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Growth Response of Juvenile Rohu (*Labeo Rohita*) Exposed to Varying Amounts of Peppermint diet with Protein

Shraddha Sharma[1] and Yogesh D. Akhare[2]

[1&2]Dept. of Zoology, Vidya Bharati Mahavidyalaya, Camp Road, Amravati, Maharashtra 444602
*Email-sharmashraddha.sm@gmail.com

ABSTRACT

The goal of the current investigation was to determine how dietary peppermint (Mentha piperita) along with protein affected Labeorohita's growing performance. Following five days of acclimation to the aquarium environment, the fingerlings were split into four equal groups, designated as groups A, B, C, and D. Each aquarium included fifteen fish. The duration of the experiment was for sixty days where one set of animals was fed a control diet devoid of peppermint and another three were fed with test diets containing varying levels of Mentha piperita and protein at 4mg,6mg and 8mg of the diet. The weight gain, body length. specific growth rates were used to evaluate fish growth. The findings showed that in Tank 4, where fish were fed a diet containing 8mg of peppermint, all growth metrics were significantly higher. When compared to the control group, the dietary inclusion of Mentha piperita in all doses increased fish appetite and may be suggested as a safe growth promoter as the outcomes demonstrated that feeding with *Mentha piperita* decreased the fatalities by enhancing fish growth and production.

Keywords : Peppermint, Labeorohita, Growth, Medicinal herbs.

INTRODUCTION

Aquaculture farming is now the fastest-growing food production industry worldwide. It offers premium animal protein, with a rise in worldwide production. Integrated fish management and polyculture are established, fruitful, and dynamic techniques that employ common fish species like mrigal (*Cirrhinus Cirrhosus*), rohu (*Labeo Rohita*), and catla (*Catla Catla*) (Uddin et al., 1994; FAO, 1997). Due to its high market value and consumer preference, rohu are currently quite popular among farmers (Dey et al., 2005; Rahman, 2006) Fresh water fish of the species *Labeo Rohita* are members of the family Cyprinidae and the class actinopterygii order Cypriniformes. It is found in freshwater environments like lakes, streams, rivers, and pools all across the world. This highly adaptable freshwater fish is found throughout Europe, Asia, and the Middle East's eutrophic waterways; it is also found in North America, Canada, and Australia (Faramarzi et al., 2012).The rohu (*Labeo Rohita*) is a highly sought-after species with significant market value. Shete et al. (2015).

Nowadays antibiotic growth promoters are being added to feed in order to achieve high production. However, the public is more receptive to natural substances because of their negative effects (residual buildup in fish tissue and, rise of antibiotic-resistant

bacteria).To deal with this the use of herbal immunostimulants has been shown to improve animal overall health , productivity and feed consumption efficiency. Medicinal plant extracts have been used for a variety of reasons since ancient times, including medications, cosmetics, and food supplements. They've been evaluated for their prospective applications as substitute remedies for the management of numerous infectious disorders and the protection of food from oxidant-induced toxicity (Kelen and Tepe 2008).

Mentha piperita, popularly known as peppermint, is a well-known perennial herb in the Labiatae family.is among the traditional medicinal herbs used worldwide (Iscan et al.,2002) Due to their extensive therapeutic range, plant extracts are frequently used in traditional and ancient medicine, typically in aqueous solutions (Zhang et al., 2002). According to available data and research, numerous studies have examined the use of dietary medicinal herbs and their extracts as possible immunostimulants in aquaculture, with eventually encouraging findings noted regarding varied fish species' disease resistance, growth patterns, feed consumption, chemical body structure, and immune response (Hoseinifar *et al*., 2016). The primary active component found in peppermint is menthol, accompanied by other elements like menthol and menthyl acetate Dried peppermint typically contains volatile oil, with varying percentages of menthol, menthone, menthyl acetate .Peppermint has been recognized for its potential as an immunostimulant and appetite enhancer (Pauli 2006). The current study aims to examine how incorporating dietary peppermint *(Mentha piperita)* affects the growth performance of the Indian major carp, rohu Labio rohita “Barbarestani *et al*. (2017)”. Depending upon the above-mentioned , this study is done to investigate the effects of mint powder dietary supplements on *Labio rohita* growth and health performance performance. Many studies have shown that the rise in feed intake could also be attributed to the effects of peppermint and the improved digestibility of food. Our study aligns with prior research findings, such as those of (Nath *et al*. (2013), which support this notion.

MATERIALS METHOD

Experimental setup

The experiment was done in a laboratory setting. The fish were collected from the adjacent fish farm, and after approximately a week of acclimation, the healthy fish were used for the experiment. The fish were housed in glass aquariums of 100 l capacity filled with regular well water that had been adequately aerated. The tanks were divided into four such as group A, group B, group C, group D where group A is for control and the remaining three are for experimental. Fish fingerlings (10 fish per tank) were added to each aquarium in an equal quantity. To prevent any fungal infection, dechlorinating solutions were added and the water was changed every day. The fish's initial body weight and general composition were established before the experiment. After the experiment growth parameters were studied by analyzing body weight, height etc.

Preparation of Experimental Diet

The Mentha piperita plants were gathered from their natural habitat Following the collection, leaves of M. piperita underwent a drying process in a well-ventilated and dark

room. Subsequently, the dried plants were finely ground using a grinder. For the preparation of experimental diet the powdered plant material in different concentration like 4mg, 6mg and 8mg was mixed with groundnut oil cake, wheat flour, soya flour, fish meal, tapioca flour, and a mix of vitamins and minerals. In all of these groundnut and soya were taken in high proportion as both of them are good sources of protein. It is widely recognized that protein is crucial and often the most costly component that needs to be provided in sufficient quantities to facilitate optimal growth while minimizing expenses (WecKL,1983). The amount of crude protein in the groundnut seed is 25–30% and oil content is 35–50% (Musa *et al.,* 2010). Whereas the control diet, devoid of peppermint, was formulated using the same ingredients.

The resulting diet was made into palette, allowed to sun-dry, and were stored.

Table 1 : Ingredients of experimental diet.

Ingredients (g/100gm)	(Group A) Control	Group B	Group C	Group D
Soya oil cake	45	45	45	45
Groundnut oil cake	25	25	25	25
Wheat flour	20	20	20	20
Corn starch	5	5	5	5
Fish meal	3	3	3	3
Mineralsand Vitamin	2	2	2	2
Peppermint powder	00	0.4	0.6	0.8

Growth performance

Growth parameters such as weight gain, and specific growth rate (SGR) were determined according to the following standard formula (Saufie *et al*., 2015).

Weight gain = Final fish weight (g) - initial fish weight(g)

$$\text{Specific growth rate} = \frac{\text{ln final weight - ln initial weight}}{\text{Number of days}} \times 100$$

Protein estimation

Lowry's technique, a commonly used method for determining total protein content in a sample. (Waterborg, & Matthews, 2002) This method's sensitivity is owed to its utilization of two color-producing reactions. This process involves Biuret Reaction which is the interaction of Cu ions with the peptide bonds of proteins under alkaline conditions. In the presence of a base like sodium hydroxide, Cu ions react with peptide bonds, leading to the reduction of cupric ions (Cu) to cuprous ions (Cu) (Lowry OH 1951). This complex formation contributes to the assay's sensitivity. In this phase, the Folin-Ciocalteu

reagent is utilized. This reagent contains a phosphomolybdic complex, which is a mixture of sodium tungstate, sodium molybdate, and phosphate, alongside copper sulfate solution and the protein sample. Upon reaction with peptide bonds in the protein sample, the Folin-Ciocalteu reagent generates a blue-purple colour. The intensity of this colour correlates directly with the protein concentration in the sample. Typically, the absorbance of the resulting colour is measured spectrophotometrically at wavelengths between 650-700 nm (Wrolstad et al., 2005). By gauging the absorbance of the produced colour in Lowry's reaction, the protein concentration in the sample can be quantified. This approach is favored for its sensitivity and reliability in determining total protein levels in biological specimens like serum.

RESULTS & DISCUSSION

Fish growth parameters

A dietary supplement of Peppermint and protein has been shown to enhance the growth performance in fish showing significant differences ($P < 0.05$) between groups, as indicated by varying superscripts within each row. There was a significant increase in weight gain, length and SGR when the mint treatment was given to the fish. There are many studies that have shown the positive effect of peppermint on aquatic animals like fish (Hong *et al*., 2012).

The body weight (in grams)

The body weight gain of *Labeo rohita* fish exhibited a notable increase in groups (C) and (D) compared to groups (A) and (B) during the initial 30 days. Among these, group (D), which received a high dose of peppermint supplement (8 mg) with their feed, recorded the highest weight in the next 30 days subsequently there was a significant increase in weight observed in groups (B), (C), and (D) in comparison to control group A. Once again, group (D) treated with a high dose of peppermint supplement mixed with feed displayed the maximum weight and length (Table 2) As mint has antioxidant properties it increases the survival rate and increases the growth performance, specific growth rate (VargaSanchez et al., 2019;)

Specific growth rate

Dietary peppermint has a positive effect on specific growth rate (Korzen *et al*., 2016) Highest specific growth rate was observed in group D which were fed with peppermint and high protein diet as compared to controls which had the lowest specific growth rate which was fed with only normal diet (Table 2).The leaves of mint has high digestive properties as also act as a good appetizer hence enhancing the SGR (Asghari *et al*., 2018)

Length of fish

Peppermint has shown a positive impact on the increase in length of fish. There's An increase in length of fish as the weight gain. The length increases gradually as the dose increases. Furthermore, morphological features also change as the fish grow in weight and length (Parvin *et al.*, 2018).

Table 2 : Growth response of juvenile rohu exposed to varying amount of peppermint diet with protein

Group	Weight (gm)	SGR	Length (cm)
Control (A)	4.36 ± 0.54	0.77±0.59	3.06±0.61
Experimental (B)	5.08±0.38	0.72±0.057	5.66±1.09
Control (A)	4.36 ± 0.54	0.77±0.59	3.06±0.61
Experimental (C)	7.44±0.75	0.92±0.071	8.32±0.23
Control (A)	4.36 ± 0.54	0.77±0.59	3.06±0.61
Experimental (D)	9.44±1.00	1.078±0.06	8.58±0.19

Data is presented as mean values with standard deviations, showing significant differences (P < 0.05) between groups as indicated by varying superscripts within each Row.

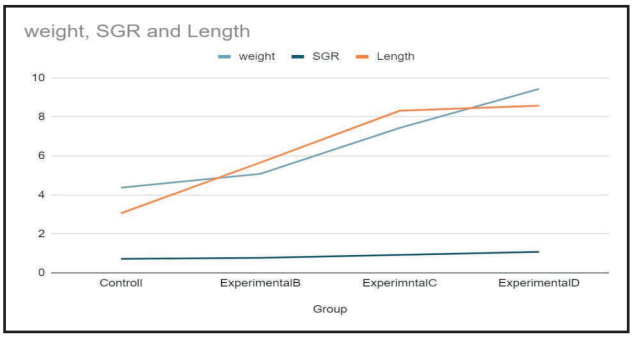


Fig 1 : Mean weight gain of fish under different experimental group

Total protein -There was a significant increase in protein and fat levels when the peppermint was given in the diet. As a result the performance of the fish was found to be enhanced.

Dietary protein plays a key role in the growth of fishes (*Barbarestan et. al. 2017)*

Table 3 : Mean value of protein (g/dL) and lipid in different experimental group

Fish group	Total protein (g/dL)	Total lipid
Control A	2.84± 0.89	2.06±0.17
Experimental B	2.52±0.88	3.70±0.32
Control	2.84± 0.89	2.06±0.17
Experimental C	3.59±0.81	4.01±0.27
Control	2.84± 0.89	2.06±0.17
Experimental D	3.48±0.81	4.20 0±.56

Data is presented as mean values with standard deviations, showing significant differences (P < 0.05) between groups as indicated by varying superscripts within each Row.

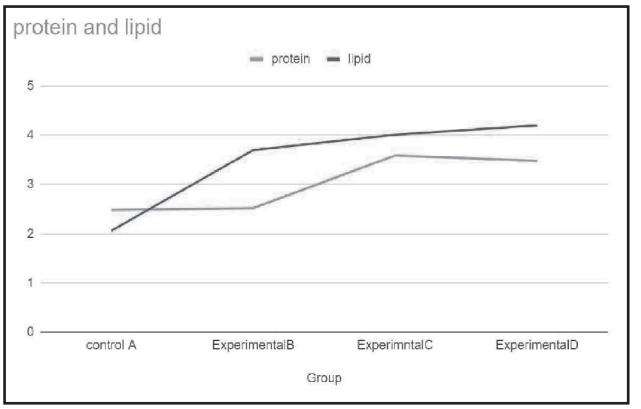


Fig 2 : Amount of protein and lipid in different experimental group

It is imperative to engage in the aquaculture sector given its pivotal role in meeting the rising global demand for seafood. Aquaculture offers a sustainable and efficient means of seafood production, crucial for feeding an expanding population. With wild fish stocks declining due to overfishing and environmental shifts, there is a pressing need for effective disease management and drug application within this important and profitable industry (Aklakur *et al.*, 2015). The discussion revolves around the potential impacts of medicinal herbs, which contain potent bioactive compounds. These compounds can influence the digestive process by either enhancing or inhibiting enzyme activity, thus affecting nutrient absorption (Talpur 2013) A particular study examines the inclusion of peppermint in fish diets, showing notable improvements in weight gain, and specific growth rate. Notably, group 4, which received a diet with 8mg of peppermint and a high protein content, experienced increases in all growth parameters. Similarly, feeding with *Mentha piperita* stimulated appetite, leading to improved weight gain and specific growth rate, as previously observed in the fingerling of *Labeo Rohita* (Adel et al., 2015).

CONCLUSION

Herbs and medicinal plants are more effective growth promoters compared to synthetic alternatives. They are cost-effective, safe, environmentally friendly, and do not have adverse effects on the fish. The study's findings indicate that incorporating Mentha piperita powder or extract into the diet of *Labeo rohita* significantly enhances their growth performance and increases their protein levels. The use of dietary peppermint was shown to positively affect various growth metrics such as weight gain, body length, and specific growth rate. Fish fed with the highest concentration of peppermint exhibited the most notable improvements, suggesting that Mentha piperita can be an effective and safe growth promoter. This herbal supplement not only improved the overall health and growth of the fish but also reduced mortality rates, making it a valuable addition to aquaculture practices.

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