Online ISSN: 2588-3526

Journal of Wildlife and Biodiversity

Volume 9(3): 374-390 (2025) (http://www.wildlife-biodiversity.com/)

Research Article

Sustainable fisheries and biodiversity conservation: The potential of insect protein to replace fishmeal in aquaculture

Nida Ismat[1*], Sikandar Hayat[1], Majid Hussain[2]

[1]Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Pakistan

[2]Department of Fisheries and Aquaculture, University of Okara, Pakistan

*Email: nidacheema7@gmail.com

Received: 01 June 2025 / Revised: 29 September 2025 / Accepted: 30 September 2025/ Published online: 17 October 2025.

How to cite: Ismat, N., Hayat, S., Hussain, M. (2025). Sustainable fisheries and biodiversity conservation: The potential of insect protein to replace fishmeal in aquaculture, Journal of Wildlife and Biodiversity, 9(3), 374-390. DOI: https://doi.org/10.5281/zenodo.17386965

Abstract

Aquaculture is an important source of food, but it is also dependent on fishmeal, a high-protein ingredient made by using wild-caught fish, posing risks of overfishing and sustainability. The present study investigated the potential of black soldier fly (BSF) and mealworm (MW) larvae as sustainable alternatives to fishmeal in aquafeeds. An experimental trial was performed in triplicate for 60 days under a completely randomized design. The results demonstrated that the highest growth performance was observed in the 75 % BSF-based diet. The 75% BSF-based diet led to the highest weight gain, SGR, and FCR of 14.53 ± 0.43 g, 1.837 ± 0.038, and 1.313 ± 0.071, respectively. Likewise, the data showed that the best growth performances were in the 50% MW-based diet group. Maximum weight gain, and better SGR and FCR were recorded in the 50% MW-based diet group as 15.56 ± 0.41g, 1.763 ± 0.029, and 1.140 ± 0.070, respectively. Enzyme activity analysis revealed that BSF 75% and MW 50% diets significantly enhanced protease activity (16.99 ± 0.39 U/mg and 16.96 ± 0.36 U/mg, respectively), lipase activity (0.68 ± 0.02 U/mg), and amylase activity (2.33 ± 0.05 U/mg) as compared to the control group. Antioxidant enzyme activities, including catalase (1.82 ± 0.05 U/mg) and superoxide dismutase (SOD) (1.84 ± 0.03 U/mg for BSF 75%), also increased significantly. The recorded water quality parameters fell within the optimal range for the growth of *Labeo rohita,* and non-significant differences were observed between the BSF and MW diet groups. The data indicated that insect-derived proteins, especially BSF and MW proteins, have the potential to replace fishmeal in aquafeeds without compromising growth performance and fish health. Further studies are required to determine optimal inclusion levels for long-term sustainability and to alleviate the pressure on wild fish stocks used for fishmeal.

Keywords: Aqua-feed, Conservation, Biodiversity, Fishmeal, Sustainability

Introduction

Aquaculture has taken a central role in global food security, having produced a sizeable proportion of the animal protein in the world. With rising global demand for fish, concerns about the sustainability of aquaculture practices are growing stronger (Pahlow et al., 2015). The most central aspect of this challenge is the dependence on fish meal, a staple protein ingredient in aqua-feeds, which has contributed towards overfishing and environmental destruction (Das et al., 2023). Fishmeal is a high-protein ingredient made out of wild-caught fish (mainly small pelagic species). It has been commonly used in aquaculture diets because of its good, balanced amino acids, digestible, and palatable properties. Nonetheless, the growing demand for fishmeal has necessitated the overexploitation of marine resources, hence its sustainability (Hancz et al., 2024). Limited capacity in fishmeal production and the associated environmental effects have forced the need to identify alternative protein sources that may satisfy the ever-expanding world market of fish products.

This has increased the search for alternative and sustainable sources of protein. Insect proteins have gained recent interest as a source, especially those of black soldier fly (*Hermetia illucens*) and mealworm (*Tenebrio molitor*) (Alfiko et al., 2022). These insects are high in proteins, essential amino acids, and also have a positive fatty acid profile, making them potential substitutes to fishmeal in fish feeds (Chen et al., 2023). Application of insect protein in fish feed has been found to provide benefits, including supplementing fish growth, nutrition utilization, and fish health (Xu et al., 2020; Secci et al., 2016). Besides, insect farming has a smaller environmental footprint compared to conventional livestock and is a very sustainable source of protein (Bai et al., 2023).

The digestive enzymes and antioxidant systems are significant demonstrations of the physiological good health of fish (Belforti et al., 2015). Lipase, amylase and protease are the digestive enzymes that hold very important roles in digestion and nutrient absorption into the body with a direct impact to the growth and feed efficiency. The antioxidant enzymes such as SOD, CAT, and peroxidase protect the cells against oxidative stress and maintain cellular integrity and functional health. Such variations in the activity of these enzymes are a possible indicator of the physiological responses to the dietary changes and are essential to assess the health implications of insect-based proteins.

Labeo rohita is an aquaculture species in Pakistan and South Asia. Its flexibility and omnivorous diet make it a good species to use when needing to check the impact of different diets. Although there is increasing popularity in insect-based aquafeeds, it has never been investigated on the possible influences that insect-based diets have on the digestive and antioxidant enzyme activities. Revealing the effect of the insect-based protein diet on the physiological parameters is essential in establishing optimal aquacultural practices so that the health and productivity of cultivated fish can be guaranteed (Lopes et al., 2015). Digestive enzymes are vital to break down the dietary macromolecules into absorbable nutrients. The three major enzymes that are involved in protein, lipid, and carbohydrate digestion include protease, lipase, and amylase, respectively. Dietary composition can modify their activity levels, and a change in this may reflect alterations in digestive efficiency and metabolic stress. Such enzymes are monitored to indicate the physiological effects of altering the diet and nutritional inadequacy of diets (Ogunji et al., 2008). Oxidative stress occurs when the antioxidant defense mechanisms do not remove reactive oxygen species (ROS) rapidly enough. Fish contain antioxidant enzymes, including SOD, CAT, and peroxidase, to ameliorate oxidative stress. These enzymes deactivate ROS to guard intracellular structures and to preserve homeostatic metabolism. Oxidative conditions and the health of the fish can be determined by changes in the activity of these enzymes since they can be affected by dietary factors. The activities of antioxidant enzymes are important in determining the overall effects of dietary modification (Hoffmann et al., 2021).

Although several studies have examined changes in the growth and feed efficiency of fish, few studies have determined the effects of insect protein on digestive and antioxidant enzymes in *L. rohita* (Kloas et al., 2015). It is important to understand the physiological response of fish to determine the acceptability of insect-based protein diets as an alternative to fishmeal. The present study was therefore planned to fill this gap by providing preliminary data on how substituting fishmeal with insect protein affects fish growth and enzyme activities.

Material and methods

Experimental Design

The experimental trials were carried out in the Fisheries Lab, University of Okara, Punjab, Pakistan. *Labeo rohita* of average weight 5.56 ± 0.04g was taken from Fish Nursery Unit, Head Balloki, Punjab, Pakistan (Hussain et al., 2025; Ajmal et al., 2025). *Labeo rohita* fingerlings were carefully transferred to the water-filled aerated bags (Xu et al., 2020). Fish were placed in two large rectangular glass aquaria for two weeks to acclimate to laboratory conditions. Two trials were carried out separately, with fifteen aquaria used for each trial. At the start of the experiment, *Labeo rohita* fingerlings (n=10) were stocked in each aquarium. An experimental trial was performed in triplicate for 60 days under a completely randomized design. Treatment 1 groups were fed with black soldier fly (BSF) larvae meal, and treatment 2 groups were fed on mealworm (MW) larvae meal, replacing fishmeal with. The composition of both diets includes CP% of 32.19 ± 0.11 (Zarantoniello et al., 2022)

Water quality parameters

The physicochemical parameters of water such as pH, dissolved oxygen (mg/L) were measured by a multimeter (HANNA (HI-98194), Romania), water temperature (°C), electrical conductivity (µS/cm) were measured by using Digital Electrical conductivity meter (AD-3000, Adwa, Romania), while total hardness (mg/L), total ammonia (mg/L), and carbon dioxide were measured by following methods explained in APHA, (2005).

Growth parameters

The growth parameters of *Labeo rohita,* such as weight gain, feed conversion ratio (FCR), and specific growth rate (SGR) were assessed using formulas following Su et al. (2017)

Enzyme activity analysis

After completing the dietary treatment experiment, three fish from each aquarium (n = 3 triplicates per treatment) were randomly selected. Fish were dissected under sterile conditions, and the liver was immediately separated and stored at -80°C until further processing. Enzyme assays were prepared by homogenizing 100mg of frozen liver was homogenized in 1 mL of ice-cold phosphate-buffered saline (PBS, pH 7.4) using a homogenizer. After that, the homogenate was spun at 10000 × g for 15 minutes at 4°C, and the supernatant was collected for further analysis (Xing et al., 2013).

Antioxidant Enzyme Activity

The peroxidase activity assay was performed by preparing a reaction mixture that included 1 mL of phosphate-buffered saline (PBS, pH 7.4), 0.5 mL of hydrogen peroxide (H_2O_2, 10 mM), and 0.5 mL of the liver homogenate supernatant. This mixture was incubated at 25°C for 10 minutes to allow the peroxidase enzyme to act on the hydrogen peroxide. After incubation, 1 mL of 3,3',5,5'-Tetramethylbenzidine (TMB) solution (1 mg/mL in DMSO) was added to the reaction mixture to initiate the colorimetric reaction. The reaction was terminated by adding 0.5 mL of 2 M sulfuric acid (H_2SO_4), which stopped the enzyme activity and allowed for the measurement of color intensity. The absorbance of the final solution was measured at 450 nm using a UV spectrophotometer (Analytik Jena, Germany). The peroxidase activity was calculated based on the change in absorbance over time (ΔA/min), and the results were expressed in units per mg of protein (Taysi et al., 2024).

For the Catalase (CAT) assay, 1 mL of the supernatant was mixed with 1 mL of hydrogen peroxide (H_2O_2, 10 mM) in a clean test tube. The reaction was initiated by the addition of hydrogen peroxide, and the change in absorbance was monitored spectrophotometrically at 240 nm every 10 seconds for 1 minute. The catalase activity was determined by measuring the rate of decrease in absorbance, which corresponds to the decomposition of hydrogen peroxide. The activity was expressed in units per mg of protein, where one unit is defined as the amount of enzyme required to decompose 1 μmol of H_2O_2 per minute at 25°C. Protein concentration was determined using the Bradford method to normalize the results (Taysi et al., 2024).

The inhibition of photo reduction of nitroblue tetrazolium (NBT) by Oxide Dismutase (SOD) was determined for measuring its activity using the Beauchamp and Fridovich technique. The perfused liver was homogenised in a 10% w/v potassium phosphate buffer (pH 7.5) and centrifuged for 15 minutes at 16000g. The supernatant was separated as an enzyme source. 100 mM phosphate buffer, 10 mM EDTA, 130 mM methionine, 750 mM NBT, 60 mM riboflavin, and the enzyme source made up the whole reaction mixture. The reaction was started by adding riboflavin, the samples were exposed to fluorescence for 30 minutes, and the colour was measured at 560 nm against a reagent blank held in the dark. The reduction of NBT is directly proportional to the activity of SOD. The activity was calculated in units per milligram of protein (Xing et al., 2012).

Digestive Enzyme Activity

Protease activity was measured using the method described by Stevens et al. (2018), with slight modifications. In a clean test tube, 0.5 mL of the liver supernatant was mixed with 1 mL of 1% casein solution in PBS (pH 7.4). The reaction mixture was incubated at 37°C for 30 minutes to allow the protease enzyme to break down casein. After incubation, the reaction was stopped by adding 2 mL of 5% trichloroacetic acid (TCA), and the mixture was centrifuged at 10,000 × g for 10 minutes. The absorbance of the supernatant was measured at 280 nm using a UV spectrophotometer (Analytik Jena, Germany). Protease activity was calculated based on the increase in absorbance (ΔA/min) and expressed in U/mg of protein. One unit of protease activity was defined as the amount of enzyme required to release 1 μmol of tyrosine per minute under assay conditions (Serafini et al., 2019).

Lipase activity was determined using the method by Taysi et al. (2024). A reaction mixture consisting of 0.5 mL of the liver supernatant, 1 mL of 0.1 M Tris-HCl buffer (pH 8.0), and 1 mL of 1% olive oil emulsion was prepared. The reaction was initiated by adding the liver supernatant to the mixture, followed by incubation at 37°C for 30 minutes. The reaction was stopped by adding 2 mL of 0.1 M NaOH to the mixture. After the reaction was stopped, the released free fatty acids (FFA) were titrated with 0.1 M NaOH using phenolphthalein as the indicator. Lipase activity was calculated based on the amount of FFA released and was expressed in U/mg of protein. One unit of lipase activity was defined as the amount of enzyme required to release 1 µmol of FFA per minute (Xing et al., 2012).

Amylase activity was determined as described by Bernfeld (1955). A clean test tube was filled with 0.5 mL of liver supernatant and 1 mL of 1% starch solution in PBS (pH 6.9), then incubated at 37 °C for 30 minutes. The reaction was stopped by adding 1 mL of DNS (3,5-dinitrosalicylic acid) reagent, and then the mixture was incubated in a boiling water bath for 5 minutes. The resulting reaction mixture was allowed to cool to room temperature, after which absorbance was read at 540 nm using a UV spectrophotometer (Analytik Jena, Germany). Amylase activity was calculated based on the change in absorbance (ΔA/min) and expressed in U/mg of protein. One unit of amylase activity was defined as the amount of enzyme required to hydrolyze 1 µmol of starch per minute under assay conditions.

Protein Quantification

For all enzyme assays (protease, lipase, and amylase), protein concentration in the liver homogenate was determined using the Bradford method to normalize enzyme activities. Protein concentration was quantified using bovine serum albumin (BSA) as the standard.

Statistical Analysis

The data thus obtained were subjected to one-way ANOVA. The significance level among parameters was compared using Duncan's Multiple Range Test at $P \leq 0.05$ (Steel et al., 1996). The statistical analysis was done using CoStat software (version 6.303).

Results

Growth Performance

The growth performance of *Labeo rohita* was assessed by varying levels (0%, 25%, 50%, 75%, and 100%) of two diets, including black soldier fly (BSF) and mealworm (MW) larvae-based diet as a replacement for fishmeal. The growth performance was assessed through parameters including weight gain (WG), feed conversion ratio (FCR), and specific growth rate (SGR). At the start of the experiment, the initial weight of *Labeo rohita* fingerlings was 5.56 ± 0.04g in all treatment groups.

The results demonstrated that the highest growth performance was observed in the 75 % BSF-based diet. The 75% BSF-based diet led to the highest weight gain, SGR, and FCR of 14.53 ± 0.43 g, 1.837 ± 0.038, and 1.313 ± 0.071, respectively. Likewise, the data showed that the best growth performances were in the 50% MW-based diet group. Maximum weight gain, and better SGR and FCR were recorded in the 50% MW-based diet group as 15.56 ± 0.41g, 1.763 ± 0.029, and 1.140 ± 0.070, respectively.

Water Quality Parameters

Water quality is a critical parameter in aquaculture as it has an impact on the health and performance of fish. The water quality parameters, such as temperature, dissolved oxygen, pH, electric conductivity (EC), total dissolved solids (TDS), total ammonia, total hardness, and carbon dioxide, were measured throughout the experiment on a fortnightly basis. The results reveal that the water quality parameters fell within the optimal range of growth of *Labeo rohita* in the three treatment groups, though non-significant differences were observed between the BSF and MW diets (Table 1). The mean water temperature was 29.62 ± 0.26°C and 29.28 ± 0.18°C in the BSF and MW diet groups, respectively. The results showed that these parameters were within the acceptable range for *Labeo rohita* growth. The dissolved oxygen levels were slightly higher in the BSF group (7.672 ± 0.03 ppm) as compared to the MW group (7.62 ± 0.02 ppm). The pH levels were nearly the same in both diet groups, as 8.116 ± 0.02 and 8.06 ± 0.02 in the BSF and MW diet groups, respectively. Electrical conductivity values were nearly the same in both BSF and MW groups at 1965 ± 3.87 µS/cm and 1962.6 ± 2.07 µS/cm, respectively. TDS values were 1656.2 ± 1.92 ppm and 1654 ± 1.87 ppm in the BSF and MW diet groups, respectively. The total ammonia and hardness values recorded were within the optimum range, ensuring no toxic effect of ammonia and water hardness applied to fish health.

Table 1. Water quality parameters during the experimental period.

Parameters	BSF	MW
	Mean ± SD	
Water Temperature (°C)	29.62 ± 0.26	29.28 ± 0.18
Dissolve oxygen (ppm)	7.672 ± 0.03	7.62 ± 0.02
pH	8.116 ± 0.02	8.06 ± 0.02

EC (µS/cm)	1965 ± 3.87	1962.6 ± 2.07
TDS (ppm)	1656.2 ± 1.92	1654 ± 1.87
Total ammonia (mgL^{-1})	0.676 ± 0.03	0.67 ± 0.03
Total hardness (mgL^{-1})	186.2 ± 1.92	183 ± 1.58
Carbon dioxide (mgL^{-1})	0.778 ± 0.02	0.758 ± 0.02

Antioxidant Enzyme Activity

The antioxidant enzyme activities, including peroxidase (POX), catalase (CAT), and superoxide dismutase (SOD), were measured to assess the physiological effects of the insect-based diets on oxidative stress.

Peroxidase Activity

The peroxidase activity was significantly higher in the BSF 75% group (3.12 U/mg) and the MW 50% group (3.23 U/mg), compared to the control diet (2.31 U/mg for BSF and 2.30 U/mg for MW) (Fig. 3). These results suggest that insect-based diets, especially BSF 75% and MW 50%, may improve the fish's ability to combat oxidative stress by enhancing the activity of peroxidase.

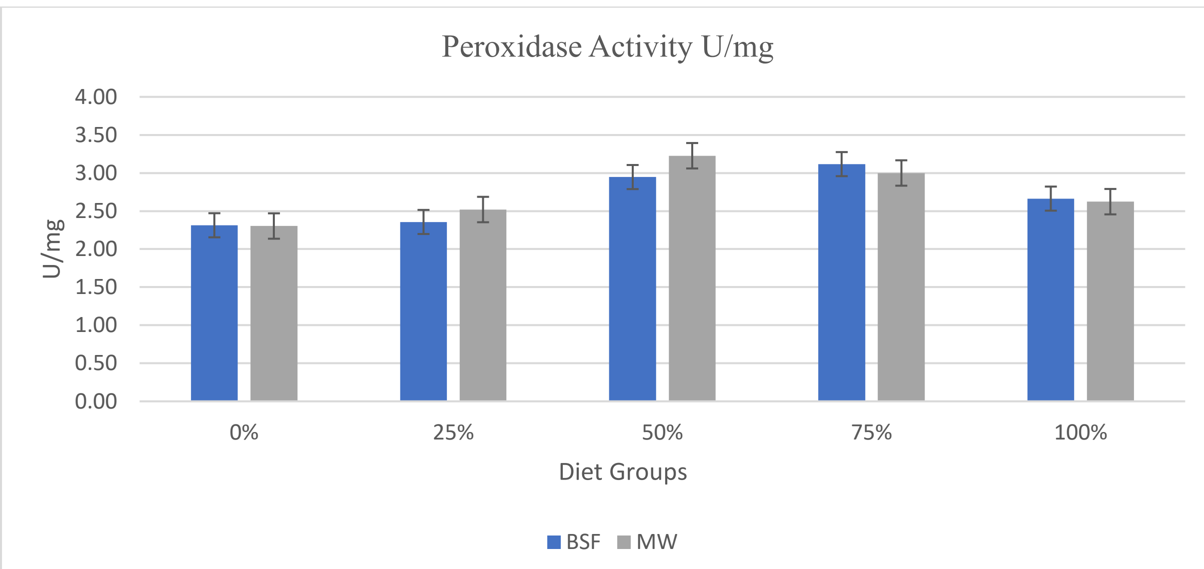

Figure 3. Peroxidase activity (U/mg) in different diet groups.

Catalase Activity

The catalase activity followed a similar pattern, with the BSF 75% and the MW 50% diet groups having the highest (1.82 U/mg and 3.23 U/mg, respectively) activities. Catalase activity showed that the MW 50% group exhibited the highest activity out of all the treatments and thus showed a greater potential to break down hydrogen peroxide and prevent the progression of oxidative damage (Fig. 4).

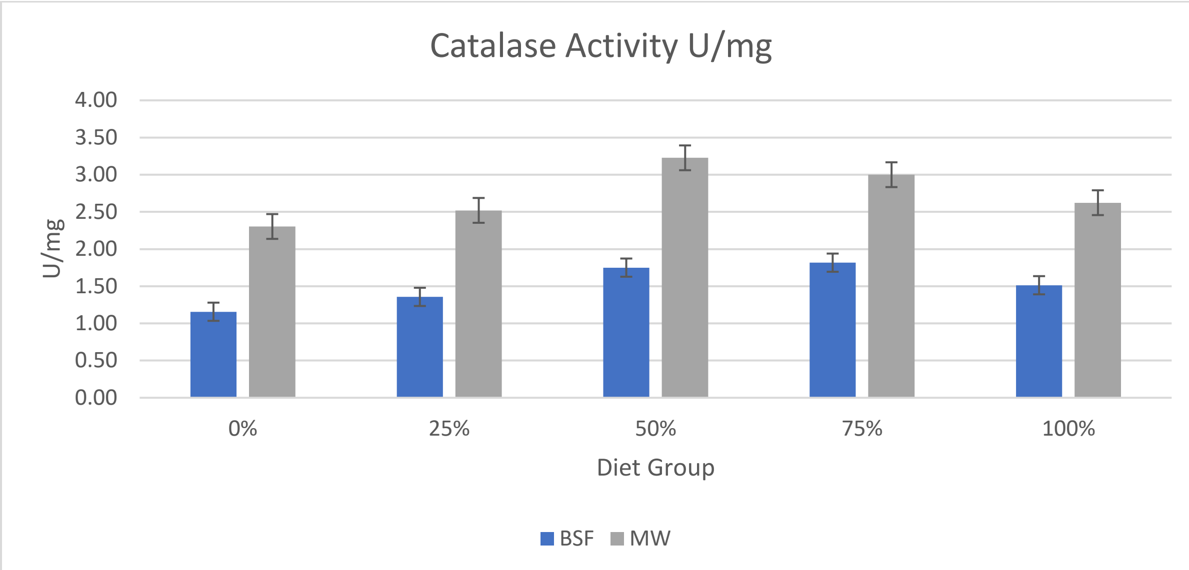

Figure 4. Catalase activity (U/mg) in different diet groups.

Superoxide Dismutase Activity

Superoxide dismutase (SOD) activity was also significantly increased in BSF 75 % (1.84 U/mg), and MW 50 (1.88 U/mg) compared to control groups (BSF: 0.92 U/mg, MW: 0.85 U/ mg) (Figure 5). These elevated values suggested that insect-based protein diets would help to enhance the antioxidant defence system of Labeo rohita These data suggested that insect proteins particularly at elevated inclusion percentages (BSF 75% and MW 50%) would help to improve the antioxidant defence system of Labeo rohita which could potentially help promote the health and performance of these fish.

Digestive Enzyme Activity

Digestive enzymes such as protease, lipase and amylase were also measured in order to evaluate the influence of insect-based proteins on the digestion and absorption of nutrients. The findings indicated that BSF 75%, and MW 50% diet treatment groups exhibited the highest activities of digestive enzymes across all enzyme types.

Protease Activity

The maximum protease activity was reported in BSF 75% group (16.99 U/mg) and in MW 50% group (16.96 U/mg), which was significantly higher than that in the control group (12.57 U/mg in BSF and 12.50 U/mg in MW). These data indicate that these diets enhance protein utilization and uptake that helps fish perform better as far as growth rates are concerned (Figure 6).

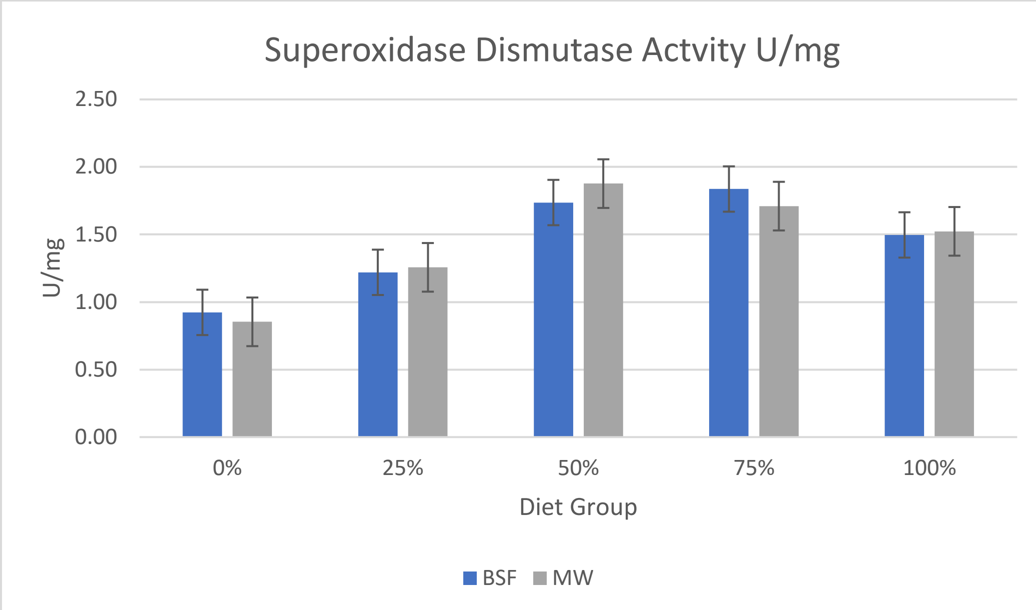

Figure 5. Superoxidase Dismutase activity (U/mg) in different diet groups.

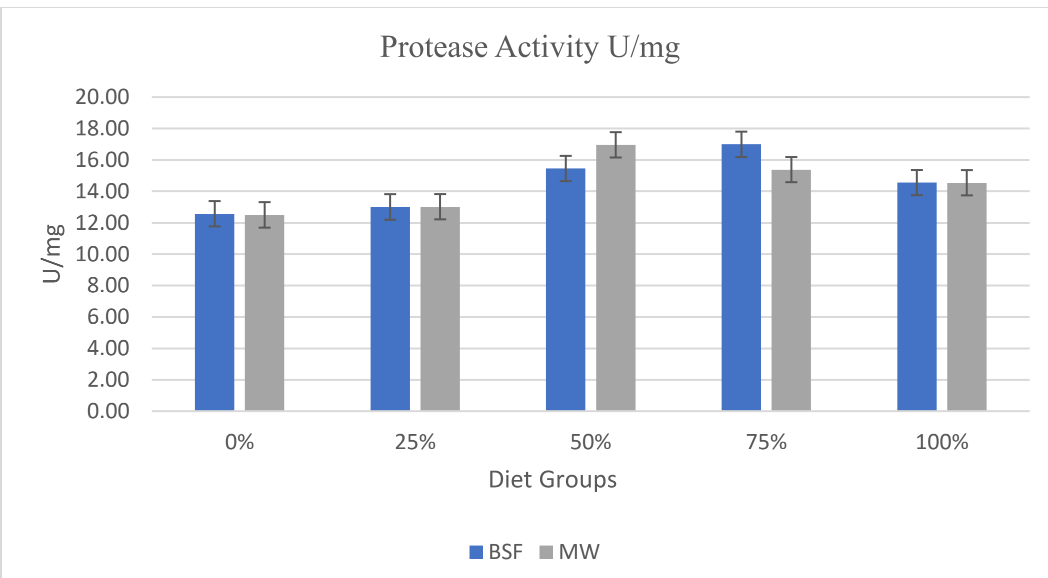

Figure 6. Protease activity (U/mg) in different diet groups.

Lipase activity

The lipase activity, indicating the decomposition of fats, was also increased in the BSF 75% (0.68 U/mg) and MW 50% (0.68 U/mg) groups compared to the control group (0.26 U/mg and 0.29 U/mg, respectively). This shows that an insect diet can enhance the digestion of lipids (Fig. 7).

Amylase activity

Amylase activity, responsible for carbohydrate digestion, was elevated in the BSF 75% (2.33 U/mg) and MW 50% (2.32 U/mg) groups, demonstrating improved carbohydrate utilization in these groups (Fig. 8). The higher digestive enzyme activities observed in the BSF 75% and MW 50% groups correlate well with the improved growth performance, suggesting that these diets enhance nutrient breakdown and absorption, leading to better feed conversion and growth.

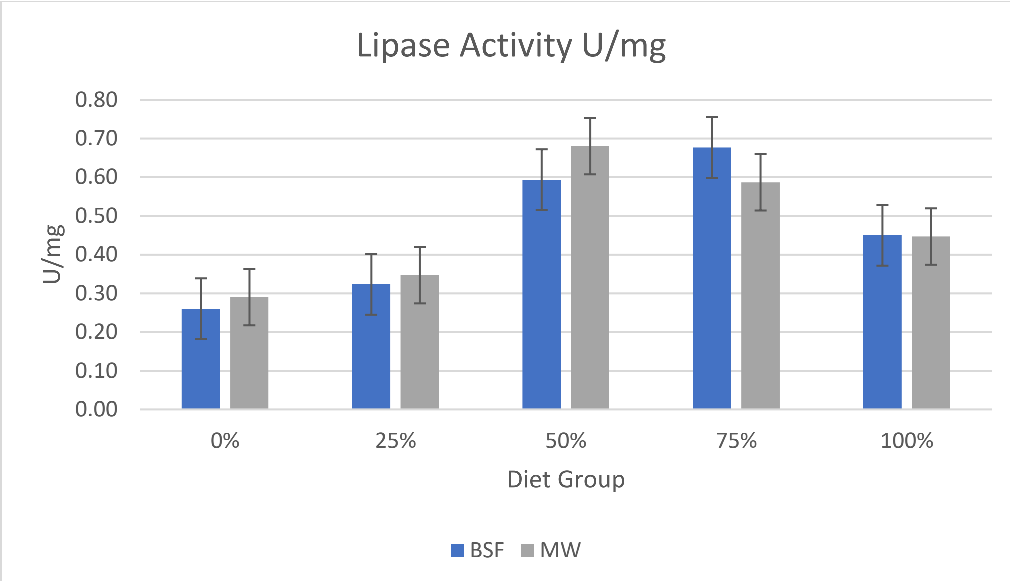

Figure 7. Lipase activity (U/mg) in different diet groups.

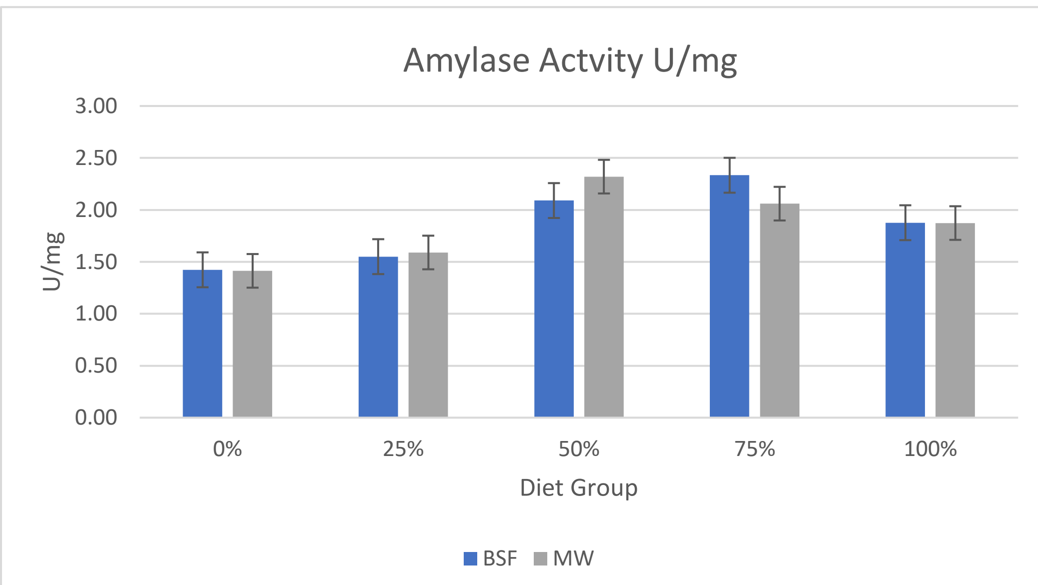

Figure 8. Amylase activity (U/mg) in different diet groups.

Discussion

The extensive growth of aquaculture and the increasing need for a sustainable source of protein in fish feed create a serious threat to wild fish stocks. Aquaculture has become an important part of the global aquatic animal production since it now provides more than 45 percent of the total global production of animal protein (Kloas et al., 2015). Conventionally used feed ingredients, including fish meal, fish oil, and soybean meal, present important environmental issues through overexploitation of marine resources and land or water requirements of crop production (Huis et al., 2017). The current study was conducted to assess the potential of insect-based protein, that is, BSF and MW larvae, related to their use as an alternative to fishmeal in fish farming. The results present valuable data concerning the effect of insect protein on the growth performance, activity of antioxidant enzymes, and activity of digestive enzymes in *Labeo rohita.* The main findings of this study suggest that the diets that incorporated insects, and particularly those with 75 percent of the BSF and 50 percent of the MW, enhance the growth performance of *Labeo rohita*. The two diets based on insect protein performed best in weight gain, SGR and FCR, and did not significantly differ in any measure, indicating insect protein as the best option to replace fishmeal. The results of our study correlate with the findings of Bai et al. (2023), Chen et al. (2023), and Belforti et al. (2015), since they found that a diet containing insect-based protein can yield similar growth performance in several fish species.

The use of the defatted yellow mealworm larvae (DYMWL) as a substitute source of protein in diets of pangasius (*Pangasianodon hypophthalmus*) was studied by Ardra et al. (2025). The results indicated that replacing fishmeal with DYMWL by up to 50 percent improved the growth performance, with the maximum weight gain recorded in the DYMWL50 group. However, an increase in DYMWL replacement reduced its growth at levels over 50%, especially at 75 and 100. The results indicated that there were no significant effects on the majority of immune parameters, digestive enzyme activities, and histological liver structure; however, high DYMWL inclusion (75 and 100) did affect the liver. The maximum inclusion of DYMWL in pangasius was found to be around 54%, hence its prospect as a sustainable alternative complete protein source in aquaculture, although further studies are required on higher inclusion rates. A study by Zhang et al. (2022) explored the impact of substituting fish meal (FM) with *Tenebrio molitor* meal (TM) on the growth performance, humoral immunity, and gut health of juvenile large yellow croakers (*Larimichthys crocea*). Four different experimental diets were created, incorporating various levels of TM replacement for FM as 0%, 15%, 30% and 45%. Juveniles (Initial weight = 11.80 ± 0.02 g) were fed the diets twice daily to apparent satiation for eight weeks. The study found no significant differences in final body weight (FBW) or weight gain rate (WG) between 0%, 15%, and 30% groups, but 45% resulted in a marked reduction in FBW and WG. Regarding immune response, serum alkaline phosphatase (AKP) activity was significantly lower in 45% compared to 0%, while 15% notably boosted lysozyme (LZM) activity. The 30% group exhibited significantly higher serum C3 levels compared to 0%, although all TM groups led to a significant decrease in C4 levels. Histological analysis revealed that TM addition increased the height and thickness of intestinal villi, as well as the thickness of intestinal muscles.

Roccatello et al. (2024) describe the usage of insect meal that can be used in aquaculture as a more sustainable and environmentally friendly choice of protein sources, compared to fishmeal and other plant-based proteins. Insects are coming into focus with increasing concerns about overfishing and resulting depletion of marine resources because of their high protein content, good amino acid balance, and the fact that they can be produced using organic waste (Salomone et al., 2017). Feeding fish with insects such as black soldier flies, mealworms, and others opens up potential advantages, such as less dependence upon wild fish stocks and less strain on the environment (Tschirner et al., 2015). Although insect meal has the potential to replace a substantial amount of fishmeal and Soy-based ingredients, issues related to scale, cost and acceptance by consumers are still emerging. The results indicate that insect farming, which does not require a high quantity of resources to convert into food, has the potential to support a more sustainable aquaculture practice that will conserve resources and the environment as well as the industry.

The results of the present study are consistent with the prior literature on the use of insect proteins in aquaculture. Researchers have proven that insect-based diets, particularly BSF and MW, provide an effective nutritional source of food for the growth of various fish species, including Labeo rohita (Salomone et al., 2018). In addition, antioxidant and digestive enzyme activities were also improved in these studies, confirming the observations by the current experiment. However, in contrast to other studies which recorded reduced growth performance in insect inclusion percentages above 25% BSF and 30% MW (Su et al., 2017), we recorded a positive linear relationship between the insect protein inclusion (up to 75% BSF and 50% MW) and improved growth. The discrepancy can be explained by the divergence in experimental conditions and the fish species and diet composition.

Although the growth of fish fed BSF 75% and MW 50% diets was significantly higher, some surprising rather than expected results were noticed in the enzyme activity levels. The greatest increase in catalase activity (3.23 U/mg) occurred in the MW 50% group, an outlier, as the BSF 75% group had superior growth dimensions. This difference might be related to the inherent dissimilarity in amino acid profile, lipid content or bioactive compounds of the two insect species used. These factors could be further analysed to explain why catalase activity is higher in the MW 50% group.

Another observation that was not anticipated was the higher activities of lipase in BSF 75 and MW 50 when compared to the control group, though their growth rates were higher. It is generally expected that the lipase activity of insect protein-based diets would reflect the positive effects on growth, as the digestion of lipids is a key factor in nutrient availability. The increased enzyme activity could imply that other components influencing growth performance, i.e., number of feed intakes and feed conversion, could have a greater effect than enzyme activity alone on digestive lipids breakdown (Stevens et al., 2018; Smetana et al., 2016). The present research highlights the prospects of using insect proteins such as BSF and MW as a possible alternative to fishmeal in aquaculture.

It was recorded that the BSF 75% and MW 50% diet groups showed high activity of peroxidase, catalase, and superoxide dismutase. Similarly, digestive enzyme activity (protease, lipase, and amylase) was also increased in the BSF 75% and the MW 50% diet groups, meaning that more nutrition was being digested and extracted. Aquafeed with an insect-based protein can help to reduce the load on the biowaste, which is a significant problem in the industrialized and developing world (Pinotti et al., 2019). This has significant implications for the sustainability of aquaculture, where insect proteins could help to reduce reliance on fishmeal to mitigate the stress on wild fish. Insect farming is also less resource-intensive and more eco-friendly in comparison to livestock farming, supplying animal food with a more sustainable source of animal protein (Huis et al., 2013).

Nevertheless, this study has some limitations despite the promising findings. First, the experiment could have lasted longer (about 60 days) to understand the extremely long-term consequences of insect-based diets on fish health and performance (Van Zanten et al., 2015). Further research can focus on a prolonged experiment to find out the effects of these diets over a longer period of time (Zhang et al., 2022). Moreover, this study aimed at the growth performance and enzyme activity. Future studies should also examine the nutritional quality of the fish grown on insect-based diets, including the effect of the diets on the fillet composition, palatability, and consumer acceptance. The other limitation is that a detailed report on the specific compositions of insect proteins is lacking, including amino acid profiles, fatty acid compositions, and other bioactive components (Wu et al., 2018). Future research should seek to define the nutritional composition of various species of insects and how this affects the growth and health of the fish. In addition, investigations on how insect proteins affect diverse fish species and in varying environmental conditions will be important in establishing the wider applicability of insect proteins in aquaculture.

Conclusions

It is concluded that preliminary data indicated that insect-derived proteins, especially BSF and MW proteins, have the potential to replace fishmeal in *Labeo rohita* without affecting the growth performance. These diets also have a beneficial effect on the activities of antioxidants and digestive enzymes in promoting health and productivity in farmed fish. Finally, additional studies are required to investigate the long-term effects and optimal inclusion levels to have better idea of various insect-based proteins. Incorporation of insect-based proteins in aquaculture feeds would be central in solving sustainability issues in the sector and reduce pressure on wild stock of fish.

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