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Influence of Spirulina on Growth and Immunity of the Fingerlings common carp, (*Cyprinus carpio*, l.1758), challenged with Pathogenic *Aeromonas hydrophila*

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Abstract

This study as carried out to evaluate the importance of dietary *Spirulina* as a growth and immunity promoter in the fingerlings of the common carp *Cyprinus carpio* (L). The laboratory maintained fingerlings are divided into four groups. The first and second groups were fed with control diets 1 and 2 and the third and fourth groups were fed with experimental diets 1 and 2 for a period of 30 days twice a day. Growth and biochemical parameters were measured in the treated fingerlings on days 1, 10, 20 and 30. Simultaneously *Cyprinus carpio* fingerlings fed on different diets for 30d were challenged individually by the pathogenic bacteria, *Aeromonas hydrophila* and kept under observation for 10d to observe clinical symptoms and to record mortality rates. The results show that *Spirulina* has a positive influence on the growth and biochemical parameters. The results also indicate that *Spirulina* enhanced phgocytic and lysozyme activities conferring protection against *A. hydrophila*. These results suggest that dietary *Spirulina* has growth promoting and immunostimulating effects in *Cyprinus carpio* fingerlings.

Key-Words: *Spirulina*, *Cyprinus carpio*, *Aeromonas hydrophila*, Immuonostimulant, Lysozyme, Phygocytosis

Introduction

Fish farming is an industrial sector that has grown extremely fast in the last decade, and improving fish performance and disease resistance of aquaculture organisms are major challenges facing fish culturists. The intensive use of antibiotics to prevent and control the bacterial diseases in aquaculture has led to an increase in antibiotic-resistant bacteria (Alderman & Hastings 1998; Teuber 2001). Accordingly several alternative strategies have been proposed, such as the use of probiotics as biological control agents. Probiotics, live microbes that may serve as dietary supplements to improve fish growth and immune responses, have received some attention in aquaculture (Gatesoupe 1999; Irianto & Austin 2002).

Spirulina, is a freshwater blue green filamentous alga that contains protein (60-70%), vitamins, minerals and essential fatty acids such as palmitic acid, linolenic acid and linoleic acid.

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Thus, it has been used as a nutrient for fish larvae (Lu et al. 2002; Lu & Takeuchi, 2004) and as an ingredient in fish diets for juveniles and adults (Nandeesha et al. 1998; Nandeesha et al. 2001; Takeuchi et al. 2002; Palmegiano et al. 2005; Palmegiano et al. 2008). Moreover, *Spirulina* received increasing attention mainly due to its bioactive components that have antioxidant activity (Cohen & Vonshak, 1991; Mahajan & Kamat, 1995; Bhat & Madyastha 2000; Madhava et al., 2000; Lin et al., 2007; Wang et al., 2007). Bermejo et al., (2008) reported that most antioxidant capacities of *Spirulina* extract are attributable to the biliproteins contained in this microalga, such as phycocyanin, and they suggested that *Spirulina* could be used to produce a natural dietary antioxidant supplement or added to healthy food products, such as cereals, fruit bars or drinks, to prevent some chronic diseases where free radicals are involved.

Cyprinus carpio is one of the most important species of exotic omnivorous carps that can utilize a wide range of food items including blue-green algae (Getabu 1994; Abdel-Tawwab & El-Marakby 2004). In this context, the present study was conducted to determine the effects of *Spirulina* on the growth performance, feed utilization, non-specific immune responses and

resistance of Common carp (*Cyprinus carpio*) to *Aeromonas hydrophila* infection.

Material and Methods

Grouping

Fingerlings of *Cyprinus Carpio* (Common carp L.1758) (5.85 ± 0.02g; 4.2 ± 0.22cm) collected from government fish farm, Tirupati, Chittoor Dist., A.P (India) were brought to the laboratory and acclimatized to the laboratory conditions (12hr:12hr L/D regime continuous aeration) for a week. Fish in group-1 (Control-1) are fed with feed containing Rice bran, Ground nut oil cake, Soybean cake and Fish meal (control diet-1). Fish in group-2 (Experimental diet-1) are fed with feed containing control diet-1+*Spirulina*. Fish in group-3 (control -2) are fed with feed containing Rice bran, Ground nut oil cake, Soybean cake, Fish meal, Coconut oil cake and Prawn meal (control diet-2). Fish in group-4 are fed with feed containing control diet-2+*Spirulina* (Experimental diet-2) (Table-1a).

Water quality parameters

The water quality parameters estimated include Dissolved Oxygen-4 to 8 ppm, Temperature-28 to 32^0C, PH-7.4 to 7.8, Salinity-0.190grams/liter, Chlorinity-0.110 grams/liter, Alkalinity-102 ppm, and Hardness of water-112 ppm. During acclimatization both control and experimental groups were fed twice daily on control and experimental diets for 30d with 25%water change daily.

Blood samples

At the end of day 1, 10, 20 and 30, blood samples were collected from replicates of different groups via the caudal vessels using disposable syringe. After each time interval, 5 fish were sampled from replicates of each group and the serum of their blood was separated and used for immune assays.

Lysozyme activity assay

Serum lysozyme activity was measured as described by Ellis (1990). Briefly, 10µl serum was mixed with 200µl of a *Micrococcus lisodeichticus* (Sigma) suspension at 0.2 mg ml in 0.05 M sodium phosphate buffer (pH 6.2). The mixture was incubated at 27°C, and its OD was detected after 1 and 6 min at 530 nm using an ELISA (enzyme-linked immunosorbent assay) plate reader. One unit of lysozyme activity was defined as the amount of enzyme that produced a decrease in absorbance of 0.001 min/ml serum. Lysozyme concentrations were calculated using a standard curve of lysozyme from chicken egg white (Sigma) concentrations.

Total serum protein and globulin

Samples were analyzed for total protein using the method outlined by Lowry et al., (1951). Albumin content was measured using a standard albumin estimation kit (Sigma) and the globulin content was estimated by subtracting albumin from total protein.

Phagocytic Activity (PA)

Percent phagocytosis and phagocytic index (PI) were determined according to Itami et al. (1994) and Weeks – Perkins et al. (1995). 0.1 ml blood was collected as described above, treated with 0.4 ml anticoagulant and centrifuged at 2500 rpm for 5 min at 4°C. Haemocytes thus separated were mixed with 0.1 ml latex beads and the mixture was incubated in a moist chamber at room temperature for 30 min. After incubation, hemocytes were fixed onto a slide with 2.5% glutaraldehyde. Slides were washed with anticoagulant solution to remove non-adhering hemocytes, air-dried, and stained with differential-quick stain (Weeks –Perkins *et al.*, 1995). Slides were then mounted using permount. Number of ingested beads and number of phagocytizing cells were counted from any 200 cells using Light microscope at 1,000 x magnification. Percentage phagocytosis and phagocytic index (PI) were calculated as follows.

$$(\%)\text{phagocytosis} = \frac{\text{No. of cells ingesting beads}}{\text{No. of cells observed}} \times 100$$

(Itami *et al.*, 1994)

$$\text{PI (Pagocytic index)} = \frac{\text{No. of cells ingesting beads}}{\text{No. of cells observed}} \times \frac{\text{No. of beads ingested}}{\text{No. of cells observed}} \times 100$$

(Weeks – Perkins *et al.*, 1995)

Growth parameters

Growth

Variations in net weight gain were calculated by the method of Sambhu and Jayaprakas (2001). Initial and final average wet weights were recorded using a standard balance. The net weight gain (g) was calculated as the difference between initial and final average wet weights.

Weight gain (g) = Mean final body weight (g) – Mean initial body weight (g)

Feed Conversion Efficiency (FCE)

FCE is defined as the fish live weight gain per unit of food fed in dry weight. FCE was calculated as follows (Sambhu and Jayaprakash, 2001).

$$\textbf{FCE} = \frac{\text{Live weight gain (g)}}{\text{Food fed (g) dry weight}}$$

Food Conversion Ratio (FCR)

Because feed is expensive, feed conversion ratio (FCR) or feed efficiency (FE) are important calculations for

the grower. They can be used to determine if feed is being used as efficiently as possible. FCR is calculated as the weight of the feed fed to the fish divided by the weight of fish growth (Mustafa and Ridzwan 2000).

$$\text{FCR} = \frac{\text{Total feed}}{\text{Wet Weight of tissue}}$$

Challenge test

After 30 days of feeding, the fish of each group were divided into two subgroups, the first subgroup of each treatment was challenged with pathogenic *A. hydrophila* (0.3 ml of 10^7 cells / ml) which was obtained from IMTECH, Chandigarh (Schaperclaus et al., 1992). The second subgroup was treated by 0.3 ml of saline as control. Both subgroups were kept under observation for 10 days to record the survival rate daily**.**

Bactericidal activity

Serum bactericidal activity to *A. hydrophila* strain (Standard strain IMTECH, Chandigarch, Dept. Of Microbilogy) was determined according to Rainger and Rowley (1993) Briefly, a 300µl of *A. hydrophila* suspension (1.5X 10^3 cells/ml) and 300µl of fresh serum were mixed in sterile ependorf tubes. The blank consisted of 300µl of bacterial suspension and 300µl of sterile PBS. The tubes were incubated at 28^0C at 24h. A 50 µl sample was removed at 0, 1, 2, 3, 4h, and different dilutions were plated on nutrient agar for 24h at 28^0C, and colony forming units (CFU) were counted. The results were recorded as survival index (SI) (Word Low & Unlles, 1978).

Survival index (SI) = CFU at end/CFU at start X 100

Results and Discussion

The percent phagocytosis, phagocytosis index, lysozyme activity and the concentrations of serum protein, albumin and globulin have been measured in the serum of *Cyprinus carpio* fingerlings fed for 30days on control (C1 and C2) and experimental (E1 and E2) diets on day 1, 10, 20 and 30 of rearing period. Results pertaining to percent phagocytosis and phagocytosis index in the serum are presented in Table-1 and Figure-1. The results clearly show that there is a significant increase in the phagocytoic activity and phagocytosis index of the serum with increase in rearing time both in control and experimental groups except on day 1. There is a significant increase ($P<0.05$) in percent phagocytosis of the serum in the experimental groups (E1 and E2) compared to control (C1 and C2) groups. For instance E1 and E2 diets enhanced phagocytic activity of the serum by 10% and 14% respectively on day 10; by 8% and 14% on day 20 and by 10% and 14% on day 30 compared to the respective C1 and C2 diets (Figure-1 and Table-1). However, there are no significant differences in serum phagocytosis of the fingerlings fed on E1 and E2 diets on all the specified rearing periods.

Figure-2 presents results on serum phagocytosis index of *Cyprinus carpio* fingerlings fed on control (C1, C2) and experimental (E1, E2) diets. It is clear from the results that there is a significant increase ($P<0.05$) in the total serum phagocytosis index with increase in rearing time both in control and experimental groups (Table-1). Apparently there are significant increases ($p<0.05$) in serum phagoctosis index of fingerlings fed on experimental diets (E1, E2) compared to those fed on control diets (C1, C2) except on day 1. Obviously E1 and E2 diets augmented total phagocytosis index by 11% and 17% on day 10; by 12% and 18% on day 20 and by 16% and 19% on day 30 compared to the respective C1 and C2 diets (Fig-2; Tab-1). However E2 diet enhanced phagocytosis index of the serum significantly than E1 on day 20 and opposite is true for day 30.

Results on the activity of serum lysozyme in the fingerlings of *C. carpio* fed on control and experimental diets are presented in Figure-3 and table-1. It is evident from the results that there is a significant increase ($P<0.05$) in the serum lysozyme activity with increase in rearing time from 1 to 30 days both in control and experimental groups. Obviously there is a significant increase ($P>0.05$) in serum lysozyme activity of the fingerlings fed on experimental diets (E1, E2) compared to those fed on control diets (C1, C2) on day 10, 20 and 30 but not on day 1. Apparently E1 and E2 diets enhanced lysozyme activity of the serum significantly by 25% and 27% on day 10; 19% and 27% on day 20 and 21% and 32% on day 30 compared to the respective C1 and C2 diets.

Results pertaining to serum protein, serum globulin and serum albumin in the fingerlings of *C. Carpio* fed on control (C1, C2) and experimental (E1, E2) diets are presented in table-2 and figures-4 (serum protein), 5 (serum globulin) and 6 (serum albumin). The results clearly show that there are significantly increases in serum protein, serum globulin and serum albumin concentrations with increase in rearing time both in control and experimental groups. Apparently there are significant increases in serum protein, serum globulin and serum albumin concentrations of fingerlings fed on E1 and E2 diets compared to those fed on C1 and C2 diets. For instance E1 and E2 diets enhanced serum protein concentration by 15% and 16% on day 10; by 12% and 13% on day 20 and by 13% and 14%

respectively on 30 compared to the respective C1 and C2 diets. Interestingly E2 diet enhanced total serum protein content significantly than E1 diet on day 10 and 20 but not on day 30 (Table-2 and Figure-4). Similarly E1 and E2 diets increased serum globulin concentrations by 11% and 19% on day 10; by 10% and 20% on day 20 and by 12% and 15% respectively on day 30 compared to the respective C1 and C2 diets. Interestingly E2 diets enhanced serum globulin content significantly than E1 diet on day 10 and 20 but not on day 30 (Table-2 and Figure-5). It is also observed that E1 and E2 diets augmented serum albumin concentrations by 11% and 13% on day 10; by 14% and 15% on day 20 and by 19% and 22% on day 30 respectively compared to the respective C1 and C2 diets. Interestingly E2 diet enhanced serum albumin content significantly than E1 diet on day 30 but on days 10 and 20 (Table-2; Figure-6).

Figure-7 and Table-3 present results on changes in weight gain of fingerlings of *C. carpio* fed for 30d on control (C1, C2) and experimental (E1, E2) diets. It is clear from the results that there is a significant increase ($P<0.001$) in the weight gain of fingerlings with increase in rearing time both in control and experimental groups with the magnitude of increase being more in fingerlings fed on E1and E2 diets than in those fed on C1 and C2 diets (Fig-8). E1 and E2 diets clearly augmented weight gain by 81% and 102% respectively on day 10; by 89% and 96% respectively on day 20 and by 91% and 97% respectively on day 30. However, the percent increase (E1-24% and E2-26%) is much less on day 1 than in other sampling periods (Fig-8 and Table-3). These results suggest that E1 and E2 diets are more effective in augmenting weight gain of fingerlings compared to C1 and C2 diets. Variations in food conversion ratio of fingerlings of *C. carpio* fed for 30d on control (C1, C2) and experimental (E1, E2) diets are presented in Figure-8 and Table-3 . The results clearly show that there is a significant decrease ($P<0.001$) in the food conversion ratio of fingerlings with increase in rearing time both in control and experimental groups with the magnitude of decrease being higher in fingerlings fed on E1, E2 diets than in those fed on C1, C2 diets. E1 and E2 diets obviously attenuated food conversion ratio by 5% and 10%respectively on day1; by 8% and 13% respectively on day 10; by 9% and 12% respectively on day 20 and by 9% and 18% respectively on day 30 with the magnitude of decrease being less on day 1 compared to other sampling periods. These results suggest that E1and E2 diets have effectively reduced food conversion ratio than C1 and C2 diets.

Changes in the food conversion efficiency of fingerlings of *C. carpio* fed for 30d on control (C1, C2) and experimental (E1, E2) diets are presented in (Fig-9 and Table-3). It is clear from the results that there is a significant increase ($P<0.001$) in the food conversion efficiency of fingerlings with increase in rearing time both in control and experimental groups. However the quantum of increase is significantly higher in the fingerlings fed E1, E2 diets than in those fed on C1, C2 diets throughout the 30 day period. E1 and E2 diets clearly enhanced food conversion efficiency by 4% and 8% respectively on day 1; by 7% and 11% respectively on day 10; by 16% and 24% respectively on day 20 and by 19% and 28% respectively on day 30; however, the magnitude of percent increase is much less on days 1 and 10 than on days 20 and 30. These results show that E1, E2 diets are more efficient in enhancing food conversion efficiency than C1, C2 diets.

Figure-10 presents results on the mortality rate *of C. carpio* fingerlings fed for 30d on control (C1, C2) and experimental (E1, E2) diets and challenged with Aeromonas hydrophila for 10d are presented in figure-10. The results clearly show that initially there is a significant increase ($P<0.01$) in the mortality rates of fingerlings fed both on control and experimental diets; however the mortality rate did not increase beyond 40/day in the fingerlings fed E1, E2 diets during 3-10d A/ hydrophila post challenge period. On the other hand in the fingerlings fed on C1, C2 diets. There was a significant increase in mortality rate from day1; reached a peak of 70/d on day 4 and stabilized at that level for the rest of the experimental period.

It is well known that phagocytosis is a process of internalization, killing and digestion of invading microorganisms and plays an important role in the prevention of infectious diseases. Several authors reported that phagocytosis is stimulated by oral administration of probiotics (Siwicki et al. 1994; Anderson et al. 1995; Rengpipat et al. 2000; Li & Gatlin III, 2003, 2004; Panigrahi et al. 2005; Taoka et al. 2006). In this study, *Spirulina* was taund to stimulate the immune system by increasing the phagocytic activity. The results presented in this study agree with Duncan and Klesius; (1996) and Qureshi and Ali, (1996), who reported that *Spirulina* had the capability of enhancing non-specific immune responses in fish. However, they demonstrated that peritoneal phagocytes from channel catfish fed on *Spirulina plantesis*, showed enhanced phagocytosis and Phagocytosis index was reported to increase chemotaxis to *Edwardsiella ictaluri* exoantigen. Watanuki et al., (2006) reported that *Spirulina*

activated the functions of leucocytes, such as phagocytosis, production of superoxide, and cytokine in the common carp, *C. Carpio*.

Application of immunostimulators, particularly herbal immunostimulants, in the aquaculture industry can be considered a remarkable advantage because they are safe and environmentally friendly (Jian and Wu, 2004). In the present study oral administration of *Spirulina* increased serum lysozyme activity to a greater extent in the fingerlings fed on experimental diets (E1 and E2) compared to those fed on control diets (C1 and C2). It has been reported that immunostimulants can enhance serum lysozyme activity (Swain et al. 2006; Yuan et al. 2007). Lysozymal activity is an important defence mechanism in fish, which causes lysis of bacteria and activation of phagocytes by acting as an opsonin. Elevated lysozyme level was reported in carp fed on immunostimulant mixed diets (Chen et al. 2003). Similar results have also been reported in *Tilapia Mosambica* following administration of *Rosmarinus officinalis* leaf powder (Selvaraj et al., 2005, Misra et al., 2006; Watanuki et al., 2006; Abutbul et al., 2004), *Eclipta alba* leaf aqueous extract (Christybapita et al., 2007) and *Zataria multiflora* essential oil (Soltani et al., 2009).

Serum total protein and globulin are considered as good indicators for determining immune system activation (Siwicki et al., 1994). Certain herbal immunostimulants have been reported to increase total protein as well as total globulin in fish (Vasudeva et al., 2004). However, other reports indicate a lack of immunostimulant influence on serum proteins in fish populations (Ispir and Mustafa, 2005; Misra et al., 2006). The results of this study clearly show that total serum protein, albumin and globulin concentrations markedly increased in fish fed on diet containing *Spirulina* compared to those fed on control diet. Suggesting an improvement in fish health after beuig fed on *Spirulina* enriched diets. Moreover, the measurement of albumin, globulin and total protein in serum is of considerable diagnostic value in fish, as it relates to the general nutritional status as well as the integrity of the vascular system and liver function (Schaperclaus et al., 1992).

In general growth rates of fish are highly variable and, in many cases, seem to be limited by the availability of food. Periods of feed restriction or feed deprivation resulting in growth retardation or loss of weight do not appear to affect the ability of fish to grow when they are returned to full rations. Conversely there is evidence to suggest that fish may show periods of rapid weight gain, commonly known as compensatory or catch-up growth, following periods of feed restriction. The importance of feed utilization in relation to compensatory growth has been high lighted in several fish species (Bull and Metcalfe, 1997; Rueda et al., 1998). A number of investigators have suggested that compensatory growth could be exploited in the commercial production of fish to manipulate rates of weight gain or to improve growth efficiency with the inclusion of herbal ingredients in the formulated feeds (Ogino, 1980).

The results of this study have clearly shown that *Spirulina* supplemented diets have significantly enhanced the total body weight of *C. carpio* fingerlings when compared to control diets. Although both control and experimental diets increased total body weight over the 30d experimental period the magnitude of increase was significantly higher in fingerlings fed on E1, E2 diets (Smith 1981; Fauconneau 1984; Soivio et al., 1989; Abdel-Tawwab et al., 2006). It is interesting to note that the total body weight at the end of 30th day increased approximately 6 times in the fingerlings fed on C1, C2 diets as against 9 times in those fed on E1, E2 diets. These results suggest that *Spirulina* a component of supplemented formulated diets might have contributed to positive growth by enhancing food conversion efficiency. Similar results have also been obtained in the present study. The results also show that there is a significant increase in the weight gain of *C. carpio* fingerlings fed on E1, E2 diets than in those fed on C1, C2 diets on par with total body weight. Interestingly the total weight gain at the end of 30th day is approximately 20 times in the fingerlings fed on C1, C2 diets as against 36 times in those fed on E1, E2 diets suggesting that *Spirulina* included in the supplemented formulated feeds might be responsible for such an increase in weight gain.

The results also show that there is a significant decrease in the food conversion ratio and a significant increase in the food conversion efficiency of *Cyprinus carpio* fingerlings fed both on C1, C2 and E1, E2 diets. However the magnitude of changes both in food conversion ratio and in food conversion efficiency is significantly higher with E1, E2 diets compared to C1, C2 diets. Similar results have been reported in other fishes as well with regard to FCR and FCE (Quinton and Blake, 1990). It is general knowledge that a lower FCR and higher FCE always promote growth in fish and the results of the present study are in consonance with this general observation. Obviously *Spirulina*, included as a supplement in formulated diet appears to influence the physiological processes in such a way

that *C. carpio* fingerlings show much better performance as far as growth is concerned.

The results of the challenge test showed that *Spirulina* supplementation provided certain amount of protection against *Aeromonas hydrophila* although not total protection. For instance mortality rate is 50% less in *C. Carpio* fingerlings fed on *Splirulina* supplanted diets than in those fed on control diets and challenged with *A. hydrophila* suggesting moderate amounts of disease resistance was reported in *C. carpio*, tilapia and rohu fed on *Spirulina s*upplemented diets and challenged with *A. hydrophila* in other studies (Selvaraj et al. 2005, Misra et al., 2006); Watanuki et al., 2006; Abdel-Tawwab and Ahmad, 2009; Andrews et al., 2011). The lack of greater protection against bacterial challenge in this study, unlike in previous studies, may be due to the lower levels of dietary *Spirulina* supplementation or the species of the *Spirulina* itself. *Spirulina* contains numerous bioactive materials including phytopigments, such as phycobilins, phycocyanin, allophycocyanin and xanthophylls, which make it a potential natural dietary source of antioxidants (Miranda et al. 1998; Bhat and Madyastha, 2000; Wang et al. 2007; Bermejo et al. 2008). The results of the present study, clearly show that the diets supplemented with *Spirulina* had attenuating effects on the mortality role due to *A. hydrophila*.

Challenge Experiments have shown that *Spirulina* increased protection against pathogenic bacteria in carp especially through activation of specific defence mechanisms (Anderson et al., 1995; Sakai, 1999). This study provides evidence that components of *C. Carpio* immune system in such as Phagoctic activity, serum phagocytic index and serum lysozyme activity could be enhanced by feeding fish with *Spirulina* incorporated diet. Incorporation of *Spirulina* into feed holds a potential for immune enhancement in *C. carpio* thereby increasing the resistance of the fish to diseases which may reduce fish mortality rates and offer economic benefits.

Conclusion

In summary it is concluded that *Spirulina* positively influenced the growth performance and feed efficiency of *Cyprinus carpio* as well as resistance to *A. hydrophila* infection.

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Table 1: Changes in the levels of Serum phagocytosis, phagocytosis index and Lysozyme activity of fingerlings of C*yprinus carpio* fed with control (C1, C2) and experimental (E1, E2) diets values are mean±SE of six observations

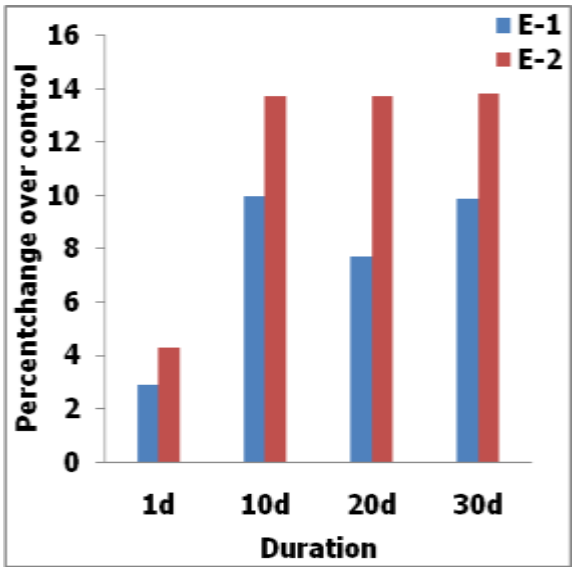
Parameter	Treatments	Duration			
		1d	10d	20d	30d
Phagocytosis	C-1	16.49 ±0.09	20.52 ±0.26	31.97 ±1.67	41.55 ±1.27
	ED-1	16.00 ±1.29*	25.57 ±0.74*	35.43 ±0.47*	45.66 ±0.1*
	C-2	16.49 ±0.12	20.40 ±0.25	34.17 ±0.63	41.79 ±1.17
	ED-2	16.67 ±0.07*	25.51 ±0.77*	38.87 ±0.55*	47.57 ±0.03*
Phagocytosis index	C-1	5.72 ±0.05	11.52 ±0.05	15.53 ±0.31	17.77 ±0.22
	ED-1	5.74 ±0.06*	13.39 ±0.43*	17.39 ±0.02*	20.60 ±0.08*
	C-2	5.83 ±0.10	12.47 ±0.10	16.75 ±0.02	22.52 ±0.06
	ED-2	5.96 ±0.03*	14.56 ±0.01*	19.75 ±0.02*	24.72 ±0.40*

	C-1	0.05 ±0.005	0.44 ±0.02	0.67 ±0.01	0.86 ±0.02
Lysozyme	**ED-1**	0.10 ±0.009*	0.55 ±0.02*	0.94 ±0.01*	1.04 ±0.02*
	C-2	0.07 ±0.005	0.49 ±0.01	0.75 ±0.05	0.93 ±0.02
	ED-2	0.15 ±0.02*	0.73 ±0.02*	0.95 ±0.01*	1.22 ±0.06*

Values similarly marked are not significantly different (P<0.001) from each other.

Table 1a: Composition on dry matter bases of the control and experimental diets

Ingredients	**Percentage %**
Rice Bran	21
Ground nut oil cake	10
Soybean cake	10
Fish meal	29
Coconut oil cake	7
Prawn meal	16
Vitamin premix	1
Mineral premix	1
Spirulina	5.00
Total	100



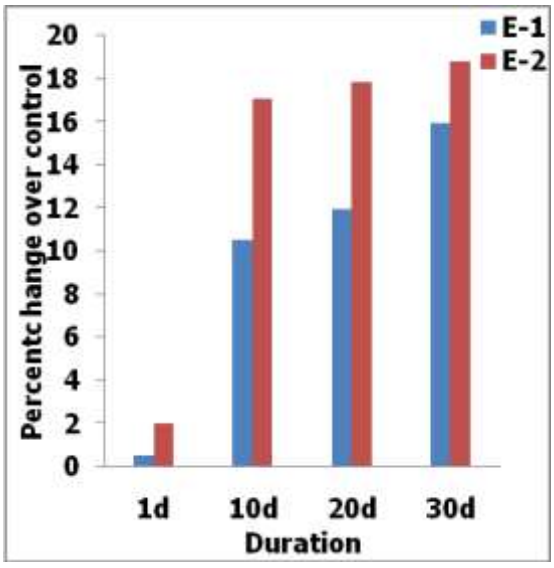


Fig. 1: % Changes in the levels of Phagocytic activity in fingerlings of *C. carpio* fed with E1, E2 diets
Fig. 2: % Changes in the levels of Phagocytosis Index in fingerlings of *C. carpio* fed with E1, E2 diets

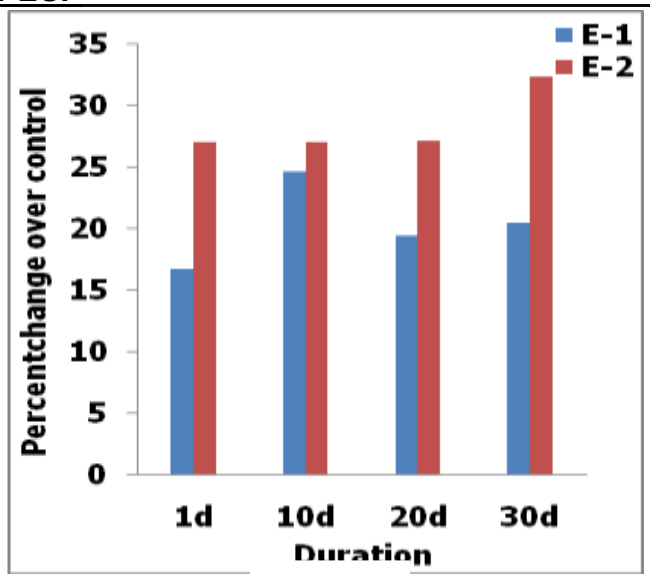


Fig. 3: % Changes in the levels of serum Lysozyme activity in the fingerlings of *C. carpio* fed with E1, E2 diets

Table 2: Changes in the levels of Serum Proteins, Albumin and Globulin of fingerlings of *C. carpio* fed with different diets (C1, C2, E1, E2). Values are mean±SE of six observations

Parameter	Treatments	Duration			
		1d	10d	20d	30d
Protein	C-1	19.67 ±0.12	22.38 ±0.07	25.37 ±0.08	30.17 ±0.08
	ED-1	22.13 ±0.05	25.68 ±0.09	28.58 ±0.13	34.23 ±0.01
	C-2	20.35 ±0.23	22.18 ±0.14	26.83 ±0.96	32.6 ±0.15
	ED-2	22.55 ±0.21	25.77 ±0.15	30.35 ±0.29	37.33 ±0.02
Globulin	C-1	9.18 ±0.22	9.67 ±0.49	9.68 ±0.89	11.48 ±0.83
	ED-1	10.02 ±0.07	10.78 ±0.09	11.05 ±0.18	13.67 ±0.18
	C-2	9.15 ±0.14	9.83 ±0.32	10.5 ±0.67	11.55 ±0.31
	ED-2	10.1 ±0.23	11.14 ±0.19	12.08 ±0.30	14.7 ±0.17
Albumin	C-1	10.38 ±0.25	12.55 ±0.02	16.02 ±0.96	15.33 ±0.05
	ED-1	11.42 ±0.62	14.9 ±1.67	17.48 ±1.43	20.57 ±1.88
	C-2	10.49 ±0.19	12.52 ±0.31	15.62 ±0.07	19.89 ±0.38
	ED-2	11.58 ±0.04	14.9 ±0.08	18.75 ±0.05	22.85 ±0.09

Values similarly marked are not significantly different (P<0.001) from each other.

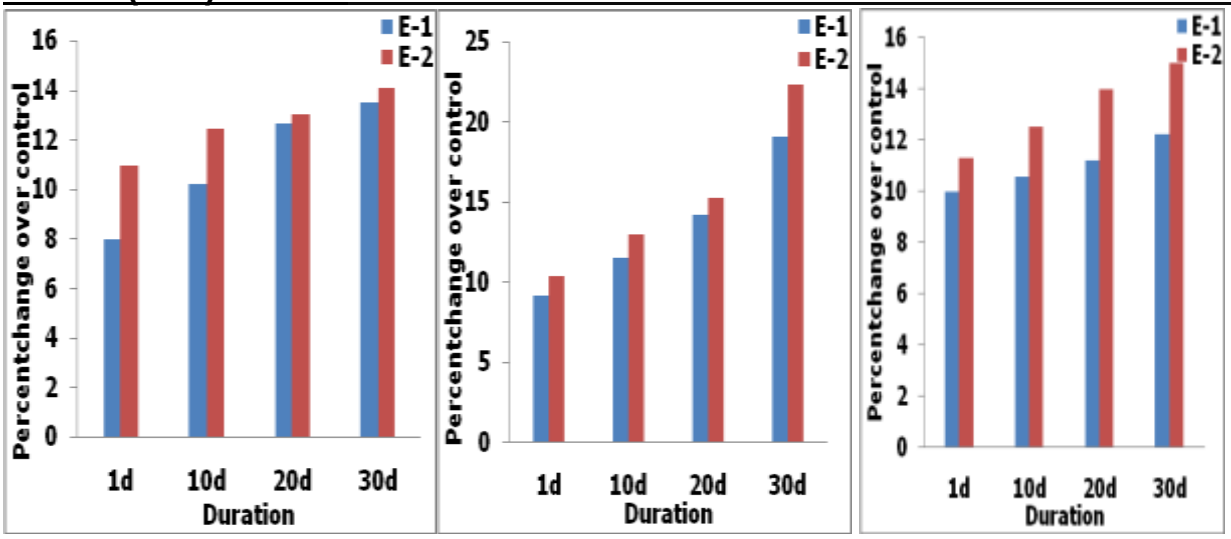
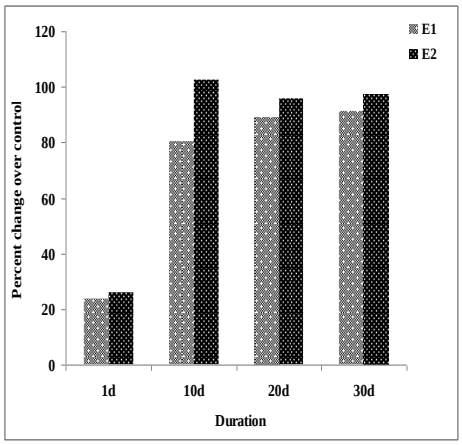


Fig. 4: % Changes in the levels of Total Serum Protein in the fingerlings of *C. carpio* fed with E1, E2 diets
Fig. 5: % Changes in the levels of globulin in the fingerlings of *C. carpio* fed with E1, E2 diets
Fig. 6: % Changes in the levels of Albumin in the fingerlings of *C. carpio* fed with E1, E2 diets

Table 3: Changes in the levels of Growth parameters of fingerlings of *C. carpio* fed with different diets (C1, C2, E1, E2) values are mean±SE of six observations

Parameter	Treatments	Duration			
		1d	10d	20d	30d
Weight Gain	C-1	3.33 ±2.37	18.44 ±1.98	35.83 ±1.78	60.25 ±1.84
	ED-1	2.24 ±2.48	31.46 ±2.44	62.92 ±1.10	104.37 ±2.16
	C-2	0.4 ±0.005	16.55 ±1.99	33.45 ±2.41	65.08 ±1.98
	ED-2	0.41 ±1.15	31.95 ±0.80	61.52 ±1.01	10.883 ±2.32
FCR	C-1	0.06 ±0.004	0.03 ±0.004	0.02 ±0.001	0.02 ±0.0002
	ED-1	0.067 ±0.007	0.037 ±0.001	0.028 ±0.0006	0.024 ±0.0002
	C-2	0.059 ±0.005	0.029 ±0.002	0.024 ±0.001	0.022 ±0.0004
	ED-2	0.063 ±0.006	0.035 ±0.0009	0.028 ±0.0003	0.023 ±0.0002
FCE	C-1	14.89 ±1.707	27.02 ±1.12	35.35 ±1.74	40.69 ±0.54
	ED-1	16.34 ±1.28	35.88 ±3.70	41.01 ±0.86	49.27 ±0.59
	C-2	15.88 ±1.82	27.98 ±1.92	35.62 ±2.10	42.00 ±1.30
	ED-2	17.07 ±1.41	34.38 ±1.91	44.70 ±0.53	51.28 ±0.44

Values similarly marked are not significantly different (P<0.001) from each other



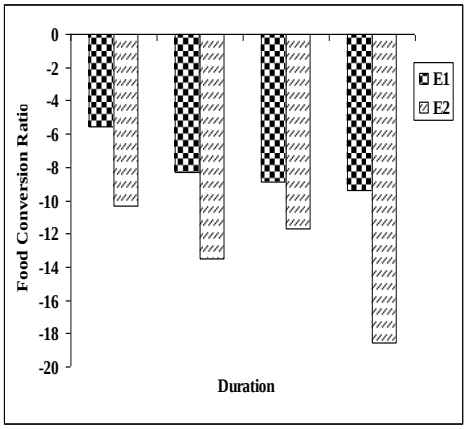
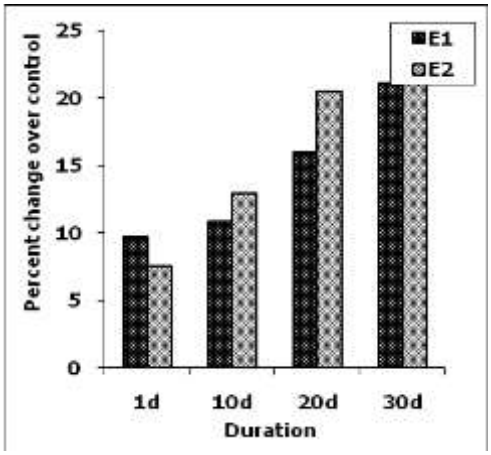


Fig. 7: % Changes in the levels of Weight Gain (gms) in the fingerlings of *C. carpio* fed with E1, E2 diets
Fig. 8: % Changes in the levels of Feed Conversion Ratio (FCR) in the fingerlings of *C. carpio* fed with E1, E2 diets



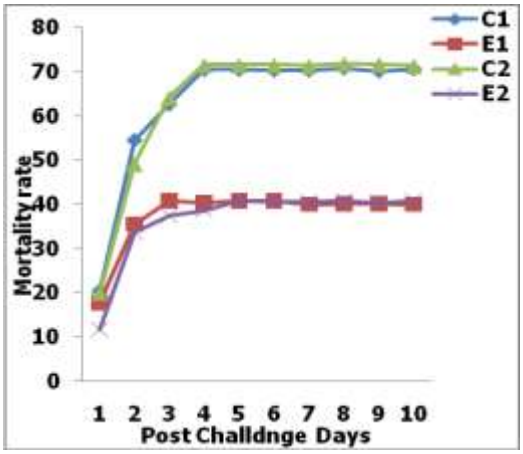


Fig. 9: % Changes in the levels of Food Conversion Efficiency (FCE) in the fingerlings of C*yprinus carpio* fed with E1, E2 diets
Fig. 10: Mortality rate (No./day) of fingerlings of *Cyprinus caarpio* fed with different diets (C1, E1 C2 and E2) at the challenged by *A. Hydrophila*

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