Journal of
Agriculture and Biology
ISSN: 3007-1755 (Print), 3007-1763 (Online)

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Research Article

Immunological and Hematological Responses of *Labeo rohita* under Starvation

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ABSTRACT

In aquaculture, fish health and food are of great concern but improper management of aquaculture leads to fasting stress and disturbed fish health. Food shortage may alter the immune system of fish affecting overall health and wellbeing of fish. The current study was designed to check the possible alterations that may occur in the immunological parameters of *Labeo rohita*. The experiment was conducted for 30 days fisheries research farm, department of Zoology, wildlife and fisheries, University of Agriculture Faisalabad. *Labeo rohita* fingerlings were collected from fish seed hatchery. The fish were procured, acclimatized and then distributed into two groups control and starved. The first group, referred as control group, fed twice a day. The second starved group, was detained in a hungry state for 28 days. Blood samples were drawn from the caudal vein of fish at the intervals of 7, 14, 21 and 28 days to examine the immunological and hematological parameters. The results showed that hematological and immunological parameters of starved group were reduced as compared to control group. The Hb, RBC, HCT, MCH, MCV, MCHC, white blood cells, monocytes, lymphocytes, eosinophils, neutrophils, granulocytes, were significantly reduced in starved group as compared to control group. The results of this study suggest fasting for period of 30 days cause significant reduction in the immunological and hematological parameters of fish.

Keywords: *Labeo rohita,* Immunological, Hematological, Starvation.

OPEN ACCESS

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Article History
Received November 06, 2024
Accepted: February 18, 2025
Published: March 06, 2025

INTRODUCTION

Aquaculture, the biggest food-producing sector in the world meets consumer needs by offering vital nutrients and premium protein (Dawood, 2021). The main obstacle facing aquaculture is improving fish nutrition to promote growth, health and value. Food stimulates the immune system and useful element in boosting growth and health. Additionally, it offers defense against stress and infections (Maundu et al., 2022). Fish is also very important source of food and the economy. Over 36 million individuals rely on fishing and aquaculture for their livelihood, and 200 million people derive direct and indirect benefits from fish.(Pauly, 2018). *Labeo rohita,* makes up 35% of all major carps (Coates et al., 2023). Many fish species experience a lack of food because of seasonal variations in water temperature or displacement from their normal habitat. Different consequences of starvation occur on morphological, biochemical (Gashkinaet al., 2022) as well as blood parameters of fish (Stepanowska et al., 2020). The primary lymphoid organs that produce immunity in fish are the thymus, head, kidney, and spleen (Lena et al., 2021). Fish immune system function is significantly impacted by stressful situations, such as fasting

(Sakyi *et al*., 2020). Stress from fasting reduces the immune system's reaction and the production of antibodies in fish. Additionally, it makes fish less resistant to diseases (Al-Sarraj et al., 2022). Fishes blood has been investigated to determine the immunological and hematological parameters normal value ranges; variations in these ranges shows irregularities in physiological processes (Mauro et al., 2022). Fish's tolerance for fasting stress is limited, and as the duration of the fasting increases, it suppresses their immunity. Fish with weakened immune systems become immotile, making them susceptible to infectious diseases (Musa et al., 2023). The objective of this study was to find out the fluctuations in immunological and hematological parameters of *Labeio rohita* under starved and normal conditions.

MATERIALS AND METHODS

The experiment was conducted at Fisheries Research Farm, Department of Zoology, Wildlife and Fisheries, University of Agriculture Faisalabad. Freshwater *Labeo rohita* fingerlings were collected from fish seed hatchery, Satiyana Road, Faisalabad. For 7 days, the fish was acclimated to the experimental setup. Two groups of fish were used for the experiment, control and treated group. Each group consists of ten fishes. The treated group was kept in a fasting state for 28 days, while the control group was provided a food equal to 3% of body weight. Daily observations were made of the physio-chemical parameters, such as: water temperature, hardness, and DO. Following the experimental trial's conclusion, fish samples were gathered for hematological and immunological investigation.

Blood collection

Blood samples were collected at the intervals of 7, 14, 21 and 28 days to examine the immunological and hematological parameters. Fish caudal veins were used to draw blood using gauge hypodermic needles2ml sterile syringes. To prevent coagulation, the syringe was flushed with EDTA until approximately 150–200 µl of anticoagulant remained in the needle before blood was extracted. The drawn blood was placed in EDTA tubes for later laboratory processing.

Immunological and hematological parameters

Different immunological and hematological parameters were evaluated, such as:

Total Red blood cells count

Using a hemocytometer, erythrocytes were counted according to the methodology of Russia and Sood (1992).

$$\text{No. of RBCs per cubic mm of blood} = \frac{\text{No. of cells counted} \times \text{dilution} \times 4000}{\text{No. of small squares counted}}$$

Total White blood cells count

Leucocytes were also counted using a hemocytometer and the Rusia and Sood (1992) method.

$$\text{No. of WBCs per cubic mm of blood} = \frac{\text{No. of cells counted} \times \text{dilution} \times 10}{\text{No. of 1 sq mm counted}}$$

Hematocrit

Hematocrit (Hct) was determined by standard microhematocrit method and expressed in percentages. Hematocrit was measured directly on a microhematocrit reader associated with the centrifuge.

Hemoglobin content count

Hemoglobin (Hb) was determined spectrophotometrically at 540nm absorbance by cyanmethemoglobin method.

Mean corpuscular hemoglobin

The mean concentration of hemoglobin (MCH) in red blood cells is the mean concentration of hemoglobin per red blood cell. It was computed with the subsequent formula:

$$\text{MCH} = \frac{\text{Hb (g/dl)} \times 10}{\text{RBC in million}} \text{ (Expressed in Pg)}$$

Mean Corpuscular Volume

MCV The volume of an average cell, or the average cell volume of all RBCs, is known as the mean corpuscular volume. It was computed with the subsequent formula:

$$\text{MCV} = \frac{\text{Hct (\%)} \times 10}{\text{RBC in million}} \text{ (Expressed in FL)}$$

Mean corpuscular Hemoglobin Concentration

The average red blood cell (MCHC) hemoglobin-containing fraction is measured in concentrations per average cell. It was computed with the subsequent formula:

$$MCHC = \frac{\text{Hb (g/dl)} \times 100}{\text{Hct(\%)}} \text{ (Expressed in g/dl)}$$

Monocytes, lymphocytes, eosinophils, neutrophils, granulocytes

Different WBCs such as monocytes, lymphocytes, eosinophils, neutrophils and granulocytes were counted manually using light microscope (Nikon E 600, Tokyo, Japan).

Statistical analyses

The obtained data was statistically analyzed in Minitab software by using two-way analysis of variance test to measure the variation among blood parameters.

RESULTS AND DISCUSSION

The goal of the current study was to determine *Labeo rohita* hematological and immunological response to fasting. In this study, there were remarkable decrease in the RBCs of fishin the third week when the fish were malnourished. According to the experiment *M. sharpeyi* demonstrated a significant drop in red blood cells (RBCs) during a 16-day food fast (p<0.05) (Najafi *et al*., 2015). Other results that were contrary. After two and four weeks without food, channel catfish (*Ictalurospanctacus*) showed no appreciable alteration in red blood cells (RBCs) (Aguirre-Guzman *et al.,* 2016).In the present study, Hb, RBC, HCT, MCH,MCHC, were reduced during the period of 28 days when fish were starved. Contrary, the study shows *Mugil cephalus* hemoglobin, MCHC, MCH and RBC were not affected during starvation for 30 days experiment (Akbary andJahanbakhshi, 2016).

Findings of the current shows there were reduction in the level of Hb, RBC, HCT, MCH, MCHC and white blood cells during 28 days' fish starvation. But in contrast the hemoglobin was not affected whereas red blood cells, white blood cells, mean corpuscular volume, mean corpuscular hemoglobin were reduced in *Salmo caspius* (Rahmati *et al*., 2019). The findings demonstrate that during the course of the 28-day experiment, the starved group's levels of Hb, RBC, HCT, MCH, MCHC, were reduced. The study found no significant differences in hematocrit, hemoglobin, RBC, MCV, MCHC, and MCH between the two sizes of fasted and fed grey mullet after 30 days (Abdel-Latif *et al*., 2022). The stress of fasting caused a considerable drop in hemoglobin level during this experiment. According to the study, *N. notopterus* demonstrated a notable decrease in hemoglobin in the starving fish (Kulkarni *et al*., 2015).

*Lutjanus guttatus was starved for 14 days and significant*reduction in hematocrit and hemoglobin were observed (Hernández *et al*., 2019). This study supports our findings that hemoglobin was significantly reduced during starvation for 28 days. RBCs were decreased when fish was starved for 28 days the results were supported by another study that showed the reduction in RBCs of all starved groups of Nile tilapia (Moustafa *et al*., 2017).

Table 1. Hematological parameters of *Labeiorohita* control and starved group.

Control Group					**Starved Group**				
Parameters	**1st week**	**2nd week**	**3rd week**	**4th week**	**Parameters**	**1st week**	**2nd week**	**3rd week**	**4th week**
Hematological					Hematological				
Hb (g/dL)	5.16±0.59	5.14±0.59	5.15±0.61	5.12±0.59	Hb (g/dL)	3.65±0.43	3.32±0.28	3.11±0.58	2.91±0.29
RBC (x10^6 µl)	1.24±0.61	1.21±0.63	1.23±0.59	1.25±0.61	RBC (x10^6 µl)	1.15±0.60	1.18±0.61	1.13±0.56	1.16±0.61
HCT (%)	16.07±1.14	16.04±0.58	16.09±0.60	16.11±0.59	HCT (%)	15.11±0.59	15.21±0.55	14.55±1.14	15.18±0.56
MCH (pg)	41.61±1.18	42.47±1.14	41.86±0.55	40.96±1.17	MCH (pg)	31.73±0.59	28.13±1.14	27.52±1.74	25.08±0.57
MCV (fL)	129.59±1.16	132.56±1.13	130.81±0.59	128.88±0.57	MCV (fL)	131.39±1.15	128.89±1.14	128.76±0.60	130.86±1.13
MCHC (g/dL)	32.10±0.59	32.04±1.17	32.00±0.58	31.78±0.57	MCHC (g/dL)	24.15±0.55	21.81±0.61	21.37±0.59	19.16±0.59

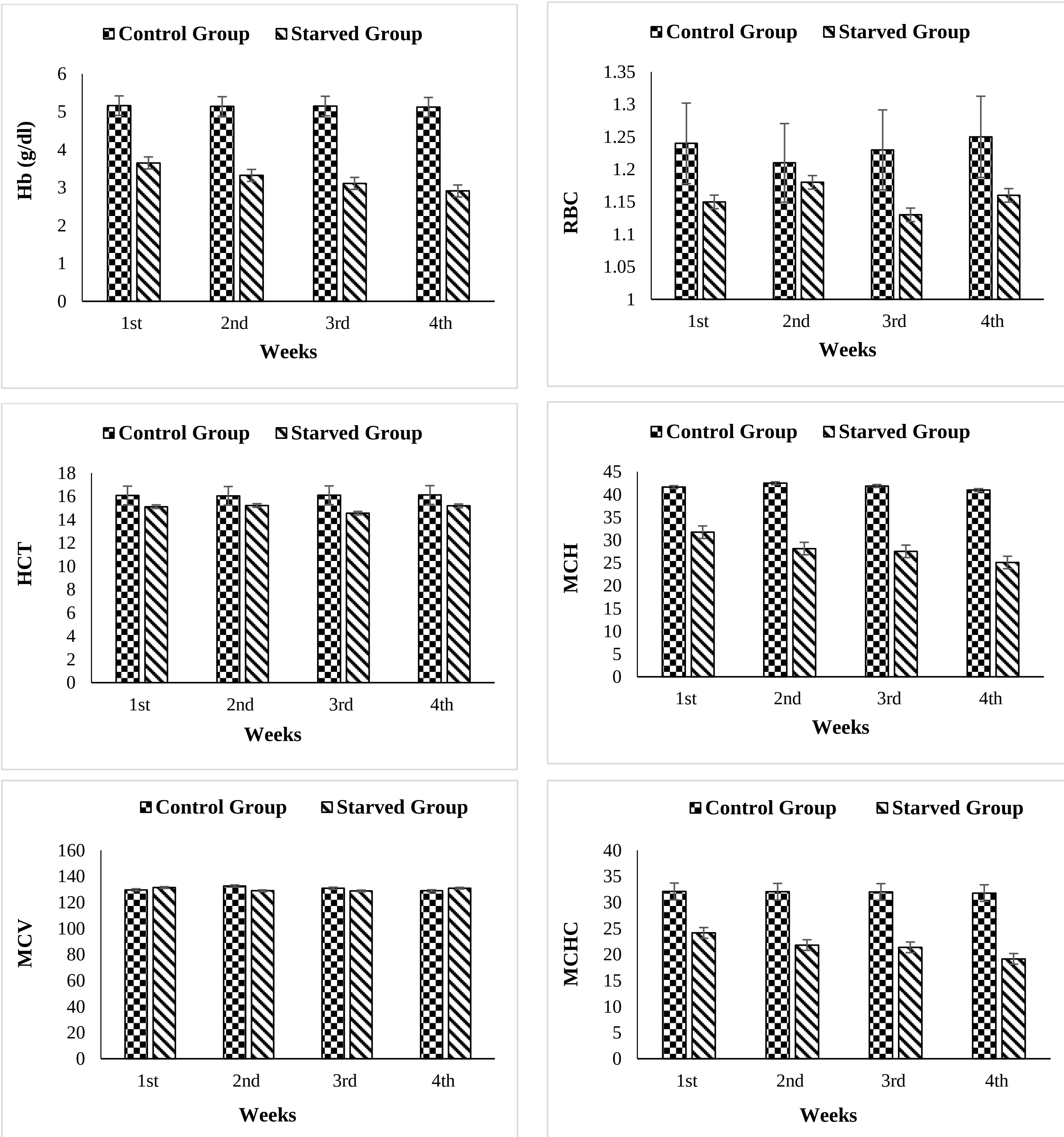

Figure 1. Comparison of hematological parameters between control and starved group. Error bars show standard deviation.

The findings demonstrated that fish undergo several alterations in their blood and immune systems during fasting. Some fishes can endure starving for longer periods of time, others cannot bear the stress of fasting. Fish immunological parameters comprise lymphocytes, monocytes, eosinophils, granulocytes, WBCs. Blood parameters showed to be an excellent technique for diagnosing the stress. This was caused by the fish's intimate relationship with its surroundings. Before any sickness symptoms showed up, the fish's blood showed even a small alteration in its body (Kumar *et al.,* 2023).

The findings of this study demonstrate a considerable decrease in white blood cells during the course of the four-week trial. Since there were differing opinions about WBCs, a counterargument was put out, stating that post-fasting leukocyte counts and the H/L ratio had increased. Conversely, dietary allowance may reduce both of these factors (Hoseini*et al*., 2019).

According to the current study, there was a decrease in monocytes, lymphocytes, eosinophils, neutrophils, and granulocytes during fasting compared to fed. Several immunological markers were monitored during the course of the 120-day or 4-month experiment, and alterations were observed in the RBC, WBC, MCV, MCH, MCHC, lymphocytes, monocytes, eosinophils, and granulocytes. During fasting, there was a drop in the of red blood cells. Reduced red blood cell counts after a 240-day of fasting leads toanemia had (Irungbam*et al.,* 2021).

There were no discernible variations in the number of *Mesopotamichthyssharpeyi* WBCs at end of the fasting time between the starting levels as well as the various fasting treatments, but upon refeeding, the starved groups' white WBC counts were noticeably greater than their starting levels. Furthermore, a comparison of the variations in WBC counts following refeeding revealed a significant rise in WBC counts (Najafi *et al*., 2015). The results of our study show the significant decrease in the 4th week of experiment during fish was starved.

The results show the reduction of white blood cells in starved group as compared to control group during 4 weeks of starvation. But in contrary, the *Salmo trutta caspius* white blood cells was increased during 6 weeks of starvation as compared to control group (Zaefarian*et al*., 2016). The current study findings show the reduction in lymphocytes, neutrophils and monocytes during starvation for 4 weeks. Another study supports our findings that revealed the significant reduction in neutrophils, monocytes but lymphocytes was not decreased during 2 days of starvation of *Oncorhynchus mykiss* (CREŢU *et al*., 2020).

It is concluded when the fingerlings of *Labeio rohita* were kept in fasting and non-fasting condition the difference in immunological and hematological parameters were observed. The fish kept in fasting condition had reduced hematological and immunological parameters as compared to the control one.

Table 2. Immunological parameters of *Labeio rohita* in control and starved group.

Control group					**Starved group**				
Parameters	**1st week**	**2nd week**	**3rd week**	**4th week**	**Parameters**	**1st week**	**2nd week**	**3rd week**	**4th week**
Immunological					Immunological				
White blood cells ($x10^3$ µl)	158.22 ±1.14	157.24± 1.72	158.21± 0.56	159.01± 0.58	White blood cells ($x10^3$ µl)	145.31 ±1.18	140.11 ±1.17	135.48± 0.59	129.46 ±1.18
Monocytes (%)	35.12± 0.59	35.14±0 .60	35.11±0 .59	35.15±0. 61	Monocytes (%)	28.44± 0.60	25.32± 0.59	31.21±0 .59	29.450 .61
Lymphocytes (%)	48.05± 0.59	48.02±0 .58	48.09±0 .58	48.07±0. 58	Lymphocytes (%)	40.13± 0.60	38.99± 0.58	42.01±0 .58	37.14± 0.54
Eosinophil (%)	5.01±0 .57	5.05±1. 17	5.03±1. 17	5.06±0.5 9	Eosinophil (%)	3.78±0 .59	2.99±0 .58	3.57±0. 58	2.39±0 .57
Neutrophils (%)	86.23± 0.55	86.26±0 .57	86.24±0 .60	86.27±0. 59	Neutrophils (%)	78.34± 1.18	80.22± 0.57	76.52±1 .14	81.43± 0.59
Granulocytes (%)	15.05± 0.57	15.09±1 .15	15.07±1 .17	15.02±0. 57	Granulocytes (%)	13.34± 0.55	11.87± 1.14	13.46±0 .61	10.99

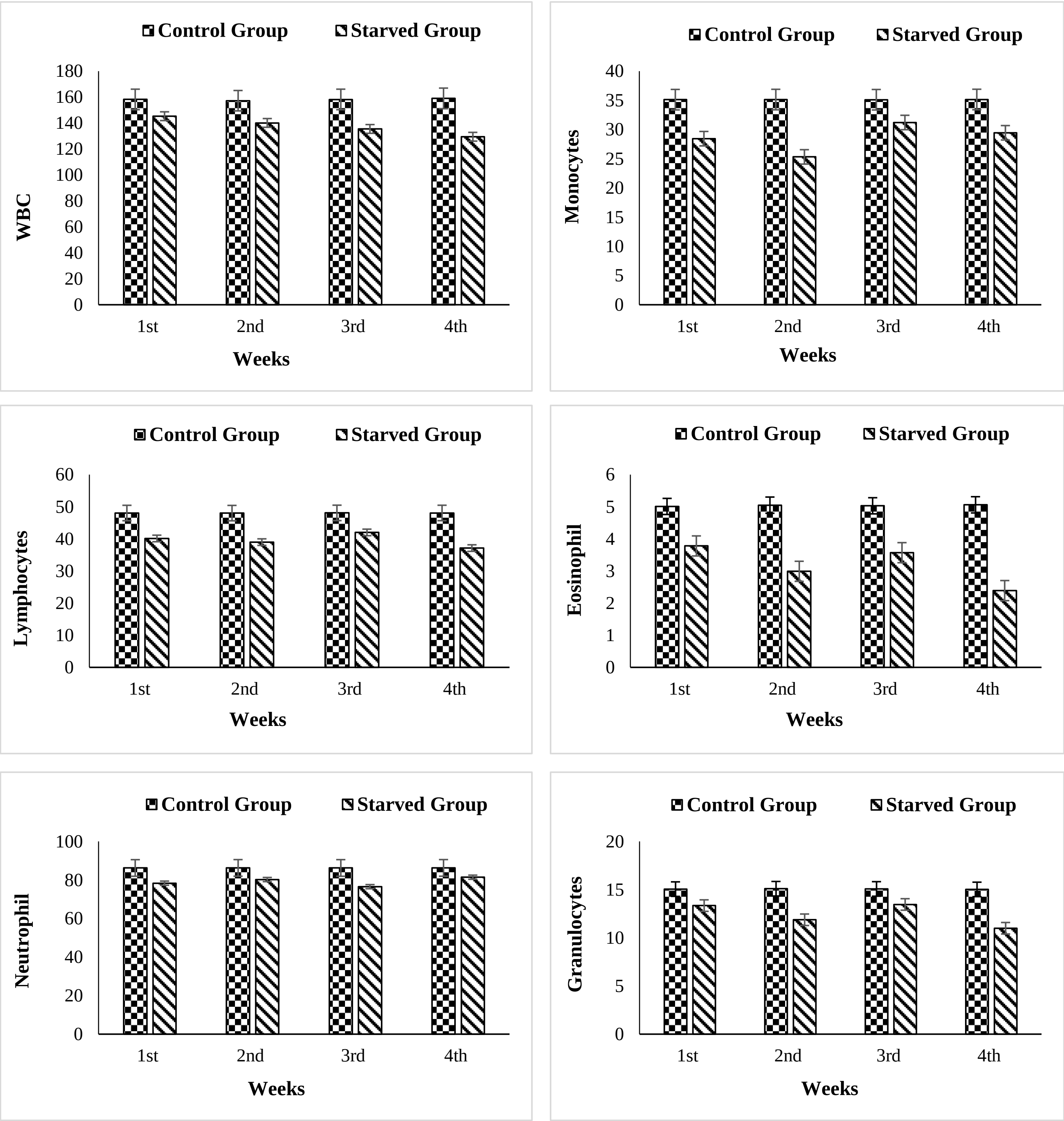

Figure 2. Comparison of immunological parameters of *Labeio rohita* in control and starved group. Error bars show standard deviation.

CONCLUSIONS

The study conclude that starvation has significant impact on the immunological and hematological parameters of *Labeo rohita*. The major impact was reduced fish growth and weaking of immune and blood parameters. As a result, while raising under unfavourable conditions, the feeding approach must be optimised.

ACKNOWLEDGEMENTS

Not applicable

AUTHOR CONTRIBUTIONS

All the authors contributed equally to this research.

COMPETING OF INTEREST

No conflicts of interest have been disclosed by the authors.

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