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Effects of curcumin on haematological values, immunity, antioxidant status and resistance of rainbow trout (*Oncorhynchus mykiss*) against *Aeromonas salmonicida* subsp. *achromogenes*

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ABSTRACT

The present study was conducted to evaluate the effects of various levels of dietary curcumin on growth performance, haematological values, immunity and protection against *Aeromonas salmonicida* subsp. *achromogenes* infection in rainbow trout, *Oncorhynchus mykiss*. Fish were fed with diets containing different levels of curcumin; 0% (C), %1 (E1), %2 (E2) and %4 (E3), as treatment groups. After 8 weeks of feeding, the growth performance [weight gain (WG), specific growth rate (SGR) and feed conversion ratio (FCR)], haematological values [the red blood cell (RBC) count, haemoglobin (Hb) concentration, haematocrit (Ht) level and erythrocyte indices: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)], various immune parameters [white blood cell (WBC) count, oxidative radical production (nitroblue tetrazolium (NBT) assay), phagocytic activity (PA) and phagocytic index (PI), total protein (TP) and immunoglobulin M (IgM) levels, serum bactericidal (BA), lysozyme (LYZ) and myeloperoxidase (MPO) activities] and antioxidant capacity [tissue malondialdehyde (MDA) levels and superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities] were analysed. In addition, fish were challenged by *Aeromonas salmonicida* subsp. *achromogenes* and survival rate was recorded for 14 days. The results indicated that the growth performance was significantly influenced by the dietary curcumin levels, the maximal WG and SGR occurred at fish fed the diet containing 2% curcumin. Fish fed the diet containing curcumin had lower FCR than those fed the control diet. The RBC count, Hb concentration, and Ht level increased in the groups fed with curcumin when compared with the control group. However, there were no significant differences in the MCV, MCH and MCHC values among experimental groups. All the chosen immune parameters were enhanced in the groups fed diets containing curcumin. Also, the relative percentage survivals were higher in the groups fed with curcumin, especially in the E2 group, compared to the control. The dietary curcumin stimulated the SOD, CAT and GSH-Px activities in liver, head kidney and spleen as compared to the control group; however, a reverse trend was observed in the MDA levels of tissues. The highest values for haematological, immunological (except TP level of E3 group) and antioxidant parameters were found in the E2 group. These results collectively suggest that curcumin can be used in aquaculture to improve the growth, haematological values, immune responses, antioxidant capacity and disease resistance of rainbow trout, *O. mykiss*.

1. Introduction

In order to control disease outbreaks in aquaculture, several veterinary drugs and antimicrobials have been used indiscriminately, which in turn have led to problems such as risk of generating resistant pathogens, drug residues accumulating in treated fish, drug toxicity and detrimental effect on the environment [1,2]. Considering the adverse effects of drugs; their use is not recommended in the aquaculture. Therefore, there is a need to develop alternative therapies for bacterial pathogens that could effectively protect fish and prevent resistance development of pathogen microorganisms [3]. On the other hand, commercial vaccines are expensive for fish producers and may not be available for all species and against emerging diseases [1]. One of the most promising methods of controlling these problems in the

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aquaculture is by strengthening the defence mechanism of fish through prophylactic administration of immunostimulants which is considered as a promising alternative to chemotherapy and vaccines because of their broad spectrum activity, cost effectiveness, and eco-friendly disease preventative measure [4]. Immunostimulants are naturally occurring substances that activate the immune system (both humoral and cellular), and thus make the animal capable of coping with diseases. Also, immunostimulants can reduce the mortalities against pathogenic challenges [5]. The roles of immunostimulants in modulating the specific immune responses and non-specific defence mechanisms have been extensively investigated and reviewed in fish [5–9].

Phytogenic dietary additives including curcumin are a very suitable choice as potential growth and health promoters in the aquaculture as, they neither cause any residue problems nor have any negative effects on fish health or to human and environment [10]. Curcumin, also known as diferuloymethane, is a yellow pigment derived from plant *Curcuma longa* [11]. The powdered rhizome of this plant, called turmeric, has been used in traditional Chinese and Hindu medicine for centuries in the treatment of different diseases [12]. Curcumin further exhibits a wide range of pharmacological effects, such as antiinflammatory, anticarcinogenic, antiinfection, antitumor, immunomodulatory [13], antioxidant, antibacterial [14], antiparasitic [15] and antidepressant [16] activities. Despite the unique biological properties of curcumin, little is known about the effects of different levels of curcumin on the growth performance, haematology, cellular and humoral immune response, and antioxidant capacity of rainbow trout, *O. mykiss*. On the other hand, the information on the use of curcumin is limited in the aquaculture and few studies have reported in the other fish species. Many authors have documented that curcumin possesses significant biological properties, such as growth promotion [17–19], hepatoprotective [20], antioxidant [10,21,22], antiparasitic [15], immunomodulatory [10] and bactericidal [23,24], for fish.

The aim of the present investigation was to evaluate the effects of dietary curcumin on the growth performance, haematological indices, immune response, antioxidant capacity and disease resistance against *A. salmonicida* subsp. *achromogenes* infection in rainbow trout, *O. mykiss*.

2. Materials and methods

2.1. Ethics statement

All procedures used in the present study were approved by the Animal Experimentation Ethics Committee of Firat University (FUAEEC, Elazig, Turkey) (Protocol number: 2014/14, 135).

2.2. Chemicals

Curcumin (CAS No: 458-37-7) and all the other chemicals were supplied by Sigma-Aldrich Chemical Co. and Merck. All reagents were of analytical grade.

2.3. Diet preparation

A basal control diet was formulated without curcumin supplementation [25] (Table 1). The experimental diets consisted of the basal diet supplemented with 1%, 2%, 4% curcumin. Dry ingredients were mixed thoroughly, then water was added and mixed thoroughly in a mixer for 20 min. The resulting dough was pelleted, dried at room temperature for 48 h and stored at 5 °C until used. Diet composition was assayed according to the procedures described by the Association of Official Analytical Chemists [26].

2.4. Experiment animals and design

O. mykiss juveniles of average weight 31.29 ± 1.17 g were procured from a trout farm (Keban, Elazig, Turkey) and transported to laboratory. Health statuses of fish were examined instantaneously upon arrival. Fish were quarantine bathed in 250 ppm formalin for 30 min [27]. Fish were acclimatised for 30 days. All fish were fed with the basal control diet until satiation twice a day in two equal parts at 9.00 a.m. and 4.00 p.m. Water quality was monitored daily throughout the experiment. Water exchange was done daily at a rate of 50% and 100% of the water was exchanged once a week. Temperature was maintained at 16 ± 1 °C, dissolved oxygen concentration 7.5 mg L^{-1} and pH 7.6 ± 0.2.

The juveniles divided randomly into 4 groups (control, E1, E2 and E3) with 75 fish in each group, maintained in 12 tanks (540 L capacity) each containing 25 fish (4 experimental groups x 3 replicates, totally 300 fish). Control was fed with the basal diet without curcumin. The remaining groups were fed with 1% (E1), 2% (E2), 4% (E3) curcumin for 8 weeks. All experimental groups were fed until satiation two times a day at 9.00 a.m. and 4.00 p.m.

Table 1
Ingredients and proximate composition of the experimental diets.

Diet ingredients	(%)	Chemical analysis	(% dry matter)
Fish meal	48.00	Dry matter	92.20
Corn gluten	10.00	Crude protein	47.42
Soy bean meal	20.00	Crude fat	10.20
Wheat flour	14.50	Crude ash	8.63
Soybean oil	6.00	Crude fibre	2.40
Vitamin mixture[a]	1.00	Metabolizable energy***	3586
Mineral mixture[b]	0.50		

[a] Vitamin mixture (IU or mg/kg diet): Vitamin A 250 000 IU, vitamin D_3 240 000 IU, vitamin E 10 000 IU, vitamin K 3000 mg, vitamin B_1 1000 mg, vitamin B_2 3000 mg, vitamin B_6 2000 mg, vitamin B_{12} 4 mg, choline chloride 100 000 mg, vitamin C 6000 mg, niacin 30 000 mg, calcium D-pantothenate 10 000 mg, folic acid 600 mg and d-biotin 200 mg.

[b] Mineral mixture (mg/kg diet): Manganese 80, iron 35, zinc 50, copper 5, iodine 2, cobalt 0.4, selenium 0.15.

2.5. Sample collection

At the end of the feeding trial, all fish in each tank were weighed and counted to calculate growth performance including weight gain (WG), specific growth rate (SGR) and feed conversion ratio (FCR) and survival. Five fish from each tank (fifteen fish per dietary treatment) were randomly captured, anaesthetized in benzocaine solution, and blood samples were taken from the caudal vein with plastic syringes. A part of the blood samples was transferred into tubes containing K_3EDTA for haematological [red blood cell (RBC) count, haemoglobin (Hb) concentration, haematocrit (Ht) level and erythrocyte indices: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)] and immunological [white blood cell (WBC) counts, oxidative radical production (nitroblue tetrazolium (NBT) assay), phagocytic activity (PA) and phagocytic index (PI)] analysis. The remaining of the blood was transferred into serum tubes. The serum tubes were allowed to clot at room temperature for 30 min and the serum samples were separated by centrifugation for 10 min at 5000 × *g* and stored at −20 °C for analysis of the other immune parameters including total protein (TP) and immunoglobulin M (IgM) levels, serum bactericidal (BA), lysozyme (LYZ) and myeloperoxidase (MPO) activities.

After the blood samples were collected, liver, head kidney and spleen were carefully removed, washed with physiological saline (0.9% NaCl) and stored at −40 °C until the biochemical assays, which were performed within 1 month after extraction. The tissue was homogenised in a Teflon-glass homogeniser in buffer containing 1.15% KCl at a 1:10 (w/v) ratio to the whole homogenate. The homogenate was centrifuged at 18 000 *g* at 4 °C for 30 min to determine malondialdehyde (MDA) level and superoxide dismutase (SOD), catalase (CAT) and

glutathione peroxidase (GSH-Px) activities.

2.6. Growth study

Calculations for the growth performance (the WG, SGR and FCR) were performed using following equations:

WG (g) = Wt − W0;

SGR (%) = 100 × (lnWt?lnW0)/t;

(Wt and W_0: Final and initial weight of fish, respectively; t: experimental days; FI: Feed intake).

2.7. Haematological analysis

The RBC count was performed using a haemocytometer and the solution developed by Natt and Herrick [28]. The Hb concentration was determined based on the absorbance of Drabkin's reagent at 540 nm [29]. The Ht level was determined through a microhematocrit centrifugation technique. The erythrocyte indices [mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC)] were calculated through standard formulas using the Ht, RBC, and Hb data [30,31].

2.8. Immunological analysis

The WBC count was simultaneously determined with the RBC count [28]. The NBT assay was used to analyse radical oxygen production by phagocytes [32]. The PA and PI were measured according to the method described by Siwicki and Anderson [33]. The TP and IgM levels in serum were determined according to the method described by Siwicki et al. [32], Siwicki et al. [34], and Yonar et al. [35]. The BA was measured following the procedure of Kajita et al. [36] with some modifications. The LYZ activity was studied following the protocol provided by Ellis [37]. The MPO activity was determined following Quade and Roth [38] with slight modification of Sahoo et al. [39].

2.9. Challenge test with A. salmonicida subsp. achromogenes

The 7 day lethal dose 50 (LD_{50}) was determined by intraperitoneal injection of graded doses of *A. salmonicida* subsp. *achromogenes* into 10 fish. *A. salmonicida* subsp. *achromogenes* NCMB 1110 was grown on blood agar at 22 °C. Fresh culture was suspended in sterile 0.85% sodium chloride (NaCl). The cell suspension was adjusted to OD 0.9 at 610 nm (10^9 cfu/ml) and logarithmically diluted to give a range of dilutions from 10^9 to 10^4 cfu/ml. The result showed that the LD_{50} on day 7 was 1.17×10^8 cfu/ml. At the end of the feeding trial, the remaining fish from each tank was experimentally exposed to live *A. salmonicida* subsp. *achromogenes*. Thirty fish (ten fish/tank) were intraperitoneally injected with 100 µL PBS containing 1.17×10^8 cfu/ml live *A. salmonicida* subsp. *achromogenes*. The challenged fish were kept under observation for 14 days of post-infection and all fish groups were fed only the basal control diet. The cause of death was confirmed by re-isolating the organism from the liver and spleen of dead fish using conventional methods.

Relative percentage survival (RPS) was quantified using the following formula [40]:

RPS (%) = (Number of surviving fish after challenge/Number of fish injected with *A. salmonicida* subsp. *achromogenes*) × 100.

2.10. Oxidative stress and antioxidant capacity

The tissue MDA level, which is an index of oxidative stress in tissues, was measured with the thiobarbituric acid (TBA) reaction [41]. The tissue SOD activity was determined according to the method described by Sun et al. [42]. The tissue CAT activity was determined according to the method previously published by Aebi [43]. The tissue GSH-Px activity was performed in compliance with the method described by Beutler [44]. The protein levels in the tissues were determined by the method described by Lowry et al. [45].

Table 2
Growth performance of rainbow trout fed with different experimental diets for 8 weeks.

	Groups			
Parameters	Control	E1 (1%)	E2 (2%)	E3 (4%)
W_0	31.10 ± 1.10^a	31.23 ± 1.25^a	31.76 ± 1.22^a	31.04 ± 1.11^a
Wt	68.44 ± 1.26^a	72.28 ± 1.47^b	73.05 ± 1.41^b	72.81 ± 1.29^b
WG	37.34 ± 1.33^a	41.05 ± 1.20^b	41.77 ± 1.27^b	41.29 ± 1.04^b
SGR	1.40 ± 0.02^a	1.51 ± 0.02^b	1.52 ± 0.02^b	1.51 ± 0.02^b
FCR	1.22 ± 0.02^b	1.13 ± 0.02^a	1.12 ± 0.02^a	1.12 ± 0.02^a
Survival	100	100	100	100

W_0: Initial weight of fish **(g)**, Wt: Final weight of fish **(g)**, WG: Weight gain **(g)**, SGR: Specific growth rate **(%)**, FCR: Feed conversion ratio.
Survival is presented as %.
The values (n = 75) are expressed as mean ± standard error. The mean values in same line with different superscripts are significantly different ($p < 0.05$).

2.11. Statistical analysis

The results are expressed as the mean ± standard error and analysed using one-way analysis of variance (ANOVA), followed by Duncan test on the SPSS (15.0) software computer program. Differences were considered statistically significant when $p < 0.05$. The LD_{50} value of bacteria was calculated according to Finney's probit analysis method [46].

3. Results

3.1. Growth study

The effects of different experimental diets on the growth performance of rainbow trout are shown in Table 2. After 8 weeks of feeding, the groups fed diets containing curcumin had significantly higher the WG and SGR than the control group ($p < 0.05$). The FCR was significantly lower in the groups fed diets containing curcumin when compared with the control group ($p < 0.05$). However, there was no statistically significant change among the WG, SGR and FCR values of the E1, E2 and E3 groups ($p > 0.05$). Survival of fish fed the experimental diets was not significantly different ($p > 0.05$).

3.2. Haematological findings

The results of different dosages of curcumin on the haematological parameters are given in Table 3. The RBC count, Hb concentration and Ht level of E1, E2 and E3 groups were found to be statistically higher than the control group ($p < 0.05$). However, the RBC count, Hb concentration and Ht level of E1, E2 and E3 groups are similar ($p > 0.05$). No significant difference in the erythrocyte indices (MCV, MCH and MCHC) was observed among the experimental groups ($p > 0.05$).

3.3. Immunological findings

The results of different dosages of curcumin on the immunological parameters are presented in Table 4. The results indicated that the immunological values of E1, E2 and E3 groups were significantly ($p < 0.05$) higher than the control group. Additionally, the immunological values of E2 and E3 groups were significantly higher than the E1 group ($p < 0.05$).

Table 3
Haematological values of rainbow trout fed with different experimental diets for 8 weeks.

	Groups			
Parameters	Control	E1 (1%)	E2 (2%)	E3 (4%)
RBC	1.60 ± 0.03[a]	1.81 ± 0.06[b]	1.85 ± 0.07[b]	1.84 ± 0.05[b]
Hb	7.04 ± 0.55[a]	7.66 ± 0.49[b]	7.99 ± 0.63[b]	7.92 ± 0.60[b]
Ht	34.27 ± 2.10[a]	41.55 ± 2.48[b]	42.35 ± 3.22[b]	41.70 ± 2.85[b]
MCV	216.47 ± 15.27[a]	227.13 ± 18.33[a]	225.04 ± 17.86[a]	225.62 ± 14.05[a]
MCH	42.13 ± 3.22[a]	42.66 ± 4.06[a]	43.85 ± 4.51[a]	43.14 ± 3.63[a]
MCHC	20.03 ± 2.39[a]	19.11 ± 3.10[a]	19.98 ± 1.89[a]	19.76 ± 2.58[a]

RBC: Red blood cell (erythrocyte) count (x10^6), Hb: Haemoglobin concentration (g/dL), Ht: Haematocrit level (%), MCV: Mean corpuscular volume (μm^3), MCH: Mean corpuscular haemoglobin (pg), MCHC: Mean corpuscular haemoglobin concentration (%).
The values (n = 15 for haematological analysis) are expressed as mean ± standard error. The mean values in same line with different superscripts are significantly different ($p < 0.05$).

3.4. Challenge test with A. salmonicida subsp. achromogenes

The RPS rates of experimental groups are given in Table 4. The RPS rate was significantly affected by different dosages of curcumin. In the E1, E2 and E3 groups, the RPS rate was significantly higher ($p < 0.05$) than in the control group. Among the groups fed diets containing curcumin, the RPS rates of E2 and E3 groups were significantly higher than the E1 group ($p < 0.05$). However, there was no significant difference between the E2 and E3 groups ($p > 0.05$). Accordingly, the highest RPS was observed in the E2 group (76.67%), followed by the E3 group (73.33%), the E1 group (60.00%) and the control group (36.67%).

3.5. Oxidative stress and antioxidant capacity

The effects of different experimental diets on the oxidative stress and antioxidant capacity in the liver, head kidney and spleen are shown in Table 5.

At the end of the trial, the groups fed diets containing curcumin had significantly lower the tissue MDA levels than the control group ($p < 0.05$). Among the groups fed diets containing curcumin, the tissue MDA levels of E2 and E3 groups were significantly lower than the E1 group ($p < 0.05$). However, there was no significant difference between the tissue MDA levels of E2 and E3 groups ($p > 0.05$).

After curcumin feeding for 8 weeks, the groups fed diets containing curcumin had significantly higher the tissue SOD, CAT and GSH-Px activities than the control group ($p < 0.05$). Among the groups fed diets containing curcumin, the tissue SOD, CAT and GSH-Px enzyme activities of E2 and E3 groups were significantly higher than the E1 group ($p < 0.05$). However, there was no significant difference between the E2 and E3 groups ($p > 0.05$).

4. Discussion

Curcumin is a popular immunostimulant that confers benefits such as growth enhancement and disease resistance in the aquaculture according to recent reports. In the present study, the growth performance, haematology, cellular and humoral immune response, and antioxidant capacity of rainbow trout were evaluated with the dietary curcumin. The results showed that the dietary curcumin positively affected the growth, haematological and immunological parameters, disease resistance, and tissue oxidant/antioxidant capacity of rainbow trout.

Cui et al. [47] found that dietary supplementation with curcumin significantly increases growth performance of juvenile gift tilapia, *Oreochromis niloticus*. Improvement in growth performance by dietary supplementation with curcumin has been reported in other aquatic animals including grass carps (*Ctenopharyngodon idells*) [17], large yellow croaker (*Pseudosciaene crocea*) [18], Asian see bass (*Lates calcarifer*) [48], crucian carp (*Carassius auratus*) [49], and Nile tilapia (*Oreochromis niloticus*) [10]. Results of this study revealed that curcumin improved the growth of rainbow trout and did not negatively affect the growth. This increase in the growth may be attributed to digestive enhancer properties of curcumin [49]. According to these results, curcumin can improve the growth of rainbow trout. Therefore, curcumin can be safely used in fish feed.

The haematological parameters are widely used to evaluate health status, stress and disease conditions of fish [50,51]. These parameters

Table 4
Immunological values of rainbow trout fed with different experimental diets for 8 weeks and effect of experimental diets on the survival of rainbow trout challenged with *A. salmonicida* subsp. *achromogenes*.

	Groups			
Parameters	Control	E1 (1%)	E2 (2%)	E3 (4%)
WBC	24.16 ± 2.17[a]	27.33 ± 1.85[b]	29.04 ± 2.10[c]	28.96 ± 1.97[c]
NBT	1.22 ± 0.14[a]	1.53 ± 0.19[b]	1.69 ± 0.22[c]	1.66 ± 0.16[c]
PA	32.45 ± 3.10[a]	36.50 ± 3.30[b]	40.30 ± 4.05[c]	39.33 ± 3.67[c]
PI	3.19 ± 0.80[a]	5.22 ± 0.90[b]	5.76 ± 1.10[c]	5.72 ± 0.90[c]
TP	28.71 ± 2.58[a]	32.79 ± 3.17[b]	36.34 ± 3.85[c]	37.22 ± 2.71[c]
IgM	16.71 ± 1.74[a]	21.29 ± 2.08[b]	24.59 ± 2.04[c]	23.90 ± 1.83[c]
BA	30.52 ± 1.52[a]	59.74 ± 2.41[b]	72.08 ± 2.83[c]	70.23 ± 1.86[c]
LYZ	110.43 ± 8.78[a]	141.67 ± 10.17[b]	178.20 ± 14.49[c]	175.96 ± 12.30[c]
MPO	0.74 ± 0.07[a]	0.87 ± 0.09[b]	0.99 ± 0.12[c]	0.96 ± 0.11[c]
RPS	36.67[a]	60.00[b]	76.67[c]	73.33[c]

WBC: White blood cell counts (x10^3), NBT: Nitroblue tetrazolium assay (mg/ml), PA: Phagocytic activity (%), PI: Phagocytic index, TP: Total protein level (mg/ml), IgM: Total immunoglobulin M level (mg/ml), BA: Serum bactericidal activity (% cfu/control), LYZ: Lysozyme activity (U/ml), MPO: Myeloperoxidase activity (Optic density), RPS: Relative percentage survivals (%).
The values (n = 15 for immunological analysis, n = 30 for RPS) are expressed as mean ± standard error. The mean values in same line with different superscripts are significantly different ($p < 0.05$).

Table 5
Effect of the experimental diets on the antioxidant capacity of rainbow trout.

	Parameters	Groups			
Tissues		Control	E1 (1%)	E2 (2%)	E3 (4%)
Liver	MDA	2.42 ± 0.55^{c}	2.28 ± 0.69^{b}	2.05 ± 0.43^{a}	2.04 ± 0.84^{a}
	SOD	3.26 ± 0.41^{a}	3.60 ± 0.74^{b}	3.98 ± 0.88^{c}	3.95 ± 0.82^{c}
	CAT	3.48 ± 0.36^{a}	3.71 ± 0.52^{b}	3.95 ± 0.71^{c}	3.94 ± 0.93^{c}
	GSH-Px	2.59 ± 0.55^{a}	2.78 ± 0.70^{b}	2.99 ± 0.87^{c}	2.98 ± 0.79^{c}
Head kidney	MDA	3.45 ± 0.62^{c}	3.20 ± 0.92^{b}	2.93 ± 0.75^{a}	2.94 ± 0.50^{a}
	SOD	2.76 ± 0.58^{a}	2.93 ± 0.51^{b}	3.17 ± 0.83^{c}	3.14 ± 0.68^{c}
	CAT	3.04 ± 0.78^{a}	3.30 ± 0.66^{b}	3.66 ± 0.92^{c}	3.65 ± 0.81^{c}
	GSH-Px	2.20 ± 0.41^{a}	2.41 ± 0.50^{b}	2.63 ± 0.72^{c}	2.60 ± 0.63^{c}
Spleen	MDA	3.19 ± 0.70^{c}	2.94 ± 0.51^{b}	2.76 ± 0.93^{a}	2.71 ± 0.78^{a}
	SOD	2.61 ± 0.57^{a}	2.89 ± 0.72^{b}	3.13 ± 0.49^{c}	3.12 ± 0.39^{c}
	CAT	2.80 ± 0.43^{a}	2.96 ± 0.61^{b}	3.15 ± 0.67^{c}	3.10 ± 0.20^{c}
	GSH-Px	2.01 ± 0.37^{a}	2.33 ± 0.50^{b}	2.58 ± 0.74^{c}	2.55 ± 0.49^{c}

MDA: Malondialdehyde level (nmol/mg protein), SOD: Superoxide dismutase activity (U/mg protein), CAT: Catalase activity (k/mg protein, k: the first-order rate constant) and GSH-Px: Glutathione peroxidase activity (U/mg protein).
The values (n = 15) are expressed as mean ± standard error. The mean values in same line with different superscripts are significantly different ($p < 0.05$).

are important tools that could be widely used in evaluating of functional and nutritional status and the capacity for fish adaptation to the external environment [52]. In addition, the haematological parameters give an indication to the health status of fish by determining any abnormality occurring owing to the use of immunostimulants [53]. The erythrocyte indices are useful in elucidating the etiology and morphological classification of anaemias. The results revealed that the RBC count, Hb concentration and Ht level of E1, E2 and E3 groups significantly increased after 8 weeks. However, in the present study no change was observed in the erythrocyte indices (MCV, MCH and MCHC). Our data showed that the dietary curcumin had a positive effect on the haematological parameters in rainbow trout and curcumin did not cause anaemia. Also, the changes in erythrocyte indices were not significant from control, which indicated the fish was not under stress.

Leucocytes are involved in the control of immunological functions. They are considered as one of the important factors of nonspecific or innate immunity and the leucocyte count/activity can be indicated as indicators of health of aquatic animals [50,54]. A respiratory burst, or oxidative burst, is an indication of the oxidative potential of reactive oxygen species produced by activated phagocytic cells. Respiratory bursts from innate immune cells, including blood neutrophils, are measured using NBT or MPO assays [55]. The NBT assay measured in terms of respiratory burst activity of phagocytes is used to detect oxidative radical production which is considered an important innate immune mechanism [56]. MPO is a haem based peroxidase enzyme released by neutrophils in fish and plays an important role in the nonspecific cellular immunity. It produces reactive oxygen species, which help in the killing of bacteria [57]. Phagocytic cells including the monocyte/macrophages and granulocytes in teleost fish are the main components of innate immune response, which regulate immune function and play a major role in combating diseases. Their phagocytic activity is a primitive defence mechanism and an important characteristic of the nonspecific immune system [58]. In addition, phagocytosis associated with respiratory burst activity is an important indicator of innate immunity in fish [59]. In this study, the administration of curcumin to rainbow trout induced the innate immunity, further improving immune responses, such as the WBC count, phagocytosis and MPO activity. Similarly, previous studies demonstrated that dietary administration of curcumin significantly affected the WBC counts in rainbow trout [60] and carp [61], the phagocytic activity and superoxide anion production in goldfish [58] and MPO activity in Indian major carp [62]. This results have showed that curcumin can stimulate the innate immunity in fish. The possible molecular mechanisms of the immunostimulating effect of curcumin have been previously described. Jageita and Aggarwal [63] have documented that curcumin enhance the innate immunity by activating macrophages and neutrophils to generate reactive oxygen species. Antony at al [64]. have also stated that curcumin increase the WBC counts, the phagocytic activity of macrophages and the production of cytokines, which play key roles in regulating immune responses.

Serum total protein including albumin and globulin plays a significant role in humoral immune response. Serum protein and globulin levels are vital fractions for sustaining healthy immune system. An increase in serum protein level shows a stronger innate immune response in fish [53,65,66]. Immunoglobulins are glycoproteins that have antibody activity and are found in the blood, lymph, and vascularized tissues. IgM, the main antibody of fish, is a major component of the teleost humoral immune system [67]. This molecule is believed to play a vital role in several immune processes such as phagocytosis, opsonisation, and neutralization of pathogenic bacteria, viruses, and toxins in the host body [68,69]. Prior researches have reported the relationship between total protein and immunoglobulin levels [59,70]. The present results indicated noticeable increase in the TP and IgM levels of fish fed with curcumin, which may be associated with induction of humoral immunity after the administration of its. This result can be explained by increasing B cells or antibody titre in circulation of curcumin [63,64]. In this sense, we suggest that curcumin may increase the TP and IgM levels by inducing humoral immunity in fish. Several other studies have also reported positive effects of immunostimulants including garlic [1,71], ginger [72,73], and curcumin [10] on the TP or Ig levels in different fish species.

The presence of protective proteins in fish blood including complements, acute phase proteins, LYZ, transferrin and anti-proteases might be analysed by serum BA, which considered as nonspecific response to inhibit the growth of pathogenic microbes [74]. LYZ is relevant as a first line barrier of defence system thus resulting in the diminution of disease by preventing bacterial pathogens [3]. LYZ, in the serum, mucus, and other body fluids, causes lysis of bacteria and activation of the complement system and phagocytes by acting as an opsonin [75]. Herbal products including curcumin are known to increase the LYZ activity in different fish species [10,58,76]. In the present study, the LYZ activity and BA were elevated significantly in the groups fed diets containing curcumin. Moreover, the group fed E2 diet had the highest serum LYZ activity, which was associated with the highest serum BA in this group. Previous studies have demonstrated LYZ activity increases following a stimulation of the immune system [77] and elevation in LYZ activity associate with increase in leucocyte number [78] or antibody titre [79]. On the other hand, many investigators have reported enhanced BA by the phagocytic cells of different fish species

treated with immunostimulants [80]. Consistent with previous investigations [77–80], these increments in LYZ activity and BA could be related to the increased WBC count, phagocytic activity or antibody titre in the groups fed diets including curcumin.

An experimental infection helps extremely to evaluate the effectiveness of dietary treatments in the resistance of host against pathogens [57]. In addition, the RPS is one of the most visual indices to evaluate the immune effect in a challenge test [65]. In the present study, after challenge with *A. salmonicida* subsp. *achromogenes*, the E1, E2 and E3 groups showed high survival when compared to the control group. Furthermore, the best RPS rate was observed in the E2 group. The increase of humoral and cellular immune parameters by curcumin is probably an important factor in reducing the percentage mortality and thereby protecting fish against *A. salmonicida* subsp. *achromogenes* challenge. Earlier studies in this line also revealed that dietary supplementation of curcumin enhanced disease resistance in *Lobeo rohita* [62,81,82] and *Oreochromis niloticus* [10] against *A. hydrophila*.

Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites, and their elimination by protective mechanisms, referred to as antioxidants [83–85]. Oxidative damage is related to the formation of reactive oxygen species and can occur when the antioxidants are deficient [86]. Lipid peroxidation is considered to be a valuable indicator of the oxidative damage in cellular components [87]. MDA as a main end product of lipid peroxidation can be used as one of the indices of lipid peroxidation [88–90]. Under normal condition, enzymatic antioxidants, such as SOD, CAT, and GSH-Px protect cells from oxidative damage due to excessive generation of reactive oxygen species. The balance between oxidant and antioxidant is fundamental for immune cell function since it preserves cell membrane integrity and functionality, cellular proteins, and nucleic acids [91]. Mahmoud et al. [10] showed that curcumin supplementation, especially at a concentration of 50 mg kg^{-1} diet, in the Nile tilapia diet resulted in an obvious elevation of both enzymatic (CAT) and nonenzymatic (GSH) antioxidant with a significant decline in the MDA level. Similarly, Jiang et al. [49] also stated that the dietary curcumin increased intestinal antioxidant capacity in crucian carp, *Carassius auratus*. In the present study, the dietary curcumin significantly increased the SOD, CAT, and GSH-Px activities while decreased the MDA levels in liver, head kidney and spleen of rainbow trout. The increase determined in this study may be explained with possibly enhancement of antioxidant capacity [10,49]. In addition to its direct antioxidant activity, curcumin may function indirectly as an antioxidant by enhancing the synthesis of glutathione, which is known to be a substrate for the GSH-Px [92,93].

In conclusion, the results of this study showed that the dietary curcumin positively influences the growth performance, haematological values, immune response, antioxidant capacity, and disease resistance against *A. salmonicida* subsp. *achromogenes* in rainbow trout. Moreover, present results demonstrated that 2% curcumin supplemented diet was the best option for this species, based on the effectiveness of curcumin on the growth, haematological indices, immunity and antioxidant capacity of rainbow trout, *O. mykiss*. These results indicated that curcumin can be considered as a beneficial dietary supplement for rainbow trout in the aquaculture.

Conflicts of interest

There is no conflict of interest to declare.

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