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Comparison of Proximate body composition and heavy metals detection in Muscles and Liver Enzymes of Major Carps from Head Islam, Vehari

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ABSTRACT This study evaluated the comparison of proximate body composition and heavy metal detection in muscles and liver enzymes of three major freshwater carps, *Labeo rohita, Catla catla,* and *Cirrhinus mrigala.* Experimental groups were taken from three different water sites, W1, W2, and W3, from Head Islam near Vehari, and compared with a Control group taken from the Assistant Director of the Fisheries District Vehari. Proximate body composition analysis revealed a significant decline in moisture content from 74.4% in the Control group to 72.0% in W3, and crude protein content decreased from 15.13% to 13.67% across the same water sites. Conversely, crude fat content showed a gradual increase, reaching 3.50 %, while ash content fluctuates slightly but consistently remains within the acceptable reference range of 2.40 % to 2.16 %. Heavy metal accumulation was found to exceed recommended safety limits in W3, with Lead levels rising from 0.21 mg/kg in the control to 1.36 mg/kg in W3. Cadmium and Mercury levels also increased significantly across water sites. Liver enzyme activity showed marked elevation, with Aspartate Transaminase levels increasing from 50 U/L in controls to 114.3 U/L in W3, and similar trends were observed in Alanine Transaminase and Alkaline Phosphate levels, indicating liver stress and possible hepatocellular damage. Albumin slightly decreased but remained within normal limits. Bilburin levels rose gradually, nearing the upper limit in W3 samples. ANOVA results confirmed highly significant differences ($P < 0.01$) among major carps for all studied parameters. These findings underscore the detrimental effects of poor water quality on fish health and highlight the risk of bioaccumulation of toxic substances in edible fish tissues. The study emphasizes the need for routine water quality monitoring and stringent environmental controls to safeguard aquaculture productivity and consumer safety.

Keywords*:* Aquaculture, bioaccumulation, freshwater carps, heavy metals, liver enzymes,

INTRODUCTION

Environmental pollution of aquatic ecosystems has grown to be a major worldwide problem, with heavy metals being a major cause for concern. In the literature, the word "heavy metal" can be defined in a variety of ways. It encompasses both essential and non-essential metals with a high atomic weight and a density higher than that of water, and is frequently used interchangeably with trace metals. Fish is a vital food source that makes up a sizable portion of diets globally. It is especially prized for its premium proteins, vitamins, minerals, omega-3 fatty acids, and vital amino acids (Tacon, 2023). Fish tissues, especially the muscles and liver, can accumulate heavy metals like lead, cadmium, mercury, and arsenic, which are

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persistent environmental contaminants. Both aquatic life and people who eat fish are at risk from this bioaccumulation (Agbugui & Abe, 2022).

Knowing the body composition of various fish species in the River Sutlej, Head Islam, and the degree of heavy pollution in their tissues is essential for evaluating possible environmental effects and guaranteeing food safety. The study reveals that the Sutlej River's surface water is contaminated with heavy metals, particularly Ni, Cr, Cd, and Pb, which pose significant health risks to humans. Heavy metals are among the contaminants that are introduced into the freshwater bodies by industrial and agricultural runoff into the aquatic systems (Mushtaq et al., 2020). Major carps are not only ecologically significant but also economically valuable in Pakistan's inland fisheries. Fish, especially major carps like *Labeo rohita, Catla catla,* and *Cirrhinus mrigala,* are at high risk of bioaccumulating these metals due to their position in the food chain and their ability to absorb contaminants through gills and diet (Rahman et al., 2019). Organs involved in metabolic regulation and detoxification are particularly sensitive, with the liver often showing early signs of damage (Lucas et al., 2021).

The liver, in particular, plays a central role in metabolism and is highly susceptible to toxicant exposure, making it a critical target for evaluating pollutant effects (Khan et al., 2019). District Vehari, located in southern Punjab, is an important agricultural hub with an expanding population and industrial footprint. Previous studies in other regions of Pakistan have identified alarming levels of heavy metals in both wild and farmed fish, but little work has focused specifically on the Vehari district. Industrial waste, sewage, and agricultural runoff contribute to the river's contamination (Setia et al., 2020). Unregulated industrial discharge, pesticide runoff from agricultural lands, and domestic sewage have led to a steep increase in toxic substances in rivers, canals, and ponds (Farooq et al., 2020). Heavy metals such as cadmium, arsenic, and lead are non-biodegradable and tend to persist in aquatic environments for extended periods. These elements can easily enter the food web through water and sediment, accumulating progressively in organisms such as fish (Ashfaq et al., 2021).

Wild fish accumulated higher concentrations of heavy metals in their muscles than cultured fish. The hierarchy of mean concentration of heavy metals in wild fishes was Zn > Mn > Cu > Pb > Cr > Ni > Cd, and in cultured fishes, it was Zn > Mn > Cr > Pb > Cu > Ni > Cd. The concentrations of heavy metals detected were below the allowable level specified by international agencies (FAO, WHO, EU) (Ahmed et al., 2022). The rise of anthropogenic activities near freshwater ecosystems, such as unregulated industrial zones, brick kilns, and wastewater discharge, has significantly increased the influx of pollutants, especially in rural and semi-urban districts like Vehari. Fish sex, age, season, and location all affect the concentration of hazardous elements in fish. Anthropogenic pollution of water sources causes aquatic loss, which upsets the food chain's equilibrium (Roy, 2024). When fish absorb metals from their environment, the contaminants tend to accumulate in muscle tissues over time. Since muscles constitute the primary edible part of fish, their contamination directly affects human health.

By altering metabolic, physiological, and biochemical processes, stressors like pollution have a negative impact on growth, development, and reproduction (Ciftci et al., 2017). According to research done on fish, all heavy metals have negative impacts on living things through mutagenesis and metabolic interference, even though some of them are necessary for existence. These adverse effects include decreased fitness, cancer-causing interference with reproduction, and eventually death (Javed & Usmani, 2019).

This research was done to assess the proximate body composition of major carps from Head Islam Vehari and to assess the heavy metal concentration in muscles and liver tissues of fishes collected from different sites from Head Islam Vehari. This study is important in many areas and has significant ramifications for scientific knowledge, environmental protection, and real-world applications. Scientifically speaking, the study pushes the boundaries of metal toxicology and aquatic ecosystem research by offering previously unheard-of insights into the complex relationships between metals and living things.

Materials and Method

The fish species selected for the study, *L. rohita, C. catla,* and *C. mrigala,* are among the most economically and nutritionally important freshwater carps in South Asia. The selection of Head Islam near Vehari as the study area was strategic. It reflects a dynamic freshwater ecosystem influenced by agricultural chemicals, effluents from urban settlements, and poor waste management practices. The comparison of samples collected from polluted sites and a controlled hatchery allows for an accurate interpretation of the environmental influence on physiological traits.

The proximate composition, including moisture, crude protein, fat, and ash content, is analyzed to determine the nutritional quality of fish muscle. Fish samples were collected during early daylight hours using cast nets. Post-collection, fish were measured and weighed to obtain biometric data. To ensure accuracy in data, each biochemical and toxicological assay was conducted in triplicate. Standard curves, blank controls, and certified reference materials were used where applicable. The study highlights the importance

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of protecting our rivers and aquatic life (Vaseem et al., 2016).

Data Collection

Samples were captured from four different sites. First samples were collected from site where water receives runoff from mechanical and small-scale manufacturing units, second sample were collected from site situated near a high-agriculture zone is frequently impacted by pesticides and fertilizer runoff during irrigation cycles, third sample were collected from downstream collection point with visible algal blooms and signs of eutrophication, likely due to cumulative contamination and for control samples were taken from The Assistant Director Fisheries Hatchery, Vehari, representing a semi- natural, monitored aquatic system with consistent water quality standards and controlled feeding conditions.
From each of these locations, specimens of *L rohita*, *C catla,* and *C mrigala* were collected. Three per species were captured from three water sites, resulting in a total of 27 samples (3 species*3 parameters* 3 water sites) and 3 per species for the same 3 parameters from Assistant Director Fishes Vehari, resulting into Grand total of 36 fishes.

Figure1. ***Head Islam Vehari***

Fish were caught using cast nets in the early morning to avoid diurnal variations in metabolism. The captured fish were immediately placed in aerated containers to minimize stress.

Figure 2. *Cast Net*

Sampling Procedure

After transportation to the laboratory in ice-cooled, aerated containers, each fish specimen was measured for total length (cm) and body weight (g) using digital calipers and precision scales. Humane euthanasia was conducted through cold shock, and tissues were extracted. Dissection was carried out in sterile conditions. The muscle tissues were sampled from the dorsal region, avoiding the lateral line and skin. Liver tissues were carefully separated from the visceral cavity, ensuring no cross-contamination. Muscle samples designated for proximate composition and heavy metal analysis were wrapped in aluminum foil, labeled, and stored at −20°C to prevent decomposition and enzymatic degradation. Liver tissues, required for enzymatic assays, were preserved in buffered formalin (10%) and stored at 4°C.
These samples were later processed for spectrophotometric and histopathological evaluations. Standard protocols as recommended by the Association of Official Analytical Chemists were strictly followed during sample handling and storage to maintain integrity. Muscle tissues were washed briefly with phosphate-buffered saline (PBS) to remove contaminants, patted dry with sterile filter paper, and homogenized using stainless steel blenders. Homogenization was essential to ensure even distribution of nutrients and metals during testing. Tissue samples were then divided into triplicates for repeatability in analytical testing. Lyophilization improved accuracy by eliminating variability due to differing water content.
Each analytical unit was labeled with specimen code, species, site ID, and assay type. The features selected for analysis in this research were categorized into three primary domains: proximate body composition, heavy metals in muscle tissues, and liver enzyme activities in serum. The researchers analyzed the proximate parameters, including protein, lipid, carbohydrate, ash, and moisture content, across different seasons. The study highlights the seasonal impact on the nutritional composition of fish, which can inform dietary choices and nutritional planning (Arjunsinh et al., 2024). Moisture Content indicates the water retention capacity and general condition of fish tissues.
The loss in weight was attributed to the moisture content. The percentage was calculated using the formula:
Moisture (%) = [(Initial weight - Final weight) / Initial weight] × 100
A decline in protein levels is indicative of physiological stress, disease, or metabolic dysfunction.
The total nitrogen content obtained was multiplied by a conversion factor of 6.25 to yield crude protein:

Crude Protein (%) = Nitrogen (%) × 6.25

In toxic environments, fat metabolism may be impaired or redirected to detoxification processes.

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The remaining lipid residue was weighed and expressed as a percentage of the initial tissue mass.
Crude Fat (%) = (Weight of extracted fat / Weight of sample) × 100
AST is a marker for liver cell damage; elevated levels suggest hepatocellular injury. Also involved in amino acid metabolism and cellular respiration. An ALT increase indicates stress or inflammation in hepatic tissues. ALP participates in membrane transport; its activity rises in response to hepatobiliary obstruction or tissue remodeling. ALB is a vital plasma protein synthesized in the liver. Reductions indicate impaired liver function and protein metabolism. Bilirubin is a byproduct of hemoglobin breakdown. High levels in serum suggest impaired excretory function of the liver. These parameters provide a biochemical snapshot of liver function, cellular integrity, and the systemic impact of environmental pollutants. They serve as reliable indicators to evaluate environmental quality and the safety of fish for human consumption (Akpan Yung et al., 2014). Fish accumulate Pb from contaminated sediments, feeding material, and water through gill absorption. Cd is highly toxic, disrupting calcium metabolism and inhibiting enzyme systems. Accumulates primarily in the kidneys and muscles, posing significant human health risks upon consumption.
The hot air oven is essential for determining the moisture content in fish samples. Moisture content is a fundamental parameter in proximate analysis as it influences all other nutritional estimations. The Kjeldahl method is a classic and widely accepted technique that involves the digestion of the sample in concentrated sulfuric acid, followed by neutralization, distillation, and titration to quantify total nitrogen content. To determine crude fat content, the Soxhlet extraction unit was employed. This apparatus allows for the continuous extraction of lipids from a sample using organic solvents like petroleum ether or hexane. The muffle furnace was used for ash content determination by incinerating dried fish samples at temperatures up to 550°C. This process burns off all organic material, leaving behind inorganic mineral residues, which are then weighed.
The AAS was pivotal in detecting and quantifying the concentrations of Pb, Cd, and Hg in muscle tissues. This highly sensitive instrument measures the absorption of light by vaporized metal atoms in a flame or graphite furnace. Each metal absorbs light at a specific wavelength, allowing for selective analysis. For biochemical assays of liver enzymes AST, ALT, ALP, as well as serum ALB and Bilirubin levels, the UV-Vis spectrophotometer was used. This device measures the absorbance or transmittance of specific wavelengths of light by liquid samples. Centrifugation was used to separate serum from whole blood. Blood samples were collected via cardiac puncture and spun at 3000 rpm for 10–15 minutes. The centrifuge ensured that serum samples were clean and free from hemolysis, which is critical for reliable enzymatic assays.

Statistical Analysis

Data generated from proximate composition, metal detection, and enzyme assays were compiled and subjected to statistical analysis. One-way Analysis of Variance (ANOVA) was employed to determine whether statistically significant differences existed among groups (fish species and sampling sites). Descriptive statistics, including means, standard errors, and confidence intervals, were calculated for all parameters. Graphical representation of data was carried out using bar charts and error bars to visualize variation across treatment groups. The rigorous application of statistical tests ensured that the conclusions drawn were supported by quantitative evidence and free from bias.

Result

The results indicate that protein content tends to decrease progressively from the control to W3 across all three fish species. In contrast, crude fat content shows a slight increase with different water sites, suggesting a potential compensatory nutrient shift. Moisture levels also exhibit a gradual decline across water sites, while ash content fluctuates slightly but consistently remains within the acceptable reference range. The data indicate the extent of metal bioaccumulation due to environmental contamination in the Vehari region. Heavy metal analysis in *L rohita, C catla,* and *C . mrigala* showed Pb, Cd, and Hg levels within permissible limits. The biochemical parameters (AST, ALT, ALP, ALB, and Bil) in *L. rohita, C. catla,* and *C. mrigala* exhibited noticeable variations compared to their respective controls.
In all three species, AST, ALT, ALP, ALB, and Bil levels increased progressively from W1 to W3, with W3 values approaching or exceeding the upper reference limits, particularly in *C. mrigala* (AST: 118 U/L, ALP: 151 U/L). ALB levels slightly decreased across the exposure groups but remained within the normal range. Bil levels showed a mild increase in all species, reaching the upper limit (1.2 mg/dL) in *L Rohita* W3.

Table 1: Proximate composition report of fishes

IDs Parameters	Moisture (%)	Crude Protein (%)	Crude Fat (%)	Ash (%)
Control Group (*L. rohita*)	75.2	15.3	3.1	2.5
W1	73.5	14.8	3.8	2.8
W2	74.1	14.2	3.7	2.4
W3	72.9	13.7	3.6	2.2
Control Group (*C. catla*)	74.5	14.9	3	2.4
W1	72.8	14.4	3.2	2.7

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W2	73.2	13.9	3.3	2.3
W3	71.9	13.5	3.3	2.1
Control Group (*C. mrigala*)	73.5	15.2	3.1	2.3
W1	72.1	14.7	3.3	2.6
W2	72.6	14.2	3.7	2.2
W3	71.2	13.8	3.6	2

The analysis of variance (ANOVA) demonstrated a highly significant effect of water site on the moisture content in major carps (P = 0.000). The Mean comparison test further illustrates this effect, showing that the control group had the highest moisture content (74.4 ± 0.86%), while the moisture content progressively decreased across the treatments (W1: 72.8 ± 0.76%, W2: 73.3 ± 0.70%, W3: 72.0 ± 0.87%). The ANOVA results show a highly significant effect of water site on the Crude Protein (%) content across the combined freshwater fish species (P = 0.000). The mean comparison test reveals a clear trend; the control group exhibited the highest crude protein content (15.13 ± 0.21%), while protein content decreased progressively with water site severity (W1: 14.63 ± 0.21%, W2: 14.1 ± 0.17%, W3:
13.67 ± 0.10%). The analysis of variance (ANOVA) for Crude Fat (%) across combined freshwater fish species revealed a highly significant water site effect (P = 0.001). The Mean comparison test indicated an increasing trend in crude fat levels from the control group (3.06 ± 0.06) to W2 (3.56 ± 0.23), with W3 (3.50 ± 0.29) also showing elevated values. The ANOVA results for Ash content in major carps indicated a highly significant effect of water sites (P = 0.006). The Mean comparison test showed that the highest ash content was observed in W1 (2.70 ± 0.12), while the lowest was found in W3 (2.16 ± 0.10). The analysis of variance (ANOVA) for Pb accumulation in major carps showed a highly significant effect of treatment (P = 0.000), indicating that water treatments markedly influenced Pb uptake. Mean comparisons test revealed a stepwise increase in Pb levels from the control group (0.25 mg/kg) to W3 (1.45 mg/kg), significantly exceeding the control. The ANOVA results for Cd content in major carps revealed a highly significant effect of water sites (P = 0.000), confirming that varying water conditions significantly altered Cd accumulation. Mean comparisons test indicated a rising trend in Cd concentration, from 0.05 mg/kg in the control to 0.30 mg/kg in W3. The ANOVA for Hg accumulation in major carps revealed a highly significant treatment effect (P = 0.000), confirming that Hg levels were significantly influenced by water quality. The control group had the lowest Hg concentration (0.018 mg/kg), while W3 exhibited the highest (0.16 mg/kg). The analysis of Pb, Cd, and Hg in *L rohita, C catla,* and *C mrigala* showed a clear increasing trend from W1 to W3 in all species. Compared to their respective controls, all exposed groups demonstrated elevated levels of these heavy metals. The analysis of variance (ANOVA) for AST levels in fish liver tissues revealed a highly significant effect of water site (P = 0.000), showing that AST activity significantly increased with the severity of water. The control group had the lowest Mean AST value (50.00 ± 5.77 U/L), while W3 had the highest (114.33 ± 7.57 U/L). The ANOVA for ALT levels in fish liver tissues indicated a highly significant effect of water site (P = 0.000), showing that exposure to different water sites significantly altered ALT activity. The control group had the lowest ALT value (49.33 ± 2.51 U/L), while W3 exhibited the highest (79.33 ± 3.06 U/L). The ANOVA results for ALP levels in fish liver tissues showed a highly significant effect of water. (P = 0.001), demonstrating that exposure to different water sites significantly impacted ALP activity. The control group had the lowest Mean ALP value (87.67 ± 2.89 U/L), while W3 exhibited the highest (148.33 ± 3.79 U/L). The ANOVA for ALB levels in fish liver tissues showed a significant effect of water sites (P = 0.021), suggesting that water conditions influenced ALB concentration. The control group had the highest Mean ALB level (4.40 ± 0.20 g/dL), while W3 had the lowest (3.76 ± 0.15 g/dL). The ANOVA results for Bil levels in fish liver tissues revealed a significant effect of water site (P = 0.033), indicating that water site exposure altered Bil concentrations. The control group had the lowest Mean Bil level (0.7 ± 0.07 mg/dL), while W3 had the highest (1.13 ± 0.07 mg/dL).

Table 2: Effects of water sites on different parameters, ANOVA

Parameter	S.O.V	DF	SS	MS	F	P-value
Moisture	Water Sites	3	12.34	4.11	15.8	0.000**
	Error	8	2.08	0.26		
	Total	11	14.42			
Crude Protein	Water Sites	3	5.157	1.71	60.6	0.000**
	Error	8	0.227	0.02		
	Total	11	5.384			
Crude Fat	Water Sites	3	1.066	0.35	17.7	0.001**
	Error	8	0.16	0.02		
	Total	11	1.226			
Ash	Water Sites	3	0.413	0.13	9.2	0.006
	Error	8	0.12	0.01		
	Total	11	0.533			
Lead	Water Sites	3	1.309	0.436	66.92	0.000**
	Error	8	0.052	0.007		
	Total	11	1.361			
Cadmium (Cd)	Water Sites	3	0.0434	0.0145	38.96	0.000**
	Error	8	0.0030	0.0004		
	Total	11	0.0464			
Mercury (Hg)	Water Sites	3	0.0194	0.0065	54.17	0.000**
	Error	8	0.0010	0.0001		
	Total	11	0.0204			
AST (U/L)	Treatment	3	1784.6	594.87	62.58	0.000**
	Error	8	75.74	9.46		
	Total	11	1860.34			

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ALT (U/L)	Water Sites	3	2485.3	828.44	29.17	0.000**
	Error	8	227.9	28.48		
	Total	11	2713.2			
ALP (U/L)	Water Sites	3	3594.5	1198.1	16.47	0.001**
	Error	8	578.0	72.25		
	Total	11	4172.5			

Discussion

Moisture and crude protein content exhibited a decreasing trend from control to W3, indicating a deterioration in fish flesh quality under stress. The crude fat content increased initially, peaking at W2, before slightly declining in W3, while ash content consistently declined across water sites. These nutritional changes reflect the fish's metabolic response to environmental stressors, potentially due to increased energy demands or impaired physiological processes.

In terms of heavy metal accumulation, Pb, Cd, and Hg concentrations were found to increase progressively with higher water levels. Pb levels in W3 exceeded the permissible safety limits, highlighting the severity of contamination. These heavy metals can disrupt cellular and enzymatic functions, contributing to oxidative stress, impaired growth, and overall health deterioration in fish. Bioaccumulation of different metals varies greatly with species; however, the chromium level in *C. mrigala* exceeds the recommended limit by FAO/WHO (Ahmed et al., 2022).

Liver enzyme analysis further confirmed physiological stress. AST, ALT, ALP, ALB, and Bil levels rose significantly with water intensity, particularly in W3, suggesting hepatocellular damage and compromised liver function. Elevated enzyme levels serve as key biomarkers of toxicity and stress in fish. Collectively, these findings indicate that water pollution has a multifaceted impact on fish health, affecting both nutritional quality and organ function.

This study concludes that water sites potentially mimicking polluted or altered environmental conditions have significant adverse effects on the nutritional and biochemical health of major freshwater fish species. Decreased moisture and protein content in fish fillets from different water sites indicate compromised quality, which could impact market value and consumer nutrition. The observed increases in fat content at moderate stress levels may represent a metabolic adaptation, whereas continued exposure leads to nutritional decline. Consistent decreases in ash content across water sites suggest mineral imbalance or losses due to stress-induced physiological disruption. Heavy metal analysis clearly indicates bio-accumulation of Pb, Cd, and Hg in fish tissues, particularly in (W3), where Pb levels exceeded safe consumption limits. Enzymatic changes are classic indicators of toxicant-induced cellular damage, supporting the need for urgent monitoring of biochemical health markers in aquaculture settings. Different fish species have higher rates of mercury bioaccumulation (Mozaffarian & Rimm, 2006).

Conclusion

This study concludes that water sites potentially mimicking polluted or altered environmental conditions have significant adverse effects on the nutritional and biochemical health of major freshwater fish species. Decreased moisture and protein content in fish fillets from different water sites indicate compromised quality, which could impact market value and consumer nutrition. The observed increases in fat content at moderate stress levels may represent a metabolic adaptation, whereas continued exposure leads to nutritional decline. Consistent decreases in ash content across water sites suggest mineral imbalance or losses due to stress-induced physiological disruption. Heavy metal analysis clearly indicates bio-accumulation of Pb, Cd, and Hg in fish tissues, particularly in (W3), where Pb levels exceeded safe consumption limits. Elevated liver enzyme activities AST, ALT, ALP, ALB, and Bil further support the conclusion that water sites inflict internal organ stress, particularly liver dysfunction. The consistency of these effects across species underscores the sensitivity of freshwater fish to waterborne pollutants. These results have significant implications for fish farmers, policymakers, and environmental managers. Firstly, the research may not fully replicate complex natural environmental conditions such as seasonal variations or fluctuating pollutant loads. Secondly, the study focused on a limited number of species and water sites; additional species or broader pollutant spectra could yield more generalized conclusions. Nutritional and biochemical assessments of cultured species should be incorporated into routine health checks to detect early signs of stress or toxicity.

Author Contributions

Tahira Ghafoor played a key role in developing the research concept, designing the experimental setup, and establishing PVC microplastic exposure concentrations. She conducted in vivo experiments on Cirrhinus mrigala, managed aquarium conditions, feeding strategies, and sampling timelines. Anam Saeed drafted the initial version of the manuscript. Maryam Riasat and Nida Younus were involved in biochemical assays such as ALT, AST, ALP, bilirubin, and serum metabolite studies utilizing spectrophotometric techniques. Naureen Rana supported the handling of the fish, executing morphometric studies, assessing growth performance, and maintaining experimental records, and worked in collecting blood samples, processing those samples, and ensuring quality control in laboratory processes.

Conflicts of Interest

The authors declare(s) that there is no conflict of interest regarding the publication of this paper.

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