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EFFICACY OF PROBIOTIC SUPPLEMENTATION ON GROWTH AND BIOCHEMICAL CHANGES IN ZEBRAFISH (*Danio rerio*)

Dr. S. SIVASURIYAN AND R. VIGNESH

P.G. and Research Department of Zoology, Rajah Serfoji Govt. College, (Autonomous) (Affiliated to Bharathidasan University), Thanjavur -613 007, Tamil Nadu, India

ABSTRACT

In the present study was to investigate the efficacy of BIFILAC probiotic on growth and biochemical changes in zebrafish (*Danio rerio*). The % of survival and growth performance were increased directly proportion to the duration of probiotic supplementation. The highest % survival and growth performance were observed in 30 days supplementation of probiotic. The biochemical analysis zebrafish indicated that probiotic diets results in significant increase in the level of carbohydrate, protein, amino acid and lipids. The hematological profile of zebrafish indicated that probiotic diets results in significant increase in the level of Hb, WBC and RBC.

Keywords: BIFILAC, *Danio rerio*, Survival and Growth performance, Biochemical analysis

INTRODUCTION

India is the third largest producer of fish in the world next only to China and Peru and it ranks second in the production of Inland fishes. Fish is very important dietary animal protein source in human nutrition. Production of aquatic species through freshwater fisheries and aquaculture for protein supply is being encouraged in developed or developing countries. According to nutritionists, fish is an excellent substitute for red meat and an excellent source of protein. Fish flesh contains all the essential amino acid and minerals viz., iodine, phosphorus, potassium, iron, copper and vitamin A and D in desirable concentrations. It serves as valuable ingredient to a healthy diet because of its low carbohydrate and unsaturated fat contents. So the inclusion of fish in our diet can make a valuable contribution to any diet that contain mainly of cereals, starchy roots and sugar for the healthy growth (FAO, 2018). In the present study to investigate the efficacy of BIFILAC probiotic on growth and biochemical changes in zebrafish (*Danio rerio*)

MATERIALS AND METHODS

Collections and Acclimatization of Experimental Animal

Zebrafish (*Danio rerio*) (Average weight 0.45 ± 0.03g) were procured from fish aquarium, Thanjavur, Thanjavur District, Tamil Nadu, India, using cast net and maintained in the laboratory in a glass aquarium tank and acclimated in aerated tap water with continuous aeration for two weeks prior to experimentation. During this period, fishes were fed with a known amount of fish food.

Experimental design

Group I : Control diet

Group II: BIFILAC probiotic supplementation

The fingerlings were fed 3% of their body weight twice a day for 30 days. Every third days, tanks were partially cleaned and water was partially changed. The temperature averaged 28±1.5°C, dissolved oxygen 7.4±0.6

mg/l, and total ammonia 0.5±0.2 mg/l. Fingerlings were weighed at 30 days intervals to determine weight gain, specific growth rate (SGR) and feed conservation ratio (FCR), survival. At the end of the experiments, the body homogenate of *D. rerio* fingerlings were carried out for biochemical analysis. The fishes are grouped into seven including control fish.

Growth Parameters

The growth parameters of *D. rerio* fingerlings were assessed by taking their body weight and length every 10 days for 30 days. The growth performance was assessed using the following formulas.

Survival percentage

Survival percentage was calculated at the end of the experiment by counting the number of fishes in each tub and is calculated as follows:

$$\text{Survival (\%)} = \frac{\text{Total number of animal harvested}}{\text{Total number stocked}} \times 100.$$

Haematological analysis

Haemoglobin, WBC and RBC were estimated by Cyanmethemoglobin method (Dacie and Lewis, 1968). Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust (1978). Protein was estimated by the method of Lowry *et al*. (1951). Total lipids in tissues were estimated by the method of Folch *et al* (1957). To estimate the amount of carbohydrate present in the given sample by using Anthrone method

Statistical analysis

Values are expressed as Mean ± SD for 10 fish. Data was calculated by student t-Test (Independent sample, P value two tail) using MS-excel ver. 2013. Statistically significant level 0.05.

RESULTS AND DISCUSSION

Zebrafish (*Danio rerio*) is a prominent model organism in biological researches in recent times. Zebrafish is a tropical freshwater fish, inhabitant of rivers (Ganges mainly) of Himalayan region of South Asia especially India, Nepal, Bhutan, Pakistan, Bangladesh, and Myanmar. It is a bony fish (teleost) that belongs to the family Cyprinidae under the class Actinopterygii (ray-finned fishes). Zebrafish was first used as a biological model by George Streisinger (University of Oregon) in the 1970s because it was simpler over mouse and easy to manipulate genetically. Streisinger's colleagues especially Chuck Kimmel in his university got much impressed by the idea of using zebrafish.

Global fishery production has been reported to be 2016 reached an all-time high of 171 million tonnes, of which 88 percent was utilized for direct human consumption, thanks to relatively stable capture fisheries production, reduced wastage and continued aquaculture growth. This production resulted in a record-high per capita consumption of 20.3 kg in 2016. Since 1961 the annual global growth in fish consumption has been twice as high as population growth, demonstrating that the fisheries sector is crucial in meeting FAO's goal of a world without hunger and malnutrition (FAO, 2018).

It has been estimated that the total fish production will be 53.64 million metric tonnes in 2030, based on annual growth rate. In contrast to world capture fisheries, which have almost stopped growing since the mid - 1980s, the aquaculture sector maintains an average annual growth rate of 8.30 % worldwide. In aquaculture the contribution of inland fishery production is 4.66 metric tonnes of which almost 90 % is contributed from freshwater aquaculture. India now ranks second and third in world fishery production and freshwater aquaculture respectively (Umaa Rani *et al.*, 2014).

Fish require adequate nutrition in order to grow and survive. Nature offers a great diversity of food to fish including plants and animals. Artificial feed plays an important role in semi intensive fish culture where it is required to maintain a high density of fish than the natural fertility of the water can support (Jhingran, 1991). The role of artificial feed in intensive fish farming cannot be ignored as nutritional requirements of fish depend upon the feed supplied. The quantity and quality of feed consumed have a pronounced effect on growth rate, efficiency of feed conversion and chemical composition of fish (Hassan *et al.*, 1996; Jena *et al.*, 1998).

Carp farming has attained commercial culture status in India and many of its neighnouring countries. With the intensification of culture, feed has become the most important component of the culture system from the viewpoint of both fish production and cost. Fish-meal-based diets generally induce good growth. However, owing to the scarcity and escalating cost of fish meal, research on alternative sources is gaining importance (Shetty and Nandeesha, 1988).

The term "probiotic" which literally means "for life" has since been employed to describe these health-promoting bacteria. The World Health Organization has defined probiotic bacteria as "live microorganisms which when administrated in adequate amounts confer a health benefits"

(FAO/WHO, 2001). Probiotics are beneficially affect the host by improving its intestinal microbial balance, are quickly gaining interest as functional foods in the current era of self-care and complementary medicine. The use of probiotics in the culture of aquatic organisms is increasing with demand for more environment friendly aquaculture practices (Gatesoupe, 1999). Use of probionts has been proposed as a measure to maintain healthy environment in aquaculture and to prevent occurrence of disease (Lipton, 1998). The microorganisms used as probiotics, including Lactobacillus, Bacillius and yeasts have been reported in penaeids and fish (Boonthai *et al*., 2011). The aim of the present study was to evaluate the effects of BIFILAC probiotics fed on survival, growth and biochemical performance of zebrafish.

Growth performance of Zebrafish

The growth performance of Zebrafish was investigated. Supplementation of BIFILAC probiotics diet was fed to Zebrafish for different durations (10, 20 and 30 days) were monitored and represent in table 1. In the present study the body weight, length was directly proportional to the duration of the exposure and stabilize the survival rate on supplementation. Among the different days, 30 days supplementation possess better growth and length were observed.

Table 1 Effect of BIFILAC probiotic supplementation on body weight (gm), length (cm) and survival of Zebrafish.

Group (Types of Feed)	Body weight (gm)	Length (cm)	Survival (%)
0 Day	0.45	1.80	-
10 Days	0.58	2.20	100
20 Days	0.69	2.80	100
30 days	0.86	3.10	100

The experiment was conducted near natural conditions, in outdoor facility using rectangular glass tanks well equipped with aerators. Both control and experimental groups were reared under similar condition and get similar feed except the presence of probiotic in the rearing water of experimental group. Thus, higher survival may be due to the supplementation of bacterial and spirulina probiotics,

The present investigation is aimed to study in the BIFILAC probiotic performance in the zebrafish is the most important fishes under the laboratory condition. Supplementation of bacterial and spirulina probiotic at the concentrations of were fed to zebrafish fish

Survival and growth of the many fishes are known to be influenced by the availability of right type of food in right concentration. Results of this study substantiate the fact that combined diets have direct growth promoting effects on zebrafish which is accordance with the reports. The significant growth performance of BIFILAC probiotics might be due to the nutrition of BIFILAC for long duration.

Artificial feed plays an important role in semi intensive fish culture where it is required to maintain a high density of fish than the natural fertility of the water can support. In the present results the final average body weight, body gain and total length of zebrafish showed a highly significant difference for the different treatments. Statistical analysis showed the significant variation among the treatments for zebrafish during the experimental period that differ from each other. Zebrafish, comparison of mean values of average body weight, body gain and total length in different treatments, showed that it appeared to attain maximum weight gain and total length under the influence of BIFILAC probiotic. Such increase in the growth of aquatic animals fed with probiotic may be attributed to the improved digestive activity by enhancing the synthesis of vitamins, enzymatic activity, with a consequent improvement of the digestibility and weight gain. While comparing overall performance on the basis of mean values, it can be concluded that this fish species gave its best performance in terms of increase in body weight, body gain and total length in the treatment of spirulina when compared to control and other diet. However the minimum increase in body weight and total length, survival were observed in BIFILAC probiotic when compared to control.

Proximate composition of Zebrafish

The proximate composition of *D. rerio* fingerlings with different duration of feeding regimes were investigated. Supplementation of probiotic diet different durations (10, 20

and 30 days) were monitored in *D. rerio* fish. Supplementation of BIFILAC probiotics diet was fed to Zebrafish for different durations (10, 20 and 30 days) were monitored and represent in table 2. The increased carbohydrate, protein, amino acid and lipid content directly proportional to the duration. . Among the different days, 30 days supplementation possess better proximate composition were observed.

Table 2 Effect of BIFILAC probiotic supplementation on carbohydrate, protein, amino acid and lipids in Zebrafish.

Group (Types of Feed)	Carbohydrate (mg/gm)	Protein (mg/gm)	Amino acids (mg/gm)	Lipids (mg/gm)
Control	391.10±20.36	2.74±1.03	21.66±2.88	0.01±0.01
10 Days	421.00±13.87	3.21±0.38	26.45±1.65	0.02±0.02
20 Days	481.00±16.54	3.84±0.48	32.75±1.89	0.03±0.003
30 days	520.00±18.78	4.10±0.52	39.16±2.10	0.04±0.004

Values are expressed as Mean ± SD for triplicates

The biochemical composition of a particular living system in the level of organic compounds like proteins, lipids, carbohydrates, amino acids and importantly nucleic acids which act as source of energy for various physiological functions. The different tissues and organs in an animal are structurally and functionally designed to carry out different physiological processes, it is possible that they have different organic compositions.

Proximate body composition is the analysis of carbohydrates, proteins and lipids contents of fish. The results on biochemical composition, such as protein, carbohydrate and lipids content of bacterial probiotic and spirulina supplementation fed were recorded. After the feeding trail experiment of every 30 days for three months, the total protein, carbohydrate and lipids content were found to be maximum in spirulina diet fed with 3 % diet, followed by bacterial probiotic a fed when compared with control. The statistical analysis made on the biochemical constituents between control and experimental diets revealed that the variation between them was significant.

Biochemical studies are very important from the nutritional point of view. Protein is essential for the sustenance of life and accordingly exists in the largest quantity of all nutrients as a component of the human body (Sudhakar *et al*., 2011). In various fish species, proteins are of important as structural compounds, biocatalysts and hormones for control of growth and differentiations (Amal and Naheb, 2012). Protein in fish is a main component constituent of tissue and organs. They are precursors of other nitrogen compounds (enzymes, hormones, slurry, neurotransmitters, cofactors, etc) and constitute an important energy source. The effect of dietary protein levels on fish growth performance varies considerably within species, size, age, diet and composition, range of proteins level tested and rearing conditions (Arredondo *et al.*, 2012). Inadequate protein levels in the diets result in a reduction of growth and loss of weight. However, when an excess of protein is supplied in the diet, only part of it is used for protein synthesis (growth) and the remaining is transformed into energy (Arredondo *et al.*, 2012). Each body cell is composed mainly of protein. Protein makes up the membrane surrounding the cell and also occurs within the cell. Protein plays a vital role in the formation of enzymes, antibodies and hormones and other substances that regulate the body process. The study revealed that high protein content were observed in soyabean supplemented fish than other diet. The high content of protein is due to rich source of protein content in soyabean. Results of the present study were agreement with earlier reports (Storebakken, 2000).

Components like carbohydrate play a vital role as energy precursors for fish under stress conditions (Umminger, 1970). Glucose is a carbohydrate that has a major role in the bioenergetics of animals, being transformed to chemical energy (ATP), which in turn can be expressed as mechanical energy (Lucas, 1996). Changes in carbohydrate metabolism measured as plasma glucose (energy substrate whose production is thought to metabolically assist the animal to cope with an increased energy demand caused by stress) used as general stress indicators in fish (Teles *et al.*, 2007). Glucose (or glucose 6-phosphate) is

released through the degradation of glycogen by glycogen phosphorylase (GP) (Roach *et al*., 1998), and energy is mainly supplied by the oxidation of glucose and lactate as a result of carbohydrate metabolism (Morgan *et al*., 1997). The glucose concentration was proposed to be mediated by endocrine release such as cortisol (Hontela *et al*., 1996). Silbergeld (1974) stated that assay of this important parameter can serve as an indicator of environmental stress. In the present study revealed that increase carbohydrate content were observed in combined diet meal fish than other diet. The increased content of carbohydrate is due to source of carbohydrate content in soyabean and spirulina. Results of the present study were agreement with earlier reports (Gumus and Ikiz, 2009).

The effect of carbohydrate and lipid levels on body composition (lipid, protein, ash and water) of fish has been investigated by a number of researchers (Kaushik and Oliva-Teles, 1985). The lipid composition of the fish body was influenced by the dietary carbohydrate and lipid levels. The lipid content of muscle was higher in fish fed with the high lipid level diet. These increments of body lipid by increasing dietary lipid level have been reported in previous studies (Kaushik and Oliva-Teles, 1985). A similar relationship between dietary carbohydrate level and whole body lipid content exists in 90 days as compared with 30 and 60 days supplementation. The increase in dietary carbohydrates in *D. rerio* results in an increases in body lipid content (Hilton and Atkinson, 1982). This effect can be due to the excesses carbohydrate of fish converted into lipids, resulting in increased weight achieved with these diets. Dietary lipid level plays an important role in influencing growth rate and muscle composition of this species

Lipids and fatty acids play a significant role in membrane and have a direct impact on membrane mediated process such as osmoregulation, nutrient assimilation and transport. On the other hand, the nature and quantity of these lipids in fish vary according to species and habit. Previous studies. (Kumaran *et al.*, 2012) correlate with our present investigation pertaining to lipid observations. Supplementation of BIFILAC probiotics in diet enhance the proximate composition compared to control.

Hematological analysis

The hematological profile of *D. rerio* fingerlings with different duration of feeding regimes were investigated. Supplementation of probiotic diet different durations (10, 20 and 30 days) were monitored in *D. rerio* fish. Supplementation of BIFILAC probiotics diet was fed to Zebrafish for different durations (10, 20 and 30 days) were monitored and represent in table 1. The increased Hb, WBC and RBC content directly proportional to the duration. Among the different days, 30 days supplementation possess better proximate composition were observed.

Table 1 Effect of BIFILAC probiotic supplementation on Hb, WBC and RBC in Zebrafish.

Group (Types of Feed)	Hb (gm/dl)	WBC (mm cc) x 10^6	RBC (mm. cc) x 10^3
Control	0.85±0.52	2.4±0.05	7.33±0.08
10 Days	1.25±0.71	2.8±0.07	7.79±0.06
20 Days	1.75±0.65	3.2±0.04	8.16±0.07
30 days	1.96±0.81	3.6±0.06	8.38±0.05

Values are expressed as Mean ± SD for triplicates

Conclusion

Hematologic evaluation is one of the diagnostic tool for identifying diseases. Normal variation from intrinsic or extrinsic factors or diseases affecting blood cells and counts may be evaluated by clinical hematology. Most of the fish have nucleated erythrocytes which play an important role in oxygen transport, which depends on the amount of hemoglobin concentration within the cell and the gas-exchange mechanism. Studies show that incorporation of probiotics stimulates the hemopoesis and also induces the nonspecific immunity in fish. Similarly, the RBC content of the probiotic supplemented fish groups was found to be higher than its non-supplemented counterparts.

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