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Studies on the ameliorative effects of a probiotic consortium on normal and *Aeromonas hydrophila* -infected freshwater fish, *Labeo rohita*

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ABSTRACT

This present study was carried out, the use of probiotic consortium in freshwater aquaculture to be highly effective in improving disease resistance, survival, growth and nutrition. In this probiotic consortium was used for different concentrations of the fish feed such as 1%, 3%, and 5%. We have shown that *Labeo rohita* fed a probiotic supplemented diet have an improved survival and growth in 60 days culture animals every 20 days *Aeromonas hydrophila* infected in fish. Maximum weight gain (21.6g) was observed after 60 days in 5% probiotic fed animals. The maximum protein content in (32.5 mg %) was observed in muscle after 60 days animals. Increasing in WBC cells in infected animals which probably indicated increased in disease fighting capability of the fish. The general conclusion obtained from the present study is that the probiotics play a vital role in growth survival, protein, and glycogen, enumeration of blood cells and protein separation of the animals compared to control animals. The supplementation of the probiotic consortium diet is probably effective for rearing conditions.

Keywords: Probiotic, Aquaculture, Growth Survival, Protein, Glycogen, Blood cells.

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INTRODUCTION

Today freshwater aquaculture fish diseases are a major problem for the fish farming industry. In India are almost the top third rank producers of fresh water fish aquaculture involved breeding of fish like as rohu, catla, carp, measure and freshwater shrimp[1]. *Labeo rohita* are affected by a huge number of environmental factors such as the problem of water pollution and food mainly affected freshwater fish and marine fish, etc., which was recently eaten the intestinal normal flora changed, tissue damage, inactivation of enzymes, and damage to genetic materials and other vital cell components. If it may be due to a variety of causes of biotoxins such as tuberculosis, eye infection, kidney diseases and various types of tumors in consuming peoples. These beauticians are not destroyed by cooking and cause illness when contaminated fish containing them are eaten. Some poisons affect the central nervous system, while others cause gastrointestinal and disorders in human beings.

Some people are allergic to freshwater *Labeo rohita* fish. Which its currently fastest growing food-protein producing sector with an annual increase of approximately 9%, among those, bacterial infections are considered as the major cause of mortality in fish hatcheries[2]. In addition, *Labeo rohita* the most important species in Indian corps. Which are highly nutritious sources of easily digestible proteins, amino acids and mineral salts and fish oils like calcium, phosphorous, iron, sodium, potassium, magnesium, and sulfur, vitamins such as A, D health promoting fat.

The *Aeromonas hydrophilia* is a gram negative, rod shaped, motile, affects a wide variety of freshwater fish species and as well as marine fish. Which is causing ulcer disease or redsoree diseases, Hem-Morhagic Septicemia etc., it is a zoonotic disease occurs worldwide. The prophylactic and therapeutic control of the bacterial diseases is based on oral administration of antibiotics. Such treatment may cause the development of antibiotic resistant bacteria. In addition, the normal (anaerobic) gut flora in the gastrointestinal tract, which is beneficial to aquaculture fish, may be inhibited due to oral chemotherapy[3]. Supposing that probiotic are being developed and marketed, they cannot be used alone as a universal disease control measure in aquaculture food.

In the modern technological approach methods, that is gaining acceptance within the industry, is the use of probiotic substance that contains live organisms thought to be highly effective in improving disease resistance survival, growth and nutrition, and is rapidly becoming a popular economically important research development in the aquaculture farming[4,5,6]

Aqualact is a powerful spore forming microorganisms; it is a possible competition with *Aeromonas hydrophilia* in the fish gastrointestinal tract, and is stimulation of the immune system, as the activation of macrophage[7]. The antibacterial effects of live microorganisms (aqualacts) are generally due to produce of antibiotics, bacteriocins, siderophores, lysozymes and proteases, and alteration of pH values by organic acid production. Which are using immunostimulants by stimulating phagocytic activity; complement mediated *Aeromonas hydrophilia* killing and immunoglobulin (Ig) production. Therefore, this present study is aimed to evaluate the role of Aqualact as growth promoters and antibacterials for *Labeo rohita* and their effect on some physiological parameters such as growth survival, protein and glycogen, enumeration of blood cells and protein separation of the animals compared to control animals. The supplementation of the probiotic consortium diet is probably effective for rearing conditions, isolation and identification of fish intestinal pathogenic organism.

MATERIALS AND METHODS

Fish collections and experimental conditions

The healthy *Labeo rohita* fishes of both sexes were collected from a fish farm at Thirumanur, Perambalur District, Tamil Nadu, India (mean individual initial weight range of 9 to 15 g and length 5 to 9 cm). Fish were maintained in the aquaria for a couple weeks for adaptation and continuous aeration was allowed to maintained as described by[8].

Preparation of Probiotic feed

Fish were fed on a balanced commercial diet at a ratio (Dry fish powder, Tapiaco floor, Wheat flour, Mineral mix, Vitamin mix, Aqualact (probiotic) in the preparations was found to be diet 3% of body weight per day. The water was partially changed daily along with waste feed and fecal material and monitored regularly; the freshwater temperature maintained at 25+5^{o}C.

Experimental set up for In-vitro studies

Growth experiments were carried out for a period of 60 days in the laboratory. The fishes were weighed accurately in digital electronic balance before the start of the experiment. Healthy live specimens of *Labeo rohita* in the weight range of 9 – 15 g and length 5 – 9 cm. The fishes were fed with test diets at 3% of their body weight daily, which was split into two rations, one feeding in the morning and another in the evening. The feed was supplied to the fish in the trough by keeping it in a glass plate.

Growth related parameters

1. Feed consumed (g) = Total amount of feed given (g) - Total amount of uneaten feed (g)

2. Mean feed intake (g) = $\frac{\text{Total feed consumed (g)}}{\text{(Initial number of animals + final numbers of animals) / 2}}$

3. Growth in terms of wet Weight gain = Final wet weight of fishes (g) after 20 days off rearing - Initial wet weight of Fishes (g)

4. Food conversion ratio (FCR) = $\frac{\text{Total feed consumed (g)}}{\text{Wet weight gained (g)}}$

5. Percentage of survival = $\frac{\text{Number of fishes survived At the End of the experiment}}{\text{A number of the fishes stocked At the start of the experiment}} \text{X100}$

6. Specific growth rate (SGR) = $\frac{\text{Line of final mean wet weight – Line of initial mean wet weight}}{\text{Days of culture}} \text{X100}$

Inoculation of Fish Pathogenic Microorganisms

Aeromonas hydrophila was used for challenging the post feed supplemented animals and control fishes. The virulence of the *Aeromonas hydrophila* was tested by determining the LD_{50} by intramuscular administration of various doses in *Labeo rohita.*

Hematological analysis

The red blood cell counts (RBC: 10^6 mm^{-3}) were determined in a 1:20 dilution of the blood sample in Hayem's solution and the white blood cell counts (WBC: 10^4 mm^{-3}) from 1:200 dilution of the blood sample in Turke's solution with a Neubauer haemocytometer. The Hb (g/dl) was determined by cyanhaemglobin method. Packed cell volume was determined by[9,10].

Biochemical composition

Liver, abdominal muscle and Kidney of *Labeo rohita*, from the various lines of experimental treatments, were analyzed for the biochemical parameters such as protein[11] and glycogen[12].

Isolation and Identification gut microorganism

The entire inner surface was scraped into 0.85% saline and uniformly mixed. The mixture was serially diluted with 0.85% saline and inoculated on nutrient agar Petri plates. Similarly the fore- and hindgut were also scraped and diluted with saline solution. The petri plates were subjected to

incubation for 24-48h.Identification of gut microbes was carried out based on following biochemical characteristics of bacterial isolates.

Analysis by SDS-PAGE

A special form of poly acrylamide gel electrophoresis is Sodium dodecyl sulfate –poly acrylamide gel electrophoresis. In this technique a protein mixture is first denatured with SDS and β-2- mercaptoethonal, which results in a reduction of the S-S-bridge in the protein and a dissociation of the polypeptide chains. The SDS forms a complex with the polypeptides.

Statistical Analysis

For all the animals under study, the mean value of glycogen and protein content in each tissue, both in control and experimental fish was estimated out and standard deviation. Two way ANOVA of the results was carried out using a statistical package (SPSS).

RESULTS AND DISCUSSION

Variation in growth parameters in relation to fees supplementation

Feeding on the fish to feed incorporated with 1%, 3% and 5% w/w of a consortium of probiotic powder. Wet weight gained (21.6g) in supplementing feed-fed fish showed a gradual increase when compared to the control, fed on normal diet. Probiotic supplemented in infected animals recorded slight decrease in wet weight-gain when compared to control animals (Table-1).

Table -1: Growth parameters in different feed supplemented group of fish *Labeo rohita*

S. No	Growth parameters	Duration of Treatment	After probiotic Supplementation				After Probiotic supplementation and Aeromonas infection			
			Control	1%	3%	5%	Control	1%	3%	5%
1	Mean feed	20days	1.07	1.05	1.08	1.16	1.2	1.2	1.10	1.23
2	intake (g)	40days	1.27	1.11	1.95	1.50	1.56	1.05	1.42	1.49
3		60days	1.44	1.20	2.64	2.82	1.65	1.10	2.30	2.5
4	Wet weight	20days	1.2	1.5	1.8	3.2	1.0	1.2	1.5	2.9
5	gained (g)	40days	9.7	10.2	11.9	13.4	8.9	7.4	9.4	10.5
6		60days	11.5	12.5	14.1	21.6	9.5	10.6	12.4	18.6
7	Food	20days	0.07	0.51	0.76	0.81	0.53	0.61	0.66	0.75
8	conversion	40days	1.27	0.78	0.88	0.93	0.65	0.67	0.71	0.82
9	ratio(FCR)	60days	1.7	1.02	1.21	1.33	1.0	0.92	1.12	1.03
10	Specific	20days	5.8	21.0	22.0	35.0	7.0	26.0	21.0	26.3
11	growth rate	40days	37.5	30.0	32.0	53.2	30.0	29.6	32.1	30.2
12	(cms)	60days	58.5	50.2	54.0	60.1	38.0	48.0	41.0	55.1
13	Feed	20days	0.79	0.75	0.87	0.97	0.88	0.69	0.72	0.83
14	consumed	40days	1.27	1.02	1.32	1.44	1.25	1.16	1.19	1.21
15	(g)	60days	1.7	1.02	1.21	1.33	1.0	0.92	1.12	1.03
16	Percentage of	60days	-	-	-	-	53.8	61.5	69.2	84.6
17	survival	60days	-	-	-	-	69.2	76.9	84.6	92.3
18		60days	-	-	-	-	92.3	100	100	100

The food conversion ratio (FCR) of probiotic supplemented fish showed a gradual increase over the control animals. Maximum FCR of 1.33 was recorded in 5% probiotic supplemented uninfected animal group a similar trend of having low FCR was evident. Infection caused a general decrease in FCR.

The specific growth rate (SGR) showed a gradual increase over the control animals. Maximum SGR of 60.0cm was recorded in 5% probiotic supplemented uninfected animals. In comparison with the infected animals after feed- supplementation for 60days, a slight decrease in SGR was evident. In probiotic incorporated feeds-fed fish, the feed consumption increased during the entire period of 60days and the maximum intake was recorded in 5% probiotic supplemented animal with 1.92g.

Tissue Protein

The protein content of the three tissues (liver, muscle, and kidney) of *Labeo rohita* was observed for 20days, 40days and 60days duration in both normal and infected fish. The values varied between 1.9 and 26.6 mg % the highest concentration in muscle and the lowest in kidney tissue. In muscle tissue, on the 20th days of supplemented feeding a gradual increase in protein could be observed, compared to those fed with normal diet. Maximum protein concentration in muscle, 32.5mg% was observed after 60days in 5% probiotic fed animals (Table-2, 3, 4).

Table. 2. Protein content (mg %) in muscle of *Labeo rohita*, supplemented with probiotic consortium (mean values ± Standard deviation)

S. No	Treatments	20days		40days		60days	
		Normal	Infected	Normal	Infected	Normal	Infected
1	Control	26.6±0.	18*±0.3	18.3*±0.2	10.1*±0.1	24.2±0.1	17.8*±0.2
2	1% Probiotic	27.9±0.	22.8*±0.4	30.2±0.2	17.2*±0.2	26.3±0.2	19.3*±0.2
3	3% Probiotic	28.8±0.2	25.0±0.9	32.6±0.6	18.8*±0.1	29.5±0.2	20.8*±0.3
4	5% Probiotic	29.3±0.2	18.4*±0.3	24.8±0.1	18.9*±0.5	32.5±0.2	21.3*±0.1

Table. 3. Protein content (mg %) in Liver of *Labeo rohita*, supplemented with probiotic Consortium (mean values ± Standard deviation)

S. No	Treatments	20days		40days		60days	
		Normal	Infected	Normal	Infected	Normal	Infected
1	Control	2.2±0.1	2.3±0.1	4.3±0.2	6.2±0.1	2.6±0.1	2.3±0.2
2	1% Probiotic	3.2±0.1	5.2*±0.1	6.2±0.2	3.6*±0.1	8.4±0.1	6.9±0.2
3	3% Probiotic	4.8±0.2	5.8±0.4	3.7±0.1	2.6±0.1	7.6±0.1	3.3*±0.2
4	5% Probiotic	4.2±0.2	6.5±0.3	3.2±0.1	6.3±0.5	4.6±0.2	3.6±0.1

The protein content in liver tissues of uninfected fish showed a gradual increase over the control animals and the values ranged between 2.2 and 8.4mg%. A maximum protein content of 8.4mg was recorded in 1% probiotic supplemented uninfected animal group on the 60th days. Generally

degrees protein content in infected animals. In kidney-tissue, there was a gradual increase in protein content corresponding to the duration of probiotic supplement feeding. Protein content ranged between 2.2 and 6.6mg % in the entire experiment period of 60days.Maximum protein content was recorded in the 3% probiotic supplemented group. Reduced level of proteins observed in the kidney tissues.

Table. 4. Protein content (mg %) in Kidney of *Labeo rohita*, supplemented with probiotic consortium (mean values ± Standard deviation)

S. No	Treatments	20days		40days		60days	
		Normal	Infected	Normal	Infected	Normal	Infected
1	Control	2.2±0.1	1.9±0.1	3.2±0.2	4.3±0.1	5.4±0.1	4.2±0.2
2	1% Probiotic	2.5±0.1	3.5±0.1	2.3±0.2	2.1±0.1	5.6±0.1	4.1±0.2
3	3% Probiotic	2.9±0.2	3.4±0.2	2.5±0.4	2.1±0.1	6.6±0.1	3.7*±0.2
4	5% Probiotic	2.3±0.2	1.8±0.3	1.8±0.1	2.2±0.5	3.2±0.2	1.2*±0.1

Note: Table 2, 3, 4; * indicates that those values are significantly different from those of the normal animals subjected to a similar experiment period (student‘t’ test, $p<0.05$

Tissue Glycogen

This variation in glycogen content in all the three tissues was in accordance with the concentration of probiotic used. In many instances there were significant variations in the glycogen content of probiotic supplemented normal animals and infected animals (t-value significantly at $P < 0.05$) (Table-5, 6, 7). In the tissues of infected fish there was a slight decrease in glycogen content as evidenced by the highly significant F-value. With regard to glycogen variation among the tissues, liver recorded maximum concentration while muscle and kidney showed lower concentrations. Among the animal feeds, 5% and3%probiotic supplemented feeds could raise the glycogen content even higher than the control. 1% probiotic ranked next among the feeds in promoting the glycogen content. Between the durations of probiotic supplementation ‘60days’ recorded maximum followed by ‘20days’ and ‘40days’ animals.

Table. 5. Glycogen content (mg %) in muscle of *Labeo rohita*, supplemented with probiotic consortium (mean values ± Standard deviation)

S. No	Treatments	20days		40days		60days	
		Normal	Infected	Normal	Infected	Normal	Infected
1	Control	2.9±0.1	1.5*±0.1	1.0±0.2	2.0±0.1	2.7±0.1	2.6±0.1
2	1% Probiotic	5.0±0.1	6.6*±0.1	2.0±0.2	1.6±0.1	4.5±0.1	2.3*±0.2
3	3% Probiotic	8.6±0.2	8.4±0.4	3.3±0.1	1.3*±0.1	5.2±0.1	5.3±0.1
4	5% Probiotic	5.5±0.2	9.0*±0.3	3.3±0.1	1.3±0.5	4.7±0.2	1.3*±0.1

Table. 6. Glycogen content (mg %) in Liver of Labeo rohita, supplemented with probiotic Consortium (mean values ± Standard deviation)

S. No	Treatments	20days		40days		60days	
		Normal	Infected	Normal	Infected	Normal	Infected
1	Control	22.3±0.1	13.3*±0.1	27.7±0.2	16.7*±0.1	31.7±0.4	17.0*±0.7
2	1% Probiotic	22.5±0.1	22.0±0.1	28.0±0.2	24.1*±0.1	31.0±1.3	25.6±0.7
3	3% Probiotic	24.5±0.2	22.8*±0.4	30.7±0.1	22.7±0.1	36.6±0.1	26.6*±0.1
4	5% Probiotic	25.7±0.2	22.0±0.3	33.6±0.1	23.6*±0.5	38.5±0.2	25.6*±0.1

Table. 7. Glycogen content (mg %) in Kidney of *Labeo rohita,* supplemented with probiotic consortium (mean values ± Standard deviation)

S. No	Treatments	20days		40days		60days	
		Normal	Infected	Normal	Infected	Normal	Infected
1	Control	2.0±0.1	1.3±0.1	1.2±0.2	1.7±0.1	1.1±0.1	1.0±0.2
2	1% Probiotic	1.5±0.1	5.6*±0.1	1.3±0.2	1.3±0.1	1.3±0.1	1.2±0.2
3	3% Probiotic	1.1±0.2	6.7*±0.2	1.1±0.4	1.5±0.1	1.7±0.1	1.7±0.2
4	5% Probiotic	1.7±0.2	7.6*±0.3	1.2±0.1	1.3±0.5	2.0±0.2	1.9±0.1

HEMATOLOGICAL ANALYSIS

R.B.C

Fish maintained on the normal probiotic supplemented feed had total RBC to the level of 1.18 – 3.0 million cells/Cu mm. RBC counts decreased in the infected animals (1.6 –2.6 million cells/Cu mm) (Table-8). When infected animals were treated with 1%, 3%, and 5% probiotics, a definite increase in RBC (1.7 –2.6 million cells/Cu mm) could be observed, though not up to the uninfected animal's level, under similar feed-regimen (Table-8). Analysis of Variance showed significant variation in RBC between infected and normal animals fed on probiotics.

Table. 8. RBC count (millions/cc) of *Labeo rohita*, supplemented with probiotic consortium (mean values ± Standard deviation)

S. No	Treatments	20days		40days		60days	
		Normal	Infected	Normal	Infected	Normal	Infected
1	Control	1.8±0.1	1.6±0.1	2.4±0.2	2.0±0.2	2.3±0.1	1.8±0.1
2	1% Probiotic	1.9±0.1	1.7±0.1	2.6±0.2	2.3±0.1	2.4±0.1	2.1±0.2
3	3% Probiotic	2.2±0.2	1.7±0.4	3.0±0.1	2.6±0.1	2.4±0.1	2.1±0.1
4	5% Probiotic	2.0±0.2	1.6±0.3	2.8±0.1	2.3±0.5	2.5±0.2	2.0±0.1

Note:'t' test showed that the differences between the normal and infected fish of each category were not significant

W.B.C

In fish fed on normal compounded feed and at feeding supplemented with 1%, 3%, and 5% probiotics, the white blood cells were to the tune of 7.2 – 11.2 thousand cells/cu.mm. WBC counts were almost equal in the fish fed on probiotics in both normal and infected animals in the

first 20 days treatment (Table-9). In infected fish treated with Probiotic incorporated feeds, a slight decrease in WBC count was observed with all the feed groups over their uninfected pairs. A maximum gain of 10.12 thousand cells/cu.mm WBC was observed with 3% probiotic fed animals, followed by the 5% probiotic fed group.

Table. 9. WBC count (Thousands/cc) of *Labeo rohita* supplemented with probiotic consortium (Mean values ± Standard deviation)

S. No	Treatments	20days		40days		60days	
		Normal	Infected	Normal	Infected	Normal	Infected
1	Control	9600±50	8200±200	7866±208	5890±60	9800±100	8600±50
2	1% Probiotic	9959±31	9233±76	9166±305	8208±102	9650±132	8700±100
3	3% Probiotic	9700±50	9216±58	11916±76	8950±50	11116±76	9870±60
4	5% Probiotic	9546±50	8800±100	10000±100	8816±76	10633±152	9700±100

Note: Values with * superscript are significantly from values for the normal animals of the same experimental animals.

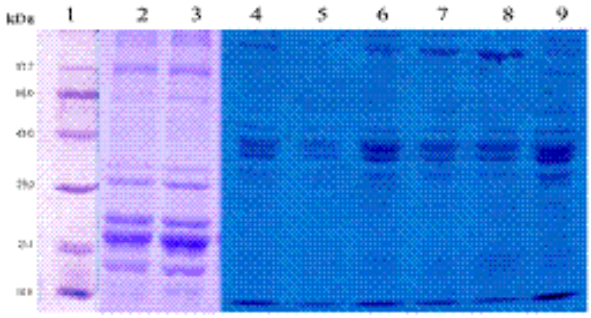
Identification of Microbes

Isolated bacterial colonies were identified based on their physical characteristics, biochemical tests and exoenzyme production. Gut microbiota of fishes collected showed the strains namely; *Bacillus* Sp, *Citrobacter*, *Staphylococcus* Sp, *Acinetobacter* Sp, *Paenibacillus* Sp, and *Corynebacterium* Sp. We're represented in gut microbiota of *Labeo rohita.*

Analysis by SDS-PAGE

Electrophorogram of muscle proteins of *Labeo rohita* both normal and *A. Hydrophila* infected and provided with three concentrations of 1%, 3% and 5% of probiotic supplementation showed distinct polypeptide fractions arranged in the order of electrophoretic migration (Figure -1). Lane -2 (Control), lane-4 (1%) lane-6 (3%) and lane-8 (5%) all represented muscle protein of a normal fish and these lanes uniformly had polypeptide fractions of 14.4-97.7 KD regions. Lane -3 (Control), lane-4 (1%) lane-6 (3%) and lane-8 (5%) all represented muscle protein of infected fish and they also uniformly had polypeptide fractions of 14.4-97.7 KD regions. Normal fish had highly pronounced polypeptide fractions between 21.1 – 43.0KD. Feed supplemented groups had several dense polypeptide fractions at the 29.9-43 KD regions probably indicating the comparatively high level of nutritional enrichment by the probiotic supplementation. There was the gradual disappearance of protein fractions after infection.

The use of probiotics for disease control in aquaculture is an area of increasing interest, as the use of antibiotics is causing concern over the possible development of antibiotic resistant bacteria. Probiotics have been defined by the world health organization food and agriculture



Lane 1: Marker
Lane 2: Control diet feed fishes (Normal)
Lane 3: Control diet feed fishes (Infected)
Lane 4: 1% Probiotic supplemented diet feed fishes (Normal)
Lane 5: 1% Probiotic supplemented diet feed fishes (Infected)
Lane 6: 3% Probiotic supplemented diet feed fishes (Normal)
Lane 7: 3% Probiotic supplemented diet feed fishes (Infected)
Lane 8: 5% Probiotic supplemented diet feed fishes (Normal)
Lane 9: 5% Probiotic supplemented diet feed fishes (Infected)

Figure- 1: Electrophorogram of Proteins in the muscle of normal and *Aeromonas hydrophila* infected *Labeo rohita*, Fed on Probiotic Supplemented diet for 60 days.

organization, as live microorganisms which when administered in adequate amounts, confer a health benefit on the host in the past decade, several gram-negative and gram-positive bacteria have been evaluated in the in vitro or in vivo for potential to inhibit- pathogenic organisms and overcome infections in fish and larvae in aquaculture[13]. In contrast to the constant habitat of terrestrial animals and resident flora in their gastrointestinal tract in aquatic animals, most microbes are transient and affected by conditions of surrounding water. During feeding experiments, it could be observed that animals were quite active and showed no and could not be related to any specific experimental situation live bacteria coated diet had a remarkable influence on the glycogen content. *B. subtilis* could bring forth a two fold increase in the whole animals. The biochemical changes induced by stress may lead to disturbance in metabolism. Changes such as reduction in protein and globulin content of the haemo lymph and inhibition of activity of certain important enzymes at cellular level lead to retardation of growth, reduction in the fecundity and longevity at the organism.

The protein content was maximum in the feed supplemented animals, particularly in their muscles. Even though there was a reduction in the protein content of infected animals in the supplemented group, they showed a higher concentration than that of the normal, and infected animals. The decrease in the total protein due to *Aeromonas hydrophilic* infection. In the present study probiotic supplementation could not effect and increase in the RBC level infected fish

nevertheless, it can be concluded that the beneficial bacterial consortium could mitigate the effects of infection by retaining the RBCs at near normal level, thus saving the fish from anaerobic conditions. Although there was declining trend in the WBCs of infected animals, the result of the present study underscores the fact that probiotics could salvage the fish from stressful conditions and retain the WBC count at near normal level.

The results of the present study that even under infection biochemical factors like protein and glycogen and hematological parameters could be retained at almost normal levels in the fish, *Labeo rohita*. These observed variations were dependent on the concentration of probiotics used. Higher concentration of probiotic had a more pronounced effect on the augmentation of tissue protein. A similar pattern was observed in the glycogen content also. Hence, from the present study, it could be inferred that the plant probiotic consortium had beneficial effects of improving the biochemical parameters of the common carp *Labeo rohita*. Adequate concentrations of these probiotics when incorporated in the regular feed of the fish will definitely improve its nutritive value and thereby its growth, as well.

CONCLUSION

This study tested the nutritional quality enhancement and disease fighting qualities of a probiotic consortium using the freshwater common carp *Labeo rohita* as the test animal. The probiotic consortium powder was incorporated separately at 1%, 3% and 5% concentrations of the fish feed. The composition of the probiotic per kilogram of the substance was as given below: *Lactobacillus sporogenes* (45,000 million cfu), *Lactobacillus acidophilus* (45,000 million cfu), *Bacillus licheniformis* (30,000 million cfu), *Bacillus subtilis* (30,000 million cfu), *Saccharomyces cerevisiae* (1, 25,000 million cfu) are still needed for better considerate of the composition and functions of the indigenous microbiota, as well as of microbial cultures of ‘‘probiotics’’. The decision of using probiotics in aquaculture has been in large part a result of chronological and experiential use and not based on scientific criteria. The use of probiotics is an important management tool, but its competence depends on sympathetic the nature of competition between species.

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