DIETARY PIPERINE IMPROVES HAEMATO-IMMUNOLOGICAL PARAMETERS, GROWTH PROFILES AND RESISTANCE AGAINST *AEROMONAS HYDROPHILA* IN *LABEO ROHITA* (HAMILTON, 1822)

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ABSTRACT

The purpose of the present study was to evaluate the effects of dietary piperine on haemato-immunological parameters, growth profile and disease resistance in Indian major carp, *Labeo rohita* challenged with *Aeromonas hydrophila*. Fish were fed with a diet containing 0 g kg^{-1} (T0), 0.5 g kg^{-1} (T1), 1.0 g kg^{-1} (T2), 2.0 g kg^{-1}(T3), 4 g kg^{-1}(T4), and 8 g kg^{-1} (T5) of piperine and blood of the experimental fish was collected on 14th, 28th, and 42nd for evaluation of haemato-immunological profiles and fishes were challenged with *A. hydrophila.* Haemato-immunological parameters at the end of 42nd day trial revealed that piperine administered through feed significantly (P<0.05) enhanced the complete blood counts, haemoglobin level, respiratory burst activity, lysozyme activity, and phagocytic activity. The percentage weight gain, feed conversion ratio and protein efficiency ratio of fishes fed with piperine were found to be significantly (P< 0.05) high compared with control. Dietary inclusion of piperine showed significantly (P<0.05) higher RPS in T3 (57.15%). The results reveal

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that 2 g kg^{-1} piperine supplemented diet has a stimulatory effect on haemato-immunological parameters along with improved growth performance and increased resistance against *A. hydrophila* infection in *L. rohita*.

Key words: Piperine, Growth, Haemato-immunological, *Aeromonas hydrophila*, *Labeo rohita*.

INTRODUCTION

The increment in aquaculture production has been achieved by the intensification of culture practices which is also increasing susceptibility of farmed animals to the problems caused due to deterioration of water quality as well as diseases. The use of antimicrobials in disease prevention though provides a promising solution but it can bring out the emergence of drug-resistant microorganisms and leave antibiotic residues in the fish and environmental issues. Therefore, several alternative eco-friendly health management strategies have been proposed and one such agent is immunostimulants. Diverse types of substances from different sources (bacterial components, chemical agents, animal or plant extracts) have been tested for aquaculture include peptides like glucan (Chen and Anisworth, 1992), chitosan (Siwicki *et al.,* 1994), levamisole (Findly and Munday, 2000; Maqsood *et al.,* 2009) including a number of plant derived immune stimulants (Logambal *et al.,* 2000). These directly activate the innate defense mechanisms acting on receptors and trigger intracellular gene activation that may result in the production of antimicrobial molecules (Logambal and Michael, 2001). A number of plant material/ products such as A*loe* (Kim *et al*., 1999), *O. sanctum* (Das *et al.,* 2013), Azadirachtin (Kumar *et al.,* 2013), *Viscum album, Urtica dioica* and *Zingiber officinale* (Dugenci *et al.,* 2003), *Radix astragalin* and *Radix angelicae sinensis* (Jian and Wu, 2004), *Astragalus radix* and *Scutellari radix* (Yin *et al.,* 2006) and *Achyranthes aspera* (Rao *et al.,* 2006) have been reported to enhance the immunity of fish against disease.

Black pepper, *Piper nigrum* belongs to the family 'Piperaceae' is a commonly available herb and considered to be a rich source of bioactive alkaloid popularly known as Piperine ($C_{17}H_{19}NO_3$). Its higher efficacy is partly attributed to its increased absorption as a result of its effect on the ultra-structure of the intestinal brush border. Black pepper or piperine treatment has also been evidenced to lower lipid peroxidation *in vivo* and beneficially influence antioxidant status in a number of experimental situations of oxidative stress. Piperine has also been found to possess anti-mutagenic and anti-tumor influences (Srinivasan, 2007). There are several reports on application of piperine in terrestrial models, however, there is a dearth of information regarding the efficacy of piperine in aquatic animals therefore, the present study provides baseline information about the prospects of application of piperine as diet supplement of a major carp *Labeo rohita*.

MATERIALS AND METHODS

Experimental animal and regime

L. rohita fingerlings procured from Malad Fish Farm, Maharashtra, India (average weight of 11.25 ± 0.75 g) were used as experimental animal. The fishes were acclimatized for 15 days in under laboratory conditions in 1000 L FRP tanks with continuous aeration. Fishes were fed with control

Table1: Composition of experimental diets (%)

Ingredients	T0	T1	T2	T3	T4	T5
Fish meal	25	25	25	25	25	25
Soya flour	20	20	20	20	20	20
Corn flour	12	12	12	12	12	12
Wheat flour	15	15	15	15	15	15
Rice bran	10	10	10	10	10	10
GOC	12	11.9	11.8	11.6	11.2	10.4
CMC	1	1	1	1	1	1
Veg. Oil	4	4	4	4	4	4
Vit & Mineral[1]	1	1	1	1	1	1
Piperine	-	0.05	0.10	0.20	0.40	0.80

a Composition of vitaminemineral mix (Agrimin) (quantity kg_1), Vitamin A e 6,25,000 IU; Vitamin D3 e 62,500 IU; Vitamin E e 250 mg; Nicotinamide e 1 g; Cu e 312 mg; Co 45 mg; Mg 6 g; Fe 1.5 g; Zn 2.13 g; I 156 mg; Se 10 mg; Mn 1.2 g; Ca 247.34 g; P 114.68 g; S 12.2 g; Na 5.8 mg; K 48.05 mg.

Table 2: Proximate composition (% fed basis) of the different experimental diets (values are mean ± SE).

Treatments	Moisture	Ash	Crude protein	Ether extract	NFE	CF	Digestible energy*
T0	7.86±0.30	9.24±0.28	32.78±0.56	7.87±0.36	39.06±0.25	0.79±0.01	370.95±4.05
T1	7.60±0.23	9.38±0.24	32.28±0.48	7.32±0.06	39.11±0.20	1.63±0.03	368.65±0.86
T2	6.89±0.10	9.45±0.15	32.57±0.21	8.09±0.08	38.07±0.20	2.43±0.03	375.09±0.26
T3	6.82±0.09	9.47±0.01	32.61±0.10	7.59±0.11	37.74±0.17	3.28±0.01	372.76±0.86
T4	7.59±0.14	9.05±0.06	32.48±0.17	8.07±0.11	36.17±0.18	4.01±0.03	373.79±0.40
T5	8.18±0.14	9.01±0.09	32.69±0.09	7.93±0.17	35.04±0.08	4.77±0.03	370.92±1.03

pelleted diet at the rate of 3% of body weight twice a day and the physico-chemical parameters of water such as temperature (25.4-26.5 ^{0}C), pH (7.1± 0.4), dissolved oxygen (6.3-6.9 mg L^{-1}), ammonia (0.01 ± 0.005 mg L^{-1}), nitrate (0.02-0.06 mg L^{-1}) and nitrite (0.001-0.004 mg L^{-1}) were maintained in optimum condition throughout the experimental period. The unconsumed feed, faecal matter and dead fish were siphoned out and 20% water exchange was provided every day.

Preparation of experimental diet

The commercial piperine EC 96% was procured from Sigma Industries Pvt. Ltd., Mumbai, India and used for preparation of six iso-nitrogenous experimental diets following the method of Rao *et al.,* (2006) with a slight modification. Experimental diets were prepared by mixing all the ingredients in required quantity along with water to form dough and calculated amount of oil was added to the dough before cooking. Further, completely cooled dough was mixed with vitamins, mineral mixture and six of the experimental diets contained with piperine in different concentrations T0 (0.0 g kg^{-1}), T1 (0.5 g kg^{-1}), T2 (1.0 g kg^{-1}), T3 (2.0 g kg^{-1}), T4 (4.0 g kg^{-1}) and T5 (8.0 g kg^{-1}) as listed in table 1. The dough was pressed through a hand pelletizer to get uniform sized pellets. These pellets were dried at 40°C for 12 hours. Proximate analysis of the diets was evaluated by standard methods (AOAC, 2005) and listed in table 2**.**

Experimental design

Three hundred twenty four *L. rohita* fingerlings were equally and randomly distributed in six distinct experimental groups in triplicate following a completely randomized design (CRD). The experimental animals were fed with the experimental dies *viz.* T0 (0.0 g kg^{-1}), T1 (0.5 g kg^{-1}), T2 (1.0 g kg^{-1}), T3 (2.0 g kg^{-1}), T4 (4.0 g kg^{-1}) and T5 (8.0 g kg^{-1}) for a period of 42 days. The animals fed with diet not supplemented with piperine were used as control. The fish were fed with the experimental diet at the rate of 3% of body weight twice a day at 09:00 and 17:00 h. Nine fish were sampled from each treatment group on 14th, 28th, 42nd day and blood was drawn for different haemato-immunological assays. After 42 days, nine fishes from each treatment were segregated for challenge study with *A. hydrophila* and post challenge study was continued up to 56 days. At the end of 56 days, relative percentage survival rate were estimated for disease resistance against *A. hydrophila*.

Collection of blood and serum separation

To estimate haemato-immunological parameters fish were randomly selected from the each experimental tanks and each fish was anesthetized with clove oil (Merck, Germany) @ 50 µl per litre of water before taking the blood. Blood was withdrawn from Vena caudalis by using a tuberculin medical syringe, which was previously rinsed with 2.7% EDTA solution. Blood collected was then transferred immediately to test tube containing thin layer of EDTA powder

(as an anticoagulant) and mixed well in order to prevent haemolysis of blood. Serum was collected without using anticoagulant and was separated from blood by keeping the tubes in slanting position for about 2 h and thereafter it was centrifuged at 1370 x *g* for 15 minutes at 4 °C followed by collection of straw colored serum with micropipette and stored at -20 °C for further analysis.

Haematological parameters

The total leukocyte counts were determined by mixing 20 µl of blood sample with 3980 µl of WBC diluting fluid (Himedia, India) and total erythrocyte counts were determined by using 20 µl of blood sample mixed with 3980 µl of RBC diluting fluid (Himedia, India) in a clean vial. The diluted fluids were observed and cells were counted using Neubauer Hemocytometer (Rohem, India). Packed Cell Volume was determined by drawing non-dotted blood by capillary action into microhaematocrit tubes. One end of the tube was sealed with synthetic sealant. The sealed tube was centrifuged in a microhaemotocrit centrifuge for 5 min at 8000 × *g*. The PCV was measured using microhaemotocrit reader and was expressed as percentage (%). Haemoglobin was analysed by the cyanomethemoglobin method using Drabkin's Fluid (Qualigens, India). Blood (20 µl) was mixed with 5 ml of Drabkin's working solution and the absorbance was measured using a spectrophotometer (Thermo Electron, Merck) at wavelength of 540 nm.

Growth parameters

To evaluate the effect of piperine on the growth and feed utilization parameters, animals were fed with supplemented experimental diets for 56 days. Fish were weighed on the 0^{th} day of the experiment and 56^{th} day of treatment. The weight was taken using an electronic balance (Citizen, India). The growth and feed utilization parameters of fish were evaluated in terms of weight gain (%), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) using the following formulae:

Weight gain (%) = (Final weight – Initial weight) x 100 / Initial weight

Specific growth rate (%) = [Ln (Final weight)-Ln (Initial weight)] x 100 /Total days of experiment

Feed conversion ratio = Feed given (dry weight) / Body weight gain (wet weight)

Protein efficiency ratio = Net weight gain (wet weight) / Crude protein fed (dry weight)

Non-specific immune parameters

Nitroblue tetrazolium assay

The respiratory burst activity of the neutrophils was measured by nitroblue tetrazolium (NBT) assay following the method of Secombes (1990). 50 µl of blood was placed into the wells of a

flat bottom micro titre plate and incubated at 37 °C for 1 h to allow adhesion of cells. The supernatant was discarded and the wells were washed three times with PBS. After washing, 100 µl of 0.2% NBT were added and incubated for 1 h. The cells were then fixed with 100% methanol for 2-3 min and washed three times with 70% methanol. The plates were air-dried and 60 µl of 2N potassium hydroxide and 70 µl dimethyl sulphoxide were added to each well. The OD was recorded in an ELISA (BioTek Power Wave 340, India) reader at 620 nm.

Serum lysozyme activity

Serum lysozyme activity was measured using colorimetric method by Anderson and Siwicki, (1995). In a cuvette, 3 ml of *Micrococcus luteus* (ATCC 7468, India) suspension in phosphate buffer (A_{450} = 0.5) was taken, to which 50 µl of diluted serum sample was added. The content of cuvette was mixed well for 15 seconds and measured using a spectrophotometer at 450 nm. The reading of lysis of the bacteria was immediately recorded at interval of 15, 30 and 270 sec. A unit of lysozyme activity was defined as the amount of sample causing a reduction in absorbance of 0.001 per minute and lysozyme activity is expressed as U/min.

Phagocytic activity (PA)

Phagocytic activity was detected using *Staphylococcus aureus* (Bangalore Geni, India) as described by Anderson and Siwicki, (1995). A sample (0.1 mL) of blood was placed in a microtiter plate well, 0.1 mL of *Staphylococcus aureus* 1×10^7 (A_{450} = 0.5-0.6) cells suspended in phosphate buffered saline (pH 7.2) were added and mixed well. The bacteria blood solution was incubated for 20 min at room temperature. 5 µl of this solution was taken on to a clean glass slide and a smear was prepared. The smear was air dried, then fixed with ethanol (95%) for 5 min and air dried. The air-dried smear was stained with 7% giemsa for 10 min. Two smears were made from each fish. The total of 100 neutrophils and monocytes from each smear were observed under the light microscope and the numbers of phagocytosing cells were counted. Phagocytic activity (*PA*) equals the number of phagocytosing cells divided by the total number of phagocyte cells counted.

$$PA = \frac{\text{Number of phagocytosing cells}}{\text{Number of total cells}} \times 100$$

Culture of pathogen and challenge study

Aeromonas hydrophila (ATCC 7966) was cultured in nutrient broth (Himedia) at 30 °C for 24 hours. The cultures were centrifuged at 3000×*g* for 10 min. The supernatants were discarded and the pellets were resuspended in phosphate buffered saline (PBS, pH 7.4). The final bacterial concentration was adjusted to 1.8×10^6 CFU ml^{-1} by serial dilution. After 42 days of experimental trials, nine fishes from each treatment group were selected randomly and *A. hydrophila* suspension of 0.2 ml (1.8×10^6 cfu ml^{-1}) was injected (intra-peritoneal) and maintained for 14

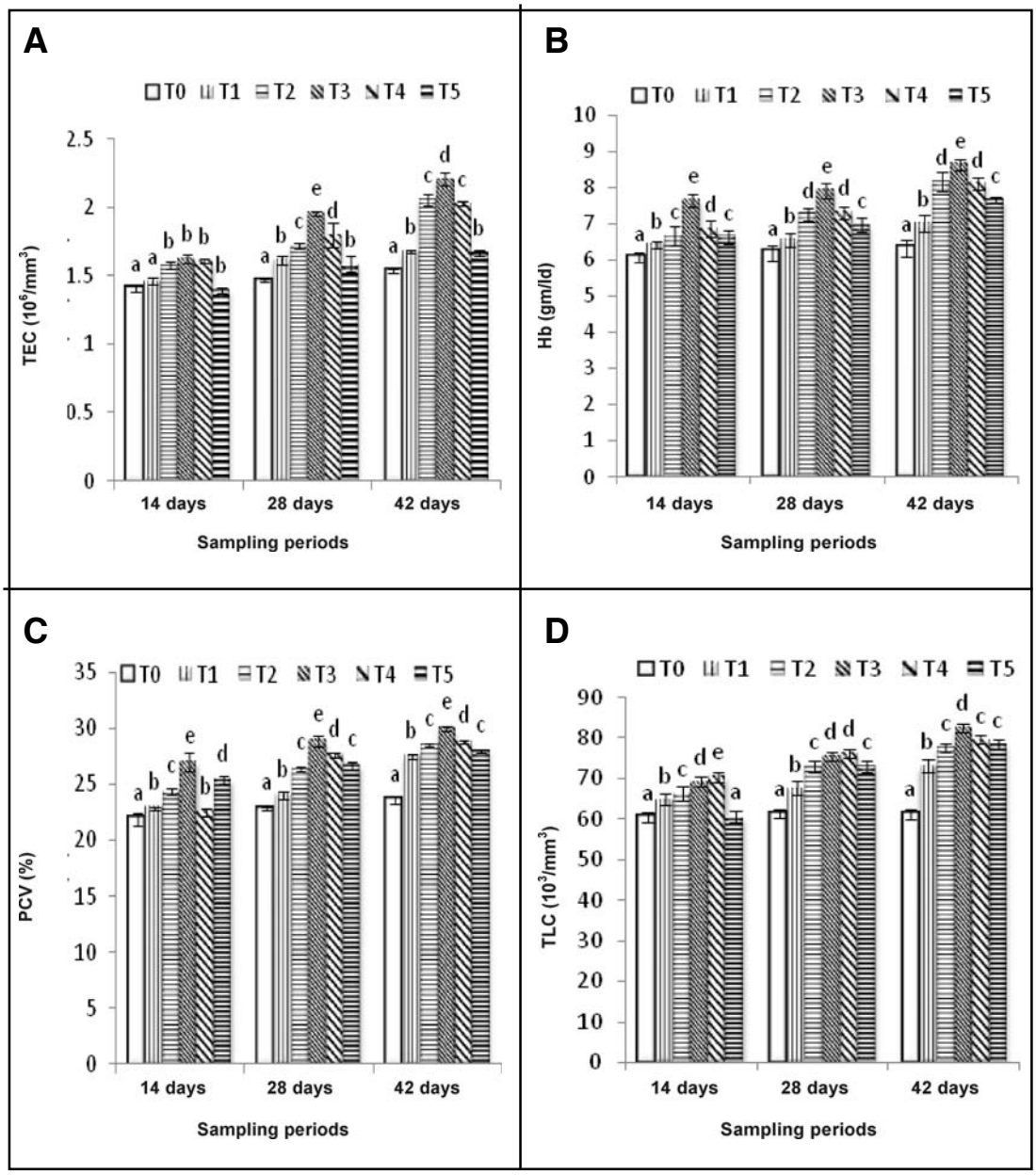

Figure 1. A. Total erythrocyte counts B. Haemoglobin levels C. Packed cell Volume D. Total leukocyte counts under different treatments fed with various levels of piperine in *L. rohita* during different sampling days (values are mean ± SE). Bars with different superscript letters indicate a significant difference (P < 0.05) in observed haematological parameters at that time point.

days. The fishes were observed regularly for any overt signs of disease including behavioural abnormalities and mortality. The causative agent was confirmed by re-isolating *A. hydrophila* from the moribund fish. Survival at the end of 14 days post infection was calculated using the following formula:

$$\text{Relative percentage survival (RPS \%)} = \frac{\text{Number of surviving fishes after challenge}}{\text{Number of fishes injected with bacteria}} \times 100$$

Statistical analysis

Table 3: Weight gain (WG in %), feed conversion ratio (FCR), protein efficiency ratio (PER) and specific growth rate (SGR) in L. rohita under different treatment groups fed with various levels of piperine (values are mean ± SE).

Treatments	WG	SGR	FCR	PER
T0	45.93^{a}±1.36	0.72^{a}±0.02	3.52^{e}±0.02	0.80^{a}±0.08
T1	50.92^{c}±2.46	0.95^{c}±0.05	2.84^{d}±0.08	1.02^{b}±0.05
T2	52.31^{d}±1.91	0.97^{c}±0.01	2.71^{c}±0.08	1.04^{c}±0.04
T3	62.04^{f}±1.15	1.05^{d}±0.08	2.44^{a}±0.03	1.20^{d}±0.05
T4	55.37^{e}±1.34	1.01^{c}±0.12	2.60^{b}±0.02	1.05^{c}±0.07
T5	49.5^{b}±1.15	0.87^{b}±0.04	2.91^{d}±0.04	0.99^{b}±0.08

The data were analysed by statistical package SPSS version 16 in which data were subjected to one-way ANOVA and Duncan's multiple range test (DMRT) was used to determine the significant differences between the means. Comparisons were made at 5% probability level.

RESULTS

Haemato-immunological parameters

The effect of piperine supplementation on the hematological parameters viz. TEC, Hb, PCV and TLC of *L. rohita* is shown in the fig 1(A-D). The above mentioned parameters increased (P<0.05) significantly in all the treatment groups compared to the control. The improvement in the hematological parameters also showed a dose response registering a highest increase in T3 beyond which it showed a decline.

Growth performance

The growth profiles of fish fed with dietary piperine of different treatment groups was significantly (P<0.05) high. The body weight gain (%) was significantly (P<0.05) higher in T3 group when compared with control. However, FCR was found to be significantly (P<0.05) improved among the treatment groups and specific growth rate was significantly (P<0.05) higher in the treatment

group T3, followed by T4, when compared to control. The protein efficiency ratio (PER) was significantly (P<0.05) higher in treatment group T3 among the other treatment groups (Table 3).

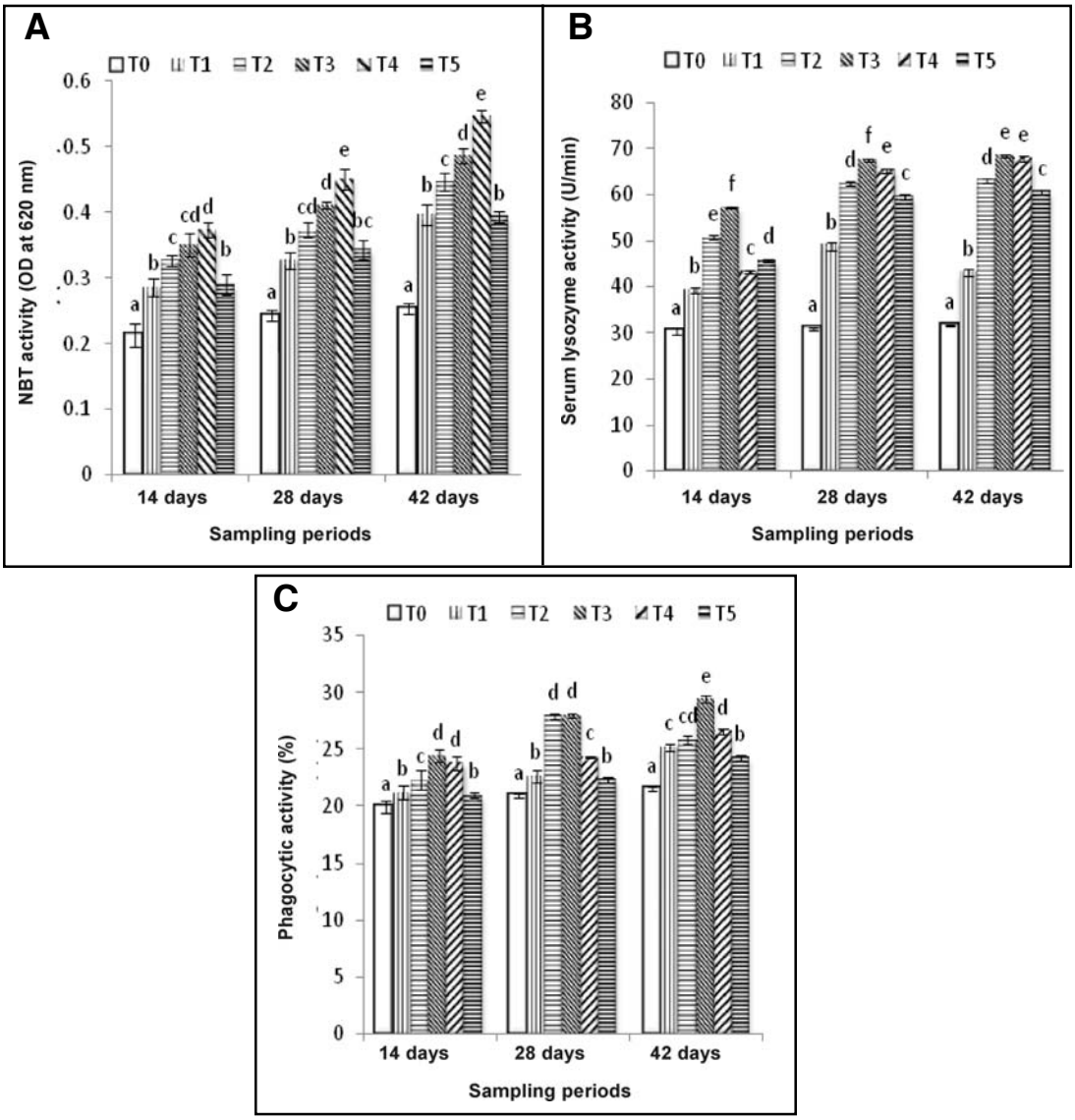

Figure 2. A. NBT activity B. Serum lysozyme activity C. Phagocytic activity under different treatments fed with various levels of piperine in *L. rohita* during different sampling days (values are mean ± SE). Bars with different superscript letters indicate a significant difference (P < 0.05) in the observed parameters at that time point.

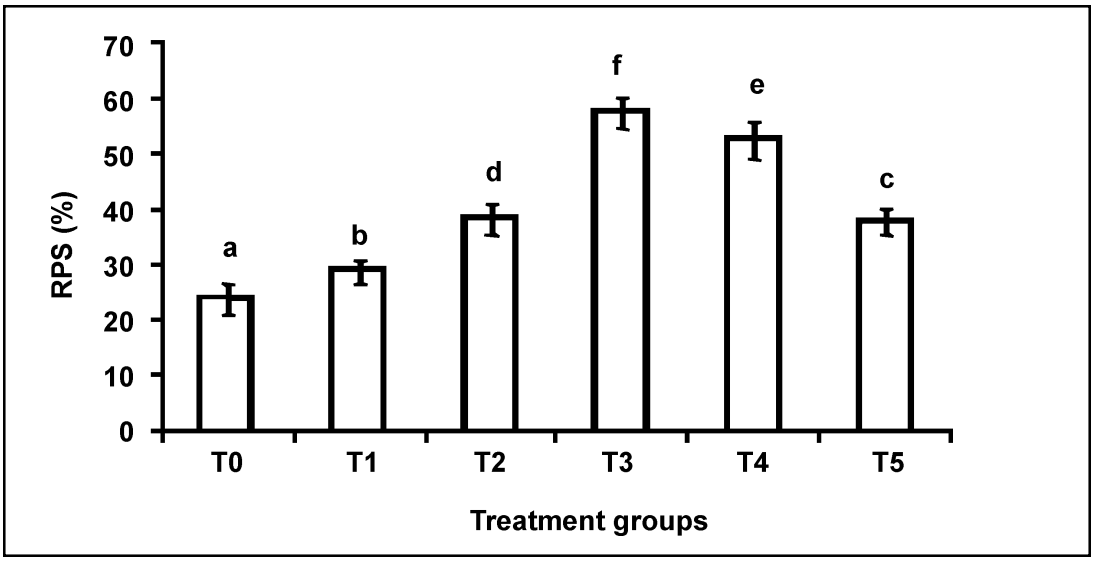

Figure 3. Relative percentage survival (RPS) under different treatments fed with various levels of piperine in *L. rohita* (values are mean ± SE). Bars with different superscript letters indicate a significant difference (P < 0.05) in RPS at that time point.

Nitroblue tetrazolium test for respiratory burst

The respiratory burst activity (NBT reduction) of neutrophils of *L. rohita* of different experimental groups is presented in fig 5. There were significant differences (P<0.05) in the respiratory burst activity among the various treatment groups on all the sampling days. The high NBT activity was found in T4 treatment group when compared with control in all sampling periods (Fig.2A). The lysozyme activity (U/min) showed an increasing trend during sampling periods in dietary fed piperine groups from 14 days to 42 days and differed significantly (P< 0.05) among the treatment groups. Moreover, the highest lysozyme activity was found in T3 group followed by T4 in 28th and 42nd sampling day (Fig. 2B). The phagocytic activity of piperine supplemented diet fed groups of fish showed significant (P<0.05) difference when compared with control during experimental periods. The phagocytic activity of *L. rohita* of different experimental groups is shown in fig. 2C and the higher activity was observed in T3 treatment groups during all sampling day.

Relative percentage survival

Relative percentage survival of *L. rohita* after challenging with *A. hydrophila* in treatment groups is shown in fig. 3. The treatment groups fed with piperine supplemented diet showed significantly (P<0.05) high disease resistance against *A. hydrophila* infection when compared with control group. The highest survival was recorded in T3 (57.15± 5.7%) group followed by T4 (52.38%) and T5 (37.63%) group.

DISCUSSION

The practical importance of non-specified defense mechanism against microbial infections has been very well documented in the case of fish, which are inevitably subjected to different stresses like excess stocking, excessive handling, poor water system and threats of exposure to various potential pathogens. For boosting the fish immune profiles, many immunostimulants are used in aquaculture that has a potential to act as an alternative to antimicrobial agents. In an earlier investigation, supplementing of diets with acetone extract of ginger was reported to enhance the growth of *Penaeus monodon* (Venkataramalingam *et al.,* 2007). Similar results were reported by Basha et al (2013) who found improved growth profiles of *L. rohita* fed with diet containing andrographolide . Rainbow trout (*Oncorhynchus mykiss*) fed with ginger also had significantly increased growth, feed conversion, and protein efficiency (Nya and Austin, 2009).

The dietary piperine appeared to significantly elicit non-specific immune parameters in terms haemato-immunological parameters in *L, rohita*. White blood cells (WBC) is known as first line of defense and play a chief role in innate immunity and increase in their numbers along with the other immunological parameters can be considered as an indicator of the improved health status of fish. Along with this, erythrocytes perform a major function of transporting necessary oxygen and a variety of gases in the vertebrates' body. In our study WBC, and RBC counts were increased significantly in T3, and T4 compared with control. Similar result has been reported by Kumar *et al.* (2013), in *Carassius auratus* fed with the diet having azadirachtin; in *L. rohita* fed with diet supplemented with i-carrageenan (Kumar *et al.,* 2013) and, in *Catla catla* juveniles fed with yeast RNA, ω-3 fatty acids and β-carotene (Jha *et al.,* 2007). However, our findings are at variance from those of Rairakhwada *et al.* (2007) in common carp, *Cyprinus carpio* juveniles with dietary microbial levan. Haemoglobin is a vital molecule present in RBC which binds with gases and transports them. Haemoglobin content in the study showed an increasing trend in concert with RBC count which is in conformity with the findings of Scott & Rogers (1988) in channel catfish *Ictalurus punctatus* and in *C. auratus* fed with azadirachtin supplemented diet, but disagreed with the findings of Jha *et al.* (2007), in *Catla catla* juveniles fed with yeast RNA, ω-3 fatty acids and β-carotene and Choudhury *et al.* (2005), in *L. rohita* fed with the diet containing yeast RNA. The PCV level in fish fed with piperine was found to be significantly higher in T3 followed by T4 in comparison to the control. The result of the present study is in agreement with the findings of Kumar *et al.* (2013) in *C. auratus* fed with azadirachtin supplemented diet and in *Oncorhynchus mykiss* fed with feed containing ascorbic acid (Dabrowski *et al.,* 2004).

It is well accepted that fish phagocytes after activation are able to generate superoxide anion (O_2^-) and its reactive derivatives (i.e hydrogen peroxide and hydroxyl radicals) during a period of intense oxygen consumption, called the respiratory burst (Secombes, 1992, 1996). These reactive oxygen species are considered to be toxic for fish bacterial pathogens (Hardie *et al.,* 2004). It is evident that increased respiratory burst activity can be correlated with increased bacterial pathogen killing activity of phagocytes (Ellis, 1990). In the present study higher respiratory

activity was observed in T4 group followed by T3 and T2. This is also in agreement with the finding of Basha *et al.* (2013) in *L. rohita* fed with varying concentration of andrographolide; and also with *O. sanctum* (Das *et al.,* 2013), but do not support the finding of Misra *et al.* (2006) in *L. rohita* fed with diet containing β glucan.

Lysozyme plays important role in innate immunity by lysis of bacterial cell wall and thus stimulates the phagocytosis of bacteria (Guobin *et al.,* 2009). Fish serum lysozyme is believed to be of leukocytic origin, and its activity increase concomitantly with leukocyte numbers (Ellis, 1990). Similarly, in our study, the treatment group with maximum lysozyme activity had the maximum leukocyte count and greater phagocytic activity. Piperine supplemented groups showed increasing trend in lysozyme activity during experimental periods with the highest activity was observed in T3 group. It was reported that herbal medicines could enhance the lysozyme activity in fish as documented by Lygren et al. (1999) in Chinese sucker treated with TCM extracts, in *L. rohita* fed with the diet containing herb *Achyranthes aspera, Ocimum sanctum* extracts and andrographolide (Rao *et al.,* 2006; Das *et al.,* 2013; Basha *et al.,* 2013) respectively; while other reported that lysozyme activity is unaffected by vitamin C in *Salmo salar* (Lygren *et al.* 1999), and also in *O. mykiss* (Verlhac *et al.,* 1996).

Phagocytic activity in the treatment groups showed significantly high values as compared with control and the highest activity was recorded in T3 followed by T4 and T2. Present findings support the report of Das et al. (2013) in *L. rohita* fed with the diet containing *O. sanctum* extracts; in greasy groupers (*Epinephelus tauvina*) fed with herbal diet containing purified active component of *Withania somnifera*, and *Myristica fragrans* (Sivaram *et al.,* 2004); in *L. rohita* fed with diet supplemented with andrographolide (Basha *et al.,* 2013).

In the present study, the relative percentage of survival was highest in T3 group followed by T4 and T5 groups, when compared to control. This is an agreement of high survivable percent in *Oreochromis mossambicus* (Logambal *et al.,* 2000) fed with diet containing *O. sanctum*. In *L. rohita* fed with diet containing herb *Achyrathes aspera* (Rao *et al.,* 2006), and andrographolide (Basha *et al.,* 2013). Similar result of high percentage of relative survival in *Cyprinus carpio* fingerlings was reported against *A. hydrophila* fed with dietary levamisole (Maqsood *et al*. 2009), similarly in *C. auratus* fed with azadirachtin supplemented diet (Kumar *et al.,* 2013).

The feed containing piperine (2 g kg^{-1}) can influence haematological, and immunological parameters and growth performances in *L. rohita*. It also provides resistance against *A. hydrophila* infection and reduces mortality in *L. rohita*. The positive immunomodulatory activities of piperine can be potentially used to develop a new immunostimulant agent in aquaculture.

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