

Extraction of Bio-Compound from Marine Waste and Its Usage as a Possible Immune Stimulant

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ABSTRACT: Protein is the major component and a growth-limiting nutrient further than denote the most expensive ingredient in aqua feeds. It has been estimated that the marine fish wastes contain considerable amount of protein in it. The study mainly focuses on the protein product extracted from the fish waste, scales of Pink perch (*Nemipterus japonicus*). Extracted proteinaceous product was used as a feed for ornamental fish (*Pangasius hypophthalmus*) at varying concentrations (5g, 10g and 15g) along with the commercially available feed. The product extracted was subjected to the quantitative and qualitative analysis of protein. The ornamental fishes fed with the extracted product at varying concentrations were checked for their growth and other morphological and physical changes. The fish were also subjected to hematological studies. The present study indicated that, there is an increase in enhancement of immune power and their phagocytic activity of the fish at 10g concentration of the extracted product along with the commercial feed. This reveals that the product could enhance the immune response. The fish fed with the proteinaceous extracted product were observed with active movements and increased body weight. Aquaculture being one of the fastest growing animal food producing sector, the present study might subsidize to the utilization of marine waste generated by the Indian population, in a useful way and could be commercialized.

KEY WORDS: Pink perch, proteinaceous product, *Pangasius hypophthalmus*, immune enhancer.

I. INTRODUCTION

Aquaculture allows for the cultivation of freshwater and saltwater fish population under controlled conditions. It has been steadily growing at a rate of 8% per year since 1980s (Food and Agriculture Organisation (FAO) of the United Nations). It is also the production sector for higher protein food. The growth of intensive aquaculture production has led to a growing interest in treating or preventing fish diseases. Protecting the fishes from disease can be done through two ways. One is by improving the immune power of the organization to fight the invasion of pathogens, and the second is through medication. Immune stimulants are now greatly used in aquaculture sector to reduce the mortality due to infections and to improve their general performance. Immune stimulants are compounds that activate the white blood cells (Leukocytes) and hence may render animals more resistant to infections to viruses, fungi, bacteria and parasites (Jan Raa, 2000). It is used in prior to the situations known to result in stress and also during developmental phases when animals are particularly susceptible to infectious agents. This project is to develop a feed from marine waste that may act as a possible immune enhancer in fishes and capable of increasing its resistance towards the adverse conditions.

As a result of heavy consumption of fish worldwide, a considerable amount of fish waste is produced every year. Fish food-processing operations produce wastes, Similar to most of the food industries both in solid (examples include scales, ssfins, tails, bones, skins, heads) as well as liquid form (Food and Agriculture Organisation (FAO) of the United Nations, 2011). It is estimated that fish processing wastes after filleting accounts for approximately 75% of the total fish weight. About 30% of the total fish weight remains as waste. Hence, there is an increasing need to address waste management of fish and fish products (Zynudheen, 2010). It is estimated that nearly 10 million tonnes of fish solid wastes are generated by Indian population every year. These waste compounds mostly include the bones, skins, heads and the scales. India being the habitat of large group of fin fish, considerable amount of fish scale wastes was produced

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per year (NSWAI, National Solid Waste Association of India). Among all the fin fish available, *Nemipterus japonicus* is the large populated and most commercially available fish in India. They are commonly called as Japanese threadfin bream, pink perch or sankara fish by Indian population. Instead of merely managing the issue of waste disposal, one promising approach is to convert these primary waste products into secondary useful products. It is to be noted that these wastes are eco-friendly recyclable compounds and excellent source of raw material for preparation of high value products including protein foods. Protein is the major component and a growth-limiting nutrient further than denote the most expensive ingredient in aqua feeds. Besides the price, availability of such high quality protein ingredients is also a problem for the feed producers. Thus the alternative protein sources of high nutritional quality have been on focus in the aquaculture sectors (Tacon 1998). Besides, fulfilling the nutritional requirements, it should also be easily digestible, under a cost-effective perspective (Francis 2001). Fish wastes can be effectively used to prepare these kinds of alternative protein rich feeds (Ghaly 2013). The fish scales are surprisingly a nutritional food source, containing a layer of proteins (mostly keratin, ichthylepidin and collagen). They are also rich sources of calcium phosphate (Janovetz 2005). The proteins present in the fish scales are generally bound with certain amount of carbohydrates (Kalyani, 2000). The proteins can be extracted from the fish scale wastes. Thus the protein feeds can also be prepared from the fish scale wastes.

In the present study, in order to isolate a novel immune stimulant for an efficient use along with the commercially available feed, we report a finest method for the isolation of immune stimulant from scale waste of *Nemipterus japonicus* to produce a protein rich food.

II. MATERIALS AND METHOD

II.1 Sample collection

Scales of fish pink perch (*Nemipterus japonicus*) were collected. The scales were washed well thrice with water and were subjected to shade dry for about a week. These dried fish scales were used for the extraction.

II.2 Extraction process

The extraction procedure generally involves the principle of pH-shift method. The dried fish scales were initially grinded well with 30% HCl of pH 1-2. After grinded well, they were subjected to the centrifugation process at 10,000 rpm for 15min. The supernatant obtained was then adjusted to the pH 9-10 by using 1N NaOH. The pH was again adjusted to neutral pH 7 using 0.1N HCl and again centrifuged at 10,000 rpm, 15min. The precipitated protein product was then collected and lyophilized.



FIGURE 1 DRIED FISH SCALES

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II.3 Protein Estimation

The protein content of the residue obtained was estimated by the method of **Lowryet al.**, (1951).

II.4 Feed Preparation

The diets were formulated to contain three different concentrations of extracted crude protein obtained. These proteins of different concentrations (5g, 10g and 15g) were mixed to dough form along with 5g of commercial feed. The dough was then kept in steaming for 20 min in a pressure cooker for proper conditioning . The pellets were prepared by hand pelletizer having 1.8mm diameter size . Finally, the pellets were air -dried for some time and kept in an oven at 50C till complete drying.

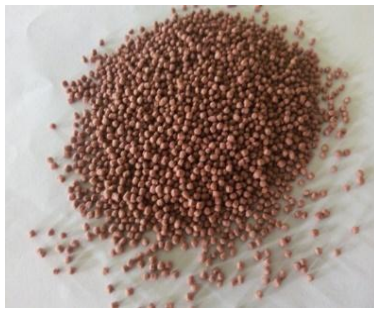


FIGURE 2.1

GROUP 1 FEED



FIGURE 2.2

GROUP 2 FEED



FIGURE 2.3

GROUP 3 FEED



FIGURE 2.4

GROUP 4 FEED

II.5 Experimental Design

The specimen fish (*Pangasius hypophthalmus*) of equal length and weight were collected and carefully transferred into the rectangular tanks. They were left for acclimatization for three days under proper aeration. The experimental design was entirely randomized with four different groups (Group:1- Normal feed, Group:2, Group:3, Group:4 in the ratio of 1:1,1:2,1:3 of normal feed and extracted product respectively) and three replications were performed. The fish were fed three times a day (10am, 1pm, 4pm), until apparent satiety during 45 days. The tanks were siphoned each day before feeding to remove faeces and uneaten feed.

II.6 Morphological Changes

After the feeding period of 45 days, the morphological changes of the fish in each group were recorded to study the significant changes in them. And the survival rates of the treated fish over the control fish were also noted visually.

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II.7 Statistical analysis

Experimental data were expressed as Mean \pm SD. Data within the groups are analyzed using one-way analysis of variants (ANOVA). Values of $p < 0.05$ were considered as statistically significant.

III. RESULTS AND DISSCUSION

The product extracted from the fish scales were quantified to contain crude protein (13.5mg) by using the Lowry method.

Growth of the fish fed with extracted feed showed an increased weight gain when compared to the control. Also, the treated groups exhibited a high survival period than the control fishes.

PARAMETERS	GROUP:1	GROUP:2	GROUP:3	GROUP:4	P VALUE
Initial Wt (g)	26.33 \pm 0.577	24.33 \pm 0.577	23 \pm 1.73	23.66 \pm 0.577	0.0045
Final Wt (g)	27.33 \pm 0.577	26.3 \pm 0.577	28.6 \pm 0.577	25 \pm 1	0.0045
Weight Gain (%)	3.79 \pm 0.08	8.22 \pm 0.19	25.03 \pm 8.14	5.61 \pm 2.35	0.0045

The values are expressed as Mean \pm S.D. Statistical significance * $P < 0.05$

TABLE 1: GROWTH OF FISH IN CONTROL AND TREATED GROUPS

The hematological parameter obtained from the blood samples proved that the feed admixed with extracted product proved an effective action on the fish immune system. Increase in the total WBC count, lymphocyte and monocytes confirmed the enhancement of immune power and their phagocytic activity. The other cells like eosinophils, basophils and neutrophils showed a decrease which stands as an evident to prove that the feed as no adverse effects.

CELLS	GROUP 1	GROUP 2	GROUP 3	GROUP 4	P VALUE
LYMPHOCYTE	48.66 \pm 0.577	50.66 \pm 0.577	51.66 \pm 0.577	46 \pm 1	0.0000
NEUTROPHILS	42.66 \pm 0.577	36.66 \pm 0.577	34.33 \pm 0.577	36.66 \pm 1.154	0.0000
EOSINOPHILS	5.33 \pm 0.577	3.33 \pm 0.577	3.66 \pm 0.577	4.66 \pm 0.577	0.0322
MONOCYTE	4.33 \pm 0.577	33.66 \pm 0.577	36.33 \pm 0.577	29 \pm 1	0.0000
BASOPHILS	3.66 \pm 0.577	2.66 \pm 0.577	1.33 \pm 0.577	2.33 \pm 0.577	0.0256
TOTAL WBC COUNT	11,100 \pm 173	16,250 \pm 250	21,367 \pm 251	12,000 \pm 100	0.0000

The values are expressed as Mean \pm S.D. Statistical significance * $P < 0.05$

TABLE 2: CELL COUNTS OF FISH IN CONTROL AND TREATED GROUPS

Though each treated groups (Group: 2, Group: 3, Group: 4) showed a positive result on comparison to the control

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(Group: 1). Group:3 is concluded to be most efficient concentration that can enhance the immunity of fish to a greater extent which was proved by growth and cell counts of treated fish groups when compared with other fish groups.

The present study exhibited the ability of the isolated product to alter the hematological parameters of the fishes and facilitated in activation of their defense mechanism against any adverse conditions. The extracted feed also decreased the mortality of the fish and proved considerable amount of weight gain, when compared to the control fish.

In conclusion, Immunostimulation is one key element in a strategy to achieve microbial control and seem to be the most fortunate method for preventing microbial diseases. Based on the present study, it is concluded that the formulated feed proves extremely beneficial to the fish feed industry to act as an effective agent to create an immune response in the fish. This study may also help in preventing the losses in aquaculture during both larval and on-growing stages. Also, in the management of enormous amount of fish wastes produced by the marine farming units and may pave way for the growth of aquaculture sector which is an emerging field.

IV. FUTURE RESEARCH

In the field of aquaculture, formulations of immunostimulants have a greater impact since it has a vital role in preventing the losses of the fish due to the adverse conditions and any pathogenic attacks. This project will also be extended to treat the fishes infected with the pathogens and too check the ability of the extracted novel product to act against those infections like ulcerative syndromes. The work will also be extended to study the several levels of immune response that are created in the fishes by the extracted product.

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