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### RESEARCH PAPER

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Enhancement of growth performance and body composition in molly fish (*Poecilia sphenops*) associated with dietary intake of garlic (*Allium sativum*)

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# **Abstract**

In recent decades, interest in using medical plants powders as growth factors and immunestimulants in aquaculture has been increased. This study was conducted to evaluate the effect of different levels of dietary supplementation of garlic, *Allium sativum* on growth factors and body composition of Molly Fish, *Poecilia sphenops*. Four diets containing supplementation at levels of 0, 5, 15 and 30 g kg<sup>-1</sup> were fed to fingerlings of Molly Fish (initial weight, 0.85 g) in triplicates tanks three daily to apparent satiation for 56 days. The result of experiment showed, growth performance and feed efficiency were improved in all treatments compared with control group. But according to the results, the best final weight, weight gain rate (WGR%), specific growth rate (SGR) and food conservation ratio (FCR) were observed in the fish fed 15 g kg<sup>-1</sup> *A.sativum* in diet. The highest protein content was obtained in the fish fed with dietary with 15 g kg<sup>-1</sup> *A.sativum* powder. Also fat tissue of fish had significantly decreasing trend compared with control group. Moisture and ash contents were no significantly affected by *A.sativum*. The results of the present study indicated clearly that the supplementation of *A.sativum* (15 g kg<sup>-1</sup> diet) enhanced the growth performance, feed utilization and body composition of Molly Fish, *P.sphenops*.

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#### Introduction

Ornamental fish is one of the important items among the various types of commercially important fishes marketed nationally and internationally. Ornamental fishes, popularly known as 'aquarium fish', or 'live jewel', (Mandal et al., 2007). The production and trade of ornamental fish is a profitable alternative in the aquaculture sector. Freshwater and marine species have been used successfully in the aquarium fish trade, being the most popular: discus (Symphysodona equifaciatus), guppy (Poecilia reticulata), swordtail (Xiphophorus helleri), molly (Poecilia sphenops, Poecilia latipinna) and goldfish (Carassius auratus) (Chong et al., 2003). Despite the economical importance of this sector, the nutritional information for ornamental fish is scarce and often few or even no data of the nutritional requirements is available (Chong et al., 2003; Blom et al., 2000). In natural conditions, fish can regulate and maintain their food intake and therefore their nutritional requirements, reducing the possibility of suffering nutritional deficiencies; however, this problem can be observed when the fish are subject to confinement conditions (Lovell, 2000). Most of the information is not specific to ornamental fish because it has been based on results from farmed fish kept under different farming conditions, nutritional requirements, feeding habits and type of food and (Velasco-Santamaria Corredor-Santamaria, 2011).

In addition, the global demand for safe food has prompted the search for natural alternative growth promoters to be used in aquatic feeds. There has been heightened research in developing new dietary supplementation strategies in which various health and growth promoting compounds as probiotics, synbiotics, phytobiotics prebiotics, and other functional dietary supplements have been used (Denev, 2008). In concerning evaluation of phytobiotics in aquaculture is a relatively new area of research showing promising results (Cristea et al., 2012). A.sativum is an important vegetable extensively cultivated in many countries. It is used as food for humans as well as some animals and as remedy for several diseases, as reported in folk medicine (Shalaby et al. 2006). A.sativum contains sulfur containing compounds. Alliin is converted to the antimicrobial active allicin when the bulb is cut or crushed. The fresh bulb contains alliin, allicin and volatile oils. When the garlic clove is crushed, the odorless compound alliin is converted to allicin, via the enzyme allinase. Also, it contains vitamins and minerals and trace elements (selenium & germanium). has A.sativum proven to be hypolipidemic, antimicrobial, antihypertensive, hepatoprotective and insecticidal. A.sativum has also been shown to reduce serum cholesterol levels and increase blood clotting time(Gabor et al., 2012).

Molly is a tropical hardy and highly adaptable fish species that comes in many different colors and varieties such as orange, green, black, sailfin, ballon, etc. Poecilia sphenops comes under Molly group. 'Poecilia' means "many coloured" and 'Sphenops' means "wedge appearance" (Nithya Jeniffer *et al.*, 2012).

So far, no trial was conducted to study the effect of dietary garlic powder on growth parameters of Molly fish till date. Therefore, this study was designed to investigate the effects of garlic powder on growth and body composition of the fish.

# Materials and methods

Diet preparation

Fresh garlic bulbs were purchased from a local market (Abadan, Khouzestan, Iran). After peeling garlic was cut into small pieces and dried in air for five days. Ingredients and nutrient contents of the experimental diets are presented in Table 1. Four diets were formulated to contain 0, 5, 15 and 30 g kg<sup>-1</sup> Garlic powder as the Control, T1, T2 and T3. All ingredients were thoroughly mixed with 300 cc kg<sup>-1</sup> distilled water, and pellets were prepared using a moist pelleting machine. The pellets were dried at room temperature for 24 h and ground into desirable particle sizes. The dried diet was packaged into plastic bag and stored frozen at -20°C until use (Maniat *et al.*, 2014).

### Growth experiment

A total number of 600 fish Molly, P.sphenops were obtained from a local commercial aquarium and transported to the Ornamental Fish Unit of Zist Pazhohan Arvand Company (Khorramshahr, Khouzestan, Iran). Molly fish (with average initial weight 0.88 g) were randomly distributed among 12 tanks (120 L circular plastic tanks) at a density of 50 fish per tank with three replicate tanks for each dietary treatment. Experimental groups were fed three daily (08:30, 11:30 and 16:30) by hand to visual satiation. Water temperature was maintained at 27.11±0.89°C, dissolved oxygen at 7.98±0.39 mg l-1 and pH at 7.41±0.11. The system was housed in a climate-controlled laboratory with photoperiod (12 h light:12 h dark). At the end of experiment, Molly fish in each tank were collectively weighed after anesthetizing with Carnation powder at a concentration of 30 mg L-1 after starvation for 24 h.

### Diets and whole body chemical analysis

Fifteen fish from each tank were randomly sampled and stored at -20°C in freezer for proximate composition at the end of experiment. Proximate analysis of diets and fish were determined according to the method of AOAC (1995). Crude protein content was determined using the Kjeldahl method using an Auto Kjeldahl System. Crude lipid was analyzed by ether extraction, moisture content by a dry oven drying at 105°C for 24 h and ash by a furnace muffler (550°C for 4 h).

#### Statistical analysis

In outline, this study was planned and executed entirely by accident. All data are collected normal distribution using the Shapiro-Wilk test was performed, and significant differences between treatments at different levels (p≤0.05) using ANOVA (One-way ANOVA) and post- Duncan test was examined. Analysis of all the data and the operations were performed by SPSS 19 software.

# Results

The growth performance and feed utilization of Molly fish fed different levels of dietary supplementation of A.sativum are given in Table 2. The result of experiment showed, the use of A.sativum in diet of fish induces to increase growth performance in all treatments compared with control group. But a group of fish fed with 15 g kg<sup>-1</sup> A.sativum in diet had significantly higher growth performance compared with control group (p<0.05). Also, 15 g kg<sup>-1</sup> A.sativum in diet of fish induced higher weight gain (gr, %) and SGR than other groups that was significantly different compared with control group (p<0.05). Results showed that FCR of all fish fed A.sativum were significantly lower than control group (p<0.05). but fish fed 15 g kg<sup>-1</sup> had the lowest FCR among treatments.

Also, the results of the body composition of Molly fish fed the experimental diets were presented in Table3. The results showed the highest amount of protein in treatment 15 g kg<sup>-1</sup> that was significantly different compared with control group (p<0.05). The use of garlic in the diet of all treatments leads to reduced significantly body fat compared with control group (p<0.05). Also, the Ash and moisture content of fish was not significantly different compared with control group (p>0.05).

# Discussion

A member of the Liliaceae family, garlic is used for centuries as a spice and also in popular medicine. It's a rich source of calcium and phosphorus; it has a high content of carbohydrates and as a consequence a high nutritive value. Garlic also contains iodine salts which have positive effects on the circulatory system and rheumatism, silicates which have a positive effect on the skeletal and circulatory system and sulfur salts with positive effects on the skeletal system, cholesterolemia and liver diseases. Garlic also contains vitamin complex B, vitamins A, C and F. Another substance with a major role is aliicin, which has anthelmintic effects (Gabor et al., 2012). Garlic powder contain 99% pure dimethyl trisulfide and a mixture of diallyl sulfides, DASS (33% diallyl sulfides DADS, 16% diallyl trisulfide DATS and 17% diallyl tetrasulfide DATTS). Many efficient organization ensures that clove defense mechanism is only

activated in a very small location and for a short duration, whereas the rest of the alliin and allinase remain preserved in their respective compartments and are available for interaction in case of subsequent microbial attacks. These phenomena explain the efficiency of garlic in improving the fish quality and shelflife, as it is well accepted that allicin which possesses a variety of biological activities is the bioactive substance(Mesalhy ALy *et al.*, 2008).

**Table 1.** Formulation and proximate composition of experimental diets (%).

	Experimental Diets				
	Control (0%)	0.5%	1.5%	3%	
Dietary composition					
fish meal	47	47	47	47	
Wheat flour	23	23	23	23	
Soybean meal	12	12	12	12	
Corn gluten meal	7	7	7	7	
Fish Oil	2	2	2	2	
Soybean oil	3	3	3	3	
Vitamin premix <sup>a</sup>	1.5	1.5	1.5	1.5	
Mineral premix <sup>b</sup>	1.5	1.5	1.5	1.5	
Filler	3	2.5	1.5	0	
Garlic powder	0	0.5	1.5	3	
Proximate Analyses (%, DM*)					
Dry Matter	84.3	84.7	85	85.3	
Crude protein	44.6	44.2	44.5	44.3	
Crude fat	9.7	9.9	9.6	9.4	
Ash	8.6	8.7	8.6	8.3	

<sup>a</sup>Vitamin premix (composition per 1kg): A=1600000 IU, D3=400000 IU, E=40000 mg, K3=2000 mg, B1=6000 mg, B2=8000 mg, B3=12000 mg, B5=40000 mg, B6=4000 mg, B9=2000 mg, B12=8 mg, H2=40 mg, C=60000 mg, Inositol=20000 mg.

<sup>b</sup>Mineral premix (composition per 1kg): Iron:6000 mg, Zinc:10000 mg, Selenium:20 mg, Cobalt:100 mg, Copper:6000 mg, Manganese:5000 mg, Iodine:600 mg, CoCl<sub>2</sub>:6000 mg.

The result of our experiment showed that 5, 15 and 30 g kg-1 A.sativum in diets has beneficial effect on growth factors and body composition of Molly fish. The final average weight, weight gain and SGR increased significantly in all treatments fed on A.sativum. But the highest growth performance was observed in fish fed 15 g kg<sup>-1</sup> A.sativum in diet. These results are in accordance with the finding of Khalil et al (2001) who reported that allicin in garlic through improving digestion and performance of intestinal flora leads to enhance the utilization of energy and better growth in Nile tilapia (Oreochromis niloticus). Also, Shalaby et al. (2006) reported significant increased WG(%) and SGR in the Nile tilapia (Oreochromis niloticus) when fed diet containing 30g kg-1 A.sativum in diet. Furthermore, the results of (Mesalhy Aly et al., 2008) confirm the positive effects of A.sativum on growth performance of Nile tilapia. Also other studies conducted by Nya and Austin (2009) on *Onchorhynchus mykiss*, (Farahi *et al.*, 2010) on rainbow trout (*Oncorhynchus mykiss*), (Lee *et al.*, 2012) on Sterlet Sturgeon (*Acipenser ruthenus*), (Guo *et al.*, 2012) on *Epinephelus coioides*, (Nwabueze *et al.*, 2012) on *Clarias gariepinus* and (Megbowon *et al.*, 2013) on cichlid fish demonstrate the use of garlic herb improves growth performance in different fish species (Maniat *et al.*, 2014).

Higher growth performance has been recorded for fish fed *A.sativum* supplemented diets (Dias, 2002; Metwally, 2009). But, higher dose of *A.sativum* in diet of different fish species induce to decline growth, due to pungent smell of garlic. Metwally (2009) reported that although growth is enhanced with *A.sativum* supplementation but high dose of *A.sativum* in fish may reduce feed intake as a result of

<sup>\*</sup>DM, dry matter.

its unpleasant odour. the result of Platel and Srinivasan(2004), Mesalhy Aly *et al*(2008) and Maniat *et al*(2014) demonstrate the use of garlic with

high levels of *A.sativum* is related to negative effect of smell and pungent taste of *A.sativum* that leads to reduce fish feed intake.

**Table 2.** Growth performance of juvenile Molly fish fed the experimental diets for 8 weeks.

Diet	Control (0%)	0.5%	1.5%	3%
Initial Weight (g)	0.87±0.032 <sup>ns</sup>	0.89±0.040	0.87±0.046	0.88±0.052
Final Weight (g)	1.73±0.081 <sup>a</sup>	1.86±0.0.068ab	2.10±0.052 <sup>b</sup>	1.85±0.098ab
Weight Gain (g fish-1)1	0.86±0.051 <sup>a</sup>	0.97±0.037 <sup>a</sup>	1.22±0.006 <sup>b</sup>	0.97±0.052 <sup>a</sup>
Weight Gain (%) <sup>2</sup>	97.96±2.602 <sup>a</sup>	109.60±4.692 <sup>a</sup>	140.63±6.729 <sup>b</sup>	110.0±4.693 <sup>a</sup>
SGR(%)3	1.22±0.025 <sup>a</sup>	1.32±0.037 <sup>a</sup>	1.56±0.049 <sup>b</sup>	1.32±0.040 <sup>a</sup>
FCR (%)4	2.70±0.152 <sup>a</sup>	2.36±0.392 <sup>b</sup>	2.26±0.317 <sup>b</sup>	2.33±0.120 <sup>b</sup>
Survival (%) <sup>5</sup>	97.76±2.233 <sup>ns</sup>	93.33±1.934	96.66±3.333	94.43±5.566

Values (means  $\pm$  SE of three replication) in the same row not sharing a common superscript are significantly different (P < 0.05).

ns = not significant (P > 0.05).

In this study, results of Molly fish body compositions showed that crude protein increased significantly with diets containing 15g kg<sup>-1</sup> garlic, although total lipid content decreased significantly with the same levels of *A.sativum*. Also, the Ash and moisture content of fish was not significantly different compared with control group. These results agree with those obtained by Shalaby *et al* (2006), Farahi *et al* (2010) and Maniat *et al* (2014), who showed that inclusion of *A.sativum* in the diet increased fish protein content and decreased whole body fat in fish. Banerjee and Maulik (2002) reported that

compounds in *A.sativum* lower the activity of lipogenic and cholestrogenic enzymes in Liver. On the other hand, Compounds in garlic increase the excretion of acidic and neutral steroids that cause the excretion of cholesterol from the body content. Water-soluble sulfur compounds such as S-alLyl Sulfur Said cysteine (SAC) and Diallyl-di-sulfide (DADS) of garlic extract inhibit the synthesis of cholesterol (Yeh and Liu, 2001; Gebhardt and Beck, 1996). As well as allicin of *A.sativum* causes inhibition of accumulation of fat in body (Elkayam *et al.*, 2003).

**Table 3.** Proximate composition (%) of the whole body of Molly fish fed the experimental diet for 8 weeks.

Diet	Control (0%)	0.5%	1.5%	3%
Crude protein	13.70±0.19 <sup>a</sup>	14.07±0.26 <sup>ab</sup>	14.87±0.17 <sup>b</sup>	14.10±0.30 <sup>ab</sup>
Crude lipid	6.56±0.09 <sup>a</sup>	6.18±0.12 <sup>b</sup>	6.10±0.11 <sup>b</sup>	6.26±0.13 <sup>b</sup>
Moisture	74.32±1.24 <sup>ns</sup>	74.20±1.26	74.23±1.42	74.25±1.21
Ash	3.43±0.12 <sup>ns</sup>	3.46±0.14	3.46±0.19	3.55±0.20

Values (mean  $\pm$  SE of three replication) in the same row not sharing a common superscript are significantly different (P < 0.05).

ns= not significant (P > 0.05).

<sup>&</sup>lt;sup>1</sup>Weight gain= final weight-initial weight.

<sup>&</sup>lt;sup>2</sup>Weight gain percent= [(final weight-initial weight)/initial weight] × 100.

 $<sup>^3</sup>$ Specific growth rate (%) = [ln (final fish wt.) - ln (initial fish wt.)] × 100/days of feeding.

<sup>4</sup>Food conversion ration= weight gain/ feed intake.

<sup>5</sup>Survival= (final fish number / initial fish number) × 100.

In generally, the result of the present experiment show that garlic (15 g kg<sup>-1</sup>) can be used as an effective feed additive to improve molly fish performance.

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