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Oral administration of garlic powder (*Allium sativum)* on growth performance and survival rate of *Carassius auratus* fingerlings

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Abstract

This study was carried out to evaluate the effect of different levels of garlic (*Allium sativum)* on growth survival and nutritional characteristics of goldfish (*Carassius auratus).* For this purpose, 180 goldfish with an average initial weight of 1.18± 0.08 g were introduced randomly into 12 aquaria (50×30×40 cm) in 4 groups, with 15 fish in each group. Treatments were fed on a basal diet for one week, followed by experimental diets for eight weeks. Garlic powder was added at levels of 0 (Control), 0.5, 1 and 1.5 mg kg^{-1} to commercial diet. At the end of the experiment, growth and survival rates and feed performance were evaluated. There was no significant difference in growth rate and feed efficiency between treatments, although they were better in the 0.5% level. The highest survival rate was achieved in the 0.5% treatment that showed a significant difference compared to the control diet (p<0.05). The best growth performance and feed efficiency were obtained in fish fed the 0.5% garlic powder diet.

Keywords: Garlic powder, Growth, Feeding, Survival, *Carassius auratus*

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Introduction

Increased demand for fish and income from fishing as well as the greater preference to fish than other animal protein sources, cultural and health reasons have accelerated the growth of the aquaculture industry. Aquaculture should be an effective, profitable industry with minimal environmental impacts (Martinez-Porchas and Martinez-Cordova, 2012). However in the fish and shrimp farming industry, infectious and noninfectious diseases are also expanding an important loss, so that every year large quantities of antibiotics and chemicals are used to control these diseases that have led to the creation of resistant bacteria against diseases, environmental pollution and residues in fish (Cermelli *et al*., 2008). Like other lower vertebrates, fish mainly rely on the non-specific immune system to combat the pathogens. Immune stimulators are able to strengthen the non-specific and specific immune system factors at the time of exposure to pathogens. Immune stimulants have been used in aquaculture including synthetic materials such as levamisole, biologic materials such as bacterial derivates, poly- saccharides, nutritional factors, animal and plant compounds (Skjermo *et al.,* 2006; Cermelli *et al.,* 2008). The annual consumption of medicinal herbs has made significant progress in recent years due to increased pathogen resistance to synthetic drugs in the European countries and developing countries (Ghasemi Pirbalouti, 2009). Traditional treatments with medicinal herbs are not fast and may not be suitable for infectious diseases. However, herbal and natural medicines have a special place in the treatment of diseases due to factors such as economic value and low-cost production, no harmful effects on the environment (organic drugs), few side effects of herbal medicines compared to chemical drugs, the relative lack of resistance to pathogens using herbal medicines, exclusivity of treatment of diseases with herbs and different clinical experiences regarding medicinal plants (Ghasemi Pirbalouti, 2009). So searching for new feed additives is still a very important aim for aquaculture researchers (Cho and Lee, 2012). Garlic (*Allium sativum*) is one of the members of family Liliaceae used as a spice and in traditional medicine. It is one of the native plants of Iran and enjoys various compounds of amino acids, minerals, vitamins, flavonoids, volatile and non-volatile compounds with medicinal value (Hussein *et al*., 2013; Najdaa *et al*., 2016). It is rich in calcium, phosphorus, carbohydrates and generally, and has a high nutritive value. Garlic also contains many valuable compounds such as iodine salts and is rich in mineral elements (iron, iodine, sodium, potassium and phosphorus) (Farahi *et al*., 2010) which have positive effects on the circulatory system, silicates which have a positive effect on the skeletal and circulatory system and sulfur salts with positive effects on cholesterolemia, skeletal system and control liver diseases and has vitamins such as vitamins A, C and B complex (Farahi *et al*., 2010) as well as linoleic

acid (Draˇgan *et al*., 2008). The presence of many useful compounds, particularly allicin in garlic has introduced this plant as a strong antimicrobial compound, growth and immune system enhancer and anthelmintic effector (Iqbal *et al.,* 2001). It has several benefits for humans and animals where it is considered to be an antimicrobial (Kumar and Berwal, 1998), an antioxidant and an antihypertensive agent (Konjufca *et al.,* 1997). Garlic powder is known as an immune stimulator (Cho and Lee, 2012).

Garlic plays a role in the control of pathogens, especially bacteria and fungi. It also increases the welfare of fish (Corzo-Martınez *et al*., 2007). So it can be said that this plant has multiple properties including its antimicrobial, anticancer and anti- fungal characteristics, promoting nutritional indices, growth and acting as an immune system enhancer with anti-stress, antioxidant and balanced blood pressure effects (Kumar and Berwal, 1998; Fazlolahzadeh *et al,* 2011). Garlic contains the most important compounds including allicin, phosphorus compounds, alliinase, peroxides and myrosinase enzymes, ajoin, citral and granyol. The use of garlic has increased cytokine production, macrophages, lymphocytes and neutrophils activity, and finally, it improves and stimulates the immune system (Khodadadi *et al*., 2013).

In previous studies the positive effects of garlic and other plant extracts have been demonstrated on growth and immune system of hamster (Yaoling *et al.,* 1998), broiler chicken (Lewis *et al.,* 2003) and also some aquatic species such as rainbow trout *Oncorhynchus mykiss* (Farahi *et al.,* 2010), *Cyprinus carpio* (Gabber Ajeel and Al-faraghi, 2013), *Lates calcarifer* (Talpur and Ikhwanuddin, 2012), *Clarias gariepinus* (Thanikachalam *et al*., 2010; Nwabueze, 2012), *Oreochromis niloticus* (Shalaby *et al*., 2006; Diab *et al*., 2007; Metwally, 2009), *Huso huso* (Tangestani *et al.,* 2011; Nobahar *et al*., 2014; Akrami *et al*., 2015), sterlet sturgeon *Acipenser ruthenus* (Lee *et al*., 2014), *Carassius auratus* (Sasmal *et al.,* 2005), *Litopenaeus vannamei* (Javadzadeh *et al.,* 2002; Zare *et al.,* 2014; Gol Aghaei *et al*., 2016), *Mesopotamichthys sharpeyi* (Maniat *et al*., 2014) and *Dicentrarcus labrax* (Norhan *et al*., 2015).

In this study, the effects of different levels of garlic powder on growth and survival rate of goldfish were evaluated. This fish was selected as an experimental model due to its high tolerance to harsh environmental conditions, high compatibility and similarity of its tissue, anatomy and physiology to other Cyprinidae species (Alishahi and Mesbah, 2012). Goldfish economically is an important ornamental fish. Its culture and propagation as an ornamental fish for the Iranian Nowruz haft- sin and enthusiasts of keeping goldfish is booming and there is an increasing demand for it in the market (Imanpoor and Kamali, 2006).

Materials and methods

This study was carried out during 8 weeks in Shahryari ornamental fish farm in Gorgan. After adaptation of fingerlings to the manual feeding on a basal diet, 180 goldfish (average initial weight of 1.18±0.08 g) were introduced randomly into 12 aquaria (50×30×40 cm) at a stocking rate of 15 fish per aquarium. All fish were fed on experimental diets for 8 weeks. A central blower was set to aerate the tanks and provide oxygen demand.

This study was designed randomly using 4 treatments each with 3 replicates. To prepare the diets firstly biomar feed (38.8% protein, 15% fat) was weighed using a digital scale (0.01g sensitivity), powdered and softened by a mixture. Then different levels of pure garlic powder including 0, 0.5, 1, and 1.5%, which was purchased from the market were added to the diet and mixed (Cho and Lee, 2012). Water (200 mL kg^{-1}) was added to the mixture to form a soft flake diet. The mixture was converted to 1mm pellets using a grinder. Obtained pellets were dried at room temperature and stored at -20 °C. Feeding was done twice a day based on 2% of fish body weight. Fish biometry was performed every two weeks. Weight and length of all treatments were measured using a digital scale (0.01 g) and ruler (0.1 mm). Growth indices were determined including an increase in body weight, specific growth rate (AOAC, 1995). Feeding parameters including feed conversion efficiency, daily consumed food, and feed efficiency were calculated according to the standard formula (AOAC, 1995; Lin and Shiau, 2005; Lin *et al*., 2013). All data were analyzed using One Way ANOVA, Duncan test in SPSS software (0.05) to determine the significant level.

Results

Growth indices

The effect of different levels of garlic powder on growth indices of *C. auratus* shown in Table 1. The final weight, increase in body weight, percent of the increase in body weight, specific growth rate (% per day) and biomass were increased in treatments fed with garlic powder compared with the control but there were no significant differences (p>0.05). Final biomass was significant in the treatment receiving 0.5% garlic powder compared to the control (p<0.05). Also, survival rate increased significantly in the treatment fed 0.5% garlic powder compared to the control (p<0.05).

Table 1: Growth indices (Mean ± SE) of *Carassius auratus* fed different levels of garlic powder during the 8-week rearing period.

Factor/ Treatment	Control	0.5% garlic powder	1% garlic powder	1.5% garlic powder
Initial weight (g)	1.25± 0.20	1.20± 4.73	1.15± 0.12	1.14± 7.59
Final weight(g)	2.91± 1.43	3.38± 0.18	3.19± 5.67	3.01± 0.32
Body weight increase (g)	1.67± 0.21	2.20± 0.17	2.05± 4.40	1.88± 0.36
% of body weight increase	136.37± 35.72	184.37± 12.67	180.87± 19.88	166.95± 38.96
Specific growth rate (% per day)	1.44± 0.26	1.76± 7.09	1.74± 0.13	1.64± 0.25

Table 1 continued:

Increase in biomass (g)	22.41± 3.81	31.60± 0.76	29.55± 0.82	27.98± 5.22
Survival %	90.42± 5.91 [a]	100± 0.02 [b]	96.52± 4.63 [ab]	96.52± 6.62 [ab]
Final biomass (g)	39.04± 2.25 [a]	48.75± 2.12 [b]	46.00± 3.08 [ab]	44.69± 4.61 [ab]

The numbers in each row with dissimilar letters have significant differences (p<0.05).

Nutritional indices: The results showed that adding different levels of garlic powder improves the nutritional indices such as the daily food intake, food conversion ratio and feed efficiency (Table 2), but there was no significant different between treatments (p> 0.05).

Table 2: Nutritional indices (Mean ± SE) of *Carassius auratus* fed different levels of garlic powder during the 8-week rearing period.

Factor/Treatment	Treatments (g. p. level)			
	Control	0.5%	1%	1.5%
Food conversion ratio	3.21± 0.62	2.48± 2.84	2.57± 4.50	2.69± 0.40
Food eaten daily (%)	4.23± 9.91	4.53± 0.17	4.33± 0.12	4.26± 0.14
Feed efficiency (%)	0.34± 5.58	0.42± 7.09	0.41± 1.43	0.40± 8.26

The numbers in each row with dissimilar letters are significant differences (p<0.05).

Discussion

In recent years, due to the emergence of antibiotic resistances in aquaculture, there has been a growing trend to use growth enhancers and immuno stimulants of plant origin (Citarasu *et al*., 1998). Many authors recorded the positive effects of administrating garlic in diets on growth and feed utilization of many fishes including; African catfish, *C. gariepinus* (Nwabueze, 2012; Agbebi *et al*., 2013), rainbow trout, *O. mykiss* (Nya and Austin, 2009; Gabor *et al*., 2012), Swordtail, *Xiphophorus helleri* (Kalyankar *et al*., 2013) and Nile tilapia, *O. niloticus* (Shalaby *et al*., 2006; Mesalhy *et al*., 2008; Metwally, 2009; Aly and Mohamed, 2010) and *C. auratus* (Sasmal *et al*., 2005).

Measured values in the present work indicate an enhancement in growth and feed utilization for all fish groups fed garlic powder specifically 0.5%, compared with the control group which is in agreement with findings of Khalil *et al*. (2001) who mentioned that allicin in garlic, promotes the performance of the intestinal flora, thereby improving digestion, and enhancing the growth and is probably related to the anti-bacterial effect of garlic extracts which had a positive effect on intestinal microbial balance (Lewis *et al*., 2003). Improvement of growth factors subsequent to the prescription of these feed complements can be attributed to their stimulating effect on the non-specific immune system of fish, in addition to the direct effect of their active ingredients on growth. This is because improvements in immune factors of fish can cause indirect growth in them (Manning and Nakanishi, 1996; Yada and Nakanishi, 2002). In other studies, adding garlic to the diet of *Onchorhynchus mykiss* (Nya and Austin, 2009; Farahi *et al*., 2010), *O.*

niloticus (Diab *et al*., 2002; Shalaby *et al*., 2006) and *C. carpio* (Khodadadi *et al*., 2013) has resulted in improved specific growth rates and increased weight gain and reduced feed conversion ratio that is similar to this study. In contrast, the studies of Ndong and Fall (2007), Sahu *et al*., (2007), Thanikachalam *et al*. (2010) and Nobahar *et al*. (2014) showed that adding different levels of raw garlic powder to the diet did not induce any significant effects on feeding and growth factors of *H. huso*, African catfish, Indian carp and tilapia hybrid. Similar results were obtained by adding different levels of garlic to diets of *Labeo rohita, O. niloticus* and *O. aureus*. Improvement in growth indices can be attributed to the presence of allicin in consumed garlic that enjoys a broad spectrum of anti microbial properties and immuno stimulation effects. With the improvement of dietary nutrient digestibility, increased intestinal function can cause better use of energy resources of the diet, inhibit and reduce harmful bacterial growth of the gastrointestinal tract and thus lead to the improvment of growth and immune parameters that are consistent with the study of Khalil *et al*., (2001).

Differences in the results of this study may be due to differences in the species, size, sex and age of the fish, diet formulation, purity and dose of this plant, the use of garlic plant in the diet (powders, oils or extracts) duration of the breeding period, physiological characteristics and storage conditions of aquatic animals.

In similar studies, subsequently increasing levels of garlic essence or extract in the diet of rainbow trout (Farahi *et al*., 2010) and Nile Tilapia (Abdelhamid *et al*., 2002; Khattab *et al*., 2004) an increase in carcas protein was observed that is similar to the results of this study. It can be attributed to more digestibility of the diet and higher energy consumption of the diet leading to improved indices of growth. On the contrary, the results of Diab *et al*. (2002) on Nile Tilapia showed that different levels of garlic in the diet, did not cause significant differences in body composition in Nile tilapia.The results of this study showed that oral supplementation of raw garlic powder affects fish survival. The survival rate of fish in treatments fed with 0.5% garlic was higher than other treatments, although this difference was not significant ($p> 0.05$). The study of Zare *et al*. (2014) showed that administration of garlic extract in the diet of *P. vannamei* post larvae increases the resistance and survival of the shrimp. Also, Thanikachalam *et al*., (2010) revealed that using 0.5% of garlic powder in the diet of African catfish increased fish survival and resistance against *Aeromonas hydrophila* bacteria. Citarasu *et al*. (2002), observed increased survival in *Penaeus monodon* larvae in stressful conditions following Artemia enrichment with herbal ingredients.

The effects of immune stimulation and increase of fish resistance following the consumption of garlic have been attributed to the presence of compounds such as allicin and vitamin

A and vitamin C in the garlic plant (Khodadadi *et al*., 2013). As a final conclusion it is obvious that raw garlic powder, especially at the level of 0.5 percent in the diet, improved growth performance of goldfish. Therefore, the low price of garlic, having no environmental concerns compared to synthetic supplements, increasing pliability and storage time due to anti-microbial effects of garlic may justify its role in fish diets and can be considered as a suitable safe supplementary food for fish diets.

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