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Research Article

Effect of different dietary levels of curcumin (*Curcuma longa*) powder on growth performance, hematology, and non-specific immune parameters in Siberian sturgeon, *Acipenser baerii*

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Abstract

This study was done to find the effect of different levels of curcumin on growth performance, immune response, and hematological markers in Siberian sturgeon, *Acipenser baerii*. For this purpose, a total number of 120 Siberian sturgeons with an average initial weight of 55.05±0.03 g (means±SD) were distributed into 12 tanks with 10 fish per tank (4 treatments with 3 replications) and fed four diets supplemented with 0 (base group, (C0), 0.5 (C0.5), 1 (C1), and 1.5 (C1.5) g kg^{-1} curcumin at 2.5% of body weight three times a day for 8 weeks. The highest final body weight (83.10 g), specific growth rate (3.12% day^{-1}), and body weight increase (51.1%) were obtained in the fish fed with 1 g kg^{-1} curcumin (p<0.05). The highest number of red blood cells and white blood cells were measured in C1 treatment. The immune responses were significantly influenced by different curcumin levels in the diets (p<0.05). The alternative complement pathway, lysozyme, and respiratory burst activity were significantly increased, especially in the fish fed with 1 g kg^{-1} curcumin. In conclusion, the results indicated that 1 g kg^{-1} curcumin notably enhances growth and non-specific immune responses in Siberian sturgeon.

Keywords: *Acipenser baerii*, Herbal extract, Growth, Immune response

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Introduction

The high quality of meat and the remarkable economic value of caviar have led to the expansion of sturgeon culture in the world (Bronzi *et al*. 2011; Bronzi and Rosenthal, 2014). Siberian sturgeon is one of the best species for aquaculture concerning high-stress resistance and great feeding adaptability being capable of growth in different situations (Adamek *et al*., 2007; Abdolahnejad *et al*., 2015; Moazenzadeh *et al*., 2017).

During recent years, growth performance and disease resistance have been considered as two primary concerns in aquaculture (Nazerian *et al*., 2017) and various chemical medications have been applied to treat bacterial infections in an aquatic organism (Bazari Moghaddam *et al*., 2017).

In aquaculture, overuse of antibiotics may lead to antibiotic resistance that can be spared t and also have negative impacts on ecology and public health (Cabello, 2006). In this regard, medicinal plants have been introduced as a new approach to prevent and treat infectious diseases in fish. During the last decades, medicinal herbs have been applied as immune stimulants to enhanced fish growth and improve specific and nonspecific defense mechanisms to increase resistance against diseases (Chakrabarti and Rao, 2006). Curcumin, a yellowish compound extracted from the rhizome of turmeric, has been commonly used as a spice, flavoring agent, food preservative, and coloring agent around the world (Prasad *et al*., 2014; Jiang *et al*., 2016). A few studies have been conducted to study its effects on fish. Manju *et al*. (2012) reported that dietary curcumin supplementation of about 5 or 10 g kg^{-1} of diet can have positive effects on growth performance in climbing perch *(Anabas testudineus*) (Manju *et al*., 2012). Also, Curcumin supplementation improved food conversion, growth performance, and biochemical parameters in Nile tilapia (*Oreochromis niloticus*) (Cui *et al*., 2013). Curcumin supplementation can enhance growth and innate immunity at a level of 2.0 g kg^{-1} diet (Tawwab and Abbass, 2017).

Over the past two decades, the number of studies has increased with the common finding that medicinal plant extracts have the potential to substitute synthetic chemicals and limit their usage in aquaculture. Application of dietary supplements such as herbal extracts has been assessed for their efficacies on sturgeons (Gholipour kanani *et al*., 2013; Nazerian *et al*., 2017). But there is not sufficient data on individual species. Thus, the current study was conducted to determine the effect of dietary curcumin on growth performance and immune response in Siberian sturgeon.

Material and methods

Experimental diets

Four isoenergetic diets were prepared (Table 1). The diet components were purchased from the Mazandaran Animal and Aquatic Feed (Sari, Mazandaran, Iran), and curcumin was bought from Soha Jissa Plantation Industries and Medicinal Plants Processing Company (Salmanshahr, Mazandaran, Iran).

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Briefly, the dry ingredients were mixed thoroughly. After the liquid ingredients were diffused into the mixture, deionized water was added (250 ml per kg of diet). The prepared mixture was extruded in an electric meat grinder (MG1400R, Pars Khazar, Tehran, Iran), and feeds were passed off based on the fish's mouth size (4 mm in diameter). Finally four experimental diets were prepared including control (0 g kg^{-1} curcumin, C0), C0.5 (0.5 g kg^{-1} curcumin), C1 (1 g kg^{-1} curcumin), and C1.5 (1.5 g kg^{-1} curcumin). Feeds were spread on separate trays (for 24 hours) and dried at room temperature. Diets were packed and stored at –20°C but daily diets were held at 4°C.

Animals and experimental condition

This experiment was carried out in Shahid Rajai Sturgeon Fish Propagation and Rearing Center of Sari (Sari, Mazandaran, Iran). A total of 120 Siberian sturgeons with initial body weights of 55.05±0.03 g (mean±SD) were randomly divided into twelve tanks (1000 L) at a density of 10 fish per tank. The fish acclimated to the experimental condition and fed with the commercial diet for two weeks. After acclimation, fish were hand-fed with experimental diets 2.5% body weight three times a day (at 08:00, 12:00, and 16:00 h) for 8 weeks. Water quality parameters were checked periodically and the flow rate was found 200 L $hour^{-1}$, dissolved oxygen was 7.4-7.8 mg L^{-1}, pH was 7.98±0.2, water temperature ranged from 22.2-23.5°C, and photoperiod was 12D:12L. The water volume was renewed one-third of the total before the first feeding time in the morning. Any mortality was removed daily and recorded survival rate.

Growth Performance

At the end of the experiment, feeding was stopped for 24 h, after that the fish in each tank were separately weighed and the growth performance was calculated as below (Mohammadzadeh *et al*., 2017):

Body weight increase (BWI, %) = 100 × [final weight (g) – (initial weight (g)] / initial weight (g)
Feed conversion ratio (FCR) = dry weight of feed given (g) / WG (g)
Specific growth rate (SGR) = Ln final weight-Ln initial weight×100/ days
Condition factor (K) = body weight/body $length^3$ × 100

Sample collection

First, fish were anesthetized with clove oil (25 ppm). Blood samples were collected by venipuncture of the caudal vein using a sterile 5-ml syringe and introduced to both heparinized and nonheparinized tubes. Non-heparinized blood was centrifuged (1,600 × g for 10 min) to obtain the serum and was separated supernatant and stored at -70°C for later analysis.

Hematological assay

Neubauer haemocytometer was applied to measure erythrocytes (RBC) and total leucocytes (WBC) (Martins *et al*., 2004).

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Deferential leucocyte counts (lymphocyte, neutrophil, monocyte, and Basophil) were determined using blood smears under a light microscope (Klontz, 1994). Cells were identified based on morphology and cell ultrastructure as documented in previous fish leucocyte studies (Ellis, 1977; Rowley, 1990).

Immunological assay

Lysozyme activity was assayed out using the method of Saurabh and Sahoo (2008) with turbidimetric essay. The first, suspension of *Micrococcus lysodeikticus* was prepared by dissolving 0.2 mg mL^{-1} in a 0.05 M sodium phosphate buffer (pH 6.2). Then, 50 µL serum was added to 2 mL suspension of *Micrococcus lysodeikticus* and the reaction mixture was divided in a 96 well microtitre plate and initial OD was measured spectrophotometrically at 450 nm immediately. Final OD was measured after incubation of the reaction mixture at 24°C for 1 h. A unit of lysozyme activity was defined as the sample amount causing a decrease in absorbance of 0.001 min^{-1}. Lysozyme of sample calibrated using a standard curve determined with hen's egg white lysozyme (Sigma) in PBS. Serum alternative complement (ACH50) activity was assayed according to Yano *et al*. (1988) in which rabbit red blood cells (RRBC) were used as a target.

The challenge trial

At the end of the experiment, all remaining fish in each tank (7 fish) were sampled and injected with *A. hydrphila* as previously described by Hoseinifar *et al*. (2017). The dead fish has removed every day and the mortality (%) was calculated after 14 days as follows:

Mortality (%) = (number of dead fish / total number of fish) ×100

Statistical Analysis

SPSS software (version 16, Chicago, IL, USA) was used to analyze data. Shapiro-Wilk and Levene's tests were applied to check the data normality and homogeneity of variances, respectively. The effect of the treatments on growth performance, immune response, and hematology marker was examined by one-way analysis of variance (ANOVA). Differences between several treatments were determined by Tukey's post-hoc tests.

Results

Growth Performance

The present results showed significant differences in FW, SGR, BWI, and CF among treatments and all curcumin treatments had better performance compared to the control treatment (Table 1; $p<0.05$). The highest FW, BWI, SGR, and CF was observed in fish fed on C1 diet. There is a significant effect in FCR among treatments and fish fed with C1 treatment had the best FCR (Table 2; $p<0.05$).

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Hematological parameter

The effect of different levels of dietary curcumin on hematological parameters of Siberian sturgeon is presented in Table 3. The RBC was influenced by dietary curcumin levels and was significantly higher in C1 treatment. WBC and lymphocyte significantly increased with increasing curcumin levels from 0 to 1 g kg^{-1} and further decreased in fish fed with C1.5 treatment. WBC had the same pattern on lymphocyte and the highest amount was observed in C1 treatment. The highest neutrophil, monocyte, and basophil amount were observed in fish fed with the control diet (Table 3; $p<0.05$).

Table 1: Experimental diets and proximate chemical composition.

Feedstuffs (g kg^{-1})	**Control**	**0.5**	**1**	**1.5**
Fishmeal[1]	150	150	150	150
Meat meal[2]	200	200	200	200
Soybean meal	230	230	230	230
Wheat meal	339	338.8	338.6	338.4
Fish oil	7	7	7	7
Soybean oil	7	7	7	7
Corn flour	50	50	50	50
L-Lysine[3]	7	7	7	7
L-Methionine 100[3]	5	5	5	5
Vitamin premix[4]	2.5	2.5	2.5	2.5
Mineral premix[5]	2.5	2.5	2.5	2.5
curcumin (g kg^{-1})	0	0.5	1	1.5
Chemical composition				
Gross Energy (Kcal kg^{-1})	4097.04	4096.25	4095.47	4094.68
Dry matter (%)	86.22	86.20	86.18	86.16
Crude protein (%)	38.21	38.21	38.21	38.20
Crude fat (%)	6.23	6.23	6.23	6.23
Crude ash (%)	6.37	6.37	6.36	6.36

[1]Pars kilka Co., Mazandaran, Iran (Kilka powder analysis; Protein: 70-72%, Fat: 8-11%, Ash: 11.6%, Moisture: 7-9%).

[2]Makianmehr Co., Golestan, Iran.

[3]Morghenojan.Co., Tehran, Iran.

[4]Vitamin premix (per kg of diet): vitamin A, 2000 IU; vitamin B_1 (thiamin), 5 mg; vitamin B_2 (riboflavin), 5 mg; vitamin B_6, 5 mg; vitamin B_{12}, 0.025 mg; vitamin D_3, 1200 IU; vitamin E, 63 mg; vitamin K_3, 2.5 mg; folic acid, 1.3 mg; biotin, 0.05 mg; pantothenic acid calcium, 20 mg; inositol, 60 mg; ascorbic acid (35%), 110 mg; niacinamide, 25 mg.

[5]Mineral premix (per kg of diet): $MnSO_4$, 10 mg; $MgSO_4$, 10 mg; KCl, 95 mg; NaCl, 165 mg; $ZnSO_4$, 20 mg; KI, 1 mg; $CuSO_4$, 12.5 mg; $FeSO_4$, 105 mg; Co, 1.5 mg.

Table 2: Effect of curcumin supplementation intake on growth performance of *Acipenser baerii*.

Factors	**C0**	**C0.5**	**C1**	**C1.5**
FW (g $fish^{-1}$)	65.45 ± 0.04^{c}	79.91 ± 0.04^{b}	83.10 ± 0.03^{a}	77.99 ± 0.01^{b}
BWI (%)	19.8 ± 0.3^{c}	45.3 ± 0.2^{b}	51.1 ± 0.1^{a}	41.8 ± 0.1^{b}
SGR (% day^{-1})	1.34 ± 0.05^{c}	2.27 ± 0.04^{b}	3.12 ± 0.01^{a}	2.12 ± 0.07^{b}
FCR	2.2 ± 0.02^{a}	1.8 ± 0.01^{b}	1.5 ± 0.05^{c}	2 ± 0.02^{b}
CF (K)	0.32 ± 0.01^{b}	0.36 ± 0.00^{a}	0.37 ± 0.01^{a}	0.36 ± 0.02^{a}

Body weight increase (BWI, %), Feed conversion ratio (FCR), Specific growth rate (SGR), Condition factor (K). Data are presented as mean±SD, n=30. Data in the same row with different superscripts are significantly different ($p<0.05$).

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Immunological parameters

Immunological parameters in fish fed various dietary curcumin levels are shown in Table 4. Alternative complement pathway and lysozyme activity of fish were affected by the dietary curcumin levels and increased with increasing curcumin levels from 0 to 1 g kg^{-1} diet and further decreased. ACH50 and lysozyme in fish-fed C1 treatment were significantly higher than other treatments (Table 4; $p<0.05$).

Table 3: hematological profile of *Acipenser baerii* fed with curcumin supplementation intake.

Factors	C0	C0.5	C1	C1.5
RBC ($\times 10^6 L^{-1}$)	0.97 ± 0.01^b	0.98 ± 0.07^b	1.25 ± 0.02^a	1.05 ± 0.06^b
WBC ($\times 10^3 \mu L^{-1}$)	8.1 ± 0.001^d	13.4 ± 0.003^b	16.2 ± 0.001^a	10.5 ± 0.002^c
Lymphocyte (%)	61 ± 0.006^c	74 ± 0.002^b	83 ± 0.007^a	75 ± 0.005^b
Neutrophil (%)	11 ± 0.004^a	8 ± 0.001^b	5 ± 0.001^c	8 ± 0.002^b
Monocyte (%)	23 ± 0.005^a	15 ± 0.002^b	11 ± 0.008^c	14 ± 0.006^b
Basophil (%)	5 ± 0.001^a	3 ± 0.002^b	1 ± 0.000^c	3 ± 0.001^b

Red blood cells (RBC), White blood cells (WBC). Data are presented as mean ± SD. Data in the same row with different superscripts are significantly different ($p<0.05$).

Table 4: Immunological responses of *Acipenser baerii* fed with curcumin supplementation intake.

Factors	C0	C0.5	C1	C1.5
ACH50 activity (U mL^{-1})	112.8 ± 0.1^d	131.5 ± 0.2^b	139.8 ± 0.4^a	123.7 ± 0.1^c
Lysozyme activity (µg mL^{-1})	18.7 ± 0.5^d	23.5 ± 0.4^b	26.7 ± 0.5^a	21.2 ± 07^c

Data are presented as mean±SD. Data in the same row with different superscripts are significantly different ($p<0.05$).

Challenge trial

Results of the challenge with *Aeromonas hydrophila* on mortality are presented in Figure 1. The lowest mortality percentage was observed in C1 treatment.

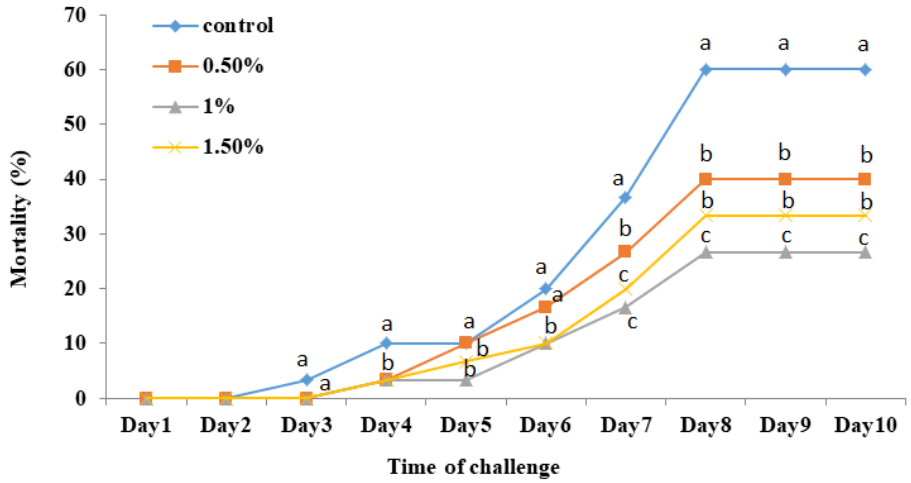

Figure 1: Cumulative mortality (%) of *Acipenser baerii* fed with curcumin supplementation intake at different levels for 60 days and subsequently challenged with *Aeromonas hydrophila*. Data are presented as mean±SD.

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Discussion

Supplementation of the Siberian sturgeon diet with curcumin improved growth performance and feed utilization over the control diet. The use of curcumin or turmeric powder as additives in aquafeeds may improve diet digestion and nutrient digestibility, which leads to improved fish growth and feed utilization (Abdel-Tawwab and Abbass, 2017). The present observations are consistent with the results of Sahu *et al*. (2008), who found that adding 1.0 g turmeric powder kg^{-1} diet improved the growth and food efficiency of rohu, *Labeo rohita*.

Dietary curcumin supplementation improved growth in Wuchang bream (*Megalobrama amblycephala*), Crucian carp (*Carassius auratus*), and Mrigala carp (*Cirrhinus mrigala*) at different levels (Xia *et al*., 2015; Jiang *et al*., 2016; Leya *et al.*, 2017).

In this study, the RBC was significantly higher in the fish-fed diet containing curcumin compared to control. According to our finding, Nazerian *et al*. (2017) reported a significant increase RBC in Beluga (*Huso huso*) juveniles fed both purple cornflower (*Echinacea purpora*) and garlic (*Allium satvium*) at 1 g kg^{-1} diet. Saeidi *et al*. (2017) showed that RBC significantly increased after 8 weeks in rainbow trout (*Oncorhynchus mykiss*) fed with 3 % nettle (*Urtica dioica*). Herbal extracts contain digested compounds that improve immune system function. The observed changes in hematological parameters may due to the long-term effects of curcumin supplementation. In this case, Soltanian and Fereidoni (2016) reported that diets supplemented with Henna (*Lawsonia inermis*) improved hematological parameters in common carp. Therefore, increasing RBC intensifies the Hb concentration and ultimately leads to the ability of fish to carry oxygen.

In the current results, several immune parameters such as WBC number, the percentage of lymphocyte, monocyte, basophil, and neutrophil were evaluated at the end of the trial. Leukocyte count was affected by curcumin levels. Similar findings were reported by Barrett (2003) and Widel *et al*. (2003), who obtained that herbal extract influenced the leukocyte count. Treatment with curcumin levels significantly increased WBC number and lymphocyte content compared to the control group. Similarly, an increased level of WBC and lymphocyte obtained in common carp fed with dietary garlic (Iranloye, 2002). Besides, similar results were reported in *Cirrhinus mrigala* fed with ginger and turmeric supplementation (Sivagurunathan *et al*., 2012) and in beluga fed with garlic and nettle supplemented diet (Nobahar *et al*., 2015). The presence of flavonoids and terpenoids in the herbal extract may increase WBC and lymphocyte numbers (Sayyah *et al*., 2005). This result is suggesting that curcumin may be involved in inflammatory responses. WBC is the first line of defense and plays a critical role in innate immunity against microbial infections. Increased WBC and Lymphocyte numbers are considered indicators of the health status

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of fish (Kumar *et al*., 2013). Neutrophil, monocyte, and basophil were decreased with increasing CM levels from 0 to 1 g kg^{-1} diet and the lowest number was observed in fish fed 1 g CM kg^{-1} diet. A similar result was found in beluga-fed herb extract. In the current study, lysozyme was significantly higher in the fish-fed diet containing curcumin compared to control. This result is according to a previous study that serum lysozyme activity could be enhanced through dietary administration by various herbs in beluga (Gholipour kanani *et al*., 2013; Nazerian *et al*., 2017) and *L. rohita* (Das *et al*., 2015) treated with herb extract. Leya *et al*. (2017) reported that serum lysozyme activity was significantly enhanced in fish fed with a curcumin diet compared to the control group in Mrigala carp. Behera *et al*. (2011) found that curcumin, the main component of turmeric powder, increased the nonspecific immunity of *Labeo rohita*. An increase in lysozyme activity can maintain the host during pathogen incursion by increasing several humoral agents (Harikrishnan *et al*., 2011). Some important cellular immune system components in fish include ACH50 (Rodríguez *et al*., 2019). In current work, ACH50 increased in fish-fed curcumin and the highest level was observed in C1 treatment.

The protective effect of curcumin was tested through challenge infection using pathogenic *A. hydrophila*. The control group had the lowest survival after the bacterial challenge. These results may be related to the potent antimicrobial properties of curcumin and could inhibit the occurrence of *A. hydrophila*. In this regard, protection Nile tilapia against pathogenic bacteria *Pseudomonas fluorescens* using turmeric supplementation was reported by Mahmoud *et al*. (2014). The obtained results are similar to Sahu *et al*. (2008), who found that feeding turmeric powder might maintain long-term protection in fish by elevating the nonspecific immune system.

In conclusion, the results indicated that curcumin notably enhances growth and improves non-specific immune responses in Siberian sturgeon.

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