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Research Article

MICROBIAL SINGLE CELL PROTEIN (SCP) FOR THE GROWTH AND FOOD CONVERSION EFFICIENCY OF SELECTED ORNAMENTAL FISH, *XYPHOPHOROUS HELLERI*

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ABSTRACT

The objective of the present study is to evaluate the effect of substituting fish meal with single cellprotien (SCP) in artificial pellated diet for the selected fish species. The ornamental fishes such as *Carassius auratus, Cyprinus carpia, Helostoma temminckii, Catla catla,* and *Pecilia sphenops,* were selected to get the gut micro flora for scp. Fish feed was prepared with five different microbial cells with various origins. Furthermore, denitrogen fixing *Azotobacter* and antagonistic *Streptomyces* were selected from the medicinal plants on the rhizosphere samples. In order to understand the food conversion efficiency and food conversion ratio in the ornamental fish, *Xyphophorus helleri* fish feed were prepared with five different microbial cells and the control feed was without the microbial cells. The total protein concentration was estimated for all the feed systems by lowry's method The rate of feeding of six fishes in each trough varied on every day. The highest rate of feeding was noticed in feed 4 and minimum in feed 2 among the microbial feeds. Feed conversion ratio and conversion efficiency revealed wider variations. The microorganism is also called as bio proteins and they could synthesize growth honnrones and vitamins. From the present observations this may be the reason for better growth rate in fish feed on single cell protein diet and have the protective mechanisms against pathogenic infections. It is a promising area of research on Microbial Biotechnology and Aquaculture.

Keywords: Fish feed, Aquaculture, Probiotics, *Streptomyces*

INTRODUCTION

Aqua culturists, being the masters of ornamental fish culture, have ever been encountered with the matter of growth rate of fish. Aquaculture is the "Farming and husbandry of economically important aquatic animals and plants under controlled conditions". Aquaculture acts as an effective solution to the consistently increasing demand for pretentious food. In India, only 1.2 million tones of fish are at present harvested by capture fisheries per year against a total world harvest of 100 million tones. Asia and Far East countries are said to be the original homes of aquaculture. To reduce the pathogenic bacteria and maintaining the bacterial load for the improvement of quality of the water, now probiotic bacteria placing a big role in aquaculture. Much more than terrestrial animals, aquatic farmed animals are surrounded by an environment that support their pathogens independently of the host animals , and so opportunistic pathogens can reach high densities around the animals[1]. It is the multi dimensional aquatic media. Along with the benefits like socioeconomic development, the aquaculture technique is alleged to have created many problems such as change in quality of water and soil, and diseaseoutbreaks [2]. Disease exerts heavy economic losses in fish and shrimp culture due to mortality, morbidity, poor product quality and costs associated with chemotherapy. Aquaculturists are, therefore, interested in developing cost effective management strategies that would prevent the outbreak and / or reduce the severity of epizootics. In many aquaculture operations today, feeding and disease resistance cost accounts for more than one half of the variable operational cost. Therefore, knowledge on quality and health improving efficiency of food is essential for a successful aquaculture. Deficiency of nutrients may cease the development of different organs including lymphoid organs. The second factor is to consider the nutrients, those are essential for the cell division of the immune system and synthesis of molecules. The third category is to maintain proper level of nutrients in the body; above which it acts as a substrate for growth of pathogens (Iron) and below which deficiency systems (anemia) in host is observed. Chinese scholars had carried out few interesting research on the bacteria of probiotics for the improvement of shrimp culture water and gained very good results.

The composition of "Probiotics" are may be bacteria, Cyanobacteria, microalgae, fungi, etc. few Chinese scholars called it as 'normal microbiota" or "effective microbiota"; it containes photosynthetic bacteria, *Lactobacillus*, *Actinomycetes,* Nitrobacteria, Denitrifying bacteria, *Bifidobacterium*, yeast etc. The emphasis in disease management should be on prevention, which is likely to be more cost-effective than cure So far, the conventional approaches, such as the use of disinfectants and antimicrobial drugs, have had limited success in the prevention or cure of aquatic diseases[3]. Which leads to poor confidence for the use of chemicals (antimicrobials, disinfectants and pesticides), it is used to treat the symptoms of the condition and not the root cause. In agriculture the value of probiotics notably Gram positive bacteria such as *Lactobacillus,* has come to be appreciated as an alternative to antibiotics in disease control strategies.

Use of microbes as food source may appear to be unacceptable to some people but the idea of consumption of microbes as food for man and animals is certainly innovative to solve the global food problem [4,5]. Algae, fungi and bacteria are the chief of microbial protein (Single Cell Protein- SCP) that can be utilized as a protein supplement. The production of such fish feed which can enhance a faster growth of fish became the substantial focus in the area of research on ornamental fish culture. There comes the prioritization of incorporation of Single Cell Protein (SCP) in aquaculture fish feed as a source of protein. The microbial SCP, especially from *Streptomyces* has a promising future in fish nutrition, which can replace the fish meal to a possible extent in formulated diets [6,7,8]. It is well accepted that microorganisms cannot be avoided in aquaculture operations. Using the probiotics with various innovative techniques the aquaculture operation can be managed successfully, even though the aquatic research has gained the momentum but, it has not reached to the level available in land animals. The application of probiotics for fish and shrimp, either as a bio control or as a bio remedial measure shows promise, but need more studies and analysis for the better understanding of probiotics and its use in aquaculture. It is very likely that non-pathogenic vibrios hold the key in isolating and developing a successful probiont for use in aquaculture. The present study aimed at the evaluation of the effect of substituting fish meal with Single Cell Protein (SCP) in artificial pelleted diet for the selected fish.

MATERIALS AND METHODS

The substituting substances included were 5 different strains of bacterial and *Streptomyces.* Four selected fresh water Ornamental fish, (1). Koi carp (*Cyprinus carpio,* Family: Cyprinidae), (2). Gold fish (*Carassius auratus,* Family: Cyprinidae), (3). Black molly

(*Poecilia sphenops,* Family: Cyprinidae) and (4). *Catla catla,* (Family: Cyprinidae).

Estimation of Microbial population:

To isolate bacteria and *Streptomyces* spp. population from the guts of Ornamental fishes, guts were removed from the above selected fishes and treated with phenol for 10mins. to get *Streptomycess* spp. and other gut flora. Phenol treated guts were then washed with distilled water and were homogenized with 9ml blank (10^{-1}). This was aseptically transferred to the dilution level of (10^{-2} to 10^{-5}). 1ml from 10^{-2} was aseptically transferred to each sterile Petri plate. About 15-20 ml of sterilized media (Nutrient Agar media, Glycerol Asparagine agar media, Actinimycetes Agar media and Potato dextrose Agar media) were aseptically transferred to each Petri plates respectively. The Petri plates were rotated clockwise and anticlockwise for uniform mixing of the sample and the medium. The Petri plates were incubated at room temperature ($28+2^0$C) till the appearance of colony forming units (7-10 days) [9, 10].

Enumeration:

The total number of bacterial and *Streptomyces* colonies in each Petri plate dish was counted and noted. The dry weight of the guts sample were also noted.

Isolation and maintenance of bacteria and *Streptomyces* spp. culture:

Streptomyces colonies appeared in media were isolated at random and subcultured on respective Medias. The stock cultures were periodically subcultured and stored at 4^0C for further studies.

Identification:

Gram staining was carried out for the randomly subcultured bacteria. In this Gram staining technique, a thin heat fixed smear of bacteria was made on a clean glass slide. To this, a few drops of crystal violet staining agent was added and left for one minute. The slide was washed in tap water. The smear was flooded with a few drops of Gram's Iodine and left for one minute. The smear was washed gently in tap water and then decolonized with 95% of ethanol. The slide was washed with running tap water and counter stain was added and observed under microscope.

Carbon sources:

Utilization and colouration expression of 7 different carbon sources were used, which includes glucose, lactose, mannitol, xylose, sucrose, rhamnose and maltose.

Feed Preparation:

Six types of feed were prepared according to the square method of[11] with the feed ingredients in F1, F2, F3, F4, F5 and C. The C is the control feed contains Bengal gram, Fish meal, Coconut oil cake, Tapioca. The F1 feed contains Bengal gram, Coconut oil cake, Tapioca and bacterial cells. The F2 feed contains Bengal gram, Coconut oil cake, Tapioca and bacterial cells. The F3 feed contains the Bengal gram, Coconut oil cake, Tapioca and *Azotobacter* cells. The F4 feed contains the Bengal gram, Coconut oil cake, Tapioca and *Streptomyces A*. The F5 feed contains the Bengal gram, Coconut oil cake, Tapioca and *Streptomyces B*. The dough was obtained with smooth appearance by grinding the ingredients to fine powder form and mixing completely with enough water. It was cooked with steam for 30 minutes and kept aside to cool and passed through pellatizer and then the wet pellets were air dried and packed in air tight container and stored in dry place at room temperature.

RESULTS

Supplementary feeding is a major expense in aquaculture operation. The commonly used conventional fed in India is a mixture of rice bran and oil cake in equal proportions. But this feed is nutritionally imbalanced to achieve the fast growth of the fish. In the present study six different diets including one control diet and five SCP diets were used for the growth of *Xhiphophorus helleri*. Table: 1 showed the protein content of different ingredients used for preparing the control and experimental diets. Ingredients used for the control diet was fish meal, groundnut, oil cake, Bengal gram, tapioca flour for the experimental diets, ingredients of control diet except fishmeal and five different types of bacteria, Azotobacter, *Streptomyces* were used.

Table: 2 showed the ingredient composition of six different diets. Here C is the control and F1 to F5 are the SCP incorporated (Single Cell Protein) diets. Diets F1 and F2 contains bacterial cells. Diet F3 contains Azotobacter, diet F4 and F5 are the *Streptomyces* spp. from different origin respectively.

Table 1: Protein Content Of Different Ingredients Used For Preparing Artificial Diets

Ingredients	Protein concentration (mg)
C-fish meal	30
F1-bacterial cells- a (*Micrococcus*)	15.88
F2-bacterial cells- b (*Bacillus)*	33.52
F3-*Azotobacter*	28.23
F4-*Streptomyces*-a (sasscbt-1)	26.47
F5-*Streptomyces*-b (sasscbt-2)	19.41

Table 2: Ingredient Composition of Diets Containing Single Cell Protein

Contents	Control	F1	F2	F3	F4	F5
Fish meal (g)	35	-	-	-	-	-
Coconut oil cake (g)	25	25	25	25	25	25
Topioca (g)	15	15	15	15	15	15
Bengal gram (g)	25	25	25	25	25	25
Bacteria (g)	-	0.77	-	-	-	-
Bacteria	-		0.44			
Streptomyces-a	-	-	-	4.18	-	-
Treptomyces-b	-	-	-	-	1.70	-
Azotobacter	-	-	-	-	-	1.55

F1, F2, F3, F4 and F5 are represents feed

Table: 3 showed the results of weight gain, food conversion efficiency and food conversion ratio of *Xhiphophorus helleri*, with six different diets. Of the six diets maximum growth was observed in the F1 and F2 diets. Table: 4 and Fig. 1&2 showed the result of variance comparing the initial weight and the final weight of control and experimental groups of the fish, *Xhiphophorus helleri.* It is clearly indicated that the test diets showed significantly better growth over the control diet. Table: 5 showed the rate of feeding by fish in various tanks. The obtained values are consumed diets by the fish in all the six tanks.

Table 3: Weight Gain, Food Conversion Efficiency and Food Conversion Ratio Of *Xyphophorus Helleri* Fed With Different Single Cell Protein Diets

Parameters	Diets					
	C	F1	F2	F3	F4	F5
Initial weight(g)	7.5	8	9.5	6	7.5	8.5
Final weight (g)	15.50	18.35	19.58	15.55	17.35	18.16
Feed given(g)	36	36	36	36	36	36
Unfeded (g)	12.07	17.04	15.08	14.08	15.15	15.07
Feed intake(g)	24.56	19.51	21.28	21.92	20.85	20.93
Food conversion efficiency (g)	32.57	53.04	47.36	43.56	47.24	46.15
Food conversion ratio (g)	2.10	2.8	1.37	2.29	2.06	1.57

Table 4: Initial and Final Weight of The Fed Fish *Xyphophorus helleri.*

Name of the fish tanks	Total no of fish fingerlings per tank	Initial weight of the fish (g)	Final weight of the fish (g)
C	6	7.5	15.50
F1	6	8.0	18.35
F2	6	9.5	19.58
F3	6	6.0	15.55
F4	6	7.5	17.35
F5	6	8.5	18.16

Table 5: Rate of Feeding In Varying Dates

Dates	Experimental tanks and feeds					
	Control (g)	F1 (g)	F2 (g)	F3 (g)	F4 (g)	F5 (g)
17.3.11	1.50	0.78	1.08	1.32	0.95	0.60
18.3.11	1.46	1.03	1.12	1.35	0.90	0.73
19,20.3.11	1.50	0.75	2.22	1.45	0.85	2.65
21.3.11	1.50	0.97	0.85	1.50	1.05	0.72
22.3.11	1.75	1.50	1.55	0.75	1.05	1.14
23.3.11	1.71	1.40	1.49	1.59	1.58	1.43
24.3.11	1.61	1.42	1.45	1.52	1.25	1.05
25.3.11	1.21	1.01	0.90	0.85	1.15	1.21
26,27.3.11	1.45	1.50	2.5	1.90	2.15	2.45
28.3.11	1.61	1.05	1.19	1.15	1.04	0.90
29.3.11	1.25	0.95	0.85	1.01	1.15	1.45
30.3.11	1.71	1.51	1.21	1.45	1.65	1.55
31.3.11	1.69	1.32	1.05	1.51	1.48	1.71
1.4.11	1.74	1.23	1.12	1.41	1.58	0.90
2.4.11	1.45	1.39	1.22	1.64	1.51	1.02
3.4.11	1.42	1.60	1.48	1.52	1.61	1.42

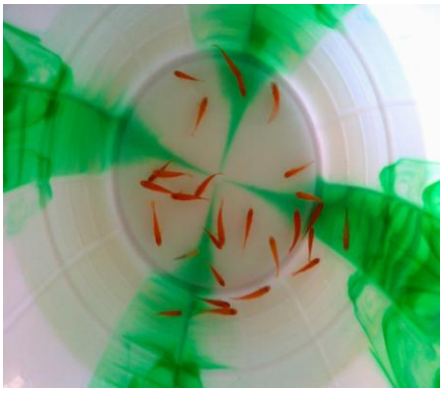

Fig. 1: Experimental Fishes

Fig. 2: Fishes Intaking Artificial Feed

DISCUSSION

Fish meal is being used extensively in aquaculture as major source of protein in formulated feeds. Because of increased cost and scarce availability, research efforts to replace fish meal partially or completely with Single Cell Protein source to bring down the cost of the feed are going on all over the world. In the present study our findings corroborated with the findings of in which the bacterial SCP as the incorporated protein[12, 13]. Moreover, Anithakumari (2000) also reported that the Single Cell Protein such as nutritional grouping bacteria and *Streptomyces* helped to replace fish meal to a certain extent in the diet of *Puntius vitatus*[14].

According to Mithun (2005) SCP diet exhibited a remarkable increase in growth and better conversion ration ratio as compared to the other diets. Here it is very clear that microbial SCP can serve to supply essential proteins to increase the survival and growth of *Xiphophorus maculates* [15]. Jagadambika Devi (2005) used SCP

individually and in mixture for replacing 30% of the fish meal in compounded diets of Barbus Schwancfeldi. Here also maximum in terms of weight was obtained in fish feed with diet containing dried SCP[16].

The protein especially in fish meal act as feed for aquaculture systems which is highly cost and is mandatory ingredient. Since the supply of fish meal has become uncertain, it is of great importance to replace the fish meal to a minimum possible extent in fish rations. Among unconventional protein sources, Single Cell Protein (SCP) of microbial origin appears to be a promising substitute for fish meal, which can replace up to 25 – 50% fish meal.

The potential of bacteria and *Streptomyces* as a probiotic feed for the growth of the Ornamental fish was investigated. Moreover carotenoides are the pigments which are the most important quality criteria determining the market value of fishes. Thousands of bacteria and *Streptomyces* are screened annually by pharmaceutical companies as potential sources as novel chemical compounds. Quite surprisingly *Streptomyces* associated with marine sponges produced potent antagonistic substance against human as well as fish and shell fish pathogen[17]. Similarly, marine ornamental fishes harbored. *Streptomyces* exhibiting antagonistic property against human and fish pathogen [18]. It is a well known fact that marine origins *Streptomyces* and bacteria are rich resources of variety of biotic compounds.

In culturing ornamental fish in captivity, nothing is more important than sound nutrient and sufficient feeding. Protein is the most important components of the diet of fish because protein in take generally determines the growth and has high cost per unit and high levels are required per unit of feeds. Quality criteria for the ingredients must be respected to insure that the final product is of consistent quality and the deleterious effects are avoided

Hence the micro organisms are rich in protein and hence they are also called bioprotein. They are able to synthesis the low molecular weight precursors of cell macromolecules and vitamins. More over they could also synthesize the growth hormones such as Indole Acetic Acid as a growth promoter [19, 20, 21]. From the present observations this may be the reason for better growth rate recorded in the fresh water fish [22] selected fed on Single Cell Protein (SCP) diets. It is a promising area of research on Single Cell Protein for the development of Microbial Biotechnology and Aquaculture Industry in the years to come.

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