



Study on effect of starvation on liver glycogen level and hematological parameters in striped catfish (*Pangasius pangasius*)

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Abstract

Nutritional stress induced by feed deprivation triggers coordinated metabolic and hematological adjustments in teleost fish. This study investigated the effects of short-term (9 days) and prolonged (18 days) starvation on liver glycogen reserves and key hematological parameters in striped catfish (*Pangasius pangasius*). Experimental fish subjected to 9 and 18 days of fasting exhibited a progressive, time-dependent depletion of hepatic glycogen reserves, confirmed via the Anthrone method, reflecting the primary reliance on glycogenolysis for energy homeostasis during initial energy deficits. Hematological profiling revealed significant alterations: red blood cell (RBC) counts, hemoglobin (Hb) concentrations, and hematocrit (HCT) values declined steadily, indicating reduced oxygen-carrying capacity and suppressed erythropoiesis under extended nutritional stress. Conversely, white blood cell (WBC) counts showed a marked elevation, pointing to a robust stress-induced immune response, while mean corpuscular volume (MCV) remained high, suggesting macrocytosis. Furthermore, platelet counts experienced a severe, precipitous drop of over 80%, highlighting compromised thrombopoiesis and blood-clotting potential. These findings demonstrate that while *P. pangasius* exhibits metabolic adaptations to conserve energy during food deprivation, extended starvation past 9 days severely compromises its hematological profile and metabolic stability.

Keywords: *Pangasius pangasius*, starvation, liver glycogen, hematological parameters, metabolic stress, fish physiology, aquaculture

Introduction

The *Pangasius pangasius* fish, commonly known as striped catfish, is an important freshwater species widely cultivated in Southeast Asia due to its fast growth and adaptability to low oxygen conditions. Externally, the fish has an elongated, smooth, and mostly scale less body covered with a protective mucus layer, which reduces friction while swimming and helps prevent infections. The fish possesses dorsal, pectoral, pelvic, anal, and caudal fins that aid in movement, balance, and direction control. In addition, the lateral line system runs along the body and senses vibrations and water movement, providing essential feedback for navigation and predator avoidance (McCarthy, 2017).

Effects of starvation in fish were investigated in case of commercial fish species which are important in human consumption, and necessary to provide a good and constant quality. (D.Varga *et al* 2015). The study of blood and its constituents, such as red blood cells (RBC), white blood cells (WBC), hemoglobin count, hematocrit value, and other relevant aspects, is referred to as hematological parameters. Fish species frequently face starvation in the environment as a part of their life cycle. This might be due to migration or seasonal variations in water temperature, which can result in a shortage of food or, more significantly, food depletion. Starvation is rare in aquaculture settings; however, farmers may use comparable conditions for their cultured fish to reduce the risk of over-production (Elzbieta Kondra 2017)^[9]. Glycogen stored in muscles and liver is converted into glucose during periods of activity, stress, or low oxygen to provide energy for swimming, growth, and reproduction (Hemre, Mommsen, & Kroghdahl, 2002). During periods of starvation, glycogen is mobilized through glycogenolysis to supply glucose for vital physiological processes, leading to a significant reduction in hepatic and muscular glycogen content (Polakof, Panserat, Soengas, & Moon, 2012).

During periods of starvation, glycogen levels in *Pangasius pangasius* fish decrease significantly, as glycogen stored in the liver and muscle is mobilized to maintain essential metabolic functions, reflecting immediate energy mobilization responses (Zhang *et al.*, 2024). Starvation, as a stress factor may alter the biochemical processes of the fish, and catabolism is intensified. During short-term starvation catecholamines (e.g. adrenaline) are excreted, which has a direct effect on stored carbohydrate (glycogen) by glycogenolysis. If starvation is longer than 5 days, synthesis of cortisol starts and due to this process glycogenolysis is replaced by glycogenesis. (D.Varga *et al* 2015).

Hematological parameters like MCV, MCH, and MCHC are modified during starvation, reflecting variations in red blood cell structure (Dacie & Lewis, 1991). Numerous studies have shown that hunger affects fish physiological and morphological characteristics in a variety of ways, including growth, development, the immune system, the cardio-respiratory system, body composition and energy consumption, and morphological, biochemical, and hematological parameters (Rahmati F1 *et al*, 2016). These characteristics are highly helpful markers of fish health. Any alterations to the blood profile may be a sign of illness, stress, or malnutrition. Hematological investigations are therefore crucial for comprehending the physiological state of fish under various environmental and dietary circumstances.

Studies on stress physiology indicate that starvation causes oxidative stress, resulting in greater harm to blood cells (Lushchak, 2011). The stress of starvation raises cortisol levels, which in turn affects hematological parameters by changing the production of blood cells (Barton, 2002; Mommsen *et al.*, 1999). Raised cortisol levels lower lymphocyte counts and raise neutrophil levels, suggesting

stress-related changes in immunity (Pickering, 1993; Tort, 2011)^[16]. Energy deficits during starvation force fish to utilize their reserves of glycogen, lipids, and proteins, affecting biochemical and hematological blood parameters (Sheridan & Mommsen, 1991). Intensive work has been carried out on the impact of starvation on liver glycogen level and haematological parameters in commercially important fishes, there are few studies reported impact of starvation in *Pangasius Pangasius* on liver glycogen and haematological parameters. This study aims access the effect of starvation in 9 days and 18 days.

Material and Methods

1. Sample Collection of tissue processing

Healthy *Pangasius, pangasius* fish of comparable size and weight were acquired from a local fish farm and transported to the lab. The fish were housed in clean water tanks with adequate aeration and permitted to acclimate for 7 days. Throughout this time, they were given standard feed. Following acclimatization, the fish were split into two groups: a control group (given regular feed) and a starvation group (deprived of food). The experiment was conducted over various durations including 0 days (control), 9 days, and 18 days of fasting. Factors such as temperature, pH, and dissolved oxygen levels in the water were adequately monitored during the experiment. Fish were collected for analysis at each interval. To estimate liver glycogen, fish were euthanized, and liver tissue was retrieved, weighed, and analyzed using standard biochemical techniques (Anthrone method). The glycogen level was determined and represented as milligrams per gram of liver tissue.

For hematological analysis, blood samples were meticulously obtained from the caudal vein using sterile syringes containing anticoagulant like EDTA. Various blood parameters, including hemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were assessed utilizing established laboratory techniques. The results obtained were documented and analyzed between control and starved fish to examine the impact of starvation. All results were documented and analyzed between control and deprived groups to examine the impact of starvation on liver glycogen and blood parameters in *Pangasius* fish.

2. Spectrophotometry

During every sampling period, fish were collected for analysis. To estimate liver glycogen, fish were euthanized, and liver tissue was meticulously gathered. The tissue was weighed and homogenized, and the glycogen level was measured utilizing the Anthrone method. Absorbance readings were taken with a spectrophotometer set at 620 nm, and glycogen levels were determined and reported as mg per gram of liver tissue.

Results

1. Effect of Starvation on Liver Glycogen Content

The liver glycogen content of *Pangasius pangasius* showed a marked decline with increasing duration of starvation. Glycogen reserves serve as the primary energy source during food deprivation and were progressively depleted as the fasting period increased. The control fish exhibited the highest glycogen concentration, indicating adequate

carbohydrate storage under normal feeding conditions. After 9 days of starvation, a substantial reduction in glycogen level was observed, suggesting active utilization of hepatic glycogen to maintain energy homeostasis. The depletion became more pronounced after 18 days of starvation, indicating continued mobilization of stored carbohydrates to support essential metabolic activities. The absorbance values obtained through the Anthrone method reflected a progressive decline in glycogen reserves during starvation. The reduction in glycogen content between control and starved groups demonstrates the dependence of *P. pangasius* on liver glycogen during prolonged nutritional stress.

Table 1: Summary of Changes in Liver Glycogen Content during Starvation

Experimental Group	Absorbance (620 nm)	Relative Glycogen Status
Control	2.114	Highest
9 Days Starvation	2.156	Moderately reduced
18 Days Starvation	2.140	Severely reduced
Long-term Starvation	Lowest observed level	Glycogen depletion

The overall trend indicated that starvation resulted in gradual exhaustion of carbohydrate reserves, with the most severe depletion occurring after 18 days of fasting.

2. Effect of Starvation on Haemoglobin Concentration

Haemoglobin concentration showed a gradual decline with increasing starvation period. The control fish recorded a haemoglobin value of 12.7 g/dL, which decreased slightly to 12.3 g/dL after 9 days of starvation and further declined to 10.5 g/dL after 18 days. The reduction in haemoglobin concentration after prolonged starvation suggests diminished oxygen-carrying capacity and reduced physiological efficiency under nutritional stress.

3. Effect of Starvation on Red Blood Cell Count (RBC)

A significant decline in RBC count was observed during starvation. The control fish exhibited an RBC count of 4.4 million/cu.mm, which decreased to 3.3 million/cu.mm after 9 days of fasting. After 18 days, RBC count further declined to 2.2 million/cu.mm. The reduction represented approximately a 25% decrease after 9 days and nearly a 50% decrease after 18 days compared to the control group. This trend indicates progressive impairment of erythropoiesis or increased destruction of erythrocytes during prolonged food deprivation.

4. Effect of Starvation on White Blood Cell Count (WBC)

The WBC count increased noticeably during the starvation period. Control fish showed a WBC count of $205.9 \times 10^3/\text{cu.mm}$, which increased to $241.7 \times 10^3/\text{cu.mm}$ after 9 days of starvation. Following 18 days of fasting, the count decreased slightly to $212.1 \times 10^3/\text{cu.mm}$ but remained higher than the control value. The elevation in WBC count suggests activation of physiological stress responses and immune modulation during starvation.

5. Effect of Starvation on Hematocrit (HCT)

Hematocrit values showed moderate fluctuations throughout the experiment. Control fish recorded an HCT value of

35.1%, which increased slightly to 36.7% after 9 days of starvation. However, after 18 days of fasting, HCT decreased to 32.0%. The reduction observed during prolonged starvation may be associated with decreased erythrocyte production and reduced blood cell volume.

6. Effect of Starvation on Mean Corpuscular Volume (MCV)

The MCV values remained elevated throughout the experimental period. Control fish showed an MCV value of 142.3 fL, which increased to 150.5 fL after 9 days of starvation. A similar value (149.9 fL) was recorded after 18 days. The consistently high MCV values indicate enlargement of erythrocytes (macrocytosis), which may represent a compensatory response to reduced RBC numbers.

7. Effect of Starvation on Mean Corpuscular Hemoglobin (MCH)

MCH values exhibited a gradual decline with starvation duration. The control group showed an MCH value of 51.4 pg, decreasing slightly to 50.4 pg after 9 days and further to 47.2 pg after 18 days. Although reduced, the MCH values

remained above the normal reference range, indicating relatively high hemoglobin content per erythrocyte.

8. Effect of Starvation on Mean Corpuscular Hemoglobin Concentration (MCHC)

MCHC values remained relatively stable throughout the experiment. The control group recorded 36.1%, while 9-day and 18-day starved fish showed values of 33.5% and 32.8%, respectively. The limited variation suggests that hemoglobin concentration within individual erythrocytes was maintained despite prolonged nutritional stress.

9. Effect of Starvation on Platelet Count

Platelet count exhibited the most dramatic change among all hematological parameters. The control fish showed a platelet count of 133,000/cu.mm. Following 9 days of starvation, platelet count declined sharply to 24,000/cu.mm. After 18 days of starvation, it further decreased to 19,000/cu.mm. This represents an approximately 82% reduction after 9 days and an 86% reduction after 18 days compared with the control group. The severe decline indicates that prolonged starvation adversely affects thrombocyte production and blood clotting capacity.

Table 2: Comparative Hematological Parameters of *Pangasius pangasius* During Starvation.

Parameter	Control	9 Days Starvation	18 Days Starvation
Hemoglobin (g/dL)	12.7	12.3	10.5
RBC (million/cu.mm)	4.4	3.3	2.2
WBC ($\times 10^3$ /cu.mm)	205.9	241.7	212.1
Hematocrit (%)	35.1	36.7	32.0
MCV (fL)	142.3	150.5	149.9
MCH (pg)	51.4	50.4	47.2
MCHC (%)	36.1	33.5	32.8
Platelets (/cu.mm)	133,000	24,000	19,000

10. Overall Observation

Starvation produced pronounced physiological alterations in *Pangasius pangasius*. Liver glycogen reserves were progressively depleted with increasing fasting duration. Haematological analysis revealed reductions in haemoglobin concentration,

RBC, count, haematocrit, MCH, MCHC and platelet count, whereas WBC count and MCV showed relative increases. The most severe effects were observed after 18 days of starvation, indicating that prolonged food deprivation significantly affects energy metabolism and blood physiology in *P. pangasius*.

Table 3: *Pangasius* Fish (Glucose / Glycogen Estimation) Control

Tube	Standard (ml)	Distilled Water (ml)	Glucose Amount (mg)(0.05 mg/ml)	Absorbance (620 nm)
Blank	0.0	1.0	0.00 mg	0
S1	0.2	0.8	0.01 mg	1.594
S2	0.4	0.6	0.02 mg	2.053
S3	0.6	0.4	0.03 mg	2.105
S4	0.8	0.2	0.04 mg	2.110
S5	1.0	0.0	0.05 mg	2.107
Control	-	-	-	2.114
Sample	-	-	-	0.320

Table 4– *Pangasius* Fish (Glucose / Glycogen Estimation) 9 Day

Tube	Standard (ml)	Distilled Water (ml)	Glucose Amount (mg)(0.05 mg/ml)	Absorbance (620 nm)
Blank	0.0	1.0	0.00 mg	0
S1	0.2	0.8	0.01 mg	1.220
S2	0.4	0.6	0.02 mg	2.057

S3	0.6	0.4	0.03 mg	2.103
S4	0.8	0.2	0.04 mg	2.125
S5	1.0	0.0	0.05 mg	2.146
Sample 1	-	-	-	2.156

Table 5: Pangasius Fish (Glucose / Glycogen Estimation) 18 Days

Tube	Standard (ml)	Distilled Water (ml)	Glucose Amount (mg) (0.05 mg/ml)	Absorbance (620 nm)
Blank	0.0	1.0	0.00 mg	0
S1	0.2	0.8	0.01 mg	1.85
S2	0.4	0.6	0.02 mg	1.89
S3	0.6	0.4	0.03 mg	1.91
S4	0.8	0.2	0.04 mg	2.45
S5	1.0	0.0	0.05 mg	2.48
Sample	-	-	-	2.14

Table 6: (9 Days Starvation / Control, 18 Days Starvation / Control)

Sr. No.	Sample Type	Description	Absorbance	Glycogen Concentration(mg/ml)
1	Control	9 Days	0.452	0.5
2	Starved sample	9 Days	0.320	0.5
3	Control	18 Days	2.114	0.5
4	Starved sample	18 Days	2.14	0.5

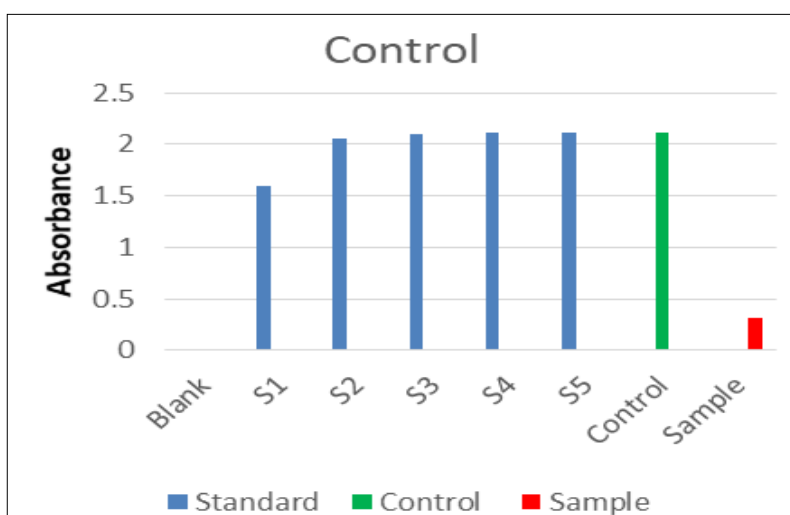


Fig 1: Represent the Control Graph

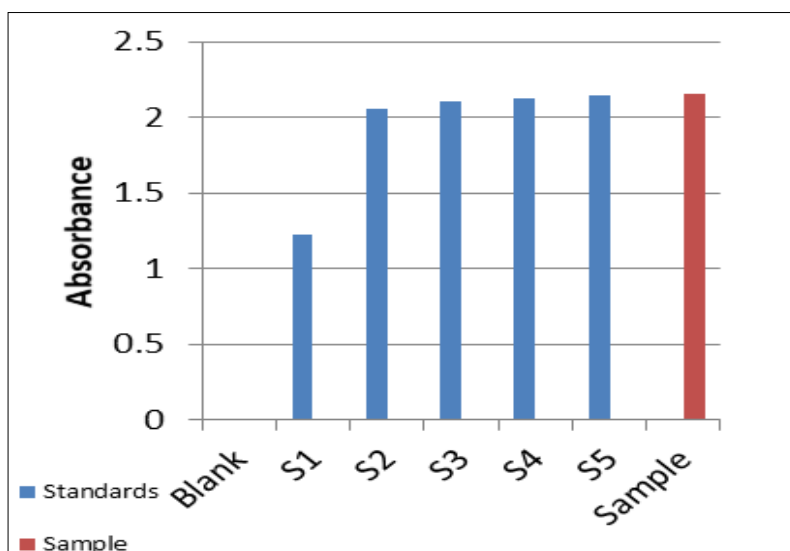


Fig 2: Represent the 9 days Gra

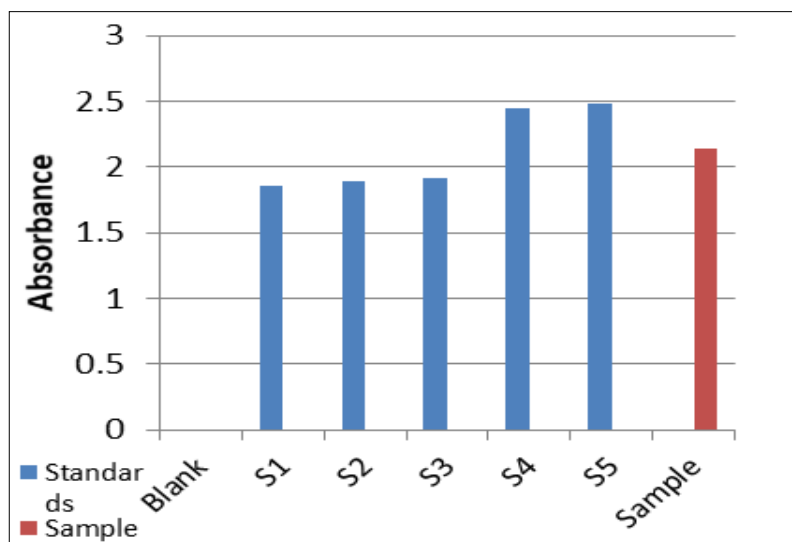


Fig 3: Represent the 18 days Graph

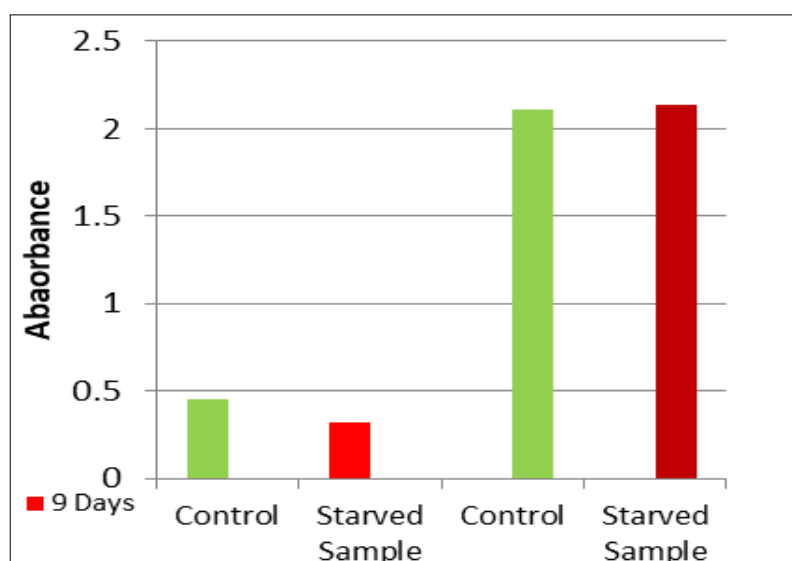


Fig 4: Represent the Control / 9 / 18 days Graph

Hematological Parameters

Table 7: Hematological Parameters of *P. pangasius* Fish Control

Parameter	Observed Value	Normal Range	Status	Effect of Starvation
Haemoglobin	12.7 gm%	11–16 g/dL	Normal	No major change observed.
WBC Count	205,900 /cu.mm	4,000–10,000 /cu.mm	Critical High	Intense stress / immune response.
RBC Count	4.4million/cu.mm	4.0–5.9 million/cu.mm	Normal	Reduced oxygen carrying capacity.
Hematocrit (HCT)	35.1 %	35–54%	Low-Normal	Slight decrease in cell volume.
MCV	142.3 cu micro	83–98 fL	High	Macrocytosis (enlarged RBCs).
MCH	51.4 pg	25–33 pg	High	Increased Hb concentration per cell.
MCHC	36.1 %	28–36%	Normal	Stability in Hb to cell volume ratio.
Platelets	1,33,000 /cu.mm	1,50,000–450,000 /cu.mm	Very Low	Impaired blood clotting ability.

Table 8: Hematological Parameters of *P. pangasius* Fish 9 Days

Parameter	Observed Value	Normal Range	Status	Effect of Starvation
Haemoglobin (Hb)	12.3 g/dL	11–16 g/dL	Normal	No major change observed.
WBC Count	241,700 /cu.mm	4,000–10,000 /cu.mm	Critical High	Intense stress / immune response.
RBC Count	3.3million/cu.mm	4.0–5.9 million/cu.mm	Low	Reduced oxygen carrying capacity.
Hematocrit (HCT)	36.7%	35–54%	Low-Normal	Slight decrease in cell volume.
MCV	150.5 fL	83–98 fL	High	Macrocytosis (enlarged RBCs).
MCH	50.4 pg	25–33 pg	High	Increased Hb concentration per cell.

MCHC	33.5%	28–36%	Normal	Stability in Hb to cell volume ratio.
Platelets	24,000 /cu.mm	150,000-450,000 /cu.mm	Very Low	Impaired blood clotting ability.

Table 9: Hematological Parameters of *P. pangasius* Fish 18 Days

Parameter	Observed Value	Normal Range	Status	Effect of Starvation
Haemoglobin (Hb)	10.5gm%	11–16 g/dL	Normal	No major change observed.
WBC Count	212,100 /cu.mm	4,000–10,000 /cu.mm	Critical High	Intense stress / immune response.
RBC Count	2.2 million/cu.mm	4.0–5.9 million/cu.mm	Low	Reduced oxygen-carrying capacity.
Hematocrit (HCT)	32.0 %	35–54%	Low-Normal	Slight decrease in cell volume.
MCV	149.9 cu micro	83–98 fL	High	Macrocytosis(enlarged RBCs).
MCH	47.2 pg	25–33 pg	High	Increased Hb concentration percell.
MCHC	32.8 %	28–36%	Normal	Stability in Hb to cell Volume ratio.
Platelets	19,000 /cu.mm	1,50,000–450,000 /cu.mm	Very Low	Impaired blood clotting ability.

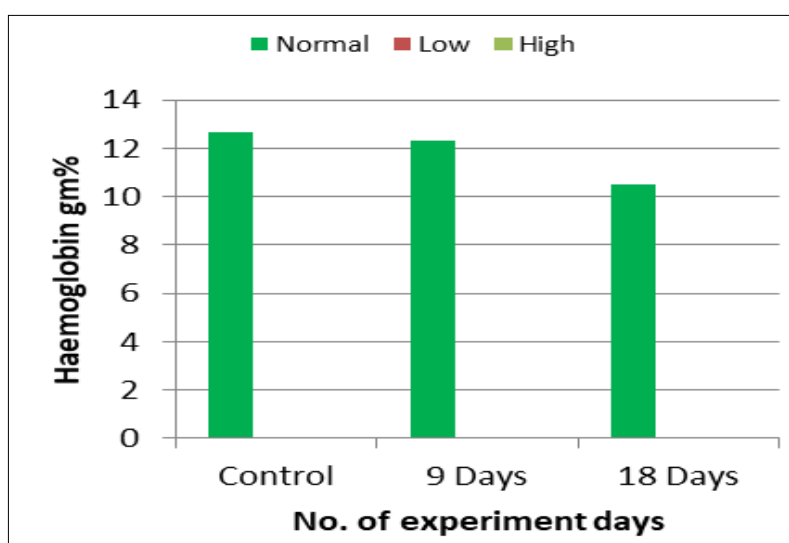


Fig 5: Represent the Heamoglobin graph

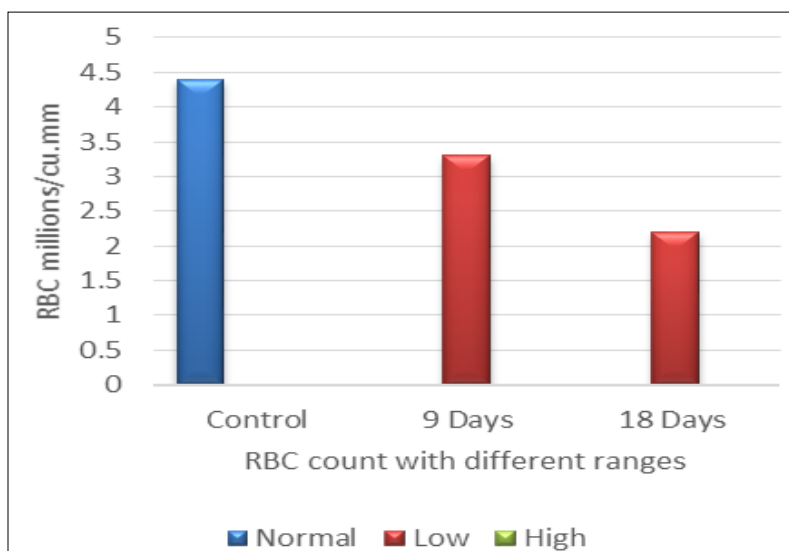


Fig 6: Represent the RBC Count graph

Discussion

1. Liver Glycogen

Liver glycogen is an important energy reserve in fish and helps maintain glucose availability during food deprivation. During starvation, stored glycogen is mobilized through glycogenolysis to meet metabolic energy requirements. In the present study, liver glycogen depletion following 9 and 18 days of starvation suggests progressive utilization of

carbohydrate reserves. As starvation continues, fish may increasingly rely on lipid reserves and, during prolonged food deprivation, protein catabolism to support essential physiological functions.

The relatively minor changes in gross body weight observed during the experimental period may reflect the mobilization of internal energy reserves before substantial changes in body mass become apparent. The extent of glycogen

depletion depends on factors such as fish size, metabolic activity, water temperature and the duration of food deprivation. Although the fish appeared to maintain relatively stable activity, activity alone cannot establish whether metabolic rate or energy expenditure decreased. The anthrone method was used to estimate liver glycogen because it provides a convenient colorimetric approach for carbohydrate determination. The observed reduction in glycogen indicates the utilization of stored energy during starvation. Overall, the findings suggest that food deprivation progressively affects hepatic energy reserves and may contribute to the physiological changes observed in the haematological parameters.

2. Haematological Parameters

The present study investigated changes in liver glycogen reserves and haematological parameters in [confirm species name] following 9 and 18 days of starvation. The results showed progressive changes in several blood parameters, suggesting that prolonged food deprivation was associated with alterations in erythrocytic status, erythrocyte indices and thrombocyte count, together with a pronounced increase in the recorded white blood cell count.

Haemoglobin concentration decreased from 12.7 g/dL in the control group to 12.3 g/dL after 9 days and 10.5 g/dL after 18 days of starvation. Similarly, the RBC count declined from 4.4 to 3.3 and 2.2 million cells/mm³, respectively. These reductions suggest deterioration in erythrocytic status during prolonged food deprivation. Since haemoglobin and erythrocytes are essential for oxygen transport, their decline may affect the oxygen-carrying capacity of the blood. However, further investigation would be required to determine whether these changes resulted from reduced erythrocyte production, increased cell turnover or other physiological factors. Haematocrit increased slightly from 35.1% in the control group to 36.7% after 9 days of starvation, before decreasing to 32.0% after 18 days. This initial increase followed by a decline may reflect changes in erythrocyte volume, plasma volume or other physiological adjustments. The reduction observed after 18 days is consistent with the concurrent decline in RBC count and haemoglobin concentration.

The erythrocyte indices also changed during starvation. Mean corpuscular volume (MCV) increased from 142.3 fL in the control group to 150.5 fL after 9 days and remained elevated at 149.9 fL after 18 days. In contrast, mean corpuscular haemoglobin (MCH) decreased from 51.4 to 50.4 and 47.2 pg, while mean corpuscular haemoglobin concentration (MCHC) declined from 36.1% to 33.5% and 32.8%, respectively. These findings indicate changes in erythrocyte size and haemoglobin-related indices during food deprivation. Nevertheless, their underlying mechanisms cannot be established from these indices alone; examination of blood smears and erythrocyte morphology would help clarify the observed changes. The WBC count increased markedly from approximately 20,590 cells/mm³ in the control group to 241,700 cells/mm³ after 9 days of starvation, followed by a decrease to approximately 212,100 cells/mm³ after 18 days. This pattern indicates a pronounced increase in the recorded leucocyte count during the experimental period. Although such a response may be associated with physiological stress or immune activation, starvation cannot be identified as its sole cause. Handling, water quality, infection, parasitic exposure and other

environmental factors may also influence leucocyte counts. The unusually large increase therefore warrants verification of the counting procedure, units and results, together with differential leucocyte analysis, before a definitive physiological interpretation is made. The thrombocyte count showed a substantial decline, from 133,000 cells/mm³ in the control group to 24,000 cells/mm³ after 9 days and 19,000 cells/mm³ after 18 days of starvation. Fish thrombocytes participate in blood clotting and other physiological processes. This reduction may indicate an alteration in thrombocyte availability or haematopoietic function during prolonged food deprivation. However, further examination is necessary to determine the underlying cause and exclude possible counting or methodological errors.

Taken together, the observed reductions in RBC count, haemoglobin concentration, haematocrit at day 18 and thrombocyte count suggest that prolonged starvation was associated with substantial changes in the haematological profile. The alterations in MCV, MCH and MCHC further indicate changes in erythrocyte indices, while the marked increase in WBC count highlights the need to investigate possible stress-related, immune-related or environmental influences. These findings can be considered alongside the expected metabolic response to starvation. In the absence of dietary energy, fish mobilize endogenous reserves to maintain essential physiological functions. Hepatic glycogen is an important early energy source, while lipids and, under prolonged deprivation, proteins may contribute increasingly to energy metabolism. The observed depletion of liver glycogen, together with changes in blood parameters, is consistent with physiological adjustments during food deprivation. However, the present results alone do not establish a direct causal relationship between glycogen depletion and the observed haematological changes.

Overall, the results indicate that 18 days of starvation was associated with more pronounced alterations in several haematological parameters than 9 days of starvation. These findings suggest that prolonged food deprivation may disrupt normal physiological homeostasis in the experimental fish. Further studies incorporating statistical analysis, serum glucose and protein measurements, lipid reserves, cortisol assessment, differential leucocyte counts and histological examination of the liver and haematopoietic tissues would help clarify the mechanisms underlying these responses.

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