Trobe indices, namely the MEV, MEH, and MEHC. However, the erythrocyte count may be determined using electronic particle detectors. Therefore, the result is significantly overestimated since it includes leukocytes and thrombocytes. Leukocytes and thrombocytes make up less than 0.1 percent of the peripheral blood cells, and manual procedures have an inherent inaccuracy of 10 percent, thus the final number is insignificant. Regardless, the traditional hemacytometer-chamber approach for manually determining the EC is the most frequent and easiest way in clinical practice. Staining solutions like Shaw, Dacie, or NatHerrickl the diluent for piscine blood should be solutions, but otherwise the technique is the same for all species. Counting both the total leukocyte and thrombocyte counts at the same time is a benefit of these solutions.

Examining blood smears that have been stained with Romanowsky stains, such as the WLG, LG, or WG solutions, can help find immature erythrocytes. Due to the variable thickness of the eosinophilic background in hemolyzed samples, these preparations should not be used to evaluate immature erythrocytes or to find the different LC12 total hemoglobin content, which includes methemoglobin (metHb). To eliminate any loose nuclei from the solution, the sample should be centrifuged before the examination [51-58].

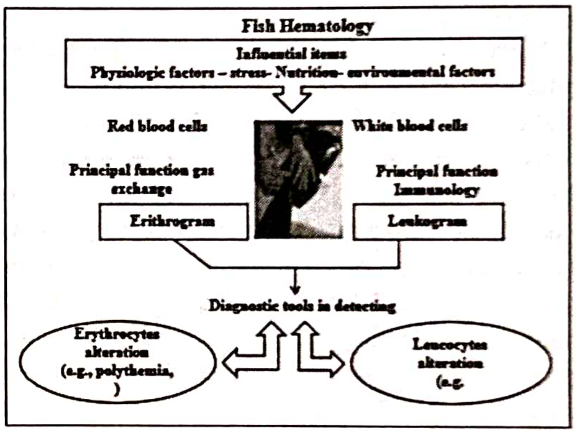

Figure 2. Hematological analyses

The acid-hematin method has also been used, even though it gives falsely high results. Samples are typically sent to a diagnostic reference laboratory for the determination of hemoglobin concentrations since this requires a centrifuge and spectrophotometer in clinical settings. As much as 25% or more of the total hemoglobin in certain fish erythrocytes includes the normal hemoglobin component metHb (methemoglobin). After the sample is collected, the Hct is measured using conventional procedures, which include centrifuging, for 5 minutes, and spinning the microhematocrit tubes at 7000 Rpm.

4. Role of Probiotics in aquaculture :

Probiotics, which include bacteria, bacteriophages, yeasts, and algae, have been examined for usage in aquaculture and have shown promising results. Many different invertebrates and vertebrates have been found to benefit from probiotics, which are beneficial to both humans and other animals. Despite these results, additional study is required to assess the significance of these findings. Probiotics have been utilized in the production of artificial feed for fish [59-61].

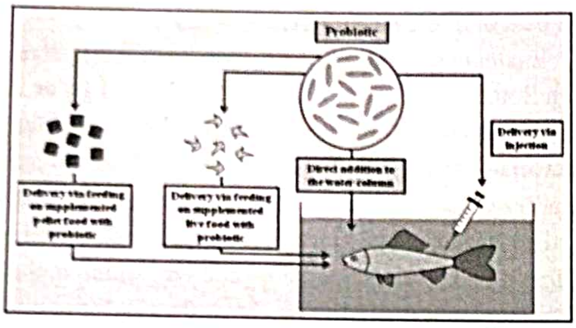

Figure 3. Probiotics in aquaculture

• Probiotic :

"Live microbial feed supplements", often known as probiotics, are cultured food that benefits the host's intestinal (microbial) balance. The use of a live microorganism as a feed supplement and its administration to the host are two of the definition's most critical elements. Although some workers have widened the scope of the term. According to Gram, a probiotic is any live microbial supplement that improves the microbial balance of the host animal while also benefiting the recipient's animal. There's nothing to feed in this case. This study said that probiotics are "any preparation of microorganisms" (but not necessarily live microorganisms) or "the parts of microorganism cells" that are good for the health of the host. In this case, humans have been left out of the need for food that has both living cells and nutrients [62,63].

In short, people have different ideas about what the word "probiotic" means. Moriarty said that the definition of a probiotic in aquaculture should include adding live naturally occurring bacteria to tanks and ponds where animals live. This is because organisms can temporarily change the bacteria in water and sediment. This is what Maeda, Nogami, Kanematsu, and Hirayama[64] have talked about when they talk about biological control. Probiotics seem to be either the whole microbe or just a part of the microbe that is good for the health of the host.

When it comes to human nutrition and food security, aquaculture has emerged as one of the most feasible and promising companies. Disease outbreaks, poor growth, and limited fish survival have hampered many intensive aquaculture operations. Fish vaccines and probiotics have been employed in aquaculture methods to reduce the risk of disease in fish populations. Not only have antimicrobial chemicals, notably antibiotics, been reduced by using probiotics in aquaculture but also the appetite and/or bio-growth performance of farmed species have been increased in an environmentally benign and sustainable way by using probiotics [65-71].

Because probiotics may be given to larvae and early fry, they are an excellent choice. Many studies have shown that fish farming may be more cost-effective if fish growth and feeding efficiency are improved. They may survive in the stomach and remain active for long periods even when stored or used in the field since they are nonpathogenic and harmless. In research by Mohanty et al., (1996)[72] a probiotic combination of bacteria and yeasts enhanced survival, weight gain, and nutrient utilization in Catla catla. According to [the kind of probiotics used affects the survival, weight gain, and nutritional utilization of fish-fed probiotic-supplemented diets.

5. Probiotics' effects on Hemoglobin concentration :

Bolliger and Everds used spectrophotometry to figure out how much total hemoglobin is in each volume of whole blood after the red blood cells have been broken down. Riggs says that hemoglobin's function is to adapt to the different metabolic needs of animals in an environment that is always changing. It also plays an important role in getting oxygen from organs that exchange gases to tissues in the body's periphery. When compared to terrestrial species, Hb establishes a boundary between the organism and the environment, particularly the change in temperature, environment, and geographical variations, as well as with O2 availability, as opposed to terrestrial species. According to several research, probiotic-enriched diets can enhance the hemoglobin levels of fish. Some probiotics, including Lactococcus sporogenes in Clarius batrachus (Indian magur), Bacillus licheniformis, Lactobacillus acidophilus, Bacillus subtilis, and Saccharomyces cervirial in Cirrihinus mrigal, have been shown to enhance the health of fish- fed a control diet. Bacteria like Lactobacillus and Bifidobacterium in Clarias gariepinus (catfish), Bacillus subtilis and Saccharomyces cerevisiae in Mori, and Lactobacillus **Acidophilus and β-glucan** in snakehead have been shown to increase the amount of hemoglobin in the fish populations tested [74,75].

6. Probiotics' effects on RBC :

The number of RBC, commonly known as **'erythrocytes', in each amount of blood, is counted** automatically using counters. Fish have oval or oblong red blood cells that may measure between 10 to 20 millimeters in length and 6 to 10 millimeters in width. High levels of the respiratory pigment and hemoglobin are the primary role of RBCs in transporting and regulating oxygen. A probiotic-supplemented diet resulted in higher levels of RBCs in fish than an unsupplemented or control diet. Bacillus subtilis, Bacillus licheniformis, Saccharomyces cervirial, and Lactococcus sporogenes were all applied to Clarius batrachus, while LAB was used in rainbow trout and Lactococcus plantarum was used in Nile tilapia, all of which were found to affect the fish's growth, development, and immune system. Probiotic supplementation has also been shown to increase the RBC count in fish [76,77].

7. Probiotics and WBC Count :

Leukocyte count (leukocyte count) is a measure of how many WBCs are present in the blood, according to Medicine Net. Fish WBCs are critical in the development and maintenance of cellular immunity and defense. Although white blood cells have been observed in organs other than the blood supply production sites (such as the spleen, kidney, and thymus), as well as their functional consequences, have been recognized. Stress and toxins may both lower leucocyte levels in fish, which governs the number of circulating white blood cells (WBCs) even though they have a modest representation (Leucocrit (Lct) = 0.3–1.0 percent). A study found that fish whose diets included probiotics could have higher white blood cell (WBC) counts than fish whose diets did not include probiotics. When compared to control diets, studies have shown that fish-fed probiotic-rich diets have better immune responses than fish-fed control diets. If probiotics are added to fish feed, the fish may have greater white blood cell levels, which might make them better able to react to stresses. Fish given probiotic-enriched diets have a stronger immune system [78].

8. Effects of Probiotics on fingerling :

Research by Subramani Munirasu et al. (2017)[79] examined the nutritional status, survival rate, and growth of freshwater fish. Labeo rohita fingerlings given false meals and additives like calcium, starch, and sardine oil were fed probiotic-enriched diets, and the results were studied. Basal with Antibiotics diet-E2 and basal diet-E1 in the control group are the experimental three groups' probiotics diets that include probiotics in their meals. To compare growth metrics, such as weight increase and specific growth rates, to control diets-E1, a 60-day feeding study was conducted. It was shown that probiotic diet (Calcium)-E3 comprised meals that included fish had considerably ($P < 0.05$) more antibacterial activity than control diets-E1. FCR values were also greater when feeding fish on experimental diet-E2 than on control diet-E1, demonstrating once again how well fish utilize their food. Freshwater fish L. rohita, according to this study, should be fed a diet high in probiotics and carbohydrates [80].

Dietary supplementation with probiotic bacteria (Bacillus pumilus) has been shown to improve the hematological health of Labeo rohita, according to a study conducted by M. Rajikkannu and colleagues (2015) [81]. For 60 days, the feeding trial was carried out. Bacillus licheniformis, Bacillus cereus, and Bacillus pumilus were given to the fish in T1, T2, and T3 in random order, and the fish in all three groups had identical body weights (5–1 gm). Similarly, the control group (T4) was served a probiotic-free diet for the same time as the treatment group. At 0, 15, 30, 45, and 60 days, researchers obtained blood samples for testing. Total RBC, Hct, Hb, and hematological indices (MCV, MCH, and MCHC) were among the measurements studied. More erythrocytes, hemoglobin, and hematocrit were found in the Bacillus pumilus-treated fish (T3) than in other groups. This study showed that Bacillus pumilus is a viable probiotic for aquaculture.

Dhanaraj M et. al. (2010) report that during a 45-day feeding study, Koi carp (*Cyprinus carpio*) fingerlings were given a diet that included *Lactobacillus acidophilus* (LAD) and/or brewer's yeast (*Saccharomyces cervisiae*). Fish with an average weight of 0.26g were fed one of four diets devised for this experiment: The simplest way is to stick to a low-calorie diet. 0.5 % LAD is the second alternative, which is the same as the first. Option three is a basic diet supplemented with 0.5 % of the SCD. Four is the standard diet + 0.25 % SCD and 0.25 %, LAD. There was a statistically significant ($P < 0.05$) difference in growth rates among the four diets tested: Diet 3, Diet 4, Diet 2, and Diet 1. Diet 3 exhibited the highest total heterotrophic count and the biggest gut microbial load of the four diets examined. When given a diet **containing 0.5% brewer's** yeast, koi carp grow quicker and have a greater gut microbial load [82].

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