



Effect of dietary supplementation of *Euglena viridis* on growth performance and selected serum biochemical parameters in *Labeo rohita* (Hamilton, 1822) fingerlings

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ABSTRACT

Fingerlings of *Labeo rohita* (Hamilton, 1822) (25±2 g) were fed on *Euglena viridis* supplemented diets @ 0 (Control), 0.1 g kg⁻¹, 0.5 g kg⁻¹ and 1.0 g kg⁻¹ for 90 days. At 30 days intervals, blood serum samples were assayed for glucose level and enzymatic parameters viz., serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (ACP) and alkaline phosphatase (ALP). Fish were challenged with a virulent strain of *Aeromonas hydrophila* after 90 days feeding period. Insignificant ($p>0.05$) differences in serum AST levels were noticed on the 30th and 60th day of feeding between the different dietary groups of fish. Serum enzymes (ALT, AST and ALP) significantly ($p<0.05$) declined in fish fed on *E. viridis* incorporated diets. On the 10th day post-challenge with *A. hydrophila*, the highest percentage of survival (75%) was recorded in the dietary group fed with 0.5 g kg⁻¹ *E. viridis*. The best feed conversion ratio (FCR) and highest specific growth rate (SGR) were recorded in the group fed with 0.5 g kg⁻¹ *E. viridis* incorporated diet. The results clearly indicated that dietary *E. viridis* promote growth rate and decreases susceptibility to *A. hydrophila* and did not negatively impact serum enzyme levels in *L. rohita*.

Keywords: *Aeromonas hydrophila*, Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), *Euglena viridis*, *Labeo rohita*

Introduction

Aquaculture is considered as the fastest growing food production sector in the world and has attracted increased importance worldwide due to increasing demand for more protein (FAO, 2011). Indian major carps are the most preferred farmed fishes in inland aquaculture sector in the Indian subcontinent owing to their rapid growth and consumer demand. This increased demand led to intensification of farming practices of many fish species especially carps leading to the spread of infectious diseases (Mokoro *et al.*, 2014).

In aquaculture, a proper diet is necessary for the maintenance of health as well as to enhance resistance to diseases in cultured animals. So, considerable attention has been given on the use of feed additives/supplements in aquaculture (Zaki *et al.*, 2012). In India, most of the farmers rely on the use of wet diet comprising rice bran and mustard oil cake together with natural pond fauna for carp culture (Biswas *et al.*, 2014). Large numbers of feed

additives are used globally for fish growth enhancement. Off late, minimal utilisation of chemicals and the use of herbal feed additives is encouraged and the global trend is to go back to nature (Mohamed *et al.*, 2003). Herbs and medicinal plant products offer an important and cheaper source for treatment in aquaculture (Madhuri *et al.*, 2012). Algae are widely accepted as natural sources for feed additives to increase feed utilisation efficiency and productivity in aquaculture (Khani *et al.*, 2017, Raji *et al.*, 2020). Microalgae comprise a wide spectrum of nutritious and bioactive compounds including vitamins, minerals, proteins, essential amino acids and pigments (Becker, 2007, Jacob-Lopes *et al.*, 2019).

Among microalgae, *Euglena* is widely dispersed in freshwater bodies. Different species of *Euglena* have been reported to have a wide range of medicinal properties, such as antimicrobial (Das *et al.*, 2005), anti-mutagenic (Foltinova *et al.*, 1994), anti-HIV (Nakashima *et al.*, 2008), immunopotentiating (Kondo *et al.*, 1992), antitumor (Quesada *et al.*, 1976) and hepatoprotective (Panja *et al.*,

2014) activity. Beta-1, 3, glucan is abundant in *Euglena* which has applications in veterinary and human therapeutics as an immunopotentiator and immunostimulant (Barsanti *et al.*, 2001; Evans *et al.*, 2019). There are reports on the use of microalgae, such as *Chlorella*, *Spirulina*, *Euglena* and *Microcystis* as feed additives for improvement of growth performance and disease resistance of fish (Das *et al.*, 2009; 2013; Sirakov *et al.*, 2012; Pradhan and Das, 2015a). It has been reported that feeding *Chlorella* enhances survival, growth rates and immunity in larval Korean rockfish, juvenile Korean rockfish and Gibel carp respectively (Bai *et al.*, 2001; Cho *et al.*, 2001; Xu *et al.*, 2014). El-Habashi *et al.* (2019) evaluated the protective effect of dietary *Spirulina*, *Chlorella* and their mixture in Nile tilapia fish against *A. hydrophila* infection. The effect of algal feed on liver enzymes in *L. rohita* has been investigated previously in detail (Pradhan and Das, 2015a,b). A recent study revealed that microalga based diet positively impacts on oxidative stress enzymes of African catfish *Clarias gariepinus* and it could supplement the requisite nutrients for their metabolic activities (Sharma *et al.*, 2019). So the application of algal products is more useful as they are safe and eco-friendly and also, they are gaining importance in aquaculture. The current study aimed to investigate the effect of dietary supplementation of *Euglena viridis* on growth performance, feed conversion, blood glucose and serum enzyme levels in fingerlings of rohu *Labeo rohita* (Hamilton, 1822).

Materials and methods

Experimental fish

Rohu fingerlings of average weight 25 ± 2 g were collected from the carp farm of ICAR-Central Institute of Freshwater Aquaculture (ICAR-CIFA), Bhubaneswar, Odisha. They were acclimatised in indoor fibre reinforced plastic (FRP) tanks holding 400 l chlorine-free tap water for 30 days and fed with commercial diet (Godrej, India). Throughout the experimental period, regular water exchange was done and continuous aeration was provided in the experimental tanks. Water quality was monitored at fortnightly intervals for different parameters and the mean values recorded were: dissolved oxygen 6.0 ± 0.4 mg l⁻¹;

temperature $28 \pm 1^\circ\text{C}$; pH 7.6 ± 0.6 ; hardness 90 ± 5 mg l⁻¹ and total alkalinity 92 ± 6.5 mg l⁻¹. Fish were fed with the experimental diets at 4% of body weight per day (Table 1).

Diet formulation

E. viridis blooms were harvested from the ponds in the farm of ICAR-CIFA, Bhubaneswar and were washed with distilled water and harvested by centrifugation at 1000 g (Sorvall CE, UK) for 5 min. The pellets were collected and shade dried for 2-3 days and ground to a fine powder form. The basal diet comprised 7.2% lipid, 39.6% protein, 14.6% ash, 3% fibre and 7.1% moisture (Table 1). Following Das *et al.* (2009), four experimental diets were prepared incorporating 0, 0.1, 0.5 and 1.0 g kg⁻¹ of powdered *E. viridis* in the basal diet. After proper mixing of the dry ingredients, binder (starch 1%) and required quantity of water were added. A dough was prepared by thoroughly mixing in a mixer and was pelletised in a manual pelletiser. The pellets were dried and kept at room temperature for further use.

Experimental design

Rohu fingerlings (n=480) were divided into 4 equal groups as group A fed basal diet and groups B, C and D fed *E. viridis* supplemented diets at 0.1, 0.5 and 1.0 g kg⁻¹ respectively. Each group of fingerlings (n=120) comprised three subgroups of 40 fish each. Throughout the 90 days of the experimental period, fish were fed @ 4% of their body weight twice daily at 09 00 hrs and 17 00 hrs with the respective diets. Unconsumed feed and faecal matter were siphoned out daily.

Bacterial pathogen for challenge experiment

Twenty-four hour broth culture of *Aeromonas hydrophila* (ATCC 49140) was centrifuged at 3000 g for 10 min. The pellet was resuspended in sterile phosphate buffered saline (PBS, pH 7.4), washed 2 times and the cells were harvested by centrifugation. *A. hydrophila* suspension (1×10^7 CFU ml⁻¹) was prepared in PBS by adjusting OD to 0.5 at 456 nm. Then the required dose was prepared using standard dilution technique for the challenge study.

Table 1. Ingredient composition of basal diet

Ingredient	Incorporation level (g kg ⁻¹ diet)
Rice bran	200
Groundnut cake	400
Fish meal	250
Soya meal	120
Vitamins and minerals (Sarabhai Chemicals, Wadi, Baroda, India)	20
Binder (Starch)	10

Note: Calculated and estimated crude protein: 40% and 39.6% respectively; Calculated and estimated lipid: 5% and 7.2% respectively.

Blood sampling and serum separation

Fish (40 from each group) were randomly picked and blood samples were collected at 30 day intervals (day 30, 60 and 90). The sampled fish were anaesthetised with MS-222 (0.1 mg l⁻¹) and blood was drawn from the caudal vein using disposable syringe (without heparin) and the fishes were released back to the respective tanks. For serum separation, blood samples were kept for an hour to clot, centrifuged at 3000 g for 5 min and the sera collected were stored at -80°C for further analyses (Das *et al.*, 2009). For estimation of serum enzymatic parameters, pooled sera from each subgroup were used.

*Estimation of serum enzyme activities**Aspartate aminotransferase (AST) activity*

Serum AST (EC 2.6.1.1) was determined following Wallnofer *et al.* (1974). To the test reagent (2 ml), serum (200 µl) was added and thoroughly mixed and incubated for 1 min at 37°C. Then α-oxoglutarate (200 µl) was added and the initial spectrophotometric reading was taken at 340 nm. At 1 min interval, three subsequent readings were taken and the activity was calculated as:

$$\text{AST activity (U l}^{-1}\text{)} = 1905 \times \Delta A$$

where ΔA = Change in absorbance at 340 nm per min

Alanine aminotransferase (ALT) activity

For estimation of serum ALT (EC 2.6.1.2) (Wallnofer *et al.*, 1974), 2 ml of test reagent and serum (200 µl) was mixed properly and was incubated for 1 min at 25°C. Subsequently, 200 µl of α-oxoglutarate was added and thoroughly mixed. After recording the initial absorbance at 340 nm subsequent readings were taken at 1 min intervals. The calculation of the final concentration was as follows:

$$\text{ALT activity (U l}^{-1}\text{)} = 1905 \times \Delta A$$

where ΔA = Change in absorbance at 340 nm per min

Acid phosphatase (ACP) activity

The activity of acid phosphatase was measured following the method of Hillman (1971). One ml of ACP working solution was mixed with 100 µl of sample. After 5 min, initial absorbance was measured at 405 nm and subsequently three more readings were taken at 1 min interval. The ACP concentration was expressed in U l⁻¹ and calculated as:

$$\text{ACP activity (U l}^{-1}\text{)} = 743 \times \Delta A$$

where ΔA = Change in absorbance at 405 nm per min

Alkaline phosphatase (ALP) activity

Serum ALP (EC 3.1.3.1) was determined as per Rosalki *et al.* (1993). Fifty microlitre of serum sample was added to 3 ml of test reagent (diethanolamine buffer, MgCl₂ and p-nitrophenyl phosphate). The initial reading was taken at 405 nm after proper mixing. The activity of

ALP was expressed as U l⁻¹ of serum and the concentration was calculated as:

$$\text{ALP Activity (U l}^{-1}\text{)} = 3300 \times \Delta A$$

where ΔA = Change in absorbance at 405 nm per min

Determination of blood glucose

Blood glucose was determined following Trinder (1969). Test reagent (1ml) was taken in all the test tubes designated as blank (B), test sample (T) and standard (S). Thereafter 10 µl of distilled water, 10 µl of serum and 10 µl of standard (100 mg dl⁻¹) were added to respective tubes and were thoroughly mixed and incubated at 25°C for 30 min. Then OD value was recorded at 546 nm using a spectrophotometer (Bio-Rad, Smartspec™3000). The final concentration of glucose was expressed in g% and it was calculated as:

$$C = n \times A_{\text{sample}} / A_{\text{standard}}$$

where A_{sample} = OD value of the sample (T); A_{standard} = OD value of standard (S) and n = Concentration of standard *i.e.*, 100.

Growth measurements

Total biomass in the tank and of the individual fish were recorded at the beginning and end of the experimental feeding. As per Ricker (1979), from the above data the specific growth rate (SGR) and feed conversion ratio (FCR) was expressed as:

$$\text{SGR (\%)} = \frac{\ln \text{ final weight (g)} - \ln \text{ initial weight (g)}}{\text{Total duration of the experiment (days)}} \times 100$$

$$\text{FCR} = \frac{\text{Feed given (dry weight, g)}}{\text{Weight gain (wet weight, g)}}$$

Challenge experiment

After 3 months of feeding an infectious dose of *A. hydrophila* (1×10⁵ CFU per fish) was injected intraperitoneally to each group of fish (n=30) in duplicate (Sahu *et al.*, 2008). Mortality was monitored and recorded up to 10 days for every 12 h interval.

Statistical analyses

The data were analysed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests (Duncan, 1955) to evaluate differences between treatments. Statistical significance was set at p≤0.05.

Results*Serum enzymatic parameters*

Modulation in serum enzymatic parameters of fish fed with *E. viridis* supplemented diets is depicted in Fig. 1 to 3. Serum AST activity was not significantly (p>0.05) different in the entire treatment groups of fish on days 30 and 60 as compared to the control fishes. Throughout

the experimental period, a gradual decrease in level of AST activity was observed in the dietary group fed with *E. viridis* incorporated diet (Fig. 1). Specifically, fish fed *E. viridis* diets presented lower ALT activity when compared with those fed the control diet (Fig. 2). The entire control group had elevated ALT activity throughout the experimental period. Likewise, significantly reduced ($p \leq 0.05$) activity of serum acid phosphatase (ACP) was observed in all the *E. viridis* diet fed groups (except group B on 60th day) in comparison to control (Fig. 3). Serum ALP activity showed significant difference between dietary groups with higher levels of *E. viridis* supplemented diet ($p \leq 0.05$) when compared with control group (except group B on 30th day) (Fig. 4).

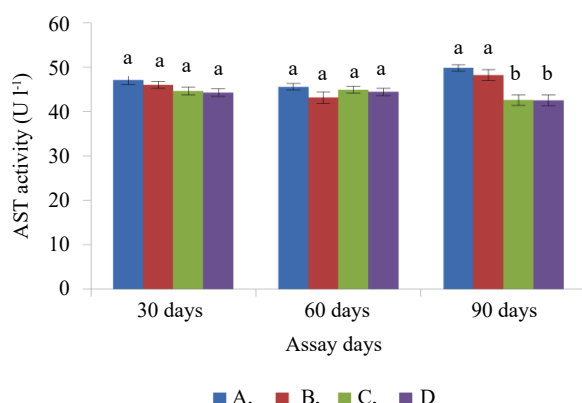


Fig. 1. Activity of AST (U l⁻¹) in *L. rohita* fingerlings fed *E. viridis* supplemented diets. Results presented as mean±S.E. Treatments bearing same alphabets are not significantly different ($p > 0.05$) (A: Control diet; B: 0.1 g *E. viridis* kg⁻¹ diet; C: 0.5 g *E. viridis* kg⁻¹ diet; B: 1 g *E. viridis* kg⁻¹ diet)

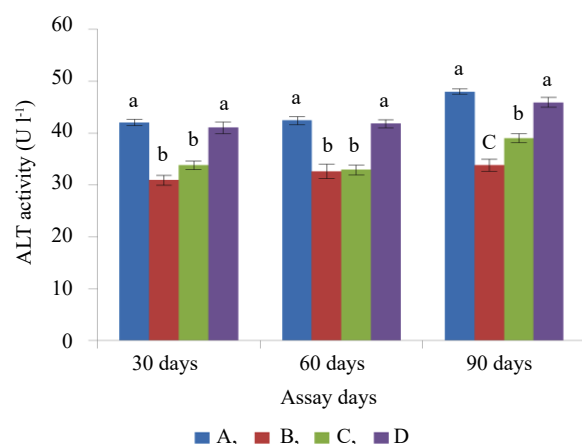


Fig. 2. Activity of ALT (U l⁻¹) in *L. rohita* fingerlings fed *E. viridis* supplemented diets. Results presented as mean±S.E. Treatments bearing same alphabets are not significantly different ($p > 0.05$) (A: Control diet; B: 0.1 g *E. viridis* kg⁻¹ diet; C: 0.5 g *E. viridis* kg⁻¹ diet; B: 1 g *E. viridis* kg⁻¹ diet)

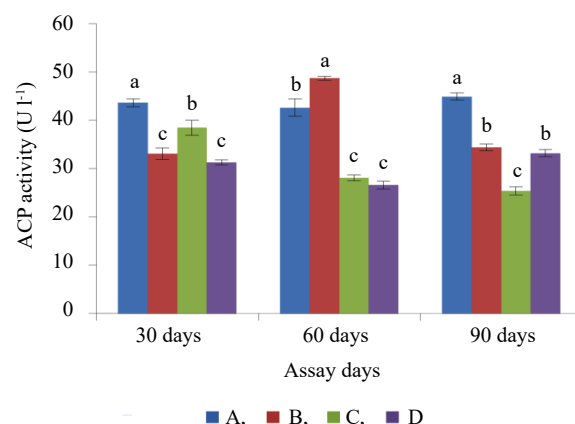


Fig. 3. ACP activity (U l⁻¹) in *L. rohita* fingerlings fed *E. viridis* supplemented diets. Results presented as mean±S.E. Treatments bearing common alphabets are not significantly different ($p > 0.05$) (A: Control diet; B: 0.1 g *E. viridis* kg⁻¹ diet; C: 0.5 g *E. viridis* kg⁻¹ diet; B: 1 g *E. viridis* kg⁻¹ diet)

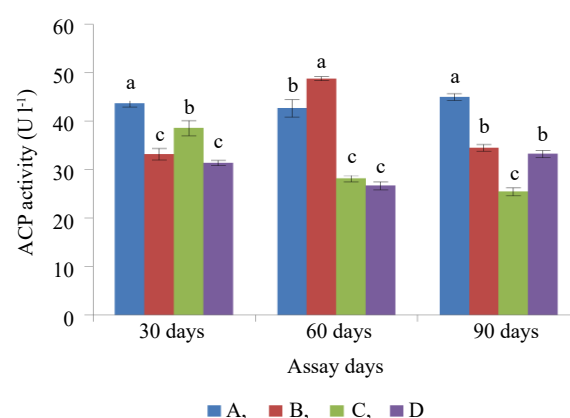


Fig. 4. ALP activity (U l⁻¹) in *L. rohita* fingerlings fed *E. viridis* supplemented diets. Results presented as mean±S.E. Treatments bearing common alphabets are not significantly different ($p > 0.05$) (A: Control diet; B: 0.1 g *E. viridis* kg⁻¹ diet; C: 0.5 g *E. viridis* kg⁻¹ diet; B: 1 g *E. viridis* kg⁻¹ diet)

Blood glucose

E. viridis supplemented diet fed groups recorded significantly ($p \leq 0.05$) lower blood glucose levels at all samplings as compared to control (Fig. 5).

Growth and feed efficiency

Increased specific growth rate (SGR) was noticed in the dietary groups fed with *E. viridis*. Elevated SGR was noticed in group C fish fed with 0.5 g kg⁻¹. There was better food conversion ratio (FCR) with lower FCR values in groups fed with *E. viridis* incorporated diets (Table 2).

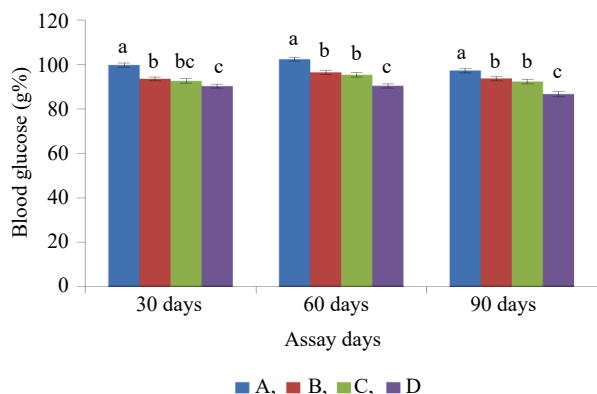


Fig. 5. Blood glucose levels in *L. rohita* fingerlings fed *E. viridis* supplemented diets. Results presented as mean±S.E. Treatments bearing common alphabets are not significantly different ($p>0.05$) (A: Control diet; B: 0.1 g *E. viridis* kg⁻¹ diet; C: 0.5 g *E. viridis* kg⁻¹ diet; B: 1 g *E. viridis* kg⁻¹ diet)

Survivability

The experimental fishes showed zero mortality up to 12 h post-challenge with *A. hydrophila*. On 10th day post-challenge, the percentage of survival was maximum (75.0%) in group of fish fed with 0.5 g per kg *E. viridis* supplemented feed, as compared with the control fish (Table 2).

Discussion

Transamination is one of the main pathways for reversible amination and deamination of amino acids and they involve in the redistribution of amino groups among amino acids. It is accomplished by transaminases and aminotransferases, which are mostly active in liver, kidney, heart and skeletal muscle. AST, ALT and ALP are liver specific enzymes and are sensitive measures of hepatotoxicity (Ozaki, 1978; Verma *et al.*, 1981; 1990; Balint *et al.*, 1997). Elevated levels may indicate destruction, degeneration and necrosis of the liver due to cellular damage. These enzymes may be used as indices to assess the toxicity of experimental material and diet treatments (Bhardwaj *et al.*, 2010). So increased attention has been given to the changing activities of the

AST and ALT, which promote the changes in activities of aminotransferase and gluconeogenesis in the liver (Rashatuar and Ilyas, 1983).

The present study revealed that the serum ALT and AST activities decreased in the *E. viridis* supplemented diet fed groups compared to control group. Statistically insignificant ($p<0.05$) serum AST levels were seen among the *E. viridis* fed groups on day 30 and day 60 as compared to control group. Similarly, significantly depleted serum AST and ALT activities were reported in fish fed with different concentrations of *Allium sativum* and chloramphenicol (Shalaby *et al.*, 2006). Results of the present study are in agreement with the reports of Bello *et al.* (2014), who found decreased level of inclusion of *A. cepa* and *T. conophorum* compared to control. El-Shatter *et al.* (1997) and Augusti *et al.* (2001), reported similar findings in rats fed on a diet containing 5% *A. sativum* where the liver enzyme (ALT, AST and ALP) activities and lipid levels decreased. Saei *et al.* (2016) also mentioned there was no significant difference in liver enzymes of rainbow trout due to the effect of different levels of Bio Acid Ultra. Algal dietary stimulation enhances the resistance against infection, thereby increasing the serum/ blood immune parameters and liver enzyme activities (Pradhan and Das, 2015a,b). So intake of *E. viridis* maintains the normal function of the liver by enhancing the regenerative capacity of its cells. This is reflected in the reduction of liver enzymes. Various types of chemical, physiological and biological factors or Krebs' cycle might alter the ALT and AST activities. So lower activity of the Krebs' cycle decreases its intermediates, thereby, α -ketoglutarate compensates ALT and AST levels (Salah and Rogers, 1993).

In pathological conditions, the acid phosphatase activity (ACP) has a significant role (Reddy and Rao, 1990). Elevated acid phosphatase activity implies the breakdown of the stored energy, for the survival and growth of fish. At an alkaline pH, ALP splits different phosphorous esterases and mediates membrane transport (Hinton *et al.*, 1973). ALP is also involved in the synthesis of protein (Pilo *et al.*, 1972), enzymes, transport of glycogen (Gupta and

Table 2. Effect of *E. viridis* supplemented diets on specific growth rate (SGR), feed conversion ratio (FCR) and survivability of *L. rohita* fingerlings

Dietary group (g <i>E. viridis</i> kg ⁻¹ diet)	SGR (%)	FCR	Final weight (g)	Survivability (on 10 th day post-challenge with <i>A. hydrophila</i>) (%)
A (0)	0.869±0.009 ^b	1.822±0.028 ^a	26.81±0.34	56
B (0.1)	0.894±0.010 ^b	1.705±0.028 ^a	27.06±0.39	68
C (0.5)	0.971±0.012 ^a	1.613±0.023 ^c	27.268±0.295	75
D (1.0)	0.953±0.014 ^a	1.645±0.027 ^{bc}	27.162±0.30	68

Note: Results presented as mean±SE. Superscript values on right hand side column-wise are significantly ($p\leq0.05$) different from the control for particular treatment.

Rao, 1974) and secretory activity. Thus, an animal may be affected in a variety of ways due to any alteration of ALP activity. It was also reported that the serum ALP activity to be an indicator of the potency of nutrient absorption in enterocytes of fish (Harpaz and Uni, 1999). Increased ALP activity has been reported while feeding rohu at various doses of turmeric for 60 days following challenge with *A. hydrophila* (Sahu *et al.*, 2008). In the present study, a similar type of observation was noticed where the group of fish exposed to *E. viridis* diet showed a significant increase in ALP activity with increasing levels of *E. viridis*. The results are also in agreement with Pradhan and Das (2015a), where they mentioned increased ALP activity ($p < 0.05$) in *Chlorella* fed fish. Similarly Abalaka *et al.* (2011) mentioned that there was significant increase in activity of ALP when *Clarias gariepinus* adults were treated with ethanolic and aqueous extracts of *Parkia biglobosa* pods, which varied with increasing concentrations of both extracts. Acid phosphatase catalyses and facilitates important physiological changes within cells and the level of expression had a considerable impact on clinical investigation. Stress-induced alterations in serum/tissue phosphatase activities have been reported in carp (Das *et al.*, 2004). African catfish *Clarias gariepinus* fishes showed significantly higher oxidative stress enzymes, catalase, glutathione-s-transferase, superoxide dismutase and lipid peroxidase activities with zero mortality when exposed to micro-alga based diet (Sharma *et al.*, 2019). Therefore monitoring these enzymatic activities in serum would provide information related to stress caused by algal feeding. Our data indicate that liver function of the fingerlings of *L. rohita* was not impaired due to feeding of *E. viridis* diet.

Blood glucose level is an indicator of non-specific stress or stress caused by physical factors (Hunn and Greer, 1991; Duncan and Klesius, 1996). Significantly decreased level of blood glucose was noticed throughout the assay days in the dietary group fed with *E. viridis* incorporated diet. This indicates that fish were without stress during treatment with optimum quantity of *E. viridis* supplemented diets. Similar kinds of results were noticed with different types of herbal diet incorporation (Sahu *et al.*, 2007; Das *et al.*, 2013; Pradhan and Das, 2015a). Our results also agreed with Shalaby *et al.* (2006), who reported a significantly low level of plasma glucose in Nile tilapia fed on different concentrations of *Allium sativum* (20, 30 and 40 g kg⁻¹) supplemented diets. Some workers investigated the protective effect of seaweed, *Gracilaria* sp. on antioxidant and immune responses in European seabass. No differences were observed in glucose, when aqueous extract of 5% *Gracilaria* sp. supplemented diet was fed to the fish after infection (Peixoto *et al.*, 2019). So the findings of our study indicate

that regular feeding of *E. viridis* diet fights against stress elements, as it was evident from the declined glucose level in treated fish which could be attributed to enhanced antioxidant levels in pancreatic cells which synthesise insulin (Metawally, 2009).

The growth-promoting activities of various algae have been tested in aquatic animals (Shimaa, 2016; Lobo *et al.*, 2018). Weight gain of fish indicates feed efficiency which improved with immunostimulants supplementation in diets (Seo *et al.*, 2009). Bai *et al.* (2001) reported that 5% dietary *Chlorella* inclusion had a positive effect on growth and feed utilisation without any negative impact on body composition and blood parameters of Korean rockfish. Commercial diet supplemented with 2% *Chlorella* powder enhanced feed utilisation, growth, serum cholesterol and whole-body fat contents in juvenile Japanese flounder (Kim *et al.*, 2002). Significantly increased weight gain and SGR was noticed in all treated groups fed on *E. viridis* in the present study. Diab *et al.* (2002) partially support these results.

In this study, a declined mortality rate was noticed in the group of fish exposed to *E. viridis* supplemented diets after experimental infection with *A. hydrophila*. Maximum survival rate was found in group C (0.5 g kg⁻¹) dietary group as compared to the control. The above findings could be attributed to dietary *E. viridis*, which would have helped to stabilise the cell membrane and provided protection to the liver against free radical mediated toxic damages and deleterious agents. Previous studies also indicate that algal diets helps to increase the survivability rate of rohu against *A. hydrophila* (Das *et al.*, 2013; Pradhan and Das, 2015a). From the results of the study, it can be concluded that dietary *E. viridis* improved growth and decreased susceptibility to *A. hydrophila* infection in *L. rohita*. Further dietary supplementation trials should focus on purification and characterisation of the bioactive compounds present in *E. viridis*. Also, appropriate feed formulation and their evaluation may enhance the quality as well as their utilisation in aquaculture feeds.

Acknowledgments

The study was supported by the Director of ICAR-CIFA, Bhubaneswar and financially assisted by the Department of Science and Technology (DST), Ministry of Science and Technology, New Delhi, India.

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