Original Research Article

Effects of Curcumin Supplemented Diet on Growth and Non-Specific Immune Parameters of *Cirrhinus mrigala* against *Edwardsiella tarda* Infection

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A B S T R A C T

The present research was conducted to evaluate the effect of curcumin supplement diet (CSD) on non-specific immunity, growth and disease resistance against *Edwardsiella tarda* infection in *Cirrhinus mrigala*. Fishes were fed with formulated diet containing curcumin in different graded levels as control (C) (0.00%), T1 (0.25%), T2 (0.5%), T3 (1.0%), T4 (1.5%), and T5 (2.0%) for 45 days. Treatments were maintained in triplicate and each tank was stocked with 20 fingerlings (10.5 g ± 1.4). Fishes were challenged with a virulent strain of *E. tarda* after 45 days of feeding and relative percentage survival was recorded over 14 days post challenge. The percentage weight gain (PWG), feed efficiency ratio (FER), feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER) of fishes fed with curcumin enriched diet were found to be significantly ($p < 0.05$) better than the control group. Dietary curcumin at the level of 1.5% (T4) showed significantly ($p < 0.05$) higher RPS (62.5%) against *E. tarda* infection than control. Blood and serum samples were collected for non-specific immune parameters on 0, 15, 30, and 45 days interval and growth performance was evaluated at every 15 days period of the experiment. The results suggest that fishes feed with curcumin enriched diet (1-1.5%) showed significantly ($p < 0.05$) higher total immunoglobulin, serum protein, serum albumin, Immunoglobulin, respiratory burst activity and myeloperoxidase activity. Hence, this study revealed that CSD has a potential stimulatory effect on non-specific immunity along with improved growth performance and increased disease resistance against *E. tarda* infection in *C. mrigala*.

Introduction

Over the past few decades, aquaculture production has increased significantly due to the intensification of culture practices. However, disease outbreaks are increasingly at the same time and are being recognized as a potential constraint on aquaculture production trade thus causes massive economic losses to the farmers. The most common strategy to combat diseases in aquaculture is the use of antibiotics; however, the use of antibiotic for controlling diseases has been widely criticized for their negative impacts (Rijkers *et al.,* 1980; Grondel *et al.,* 1987; Ellis, 1988). In order to reduce the risk of disease, the level of resistance to infection in the cultured organisms should be increased by the use of better feeds, vaccines, and immunostimulants and by selective breeding for higher disease resistance (Raa *et al.,* 1992). Immunostimulation is considered as a

promising alternative therapy to antibiotics because of their broad spectrum nature, cost effectiveness, and eco-friendly disease preventive measure. The immunostimulant can be derivative of biological agents and synthetic drugs. Among the biological agents, plant derivatives are most commonly used for disease treatment because it is economical and versatile in nature.

Curcumin, derived from the rhizome of turmeric plant (*Curcuma longa*), is one of the best-studied natural compounds. There are several reports indicating a wide range of pharmacological activities of turmeric reported as anti-microbial (Negi *et al.,* 1999), antitumour (Ozaki *et al.,* 2000), anti-allergic (Yano *et al.,* 2000), anti-inflammatory (Surh *et al.,* 2001) and antioxidant (Masuda *et al.,* 2002). Most of these effects were observed in studies conducted earlier in mammals or in cell lines.

Edwardsiella sp. are the most significant disease-causing microbes (Mohanty and Sahoo, 2007) which causes edwardsiellosis leading to mass mortality in freshwater and marine fish (Ewing *et al.,* 1965) with extensive skin lesions, affecting internal organs such as liver, kidney, spleen and muscle (Nucci *et al.,* 2002) and also affects human beings by causing gastroenteritis and generalized infections mainly occurs among individuals with impaired immune systems (Plumb, 1994; Thune *et al.,* 1993; Rao *et al.,* 2001). Therefore, the objective of the present study was to evaluate the effect of curcumin-supplemented diet on the non-specific immunity, growth performance, and disease resistance against *Edwardsiella tarda* in *Cirrhinus mrigala*.

Materials and Methods

C. mrigala fingerlings with an average weight of (10.5 ± 1.4 g) were procured from Namdev Fish Farm, Mumbai, India. Fishes were acclimatized under well-aerated conditions in the wet laboratory 1000 L FRP tanks at 25-28 0C fed with a commercial carp diet containing 45% protein @ 4% of their body weight.

Experimental design and diet formulation

Curcumin was brought from Mark, India. The diets were prepared using ingredients such as Corn flour, Soya flour, fish meal, wheat flour, cellulose, cod liver oil, vitamin and mineral mixture premix etc. The required ingredients were mixed to form dough with the addition of a necessary quantity of water and the same was cooked. The steamed dough was then completely cooled. In various concentrations the vitamins and mineral mixture along with curcumin were added and six experimental diets were made as C (0.0%), T1 (0.25%), T2 (0.5%), T3 (1.0%), T4 (1.5%), and T5 (2.0%) (Manju, Vijayasree, Akbarsha and Oommen 2013) as listed in table 1. The pellets were prepared and dried properly before use. After drying, pellets were packed in polyethylene bags, sealed airtight and labeled. The feed having (43%) crude protein, (11%) lipid and (36%) carbohydrate were prepared. *C. mrigala* fingerlings (n=360) of mean weight (10.5 g ± 1.4) were randomly distributed into 18 tanks to form six experimental groups in triplicate following a completely randomized design (CRD). The fish were fed with the experimental diet to satiation for 45 days. The physicochemical parameters of water were maintained optimum during the experimental period.

Sampling

Six fishes were sampled from each treatment group on the 0th day (before feeding trial), 15th, 30th and 45th day and the blood were collected with and without anticoagulant. Blood without anticoagulant was allowed to clot for 2 h for the separation of serum. It was

then centrifuged at 4500 rpm for 5 min. Serum and plasma were collected and stored at −80 °C for further analysis.

Growth parameters

Fish (n=5) were weighed biweekly from different treatment groups. The growth performance of fish was evaluated in terms of percentage weight gain (%), feed conversion ratio (FCR), feed efficiency ratio (FER), specific growth rate (SGR), and protein efficiency ratio (PER) using the following formulae:

Weight gain (%) = (FW-IW) × 100/IW, FCR= Feed gain (DW)/body weight gain (WW), FER=1/FCR, SGR (%) = [ln (FW)-ln (IW)/N] ×100 and PER= body weight gain (WW)/ crude protein fed. Where FW= final weight, IW= initial weight, DW= dry weight, WW= wet weight gain, ln=natural log and N= number of cultural days.

Non-specific immune parameters

Nitroblue tetrazolium assay (NBT) was done following the method of Secombe (1992). The lysozyme activity level was measured using the turbidimetric assay following Sankaran and Gurnani (1972), hen egg white lysozyme (Sigma) taken as standard. Phagocytic activity was detected using *Staphylococcus aureus* (Hi-Media Mumbai, India) as described by Siwicki and Anderson (1994). Total myeloperoxidase (MPO) content present in serum was estimated according to Quade and Roth (1997). Using total protein kit (Merck, India) Serum protein was estimated by biuret and BCG dye binding following the method of Reinhold (1953). Albumin was estimated by Bromo cresol green binding method developed by Doumas, Watson and Biggs (1971) using albumin kit (Merck, India). Globulin was calculated by subtracting albumin values from total serum protein values. Immunoglobulin plasma was separated by precipitation with polyethylene glycol (PEG) as described by Anderson and Siwicki (1995).

Edwardsiella tarda lethal dose 50 (LD50) estimation

E. tarda (ATCC 15947) was cultured in 5 ml BHI broth at 28ºC and centrifuged at 5000 rpm. The pellet was dissolved in 5 ml sterile PBS (pH 7.3). Separately 9 ml of sterile PBS was dispensed into 10 sterile tubes. To the first tube, 1 ml of neat and clean culture *E. tarda* was added and the contents were mixed thoroughly. 1 ml inoculum of the first tube was then transferred to the next tube and serial dilution was done. This procedure was repeated up to the last tube. 0.1 ml of sample from each of the dilution was inoculated to sterile Petri plates containing BHI agar and incubated at 28°C for 16–18 hrs. Plates having 30-300 round tiny cream colonies were selected for counting by using the formula and expressed as CFU per ml.

The number of bacteria was calculated as follows:

CFU/ml = No. of colonies × Reciprocal of dilution factor × 10

6 fishes in each group (six groups) were administered with 100μl of different dilutions of the bacteria ranging from 1×10^3 to 1×10^7 intra-peritoneally into each treatment using 1ml tuberculin syringe. One group was injected with PBS (pH 7.3) and was taken as control. Mortality was recorded daily for 72 h and the mortality that occurred post 12 h was counted and subsequently, the LD50 value was calculated by using the method of Reed and Muench (1938).

Challenge test

E. tarda (ATCC 15947) was cultured in nutrient broth (Himedia) at 37 0C for 24 h.

The cultures were centrifuged at 3500 rpm for 10 min. The supernatant was discarded and the pellets were suspended in phosphate buffered saline (PBS, pH 7.3).The final bacterial concentration was adjusted to 5.4×10^5 CFU per ml by serial dilution.

After days 45 days of feeding 5 fishes from each replicate (n=15) were selected for the challenge test. The fishes were injected with the virulent strain of *E. tarda* suspended in 0.2 ml (5.4×10^5 CFU-1). The challenged fishes were kept under observation for 14 days and subsequently, RPS was recorded.

Relative percentage survival (RPS) calculation

Survival was calculated at the end of the 14 days post infection, using the following formula (Amend, 1981)

Relative percentage survival (RPS %)

$$= [1- (\frac{\% \text{ of mortality fishes after challenge}}{\% \text{ of mortality in control}})] \times 100$$

Statistical analysis

One way ANOVA was used for the analysis of the raw data and Duncan's multiple range tests were performed to analyze the statistical difference between the treatments. SPSS version 16 was used to perform all the analysis.

Results and Discussion

Growth performance

The effect of Curcumin fed diets to the fishes at different levels on the growth performance is presented in table 2. At the end of the experiment period, the highest percent weight gain (PWG) was recorded in treatment T4

(1.5 % curcumin). FCR was significantly lower (p<0.05) in treatment T4 (2.00 ± 0.11) than control group while FER and SGR were significantly higher in T4 compared to control.

Non-specific immune parameters

The NBT activity (OD at 620 nm) among different treatment groups ranged from 0.14 ± 0.01 to 0.18 ± 0.03 including control groups (Fig. 1). NBT activity increased significantly (p <0.05) with time in fishes fed with different doses of curcumin and the highest activity of NBT was observed in the group fed with 1% to 1.5% curcumin supplement diet (CSD).

Figure 2 shows the Serum lysozyme activity (units/min) of *C. mrigala* during 45 days of feeding in different curcumin treatment groups and control. The results showed the serum lysozyme activity was significantly (p <0.05) highest in treatment T4.

The phagocytic activity (%) was found to be significantly (p>0.05) higher in different curcumin-fed groups compared to control. The highest phagocytic activity was observed in T3 and T4 curcumin supplemented groups (Fig. 3). The Myeloperoxidase activity (OD at 450 nm) of the different treatment groups varied significantly (p<0.05) and showed an increasing trend from 15 days to 45 days of sampling (Fig. 4). The highest MPO activity was found to be in the treatment T2 throughout the experiment.

Table 3 shows the levels of serum protein, albumin, globulin and total immunoglobulin of *C. mrigala* during 45 days of feeding trail with treatment and control diets. At the end of 45-day serum protein in T3 (5.82 ± 0.39) were improved with no significant difference with treatment T4 and T5. Similar results were also observed for globulin in different treatments.

However, the albumin content was highest in treatment T5 (3.12± 0.15) on 45 days of sampling. Total immunoglobulin values in treatment group T1 (1.48 ± 0.07) and T4 (1.53 ± 0.20) were significantly (p<0.05) higher with no significant difference than rest of the treatment groups.

LD50 of *E. tarda*

The first mortality was observed after 24 h of intraperitoneal injection with *E. tarda* $1x10^3$ CFU / ml. After 72 h the cumulative percentage mortality was recorded as $1x10^3$ (83%), $1x10^4$ (66%), $1x10^5$ (50%), $1x10^6$ (33%) and $1x10^7$ (16%) respectively. However, intraperitoneal injection with PBS (pH 7.3) in control group shows no mortality (Table 4). In the current study, it was determined that for *C. mrigala* the lethal dose (LD50-72h) of *E. tarda* was recorded as $5.4x10^5$cfu/ml. The *E. tarda* was re-isolated from dead fish by dissecting the internal organ (Kidney) for further confirmation in SS- agar (Fig. 5 and plate 1).

Challenge study

The experimental fish in each group was challenged with the LD50 dose of 5.4×10^5 CFU / fish by intraperitoneal injection.

Mortality was recorded twice a day for 14 days. Accordingly, the RPS in the different treatment groups post challenge were calculated and the highest RPS was recorded in T4 (62.5%) followed byT2 (50%) respectively (Table 5).

It is a well-known fact that the supplementation of the dietary bioactive compounds such as immunostimulants and probiotics improves the growth, immunity performance and resistance to infections (Ramasamy *et al.,* 2011).

Fig.1 NBT activity under different treatments fed with various levels of curcumin in *C. mrigala* during different sampling days. Values are mean ±SE. Mean values with different superscript for a parameter is significantly different, (p < 0.05)
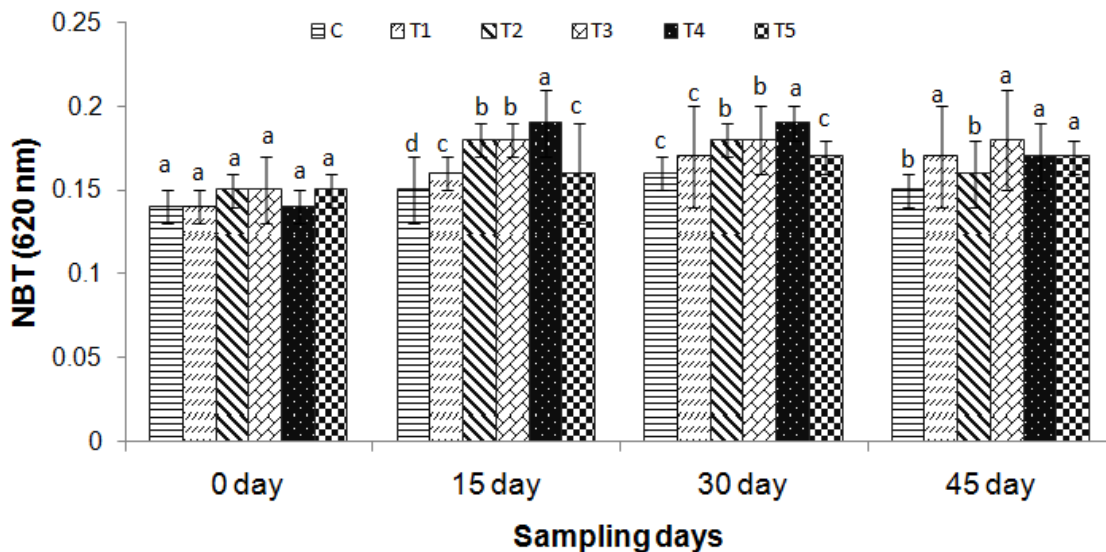
Fig.2 Serum lysozyme activity under different treatments fed with various levels of curcumin in *C. mrigala* during different sampling days. Values are mean ±SE. Mean values with different superscript for a parameter is significantly different, (p < 0.05)
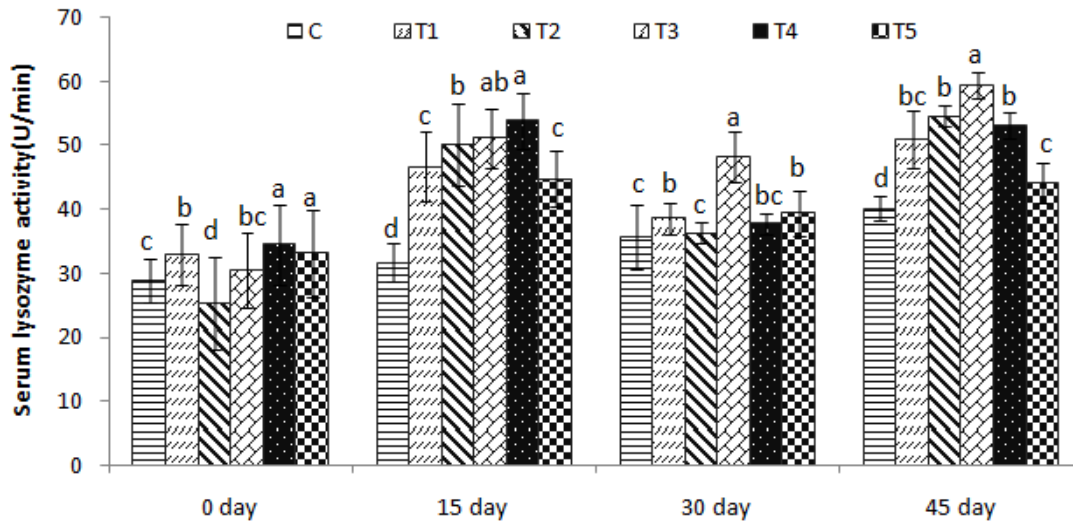

Fig.3 Phagocytic activity under different treatments fed with various levels of curcumin in *C. mrigala* during different sampling days. Values are mean ±SE. Mean values with different superscript for a parameter is significantly different, (p < 0.05)
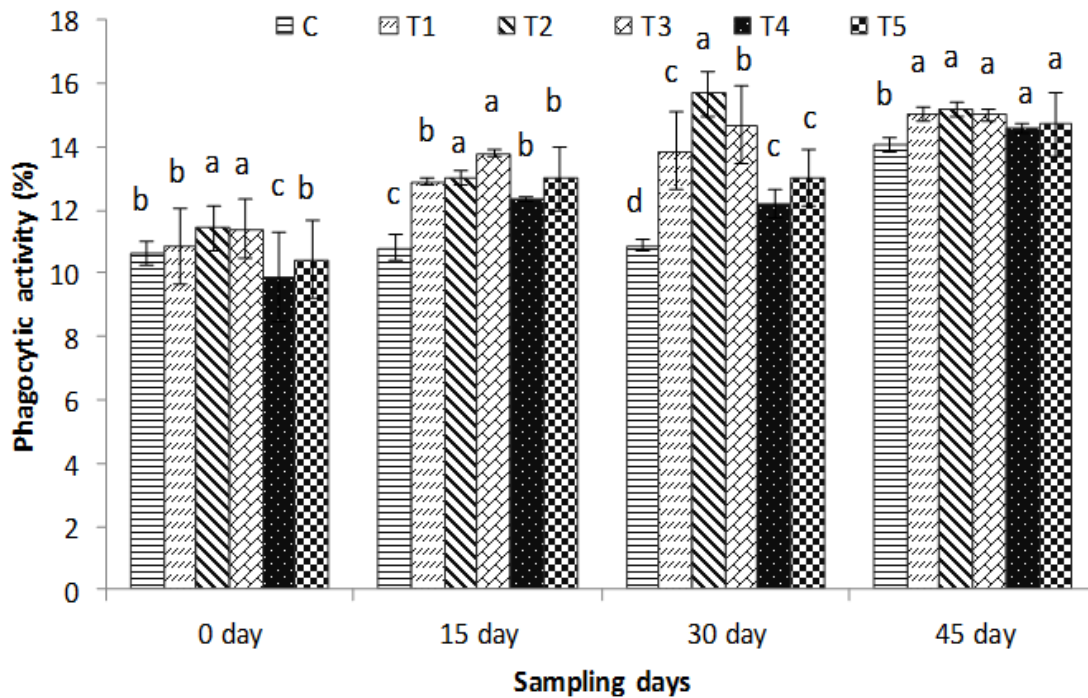
Fig.4 Myloperoxidase activity (MPO) under different treatments fed with various levels of curcumin in *C. mrigala* during different sampling days. Values are mean ±SE. Mean values with different superscript for a parameter is significantly different, $(p < 0.05)$
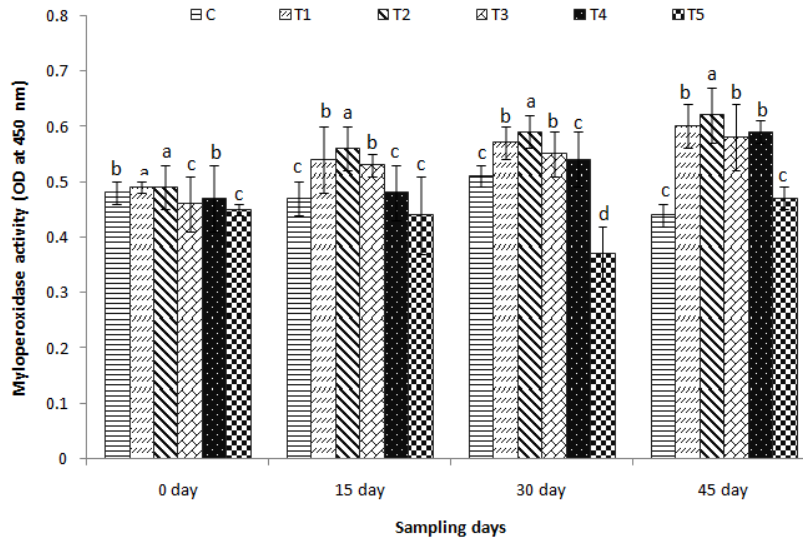

Fig.5 Clinical sign of *E.tarda* infected mrigal fish

Haemorrhage

Gross haemorrhagic lesions on ventral surface of body

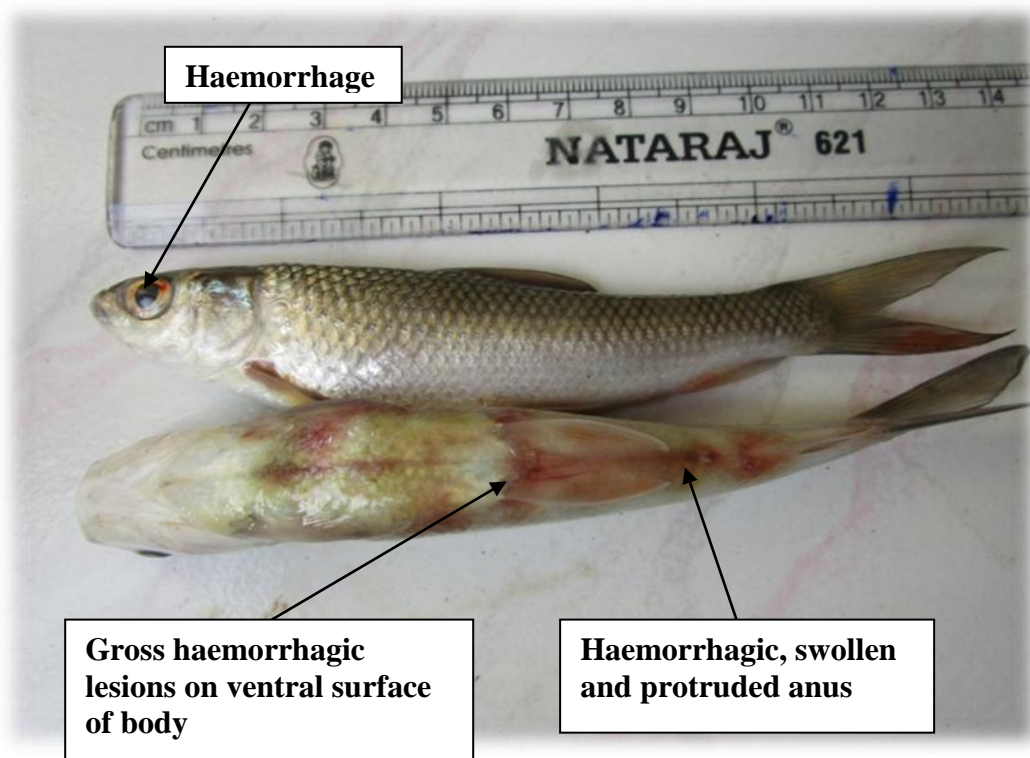Haemorrhagic, swollen and protruded anus

Plate.1 *E. tarda* colonies on SS agar varied from 1-3mm diameter and showed small black centers to predominantly black colonies indicating H_2S production

Table.1 Formulation of experimental diets (in 100 grams)

Ingredients (g)	Control	T1	T2	T3	T4	T5
Fish meal	25	25	25	25	25	25
Soya flour	32	32	32	32	32	32
Corn flour	12	12	12	12	12	12
Wheat flour	15	15	15	15	15	15
Cellulose	6	5.5	5	4.5	4	3.5
CMC	2	2	2	2	2	2
Vegetable oil	6	6	6	6	6	6
Vitamin and Mineral premix	2	2	2	2	2	2
Curcumin	0	0.25	0.5	1.0	1.5	2.0

Composition of Vitamin-mineral mix (Agrimin) (Quantity kg^{-1}), Vitamin A-6,25,00 IU; Vitamin D3- 62,500 IU; Vitamin E- 250 mg; Nicotinamide-1 g; Cu-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 mg; Ca- 247.34 g; P- 114.68 g; S- 12.2 g; Na- 5.8 mg; K- 48.05 mg.

Table.2 Effect of curcumin fed diet on the growth performance of *C. mrigala*

Parameter	Control	T1	T2	T3	T4	T5
IW(g)	12.30	12.57	12.57	12.21	12.30	12.50
FWG(g)	16.70	16.63	17.00	15.77	17.47	17.77
PWG (%)	$35.79^b \pm 1.16$	$32.37^b \pm 1.24$	$35.44^b \pm 5.64$	$39.17^a \pm 0.08$	$42.10^a \pm 2.63$	$37.14^b \pm 3.00$
FCR	$2.36^{abc} \pm 0.07$	$2.19^{bc} \pm 0.06$	$2.35^{bc} \pm 0.02$	$2.00^c \pm 0.18$	$2.00^c \pm 0.11$	$2.74^a \pm 0.05$
FER	$2.12^d \pm 0.06$	$2.19^{bc} \pm 0.06$	$2.34^c \pm 0.312$	$2.70^a \pm 0.177$	$2.60^b \pm 0.109$	$2.31^c \pm 0.049$
SGR	$1.43^{abc} \pm 0.03$	$1.35^{bc} \pm 0.03$	$1.42^{abc} \pm 0.13$	$1.57^c \pm 0.09$	$1.58^{ab} \pm 0.08$	$1.21^a \pm 0.03$

Note: IW= Initial weight; FWG = final weight gain; PWG = percent weight gain; FCR =feed conversion ratio; FER= feed efficiency ratio; SGR = specific growth rate; Values in the same column with different superscripts letters are significantly different ($P < 0.05$). Values are presented as mean ± SEM (n = 5).

Table.3 Non-specific immune parameters in *C. mrigala* fed with curcumin

Parameter	Treatment	0 day	15 day	30 day	45 day
Protein (mg/dl)	Control	$2.58^a\pm0.17$	$2.47^b\pm0.38$	$3.88^d\pm0.66$	$4.33^b\pm0.56$
	T1	$2.57^a\pm0.12$	$3.03^a\pm0.42$	$5.21^b\pm0.87$	$5.52^b\pm0.20$
	T2	$2.61^a\pm0.42$	$2.53^b\pm0.04$	$4.71^c\pm0.55$	$4.98^b\pm0.10$
	T3	$2.85^a\pm0.56$	$3.17^a\pm0.45$	$6.23^a\pm0.76$	$5.82^a\pm0.39$
	T4	$2.42^a\pm0.31$	$2.58^b\pm0.15$	$6.50^a\pm0.53$	$5.57^a\pm0.21$
	T5	$2.35^a\pm0.48$	$2.93^a\pm0.45$	$4.93^c\pm0.55$	$5.77^a\pm0.11$
Albumin (mg/dl)	Control	$1.02^b\pm0.11$	$1.21^c\pm0.20$	$2.17^c\pm0.11$	$2.86^b\pm0.01$
	T1	$1.13^b\pm0.06$	$1.20^b\pm0.18$	$2.75^b\pm0.09$	$3.26^b\pm0.21$
	T2	$0.98^b\pm0.16$	$1.17^b\pm0.12$	$3.09^b\pm0.05$	$3.23^b\pm0.06$
	T3	$1.07^b\pm0.10$	$1.20^b\pm0.03$	$2.59^b\pm0.10$	$2.97^b\pm0.10$
	T4	$1.06^b\pm0.05$	$1.89^b\pm0.14$	$3.20^b\pm0.13$	$3.07^b\pm0.24$
	T5	$1.04^a\pm0.18$	$1.92^a\pm0.16$	$2.62^a\pm0.16$	$3.12^a\pm0.15$
Globulin (mg/dl)	Control	$0.69^a\pm0.12$	$0.52^c\pm0.08$	$1.70^d\pm0.27$	$1.47^d\pm0.24$
	T1	$0.93^a\pm0.08$	$1.05^a\pm0.17$	$2.46^b\pm0.39$	$2.26^c\pm0.16$
	T2	$0.86^a\pm0.13$	$0.91^b\pm0.09$	$1.62^d\pm0.29$	$1.76^d\pm0.16$
	T3	$0.91^a\pm0.17$	$0.86^b\pm0.25$	$2.48^b\pm0.26$	$2.85^a\pm0.35$
	T4	$0.74^a\pm0.16$	$0.69^c\pm0.08$	$2.95^a\pm0.35$	$2.50^b\pm0.45$
	T5	$0.50^a\pm0.06$	$1.01^a\pm0.05$	$2.31^c\pm0.26$	$2.65^a\pm0.15$
Total Immunoglobulin (mg/dl)	Control	$0.49^d\pm0.07$	$0.55^b\pm0.01$	$1.14^c\pm0.12$	$1.24^c\pm0.02$
	T1	$0.56^{cd}\pm0.05$	$0.57^b\pm0.02$	$1.56^{ab}\pm0.32$	$1.48^a\pm0.07$
	T2	$0.76^{bc}\pm0.04$	$0.46^c\pm0.12$	$1.63^a\pm0.24$	$1.36^c\pm0.02$
	T3	$0.85^a\pm0.03$	$0.53^b\pm0.01$	$1.48^b\pm0.17$	$1.43^{ab}\pm0.17$
	T4	$0.72^{bc}\pm0.01$	$0.69^a\pm0.03$	$1.53^{ab}\pm0.27$	$1.53^a\pm0.20$
	T5	$0.82^a\pm0.13$	$0.73^a\pm0.05$	$0.93^d\pm0.15$	$1.31^c\pm0.06$

The means with no superscript letter in common per factor indicates significant difference. If the effects were significant, ANOVA was followed by Duncan multiple range test. $p< 0.05$.Values are presented as mean ± SE.

Table.4 Mortality of *C. mrigala* during the DL_{50-72h} of *Edwardsiella tarda*

Bacteria concentration (cfu / mL)	24 h	48 h	72 h	Mortality%
PBS(pH7.3)	0	0	0	0
1×10^3	1	2	2	83
1×10^4	0	1	3	66
1×10^5	0	1	2	50
1×10^6	0	0	2	33
1×10^7	0	1	0	16

Table.5 Relative percentage Survival of *C. mrigala* after challenge study with *E. tarda*

Treatment	No. of fish injected	No. of fish death	Cumulative mortality (%)	RPS (%)
Control	15	8	53.33	----
T1	15	5	33.33	37.5
T2	15	4	26.67	50
T3	15	5	33.33	37.5
T4	15	3	20.00	62.5
T5	15	6	40.00	25

The means with no superscript letter in common per factor indicates significant difference. If the effects were significant, ANOVA was followed by Duncan multiple range test. $p< 0.05$.Values are presented as mean ± SE.

Immune system stimulation of fishes through novel drugs, especially from plant sources, is of great interest for commercial aquaculture. Keeping in view the above facts the present study evaluates the effect of active compound from turmeric *Curcuma longa* (curcumin) on growth performance, non-specific immune parameters and disease resistance against *E. tarda* of *C. mrigala*.

The present study demonstrates that dietary supplementation with curcumin enhances growth and disease resistance of *C. mrigala* during the experimental period. The results are in agreement with the previous findings of Curcumin, Andrographolide, and ginger extract dietary inclusion has been shown to improve the growth performance of *Carassius auratus*, *Labeo rohita* and tilapia (Jiang *et al.,* 2016; Basha *et al.,* 2013; Immanuel *et al.,* 2009).

NBT activity is typically associated with respiratory burst activity, characterized by intracellular localization of super oxide anion in phagocytes (Sahoo *et al.,* 2005). Phagocytes after activation generate super oxides and other reactive compounds (*i.e.* hydrogen peroxide and hydroxyl radical) during the period of intense oxygen consumption called respiratory burst (Secombes and Fletcher, 1990). These oxygen reactive radicals are considered toxic for bacterial fish pathogens (Hardie *et al.,* 1991). In the present study, the highest activity of NBT was observed in the group fed with 1% to 1.5% CSD which was beneficial in protecting them from the disease-causing organism (*E. tarda*). Other immunostimulant such as β-glucan (Jeney and Anderson, 1993), chitin (Sakai *et al.,* 1992), azadirachtin (Kumar *et al.,* 2013), garlic (Sahu *et al.,* 2007), Andrographolide (Basha *et al.,* 2013) are also known to stimulate phagocytes in fishes.

It is well known that lysozyme is a primary marker of fish defense system, which causes of lysis of pathogens, activation of the complement system and phagocytes by acting as opsonin (Magnadottir, 2006). Lysozyme is an enzyme that breaks down the peptidoglycan (β-1, 4-glycosidic linkages) of the bacterial cell wall thereby controlling the infection. An increase in the lysozyme activity suggests elevation of various humoral factors that protect the host during pathogen invasion (Harikrishnan *et al.,* 2010). It was also previously reported that serum lysozyme activity could be enhanced through dietary administration by various herbs in Chinese sucker (Zhang, Gong, Yu and Yuan 2009) and *L. rohita* (Das *et al.,* 2015) treated with tulsi (*Ocimum sanctum*) extracts; in yellow crocker supplemented with Astragalus root (Jian and Wu, 2003) and *L. rohita* fed with Andrographolide (Basha *et al.,* 2013). In present finding, Serum lysozyme activity was significantly enhanced in fishes fed with curcumin diet in compared to control group. The results showed the serum lysozyme activity was significantly ($p < 0.05$) highest in treatment T4 (1.5%).

Myeloperoxidase (MPO) is considered to be abundantly expressed in neutrophil granulocytes which play an important role in the defense of the organism. MPO is stored in primary, azurophilic granules of neutrophils and having broad bactericidal armamentarium. It utilizes hydrogen peroxide during the respiratory burst to produce hypochlorous acid (Dalmo *et al.,* 1997). In our finding, the treatment groups showed higher MPO activity (except T5) compare to control. A similar agreement was reported in *L. rohita* fed with levamisole and *Allium sativum* respectively (Wijendra and Pathiratne, 2007; Sahu *et al.,* 2007)

Serum protein, albumin, and globulin are important factors in immune response enhancement and are thought to be associated

with stronger non-specific immune responses in fishes (Wiegertjes *et al.,* 1996). In the present study the serum protein, albumin and globulin values in the fishes fed with curcumin supplement diet were significantly higher than control. Similarly, higher total serum protein, albumin, and globulin were observed in *Labeo rohita* fed with tulsi (*Ocimum sanctum*) (Das *et al.,* 2013).

Proteins include the humoral elements of the nonspecific immune system, e.g. immunoglobulin, transferrin, agglutinins or precipitins (Magnadottir, 2010). Fish-immunoglobulin is a tetrameric molecule and is far for the most prevalent immunoglobulin in plasma (Warr, 1997). The total immunoglobulin level showed a significant increase with a graded increase in the concentration of curcumin up to T4 group. The present finding of higher total immunoglobulin is supported with the findings in greasy groupers (*Epinephelus taurine*) fed with herbal diet containing purified active component of *Ocimum sanctum* (Sivaram *et al.,* 2004), *Withania somnifera*, and *Myristica fragrans* in Siberian sturgeon, (*Acipenser baeri*) fed with epin and glucan respectively also reported higher level of total Ig (Kolman *et al.,* 2001). Also in Indian major carp (*Labeo rohita*) dietary incorporation of *Achyranthes aspera*, coated feed (Rao *et al.,* 2004) and levamisole (Wijendra *et al.,* 2007), supports the present findings of higher total Ig in subsequent treatment groups.

Edwardsiellosis is one of the major threats to fish culture (Sahoo and Mukherjee, 2002), which caused heavy mortality in aquaculture farms. In the present study, the highest relative percentage survival (RPS) was recorded in T4 (62.5%) followed by T2 (50%), groups respectively. The T4 and T5 groups showed better performance in terms of overall growth parameter than the control group which is an agreement with previous study conducted in *L. rohita* fed with diet containing herb *Achyrathes aspera* (Rao *et al.,* 2004) and in *P. monodon* fed with *Solanum tribbatum*, *Andrographis*

paniculata and *Psoralea corylitolia* (Citrasu *et al.,* 2003a). Since fish require a long time to produce antibody, therefore, the decreased mortality rate should be considered mainly due to increased non-specific immunity (Anderson, 1992) as evident from the present study were increased NBT activity, phagocytic activity, lysozyme activity suggesting Curcumin supplement diet (CSD) is responsible for enhancing of humoral immune responsive.

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