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# Alterations In Certain Biochemical Parameters Due To Helminth Infection In Labeo Rohita At Bhojpur, India

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***Abstract***
***Background:*** *The current investigation was conducted to examine alterations in certain biochemical parameters resulting from helminth parasites in Labeo rohita collected from various sites along the Ganga River at Bhojpur, Bihar, India.*
***Materials and Methods:*** *The host fish were found to harbor various helminth parasites in the intestine, stomach, gills, and skin. Biochemical parameters, including glycogen, protein, and lipid, were measured using standard methods.*
***Observations and Results:***
*The glycogen content of uninfected Labeo rohita ranged from 11.80 mg/g in March 2023 to 24.10 mg/g in December 2024. In comparison, infected fish values range from 6.10 mg/g to 19.0 mg/g. When evaluating the impact of parasitic infection, the percentage reduction in glycogen varied from 20.90% to 48.74% indicating a moderately significant change (t = 3.64, p<0.01). Controlled fish showed a range from 12.90 g/100g to 21.63 g/100g. But, in infected fish, the range of protein content was from 6.09 g/100g to 14.62 g/100g. These findings indicate that the percentage reduction in protein due to parasitic infection ranged from 31.23% to 52.79%. Correspondingly, these variations in protein content due to infection were highly significant (t = 5.99, p<0.001). The lipid content in Labeo rohita ranges from 10.39% to 14.86%. In contrast, infected fish showed variations from 7.15% to 9.80%. The study also quantified the reduction in lipid content due to parasitic infections from 27.88% to 42.38%. The percentage variation due to parasitic infections was found to be moderately significant (t = 3.28, p<0.01).*
***Conclusions:*** *The variation in biochemical reactions in response to the parasitic infection Labeo rohita confirms resistance to the potentially stressful conditions. These parameters were studied further to determine their potential as markers of infection.*

## INTRODCUTION

Fish has long been recognized as a vital component of a balanced and complete human diet, providing a unique combination of high-quality protein, essential fatty acids, vitamins, and minerals that are vital for growth, health, and disease prevention. Its significance in human nutrition spans biological, economic, cultural, and public health perspectives, making it a cornerstone of both traditional and modern diets worldwide. The need for live and disease-free major carps has become increasingly critical due to their economic, nutritional, and ecological importance in freshwater aquaculture. The Major carp Catla catla, Labeo rohita, and Cirrhinus mrigala form the backbone of inland fish production in South and Southeast Asia, contributing significantly to food security, rural livelihoods, and the supply of affordable animal protein. A proximate composition of 14-19% protein and 1-7% lipid occurs in major carp muscle. Glycogen content in major carp varies significantly by species, tissue, environmental factors, season, diet, and water quality. The liver and muscle tissues generally have the highest glycogen concentrations, which are used for energy storage and mobilization, especially during periods of stress or low oxygen. However, disease outbreaks, poor brood stock management, and lack of quality seed production threaten their sustainability, productivity, and safety. Helminth parasite infection significantly alters the chemical composition of major carps, typically leading to a decrease in protein, lipid, and glycogen content, while moisture content generally remains unchanged. However, few workers have focused on the biochemical aspects of the life of fish helminths and their hosts' responses to infection [1 - 4].

The current study aims to examine alterations in certain biochemical parameters resulting from helminth parasites in Labeo rohita collected from various sites along the Ganga River at Bhojpur, Bihar, India.

## MATERIALS AND METHODS

The study was conducted at the Department of Zoology, Maharaja College, Ara. Bihar, India. Labeo rohita were collected from the River Ganga between 2022 and 2025. The fish were caught using local fishing gear and transported to the laboratory in aerated containers. The fish were examined for helminth parasites. The changes in biochemical parameters were studied using the following methods.

1. The Kemp et al. (1954)[5] method is a colorimetric method for glycogen in fish liver or muscle. It is based on the principle of converting glycogen into glucose units (by acid hydrolysis) and then measuring the glucose using

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anthrone reagent. A glucose standard curve was used to calculate the amount of glycogen. The glycogen (mg/g tissue) content was expressed as:

$$\frac{\text{Sample Absorbance}}{\text{Stnadard Absorbance}} \times \text{Std. Glucose Conc.} \times \text{Dilution Factor} \div \text{Weight of Tissue (g)}$$

**2.** Gornall et al. (1949)[6], Biuret method for protein estimation. This is a robust, low-interference colorimetric assay—great for fish muscle/tissue extracts. In a strongly alkaline solution, $Cu^{2+}$ forms a violet chelate with peptide bonds. The color intensity is proportional to protein concentration and is read at ~540–560 nm. $A_{540}$ vs. protein concentration (mg/ml) for the standards was plotted as a straight line through the origin. The protein concentration of the samples from the curve was determined.

$$\text{Protein (mg/ml)} = \frac{\text{Sample Absorbance}}{\text{Stnadard Absorbance}} \times C \text{ std}$$

3. The Folch et al. (1957)[7], method was applied for the estimation of lipid. Lipids were efficiently extracted from tissues with a 2:1 (v/v) mixture of chloroform: methanol. The addition of a small volume of salt/water causes a biphasic separation; most lipids partition into the lower chloroform phase, which is then collected and evaporated to determine the total lipid weight. It was expressed as the % of lipid (wet weight).

This study follows the proposed recommendations of the Departmental Ethical Committee for Experiments of the Department of Zoology, Maharaja College, Arrah, and the ethical guidelines followed for fish sampling and other purposes.

The data were analyzed using Excel and AtoZmath.com software to determine the significant levels of changes in glycogen, protein, and lipid due to infection of helminth parasites in Labeo rohita.

## RESULTS AND DISCUSSION

(A) The glycogen content of Labeo rohita showed variation throughout the year. In the control group, average values were 17.39 mg/g, ranging from 11.80 mg/g in March 2023 to 24.10 mg/g in December 2024. Notably, a one-tailed F test indicated an insignificant yearly effect on glycogen content ($F = 0.28$; $p > 0.05$). In comparison, infected fish exhibited an average glycogen content of 12.25 mg/g, with values ranging from 6.10 mg/g in April 2024 to 19.0 mg/g in December 2024, and also demonstrated insignificant variation ($F = 0.16$; $p > 0.05$). When evaluating the impact of parasitic infection, the percentage reduction in glycogen varied from 23.71% to 45.76% across different months from 2022 to 2025, indicating moderate significance ($t = 3.53, 3.64, 3.55$; $p<0.01$) (Table 1). Kumar (2023)[8] observed that the carbohydrate content of Cyprinus carpio was lower in infected muscle (36.1 mg/g), gut (35.7 mg/g), liver (29.4 mg/g), and gill (28.5 mg/g) when compared to uninfected fish organs (39.2 mg/g, 41.4 mg/g, 36.6 mg/g, and 31.2 mg/g) respectively. Manoonkar et al. (2024)[9] observed that the glycogen content of various infected fish had lower glycogen content in their tissues from 17.57 to 20.27 mg/100ml, compared to the control tissue from 21.17 to 23.42 mg/100ml. Helminth infections lead to a reduction in the glycogen content of freshwater fish. This occurs because the fish use their energy reserves to fight the infection and repair tissue, while the parasites consume host nutrients, such as glycogen, for their own growth and development. Consequently, helminth infection negatively impacts the host's energy stores, potentially leading to tissue damage and a decrease in digestive enzyme activity (B) Controlled fish showed an average protein content of 17.10 g/100g, with values ranging from 12.90 g/100g in January 2025 to 21.63 g/100g in October 2022. This variation was significantly affected by yearly differences ($F = 86.09$; $p < 0.001$). Similarly, in infected fish, the average protein content was 10.56 g/100g, varying from 6.09 g/100g in January 2025 to 14.62 g/100g in December 2022. This, too, was significantly affected ($F = 48.28$; $p < 0.001$). These findings indicate that the percentage reduction in protein due to parasitic infection ranged from 31.23% in December to 40.33% in June 2022-2023, peaking at 52.79% in January 2025. Correspondingly, these variations in protein content due to infection were highly significant ($t = 4.36, 5.99, 4.03$; $p<0.001$) (Table 2). In fish muscle, water content reached its peak levels while muscle protein content decreased during the monsoon. Jagtap (2023) [10] recorded protein content in infected and non-infected fish 19.28 mg/gm of dry tissue in Mystus seengala and in non-infected protein content 21.78 mg/gm, 20.18, 22.09 and 18.12, 20.21 respectively was higher than infected one between period of March to Dec-2019 respectively comparatively low in infected fish than non-infected one showing an increasing trend from summer to monsoon and decrease in winter months. Arjunsinh et al. (2024)[11] observed that Pangasianodon hypophthalmus displayed the lowest protein content at 9.72 g/100g, while Rastrelliger kanagurta exhibited the highest of 18.36 g/100g. Mishra (2021)[12] documented an average protein content of 15.95% during the monsoon season and 16.99% during the post-monsoon season in Labeo rohita sourced from various fish markets in Sultanpur, Uttar Pradesh. The observed increase in protein content during the post-monsoon season suggests a recovery phase for the fish following the demanding breeding activities. The decrease in total protein can be attributed to the consumption of nutrients by parasites and inhibition of protein and nutrient absorption in the nutrient materials. Also, Protein level loss arising from cell destruction, malabsorption, and fasting may reflect the common impact of decreased total protein.

(C) The study on lipid content in Labeo rohita reveals that the average lipid content of healthy specimens was 12.15%, with a range from 10.39% in August 2024 to 14.86% in December 2024. Analysis using a one-tailed F-test revealed a highly significant yearly variation ($F = 11.86$; $p < 0.001$). In contrast, infected Labeo rohita had an average lipid content of 7.91%, with variations from 7.15% in August 2024 to 9.80% in December 2024. The F-test indicated a significant effect of this variation ($F = 3.11$; $p < 0.05$). The study also quantified the reduction in lipid content due to parasitic infections; reductions ranged from 27.88% in December to 38.47% in April during 2022-2023, 31.10% in May to 37.85% in December during 2023-2024, and 29.16% in July to 42.38% in March during 2024-2025. The percentage variation due to parasitic infections was noted to be moderately significant ($t = 2.97, 2.85, 3.28$; $p<0.01$) (Table 3). Arjunsinh et al. (2024)[11] observed that in Wallago attu, Labeo rohita, Pangasianodon hypophthalmus and Rastrelliger kanagurta had the lowest lipid content during the monsoon season, measuring at 0.69%, 1.59%, 9.33%, and 2.47%, respectively, contrasting with their highest levels during the winter season at 1.07%, 2.05%, 11.04%, and 4.05%, respectively. Jawale (2023)[13] observed that Claraias batrachus with cestode parasites have 0.18 mg/g lipid (weight of tissue) in infected and in non-infected 0.22 mg/g, showing a decrease of 18.18%. Kadu (2024)[4] observed that Mystus seenghala with cestode parasites have 11.23 mg/g, 12.15 mg/g, and 10.14 mg/g lipid (weight of tissue) in infected and in non-infected 12.90 mg/g, 13.20 mg/g, and 11.05 mg/g, showing a decrease of 07.95 to 12.94%. Lipids are of great importance to the body of cestodes as the chief concentrated storage form of energy, and they play a role in cellular structure and various other biochemical functions.
Therefore, the variation in biochemical reactions in response to the parasitic infection Labeo rohita confirms resistance to the potentially stressful conditions. These parameters were studied further to determine their potential as markers of infection.

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**Table 1: Glycogen content of selected control and infected Labeo rohita during 2022-2025**

| Month/Year | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Total | Avg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2022-2025 (Control) | 19.2 | 19.4 | 19.7 | 21.9 | 16.4 | 13.6 | 11.8 | 15.6 | 16.4 | 17.8 | 18.7 | 18.3 | **208.8** | **17.40** |
| | 20.1 | 20.4 | 20.3 | 20.2 | 14.6 | 13.9 | 12.8 | 11.9 | 14.6 | 17.9 | 17.6 | 18.1 | **202.4** | **16.87** |
| | 20.3 | 22.6 | 23.3 | 24.1 | 12.6 | 13.7 | 12.9 | 13.6 | 17.3 | 18.6 | 17.4 | 18.3 | **214.7** | **17.89** |
| **Average** | **19.87** | **20.80** | **21.10** | **22.07** | **14.53** | **13.73** | **12.50** | **13.70** | **16.10** | **18.10** | **17.90** | **18.23** | 208.63 | 17.39 |
| **Range** | **±0.55** | **±1.60** | **±1.80** | **±1.10** | **±1.90** | **±0.05** | **±0.55** | **±1.00** | **±0.45** | **±0.40** | **±0.65** | **±0.00** | | |
| | 14.6 | 14.8 | 14.6 | 16.4 | 11.9 | 8.7 | 6.4 | 10.2 | 11.3 | 12.7 | 13.8 | 13.4 | **148.8** | **12.40** |
| | 23.96% | 23.71% | 25.89% | 25.11% | 27.44% | 36.03% | 45.76% | 34.62% | 31.10% | 28.65% | 26.20% | 26.78% | | |
| | 15.9 | 15.8 | 14.9 | 14.6 | 9.2 | 8.3 | 7.4 | 6.1 | 9.6 | 12.9 | 12.9 | 13.6 | **141.2** | **11.77** |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2022-2025 (Infected) | 20.90% | 22.55% | 26.60% | 27.72% | 36.99% | 40.29% | 42.19% | 48.74% | 34.25% | 27.93% | 26.70% | 24.86% | | |
| | 15.7 | 17.7 | 18.1 | 19 | 7.1 | 8.3 | 7.3 | 7.6 | 10.3 | 13.4 | 12.6 | 13.9 | **151** | **12.58** |
| | 22.66% | 21.68% | 22.32% | 21.16% | 43.65% | 39.42% | 43.41% | 44.12% | 40.46% | 27.96% | 27.59% | 24.04% | | |
| Average | **15.40** | **16.10** | **15.87** | **16.67** | **9.40** | **8.43** | **7.03** | **7.97** | **10.40** | **13.00** | **13.10** | **13.63** | 147.00 | 12.25 |
| Range | ±0.55 | ±1.45 | ±1.75 | ±1.3 | ±2.4 | ±0.2 | ±0.45 | ± 1.3 | ± 0.5 | ±0.35 | ±0.6 | ±0.25 | | |

**F = 0.28 (df = 2 and 33) for control fish F=0.16(df=2and 33) for infected fish**
**'t' = 3.53 (df=14) for first group fish 't'=3.64(df=14) for second group fish 't' = 3.55 (df=14) for third group fish**

| Table 2: Protein content of selected control and infected Labeo rohita during 2022-2025 | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Month/Year | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Total | Avg |
| 2022-2025 (Control) | 21.32 | 21.63 | 21.41 | 21.26 | 18.4 | 19.7 | 19.81 | 19.93 | 19.21 | 18.92 | 18.99 | 20.15 | **240.73** | **20.06** |
| | 17.34 | 17.47 | 17.52 | 17.69 | 15.63 | 18.85 | 16.06 | 16.95 | 16.09 | 15.35 | 15.94 | 16.53 | **201.42** | **16.79** |
| | 15.42 | 15.69 | 15.87 | 14.15 | 12.9 | 13.75 | 14.54 | 15.12 | 15.55 | 13.89 | 13.94 | 14.67 | **175.49** | **14.62** |
| Average | **18.03** | **18.26** | **18.27** | **17.7** | **15.64** | **17.43** | **16.08** | **17.33** | **16.95** | **16.05** | **16.29** | **17.12** | 205.15 | 17.10 |
| Range | ±2.95 | ±2.97 | ±2.77 | ±3.56 | ±2.75 | ±2.98 | ±2.64 | ±2.41 | ±1.83 | ±2.52 | ±2.53 | ±2.74 | | |
| 2022-2025 (Infected) | 14.23 | 14.36 | ±.14 | 14.62 | 11.04 | 12.07 | 12.81 | 12.39 | 12.12 | 11.29 | 11.54 | 13.51 | **154.12** | **12.84** |
| | 33.26% | 33.61% | 33.96% | 31.23% | 40.00% | 38.73% | 35.34% | 37.83% | 36.91% | 40.33% | 39.23% | 32.95% | | |
| | 11.47 | 11.01 | 11.25 | 11.11 | 9.36 | 12.58 | 10.6 | 10.45 | 10.04 | 9.53 | 9.47 | 10.05 | **126.92** | **10.58** |
| | 33.85% | 36.98% | 35.79% | 37.20% | 40.12% | 33.26% | 34.00% | 38.35% | 37.60% | 37.92% | 40.59% | 39.20% | | |
| | 8.24 | 9.96 | 9.78 | 8.52 | 6.09 | 7.57 | 7.45 | 9.21 | 9.47 | 7.45 | 7.49 | 7.75 | **98.98** | **8.25** |
| | 46.56% | 36.52% | 38.37% | 39.79% | 52.79% | 44.95% | 48.76% | 39.09% | 39.10% | 46.36% | 46.27% | 47.17% | | |
| Average | **11.31** | **11.78** | **11.72** | **11.42** | **8.83** | **10.74** | **10.29** | **10.68** | **10.54** | **9.42** | **9.50** | **10.44** | 126.67 | 10.56 |
| Range | ±3.00 | ±2.20 | ±2.18 | ±3.05 | ±2.48 | ±2.25 | 2.68 | ±1.59 | ±1.33 | ±1.92 | ±2.03 | ±2.88 | | |

**F = 86.09 (df = 2 and 33) for control fish F=48.28 (df=2and 33) for infected fish**
**'t' = 4.36 (df=14) for first group fish 't'=5.99 (df=14) for second group fish 't' = 4.03 (df=14) for third group fish**

| Table 3: Lipid content of selected control and infected Labeo rohita during 2022-2025 | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Month/Year | Sep | Oct | Nov | Dec | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Total | Avg |
| 2022-2025 (Control) | 11.62 | 11.83 | 11.95 | 12.05 | 11.98 | 11.96 | 11.94 | 11.8 | 11.67 | 11.59 | 11.58 | 11.58 | **141.55** | **11.80** |
| | 11.52 | 11.38 | 11.42 | 13 | 12.8 | 12.18 | 11.54 | 11.47 | 11.51 | 11.48 | 11.73 | 10.39 | **140.42** | **11.70** |
| | 12.38 | 13.54 | 13.55 | 14.86 | 14.08 | 13.81 | 12.93 | 12.48 | 12.14 | 11.97 | 11.83 | 11.94 | **155.51** | **12.96** |
| Average | **11.84** | **12.25** | **12.31** | **13.30** | **12.95** | **12.65** | **12.14** | **11.92** | **11.77** | **11.68** | **11.71** | **11.30** | **145.83** | **12.15** |
| Range | ±0.38 | ±0.86 | ±0.80 | ±1.41 | ±1.05 | ±0.93 | ±0.50 | ±0.34 | ±0.24 | ±0.19 | ±0.13 | ±0.18 | | |
| 2022-2025 (Infected) | 7.49 | 7.69 | 7.89 | 8.69 | 7.5 | 7.59 | 7.38 | 7.26 | 7.85 | 7.95 | 7.76 | 8.08 | **93.13** | **7.76** |
| | 35.54 % | 35.00 % | 33.97 % | 27.88 % | 37.40 % | 36.54 % | 38.19 % | 38.47 % | 32.73 % | 31.41 % | 32.99 % | 30.22 % | | |
| | 7.74 | 7.45 | 7.85 | 8.08 | 8 | 8.24 | 7.83 | 7.25 | 7.93 | 7.37 | 7.84 | 7.15 | **92.73** | **7.73** |
| | 32.81 % | 34.53 % | 31.26 % | 37.85 % | 37.50 % | 32.35 % | 32.15 % | 36.79 % | 31.10 % | 35.80 % | 33.16 % | 31.18 % | | |
| | 7.41 | 8.84 | 8.18 | 9.8 | 9.68 | 8.55 | 7.45 | 7.83 | 7.49 | 7.38 | 8.38 | 7.97 | **98.96** | **8.25** |
| | 40.15 % | 34.71 % | 39.63 % | 34.05 % | 31.25 % | 38.09 % | 42.38 % | 37.26 % | 38.30 % | 38.35 % | 29.16 % | 33.25 % | | |
| Average | **7.55** | **7.99** | **7.97** | **8.86** | **8.39** | **8.13** | **7.55** | **7.45** | **7.76** | **7.57** | **7.99** | **7.73** | 94.94 | **7.91** |
| Range | ±0.04 | ±0.58 | ±0.15 | ±0.56 | ±1.09 | ±0.48 | 0.04 | ±0.29 | ±0.18 | ±0.29 | ±0.31 | ±0.06 | | |

**F=11.86(df=2and 33) for control fish F= 3.11 (df = 2 and 33) for infected fish**
**'t' = 2.97 (df=14) for first group fish 't'=2.85(df=14) for second group fish 't' = 3.28 (df=14) for third group fish**