EFFECTS OF BARLEY GRASS SUPPLEMENTATION ON DIGESTIVE ENZYME ACTIVITY IN FRESHWATER FISH *CHANNA PUNCTATUS* (BLOCH, 1793)

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Abstract
To improve fisheries and achieve maximum yields from freshwater resources, it is essential to provide artificial feeds that promote rapid fish growth and enable fish to reach their maximum weight in the shortest possible time. One effective strategy involves incorporating novel substances into fish diets to enhance feed conversion efficiency and improve overall conditions for growth and maintenance. Although hormones, antibiotics, and various chemicals have been tested as growth promoters and antibacterial agents in aquatic animals, their use is not recommended due to residual effects in the muscle tissues of fish and prawns. Plants, on the other hand, offer natural, safer, and more cost-effective alternatives. Barley grass (Hordeum vulgare), the young grass of the common barley plant belonging to the Poaceae family, is rich in bioactive compounds with diverse nutritional and pharmacological benefits. These properties suggest its effective potential for use in freshwater fish culture. The present study investigates the effect of dietary barley grass supplementation on the digestive enzyme activity of the freshwater fish Channa punctatus (Bloch, 1793). The activities of amylase, lipase, and total protease were found to be significantly increased in groups fed with barley grass-supplemented diets. Based on these findings, barley grass supplementation is recommended as a valuable dietary additive for the successful aquaculture of this important fish species.
Keywords: *Barley grass, Channa punctatus, Digestive enzymes, Feed, Freshwater Fish.*

Introduction

Freshwater fish culture is a important system in fish production, involving the commercial cultivation and rearing of fish in freshwater environments such as tanks, ponds, and other enclosures for food production. Fish serves as a highly nutritious and rich source of animal protein. To improve fisheries and maximize yields from freshwater resources, it is essential to provide artificial feeds that promote rapid growth and enable fish to reach maximum weight in the shortest possible time. One effective strategy is to incorporate novel substances into fish diets that enhance feed conversion efficiency and improve overall conditions for growth and maintenance (Bhosale *et al.*, 2010; Shrivastava *et al.*, 2012; Joshi *et al.*, 2015).

While hormones, antibiotics, and various chemicals have been tested as growth promoters and antibacterial agents in aquatic animals, their use is generally discouraged due to residual effects in the muscle tissues of fish and prawns. In contrast, plants offer safer and more cost-effective alternatives as sources of bioactive compounds. These plant-derived substances can stimulate appetite and feed intake, promote growth, enhance endogenous enzyme secretion, and activate immunostimulatory and antioxidant responses within aquaculture systems (Hess and Sherma, 2004; Lee *et al.*, 2012; El-Gawad *et al.*, 2016; Joshi, 2017; Makode *et al.*, 2018).

Barley grass (*Hordeum vulgare*), the young grass of the common barley plant from the Poaceae family, is one of the most widely available herbs in India. It is commonly consumed fresh as juice or dried into powder for both animal and human use. According to Yawen *et al.* (2018), barley grass powder is an excellent functional food that not only provides essential nutrition but also helps eliminate cellular toxins in humans, thanks to its rich functional components. Barley grass contains numerous bioactive compounds, including gamma-aminobutyric acid (GABA), flavonoids, saponarin, lutonarin, superoxide dismutase (SOD), potassium, calcium, selenium, tryptophan, chlorophyll, vitamins A, B1, C, and E, dietary fiber, polysaccharides, alkaloids, metallothioneins, and polyphenols.

The health benefits of barley grass are extensive: it promotes sleep, exhibits antidiabetic effects, regulates blood pressure, enhances immunity, protects the liver, provides anti-acne and detoxifying properties, acts as an antidepressant, improves gastrointestinal function, and offers anticancer, anti-inflammatory, antioxidant, hypolipidemic, and antigout effects. Additionally, it reduces hyperuricemia, prevents hypoxia and cardiovascular diseases, alleviates fatigue and constipation, soothes atopic dermatitis, supplements calcium, and improves cognition. These attributes position barley grass as a promising functional food for preventing chronic diseases and a valuable raw material for modern dietary structures aimed at advancing the health industry. The preventive and therapeutic roles of its key components like GABA, flavonoids, SOD, potassium, calcium, vitamins, and tryptophan that have been well documented (Panthi *et al.*, 2020). Given these beneficial properties, barley grass holds great potential for application in freshwater fish culture. The present study takes a novel approach to investigate the effects of dietary organic barley grass supplementation on the digestive enzyme activity of the freshwater fish *Channa punctatus* (Bloch, 1793).

Materials and Methods

The protocol of Joshi (2021) was adopted during the evaluation of the dietary barley grass induced effects on Digestive Enzyme activity in freshwater fis*h Channa striata* (Bloch, 1793).

Experimental containers

The experimental containers were rectangular plastic aquaria of 10-liter capacity (100 X 100 X 100 Cm). The container had flat bottom. They were easy to clean, and no material collected at corners and cracks. They were rinsed with tap water, cleaned with detergent and rinsed once with 10% of HCl and then rinsed twice with tap water before use in each experiment. Each container was covered with a mosquito net to prevent the fish from escaping. The containers were divided into the following 6 groups with 20 fish each and observed for the next 60 days.

Maintenance and acclimatization

The collected fish were disinfected with 0.1% $KMNO_4$ solution to avoid fungal infection. These fish were acclimatized for a week and maintained glass aquarium. Aquariums were aerated by air pump for supply of oxygen to individuals. The drain settled in tanks is collected by filtration net. Water quality was maintained during the feeding trial with light: dark cycle of 12:12 h during study. The specimens were fed on a control diet during the course of the experiment.

Water quality standards

The water analysis is performed according to American Public Health Association (Anonymous, 2000). The aquarium water was aerated continuously during experimental tenure. The water composition and characteristics were maintained within the effective range (Bhatnagar and Devi, 2013).

Fish feed Formulation

For the experiment, barley grass from conventional organic farms were used. A crop of mature grass trimmed to 1/2-inch above the soil. It took 6 to 9 days for grass to mature. Then the fresh meat was thin sliced. The harvested grass blades and meat slices were laid on a clean baking sheet separately and placed into food dehydrator. The temperature was set to 150^{O} F for eight hours or until dry; then grinded in food processor. The ingredients were weighed, mixed and pelleted. After pelleting, the feeds were air dried and put in an air-tight container. The proximate compositions of feed were estimated by using the Association of Analytical Chemists (Anonymous, 1995) methods with some modifications (Mohammad *et al.,* 2019). The composition of experimental diet is given in Table 1 and 2.

Feeding regime

During the acclimation, fish were fed the control diet to satiation twice a day at 09:00 and 15:00 hours. After acclimation, fish were fasted for one day; batch weighted and randomly distributed among density of 10 fish $tank^{-1}$. During the experiment, fish were fed on experimental diet to satiation third a day at 08:00, 12:00 and 16:00 hours.

Table 1. Ingredient and Proximate composition of the experimental feeds (on % basis)

Ingredients (%)	**B0**	**B1**	**B2**	**B3**	**B4**
Wheat flour	45	42.5	40	37.5	35
Soybean flour	24	24	24	24	24
Corn flour	10	10	10	10	10
Meat powder	15	15	15	15	15
Soybean oil	05	05	05	05	05
Watrmin® Forte*	01	01	01	01	01
Barley grass Powder	00	2.5	5.0	7.5	10
Proximate Composition (%)					
Moisture	5.13	4.34	3.94	3.51	3.20
Crude protein	33.48	34.29	35.42	37.88	41.36
Crude lipid	6.37	6.16	6.11	5.21	5.19
Crude fibre	19.03	21.49	23.08	24.19	25.23
NFE	18.59	16.86	15.24	13.4	11.87
Crude Ash	17.40	16.86	16.21	15.81	13.15
Gross Energy (KJ/g)	13.88	14.39	14.89	15.29	16.27

* Vitamins, Minerals and Amino Acids supplements for Aquatic feed. Manufactured by Virbac Animal Health India Pvt. Ltd.

Digestive Enzyme activity

For Analysis, after each week of treatment, the blood samples of fish were collected directly from heart with the help of syringe (Hassaan and Soltan, 2016; Joshi, 2021). Blood samples were collected 18 h after the final feeding for the biochemical assay. The samples were collected in non-heparinized tubes and stored in freezer at 4-8°C up to 24 hrs. The blood samples were allowed to clot and then centrifuged at 4000 rpm for 10 minutes to separate the serum (Hassan and Sotan, 2016; Pradhan *et al.*, 2021). The Digestive Enzyme activity were estimated with help of Automated Analyzer (Mispa Ace Clinical Chemistry Analyser, Agappe Diagnostic Ltd. India).

In digestive enzyme activity, analyzers typically measure the levels of amylase and lipase. While both enzymes play crucial roles in carbohydrate and fat digestion respectively, protease functions distinctly by breaking down proteins. Therefore, the accurate determination of protease activity requires separate analysis, as it cannot be directly inferred from amylase and lipase values alone.

However, to estimate an approximate value of protease activity based on the available data, the following empirical formula suggested by Joshi (2025) can be applied:

The approximate formula to estimate Protease based on Amylase and Lipase values is:

$$\textbf{Protease} \approx 0.310 + (1.337 \times \textbf{Amylase}) - (0.094 \times \textbf{Lipase})$$

This formula is based on the general observation that protease increases significantly with amylase while lipase has a slight negative influence in this model.

Statistical analysis

Results were recorded as Mean ± Standard Deviation of triplicate (Joshi *et al.,* 2015).

Results and Discussion

The present study aimed to evaluate the effect of dietary barley grass supplementation on digestive enzyme activities of amylase, lipase, and total protease in the freshwater fish *Channa punctatus*. The results clearly demonstrate a positive correlation between the level of barley grass in the diet and the activity of digestive enzymes (Table 1). Amylase activity showed a progressive and significant increase across all treatment groups compared to the control (B0). The activity rose from 0.354 ± 0.035 U/mg protein in the control group to 2.372 ± 0.378 U/mg protein in the B4 group, indicating a nearly 7.5-fold enhancement. This suggests that barley grass inclusion markedly enhances carbohydrate digestion efficiency in *Channa punctatus*, possibly due to the presence of functional bioactive compounds such as flavonoids, saponarin, and enzymes which may stimulate pancreatic secretion or directly contribute to enzymatic activity.

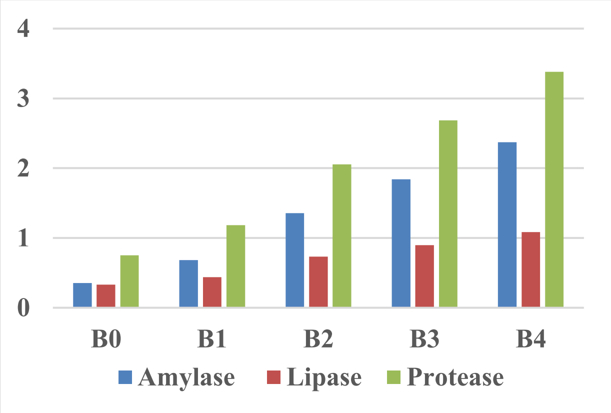
Similarly, lipase activity followed a rising trend from 0.330 ± 0.130 U/mg protein (B0) to 1.086 ± 0.342 U/mg protein (B4), indicating improved lipid digestion capacity in fish fed with barley grass-enriched diets. Lipase activity nearly quadrupled in the B4 group. This enhancement could be attributed to the rich nutritional profile of barley grass, including essential fatty acids, vitamins (e.g., E and B-complex), and antioxidants which may optimize lipid metabolism and cellular function.

The most pronounced effect was observed in total protease activity, which increased from 0.752 ± 0.260 U/mg protein in the control (B0) to 3.379 ± 0.437 U/mg protein in the highest supplementation group (B4). This fivefold increase indicates a substantial improvement in the fish's protein digestion capability, likely due to barley grass's ability to stimulate endogenous enzyme production or provide protease-like bioactive compounds. The control and test feed groups for all parameters were significantly different (*P<0.05*) from each other.

Table 2: Digestive enzyme activity in fish fed on barley grass powder-based feed

Parameter		B0	B1	B2	B3	B4
Amylase (U/mg protein)	Mean	0.354	0.684	1.357	1.841	2.372
	±SD	0.035	0.130	0.472	0.271	0.378
Lipase (U/mg protein)	Mean	0.330	0.437	0.732	0.897	1.086
	±SD	0.130	0.071	0.118	0.307	0.342
Total Protease (U/mg protein)	Mean	0.752	1.183	2.056	2.687	3.379
	±SD	0.260	0.425	0.224	0.212	0.437

Figure 1: Digestive enzyme activity in fish fed on Barley grass powder-based feed



Overall, the consistent increase in the activity of all three digestive enzymes—amylase, lipase, and protease—from groups B1 to B4 indicates that dietary supplementation with barley grass significantly enhances the digestive physiology of *Channa punctatus*. This enhancement is likely to contribute to more efficient nutrient assimilation, improved growth performance, and better overall health status in the fish. These findings align with previous research highlighting the positive effects of plant-based feed additives on digestive enzyme activity in aquatic species.

Digestive enzymes play a crucial role in breaking down nutrients in feed, thereby increasing feed utilization efficiency (Widanarni and Jusadi, 2015). In recent years, digestive enzyme activity has been employed as a reliable indicator for assessing nutrient utilization and correlating it with growth performance under various feeding regimes (Wu *et al.*, 2007; Jun *et al.*, 2009; Xie *et al.*, 2011). Among these, the specific activity of amylase is particularly important for evaluating the efficiency of carbohydrate digestion (Thongprajukaew *et al.*, 2011).

In the present study, significant improvements were observed in the activity levels of protease, amylase, and lipase in fish fed barley grass-supplemented diets. This suggests a strong link between the enhanced utilization of carbohydrates and proteins and the growth of *Channa punctatus*. These results are supported by earlier studies demonstrating that increased digestive enzyme activity is associated with better nutrient absorption and growth (Kumar *et al.*, 2006; Thongprajukaew *et al.*, 2011; Karun *et al.*, 2017; Shabana *et al.*, 2019; Goswami *et al.*, 2020; Pradhan *et al.*, 2021).

The findings imply that barley grass supplementation stimulates the activity of digestive enzymes, thereby promoting efficient digestion and utilization of dietary nutrients. This improved digestive function ultimately contributes to enhanced growth and development in *Channa punctatus*, making barley grass a valuable natural feed additive in sustainable aquaculture.

Conclusion

This study evaluated the effect of barley grass (*Hordeum vulgare*) supplementation on digestive enzyme activity in *Channa punctatus* (Bloch, 1793). Results showed significant increases in amylases, lipase, and protease activities in fish fed barley grass diets, indicating enhanced nutrient digestion and utilization. Consequently, barley grass improves growth and development, making it a natural, sustainable feed supplement recommended for successful freshwater aquaculture.

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