

Effects of dietary Garlic extract on some blood, immunity and growth parameters of Common Carp fingerlings (*Cyprinus carpio*)

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Abstract

This research was carried out to study the effect of dietary garlic extract on some blood, immunity and growth parameters in common carp fingerlings. One hundred thirty five fish with an average weight of 15 ± 3.4 g were cultivated in 9 aquariums of 20 liters, with a density of 15 in each. Fish were fed with two diets of 1 and 5 g kg⁻¹ garlic extract for 8 weeks. By the end of the eighth week; growth, blood and immunity parameters were measured in fish. Mean temperature, dissolved oxygen and pH during the experiments were 17.01 ± 1.7 °C, 7.23 ± 0.41 mg L⁻¹ and 7.51 ± 0.81 , respectively.

Fish were fed with 5 g kg⁻¹ garlic extract had lower FCR (Feed Conversion Ratio) than the control group ($p < 0.05$). Final weight, weight rate, Specific Growth Rate (SGR), and mean daily growth in treatment groups did not show a significant difference with the control group ($p > 0.05$). The highest number of White Blood Cells (WBC), Lymphocyte, the highest amount of Lysozyme and IgM were reported in fish receiving the 5 g kg⁻¹ garlic extract compared to the control ($p < 0.05$). Based on the results, garlic extract at 5 g kg⁻¹ supplemented diet can improve some parameters of the blood, immune and growth of Common Carp fingerlings.

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Introduction

The common carp (*Cyprinus carpio*) is the most important fish in Cyprinidae. The origin of this fish return to the Black Sea and is now widely used as culture species, which covers about 25-35% of warm water fish production (Sanders, Batts & Winton 2003). Generally, this advantage could be due to rapid growth and resistance to pathogens, which are the most desirable characteristics of fish for aquaculture industry (Genc, Aktas, Genc & Yilmaz 2007). Unsuitable breeding conditions, inappropriate nutrition, stress and tension in fish result in growth reduction, immune system suppression and the development of various diseases in fish (Li & Gatlin 2005). Recently, the use of immune stimulants, especially plant-based stimulants, has expanded to increase the body's resistance, increase the growth, decrease food conversion rate (FCR) and their survival to diseases (Hoseinifar, Zare & Marrifield 2010; Sheikhzadeh, Karimi pashaki, Heidarieh, Nofouzi & Tayefi-Nasabadi 2012). Plant-based immune and growth stimulants have advantages; these include availability, less damage to the environment and animals, and the possibility of generating at a widespread low-cost basis. (Francis, Makkar & Becker 2001). Garlic (*Allium sativum*) of the Alliaceae family is one of the most important native plants in Gilan province and has significant therapeutic value (Hussein, Hamdy & Ibrahim 2013). This herb has several anti-microbial, anticarcinogenic, antifungal and anti-stress properties and also known as a factor in improving nutritional indices, immune and

growth stimulants, antioxidants, and also blood pressure stability (Fazlolahzadeh, Keramati, Nazifi, Shirian & Seifi 2010; Kumar & Berwal 1998). Among the most important garlic compounds, allicin, phosphoric compounds, alkaline enzymes, peroxidase, ajoene, citral and granulated are mentioned. Some studies have shown that garlic consumption increases the production of cytokines, the activity of macrophages and lymphocytes, and ultimately improves and stimulates the immune system (Gholipour, Nobahar, Kakoolaki & Jafarian 2013; Khodadadi, Peghan & Hamidavi 2013). Garlic is rich in minerals (iron, iodine, sodium, potassium and phosphorus), and useful vitamins (A and C) for the body of the fish (Farahi, Kasiri, Sudagar, Iraei & Shahkolaei 2010). The presence of beneficial compounds in garlic, especially allicin, has introduced this plant as a strong antimicrobial and immune-growth enhancer. In this study, the effect of dietary garlic extract on growth performance, some blood and immune parameters of the common carp (*Cyprinius carpio*) was studied.

Materials and Methods

Preparation of Garlic extract

Fresh garlic was collected from one of the farms in the province of Gilan located in Astaneh Ashrafieh and was kept open for 30 minutes after sectional cut, Then the garlic was mixed with Phosphate Buffered Saline (PBS) (pH 7.2) in a blender in equilibrium proportion and passed through two-layer sterile gas and the output centrifuged at 3400 g for 10 min at 4 °C,

Finally, the supernatant was dried with a Spray Dryer (Buchi Mini Spray Dryer B-290) and kept until the time it was used in a refrigerator at a temperature of 4 °C (Ghazanfari, Hassan & Khamesipour 2006).

Supplementation of the normal diet with Garlic extract

The basic diet was prepared from livestock, poultry and Aquaculture Company of Rasht (Roohin), which contains 34.9% protein, 12.8% fat, 11.2% ash, 10% moisture and 31.1% total carbohydrate. Garlic extract was added to the basic diet at 1 and 5 g kg⁻¹. The amounts mentioned are mixed with 50 g of the ration and then added to the rest of the diet and mixed with an electric mixer for 20 minutes until homogenized. After adding some water to the composition and forming the dough, the meat grinder was used to turn the food into pellets. Finally, the pellets dried at 30 °C for 24 hours and then packed and kept in a refrigerator at a temperature of 14 °C.

Fish and experimental conditions

This research was conducted at the Laboratory of Aquaculture and Aquatic Diseases at Anzali Inland Aquaculture Research Institute. One hundred thirty five *Cyprinus carpio* fingerlings were taken from a farm located in Rasht and transferred to the laboratory. These fish with an average weight of 15 ± 3.4 g and a mean length of 9.8 ± 0.78 cm were introduced to 9 aquariums of 20 liters and stocking of 15 fish each. This Research was carried out with one control group (not garlic extract) and 1, 5 g Kg⁻¹ garlic extract groups. Mean temperature, dissolved oxygen and pH during the culture

period were 17 ± 1.7 °C, 7.23 ± 0.41 mg L⁻¹ and 7.51 ± 0.81 respectively. The fish were fed for 8 weeks.

Growth indices

Measurements of growth indices including weight rate, specific growth rate (SGR), food conversion ratio (FCR) and survival rate were performed (Luo, Xu, Teng, Ding & Yan 2010):

$$\text{Weight rate} = \frac{\text{End weight (g)} - \text{Primary weight (g)}}{\text{Primary weight (g)}} \times 100$$

$$\text{Special growth rate (SGR)} = \frac{(\ln, \text{end weight} - \ln, \text{primary weight})}{\text{Number of breeding days}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{End weight (g)} - \text{Primary weight (g)}}{\text{Amount of food eaten}}$$

$$\text{Survival rate} = \frac{\text{Number of fish at the end of the experiment} \times 100}{\text{Number of fish at the beginning of the experiment}}$$

Blood sampling

At the end of the 8 weeks, blood samples were collected. From each group, 9 fish were randomly assigned. Feeding was discontinued 24 hours before blood collection, and then blood samples were taken using a 1 ml syringe and through a dermal vein behind the dorsal fin. For blood sampling, anesthetics were not used due to the possibility of affecting blood parameters (Torrecillas, Makol, Caballero, Montero, Gines, Sweetman & Zquierdo 2011). One ml for serum separation in eppendorf tubes without an anticoagulant of heparin and 0.5 ml in an eppendorf tubes containing an anticoagulant of heparin, were collected. Then the samples were centrifuged (model 5810R-eppendorf) at 3000 g for 10 minutes. The serum

was separated and stored at -80 °C. Blood parameters include Red Blood Cell (RBC), hematocrit, hemoglobin, White Blood Cell (WBC), differential WBC count including Lymphocyte, Eosinophil, Neutrophil and Monocyte, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), lysozyme and Immunoglobulin M (IgM) were measured using the standard methods.

Haematological assay

The total RBC counts ($\text{RBC} \times 10^6$ per ml) were determined in a 1:200 dilution of the blood sample in Ressa solution and total WBC counts ($\text{WBC} \times 10^3$ per ml) in a 1:20 dilution of the blood sample with a Neubauer hemocytometer. The hematocrit (Hct) concentrations were determined by using the micro hematocrit method (Houston, 1990; Klontz, 1994). The hemoglobin (Hb) concentrations were determined by the Cyanmethemoglobin method (Klontz, 1994) using a haemoglobin reagent set (Pars Azmun Diagnostics). All the values of RBC indices, MCH (pg), MCHC (%) and MCV (fl) were calculated according to Wintrobe formulae (Anderson & Klontz 1965). The differential leukocytes count was carried out using blood smears stained with Wright-Gimsa (Klontz, 1994).

Immunological assay

The turbidimetric assay for lysozyme was carried out according to Sahoo, Mahapatra, Saha, Barat, Sahoo, Mahanty, Gjerd, Qdegard, Rye & Salte (2008). The nephelometric method for IgM was recommended by Yeh, Chang, Li

& Chang (2008). In this method, the IgM contained in the blood serum sample with a polyclonal anti-IgM antibody forms a complex and causes clouding of the solution. The monochromatic light photomultiplier scans the solution in wavelengths between 400 and 840 nm, which after the collision the antibody complex and the antigen are dispersed, and the degree of differentiation is proportional to the amount of IgM.

Statistical analysis

The data were normalized by Kolmogorov-Smirnov test and homogeneity test was performed by Levene test. In the case of homogeneity of data, one way ANOVA was used to compare the mean of nutritional treatments and the Duncan test at 95% probability level was used to isolate homogeneous groups. Non-parametric Kruskal-Wallis test was used for non-homogeneous data. The significance of the groups was determined using the Mann-Whitney test at a probability level of 95%. The SPSS statistical software, 19th edition, was used for data analysis.

Results

Tables 1, 2 and 3 show the results of growth, blood and immunity indices of fish at the end of the eighth week. FCR in the treatment of 5 g kg⁻¹ supplemented diet showed a significant decrease compared to the control group ($p < 0.05$). Including final weight, weight rate, SGR in treatments 1 and 5 g kg⁻¹ of garlic extract, were not significantly increased compared to the control group ($p > 0.05$). There

was no significant difference in Hb, Hct, RBC, MCV, MCH and MCHC indices between the treatments and the control group ($p>0.05$). WBC in fish fed diets containing both concentrations of garlic extract showed a significant increase compared to the control

group ($p<0.05$). There was a significant increase in lymphocyte count in fish fed with extract of 5 g kg⁻¹ garlic ($p<0.05$). Fish fed with 1 and 5 g kg⁻¹ of garlic extract in lysozyme and IgM indices increased significantly compared to the control group ($p<0.05$).

Table 1. Comparison of carp fingerlings growth parameters (*Cyprinus carpio*) in dietary garlic extract concentration at the end of the eighth week

Growth parameters	0 g kg ⁻¹ (garlic extract)	1 g kg ⁻¹ (garlic extract)	5 g kg ⁻¹ (garlic extract)
Primary weight (g)	15 ± 4.3 ^b	15.1 ± 3.1 ^b	15.1 ± 2.1 ^b
Final weight (g)	28.5 ± 3.1 ^b	28.9 ± 2.1 ^b	29.3 ± 4.7 ^b
Primary total length (cm)	9.8 ± 0.78 ^b	9.8 ± 0.4 ^b	9.7 ± 0.5 ^b
Total length (cm)	11.9 ± 1.1 ^b	12.1 ± 0.2 ^b	12.7 ± 1.01 ^b
weight rate	47.5 ± 10.72 ^b	47.9 ± 2.1 ^b	48.5 ± 1.03 ^b
FCR	18.51 ± 0.26 ^b	18.11 ± 0.22 ^b	17.73 ± 1.5 ^a
Average daily growth (g per day)	0.42 ± 0.2 ^b	0.43 ± 11.2 ^b	0.45 ± 0.66 ^b
SGR (percent per day)	2.03 ± 1.1 ^b	2.06 ± 1.56 ^b	2.09 ± 0.2 ^b
Survival rate (%)	100 ^b	100 ^b	100 ^b

The numbers (Mean ± SD) with different letters in each row have a statistically significant difference ($p<0.05$).

Table 2. Comparison carp fingerlings blood parameters (*Cyprinus carpio*) in different dietary garlic extract concentration at the end of the eighth week

Blood parameters	0 g kg ⁻¹ (garlic extract)	1 g kg ⁻¹ (garlic extract)	5 g kg ⁻¹ (garlic extract)
Hct (%)	35 ± 3 ^b	36.1 ± 0.1 ^b	38.1 ± 2.3 ^b
Hb (g dl ⁻¹)	7.4 ± 0.05 ^b	7.6 ± 3.2 ^b	7.7 ± 1.2 ^b
RBC (number×10 ⁶)	0.79 ± 0.05 ^b	0.8 ± 0.4 ^b	0.81 ± 2.3 ^b
WBC (number×10 ⁴)	3.6 ± 0.76 ^b	4.4 ± 1.2 ^a	4.8 ± 2.3 ^a
MCV (fl)	432.5 ± 7.11 ^b	435 ± 8.1 ^b	435 ± 0.11 ^b
MCH (pg)	87 ± 1.1 ^b	88 ± 0.11 ^b	89 ± 2.1 ^b
MCHC (%)	19 ^b	19.4 ± 1.1 ^b	20 ± 3.1 ^b
Lymphocyte (%)	65 ± 3.6 ^b	66.6 ± 0.51 ^b	78 ± 1.1 ^a
Monocyte (%)	2.66 ± 1.15 ^b	2.4 ± 0.11 ^b	2.3 ± 8.2 ^b
Neutrophil (%)	27.66 ± 2.51 ^b	28.26 ± 1.2 ^b	29.1 ± 0.8 ^b
Eosinophil (%)	1.66 ± 0.57 ^b	1.4 ± 0.11 ^b	1.3 ± 0.81 ^b

The numbers (Mean ± SD) with different letters in each row have a statistically significant difference ($p<0.05$).

Table 3. Comparison of carp fingerlings Immune parameters (*Cyprinus carpio*) in dietary garlic extract concentration at the end of the eighth week

Immune parameters	0 g kg ⁻¹ (garlic extract)	1 g kg ⁻¹ (garlic extract)	5 g kg ⁻¹ (garlic extract)
Lysozym (µg ml ⁻¹)	39.33 ± 12.51 ^b	42.51 ± 11.1 ^a	49.51 ± 2.1 ^a
IgM (mg dl ⁻¹)	29 ± 2.8 ^b	33.41 ± 0.87 ^a	40 ± 0.05 ^a

The numbers (Mean ± SD) with different letters in each row have a statistically significant difference ($p<0.05$).

Discussion

In the present study, fish fed garlic extract of 1 and 5 g kg⁻¹, weight rate, SGR and mean daily growth were not significantly different from the control group ($p>0.05$). The treated fish with 5 g kg⁻¹ garlic extract had a reduced FCR compared to the control group ($p<0.05$). Jayaprakas & Eupharsia (1995) showed the effect of dietary garlic for 2 months on the survival, growth, and resistance parameters of Nile tilapia, although, fish did not have casualties during 2 months and were resistant to some pathogens, any significant increase was not observed in weight. By increasing the feeding of fish with garlic food for up to 8 months, weight rate was significantly increased in treatment groups compared with the control group ($p<0.05$). In 2001, Khalil, Nadia & Suleiman observed that allicin enhances intestinal function, improves nutrition and better utilizes energy, and ultimately improves growth. Kim, Chum, Koo, Choi, Kim, Kwon, Chung, Billiarand & Kim also reported in 2001 that the use of low garlic (1%) in fish meals reduced the mortality in these fish for one month and its increase to 2 months improved the growth parameters, including the average weight. Cullen, Monahan, Callan & Doherty (2005) reported that when the garlic was used from 1 to 10 g kg⁻¹ in fish food, there was a reduction in fish mortality at the end of this period. In 2006, Shalaby, Khattab & Abdolrhahman Added a diet of 10 to 40 g kg⁻¹ of garlic (1 to 4 % of garlic), in fish fed with 1 and 2 percent garlic, growth rate was low, while in the group that added 3% of garlic to their diet,

growth rate was significantly increased. Farahi *et al.* (2010) studied the effect of garlic on rainbow trout growth parameters, reported that in fish fed 1 and 2 % of garlic to their diet, the growth rate progressed gradually, but in fish with 3% of garlic, a faster growth rate was observed, there was no significant difference in SGR between 1% and 2% garlic groups and control group, but FCR in the 1% and 2% garlic groups was significantly different from the control group. Khodadadi *et al.* (2013) observed that by adding a diet containing 0, 0.1 and 1% garlic to common carp diet with a weight of 25 g in 8 weeks, weight rate and FCR in 1% garlic fish group were significantly increased and decreased respectively ($p<0.05$). In the study of Lee, Ra, Song, Sung & Kim (2012), the effect of garlic extract on growth and body composition in Sterlet sturgeon (*Acipenser ruthenus*) was investigated, the results indicated an improvement in the growth parameters and physical composition in fish. In a study by Manoppo, Kolopita & Malatundah (2016), the effect of garlic on common carp was investigated in Indonesia. In this study, Garlic was used in fish, 5, 10, 15 and 20 g kg⁻¹. The results showed that as the amount of garlic was increased, the weight rate and SGR increased. Ebrahimi, Tangestani, Alizadeh Doughikolae & Zare (2011) investigated the effects of levels of garlic essential oils (50, 100, 150 and 200 mg kg⁻¹) on growth, nutrition and chemical composition of *Huso huso* during 8-week experiment. A slight increase in growth indices was reported and a decrease in the FCR.

In our study, the number of WBC in experimental groups fed with 1 and 5 g kg⁻¹ garlic extract increased significantly compared to the control group ($p < 0.05$). In 2010, Ndong & Fall reported that adding garlic to 0.5% diet for more than 4 weeks could improve the total white WBC and phagocytic index.

In this study, the fish receiving the garlic extract (both concentrations) did not show a significant increase in hematocrit, hemoglobin, and RBC in comparison with the control group ($p > 0.05$). Contrary to our study, Shalaby *et al.* Reported in 2006 that increased levels of garlic in the diet increased the level of RBC in the Tilapia fish. Nwabueze (2012) also observed that addition 0, 0.5, 1, 3 % garlic to the *Clarias garepinus* diet, caused significantly increased Hct, RBC and Hb indices in 0.5% garlic group.

In this research, in fish fed with garlic diets, 5 g kg⁻¹, compared with the control group, a significant increase in lymphocyte percentage was reported ($p < 0.05$). Tangestani, Alizadeh Doughikolae, Ebrahimi & Zare (2010) observed that the addition of garlic essential oil at a level of 0.15 g kg⁻¹ to the diet had a positive effect on the blood immune parameters in *Huso huso* and caused a significant increase in WBC, lymphocytes, monocytes, eosinophils and neutrophils but there was no significant difference in the number of Hct, Hb and RBC.

In the present study, the highest levels of lysozyme and IgM were identified in fish fed with a diet containing 5 g kg⁻¹ of garlic extract. In 2010, Ndong & Fall reported that the addition of garlic to the diet of 0.5% over 4 weeks increased the activity of lysozyme in

Tilapia fish, while Tilapia fish that fed a 1% concentration of garlic in the diet had no improvement in the lysozyme index. Talpur & Ikhwanuddin (2012) observed that *Lates calcarifer* fed 10 g kg⁻¹ garlic, had a significant increase in the activity of lysozyme and IgM.

In general, in the past studies about the effects of garlic or its extract, incremental effects on growth rate and SGR and reducing effect on FCR were reported, in present study, maybe due to low concentrations of garlic used in the test, there was no significant increase in weight indices, however the significant decrease was observed in FCR parameter. In previous studies, there were increasing effects or some reducing effects on RBCs as well as incremental effects on WBCs, we reported positive effects in WBCs too but in the indicators related to RBCs, no significant effects were observed.

Conclusion

According to the results of this study, the level of 5 g kg⁻¹ of dietary garlic extract is the most desirable concentration for carps, because it reduced FCR, increased WBC and lymphocyte and enhanced lysozyme and IgM activity in common carp fingerlings.

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Conflict of interests

The authors declare that there is no conflict of interest.

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بررسی اثرات عصاره سیر جیره غذایی بر برخی از فراسنجه های خونی، ایمنی و رشد بچه ماهیان قدانگشتی کپور معمولی (*Cyprinus carpio*)

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چکیده

این تحقیق به منظور بررسی تأثیر جیره غذایی حاوی مکمل عصاره سیر بر روی برخی فراسنجه های خونی، ایمنی و رشد در بچه ماهیان انگشت قد کپور معمولی صورت گرفت. بدین منظور ۱۳۵ بچه ماهی کپور با وزن متوسط $3/4 \pm 15$ گرم در ۹ آکواریوم ۲۰ لیتری با تراکم ۱۵ بچه ماهی در هر آکواریوم نگهداری شدند. بچه ماهی ها با دو جیره غذایی ۱ و ۵ گرم عصاره سیر در هر کیلوگرم به مدت ۸ هفته تغذیه شدند. در پایان هفته هشتم، فراسنجه های خونی، رشد و ایمنی آنان مورد سنجش قرار گرفت. دمای متوسط، اکسیژن محلول و pH طی ۸ هفته، به ترتیب $17/01 \pm 1/7$ درجه سانتی گراد، $7/23 \pm 0/41$ میلی گرم بر لیتر و $7/51 \pm 0/81$ بود. ضریب تبدیل غذایی (FCR) بچه ماهی های تغذیه شده با ۵ گرم عصاره سیر بر کیلوگرم کمتر از گروه شاهد بود ($p < 0/05$). شاخص هایی مانند وزن نهایی، میزان وزن، نرخ رشد ویژه (SGR) و میانگین رشد روزانه در تیمارهای مورد نظر تفاوت معنی داری با گروه شاهد نداشت ($p > 0/05$). بیشترین تعداد گلبول سفید، لنفوسیت و بالاترین مقدار لیزوزیم و IgM در ماهیانی که عصاره سیر ۵ گرم در کیلوگرم دریافت کرده بودند در مقایسه با گروه شاهد گزارش گردید ($p < 0/05$). بر اساس نتایج بدست آمده، عصاره سیر به میزان ۵ گرم در کیلوگرم در جیره غذایی می تواند برخی از فراسنجه های خونی، ایمنی و رشد را در بچه ماهیان انگشت قد کپور معمولی، بهبود بخشد.

کلمات کلیدی: فراسنجه های رشد، ایمنی و خونی، عصاره سیر، کپور معمولی، نرخ رشد ویژه

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