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ORIGINAL ARTICLE

Enzyme Assay Inliver and Muscle of fresh Water Fish *Heteropneustes fossilis* (Bloch.) after Feeding of an Alga, Spirulina

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ABSTRACT

Problem of nutritious food is increasing day by day specially for protein. Protein deficiency cause serious growth disorders in children and adults too. Spirulina is looking a great source of protein for increasing population. It also provides nutrition to fishes to enhance protein and other contents which Nin turn going to human via food. Keeping these points in view, it is justified to study the effect of Spirulina feeding on biochemical parameters of liver and muscles of Heteropneustes fossilis.

Key words: *Liver, Muscle, Heteropneustes fossilis*

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INTRODUCTION

Any fish cultured with artificial feeds need high percentage of protein in the diet for fast growth and better growth performance. However requirement of protein by a particular species of fish vary with others. It has been established that protein is required by all animals for body maintenance and growth, and that the protein level needed for these functions varies with the species and culture environment (Delong, *et al*., 1958, Lovell, 1972). For fish, the optimum amounts of protein in formulated feeds are important because either low or high levels of protein may lead to poor growth. As well, excess protein in fish diet may be wasteful and cause the diets to be unnecessarily expensive. Therefore, in the present study attempts were taken to investigate the growth performance in formulated feed for *H. fossilis* fish. For carrying out feeding trial under laboratory conditions, rearing facilities were created and *H. fossilis* fish has been selected as the experimental fish.

Spirulina (Arthrospira) is cultured commercially in China and in some other parts of the world as this cyanobacterium has been proved to be a valuable source of food supplement not only for human but for other farm animals too. Furthermore, it has been found that microalgae to be very effective bio absorbents, as they possess a large surface area and high binding affinity. Cell wall of these microalgae consists of polysaccharides, proteins and lipids having lots of negative groups which are the dominant binding sites of toxic metal cations.

Spirulina is one of the most concentrated natural sources of nutrition for all animals. Early interest in *Spirulina* focused mainly on its potential as a source of protein and vitamins. *Spirulina* contains 60-70% protein by weight, is the richest source of vitamins B12 and beta carotene (20 times more than carrots), and is loaded with essential fatty acids and minerals. Essential amino acids (62%) such as isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane, and valine are present in *Spirulina*. *Spirulina* improves the intestinal flora in fish by breaking down indigestible feed components to extract more nutrients. *Spirulina* stimulates the production of enzymes that transport fats within the fish for growth instead of storage. The cell wall of *Spirulina* is rich in muco-proteins that enhance the natural mucus layer of the skin, resulting in the shiny appearance of fins and skin and improving resistance to skin infections. More recently, new interests concern the therapeutic effects of *Spirulina* as a growth promoter and probiotic or booster of the immune system in all animals including fish.

MATERIALS AND METHODS

SELECTION, REARING AND MAINTENANCE OF FISH:

Heteropneustes fossilis a catfish belongs to the family Heteropneustidae. It is commonly known as Asian Stinging catfish or fossil cat. It is found in India, Pakistan, Nepal, Srilanka, Thailand and Myanmar. In Sri Lanka, this fish is called Hunga by the Sinhala speaking community, in India it's called singhi.

Catfish species live in inland or coastal waters of every continent except Antarctica. Catfish have inhabited all continents at one time or another. Catfish are most diverse in tropical South America, Africa, and Asia. More than half of all catfish species live in the America. They are the only ostariophysans that have entered freshwater habitats in Madagascar, Australia, and New Guinea.

Juvenile live fishes were purchased from the local fish market during September to April when the room temperature ranges from 25 to 36^{0}C and water temperature from 20 to 25^{0}C. The fish averaging 6-10cm standard length and average body weight of 60-70gm were used for the study. The fishes were conveyed to fisheries laboratory in the portable well aerated colourless polythene bags containing water. After examining carefully for any injury they were kept in one percent solution of potassium permagnate for few minutes to get rid of any dermal infection. After acclimatization for 15days they were reared in large glass aquaria measuring 75 x 37.5 x 37.5 cm and fed on boiled egg yolk and fish food. Tap water stored in large aquariam for dechlorination was used as a diluent medium. The water of aquarium was changed after every two days or even earlier when it gave foul smell.

EXPERIMENTAL PROTOCOL:

To assess the effect of spirulina the fish, *Heteropneustes fossilis* were grouped in to five sets, four acute and one control each consisting six.The *Heteropneustes fossilis* were taken live and dissected carefully and muscles were taken out for biochemical estimation.

EXPERIMENTAL MATERIAL:

For feeding of *spirulina* was collected in form of powder from Recon Ltd., Bangalore, India. It was mixed with water and released in aquarium (10mg /25L).

ED_{50} DETERMINATION:

Effective dose is the dose at which 50% animals show effect if known as effective dose. For ED_{50} determination of spirulina the fishes are divided into four groups (I, II, III and IV). Each group consisting of 4 individuals. Different doses of spirulina were administered to fishes of each group. The standard solution of spirulina prepared fresh daily and prepared by diluting it with distilled water. The effectiveness was noted after 96 hours in all the groups. The dose at which 50% effectiveness occurred was noted. The doses were converted mg to µg and then to log dose and then graph has been plotted. The calculation of ED_{50} was done by Thompson and Weil method (1952), which is most efficient, accurate and shortest way to calculate effective dose. The doses are converted to logarithms and other values obtained from table (Biometrics, 1952) and then a graph was plotted in which concentration (first converted mg to µg and then to log dose) is on X-axis and on Y-axis % of response. ED_{50} was calculated by the formula

$\log ED_{50} \simeq \log D_a + d\,(f+1)$

D_a = lowest dose

d = log of constant rates between dosage level = 0.30103

f = from table (which require r, n, k)

n = number of animals at particular level

k = number of doses –1 [(i.e. 4-1) = 3]

r = number of animals affected at particular level

EXPERIMENTATION AND BIOCHEMICAL ESTIMATION:

Six fishes from each set (control set and experimental sets) were sacrificed for the biochemical studies after 24, 48, 72 and 96 hrs after feeding of Spirulina.The alkaline phosphatase and acid phosphatase were estimated by the kit method (Kind and King, 1954 & King's 1959). Statistical calculations: In the present investigation, the following formulae were used for different statistical calculations through SPSS 17.0.

RESULTS AND DISCUSSION

Table 1: Alkaline Phosphatase (U/L) in liver and muscle of *Heteropneustes fossilis*after feeding of *Spirulina*

S.No.	Duration	No. of fishes	Liver		Muscle	
			Control	Treatment	Control	Treatment
			Mean±S.Em.	Mean±S.Em.	Mean±S.Em.	Mean±S.Em.
1.	24hrs	6	18.20±0.78	16.48±0.57*	13.15±0.70	11.40±0.54*
2.	48hrs	6	18.20±0.78	15.52±0.68*	13.15±0.70	11.02±0.65*
3.	72hrs	6	18.20±0.78	14.56±0.96*	13.15±0.70	10.16±0.95*
4.	96hrs	6	18.20±0.78	12.52±1.12**	13.15±0.70	9.44±0.65**

* Non-significant, **Significant, *** Highly significant, **** Very highly significant

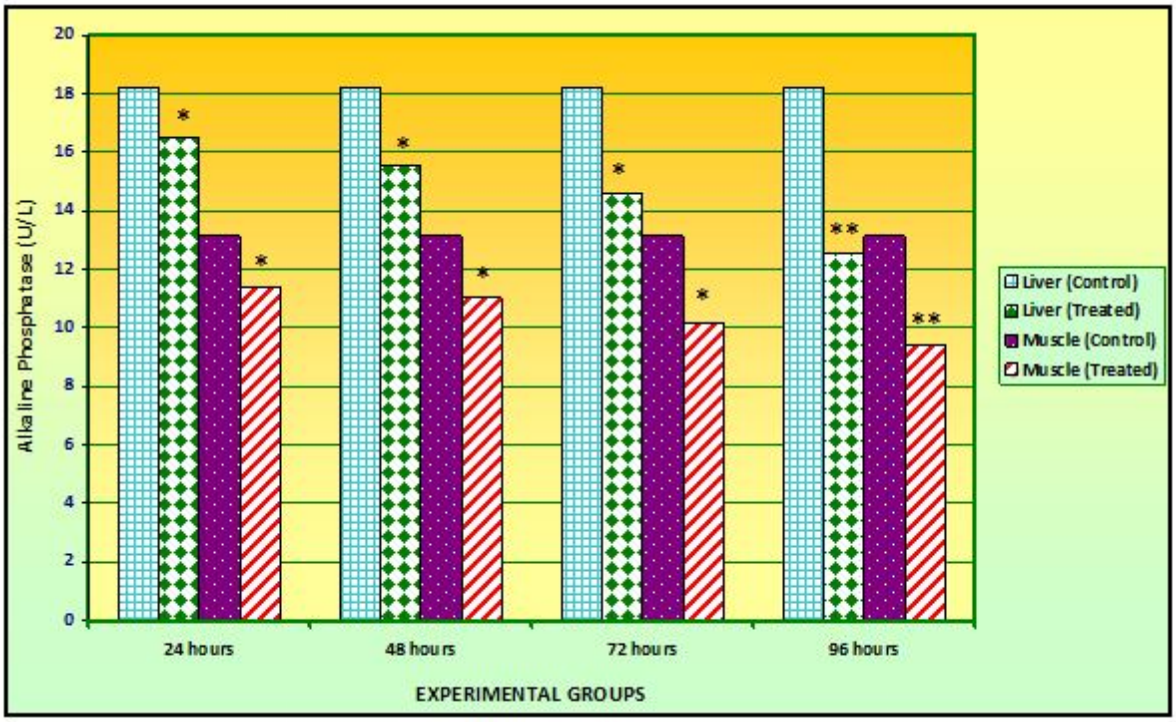
Table 2: Acid Phosphatase (U/L) in liver and muscle of *Heteropneustes fossilis*after feeding of *Spirulina*

S.No.	Duration	No. of fishes	Liver		Muscle	
			Control	Treatment	Control	Treatment
			Mean±S.Em.	Mean±S.Em.	Mean±S.Em.	Mean±S.Em.
1.	24hrs	6	7.36±0.52	6.95±0.69*	5.30±0.50	5.00±0.60*
2.	48hrs	6	7.36±0.52	6.66±0.57*	5.30±0.50	4.62±1.07*
3.	72hrs	6	7.36±0.52	5.89±0.56*	5.30±0.50	4.09±0.54*
4.	96hrs	6	7.36±0.52	5.68±0.52*	5.30±0.50	4.10±0.58*

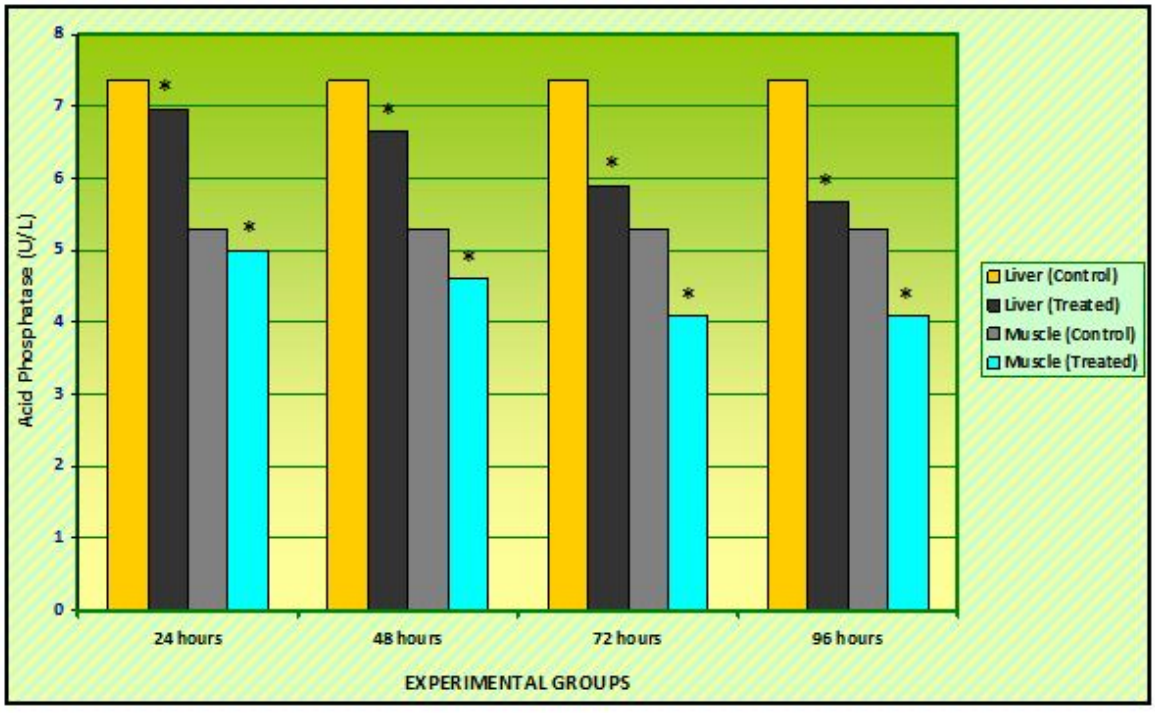
* Non-significant, **Significant, *** Highly significant, **** Very highly significant

Acid and Alkaline phosphatase activities shows decreasing trend in 24hr, 48hr, 72hr and 96hr feeding of spirulina as compared to control both in liver and muscles. The decrease is more in muscles as compared to liver.

Graph 1: Alkaline Phosphatase (U/L) in Liver and Muscle of *Heteroppeustes fossils* after Spirulina Feedimg



Graph 2: Acid Phosphate (U/L) in Liver and Muscle of *Heteroppeustes* fossils after Spirulina Feeding



Spirulina reduced the toxicity in water and fish which in turn normalize the ALP activity. On other hand, The ACP activity normalized significantly in fish exposed to spirulina alone more than control. The obtained results showed that all the tested biochemical parameters were improved in due to *Spirulina platensis* 10 mg L-1 which is considered as an optimum dose could improve the health status of fish.

These alterations in acid phosphatase level may be attributed to destruction of hepatocytes and inhibition of bile on account of alkylbenzenesulphonate toxicity (Yilman, *et al*., 2004). In agreement to the present findings, decrease in the acid phosphatase content in serum of *Channapunctatus* due to kinadon toxicity (Ololade and Oginni, 2010).

Phosphatase activity is of significance in pathological conditions (Velisek*et al*., 2007). Increased ALP activity ($p<0.05$) was marked in the group of fish fed with *Chlorella* over different days except 30 days of feeding and at 10 day post-challenge in groups B and D. Increase in phosphatase activity indicates higher breakdown of the energy reserves, which is utilized for the growth and survival of fish. ALP is the brush border enzyme, which splits various phosphorous esterases at an alkaline pH and mediates membrane transport (Stara, *et al*., 2012). ALP is also involved in transport of glycogen, protein synthesis and synthesis of certain enzymes and secretary activity. Thus, any alteration in the activity of ALP may affect an animal in a variety of ways.

Increased ALP activity was reported while feeding rohu at various doses of turmeric for a period of 60 days following challenge with *A. hydrophila*. In the present study, similar types of observations were noticed (Saravanan, *et al*., 2011). Prusty, *et al*. (2011) reported that optimum dietary *Chlorella* supplementation at 5% of diet had positive effect on growth and feed utilisation without any negative effect on blood parameters and body composition of Korean rock fish. Dietary supplementation of 2% *Chlorella* powder in the commercial diets improved growth, feed utilisation, serum cholesterol and whole body fat contents in juveniles Japanese flounder (Kadry, *et al*., 2012). It can be inferred from the challenge study that the increased protection against the pathogen could be due to the enhancement in the defense system, as is evidenced with increase in different immune parameters in fish, post pathogen challenge

The alkaline phosphatase activity showed decreasing trend which may be due to less secretion from hepatocytes, inhibition of bile or decreasing of alkaline phosphatase by normalizing the secretory function of liver cell.

These results suggest that Spirulina could chelate toxic ions producing a stable complex, thus reducing the chance for toxic substance uptake. The formation of metal- chelate complex in water evidently reduced the metal burden in tissues and thereby improved the biochemical parameters of fish exposed to polluted water. *Lemnagibba* L (weed and extract) were effective in removing Hg from water and reducing Hg bioaccumulation in liver and muscular tissues of *O. niloticus* fish (Hamed, 2015). Finally, we could conclude that Spirulina provided protection against the toxic action of pollutants present in water and increased the chance of biochemical and enzymes regeneration (Gaber, *et al*., 2013).

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