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Effect of garlic (*Allium sativum*) on growth factors, some hematological parameters and body compositions in rainbow trout (*Oncorhynchus mykiss*)

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Abstract. The aim of this study was to assess the effect of garlic (*Allium sativum*) on growth factors, some hematological parameters and body compositions in rainbow trout (*Oncorhynchus mykiss*). A total number of 360 fish (average weight 20.88±0.25 g) was used. Fish were divided into four groups fed on diets containing garlic in different levels; 10 g kg^{-1}, 20 g kg^{-1}, 30 g kg^{-1} diet and the control group diet was without garlic. The experiment extended for two months. The results showed that, weight gain and growth performance of *O. mykiss* significantly ($p<0.05$) increased in all groups fed on garlic. There was significant decreases of plasma glucose in fish fed on diets containing garlic. Mean values of total plasma protein increased significantly in all treatments when compared to control group ($p<0.05$). Protein content and ash contents in fish body were significantly higher in the group fed on diet containing 30 g kg^{-1} diet of garlic than all other groups. Total lipids content in fish body decreased in treatments and it was lower in fish fed on 30 g kg^{-1} diet of garlic. The results of this study show that addition of garlic *Allium sativum* to fish diet can promote growth and improve fish health.

Key words: garlic (*Allium sativum*), growth factors, hematological parameters, body compositions, rainbow trout (*Oncorhynchus mykiss*).

چکیده. هدف از این مطالعه بررسی اثر سیر (*Allium sativum*) روی فاکتورهای رشد، برخی پارامترهای خونی و ترکیبات بدن در ماهی قزل آلای رنگین کمان (*Oncorhynchus mykiss*) بود. تعداد 360 عدد ماهی (میانگین وزن 0.25± 20.88 گرم) مورد استفاده قرار گرفت. ماهیان در سه گروه توسط جیره حاوی سیر در سطوح مختلف 10، 20 و 30 گرم در کیلوگرم تغذیه شدند و جیره گروه شاهد بدون سیر بود. آزمایش به مدت 2 ماه طول کشید. نتایج نشان داد که وزن بدست آمده و کارایی رشد قزل آلای رنگین کمان به طور معنا داری در گروه های تغذیه شده با سیر افزایش یافت ($p<0.05$). کاهش معنا داری در گلوکز سرم خون در ماهیان تغذیه شده توسط جیره های حاوی سیر وجود داشت. میانگین مقادیر پروتئین کل سرم خون به طور معنا داری در تمامی تیمارها در مقایسه با گروه شاهد افزایش یافت ($p<0.05$). محتویات پروتئین و خاکستر بدن ماهیان در گروه تغذیه شده توسط جیره حاوی 30 گرم در گیلوگرم سیرنسبت به سایر گروه ها به طور معنا داری بالاتر بود. محتویات چربی کل بدن در تیمارها کاهش یافت، به طوریکه در ماهیان تغذیه شده توسط جیره حاوی 30 گرم در گیلوگرم سیر پایین تر بود. نتایج این مطالعه نشان می دهد که افزودن سیر (*Allium sativum*) به جیره ماهیان می تواند رشد را افزایش دهد و سلامتی ماهیان را ارتقاء بخشد.

کلمات کلیدی: سیر (*Allium sativum*)، فاکتورهای رشد، پارامترهای خونی، ترکیبات بدن، ماهی قزل آلای رنگین کمان (*Oncorhynchus mykiss*)

Introduction. Feed and feeding are among the most important factors influencing growth, feed utilization and tissue composition of the fish in intensive culture (Okumus & Mazlum 2002).

Garlic 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). It is probably one of the earliest known medicinal plants.

In recent years, the concern about bacterial resistance to antibiotics in livestock industry has led to legislation minimizing/eliminating the use of such compounds. The use of the immunostimulants in aquaculture is becoming popular, enhancing the activity of the non-specific defense mechanisms and increasing disease resistance (Dalmo & Seljelid 1995).

Garlic contains sulfur containing compounds. Alliin, is converted to the anti-microbial active allicin, when the bulb is cut or bruised. 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. Allicin gives garlic its characteristic pungent smell (Williamson 2003). Also, it contains vitamins and minerals (Gruenwald 2004) and trace elements (selenium & germanium) (Skidmore-Roth 2003). Allicin (diallythiosulfinate) is the most abundant compound representing about 70% of all thiosulfinates present, or formed in crushed garlic (Block 1992; Han et al 1995). Garlic has proven to be hypolipidemic (Sumiyoshi 1997), antimicrobial (Kumar & Berwal 1998), antihypertensive (Suetsuna 1998), hepatoprotective (Wang et al 1998) and insecticidal (Wang et al 1998). Garlic extract has also been shown to reduce serum cholesterol levels (Bordia et al 1975; Augusti & Mathew 1974) and increase blood coagulation time (Bordia et al 1975).

Using of garlic in fish farming has become popular for enhancing the activity of non-specific defense systems and conferring protection against diseases and it was used as a growth promoter in *O. niloticus* culture (Diab et al 2002; Metwally 2009) also it increased body gain, feed intake and feed efficiency ratio (Abd-El Allatif & Ebraheem 1996; Metwally 2009).

This work was carried out to study the effect of different values of garlic on growth factors, some hematological parameters and body composition in rainbow trout (*Oncorhynchus mykiss*).

Material and Method. Experimental fish: the rainbow trout (20.88±0.25 g) were obtained from a commercial farm in Haraz, Iran and were transferred to the place of experiment and acclimated for 2 weeks. During the acclimation, fish were fed the experimental diet to satiation twice a day at 09:00 and 15:00 hours. After acclimation, fish were fasted for one day, batch weighted and randomly distributed among 12 troughs at a density of 30 fish per tank.

Experimental diet and feeding regime: the basal experimental diets were formulated with the commonly available ingredients (see Table 1). The formula and analyzed proximate composition of the basal diet are shown in Table 1. The ingredients were grinded, milled, weighed, mixed and pelleted with meat mincer through a 2 mm die. After cold pelleting, the feeds were air dried and put in an air-tight container. All diets were stored at -20 °C until fed. During the experiment, fish were fed the experimental diet to satiation third a day at 08:00, 12:00 and 16:00 hours.

Measurements and sample analysis: sampling was carried out each 20 days. Water temperature was 15ºC, O_2 7-8 mgl^{-1}, pH 7-8 and light:dark cycle of 12:12 h was maintained during the feeding trial. Proximate composition of diets and tissues were carried out using the Association of Analytical Chemists (AOAC 2000) methods. Protein was determined by measuring nitrogen (N×6.25) using the Kjeldahl method; Crude fat was determined using petroleum ether (40–60 Bp) extraction method with Soxhlet apparatus and ash by combustion at 550 °C. Blood samples were collected from the fish caudal vein and placed into heparinized tubes and by ice flask were rapidly transferred to the laboratory. The indices used to evaluate the haematological profile included the erythrocyte count (RBC), haemoglobin concentration (Hb), haematocrit (HT), mean erythrocyte volume (MCV), mean colour concentration (MCHC) and erythrocyte haemoglobin (MCH). The procedures were based on Unified Methods for Haematological Examination of Fish (Svobodova et al 1991). Blood plasma was obtained by the centrifugation of blood samples in a cooled centrifuge (4°C, 837 × g). Biochemical indices determined in the blood plasma included glucose (GLU), total proteins (TP), albumins (ALB), calcium (Ca^{2+}) and inorganic phosphate (PHOS).

Calculations and statistical analysis: the following variables were calculated:

Body weight increase (BWI) = $W_t - W_0$ (Tacon 1990)

Specific growth rate (SGR) = $(\ln W_t - \ln W_0) \times 100\ t^{-1}$ (Hevroy et al 2005)

Feed conversion ratio (FCR) = total dry feed consumed (g) / total wet weight gained (g) (Shalaby et al 2006)

W_t and W_0 were final and initial fish weights (g), respectively; and t is the experimental period in days. The number of newborn fish in each aquarium in each day was counted, and they transfered into related larval aquaria; and finally the sex ratio of

newborn fish after they displayed the morphological charactristics of male or female were calculated. The data obtained from the trial were subjected to one-way analysis of variance (ANOVA) (using SPSS 16.0 programme) to test for effects of dietary treatments. When ANOVA identified significant difference among groups, multiple comparison tests among means were performed using Duncan's new multiple range test. For each comparison, statistically significant differences were determined by setting the aggregate type I error at 5% ($P<0.05$).

Table 1

Formulation and proximate composition of the basal diets

Ingredients	Control	Allium sativum diets (g kg^{-1} diet)		
	0	10	20	30
Fish meal	50	50	50	50
Wheat Meal	20	20	20	20
Soybean meal	12	12	12	12
Fish oil	10	10	10	10
Garlic	-	1	2	3
Vit. premix [a]	1.5	1.5	1.5	1.5
Min. premix [b]	1.5	1.5	1.5	1.5
Filler	5	4	3	2
Proximate composition	*(%)*			
Crude protein	40.48			
Crude lipid	17.60			
Ash	11.26			
Fiber	1.48			

[a] Vitamin A, 3600000 IU; Vitamin D_3, 800000 IU; Vitamin E, 14.4 g; Vitamin K_3, 0.8 g; VitaminB_1, 0.71 g; Vitamin B_2, 2.64 g; Vitamin B_6, 1.176 g; Vitamin B_9, 0.4 g; Niacine, 11.88; Ca D-pantothenate, 3.92 g; Choline chloride, 100 g; Vitamin B_{12}, 6 mg; H_2, 4mg. [b] Mn, 39.68 g; Zn, 33.88 g; Fe, 20 g; Cu, 4 g; I, 397 mg; Se, 80 mg; Choline chloride, 100 g.

Results. Growth performances: growth performances of the fishes after 60 days of feeding are summarized in Table 2; fish group fed on 30g/kg garlic had higher final weight, weight gain, and SGR than fish fed on other levels of garlic and control. The highest amounts of dry feed intake (g/fish/day) were seen in fish groups fed on 30 g kg^{-1} garlic. Results in Table 2 show that FCR decreased significantly to 1.36±0.01 30 g *Allium sativum*/kg diet.

Table 2

Effects of garlic on growth parameters in rainbow trout (*O. mykiss*) fed on experimental diets

Parameters	Control	10 g kg^{-1} garlic	20 g kg^{-1} garlic	30 g kg^{-1} garlic
Initial weight (g)	20.96 ± 0.30^{a}	20.81 ± 0.09^{a}	20.87 ± 0.34^{a}	20.87 ± 0.33^{a}
Final weight (g)	96.04 ± 3.10^{c}	107.67 ± 4.17^{b}	110.50 ± 4.33^{b}	117.99 ± 2.57^{a}
BWI (g)	75.08 ± 3.40^{c}	86.85 ± 4.10^{b}	89.63 ± 4.46^{b}	97.12 ± 2.50^{a}
SGR	1.52 ± 0.05^{c}	1.64 ± 0.04^{b}	1.67 ± 0.05^{ab}	1.73 ± 0.02^{a}
FCR	1.60 ± 0.05^{b}	1.44 ± 0.04^{a}	1.41 ± 0.05^{a}	1.36 ± 0.01^{a}
Feed intake (g)	120.09 ± 1.38^{c}	124.63 ± 2.51^{b}	126.42 ± 1.63^{b}	132.27 ± 2.19^{a}

Groups with different alphabetic superscripts differ significantly at $p<0.05$ (ANOVA)

Body compositions: protein content in fish body were significantly higher in the group fed on diet containing 30 g kg^{-1} diet of garlic than all other groups ($p<0.05$). Total lipids

content in fish body decreased in treatments and it was significantly lower ($p<0.05$) in fish fed on 30 g kg^{-1} diet of garlic. Ash content was significantly higher ($p<0.05$) in fish fed on 30 g garlic kg^{-1} diet, and the lowest values were obtained with 10 g kg^{-1} *Allium sativum* and control.

Table 3

Chemical compositions of whole body (% of wet sample) of rainbow trout (*O. mykiss*) under different treatments.

Parameters	control	10 g kg-1 garlic	20 g kg-1 garlic	30 g kg-1 garlic
Crude protein	18.16 ± 0.06^{c}	18.22 ± 0.01^{c}	18.41 ± 0.04^{b}	18.82 ± 0.10^{a}
Total fat	5.31 ± 0.30^{a}	5.05 ± 0.23^{ab}	4.74 ± 0.06^{b}	4.33 ± 0.18^{c}
Ash	1.32 ± 0.01^{c}	1.32 ± 0.01^{c}	1.37 ± 0.02^{b}	1.41 ± 0.02^{a}

Groups with different alphabetic superscripts differ significantly at $p<0.05$ (ANOVA)

Hematological parameters: results of erythrocyte count (RBC), hemoglobin content, and hematocrit (HT) are given in Table 4. It shows that diets containing 20 and 30g/kg diet of *Allium sativum* increased all the examinad blood parameters, which were significantly different from those of control. Erythrocyte count and hemoglobin content increased in fish fed on diets containing 20 and 30 g garlic. Also, hematocrit values increased significantly in fish fed on 30 g *Allium sativum*. The values recorded for MCV and MCHC were comparable in groups under study.

Table 4

Haematological parameters in rainbow trout (*O. mykiss*) under different treatments

parameters	Control	10 g kg-1 garlic	20 g kg-1 garlic	30 g kg-1 garlic
RBC (T/l)	1.18 ± 0.07^{c}	1.35 ± 0.07^{b}	1.39 ± 0.04^{ab}	1.51 ± 0.12^{a}
Hb (g/l)	47.81 ± 1.43^{b}	42.11 ± 2.06^{c}	52.35 ± 1.69^{ab}	54.49 ± 3.86^{a}
HT (l/l)	0.34 ± 0.05^{b}	0.32 ± 0.05^{b}	0.38 ± 0.01^{ab}	41 ± 0.01^{a}
MCV (fl)	278.96 ± 16.71^{a}	261.73 ± 13.75^{a}	277.35 ± 13.75^{a}	284.79 ± 15.85^{a}
MCH (pg)	32.93 ± 1.52^{b}	33.66 ± 1.71^{b}	38.52 ± 2.32^{a}	40.24 ± 3.63^{a}
MCHC (g/l)	124.82 ± 19.69^{a}	123.35 ± 15.04^{a}	131.97 ± 12.09^{a}	135.82 ± 18.90^{a}

Groups with different alphabetic superscripts differ significantly at $p<0.05$ (ANOVA)

Biochemical blood plasma profile: plasma glucose in fish fed on diets containing garlic showed significantly difference with control group ($p<0.05$). Mean values of total plasma protein increased significantly in all treatments when compared to control group ($p<0.05$). Furthermore other indices of blood plasma were higher in treatments.

Table 5

Some biochemical indices of blood plasma in rainbow trout (*O. mykiss*) under different treatments

parameters	Control	10 g kg-1 garlic	20 g kg-1 garlic	30 g kg-1 garlic
GLU (mmol/l)	5.01 ± 0.17^{a}	4.73 ± 0.48^{a}	4.93 ± 0.33^{a}	3.93 ± 0.17^{b}
TP (g/l)	25.17 ± 0.76^{c}	33.63 ± 1.96^{b}	39.51 ± 2.12^{b}	45.98 ± 5.49^{a}
ALB (g/l)	8.38 ± 17^{b}	8.45 ± 13^{b}	8.62 ± 0.08^{ab}	8.90 ± 0.20^{a}
Ca^{2+} (mmol/l)	2.47 ± 0.19^{b}	2.62 ± 0.13^{b}	2.72 ± 0.07^{b}	3.04 ± 0.15^{a}
PhOS (mmol/l)	3.68 ± 0.12^{a}	3.73 ± 0.25^{a}	3.79 ± 0.35^{a}	3.93 ± 0.35^{a}

Groups with different alphabetic superscripts differ significantly at $p<0.05$ (ANOVA)

Discussion. Garlic is a main 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). Now days antibiotics are largely used for treatment and control or reduce harmful bacterial contamination, so need to replace them with natural substances to avoid from bad effects of them.

In this study the highest growth performance was observed in fish fed diets containing garlic, specialy on 30 g garlic. It agrees with studies results of Diab et al (2002), Abou-Zeid (2002), Shalaby et al (2006).

Feed intake increased with increasing *Allium sativum* levels. Feed conversion ratio decreased with increasing *Allium sativum* levels. These results are also in agreement with those obtained by Khattab et al (2004), Gomes et al (1993), Degani et al (1997).

In this study, results of *O. mykiss* body compositions showed that crude protein and ash increased significantly with diets containing 30g *Allium sativum*, although total lipid content decreased significantly with the same levels of *Allium sativum*. These results agree with those obtained by Abdelhamid et al (2002), Khattab et al (2004), and Shalaby et al (2006), who showed that inclusion of Biogen in the diet increased fish protein content and decreased whole body fat in fish. However, Diab et al (2002) reported that there were no significant changes in fish body composition caused by different garlic levels.

The present study demonstrated that administration of garlic induced significant increases in all blood parameters (erythrocyte count, haemoglobin content and hematocrit value) in treated fish, which agrees with the results of Martinz et al (2002) and Shalaby et al (2006). Also plasma glucose concentration reduced significantly in fish fed on diets containing the *Allium sativum*. These results agree with those of Kumar & Reddy (1999), Thomson & Ali (2003) and Shalaby et al (2006). Total protein of plasma increased in treatments wich agrees with Hussein et al (2001) but Shalaby et al (2006) said it was not significantly high in treatments. The results of the study showed that use of garlic can effectively improve growth performance and fish health.

Conclusions. At the end, from the obtained results it could be recommended that garlic (*Allium sativum*) may be used as a growth promoter and antibiotic for the treatment or prevention of diseases and for enhancing fish tolerance to environmental stressors (Sivam 2001); so garlic should be added to the diets of fish.

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