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Article

Impacts of food and habitat on blood profile of rohu *Labeo rohita* (Hamilton, 1822)

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Abstract: Food and habitat are important environmental factors, which are play significant role in the physiology of fish. The present experiment was conducted to ascertain the effects of food and habitat on hematological parameters in rohu *Labeo rohita* reared from first feeding in pond with natural foods and in aquarium using formulated feed for three months (90 days). Fish were sacrificed after the rearing period and hemato-biochemical parameters (hemoglobin - Hb, red blood cell - RBC, white blood cell - WBC and glucose) were measured. Hb (%) of the fish in ponds was slightly higher than in the fish of aquarium. RBC ($\times10^6$/mm^3) in the fish reared in aquaria showed a significant decline in comparison to the pond reared fish, whereas WBC ($\times10^3$/mm^3) showed opposite scenario. Blood glucose was slightly higher in the fish of aquarium than in the fish of pond. The water quality parameters showed no significant difference between the two habitats. The data reported in this study contributes towards the knowledge of hematological evaluation in *Labeo rohita* and showed that fish food and habitat were crucial in establishing the physiological range supporting the accurate interpretation of hematological parameters for use in examining the health status of this species.

Keywords: aquaculture; water quality; hematology; Indian major carp; physiology

1. Introduction

The development of fishery resources and the rehabilitation of ecosystems are seen to be possible through aquaculture activities (Hossain *et al*., 2020a, b). One of the riskiest aspects of aquaculture is larval rearing. The creation of commercial diets with a nutritionally balanced can affect the growth performances and survival of aquatic organisms. To reduce the danger of high mortality during this stage of culture, special planning and methods are needed. Many farmed fish need zooplankton as their first food source, while others use it to grow more quickly and have greater survival rates. Fish and shellfish larvae cannot consume feed with artificial supplements. For their sustenance, they need tiny live foods. Live foods are a simple to digest source of protein for fish and shellfish. Because physiological capacities and tolerances are the transfer functions that stably link environment and individual choices with fitness consequences, biology offers a "fish-eye" view of the environment and the tradeoffs that individuals face when making decisions with fitness implications (Ashaf-Ud-Doulah *et al*., 2021). According to Weissburg and Browman (2005), physiological processes are a reflection of an organism's internal ecology, which is made up of interacting cells, tissues, and organ systems, all of which have specific functions to play within a larger ecosystem known as the individual. These interactions can scale according to an individual's fitness. Because fish have such close physical and chemical ties to their surroundings, their blood components are often a good indicator of these changes.

For the purpose of determining and controlling aquatic species' health state in natural settings, knowledge of their hematological and biochemical parameters is crucial (Shahjahan *et al*., 2021). Hematologic and plasma chemistry markers can provide predictive, albeit very varied, information based on the fish species, age, cycle of sexual maturity, and state of health (Shahjahan *et al*., 2022a,b). The effects of environmental husbandry conditions on stress, metabolic disorders, reproductive dysfunctions, and disease in wild and cultured fish can be easily assessed using periodic hematological analyses (Filiciotto *et al*., 2012).

The rohu (*Labeo rohita*) is a carp fish found in South Asia. The Rohu is a freshwater omnivore that exhibits a varied diet throughout its life cycle. During its initial developmental stages, it primarily feeds on zooplankton; however, it progressively increases its consumption of phytoplankton as it grows. As a juvenile or adult, it is a herbivorous column feeder, primarily feeding on phytoplankton and submerged vegetation. The Rohu's feeding mechanism involves utilizing modified, thin hair-like gill rakers, which allow it to filter out food particles from the water. This species is extensively cultured in South Asia and is known for its fast growth, with individuals reaching up to 35-45 cm in total length and 700-800 g in weight within a year, under normal growth conditions. It is the most extensively grown freshwater fish in Bangladesh due to its great growth potential, healthy and exquisite taste, and high market value, as well as its readily available fry and fingerlings for culture and consumer preferences. Due to its favorable compatibility with other carp species like Catla (*Catla catla*) and Mrigal (*Cirrhinus mrigala*), the Rohu has become a favored option for carp polyculture systems. Bangladesh's overall rohu production is predicted to be about 10.50% (DoF, 2018). Almost 90% of the products are consumed domestically, ensuring a year-round supply of animal protein to low and middle-income individuals. The traditional aquaculture of this carp has been practiced for several centuries in the small ponds of South Asian regions. In this experiment, we investigated the impact of food and habitat on the blood profile of rohu.

2. Materials and Methods

2.1. Ethical approval

The experiment was carried out in accordance with the guidelines for Animal Experiments at the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh.

2.2. Experimental design

The rohu, *Labeo rohita* was selected for the experiment. Hatchlings of the rohu were collected from Bangladesh Fisheries Research Institute (BFRI), Mymensingh. The collected hatchlings were reared in three aquaria fed with only artificial feed and in three ponds fed with only natural foods from their first feeding for three months.

2.3. Blood sampling

After three months of rearing, a total of eight fish from each treatment were selected for slaughtering, and their blood samples were collected through the caudal vein using a micropipette. The blood withdrawal process was completed within a minute per fish, to minimize stress and ensure accurate readings of the normal blood values. Each blood sample was collected in a sterile eppendorf tube containing ethylene diamine tetraacetic acid (EDTA) as an anticoagulant. Samples that were difficult to collect or showed signs of clotting during laboratory screening were discarded after gentle mixing.

2.3.1. Measurement of hemoglobin (g/dL)

Hemoglobin (Hb) levels (g/dL) in the blood were tested using hemoglobin strips in a digital Easy Mate® GHb, blood glucose/hemoglobin dual-function monitoring system.

2.3.2. Red blood cell (RBC) and white blood cell (WBC) count

Blood samples were examined to estimate the numbers of erythrocytes (RBC, $\times 10^6/mm^3$) and leucocytes (WBC, $\times 10^3/mm^3$), using a research microscope (OLYMPUS-CX21, Japan) at 4X and 10X magnification. Counted blood cells then estimated by the following formula to obtain required value,

$$\text{RBC} (\times 10^6/\text{mm}^3) = \frac{\text{Total no. of cell in 5 large squares} \times \text{dilution factor} \times \text{depth factors}}{\text{No. of small square counted}} \times 16$$

$$\text{WBC} (\times 10^3/\text{mm}^3) = \frac{\text{Total no. of cell in 1 large square} \times \text{dilution factor} \times \text{counting factors}}{\text{Volume factor (0.1)}}$$

2.3.3. Measurement of glucose (mg/dL)

Glucose levels (mg/dL) in the blood were monitored using glucose strips in a computerized EasyMate® GHb blood glucose/hemoglobin dual-function monitoring system.

2.4. Monitoring water quality parameters

Temperature, dissolved oxygen - DO, pH, free CO_2, and total alkalinity were among the water quality characteristics measured in the ponds and aquarium. A Celsius thermometer was used to determine the temperature (°C). A DO meter (Model DO5509, Lutron, Taiwan) was used to estimate DO. The pH of the water was measured using a portable pH meter (Model RI 02895, HANNA Instruments Co.). The free CO_2 in water was measured using a titrimetric method that included phenolphthalein and a 0.0227N NaOH titrant. Water total alkalinity was evaluated using a titrimetric method with methyl orange indicator and 0.02N H_2SO_4 titrant.

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was conducted, and Tukey's post hoc test was utilized to identify any statistically significant differences between the treatments. The values were expressed using means and standard deviations (SD). A significance level of $P<0.05$ was used to define statistical significance. The IBM SPSS Statistics program (Version 25.0) provided by IBM in Chicago, USA, was employed to conduct the statistical analyses.

3. Results

3.1. Effects of food and habitat on hemoglobin (Hb)

The value of Hb (%) in pond fish (12.10 ±1.25) and the aquarium fish (11.23 ± 1.05) showed no significant different during the experimental period (Figure 1).

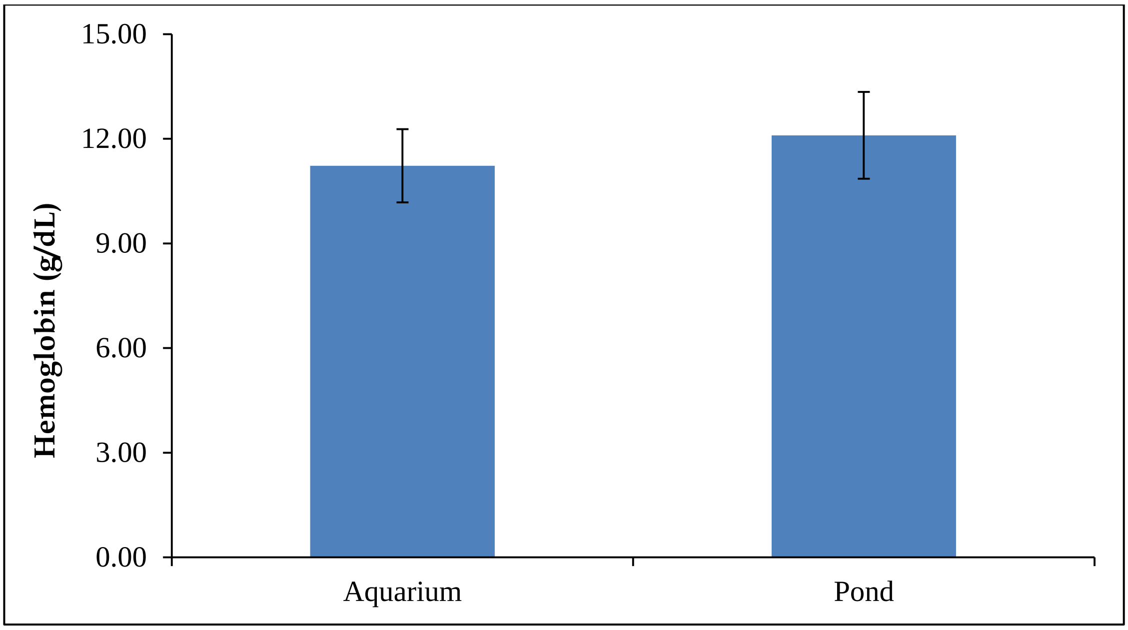

Figure 1. Blood hemoglobin (mean ± SD) of fish reared in aquarium with artificial feed and pond with natural foods.

3.2. Effects of food and habitat on red blood cell (RBC)

The variation of red blood cell between pond fishes ($1.99 \pm 0.27 \times 10^6$) and aquarium fishes ($1.41 \pm 0.20 \times 10^6$) (Figure 2). Fish in pond habitat showed significantly ($P<0.05$) higher amount of red blood cell than the aquarium fishes during the experimental periods.

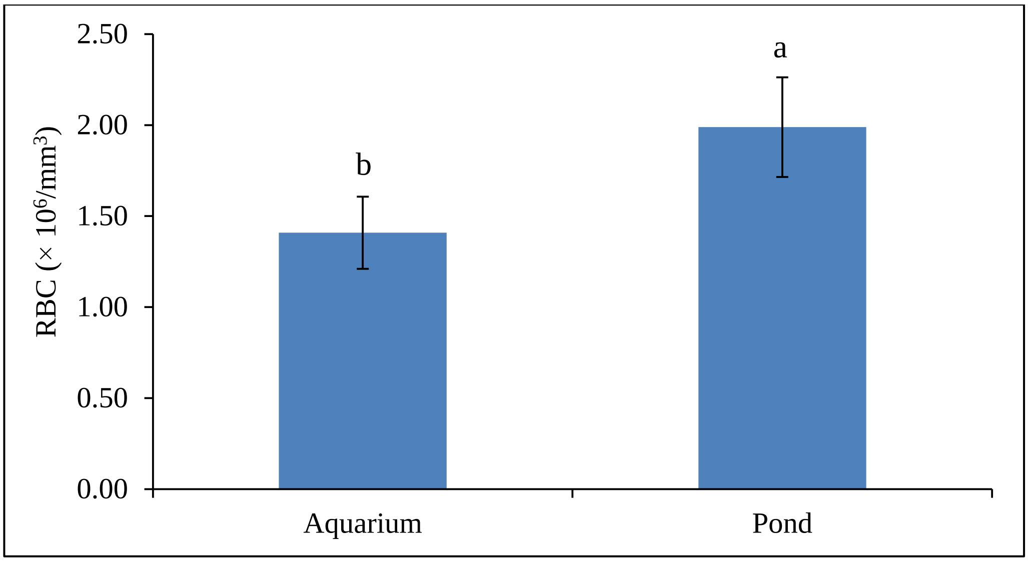

Figure 2. Red Blood Cells values (mean ± SD) of fish reared in aquarium with artificial feed and pond with natural foods. Values accompanied by different letters are significantly different.

3.3. Effects of food and habitat on white blood cell (WBC)

WBC of the blood acts as defensive element against the external and unwanted intrusive element. The values of WBC ($\times 10^3/mm^3$) showed opposite scenario with RBC values (Figure 3). The values of WBC in aquarium fishes ($1.66 \pm 0.26 \times 10^3$) were significantly ($P < 0.05$) higher than the pond fishes ($1.19 \pm 0.14 \times 10^3$).

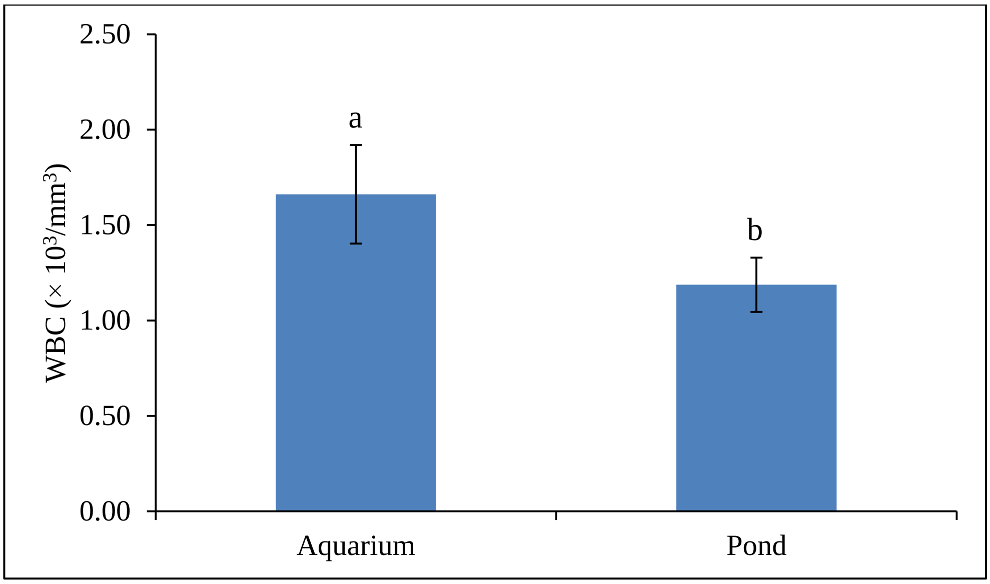

Figure 3. White blood cells values (mean ± SD) of fish reared in aquarium with artificial feed and pond with natural foods. Values accompanied by different letters are significantly different.

3.4. Effects of food and habitat on blood glucose

The value of blood glucose in aquarium fishes (173 ± 28.60) and the pond fishes (150 ± 33.34) showed no significant difference (Figure 4).

3.5. Water quality parameters

Water quality characteristics are critical for aquatic creature growth and development. Throughout the study period, water quality parameters (temperature, dissolved oxygen, free CO_2, pH, and total alkalinity) were assessed at various food and habitat conditions (Table 1).

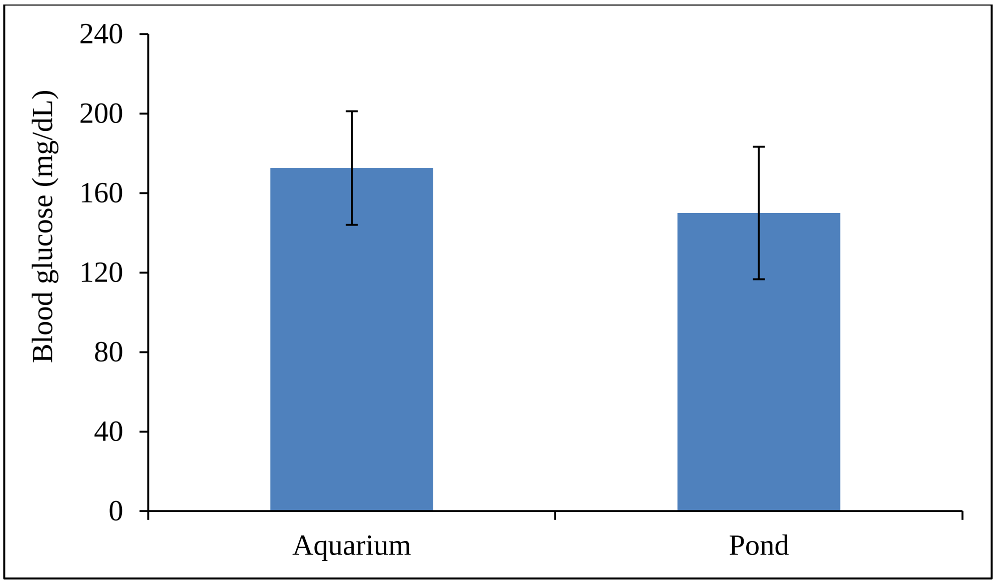

Figure 4. Blood glucose level (mean ± SD) of fish reared in aquarium with artificial feed and pond with natural foods.

Table 1. Water quality parameters during the study period.

Water quality parameters	Aquarium/artificial feed	Ponds/natural foods
Temperature (°C)	30.97 ± 0.06	33.47± 0.06
Dissolved oxygen (mg/L)	5.06 ± 0.96	4.50 ± 0.36
Free CO_2 (mg/L)	11.67 ± 1.53	13 ± 3.00
pH	7.71 ± 0.63	7.19 ± 0.18
Total alkalinity (mg/L)	202.67 ± 6.58	215.67 ± 7.89

4. Discussion

Knowledge of hematological features is an important tool that can be utilized as an effective and sensitive gauge to monitor physiological changes in fishes (Shahjahan *et al*., 2022a, b). Stress and other environmental factors commonly alter many blood parameters (Bani and Vayghan, 2011; Islam *et al*., 2020). Blood parameters are increasingly being used to identify the physiological condition or stress response in fishes to either endogenous or exogenous alterations (Shahjahan *et al*., 2021). Environmental changes can easily alter the physiological and biochemical properties of fish blood (Shahjahan *et al*., 2020). The interaction between dietary crude protein, citric acid, and microbial phytase affects the hemato-immunological parameters in young rohu, *L. rohita*. The level of dietary protein and red blood cell characteristics were found to be invertedly related. When compared to fish fed feed containing 35% protein, *L. rohita* demonstrated greater Hb on a diet containing 25% protein. These findings imply that there may be species-specific variations in the association between the values of red blood cell parameters and dietary protein levels in fish. In the current study, pond fish fed a lower protein diet showed higher levels of RBC and Hb than aquarium fish fed a higher protein diet. Iron serves as the fundamental component of the red pigment in mature erythrocytes (RBCs), which deliver oxygen to diverse tissues (Rehulka, 2002). Reduction in RBCs is a sign of anemia brought on by stressful circumstances (Li *et al*., 2011). RBC count decreases as a result of hemolysis brought on by the quantity of toxicants in the diet (Kavitha *et al*., 2010; Saravanan *et al*., 2011). Leukocytosis, an adaptive response by the body to chemical stress on the tissues, is indicated by a higher White Blood Cell (WBC) count. According to Ahmed *et al.* (2016), exposure to sub-lethal concentrations of toxins causes an increase in WBC count, which is accompanied by an increase in antibody synthesis. This increase in antibody synthesis helps fish to survive and recover from the toxic effects. Additionally, it speeds up the clearance of cellular debris from necropsied tissue (Hossain *et al.*, 2016). In order to protect itself against stress, the body's WBC rises through stimulation of the leukopoietic process and increased leukocyte release into the blood circulation (Shahjahan *et al.*, 2018). In the current study, pond fishes had lower WBC levels than aquarium fishes. Fish may suffer injury if their WBC levels drop because stress causes their immune systems to become activated in polluted environments (Suchana *et al*., 2021). Fish that are under stress typically show elevated blood glucose levels. Increased glucose levels in the fish exposed to pond

water in the current study may be the result of glycogen being mobilized into glucose to fulfill the increased demand for energy in a stressful environment. Stress-related stimuli cause the fish's adrenal tissue to release these hormones quickly. Glucocorticoids hormone is known to cause hyperglycemia in animals. Such an increase may be the result of stressed fish gluconeogenically responding more quickly in an effort to meet their increased energy needs (Suchana *et al*., 2021).
Water quality characteristics have a large impact on aquatic species. Temperature is the most critical parameter that directly affects the physiology of aquatic creatures. It has an impact on metabolism, growth, and reproduction of fishes. Many aquatic organisms can only thrive in a narrow temperature range. In the current study, high water temperature in a pond showed a higher development rate of fishes than aquarium fishes. A healthy aquatic ecosystem requires dissolved oxygen. The concentration of CO_2 in water is influenced by a variety of factors, such as respiratory and photosynthetic activities of living organisms, organic matter decomposition, and fish respiration. If water circulation is poor in a fish habitat with a high population density, CO_2 levels can increase considerably. This rise in CO_2 content can impede CO_2 expulsion from fish blood through their gills, resulting in an increase in CO_2 concentration in the blood (known as hypercapnia) and a reduction in blood pH (known as acidosis). Furthermore, the oxygen-carrying ability of hemoglobin in the blood reduces. However, in the current investigation, dissolved oxygen in pond water declined dramatically while free CO_2 increased as temperature rose. However, temperature change is also directly connected to DO concentration (Boyd and Tucker, 1998). As the temperature rises, so does the oxygen demand of the fish (Ravichandra, 2012; Uddin *et al*., 2016). The effect of alkalinity and acidity on fish has also been studied, particularly in terms of their responses. The pH and total alkalinity levels in aquarium and pond water differed in the current investigation. These might be caused by organic matter decomposition, and pond soil quality. It is also important that fish tolerance to habitat varies according to species, developmental stage, environmental temperature, DO, pollution, season etc. (Amin *et al*., 2005; Rahman *et al*., 2021).

5. Conclusions

The findings of this study reveal the characteristics of rohu's blood profile under two different conditions (pond fish culture with a natural diet and aquarium fish culture with an artificial diet). By highlighting the impact of multiple physico-chemical factors of water and the protein content of feed on the hematology and serum biochemistry ranges of fish belonging to the same species, it is demonstrated that blood parameters could be useful in monitoring the effects of different treatments.

Data availability

Corresponding author (Md Shahjahan) is bound to provide data on request.

Conflict of interest

None to declare.

Authors' contribution

Saad Abu Wakkas performed the experiment, collected data and prepared the draft. Md Iqramul Haque and Mohammad Ashaf-Ud-Doulah assisted in data collection. Md Shahjahan conceptualized the experiment, supervised and edited the manuscript. All authors have read and approved the final manuscript.

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