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Effect of *Allium sativum* Supplemented Diets on Growth and Haematological Responses in Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

The present work was designed to evaluate the effect of garlic (*Allium sativum*) as a natural feed ingredient on growth performance and immune response in Nile tilapia (*Oreochromis niloticus*). A total of 150 juvenile's tilapia were taken randomly and divided into five experimental units (10 fishes per unit) with three replicates. Fish was hand feeded containing five different levels of garlic (T_1, control) 2.5 (T_2), 5.0 (T_3), 7.5 (T_4), and 10g/500g (T_5) in powdered form. Fish were fed at 6% of body weight twice, daily. Feed utilization was estimated in terms of feed conversion ratio (FCR). The growth was measured in terms of total weight gain (TWG) and length of the body was calculated. At the end of the trial, immune response monitored in terms of hemoglobin (Hb), hematocrit (Ht), white blood cells (WBC), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Average mean weight gain (11.03±0.8g) and average mean length (7.8±0.4cm) was observed maximum in T_4 as compared to other dietary treatments. There observed a significant difference ($P<0.05$) in FCR among all the dietary treatments with highest in T_4 (0.59±0.32). Dietary treatment T_5 (10/500g) showed significance difference ($P<0.05$) in red blood cells (RBC) count (2.82±0.33), white blood cells (WBC) count (6.56±0.83), hemoglobin (24.89±2.67) and hematocrit (37.27±3.83) as compared to other treatment groups including control. The results of our study showed that dietary treatment T_4 (7.5g/500g) showed maximum growth and FCR while dietary treatment T_5 (10/500g) showed better immune response in tilapia over other dietary treatments. So, it is concluded that garlic can be used as immunity stimulant and to enhance growth performance in Nile tilapia.

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Authors' Contribution
JAN conceived the study design, collected and analyzed data and wrote the manuscript. SH and AT assisted in data collection and statistical analysis. AM, DH and HMT helped in designing the study and preparation of the manuscript.

Key words
Nile tilapia, Garlic, Weight gain, Immunity, FCR.

INTRODUCTION

Aquaculture is best site to earn in developing countries (Manoj and Vasudevan, 2009) and it is fastly growing sector in the world (Harikrishnan *et al*., 2011). Due to continuously increasing trend of culturing fish and shellfish species, aquaculture has become pivot component for animal health in industry (Kolkovski and Kolkovski, 2011). Fish as a source of food has a lot of potential to reduce the risk of food shortage, mainly in the countries like Pakistan. It provides a high quality protein and balanced essential amino acids, necessary minerals and fatty acids than that of meat, milk and eggs (Hossain, 1996).

Tilapia is second most important fish formed at large scale after carps in all over the world and described as 21st century most important fish (Shelton, 2002). In order to raise fish production, there is a need of research in the feeding practices, to increase the growth of fish and to decrease the waste materials in the water (Singh *et al*., 2005). Inclusion of new substances in fish diet is another approach to elevate general conditions for fish maintenance and growth or to improve feed conversion efficiency (Fernández-Navarro *et al*., 2006). To attain high production is necessary in improvement of fisheries in fresh water sources. For improvement it is necessary to prepare and provide synthetic or formulated feed. Because of this fish grow faster and gain high weight in shortest possible time (Bhosale *et al*., 2010). Pathogenic diseases are the major problems for aquaculture (Rahman *et al*., 2009). Bacteria cause bacterial diseases which are responsible for large scale mortalities in both fish farming and as well as in wild fisheries in all over the world. These are opportunistic pathogens which invades at tissue of host fish and cause there infection. *Edwardsiella tarda,* Aeromonad and Pseudomonad are the major pathogenic bacteria among all the bacteria which widely cause pathogenicity in aquatic organisms (Rahman *et al*., 2009). The excessive use of antibiotics in treatment has developed resistance and it is

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also difficult, non-effective, costly and environmentally hazardous (Cañada *et al*., 2009). To control fish diseases and enhance growth, using of immune stimulus chemicals seems to be an attractive alternatives (Raa, 1996). Effect of immune stimulants depends upon different factors such as dosage, method of administration, time and physiological condition of fish. To control several diseases, large numbers of plants have been used for treatments in medicines (Chakrabarti *et al*., 2012).

Inclusion of new substances in fish diet is another approach to elevate general conditions for fish maintenance and growth or to improve feed conversion efficiency (Fernández-Navarro *et al*., 2006). Recently it has been shown that medicinal plants are used in fish diets for stimulation of immune system and increase disease resistance in fish (Chakrabarti *et al*., 2012). To enhance the color attributes and flavor of food stuff are generally some spices, herbs and plants are used in feed. On the other hand some species of plants and herbs also have antimicrobial and antioxidant activities (Bayder *et al*., 2004). It is estimated and stated that products obtained from plants are used to improve and enhance different activities e.g. growth promotion, anti-stress, appetite stimulation and immune system in aquaculture (Citarasu *et al*., 2001, 2002; Sivaram *et al*., 2004). Natural plant products have antimicrobial characteristics in some aquatic animals due to their bio active compounds such as polypeptide, terpinoides, quinones, lectines, alkaloids, polyphenols and phenolics and other synthetic compounds and effective alternatives of antibiotics. Oral intake of these compounds increase growth, stimulation of appetite immune and tonic (Olusola *et al*., 2013). To improve immunity and increase growth rate, different medicinal plants are used in birds, fishes, and animals diet. In India most widely selling medicines are prepared from herbs, three of most prepared from *Allium sativum,* Panax species and *A. barbedensis.* Medicinal plants are more valuable because of low cost but provide more protection and fewer side effects due to, due to minimum cost potency, enhanced tolerance, complete accessibility or more advantageous and a recycle able (Parveen and Shrivastava, 2012).

Garlic (*Allium sativam)* control pathogenicity of bacteria and fungi as a result improve health of fish (Corto-Martinez *et al*., 2007). The allium species are used to increase immune system activity and promote lymphocyte production, release of cytokine natural killer cell activity and phagocytosis (Kyo *et al*., 1998). Garlic was used in diet of rainbow trout to effectively enhance their immune system (Nya and Austin, 2009) and also was used in *Labeo rohita* diet against *A. hydrophila* infection (Sahu *et al*., 2007). Garlic has been used as a functional food and flavoring agent. Various extract forms are obtained from garlic (*Allium sativum*) like as ethanol, aqueous and dried powder (Shin and Kim, 2004). Garlic possesses higher nutritive value than any other bulbous crops (Sood *et al*., 2003) its extracts also known to enhance glutathione peroxidase activity (Pedraza-Chaverrí *et al*., 2001). Garlic supplemented diet of Nile tilapia has shown better growth performance, PER and FCR (Shalaby *et al*., 2006). The present work has been conducted to monitor the effect of garlic supplemented diets on growth performance, FCR and immune response in Nile tilapia.

MATERIALS AND METHODS

Diet preparation and formulation

Commercial feed was taken from Fisheries Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad in the form of small pellets. Feed was grinded to powder form by a locally available grinder. Garlic was supplemented in powder form in the remaining ingredients of diets. For that, garlic peeled and cut it into small pieces, dried and soaked in air over night. When dried, it was grinded to powder form in a grinder and packed in poly ethylene bags. Garlic was supplemented into commercial feed to achieve the following; (1) T_1 (0g/500g, control); (2) T_2 (2.5g/500g); (3) T_3 (5.0g/500); (4) T_4 (7.5g/500g); (5) T_5 (10g/500g).

Table I.- Basal diet composition (% dry matter basis) fed tilapia for 12 weeks.

Ingredients	Dry matter basis (%)	Ingredients	Dry matter basis (%)
Wheat flour	16	Fish meal	15
Starch	20	DCP	1
Canola meal	14	Soya oil	1
Sunflower meal	12	Premix	2
Corn gluten 30%	19		

Fish and experimental conditions

The fingerlings of Nile tilapia (*Oreochromis niloticus)* were obtained from the Fisheries Research Farms, Department of Zoology, Wildlife and Fisheries, University of Agriculture Faisalabad. Prior to the start of the experiment, the fish were acclimatized in the glass aquaria filled with tap water with standard values of pH 7, temperature 26±1°C and dissolved oxygen 5.0±0.3mg/L for two weeks. During acclimatization, the fingerlings were fed with corn glutton and commercial diet (control diet). 150 tilapia fingerlings of equal size were randomly divided in 5 experimental units (10 fish/unit) in triplicates for a period of 5 weeks. Fish were fed at 6% body weight at 8:00 AM and 4:00 PM twice, daily.

Table II.- Weekly increase in average body weight (g) and average total length (cm) of Nile tilapia (*Oreochromis niloticus*) fed different levels of garlic supplemented diets.

Weeks	T_1	T_2	T_3	T_4	T_5	Mean
Average body weight (g)						
1	2.78±0.88	4.04±1.72	3.02±1.06	10.05±2.59	7.51±2.1	5.48±2.8
2	2.83±0.89	4.31±1.91	3.56±1.41	10.55±1.33	6.82±1.8	5.61±2.8
3	2.9±0.82	3.96±1.82	3.46±1.28	10.5±1.3	7.14±1.8	5.59±2.9
4	3.83±1.30	4.81±2.22	4.58±1.53	11.9±3.04	7.81±1.7	6.58±3
5	4.14±1.31	5.12±2.21	4.89±1.53	12.16±3.1	8.23±1.2	6.91±3
Mean	3.27±0.6	4.45±0.4	3.9±0.7	11.03±0.8	7.5±0.5	
Average total length (cm)						
1	5.71±0.94	6.02±0.91	5.59±0.76	7.93±2.54	6.84±1.73	6.4±0.98
2	5.18±0.62	5.76±0.84	5.25±1.02	7.12±2.86	6.61±1.89	6.0±0.85
3	5.54±0.46	6.08±0.92	5.75±0.78	7.65±2.62	7.18±1.70	6.4±0.93
4	5.55±0.62	6.12±0.87	6.17±0.77	7.89±2.43	7.28±1.62	6.6±0.95
5	5.75±0.62	6.36±0.86	6.41±0.80	8.16±2.45	7.58±1.62	6.9±0.99
Mean	5.5±0.23	6.1±0.22	5.8±0.46	7.8±0.4	7.1±0.38	

Growth performance

Growth performance was calculated in terms of % weight gain (percentage weight gain = (final weight-initial weight) / (initial weight) × 100), specific growth rate (SGR = ln (final wet body weight) – ln (initial wet body weight) × 100, time duration (days) and feed conversion ratio (FCR = feed intake (g) / total weight gain (g)).

Table III.- Weekly changes in feed conversion ratio (FCR) and specific growth rate (SGR) of Nile tilapia (*Oreochromis niloticus*) fed different levels of garlic supplemented diets.

Weeks	T_1	T_2	T_3	T_4	T_5
FCR					
1	-	-	-	-	-
2	1.13	0.32	0.13	0.42	0.28
3	0.83	0.27	0.38	0.77	0.35
4	0.08	0.64	0.11	0.22	0.42
5	0.27	0.33	0.32	0.94	0.39
Mean	0.58±0.49	0.39±0.17	0.23±0.13	0.59±0.32	0.36±0.06
SGR					
1	-	-	-	-	-
2	0.06	0.22	0.55	0.16	0.25
3	0.08	0.26	0.18	0.09	0.19
4	0.93	0.11	0.66	0.31	0.16
5	0.26	0.21	0.22	0.07	0.17
Mean	0.33±0.41	0.20±0.07	0.40±0.24	0.16±0.11	0.20±0.04

Haematological responses

Collection of blood samples

At the end of trial, blood samples were collected from all the experimental groups. Fish were gently netted out avoiding stress, anesthized by dipping it in water containing few drops of clove oil for 3 min. Blood was collected from the heart ventricle following the established protocol and stored in heparin for further use.

Measurement of blood parameters

At the end of the trial, immune response of tilapia measured in terms of hemoglobin (Hb), hematocrit (Ht), white blood cells (WBC), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) by following the standard protocols.

Statistical analysis

All the parameters were computed by analysis of variance (ANOVA). To compute all the statistical data, Statistix, software program version 8.1 (Analytical Software, Tallahassee, FL) was used. The significance level was set at $P \leq 0.05$.

RESULTS

Growth performance

Fish treated with 7.5g/500g (T_4) garlic supplemented diets showed maximum increase in average weight gain (11.03±0.8) and average increase in total length (7.8±0.4) compared to other dietary treatments with minimum (3.27±0.6) and (5.5±0.23) in control group, respectively (Table II). FCR was also observed maximum (0.59±0.32) in T_4 compared to other dietary treatments (Table III). Specific growth rate was observed maximum (0.40±0.24) in T_3 while minimum (0.16±0.11) in T_4 (Table III).

Table IV.- Mean±SD values of different blood parameters as an indicator of immunity in Nile tilapia (*Oreochromis niloticus*) fed different levels of garlic supplemented diets.

Blood parameters	T_1	T_2	T_3	T_4	T_5
RBC (million/mm^3)	1.26±0.20	1.55±0.28	1.79±0.28	2.07±0.30	2.82±0.33
WBC ($10^3.\mu^{-1}$)	2.94±0.31	3.24±0.33	4.24±0.46	5.3±0.48	6.56±0.83
Hb (g/dl)	7.02±0.52	9.01±0.36	12.23±0.42	15.17±0.44	24.89±2.67
Ht (%)	13.34±1.02	17.11±1.99	22.66±3.20	26.63±2.92	37.27±3.83
MCV (µm^3)	106.31±5.12	111.06±5.41	126.60±1.55	128.82±2.13	132.22±1.77
MCH (µg)	56.04±3.65	59.09±6.51	68.92±5.68	73.80±5.74	88.19±1.26
MCHC (%)	5.26±0.11	5.08±0.65	5.42±0.42	5.75±0.37	6.67±0.12

RBC, Red blood cells; WBC, White blood cells; Hb, hemoglobin; Ht, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

Hematology

There observed an increasing trend in all the hematological parameters from T_1 to T_5 (Table IV). Highest mean average value of RBC (2.82±0.33) and WBC (6.56±0.83) was found in T_5 while minimum (1.26±0.20) and (2.94±0.31) in control group, respectively. Hemoglobin (24.89±2.67), hematocrit (37.27±3.83), MCV (132.22±1.77), MCH (88.19±1.26) and MCHC (6.67±0.12) were also found maximum in dietary treatment group T_5.

DISCUSSION

The results of present study showed a significant difference ($p<0.05$) in growth performance and hematological parameters among all the dietary treatment groups supplemented with different levels of garlic. Dietary treatment group T_4 (7.5/500g) showed maximum performance in terms of growth hematology compared to other dietary treatments. Similar results were found by the study of Mesalhy *et al*. (2008) who fed 10, 20g/kg diet to Nile tilapia for one month and observed significant increase in final body weight gain as compared to control group for a period of 8 weeks. The results of present study also are in line with the findings of Ahmed *et al*. (2008) who observed same significant ($p<0.05$) difference in growth among control and garlic supplemented fish.

Nya and Austin (2009) used different diets with garlic supplementation against *Aeromonas hydrophila* in rainbow trout (*Oncorhyncus mykiss*) and found significant results in FCR and weight gain in experimental fish. Abdel-Hakim *et al*. (2010) concluded similar trends in growth performance of tilapia containing garlic supplemented diets. Abdel-Tawab (2010) researched out similar results in Nile Tilapia with garlic supplementation that improved survival rate, growth, and feed utilization, especially at higher concentration of 2g of garlic. Farahi *et al*. (2010) concluded similar results in which garlic supplemented diets significantly ($p<0.05$) increased growth performance in *O. mykiss*. Mehrim *et al*. (2014) concluded similar results in tilapia treated with garlic showed significant ($p<0.05$) increase in weight and body composition. Maniat *et al*. (2014) concluded same results with *Mesopotamichthys sharpeyi* in which feed efficiency and growth performance were improved in groups treated with garlic supplemented diets. But the best results of FCR, SGR were found in the group fed with higher concentration (10g/kg) of garlic powder.

Hematological parameters (RBC, WBC, Hb, Ht, MCV, MCH and MCHC) were measured at the end of the trial and found significant ($p<0.05$) difference in all the parameters among all the dietary groups with best results at higher garlic supplementation level (10g/500g). Al-Salahy and Mahmoud (2003) have shown a similar trend in blood serum and RBC in orally administrated garlic in *Chrysichthys auratus*. The results of the study of Shalaby *et al*. (2006) are in accordance with the findings of present study in which erythrocytes numbers and hemoglobin contents were found significantly ($p<0.05$) higher in garlic treated fish compare to control group. Nya and Austin (2009) used different diets with garlic supplementation against *Aeromonas hydrophila* in rainbow trout *(Oncorhyncus mykiss*) and found similar results with increasing leucocytes, erythrocytes, hematocrit, lysozyme level and anti-protease activity along with antioxidant activity stimulations. Thanikachalam *et al*. (2010) concluded that hematological parameters like RBCs and WBCs was also increased in garlic treated groups as compared to control group. The results proved that the *Clarias gariepinus* have increased RBCs and WBCs and more immune potent against *Aeromonas hydrphila* even at 0.5% garlic supplementation.

Fazlolahzadeh *et al*. (2011) described increased immunity, lymphocyte and erythrocytes activities and decreased mortality rate in fish groups treated with 0.45g and 0.6g of garlic compared to the 0.3g of garlic diet and the control. Similar results were reported by Talpura and

Ikhwanuddin (2012) in which erythrocytes, leucocytes, hematocrit, hemoglobin, phagocytic activity, respiratory burst, lysozyme, anti-protease and bactericidal activities were enhanced following feeding with garlic. Our findings also confirmed the previous published results of Atea and Ajeel (2012) who reported significant difference ($P<0.05$) in Hb, PCV, WBCs, and RBCs values of 10g/kg garlic supplemented diets and control. Nwabueze (2012) concluded that in *Clarias gariepinus*, addition of garlic in the diets at 3% of body weight significantly ($P<0.05$) increased hemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC) compared to control group in. Tanekhy and Fall (2015) concluded that garlic increased immunity level in all garlic supplemented dietary group as compared to control.

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Statement of conflict of interest

The authors declare no conflict of interest.

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