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Research article

Effect of Bromelain-Fermented Diets on Digestive Enzyme Activities and Muscle Proximate Composition of *Labeo Rohita*

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Abstract

Plant proteins are considered most suitable to replace fish meal because they are cheap, readily available, and abundant. However, plant proteins are not digested efficiently in fish due to the presence of complex protein structures. Therefore, this preliminary study was conducted to evaluate the effect of bromelain-fermented plant diets on the digestive enzyme activities and muscle proximate composition of *Labeo rohita*. For this purpose, healthy fingerlings with an average initial weight of 10±0.2 g were procured and acclimatized under laboratory conditions. Then, 20 fingerlings were transferred to each of 20 glass aquaria (160 L capacity) in three replicates. Five diets were prepared using plant meals and were fermented using 10, 20, 30 and 40% bromelain powder for 48 hours at 55°C. The control diet was not fermented. Fermented diets were fed to fish for 90 days, and then digestive enzyme activities and muscle proximate composition were determined. Fish fed bromelain-fermented diets (10-40%) showed a significant increase in digestive enzyme activities (protease and lipase) compared with the control group. However, no significant effect was observed on the amylase activities in fish fed bromelain-fermented diets. Muscle proximate composition revealed that crude protein (CP) contents were increased while crude fat (CF) contents were decreased in fish fed fermented diets compared with the control group. However, no significant differences were observed in moisture and ash contents. In conclusion, fish fed fermented diets showed enhanced activities of digestive enzymes (protease and lipase) and crude protein contents in the muscle of *L. rohita*. Therefore, it is recommended to conduct a detailed trial on bromelain fermentation.

Keywords: Bromelain, digestive enzyme activities, fermentation, muscle proximate, plant protein

1. Introduction

Proteins constitute a crucial component of fish diets, serving as the primary source of essential amino acids required for growth, tissue repair, and metabolic activities. Traditionally, fish meal has been the predominant protein source in aquafeed due to its well-balanced amino acid profile and palatability [1]. However, the escalating costs and limited availability of fish meal, coupled with concerns over its sustainability and environmental implications, have driven researchers and feed manufacturers to explore alternative protein sources [2].

The quest for viable alternative protein sources has led to investigations into plant-based ingredients. Plant meals derived from various sources possess the potential to replace or supplement fish meal in aquafeed formulations [3]. However, the full substitution of fish meal with plant meals presents challenges, mainly related to differences in amino acid profiles, anti-nutritional factors, and digestibility. These factors can

potentially hinder the growth and performance of farmed fish when plant-based ingredients dominate the feed composition [2, 3].

One promising strategy to overcome the limitations of plant-based ingredients is fermentation. Fermentation has gained attention as a means to improve the nutritional quality and digestibility of plant protein sources in aquafeeds [4]. Exogenous proteases such as bromelain breakdown complex carbohydrates and enhance the availability of nutrients for fish digestion and absorption. The utilization of fermentation techniques in digesting plant protein sources holds the potential to create more balanced and sustainable aquafeeds, thereby contributing to the growth and well-being of farmed fish [5, 6].

Labeo rohita, commonly known as Rohu, is a notable freshwater species in the aquaculture sector that belongs to the *Cyprinidae* family. It is the most widely cultured fish species in the Indian subcontinent due to its fast growth rate. Furthermore, this species has a delicious taste, consumer preference and enormous demand in the local market [7]. This study was performed to evaluate the effects of bromelain fermentation on the digestive enzyme activities and proximate composition of *L. rohita*, shedding light on the potential benefits and implications of incorporating fermented plant protein sources in the diets of this commercially important fish.

2. Material and Methods

2.1 Feed formulation and fermentation process

Feed ingredients were taken from local stores and were processed to a fine powder. Then, ground feed ingredients were subjected to proximate composition analysis [8]. Then, the ingredients (other than vitamin premix, mineral mixture, and fish oil) were mixed with water (1:1) and heated to 55°C. Bromelain enzyme powder was added to the mixture at 1%, 2%, 3% and 4% concentrations and then incubated for 48 hours at 55°C. After the completion of the incubation period, the mixture was subjected to 90°C for 5 minutes for inactivation of the enzyme and then dried to formulate feed [9]. Control diet ingredients were not fermented. Fish oil, vitamin premix and mineral mixture were mixed with fermented ingredients in a mixer. Water was added to make a stiff dough that was pelleted through meat mincer (Anex, AG3060). The pellets were shade-dried, weighed and packed in self-sealing bags. Five experimental diets containing 0, 10, 20, 30 and 40 g/kg fermented bromelain were prepared. The experimental diet was given to fingerlings throughout the 90 days.

Table 1. Composition of the experimental diets.

Ingredients (%)	**Percentage**
Fish Meal	10
Soybean Meal	25
Sunflower Meal	25
Wheat Flour	10
Rice Polish	20
Fish Oil	7.0
Mineral Mixture*	1.0
Vitamin premix**	1.0
Choline Chloride	1.0
Proximate composition%	
Dry matter	91.23±0.15
Crude protein	30.45±0.34
Crude fat	12.04±0.12
Ash	4.54±0.05

*Mineral mixture contained the following per kilogram: selenium 100 mg, manganese 23750 mg, iodine 2750 mg, copper 5000 mg, zinc 75000 mg, magnesium 200000 mg, and cobalt 2000 mg.

**Vitamin premix contained the following per kilogram: 60000 mg inositol, 2400 mg vitamin E, 4000000 IU, 2400 mg vitamin K3, 4000 mg vitamin B1, 4000 mg vitamin B6, 1200 mg folic acid, 40000 mg vitamin C, 10 mg vitamin B12, vitamin A 10000 mg, 100 mg D-biotin, 4000 mg niacin, Cal. D. Pantothenate, vitamin D3 480000 IU.

2.2 Fish procurement and rearing conditions

L. rohita (10 ± 0.2 g) were obtained from the Department of Fisheries and Aquaculture, UVAS, Ravi Campus, Pattoki. Fish were acclimatized to laboratory conditions after treatment with $KMnO_4$. After acclimatization, the fish were distributed to glass aquaria at a stocking rate of 20 fish per aquarium in triplicate (a total of 45 aquaria). During the 3 months of the experimental trial, the fish were fed two times a day at 3% of

their body weight. During the trial, water quality parameters (pH, temperature, DO) were checked throughout the experiment, and their mean values were 28±0.7 ℃, 7.2±0.2 and 7.6±0.3 mg per liter, respectively.

2.3 Fish harvesting

At the end of the feeding trial, 5 fish were anaesthetized with clove oil (5 mg/L) and dissected. Hepatopancreases of these fish were collected and stored in distilled water. Another five fish from each treatment were sacrificed for muscle proximate analysis.

2.4 Digestive enzyme activities

Hepatopancreas samples (1 g) were homogenized with 5% sucrose solution (0.25 M) from each treatment. The homogenate was centrifuged at 5000 × g for 15 minutes, and the supernatant was collected for further analysis of digestive enzymes. Amylase activities were determined using the start solution following Rick and Stegbauer [10]. The casein digestion method of Kunitz [11] was used to calculate the protease enzyme activity. Lipase activities were determined using p-nitrophenylpalmitate (pNPP) following the method of Mahadik, Puntambekar [12].

2.5 Muscle proximate composition analysis

The muscle proximate composition was determined following the standard protocols of AOAC [8].

2.6 Statistical analysis

The obtained data were subjected to one-way analysis of variance (ANOVA) using SPSS (version 23). Means of the parameters were compared using Tukey's HSD test. The results were considered significant at $p < 0.05$.

3. Results

3.1 Digestive enzyme activities

Fermented diets with different levels of bromelain showed significant improvements in digestive enzyme activities (Table 2). Diets fermented with all bromelain levels showed higher protease activities compared with the control group. However, all bromelain levels showed nonsignificant differences from each other. Similarly, lipase activities were enhanced in fish fed diets fermented with graded levels of bromelain compared to the control group. Amylase activities were not affected by different levels of bromelain in fermented feeds.

3.2 Muscle proximate composition

The proximate muscle composition of *L. rohita* fed fermented diets containing various levels of bromelain is presented in Table 3. Moisture contents were not affected in the muscle of fish. Significantly higher crude protein contents were observed in fish fed fermented diets containing 10-40% bromelain compared with the control group. Fish fed a fermented diet with 10-40% bromelain showed a significant reduction in crude lipid content compared with the control group. Ash contents were not affected by fermented diets.

4. Discussion

The main challenges in the aquaculture sector are the consistent availability and high cost of feeds [13]. The cost of feed is critical since it accounts for 30-70% of overall expenses and helps in determining the sustainability of aquaculture success [14]. To decrease the cost of feed, plant protein sources are excellent options and are available abundantly. However, plant proteins have lower digestibility than fishmeal due to the presence of complex protein structures, high carbohydrate levels and antinutritional factors [3]. Therefore, fermentation with exogenous enzymes can be useful to enhance the digestibility of plant proteins. The bromelain enzyme is present in natural compounds such as pineapple (*Ananas comosus*) fruit peels and stems and is a blend of proteolytic enzymes [15].

In the current study, digestive enzyme activities were enhanced significantly in fish fed fermented diets with bromelain, except amylase activities. This might be because bromelain primarily aids in protein digestion by partly hydrolysing protein molecules into smaller units and boosting their bioavailability [16]. Fermentation is an efficient method to enhance the quality of feed ingredients [17] by inactivating anti-nutritional components [18] and increasing the absorption of nutrients [19]. Digestive and brush border enzyme activities are closely related to fish digestive and metabolic capabilities [20]. The findings of our study are in line with the results of [21], who reported that dietary fermented bromelain supplementation had no effect on amylase activity in the mid-

Table 2. Effect of bromelain-fermented diets on the hepatopancreatic digestive enzyme activities of *Labeo rohita*.

Parameters	Diets fermented with different levels of bromelain				
	0%	10%	20%	30%	40%
Amylase	2.76±0.04	2.79±0.04	2.78±0.03	2.79±0.03	2.79±0.04
Protease	0.76±0.04^{b}	0.89±0.05^{a}	0.88±0.04^{a}	0.89±0.05^{a}	0.9±0.04^{a}
Lipase	0.49±0.03^{b}	0.71±0.04^{a}	0.7±0.04^{a}	0.69±0.06^{a}	0.68±0.05^{a}

The data presented are the mean ± SD of three replicates. Values with different superscripts in a row indicate significant differences ($p < 0.05$).

Table 3. Effect of bromelain-fermented diets on the muscle proximate composition of *Labeo rohita*.

Parameters	Diets fermented with different levels of bromelain				
	0%	10%	20%	30%	40%
Moisture	75.12±0.07	75.14±0.06	75.11±0.06	75.12±0.07	75.12±0.06
Crude protein	17.19±0.06^{b}	17.41±0.07^{a}	17.4±0.05^{a}	17.37±0.05^{a}	17.38±0.07^{a}
Crude fat	4.39±0.04^{b}	4.23±0.06^{a}	4.21±0.06^{a}	4.23±0.06^{a}	4.24±0.06^{a}
Ash	2.55±0.03	2.55±0.03	2.54±0.03	2.54±0.03	2.53±0.02

The data presented are the mean ± SD of three replicates. Values with different superscripts in a row indicate significant differences ($p < 0.05$).

gut and hindgut of Gibel carp. Similarly, protease and lipase activity increased in Nile tilapia intestines given a diet combined with exogenous enzymes compared to the control [22].

Proximate composition is an excellent indicator of the nutrient profile in the body [23]. In the current study, crude protein contents were significantly increased while crude fat contents were decreased in fish fed diets fermented by bromelain. The enhancement of protease activity indicates that fish effectively utilized proteins in the muscle compared with the control group, which is in line with the study of [24]. This can be attributed to the enhancement of protease activities, which can lead to better nutrient utilization. A decrease in crude fat content in the muscle of fish indicates a better nutrient profile, as the aim is to increase the protein and decrease the fat contents. This might be due to an increase in lipase activities, which enhanced the utilization of lipids in the body instead of their deposition. No differences were observed in moisture and ash contents, which is supported by [25].

5. Conclusion

In conclusion, *Labeo* rohita fed diets fermented by bromelain showed significantly enhanced digestive enzyme activities compared to the control group. Protease and lipase activities were enhanced, while amylase activities were not affected. A significant increase in crude protein and a decrease in crude lipid contents in muscles were observed. However, no alterations were noted in moisture and ash contents. This study is helpful for the aquafeed industry to solve the problem of expensive and scarce fishmeal using fermented plant-based diets. However, the current study is limited to fermentation of the whole diet, including fishmeal and other ingredients. Further studies need to be conducted on separate fermented plant ingredients and their replacement with fishmeal.

Data Availability statement

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

All authors declare that, they have no conflict of interest.

Author Contributions

TY: Investigation; MF: Conceptualization, Methodology, Supervision; SZHS: Conceptualization, Methodology, Writing - Review & Editing; WA: Writing - Original Draft, Formal analysis; SQ: Data Curation

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