Impact of Spirulina Supplementation on Biochemical Changes in Fingerlings of *Labeo rohita* (Rohu Fish)

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ABSTRACT

Food is a major requirement for all living organisms including fish for reproduction, growth and maintenance. Feeding management plays a critical role in the success of fish culture. The current trend in fish culture is towards increased intensification whereby, provision of feeds becomes necessary and success depends significantly on the availability of well-balanced nutritionally complete and cost effective compounded feeds The aim of the study was to evaluate the effects of Spirulina on Growth performance, Biochemical and Haematological of common carp as Labeo rohita. The present study demonstrated that the fingerlings of Labeo rohita fed on 1% of Spirulina diet of each, it is clear that incorporation of Labeo rohita considerably enhanced the growth, biological and hematological performance of fish as compared with that of control diet. The potential health benefits of Spirulina diet may be due to the micro and macro nutritional compositions as compared to other diet supplementations. So the present study recommends the use of Spirulina and in fish feed in modest amount as it enhances the feed quality without much affecting the cost factor.

KEYWORDS: *Labeo rohita, Spirulina, Biochemical and Hematology*

INTRODUCTION

Food is a major requirement for all living organisms including fish for reproduction, growth and maintenance. Feeding management plays a critical role in the success of fish culture. The current trend in fish culture is towards increased intensification whereby, provision of feeds becomes necessary and success depends significantly on the availability of well-balanced nutritionally complete and cost effective compounded feeds. In fish culture systems, the importance of artificial feed supplementation cannot be over emphasized. Since feed is the main variable cost in culture fish production, precise information on the nutritional requirement is necessary in order to formulate and produce economical and nutritionally balanced and complete diet tailored towards the needs of the fish (Al Ogaily *et al*, 1996).

Carp farming is the backbone of Indian aquaculture contributing more than 85% of the total production. In recent years, technological advancement and modification of supplementation of feed, culture practice from traditional to semi-intensive and intensive system has led to various environmental related stressors in the culture species (Eddy and Williams, 1987). The Indian major carps, *catla*, rohu, mrigal and kalbasu are fast growing and highly preferred food fishes in India. These carps have also gained popularity in other Southeast Asian countries. The Indian major carps *Catla catla, Labeo rohita and Cirrhinus mrigala* are the most important commercial fishes in India with a maximum market demand and acceptability as food by the consumers due to their taste and flesh. They contribute about 67% of total freshwater fish production (ICLARM, 2001). In India, the aquaculture practices mainly revolve around a few species of finfish and shellfish, among which the Indian Major Carp's viz. *Catla catla* and *Labeo rohita* contribute substantially to the inland production. Although carp culture is widely practiced, the non- availability of appropriate compounded feed to meet the demands of the species still remains as a major constraint.

Fish require adequate nutrition in order to grow and survive. Survival and growth of the fish was depending on the feed and fertilizer in cultural practices. 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. 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 biochemical composition of fish. Development of aquaculture will be greatly enhanced by finding alternative and less expensive ingredients. Therefore the present study was focused to evaluate the effects of *Spirulina* on Growth performance, Biochemical changes of common carp as *Labeo rohita.*

MATERIALS AND METHODS

Preparation of Spirulina extract:

2 gram of the powder of *Spirulina* were transferred in to conical flask (250ml). The conical flask containing 50ml of water. The conical flask containing Spirulina were shake it well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using whatman filter paper No.1 and filtrate used for further analysis.

Phytochemical screening:

Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973 and 1984).

Collection and acclimation of experimental fishes:

Fingerlings of *Labeo rohita* (average weight 8.43 ± 1.59g) were procured from Fish farm, Thitta, 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.

Distribution of Indian Major carps:

The Indian major carps Rohu (*Labeo rohita*) which are also known as the gangetic carps are the natural inhabitants of the Ganga river network, namely the Ganga, the Gomati, the Yamuna and the Brahmaputra and the Indus river system in North India. The Ganga river system running to a total length of about 8,047 km, besides the major carps, harbors the richest freshwater fauna of India, ranging from mahaseers and the torrential fishes of hills to a wide array of other fishes of great commercial value.

Preparation of Diet:

The Spirulina was obtained from PARRY Nutraceuticals Division of EID Parry (India) Ltd. at Pannangudi, Pudukkottai Dist. Tamilnadu, India. The fingerlings were fed 1% of their body weight twice a day for 30 days. Every ten 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

Sampling of the fish:

Fishes were sampled once a month for six months using drags net. Length and weight of ach species were measured separately to assess the health of condition of fish and their growth. The length (mm) and weight (g) of individual of fish were recorded separately on treatment wise with the help of measuring scale and portable sensitive balance. Fishes were captured randomly from each experimental treatment and these fishes were used for hematological and serological analysis.

Growth Parameters:

The growth parameters of the Labeo rohita fingerlings were assessed by taking their body weight at every 10 days.

Preparation of homogenate:

The 1g tissues was weighed and homogenized using a Teflon homogenizer. Tissue homogenate was prepared in 0.1 M Tris Hcl buffer (pH 7.4) and used for the estimation of various biochemical parameters.

Biochemical estimations:

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. Amino acid in tissues were estimated by the method of Rosen (1957).

Statistical Analysis:

Values were expressed as mean ± SD for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey's test for multiple comparisons. The results were statistically analyzed by Graphpad Instat Software (Graphpad Software, San Diego, CA, USA) version 3 was used and $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Phytochemicals analysis:

In the present study was carried out on the *Spirulina* revealed the presence of medicinally active constituents. The phytochemical characters of the *Spirulina* investigated and summarized in Table 1. The phytochemical screening *Spirulina* showed that the presence of tannin, saponins, flavonoids, terpenoids, triterpenoids, steroids, polyphenol, alkaloids, protein, carbohydrate, anthroquinones and glycoside present where phlobatannin was absent in aqueous extract.

Table 1. Phytochemicals qualitative analysis of *Spirulina*

S.No	Test analysis	Aqueous extract
1	Tannin	+
2	Phlobatannin	-
3	Saponin	+
4	Flavonoids	+
5	Steroids	+
6	Terpenoids	+
7	Triterpnoids	+
8	Alkaloid	+
9	Carbohydrate	+
10	Protein	+
11	Anthroquinone	+
12	Polyphenol	+
13	Glycoside	+

(-) Indicates Absence; (+) Indicates Presence;

Phytochemicals, as plant components with discrete bio-activities towards animal biochemistry and metabolism are being widely examined for their ability to provide health benefits. It is important to establish the scientific rationale to defend their use in foods, as potential nutritionally active ingredients. Phytochemicals could provide health benefits as: (1) substrates for biochemical reactions; (2) cofactors of enzymatic reactions; (3) inhibitors of enzymatic reactions; (4) absorbents/ sequestrates that bind to and eliminate undesirable constituents in the intestine; (5) ligands that agonize or antagonize cell surface or intracellular receptors; (6) scavengers of reactive or toxic chemicals; (7) compounds that enhance the absorption and or stability of essential nutrients; (8) selective growth factors for beneficial gastrointestinal bacteria; (9) fermentation substrates for beneficial oral, gastric or intestinal bacteria; and (10) selective inhibitors of deleterious intestinal bacteria. Such phytochemicals include terpenoids, phenolics, alkaloids and fiber (Harborne 1984). The rich nutrients present in the *Spirulina* possesses potential health benefits.

Morphometric & growth parameters of freshwater fish Labeo rohita (Fingerlings) with Spirulina supplemented fish at 30 days:

The results of the morphometric and growth parameters of *Labeo rohita* fingerlings with different feeding regimes are presented in Table 2. Supplementation of, *Spirulina* at the concentration of 1% were fed to *Labeo rohita* fish for different

durations (10, 20 and 30 days). Among the different durations, the *Spirulina* supplemented fish has potential morphometric and growth performances were observed at 30 days.

Table 2. The morphometric and growth Parameters of freshwater fish *Labeo rohita* (Fingerlings) with different feeding regimes

Parameters	Growth parameters							
	0 days		10 days		20 days		30 days	
	Control	Test	Control	Test	Control	Test	Control	Test
Length (Cm)	9.85±1.12	9.92±1.23	10.50±1.36	11.27±1.42	12.15±1.68	12.83±1.79	13.80±1.81	14.10 ± 1.95
Weight (Kg)	8.43±1.59	8.57±1.84	9.64±2.05	10.37±2.26	10. 73±2.14	11.54±2.35	12.08±2.49	12. 89 ± 2.97

Values are expresses as Mean ± SD for 10 fishes

The significant growth performance of *Spirulina* diet. This study also substantiates many other earlier reports on benefits of using *Spirulina* as part of aquaculture diet for various commercially important food fishes for growth performance (Tongsiri *et al.*, 2010; Biswas *et al*., 2007; Hernandez *et al*., 2007; Tomas *et al*., 2009; Piccolo *et al*., 2011).

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 and total length of *Labeo rohita* showed a highly significant difference for the different treatments. Statistical analysis showed the significant variation among the treatments for the *Labeo rohita*, during the experimental period that differ from each other. In case of *Labeo rohita*, comparison of mean values of average body weight and total length in *Spirulina* treatments, showed that it appeared to attain maximum weight gain and total length under the influence of *Spirulina* diet. 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 and total length in the treatment of *Spirulina* diet when compared to control diet.

Proximate composition of freshwater fish Labeo rohita (Fingerlings) with Spirulina supplemented fish at 30 days:

Labeo rohita fingerlings showed (Table 3) the content of carbohydrate (25.86 ± 2.41), protein, (28.32 ± 1.43), amino acid and (21. 37 ± 1.73) and lipid (9.43 ± 1.52) were observed in control fish while carbohydrate (27.32 ± 2.53), protein, (30.21 ± 1.52), amino acid and (24.86 ± 2.31) and lipid (9.85 ± 1.76) content were increased in 10 days *Spirulina* supplemented fish. The content of carbohydrate (30.71 ± 2.58), protein, (33.76 ± 1.63), amino acid and (27.44 ± 2.48) and lipid (10.06 ± 1.81) were observed in 20 days *Spirulina* supplemented fish while carbohydrate (32.98 ± 2.64), protein, (40.87 ± 1.78), amino acid and (31.83 ± 2.51) and lipid (10.35 ± 1.94) content were increased in *Spirulina* supplemented fish 30

days. . The proximate composition of freshwater fish *Labeo rohita* was highest in *Spirulina* diet in 30 days.

Table 3. Proximate composition of freshwater fish *Labeo rohita* (Fingerlings) with *Spirulina* supplemented fish at 30 days

Test	Biochemical estimations			
	Control fish	**10 Day**	**20 Day**	**30 Day**
Carbohydrate (mg/gm)	25.86 ± 2.41	27.32 ± 2.53	30.71 ± 2.58	32.98 ± 2.64
Protein (mg/gm)	28.32 ± 1.43	30.21 ± 1.52	33.76 ± 1.63	40.87 ± 1.78
Amino acids (mg/gm)	21. 37 ± 1.73	24.86 ± 2.31	27.44 ± 2.48	31.83 ± 2.51
Lipids (mg/gm)	9.43 ± 1.52	9.85 ± 1.76	10.06 ± 1.81	10.35 ± 1.94

Values are expresses as Mean ± SD for 10 fishes

The biochemical analysis *Labeo rohita* fingerlings indicated that *Spirulina* diets results in significant increase in the level of carbohydrate and lipids in 30 days when compared to control, 10 and 20 days supplementation of *Spirulina.* The changes in carbohydrate, protein and lipid is contents of fish body could be related to the changes in their synthesis and deposition rate in fish muscles (Abdel-Tawwab *et al.,* 2006). The biochemical analyses often provide vital information for health-assessment and management of cultured fish (Cnaani *et al.,* 2004; Ghosh *et al.,* 2003).

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 *Spirulina* supplementation fed were recorded. After the feeding trail experiment of every 30 days, the total protein, carbohydrate and lipids content were found to be maximum in *Spirulina* diet fed with 1% diet 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). 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*., 2013) each body cell is composed mainly of protein.

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.

Components like carbohydrate play a vital role as energy precursors for fish under stress conditions (Umminger, 1970). 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). 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 *Spirulina*. Results of the present study were agreement with earlier reports (Gumus and Ikiz, 2009).

CONCLUSION

The above data demonstrated that the fingerlings of *Labeo rohita* fed on 1% of *Spirulina* diet of each, it is clear that incorporation of *Labeo rohita* considerably enhanced the growth, biological performance of fish as compared with that of control diet. The potential health benefits of *Spirulina* diet may be due to the micro and macro nutritional compositions as compared to other diet supplementations. So the present study recommends the use of *Spirulina* and in fish feed in modest amount as it enhances the feed quality without much affecting the cost factor.

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