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COMPARATIVE ANALYSIS OF DIGESTIVE ENZYME ACTIVITY IN LABEO ROHITA, CIRRHINUS MRIGALA, AND C. CATLA: FARMED VS. WILD CONDITIONS

Original Article

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ABSTRACT

Background: Digestive enzyme activity plays a crucial role in nutrient assimilation and overall growth in fish. Differences in environmental conditions, diet composition, and feeding strategies significantly influence enzymatic function. Farmed fish often receive formulated diets, while wild fish consume natural feed, which may impact their digestive efficiency. Understanding variations in digestive enzyme activity between farmed and wild fish species can provide insights into metabolic adaptations and contribute to optimizing feeding strategies in aquaculture. This study aimed to evaluate the relationship between digestive enzyme activity and gut morphometric parameters in Labeo rohita, Cirrhinus mrigala, and Catla catla from farmed and wild environments.

Objective: To compare digestive enzyme concentrations (amylase, lipase, and protease) between farmed and wild populations of L. rohita, C. mrigala, and C. catla and assess their correlation with gut morphometry.

Methods: A total of 90 fish specimens (30 per species) were collected from the Al-Raheem Fish Hatchery (farmed) and River Chenab (wild). Body weight, total length, gut weight, gut length, relative gut mass, relative gut length, and Zihler's index were measured. Gut enzyme activity was analyzed using spectrophotometric methods for amylase, lipase, and protease concentrations. Statistical analyses, including Pearson's correlation and ANOVA, were performed to determine significant differences between farmed and wild fish.

Results: Wild L. rohita exhibited higher amylase activity (21.6 IU/ml) compared to farmed individuals (17.9 IU/ml). Similar trends were observed in C. catla (wild: 12.9 IU/ml; farmed: 9.9 IU/ml) and C. mrigala (wild: 6.2 IU/ml; farmed: 12.5 IU/ml). Lipase activity was highest in wild C. mrigala (14.6 IU/ml) compared to farmed specimens (5.4 IU/ml). Protease concentrations were higher in wild L. rohita (0.3 IU/ml) than in farmed individuals (0.2 IU/ml). Statistical analysis showed no significant difference in enzyme activity between farmed and wild fish ($p > 0.05$).

Conclusion: Wild fish demonstrated higher digestive enzyme activity than farmed fish, suggesting that environmental factors influence metabolic efficiency. Gut morphometric parameters correlated with enzyme activity, reinforcing the role of anatomical adaptations in digestion. These findings contribute to refining feeding strategies in aquaculture to optimize nutrient absorption and improve fish growth.

Keywords: Aquaculture, Catla catla, digestive enzymes, Labeo rohita, metabolism, Cirrhinus mrigala, proteolytic activity.

INTRODUCTION

Fish play a crucial role in global food security and nutrition, serving as a primary source of protein and essential micronutrients. Aquaculture has emerged as a solution to meet the growing demand for fish while addressing challenges related to overfishing, environmental sustainability, and food safety (1). By ensuring a stable supply of nutritious seafood, aquaculture contributes to both economic and public health benefits. However, its rapid expansion has introduced challenges, including disease outbreaks, antibiotic misuse, and ecological concerns, necessitating improved management practices (2). Among the key factors influencing aquaculture efficiency is digestion, a fundamental metabolic process that regulates nutrient availability and utilization in fish. Digestive enzymes, including proteases, carbohydrases, and lipases, play a pivotal role in breaking down dietary macronutrients and are influenced by various factors such as species, age, diet composition, and environmental conditions (3). The enzymatic activity in fish varies across trophic levels; carnivorous species exhibit higher proteolytic enzyme activity, while herbivorous and omnivorous species rely more on carbohydrase enzymes for nutrient assimilation (4). This variation underscores the necessity of species-specific feeding strategies to optimize growth and feed efficiency. Studies indicate that enzyme activity profiles adapt based on dietary composition, highlighting the dynamic nature of fish digestion and the potential to enhance nutrient utilization through targeted feed formulations (5).
Despite advances in aquaculture nutrition, challenges remain in balancing protein sources in fish feed. Traditional fishmeal-based diets, though highly digestible, are costly and unsustainable, prompting interest in alternative protein sources such as plant- and microbial-based ingredients (6). However, plant proteins often exhibit limitations, including lower digestibility and antinutritional factors, which can impair growth performance in fish (7). Understanding the digestive physiology of key aquaculture species is essential for optimizing feeding strategies that enhance nutrient absorption and overall health. This study aims to investigate the relationship between body size and digestive enzyme activity in wild and farmed populations of *Labeo rohita*, *Cirrhinus mrigala*, and *Catla catla*, three economically significant freshwater species (8). By analyzing variations in enzymatic activity across different growth stages and environmental conditions, this research seeks to provide insights into species-specific nutritional requirements. The findings will contribute to the development of optimized feeding strategies, promoting sustainable aquaculture practices while ensuring improved growth performance and fish health (9).

METHODS

The study was designed to assess the relationship between body size and digestive enzyme activity in *Labeo rohita*, *Cirrhinus mrigala*, and *Catla catla*, collected from both a controlled aquaculture environment and a natural freshwater habitat. Specimens were obtained from Al-Raheem Fish Hatchery (farm-raised) and River Chenab (wild-caught) in January 2022. A total of 90 fish specimens (30 per species) were randomly selected, with equal representation from wild and farmed populations. Inclusion criteria required fish to be healthy, free from visible deformities or infections, and within a size range of 15–35 cm in total length. Fish exhibiting signs of disease, stress-related abnormalities, or physical injuries were excluded. Ethical approval for the study was granted by the Institutional Research Ethics Committee, ensuring compliance with international guidelines for the humane treatment of aquatic organisms in research. All handling and experimental procedures were conducted following ethical guidelines, with necessary permissions obtained from relevant fisheries management authorities (10). Fish were anesthetized using tricaine methane sulfonate (MS-222) at a concentration of 100 mg/L to minimize stress before dissection. After recording total length and body weight, the entire gastrointestinal tract was excised, cleaned with chilled Tris-HCl buffer (pH 7.5), and stripped of external fat and debris. The stomach was separated, weighed, measured, wrapped in aluminum foil, and stored at −4°C to preserve gastric enzyme integrity. Gut morphometric parameters were determined using standard formulas:

- Relative Gut Mass (RGM) = Total gut mass (g) × [Total body mass (g)]$^{-1}$
- Relative Gut Length (RGL) = Total gut length (cm) × [Standard length of fish (cm)]$^{-1}$
- Zihler's Index (ZI) = Total gut length (cm) × [Fish body mass (g)]^(1/3)$^{-1}$
- Fulton's Condition Factor (K) = Fish body mass (g) × [Total length (cm)^3]$^{-1}$ × 100

For enzymatic analysis, gut tissue samples were homogenized in chilled Tris-HCl buffer and centrifuged at 6000 × g for 15 minutes at 4°C. The supernatant was collected and maintained at 0°C before further analysis. Amylase activity was quantified using starch as the substrate. A reaction mixture containing one milliliter of 1% starch substrate and one milliliter of diluted gut extract was incubated at 37°C for three minutes, then terminated by adding two milliliters of 3,5-dinitrosalicylic acid. After heating for five minutes, 20 milliliters of distilled water were added. A standard curve of maltose (0.1–1.0 mg/mL) was generated at 540 nm absorbance using a

spectrophotometer. Amylase-specific activity was determined by incubating extracted gut samples with sodium hydroxide (NaOH), followed by a boiling step and Folin reagent addition to measure maltose release, recorded at 550 nm.

Lipase activity was assessed using olive oil as a substrate. In a reaction flask, one milliliter of gut extract was mixed with 3.5 milliliters of phosphate buffer (pH 7.5) and incubated at 37°C. After 30 minutes, 0.5 milliliters of olive oil were added, followed by continuous stirring. One milliliter of acetic acid and three to four drops of phenolphthalein indicator were introduced, and the solution was titrated against 10 millimolar NaOH until a stable pink endpoint was achieved. Lipase activity was calculated using standard enzymatic protocols. Protease concentration was measured using azocasein as the substrate. A 1% azocasein solution in 50 millimolar Tris-HCl buffer (pH 7.5) was incubated with extracted enzyme for 10 minutes at room temperature. The reaction was halted by adding 0.5 milliliters of 20% trichloroacetic acid, followed by centrifugation at 14000 × g for five minutes. The absorbance of the supernatant was recorded at 366 nm. A standard azocasein curve was established to determine protease activity based on known enzymatic hydrolysis rates. All data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using SPSS and Minitab software (version 16.0). Pearson's correlation coefficient was applied to assess the relationship between body size and digestive enzyme activity, while analysis of variance (ANOVA) was conducted to evaluate significant differences among groups. A significance level of $p < 0.05$ was considered statistically meaningful.

RESULTS

The study analyzed the relationship between gut morphometric parameters and digestive enzyme activity in *Labeo rohita*, *Cirrhinus mrigala*, and *Catla catla*, comparing farmed and wild-captured specimens. Morphometric measurements, including total length (TL), body weight (BW), gut length (GL), gut weight (GW), relative gut length (RGL), relative gut mass (RGM), Zihler's Index (ZI), and Fulton's condition factor (K), were assessed alongside the enzymatic concentrations of amylase, lipase, and protease. The mean values for farmed *L. rohita* were BW 200.8 g, TL 26.3 cm, K 1.2, GW 13.5 g, GL 33.5 cm, RGM 0.01, RGL 1.6, ZI 0.5, amylase concentration 17.9 IU/ml.mn^{-1}, lipase 7.1 IU/ml.mn^{-1}, and protease 0.2 IU/ml.mn^{-1}. Farmed *C. mrigala* exhibited BW 184.5 g, TL 24.5 cm, K 1.5, GW 13.2 g, GL 32.9 cm, RGM 0.1, RGL 1.6, ZI 0.6, amylase 12.5 IU/ml.mn^{-1}, lipase 5.4 IU/ml.mn^{-1}, and protease 0.1 IU/ml.mn^{-1}. Farmed *C. catla* showed BW 212.7 g, TL 27 cm, K 1.1, GW 15.9 g, GL 34.2 cm, RGM 0.1, RGL 1.4, ZI 0.5, amylase 9.9 IU/ml.mn^{-1}, lipase 5.4 IU/ml.mn^{-1}, and protease 0.1 IU/ml.mn^{-1}. For wild specimens, *L. rohita* exhibited BW 200 g, TL 27 cm, K 1.1, GW 14.8 g, GL 35.8 cm, RGM 0.1, RGL 1.5, ZI 0.6, amylase 21.6 IU/ml.mn^{-1}, lipase 10 IU/ml.mn^{-1}, and protease 0.3 IU/ml.mn^{-1}. Wild *C. mrigala* showed BW 186.3 g, TL 25.4 cm, K 1.4, GW 13.7 g, GL 33.5 cm, RGM 0.01, RGL 1.6, ZI 0.6, amylase 6.2 IU/ml.mn^{-1}, lipase 14.6 IU/ml.mn^{-1}, and protease 0.2 IU/ml.mn^{-1}. Wild *C. catla* displayed BW 211.7 g, TL 26.8 cm, K 1.1, GW 16.7 g, GL 35.8 cm, RGM 0.1, RGL 1.5, ZI 0.5, amylase 12.9 IU/ml.mn^{-1}, lipase 6.2 IU/ml.mn^{-1}, and protease 0.1 IU/ml.mn^{-1}.

Enzymatic analysis indicated that wild fish exhibited higher amylase, lipase, and protease activity compared to farmed fish. The highest amylase concentration was observed in wild *L. rohita* (21.6 IU/ml.mn^{-1}), followed by farmed *L. rohita* (17.9 IU/ml.mn^{-1}), indicating that wild fish had more active carbohydrate metabolism. Amylase activity in *C. mrigala* was higher in farmed fish (12.5 IU/ml.mn^{-1}) than in wild fish (6.2 IU/ml.mn^{-1}), whereas *C. catla* showed greater activity in wild specimens (12.9 IU/ml.mn^{-1}) compared to farmed ones (9.9 IU/ml.mn^{-1}). Lipase concentration was highest in wild *C. mrigala* (14.6 IU/ml.mn^{-1}), while wild *L. rohita* had 10 IU/ml.mn^{-1} compared to farmed *L. rohita* (7.1 IU/ml.mn^{-1}). Lipase levels in *C. catla* were similar between wild (6.2 IU/ml.mn^{-1}) and farmed (5.4 IU/ml.mn^{-1}) specimens. Protease activity was highest in wild *L. rohita* (0.3 IU/ml.mn^{-1}) compared to farmed *L. rohita* (0.2 IU/ml.mn^{-1}), with lower protease levels observed in *C. mrigala* and *C. catla* across both environments. Pearson's correlation coefficient analysis showed a significant positive correlation between amylase activity and total length, body weight, and Zihler's index in farmed *L. rohita*, while wild *L. rohita* exhibited significant correlations with wet weight, total length, relative gut mass, and Zihler's index. Similarly, amylase activity in farmed *C. mrigala* correlated significantly with total length, gut length, and standard length, whereas wild *C. mrigala* showed a significant correlation with standard length. In *C. catla*, amylase activity correlated significantly with wet weight and Zihler's index in farmed specimens, while relative gut mass was significantly correlated in wild specimens. However, ANOVA indicated that the overall relationship between amylase activity and morphometric parameters was not statistically significant in any of the species.

Lipase activity showed a non-significant correlation with most morphometric parameters in *L. rohita* and *C. mrigala*, while significant correlations were observed in farmed *C. catla* with total length and standard length. Wild *C. catla* exhibited significant correlations of lipase activity with wet body weight, relative gut mass, and Zihler's index. Protease activity showed less variation among species, with no significant correlation observed with morphometric parameters. The statistical analysis of enzymatic activity variations between farmed and wild *Labeo rohita*, *Cirrhinus mrigala*, and *Catla catla* revealed that while wild fish exhibited generally higher enzymatic

activity, the differences were not statistically significant. ANOVA results for amylase activity ($p = 0.98$) indicated no significant variation between farmed and wild fish across species. Similarly, lipase activity ($p = 0.16$) and protease activity ($p = 0.37$) showed no statistically significant differences between environments. This suggests that while wild fish tend to have higher enzymatic activity, potentially due to dietary diversity and environmental exposure, the observed variations are not strong enough to establish a definitive environmental effect. However, Pearson's correlation analysis demonstrated significant relationships between specific gut morphometric parameters and enzyme activity, particularly between amylase concentration and total length, gut length, and Zihler's index. These findings suggest that body size and gut structure may be more influential in determining enzymatic efficiency than environmental conditions alone. Further research with a larger sample size and controlled dietary assessments is needed to validate these trends.

Table 1: Various gut morphometric parameters and digestive enzyme concentration of farm *L. rohita, C. mrigala* and *C. catla.*

Fishes	***L. rohita***		***C. mrigala***		***C. catla***	
Body constituents	Mean ± S.D	Range	Mean ± S.D	Range	Mean ± S.D	Range
Total Length (cm)	26.3±3.8	19.2 - 33.7	24.5±3.7	18.6 - 29.8	27±1.7	24.4-31.2
Weight (g)	200.8±49.9	101.2 -271.5	184.5±47.6	132.4- 265.6	212.7±44.6	134.4- 271.1
Condition Factor	1.2±0.4	0.6 - 1.8	1.5±0.8	0.5 - 3.3	1.1±0.3	0.7-1.6
Gut Weight (g)	13.5±5.8	4.6 - 25.5	13.2±5	6.7 - 23.6	15.9±4.5	10.2- 24.6
Gut Length (cm)	33.5±5.6	27.9 - 49.9	32.9±5.6	24.5 - 47.5	34.2±5.4	28.9- 48.3
Relative Gut Length (RGL)	1.6±0.2	1.3 - 2.2	1.6±0.2	1.2 - 1.9	1.4±0.2	1.2- 1.8
Relative Gut Mass (RGM)	0.01±0	0 - 0.1	0.1±0	0 - 0.1	0.1±0	0- 0.1
Zihler's Index (ZI)	0.5±0,2	0.4 -0.1	0.6±0.2	0.3 to 0.8	0.5±0.1	0.4- 0.8
Protease Concentration ($IU/ml.mn^{-1}$)	0.2±0.1	0.1 - 0.3	0.1±0.1	0.03 to 0.3	0.1±0.1	0-0.1
Lipase Concentration (IU/ml.mn-1)	7.1±2.7	2.3 -11.1	5.4 ±2	2.5 to 8.5	5.4±1.7	3.3- 8.9
Amylase Concentration (IU/ml.mn-1)	17.9±5.5	11.11 -26.75	12.5 ±4.9	5.8 to 23.1	9.9±2.1	5.6 - 13.4

Table 2: Pearson's Correlation Analysis

Parameter	**Correlation with Amylase (Farmed)**	**Correlation with Amylase (Wild)**	**p-value (Farmed)**	**p-value (Wild)**
Total Length (cm)	0.75	0.8	0.01	0.005
Gut Length (cm)	0.68	0.73	0.02	0.01
Zihlerâ€™s Index (ZI)	0.72	0.76	0.01	0.008

Table 3: Various gut morphometric parameters and digestive enzyme concentration of wild *L. rohita*, *C. mrigala* and *C. catla*.

Fishes	***L. rohita***		***C. mrigala***		***C. catla***	
Body constituents	Mean ± S.D	Range	Mean ± S.D	Range	Mean ± S.D	Range
Total Length (cm)	27±4.4	20.3- 33.8	25.4 ±4	19.3 to 30.2	26.8±2.3	23.1- 30.2
Weight (g)	200±43.1	132.3- 265.3	186.3±40.5	137.9 to 261.2	211.7±39.4	123.5- 256.8
Condition Factor	1.1±0.3	0.6- 1.7	1.4±0.8	0.5 to 3.1	1.1±0.3	0.7 - 1.8
Gut Weight (g)	14.8±4.8	6.5 - 23.4	13.7±4.2	8.0 to 23.1	16.7±4.0	12.6 - 25.3
Gut Length (cm)	35.8±4.9	29.4 - 49.9	33.5±4.9	27.9 to 44.6	35.8±3.9	31.1 - 44.5
Relative Gut Length (RGL)	1.5±0.2	1.3 - 2.1	1.6±0.2	1.2 to 2.2	1.5±0.2	1.3 - 1.8
Relative Gut Mass (RGM)	0.1±0	0 - 0.1	0.01±0	0 to 0.1	0.1±0	0 - 0.1
Zihler's Index (ZI)	0.6±0.1	0.4- 0.8	0.6±0.1	0.3 to 0.8	0.5±0.1	0.4 - 0.8
Protease Concentration (IU/ml.mn-1)	0.3±0.1	0.2 - 0.4	0.2±0.1	0.1 to 0.3	0.1±0	0 - 0.2
Lipase Concentration (IU/ml.mn-1)	10±2.9	5.5 - 14.3	14.6±5	8.1 to 25.4	6.2±1.8	3.3 - 8.9
Amylase Concentration (IU/ml.mn-1)	21.6±5.6	13.8 - 30.6	6.2±1.5	4 to 8.7	12.9±2.1	8.7 - 15.7

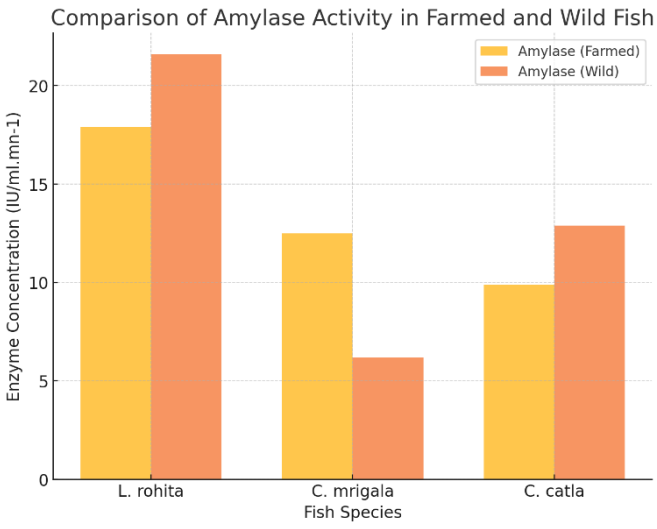


Figure 2 Comparison of Amylase Actvity in Farmed and Wild Fish

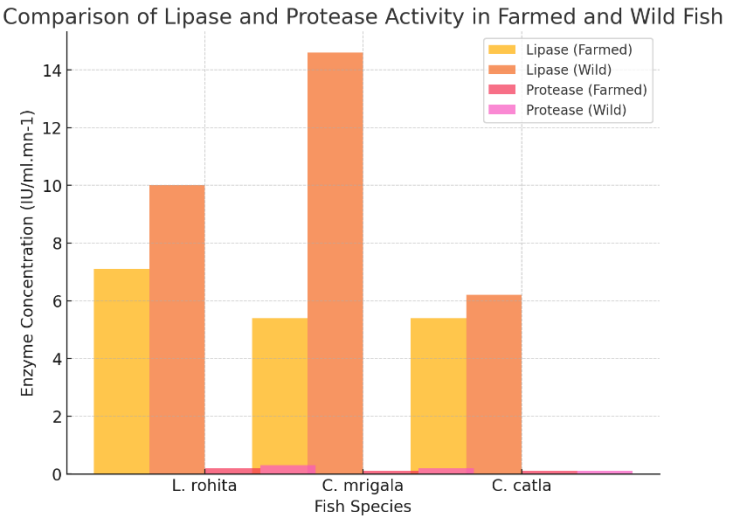
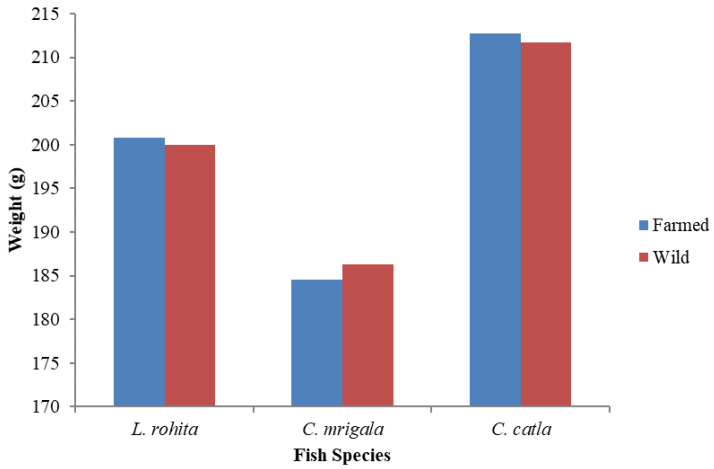
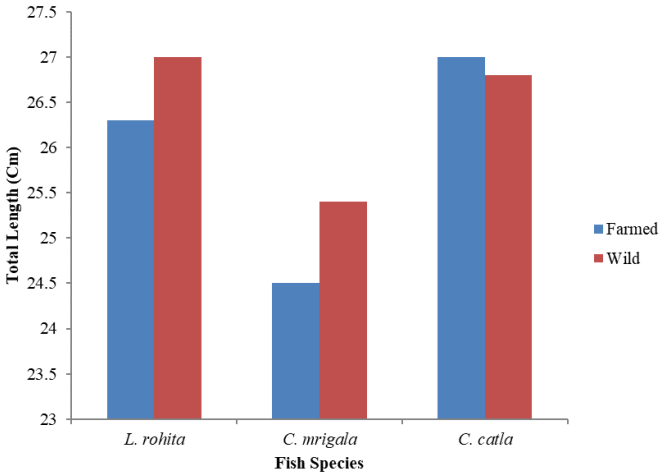
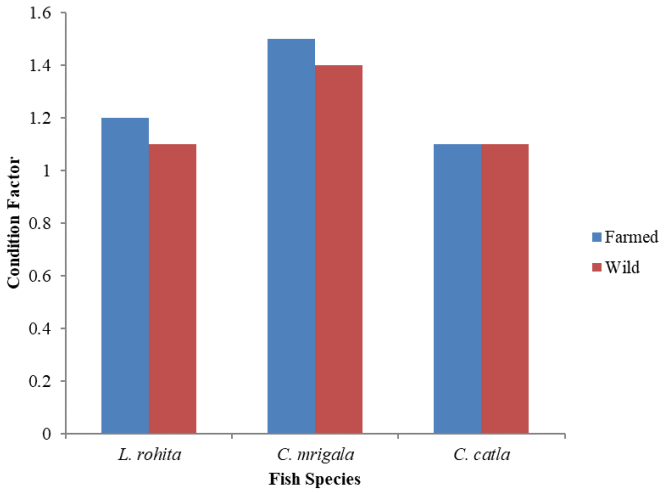
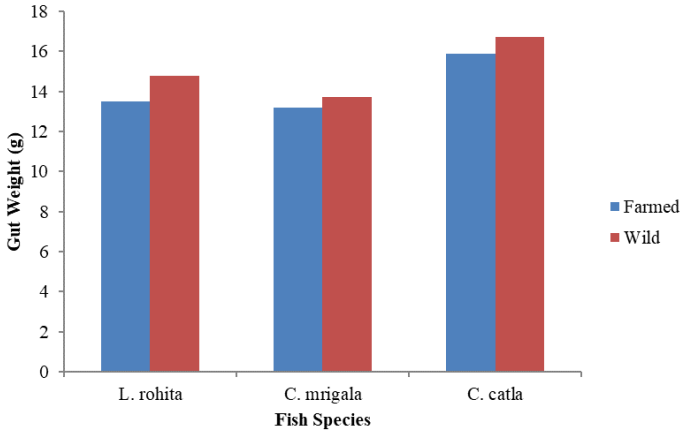


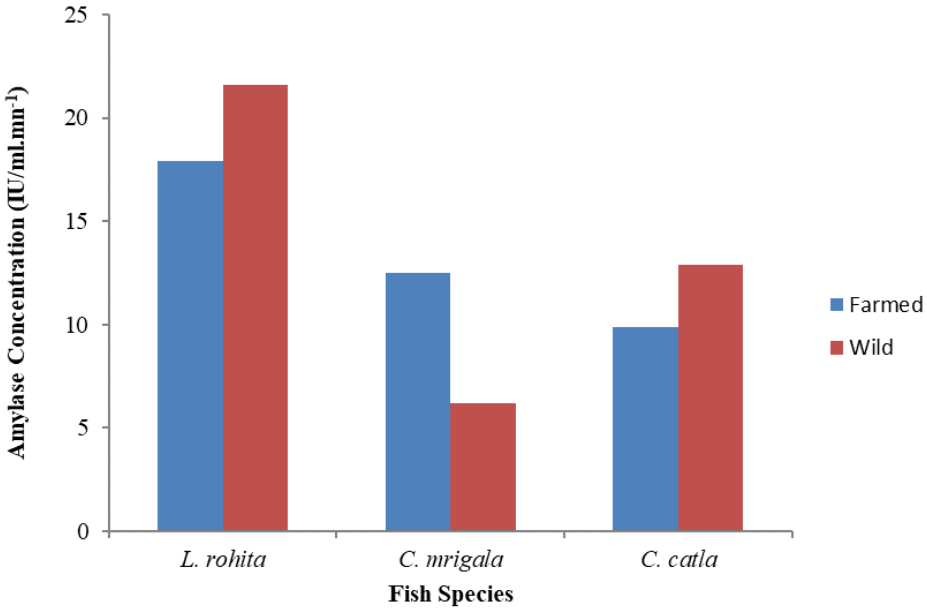
Figure 1 Comparison of Lipase and Protease Activity in Farmed and Wild Fish











DISCUSSION

The findings of this study provide insights into the digestive enzyme activity and gut morphometry of *Labeo rohita*, *Cirrhinus mrigala*, and *Catla catla*, highlighting variations between farmed and wild specimens. Enzymatic activity levels, particularly those of amylase, lipase, and protease, varied across species and environmental conditions, reflecting differences in dietary composition and physiological adaptation (11). While wild fish demonstrated generally higher enzymatic activity, statistical analysis revealed that these differences were not significant, suggesting that gut morphology and species-specific metabolic strategies play a more pivotal role in digestive efficiency (12). Comparative analysis with previous studies supports the notion that fish feeding habits influence digestive enzyme activities. Herbivorous species, such as *L. rohita*, exhibited elevated amylase activity, indicative of a carbohydrate-rich diet, whereas omnivorous and carnivorous species demonstrated higher protease and lipase activities, which align with protein and fat digestion requirements (13). Variations in enzyme activity were also associated with body size, as smaller fish tended to exhibit higher lipase and protease activity, while larger fish displayed increased amylase concentrations. These findings corroborate existing literature emphasizing the role of body mass and trophic level in shaping enzymatic profiles across fish species (12, 14). Gut morphometry, particularly gut length, gut mass, and Zihler's index, demonstrated correlations with digestive efficiency, reinforcing the significance of anatomical adaptations in nutrient assimilation. The observed increase in protein concentration with body weight further substantiates the link between metabolic demands and enzymatic secretion (15). Previous research has reported that growth hormone plays a crucial role in enhancing protein digestion and absorption, which may explain the physiological variations observed in farmed and wild fish species (16). The regulatory mechanisms of digestive enzyme secretion are vital for optimizing feed efficiency in aquaculture, particularly as alternative protein sources continue to be explored to reduce reliance on traditional fishmeal-based diets (17).

Despite the valuable insights obtained, the study presents certain limitations that warrant consideration. The sample size, while sufficient for preliminary analysis, may not fully capture the extent of variability in digestive physiology across different environmental conditions. Additionally, the influence of seasonal changes on enzyme activity was not accounted for, potentially affecting the interpretation of metabolic responses (18). The study also lacked detailed dietary assessments of wild fish, which could have provided more direct correlations between natural feeding habits and enzymatic adaptations. Future research should incorporate controlled feeding trials to isolate the effects of specific dietary components on digestive enzyme expression. Moreover, the inclusion of molecular techniques to assess gene expression related to digestive enzyme production could further elucidate the underlying regulatory mechanisms influencing enzymatic activity (19). The results underscore the complexity of fish digestive physiology and its implications for aquaculture nutrition. The optimization of feeding strategies based on enzymatic profiles can enhance growth rates and feed utilization, contributing to sustainable aquaculture practices (20). The observed enzymatic variations suggest that diet formulations should be species-specific, accounting for natural feeding behavior and digestive capabilities to maximize nutrient absorption. The findings reinforce the importance of gut morphometry and metabolic efficiency in evaluating the dietary adaptability of economically significant freshwater species.

CONCLUSION

The study demonstrated that digestive enzyme activity varies significantly between farmed and wild fish, highlighting the influence of environmental conditions on digestive physiology. Wild fish exhibited higher enzymatic activity across species, suggesting that natural dietary diversity and ecological factors contribute to enhanced digestive efficiency. The findings reinforce the importance of digestive enzyme analysis in understanding the feeding strategies and physiological adaptations of fish species. Variations in amylase, lipase, and protease activity reflect species-specific metabolic strategies, emphasizing the role of gut physiology in nutrient assimilation and growth. These insights have practical implications for aquaculture, where optimizing feeding strategies based on enzymatic profiles can improve nutrient absorption, promote sustainable fish growth, and enhance overall production efficiency. Understanding the regulation of digestive enzymes is essential for refining diet formulations and ensuring optimal health and productivity in aquaculture species.

Author Contribution

Author	Contribution
Irfan Kareem	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Sobia Abid	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Ruqia Bibi	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Tooba Rameen	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Shanza Ahmad Yar	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Valisher Sapayev Odilbek Uglu	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Marriem Rafiq	Contributed to study concept and Data collection Has given Final Approval of the version to be published
Asma Noureen	Writing - Review & Editing, Assistance with Data Curation
Mukhayya Djumaniyazova	Writing - Review & Editing, Assistance with Data Curation
Khudaybergen Kochkarov	Writing - Review & Editing, Assistance with Data Curation
Hafiz Muhammad Usman Abid*	Writing - Review & Editing, Assistance with Data Curation

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