EFFECT OF GARLIC ON THE SURVIVAL, GROWTH, RESISTANCE AND QUALITY OF *OREOCHROMIS NILOTICUS*

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Abstract

Sixteen hundred *Oreochromis niloticus* were divided equally among five groups, each comprising four equal sized replicates, to evaluate the efficiency of a garlic-supplemented diet (10 and 20 g kg^{-1} diet fed) in the performance of *O. niloticus*. Group (1) was the control (fed on base diet). Groups (2 & 3) were fed on garlic-supplemented diet (10 and 20 g kg^{-1} diet fed) for one month, respectively, and groups (4 & 5) were fed on same doses of garlic-supplemented diet for two month, respectively. All groups were fed on the base diet after the periods of garlic-supplementation until Month 8. Body weight and blood parameters were recorded. Challenge infection with pathogenic *Aeromonas hydrophila,* was carried out immediately after feeding the test diets as well as Month 8. Survival and individual body weights were recorded at the end of the trial. Studies on fish quality and shelf-life were also carried out. A non-significant increase was seen in the hematocrit values among all treatment groups. Statistically significant increases in total leukocytic count in treatment group 2 were also observed. Moreover, the nitroblue tetrazolium value was significantly elevated after 2 month and the monocytic count was significantly increased in groups 2-5. There was no statistically significant increase in mean individual body weights of treatment groups 2-5 after one and two months, but there was a statistically significant increase after eight months in all treatments except group 2. Survival rates were significantly higher in all treatments compared to the control. The relative level of protection against the challenge infection was higher in all treatment groups than in the control. The quality and shelf-life of the garlic supplemented tilapia was better than those of the control. Both dose rates of garlic induced a similar effect, enhancing immunity and health status. Consequently garlic improved the growth performance. Moreover, fish quality and shelf-life were improved. However, further extensive testing, including full commercial cost benefit analysis, is necessary before recommending its application in aquaculture.

KEY WORDS: Garlic, survival, growth, resistance, challenge, shelf life, *Oreochromis niloticus.*

INTRODUCTION

Disease outbreaks were recently identified as a major constraint to aquaculture production and trade, with a consequent effect on the industry's economic

development (Yunxia *et al,* 2001). The use of disinfectants and antimicrobials has shown limited success in preventing or curing aquatic diseases (Subasinghe 1997). Furthermore, there is a growing concern about the use and abuse of the antimicrobials in aquaculture, as they increase the selective pressure exerted on the microbes and encourage the emergence of resistant bacteria by transferring resistant-genes to bacteria not exposed to antibiotics. Moreover, the antimicrobials lead to drug residues in the treated fish, besides having a negative impact on the environment (FAO/WHO/OIE 2006). Antimicrobials can generate cross-resistance against human antimicrobials, which could pose a hazard (Witte *et al,* 1999). Moreover, commercial vaccines are expensive for fish producers, and may not be available against the encountered and emerging diseases (Raa *et al,* 1992). Currently, 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 and Seljelid 1995; Raa 1996).

In addition to sensory evaluation, total psychrotroph and mould counts are key indicators in the determination of fish quality and consumer acceptability (Shewan *et al,* 1953). Harvested fish exhibited collapse of the immune system, which allows the proliferation and colonization of bacteria and fungi on the skin surface, followed by invasion of the fish-flesh (Murry and Shewan 1979). These microorganisms are the major cause of the edible fish spoilage.

Garlic can help in the control of pathogens, especially bacteria and fungi, and increase the welfare of fish (Corzo-Martinez *et al,* 2007; Adetumbi *et al,* 1986; Ress *et al,* 1993). Garlic, *Allium sativum* L., has been used for the treatment of many diseases since ancient times as reported in the Codex Ebers (1550 BC), where an Egyptian medical papyrus described several therapeutic formulas based on the garlic as a useful remedy for a variety of diseases such as heart problems, headache, bites, worms and tumors (Block 1985). Cloves of garlic were found in the tomb of Tutankhamen and in the sacred underground temple of the bulls of Saqqara. It has long been considered that garlic (*Allium sativum*) has several beneficial effects for human and animals, exhibiting antimicrobial, antioxidant, and antihypertensive properties (Konjufca *et al,* 1997; Sivam 2001). Previous research suggested that those functions are mainly attributed to the bioactive components of garlic, including sulphur containing compounds, such as allin, diallylsulphides and allicin (Amagase et al, 1993). Many beneficial health properties of garlic are attributed to organosulphur compounds, particularly to thiosulfinates (Block 1992). 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 and 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 1977) and increase blood coagulation time (Bordia *et al,* 1975). An antifungal activity has been identified in garlic bulbs (Fromthing and Bulmer 1978). S-allyl cysteine, present in the crushed garlic, was found to inhibit tumor metabolism and enhance the immune response (Sumiyoshi 1997). The allyl sulfides enhance the glutathione S-transferase enzyme systems, which through their dependent biochemical pathways enhance the liver's detoxification of carcinogenic substances. The allium species show immune enhancing activities that include promotion of lymphocyte synthesis, cytokine release, phagocytosis and natural killer cell activity (Kyo *et al,* 1998).

The present study was conducted to evaluate the efficiency of the garlic (*Allium sativum* L) in improving the immune response, survival, growth and disease resistance in Nile tilapia (*O. niloticus*).The effects on quality and shelf-life of fish were also considered.

MATERIALS AND METHODS

1. Fish

A total of 1600 Nile tilapia (*O. niloticus)* fry (mean individual initial weight = 6.5 ± 1.0 g) were divided into five equal treatments, including the control. Each treatment and the control consisted of four equal replicates (80 fish per replicate) that were randomly assigned to 20 hapas (3 x 2 x 1 m, each) fixed in earthen pond in four rows of 5 hapas each. Fish were fed on a base diet of 25% protein at 3% (summer) and 1% (winter) body weight per day, divided into two feeding times. Feed was placed in plastic trays fitted in hapas (one per hapa).

2. Garlic

Garlic (*Allium sativum* L) was procured from the local market, crushed and two doses, i.e. 10 and 20 g of garlic kg^{-1} feed were mixed with the balanced diet in pellets. The pellets were prepared biweekly, air-dried at room temperature for 24 hours and stored in a refrigerator (4°C). The chemical analyses of garlic cloves revealed sulfer-containing amino acids (1-3%) named alliin which is the stable precursor that is converted to allicin by the action of allinase enzyme which represent 10 mg/g garlic cloves fresh weight (Ellmore and Feldberg 1994).

3. Diets

A balanced ration was prepared (Table 1). The ingredients were obtained from several specialized suppliers and prepared locally in the WorldFish Center in the form of pellets. The basal diet was prepared by grinding the corn to granules using 0.5 mm mesh (Thomes-Willey Laboratory Mill Model 4). The ingredients were mixed

mechanically by horizontal mixer (Hobart model D300T) at a low speed for 30 min. Oil (vegetable & cod liver) was added gradually to assure the homogeneity of the ingredients. The mixing speed increased for 5 min during the addition of water (600 ml) until the mixture began to clump. Pellets were then prepared using a pellet machine (CPM California pellet mill Co.) with 0.5 cm diameter. Batches of feed were prepared every two weeks and pellets left for 24 h to air dry, and stored in a refrigerator (4°C) for daily use.

4. Experimental design (Table 2)

The study was conducted over an eight-month period (September 2005 to April 2006) to evaluate the efficacy of garlic in promoting production of the cultured *Oreochromis niloticus*. Sixteen hundred *O. niloticus* fry were divided into five equal groups (groups 1-5; see above). Group 1 was fed a base diet for 8 months (control). Garlic supplemented base diets (10 and 20 g kg^{-1} diet fed) were given to groups 2 and 3 for 1 month and to groups 4 and 5 for 2 months, respectively. Four replicates were used in each group and were randomly assigned to hapas fixed in four rows of earthen bottom ponds in such a manner that each row represented the replicate of each treatment group. Hapas were observed daily and any dead fish removed.

The trial was conducted in three stages, (first, second and third phases). Fish were given the garlic-supplemented basal diet for one and two months, phase 1 and 2 respectively. The base diet was fed to all treatment and control groups throughout the third phase until the end of the experiment (8 months). The third phase was designed to evaluate the possible continuity of the efficacy of the garlic, administered in the first and second phases. At the end of the feeding period, the fish were examined in the laboratory for the various parameters.

Table 1.Composition of the basal diet used throughout the experiment.

Ingredients	Diet (%)	Protein (%)		Metabolic energy (Joules)	
		ingredients	feed	Ingredients	feed
Fish meal	8.00	0.72	5.76	4000	32000
Soybean meal	52.9	0.48	25.392	2870	151823
Ground corn	29.1	0.109	3.1719	1240	36084
Wheat flour	5.00	0.134	0.67	2700	13500
Vegetable oil	2.00	0.00	0.00	9100	18200
Cod liver oil	2.00	0.00	0.00	9100	18200
Di calcium phosphate	1.00	0.00	0.00	0.00	0000
Mineral mix.	0.07	0.00	0.00	0.00	0000
Vitamin mix.	0.05	0.00	0.00	0.00	0000
Vitamin C	0.03	0.00	0.00	0.00	0000
Total	100.15	0.00	34.9939	0.00	269807

Ingredients were obtained from local markets.

Table 2. Design of experiment and parameters measured along the experiment.

Group *	Treatment (phases) & duration in months			Hematology (phases)			Growth (phases)			Challenge (phases)			S, Q & Sl (phases)
	1st 1st month	2nd 2nd month	3rd 6 more months	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	3rd
1	BD	B.D.	B.D.	M.	M.	nm	M.	M.	M.	D.	D.	D.	M.
2	10g**	B.D.	B.D.	M.	nm	nm	M.	nm	M.	D.	nd	D.	M.
3	20g**	B.D.	B.D.	M.	nm	nm	M.	nm	M.	D.	nd	D.	M.
4	10g**	10g**	B.D.	nm	M.	nm	nm	M.	M.	nd	D.	D.	M.
5	20g**	20g**	B.D.	nm	M.	nm	nm	M.	M.	nd	D.	D.	M.

Each group had 320 Oreochromis niloticus with 4 replicates ie; 80 fish/replicate. Group 1 served as a control.

dose of garlic/ kg of feed. B.D.= basal diet; M= measured; nm= not measured; D= done; nd= not done, S= survival, Q=quality & Sl= Shelf-life.

5. Laboratory tests

(i). Body weight gain: All fish from all treatment replicates were weighed individually after 1, 2 and 8 months.

(ii). Survival: Survival rate was recorded during the course of the feeding experiment for all treatment replicates.

(iii). Some hematological and immunological parameters: The packed cell volume, total and differential leukocytic counts (TLC & DLC) were carried out according to the method of Stoskoph (1993). Nitroblue tetrazolium (NBT) was measured after Siwicki *at al.* (1985). These parameters were determined from blood samples, collected after the first and second phases from the caudal vein of 20 fish from each treatment group (5 from each replicate) using sterile syringes with saturated EDTA.

(iv). Challenge infections: These were carried out three times on the treatment groups: after feeding on the test diets for one and two months and at the end of the experiment (month 8). Twenty fish from each treatment group and from the control (5 from each replicate), were clinically examined and blood samples bacteriologically tested and determined to be free from bacterial infection, were then artificially infected by intraperitoneal injection with 0.5 ml of culture suspension of pathogenic *Aeromonas hydrophila* containing 10^8 bacteria ml^{-1} that were previously isolated from moribund fish and studied for pathogenicity. A culture suspension of *Aeromonas hydrophila* was prepared by culturing in agar for 24 h, washed and suspended in saline (0.85%) and counted using MacFirland standard tubes (No.1). The relative level of protection (RLP), among the challenged fish was determined according to Ruangroupan *at al.*, (1986) using the following equation.

RLP = 100 - percentage of immunized mortality ÷ percentage of control mortality x100.

(v). Sensory evaluation: The sensory evaluation was done to detect the consumer acceptability for fish odor or taste. Ten fish from each garlic supplemented and control groups were evaluated for odor and taste by three panelists after roasting at an internal temperature of 48°C in a 165°C oven. Odor and taste were evaluated by 5 point scale; 1(very bad), 2 (bad), 3 (fair), 4 (good) and 5 (very good). The fish were considered unacceptable when their sensory score was below 3, according to Shewan *et al*, (1953).

(vi) Shelf-life: This was determined for all fish groups at harvest time where 20 fish per group (5 fish per replicate) were used to determine the total psychrotroph and mould counts in the fish-flesh 0, 48, 96 and 144 h after harvest. Throughout this period, fish were stored in an ice-container, the crushed ice being replaced daily. For determining total psychrotrophs, 10 g sample of the fish-flesh was transferred to a sterile blender with 90 ml sterile peptone water. The blender was operated at a high speed for 2 min. Decimal dilutions were prepared and 1 ml from each dilution of muscles was inoculated into duplicate plates, on standard plate count agar. The inoculated plates were incubated at 20°C for 48 h. The total colony count was calculated (Thatcher and Clark, 1975). Fish-flesh samples (50 g) were homogenized in a warning blender with 450 ml sterile peptone water to give 0.1 dilutions for counting the total mould. Serial dilutions to 10^{-6} were then made. One ml of each dilution was poured in a sterile Petri dish and mixed carefully with 15 ml acidified malt extract agar (pH 4.5). The inoculated plates were incubated at 25°C for 5-7 days. Incubated plates were examined daily by stereomicroscope in order to enumerate the characteristic star shaped mould growths (APHA 1992).

6. Statistical analysis

One way and two way analysis of variance (ANOVA) and Duncan's Multiple Range Test (Duncan 1955) were used to determine differences among treatments (mean at a significant level of $P<0.05$). Standard errors were also estimated. Analysis was carried out using the SAS package (SAS 2005).

RESULTS

In the first phase of the experiment, groups 2 and 3 revealed a non significant increase in the hematocrit values without any significant change in the NBT when compared with the control (group 1). A statistically non significant change in total leukocytic counts was seen in groups 2 and 3 when compared with the control group. However, a significant increase in monocytes was evident in both the treatment groups. In the second phase, groups 3 and 4 revealed a non-significant increase in hematocrit values. The NBT value was significantly elevated in groups 4 and 5 when

compared to the control. The total leukocytic count of groups 4 and 5 showed no change despite the significantly elevated monocytes, compared to controls (Table 3).

A trend of increased growth rates among experimental fish (groups 2 and 3) was apparent. . The relative level of protection, after challenge infection, was the highest in group 3 (62.5%) followed by group 2 (50%). At the end of the second phase of the experiment, a non-significantly increased growth rate was observed in groups 4 and 5 (Graph 1). The relative level of protection after challenge was best (44.4%) in group 5 followed by group 4 (33.3%) (Table 4, Graph 2)

Table 3. Hematological and immunological parameters of *Oreochromis niloticus* at the end of the first (1 month) & second phase (2 months) of garlic supplement compared with the control.

Parameters	One month			Two months		
	Control (gp. 1)	Garlic 10g/kg (gp. 2)	Garlic 20g/kg (gp. 3)	Control (gp. 1)	Garlic 10g/kg (gp. 4)	Garlic 20g/kg (gp. 5)
HCV (%)	$30.6^{A}\pm2.09$	$32.1^{A}\pm1.77$	$32.7^{A}\pm1.33$	$28.8^{A}\pm1.03$	$31.7^{A}\pm1.57$	$32.3^{A}\pm1.54$
NBT (mg/ml)	$0.26^{A}\pm0.04$	$0.26^{A}\pm0.02$	$0.26^{A}\pm0.03$	$0.11^{B}\pm0.02$	$0.13^{A}\pm0.03$	$0.13^{A}\pm0.01$
TLC (10^3/µl)	$36.5^{AB}\pm1.45$	$37.7^{A}\pm1.25$	$32.8^{B}\pm1.74$	$41.6^{A}\pm1.09$	$37.4^{B}\pm0.76$	$41.2^{A}\pm0.79$
Neutrophils (10^3/µl)	$11.85^{A}\pm0.15$	$11.63^{A}\pm0.23$	$11.29^{A}\pm0.20$	$12.35^{A}\pm0.14$	$12.06^{A}\pm0.36$	$12.1^{A}\pm0.24$
Lymphocytes (10^3/µl)	$23.4^{AB}\pm1.14$	$24.42^{A}\pm0.93$	$20.21^{B}\pm1.45$	$27.62^{A}\pm0.91$	$23.77^{B}\pm0.61$	$27.13^{A}\pm0.62$
Eosinophils (10^3/µl)	$0.31^{A}\pm0.08$	$0.31^{A}\pm0.08$	$0.18^{A}\pm0.06$	$0.6^{A}\pm0.08$	$0.45^{A}\pm0.08$	$0.49^{A}\pm0.06$
Basophils (10^3/µl)	$0.04^{A}\pm0.04$	$0.15^{A}\pm0.06$	$0.08^{A}\pm0.05$	$0.09^{A}\pm0.06$	$0.19^{A}\pm0.09$	$0.25^{A}\pm0.07$
Monocytes (10^3/µl)	$0.9^{B}\pm0.12$	$1.19^{A}\pm0.18$	$0.97^{A}\pm0.11$	$0.95^{B}\pm0.14$	$1.31^{A}\pm0.09$	$1.19^{A}\pm0.11$

Mean ± S.E. having the same letter in the same row are not significantly different at P<0.05.

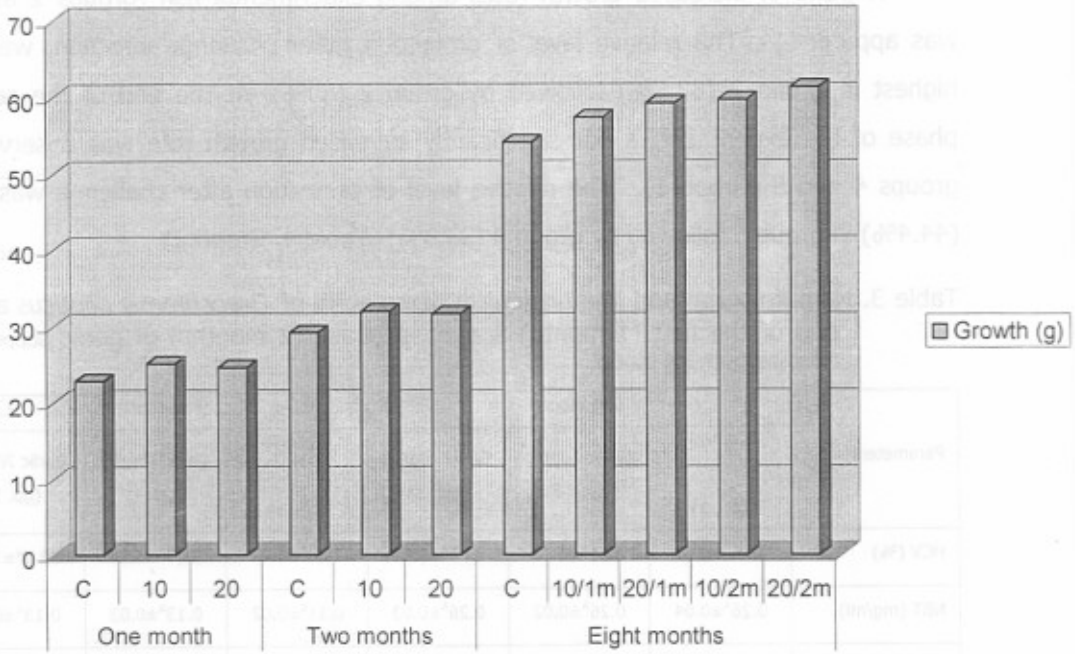
Table 4. Growth and relative level of protection after challenge infection of *Oreochromis niloticus* at the end of the first and second months on garlic-supplemented diet compared with control.

Parameters	One month			Two months		
	Control (gp. 1)	Garlic 10g/kg (gp. 2)	Garlic 20g/kg (gp. 3)	Control (gp. 1)	Garlic 10g/kg (gp. 4)	Garlic 20g/kg (gp. 5)
Growth (g)	$22.81^{A}\pm0.76$	$25.08^{A}\pm2.77$	$24.61^{A}\pm0.93$	$29.12^{A}\pm1.1$	$31.93^{A}\pm1.23$	$31.57^{A}\pm1.26$
RLP* (%)	0	50.0	62.5	0	33.3	44.4

Mean ± S.E. having the same letter in the same row are not significantly different at P<0.05.

RLP= Relative level of protection.*

Graph (1) The body weight gain of Oreochromas niloticus supplemented with garlic (g/kg) after three phases of the study



A significant increase, in survival rate was seen in fish of groups 2 - 5 during the third phase of the experiment. The growth rate was significantly increased in groups 3, 4 and 5. The relative levels of protection, after the challenge infection was 5.3% for groups 2 and 3. It was higher in group 4 (10.53%) and highest in group 5 (15.79 %) (Table 5 and Graph 2).

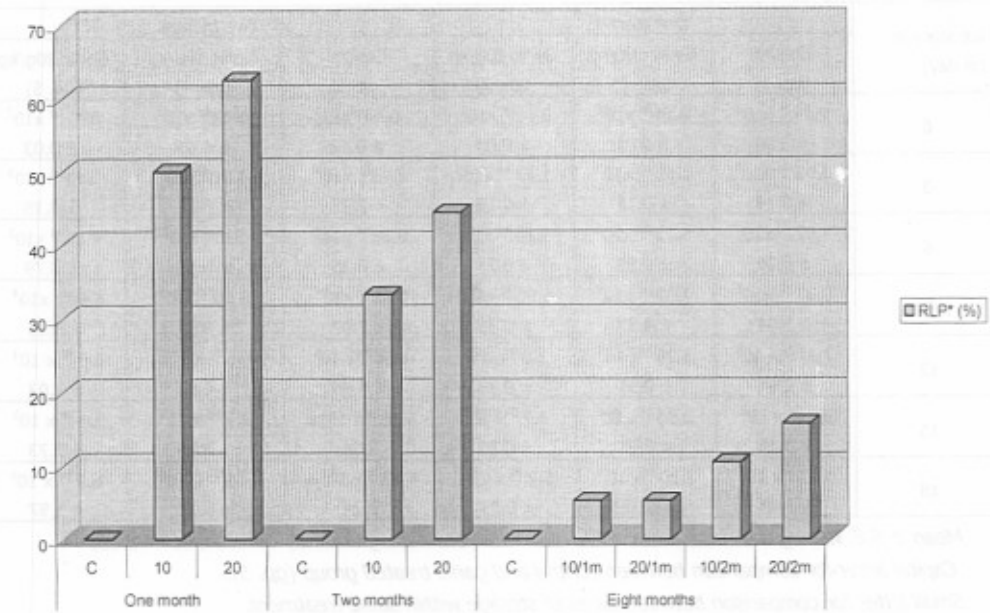
Table 5. Growth and survival rates besides the relative level of protection after challenge of *Oreochromis niloticus* at the end of the third phase (8 months) compared with the control group.

Parameters	One month			Two months		
	Control (gp. 1)	Garlic 10g/kg (gp. 2)	Garlic 20g/kg (gp. 3)	Control (gp. 1)	Garlic 10g/kg (gp. 4)	Garlic 20g/kg (gp. 5)
Growth (g)	$54.1^{B}\pm 1.48$	$57.3^{AB}\pm 0.86$	$59.14^{A}\pm 1.06$	$54.1^{B}\pm 1.48$	$59.6^{A}\pm 1.97$	$61.2^{A}\pm 1.07$
Survival (%)	$68.9^{B}\pm 2.24$	$79.56^{A}\pm 1.47$	$78.31^{A}\pm 1.64$	$68.88^{B}\pm 2.24$	$76.88^{A}\pm 6.05$	$80.13^{A}\pm 3.17$
RLP* (%)	0.00	5.26	5.26	00.00	10.53	15.79

Mean ± S.E. having the same letter in the same row are not significantly different at P<0.05.

RLP= Relative level of protection.*

Graph (2) Relative level of protection in Oreochromas niloticus supplemented with garlic and challenged with Aeromonas hydrophila after three phases of the study



The sensory evaluation of the harvested fish revealed mean odor values of 5, 4.9, 4.5, 4.5 and 4.0 for groups 1, 2, 3, 4 and 5, respectively) and mean taste values of 5.0, 4.8, 4.5, 4.4 and 4.1 for the same groups, respectively (Graph 3). The mean value for the total psychrotroph and mould counts of the harvested fish showed a non-significantly deceased values in the garlic supplemented groups for 1 and 2 month when compared with the control. This effect was maintained till the 9^{th} and 12^{th} day of ice storage, for the psychrotrophs and moulds respectively. A significant decrease in the mean value of the total psychrotroph count was noticed in all the garlic supplemented groups, except group 2 (10 g kg^{-1} for 1 month), from the 12^{th} until the 18^{th} day of ice-storage. A significant decrease in the mean value of the total mould count was noticed in all garlic supplemented groups (2-5) from the 15^{th} until the 18^{th} day of ice-storage, in comparison to controls. The mean value of the total psychrotroph and mould counts, in all groups including the controls, was increased by prolonging the period of the ice-storage. Groups 1 & 2 exceed the permissible limit of the Egyptian standard for the total psychrotroph count after 15 day ice-storage while groups 3, 4 and 5 exceeded that limit on the 18^{th} day of ice-storage (Tables 6 & 7).

Table. 6. Mean value of the total psychrotroph count in the flesh of *Oreochromis niloticus* at the harvest time (end of the third phase = 8 month), compared with the control group.

Ice storage (in day)	One month			Two months		
	Control (gp. 1)	Garlic 10g/kg (gp. 2)	Garlic 20g/kg (gp. 3)	Control (gp. 1)	Garlic 10g/kg (gp. 4)	Garlic 20g/kg (gp. 5)
0	$0.14^{Af} \times 10^2 \pm 0.02$	$0.14^{Af} \times 10^2 \pm 0.01$	$0.11^{Ae} \times 10^2 \pm 0.03$	$0.44^{Af} \times 10^2 \pm 0.12$	$0.12^{Ae} \times 10^2 \pm 0.01$	$0.12^{Ae} \times 10^2 \pm 0.03$
3	$2.53^{Ae} \times 10^3 \pm 0.14$	$1.90^{Ae} \times 10^3 \pm 0.12$	$1.17^{Ad} \times 10^3 \pm 0.18$	$2.73^{Ae} \times 10^3 \pm 2.22$	$2.10^{Ad} \times 10^3 \pm 0.12$	$2.17^{Ad} \times 10^3 \pm 0.18$
6	$7.87^{Ae} \times 10^4 \pm 0.20$	$5.72^{Ae} \times 10^3 \pm 0.55$	$5.20^{Ad} \times 10^3 \pm 0.75$	$6.89^{Ad} \times 10^4 \pm 0.25$	$5.6^{Bd} \times 10^3 \pm 0.55$	$4.31^{Bd} \times 10^3 \pm 0.79$
9	$8.33^{Ad} \times 10^4 \pm 2.44$	$7.00^{Ad} \times 10^4 \pm 4.57$	$6.00^{Ac} \times 10^4 \pm 2.35$	$9.13^{Bd} \times 10^4 \pm 2.07$	$8.80^{Bc} \times 10^4 \pm 2.57$	$8.60^{Bc} \times 10^4 \pm 3.35$
12	$7.31^{Ac} \times 10^5 \pm 0.64$	$5.20^{Ac} \times 10^5 \pm 0.55$	$3.0^{Bc} \times 10^4 \pm 0.33$	$8.30^{Ac} \times 10^5 \pm 0.64$	$6.6^{Bc} \times 10^4 \pm 0.52$	$6.0^{Bc} \times 10^4 \pm 1.03$
15	$5.6^{Ab} \times 10^6 \pm 3.14$	$2.90^{Ab} \times 10^6 \pm 0.01$	$4.7^{Bb} \times 10^5 \pm 0.28$	$6.16^{Ab} \times 10^6 \pm 3.54$	$8.2^{Bb} \times 10^5 \pm 1.19$	$7.4^{Bb} \times 10^5 \pm 1.73$
18	$4.23^{Aa} \times 10^7 \pm 3.44$	$2.70^{Aa} \times 10^7 \pm 0.12$	$5.2^{Ba} \times 10^6 \pm 0.39$	$6.63^{Aa} \times 10^7 \pm 2.24$	$6.1^{Ba} \times 10^6 \pm 0.72$	$6.2^{Ba} \times 10^6 \pm 1.97$

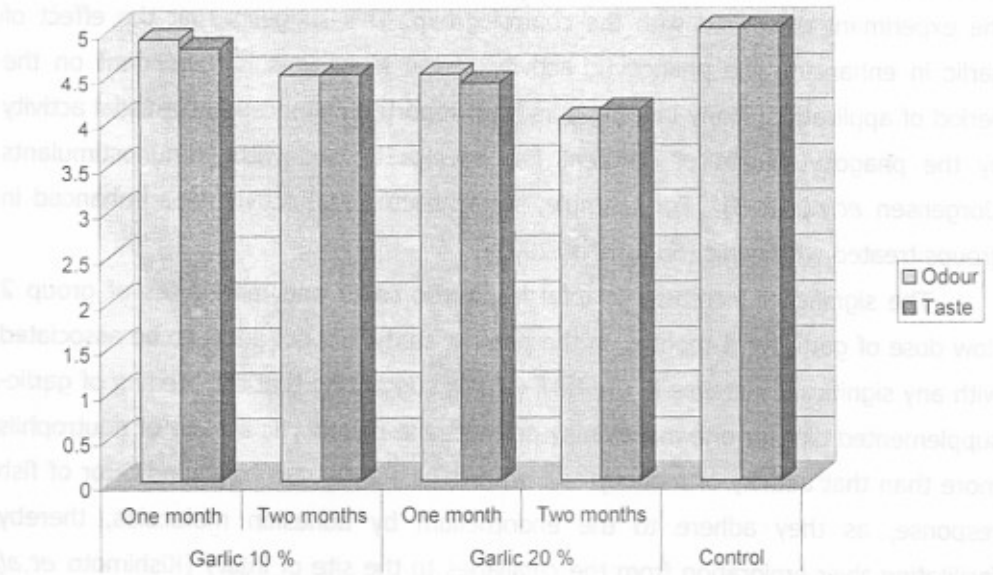
Mean ± S.E. having the same letter in the same row are not significantly different at P<0.05.
Capital letter for comparison between control and garlic treated group (gp. 5).
Small letter for comparison between times of storage within same treatment.

Table 7. Mean value of the total mould count in the flesh of *Oreochromis niloticus* at the harvest time (end of the third phase = 8 months), compared with the control group.

Ice storage in day	One month			Two months		
	Control (gp. 1)	Garlic 10g/kg (gp. 2)	Garlic 20g/kg (gp. 3)	Control (gp. 1)	Garlic 10g/kg (gp. 4)	Garlic 20g/kg (gp. 5)
0	$2.2^{Ae} \times 10^1 \pm 0.01$	$2.0^{Ad} \times 10^1 \pm 0.51$	$1.8^{Ad} \times 10^1 \pm 0.75$	$2.5^{Ae} \times 10^1 \pm 0.22$	$1.6^{Ad} \times 10^1 \pm 0.01$	$2.1^{Ad} \times 10^1 \pm 0.05$
3	$4.0^{Ad} \times 10^2 \pm 0.17$	$3.6^{Ac} \times 10^2 \pm 0.42$	$2.8^{Ac} \times 10^2 \pm 1.21$	$2.3^{Ad} \times 10^2 \pm 0.43$	$2.8^{Ac} \times 10^2 \pm 0.17$	$2.5^{Ac} \times 10^2 \pm 0.06$
6	$6.6^{Ad} \times 10^2 \pm 0.25$	$6.4^{Bc} \times 10^2 \pm 0.21$	$6.2^{Bc} \times 10^2 \pm 0.79$	$6.3^{Ad} \times 10^2 \pm 0.13$	$5.6^{Bc} \times 10^2 \pm 0.23$	$5.8^{Bc} \times 10^2 \pm 0.81$
9	$5.0^{Ac} \times 10^3 \pm 0.74$	$4.2^{Ab} \times 10^3 \pm 0.23$	$3.5^{Ab} \times 10^3 \pm 1.45$	$6.1^{Ac} \times 10^3 \pm 0.36$	$3.7^{Ab} \times 10^3 \pm 2.61$	$3.0^{Ab} \times 10^3 \pm 6.37$
12	$6.6^{Ac} \times 10^3 \pm 0.55$	$5.4^{Bb} \times 10^3 \pm 0.37$	$4.2^{Bb} \times 10^3 \pm 1.57$	$5.9^{Ac} \times 10^3 \pm 0.22$	$4.6^{Ab} \times 10^3 \pm 0.42$	$4.1^{Ab} \times 10^3 \pm 1.07$
15	$3.2^{Ab} \times 10^4 \pm 0.54$	$7.2^{Bb} \times 10^3 \pm 0.54$	$5.7^{Bb} \times 10^3 \pm 0.87$	$5.4^{Ab} \times 10^4 \pm 0.12$	$6.6^{Bb} \times 10^3 \pm 2.12$	$5.2^{Bb} \times 10^3 \pm 0.69$
18	$2.5^{Aa} \times 10^5 \pm 0.34$	$6.6^{Ba} \times 10^4 \pm 0.37$	$4.6^{Ba} \times 10^4 \pm 1.28$	$7.5^{Aa} \times 10^5 \pm 0.17$	$4.8^{Ba} \times 10^4 \pm 0.76$	$4.4^{Ba} \times 10^4 \pm 0.95$

Mean ± S.E. having the same letter in the same row are not significantly different at P<0.05.
Capital letter for comparison between control and garlic supplemented group .
Small letter for comparison between times of storage within same treatment.

Graph (3) Sensory evaluation in the flesh of Oreochromis niloticus supplemented with garlic at end of the third phase of the study



DISCUSSION

The hematocrit values of the experimental fish showed a non significant increase during the first and second phases of the experiment, which support the contention that the garlic doses tested were both safe and efficacious. Sahu (2004) also observed an increase in erythrocytic count after administering garlic. A marked increase in the total leukocytic count was seen in group 2.and was associated with a high number of monocytes and lymphocytes during the first phase. Iranloye (2002) also reported increases in total white blood cell count, neutrophils, lymphocytes and monocytes following 30-day of feeding garlic, illustrating the anti-infection properties of garlic. Fish sampled during the second phase in the present study showed a non significant change in TLC as well as significant increases in monocytes. This suggests that the feeding of garlic-supplemented diet for 2 months may be less effective in improving the TLC but may improve the monocytes. Hematological findings showed that small doses of garlic (10 g kg^{-1}) administered for one month were sufficient to induce the most promising results. Heo *et al,* (2000) reported that the only changes in fish blood profiles, observed when l-carnitine was included in diet, were increased concentrations of WBC and lymphocytes, although there is no direct evidence from the literature on the effect of l-carnitine supplementation on immune-related blood cell counts. However, many previous reports have suggested that l-carnitine supplement may influence lipid metabolism. Dietary lipid may affect a great number of immune parameters, such as lymphocyte proliferation, cytokine synthesis, natural killer cell activity and phagocytosis (De Pablo and De Cienfuegos 2000).

The NBT test is used to determine phagocyte activity as an indicator of immune response, especially neutrophils and monocytes (Jabs et al, 1980). A significant increase in NBT values was detected in fish during the second phases of the experiment compared with the control group. This suggests that the effect of garlic in enhancing the phagocytic activity of the leukocytes is dependent on the period of application. Many investigators have reported enhanced bactericidal activity by the phagocytic cells of different fish species treated with immunostimulants (Jorgensen *et al*, 1993). For example, serum bactericidal activity was enhanced in groups treated with garlic (Sahu *et al*, 2007).

The significant increases in total leukocytic count and monocytes of group 2 (low dose of garlic for 1 month), in the present study, did not seem to be associated with any significant increase in the NBT values, suggesting that the feeding of garlic-supplemented diet for one month may promote the phagocytic activity of neutrophils more than that activity of monocytes. Neutrophil activity can be an indicator of fish response, as they adhere to the endothelium by adhesion molecules, thereby facilitating their emigration from the capillaries to the site of injury (Kishimoto *et al*, 1989; Magnuson et al, 1989). Neutrophils also exhibit increased production of oxygen radicals which are potentially capable of destroying invading pathogens (Hassett and Cohen 1989).

While no significant increase in total leukocytic count was seen in groups 4 and 5, the monocyte numbers significantly increased. The latter seem to be associated with the significant increase in NBT values, suggesting that the application of garlic for two months may be more effective on the phagocytic activity of the monocytes. However, other studies claim that the protective effect of garlic may be associated with its antioxidant properties (Pedraza-Chaverri *et al*, 2000; Rahman 2003).

Several herbs have been tested for their growth-promoting activities in aquatic animals (Jayaprakas and Eupharsia 1996; Citarasu *et al*, 2002; Sivaram *et al*, 2004). Our observations showed non-significant increases in growth rates after one or two months of feeding with garlic. Horton *et al*, (1991) reported no effects of feeding 1 or 10 g garlic kg^{-1} diet on the growth performance of pigs. Other studies have shown that garlic did not affect growth performance of broilers (Freitas *et al*, 2001) or growing lambs (Bampidis *et al*, 2005). The pungent smell of garlic, may lead to lower diet palatability. However, in the present study a marked increase in growth rate was noticed after the third phase (8 months) in groups 2-5. The increase, after 8 months, was significant in groups 3, 4 and 5. Cullen *et al*, (2005) also found improved feed conversion rates when garlic was added to the diet for grower-finisher pigs at the level of 1 or 10 g kg^{-1} diet. Khalil *et al*, (2001) mentioned that garlic contains allicin, which promotes the performance of the intestinal flora, thereby improving digestion, and enhancing the utilization of energy, leading to improved growth.

Reduced mortalities following pathogenic challenges in the presence of a low dose of herbal principals, have been reported by Kim *et al*, (2001) and Jain and Wu (2003). The relative level of protection against challenge infection was fairly good during both phases 1 & 2 of the present study. However the response during the first phase (groups 2 & 3) was better than that of the second phase (groups 4 & 5). Immunostimulants can increase non-specific immunity by either increasing the number of phagocytes or activating phagocytosis (Shoemaker *et al,* 1997), as seen in our study. Many defense mechanisms activated by garlic counteract the challenge infection including the production of superoxide anions against the *A. hydrophila* infection. It has been found that the aqueous extract of raw garlic and dried powder scavenge hydroxyl radicals (Yang *et al,* 1993; Kim *et al*, 2001), and superoxide anions (Kim *at al.*, 2001). Similar phagocytic activities may have occurred in the present work and enhanced protection against the challenge infection. Gildberg and Mikkelsen (1998) fed Atlantic cod (*Gadus morhua*) fry on a commercial feed, supplemented with *Carnobacterium divergens* alone or combined with immuno-stimulating peptides for 3 weeks, then challenged with *V. anguillarum* (10^7 ml^{-1}, for 1 h). The fry showed reduced mortality. In the present study the relative level of protection of fish, challenged after 8 month of experiment (third phase), was better than that of the control group, but lower than that of the first and second phase.

Garlic has been used for centuries in many societies against parasitic, fungal, bacterial and viral infections. The recent chemical characterization of their sulphur compounds has promoted claims that such compounds are the main active antimicrobial agents (Rose *et al*, 2005). However, the level of protection, during the 3^{rd} phase, was higher in groups 4 and 5 than groups 2 and 3. Moreover, the high garlic dose afforded greater protection than the low dose. These findings can be used to explain the low levels of protection seen during the third phase (8 months), when compared with the first and second phases in the current experiment. Feeding of the garlic-supplemented diet for one month was effective against the immediate challenge infection while feeding for 2 months proved more effective against the later challenge (8 months).

The survival rate was significantly greater in all garlic-supplemented groups when compared with the control group at the end of the experiment (8 months). However, it was significantly higher in group 5 than the other groups. Although the use of garlic resulted in good survival rates, feeding the higher doses of garlic for extended periods gave better results. Using a combination of five herbs developed an Artemia-enriched herbal diet for *Penaeus monodon*, which significantly increased survival rate during stress conditions (Citarasu *et al*, 2002). The current results showed the stimulatory effect of garlic on the immune system that correlates with improved fry survival. The improved survival rate may be due to the enhanced immune response resulting from increased numbers of monocytes (groups 2-5),

increases in phagocytic activity (groups 4-5), or other defense mechanisms. Immunostimulants can also increase serum lysozyme activity by increasing the number of phagocyte-secreting lysozyme, or by increasing the amount of lysozyme synthesized per cell (Engstad *at al.*, 1992).

Many studies on garlic product have been published; however, there are very few reports on its effects on flesh quality. Kwon *et al*, (2005) reported that garlic improved the meat quality of growing-finishing pigs. Sensory evaluation is used as an indicator for sea-food quality and consumer acceptability. Both the odor and taste of groups 2-5 were within acceptable limits when compared with the control. Groups 3-5 achieved higher evaluation grades than groups 1 and 2. Total psychrotroph and mould counts increased in all groups (1-5) with increasing periods of storage. However, the total psychrotroph count was still within the accepted level of the Egyptian Standard (2000), when the fish were stored on ice until day 12 for groups 1 & 2 and day 15 for groups 3, 4 and 5. Garlic is known to have medicinal properties and is useful in combating bacteria (Ress *et al*, 1993) and fungi (Adetumbi *at al.*, 1986). Garlic contained 1-3% alliin which converted to allicin by the action of allinase (10 mg/g garlic cloves) (Ellmore and Feldberg 1994). It is also known that, 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 (Miron *et al*, 2004), is the bioactive substance. However, several reports suggest that garlic also has a lipid-lowering action. On the other hand, Yeh and Liu (2001) suggested that garlic inhibits the synthesis of cholesterol and fatty acids in the liver; however, the exact mechanisms are not well understood. Therefore, further studies should be done to establish the relationship between the dose used, period of application and time of withdrawal to obtain the best evaluation for the fish quality and shelf-life.

CONCLUSION

It can be concluded that garlic improves the immune response of *O. niloticus* through a rapid increase in monocytes and over a longer time frame enhanced phagocytic activity which affords increased protection against immediate challenge infection. High doses also appear to enhance growth rate, particularly after 8 months, dietary inclusion. A significant improvement was seen in the survival of all treatment. It seems beneficial to use garlic at lower doses for one month, in short-

term operations, such as in the hatchery, and for two months to improve aquaculture production. Post-harvest flesh quality and shelf-life of fish fed on garlic supplemented diets for both 1 month or low dose for 2 month was improved. More extensive field trials and economic studies are, however, necessary before large scale, commercial use could be recommended.

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REFERENCES

1. Adetumbi M., G. T. Javor and B. H. Lau. 1986. *Allium sativum* (garlic) inhibits lipid synthesis by *Candida albicans*. Antimicrobial Agents and Chemotherapy, 30: 499–501.
2. Amagase H. and J. A. Milner. 1993. Impact of various sources of garlic and their constituents on 7, 12-dimethylbenz[a]anthracene binding to mammary cell DNA. Carcinogenesis, 14: 1627–1631.
3. APHA (American Public Health Association). 1992. Compendium of Methods for the Microbiology Examination of Food. 3rd Ed., Edward Brothers, Washington, USA.
4. Augusti K. T. 1977. Hypocholesterolaemic effect of garlic, Allium sativum, Linn. Indian J. Exp. Biol., 15, 489–490.
5. Bampidis V. A., V. Christodoulou, E. Christaki, P. Florou-Paneri, A. B. Spais. 2005. Effect of dietary garlic bulb and garlic husk supplementation on performance and carcass characteristics of growing lambs. Anim. Feed Sci. Technol., 121, 273–283.
6. Block E. 1985. The chemistry of garlic and onion. Sci. Am., 252: 114–119.
7. Block, E. 1992. The organ sulfur chemistry of the genus Allium implications for the organic chemistry of sulfur. Angew. Chem. Int. Ed., 31, 1135–1178.
8. Bordia A., H. C. Bansal, S. K. Arora and S. V. Singh. 1975. Effect of essential oils of garlic and onion on alimentary hyperlipemia. Atherosclerosis, 21, 15–19.
9. Citarasu T., M. M. Babu, R. Raja Jeya Sekar and M. P. Marian. 2002. Developing Artemia enriched herbal diet for producing quality larvae in *Penaeus monodon* Fabricius. Asian Fish Sci., 15, 21–32.
10. Corzo-Martinez M., N. Corzo and Mar Villamiel. 2007. Biological properties of onions and garlic, Trends in Food Science & Technology, 18, 609-625.

11. Cullen S.P., F. J. Monahan, J. J. Callan and J. V. O'doherty. 2005. The effect of dietary garlic and rosemary on grower-finisher pig performance and sensory characteristics of pork. Ir. J. Agric. Food Res., 44, 57–67.
12. Dalmo R. A. and R. Seljelid. 1995. The immunomodulatory effect of LPS, laminaran and sulphated laminaran [b (1, 3)-D-glucan] on Atlantic salmon, *Salmo salar* L., macrophages in vitro. J. Fish Dis., 18, 175–185.
13. De Pablo M. A. and G. A. De Cienfuegos. 2000. Modulatory effects of dietary lipids on immune system functions. Immunol. Cell Biol., 78, 31.
14. Duncan, B. 1955. Multiple Range and Multiple (F) test". Biometrics, 11: 1- 2.
15. Egyptian Standards. 2000. Egyptian Standard for Chilled Fish. Ministry of Industry Technology Improvement. ES, 3494.
16. Ellmore, G. S. and R. S. Feldberg. 1994. Alliin lyase localization in bundle sheaths of garlic clove (*Allium sativum*). American J. Bat. 81, 89 – 94.
17. Engstad, R. E., B. Robertson and E. Frivold. 1992. Yeast glucan induces increase in activity of lysozyme and complement mediated haemolytic activity in Atlantic salmon blood. Fish Shellfish Immunol., 2, 287–297.
18. FAO/WHO/OIE, 2006. Expert Consultation on antimicrobial use in aquaculture and antimicrobial resistance. Seoul, Republic of South Korea, June 13 – 16.
19. Freitas, R., J. B. Fonseca, R. T. Soares, H. S. Rostagn and P. R. Soares. 2001. Utilization of garlic (*Allium sativum* L.) as growth promoter of broilers. Rev. Bras. Zootec., 30, 761–765.
20. Fromthing, R. A. and G. S. Bulmer. 1978. In vitro effect of aqueous extract of garlic (*Allium sativum*) on the growth and viability of Cryptococcus neoformans. Mycologia, 70, 397– 405.
21. Gildberg, A. and H. Mikkelsen. 1998. Effects of supplementing the feed to Atlantic cod (*Gadus morhua*) fry with lactic acid bacteria and immuno-stimulating peptides during a challenge trial with *Vibrio anguillarum*. Aquaculture, 167(1-2): 103-113.
22. Han, J., L. Lawson, G. Han and P. Han. 1995. A spectrophotometric method for quantitative determination on allicin and total garlic thiosulfnates. Anal. Biochem., 225, 157–160.
23. Hassett, D. J. and M. S. Cohen, 1989. Bacterial adoption to oxidative stress: implications of pathogenesis and interaction with phagocytic cells. Fed. Am. Soc. Exp. Biol., 3, 1574–1581.
24. Heo, K., J. Odle, I. K. Han, W. Cho, S. Seo, E. van Heugton and D. H. Pilington. 2000. Dietary l-carnitine improves nitrogen utilization in growing pigs fed low energy, fat-containing diets. J. Nutr., 130, 1809–1814.
25. Horton G. M. J., D. B. Blethen and B. M.Prasad. 1991. The effect of garlic (*Allium sativum*) on feed palatability of horses and feed consumption, selected

performance, and blood parameters in sheep and swine. Can. J. Anim. Sci., 71, 607–610.

26. Iranloye, B. O. 2002. Effect of chronic garlic feeding on some haematological parameters. Afr. J. Biomed. Res., 5, 81–82.
27. Jabs, D., M. Regan, M. Horita, M. Yokoyama and C. Tseng. 1980. Assaying of human neutrophil function. Laboratory Management, 18: 37-41.
28. Jain, J. and Z. Wu. 2003. Effect of traditional Chinese medicine on nonspecific immunity and disease resistance of large yellow croaker *Psedcosciaena crocea* (Richardson). Aquaculture 218, 1–9.
29. Jayaprakas, V. and J. Eupharsia. 1996. Growth performance of Labeo rohita (Ham.) Livol (IHF-1000), an herbal product. Proc. Indian Natl. Sci. Acad, 63, 1–10.
30. Jorgensen, J. B., G. J. E. Sharp, C. J. Secombes and B. Robertsen. 1993. Effect of a yeast cell wall glucan on the bactericidal activity of rainbow trout macrophages. Fish & Shellfish Immunol., 3, 267–277.
31. Khalil, R. H., B. M. Nadia and M. K. Soliman. 2001. Effects of Biogen and Levamisol Hcl on the immune response of cultured *Oreochromis niloticus* to *Aeromonas hydrophila* vaccine. Beni-Suef Vet. Med. J., Egypt, XI (2): 381-392.
32. Kim, K. M., S. B. Chun, M. S. Koo, W. J. Choi, T. W. Kim, Y. G. Kwon, H. T. Chung, T. R. Billiar and Y. M. Kim. 2001. Differential regulation of NO availability from macrophages and endothelial cells by the garlic component S-allyl cysteine. Free Radic. Biol. Med., 30, 747–756.
33. Kishimoto, T. K., M. A. Jutila, E. L. Berg and E. C. Butcher. 1989. Neutrophil MAC-1 and MEL-14 adhesion proteins inversely regulated by chemotactic factors. Science, 245, 1238–1241.
34. Konjufca, V. H., G. M. Pesti and R. I. Bakalli. 1997. Modulation of cholesterol levels in broiler meat by dietary garlic and copper. Poultry Science, 76: 1264-1271.
35. Kumar. M. and J. S. Berwal. 1998. Sensitivity of food pathogens to garlic (*Allium sativum L.*). J. Appl. Microbiol., 84, 213–215.
36. Kwon. O. S., J. H. Cho, B. J. Min, H. J. Kim, Y. G. Chen, J. S. Yoo, I. H. Kim, J. C. La and H. K. Park. 2005. Effect of supplemental medicinal plants (*Artemisia, Acanthopanax* and Garlic) on growth performance, IGF-1 and meat quality characteristics in growing-finishing pigs. Kor. J. Food Sci. Ani. Resour., 25, 316–321.
37. Kyo. E., N. Uda, A. Suzuki, M. Kakimoto, M. Ushijima, S. Kasuga and Y. Itakura. 1998. Immunomodulation and antitumor activities of aged garlic extract. Phytomedicine, 5, 259–267.
38. Magnuson. D. K., A. Weintraub, T. H. Pohlman and R. V. Maier. 1989. Human endothelial cell adhesiveness for neutrophils, induced by Escherichia coli

lipopolysaccharide in vivo, is inhibited by Bacteroides fragilis lipopolysaccharide. J. Immunol., 143, 3024–3033.

39. Miron, T., Bercovici T., Rabinkov A., Wilchek M. and Mirelman D., 2004. Allicin: preparation and applications. Anal. Biochem. 331:364–369.
40. Murray C. K. and T. C. Shewan. 1979. The microbial spoilage of fish with special reference to the role of psychrotrophs. In: Russel A.D. & Fuller R. (eds) cold tolerance microbes in spoilage and environment. Academic Press, 117-136.
41. Pedraza-Chaverri J., P. D. Maldonada, O. N. Medina-Campos, I. M. Olivares-Corichi, M. A. Granados-Silvestre, R. Hernandez- Pando and M. E. Ibarra-Rubio. 2000. Garlic ameliorates gentamicin nephrotoxicity: relation to antioxidant enzymes. Free Radic. Biol. Med., 29, 602–611.
42. Raa, J. G., G. Rorstad, R. Engstad and B. Robertson. 1992. The use of immunostimulants to increase resistance of aquatic organisms to microbial infections. In: Diseases in Asian Aquaculture. M. Shari., R. P. Subasinghe, and J. R. Arthur (Eds). Fish Health Section, Asian Fisheries Society, Manila, Phillippines, 26–29 November 1990, vol. 1, pp. 39–50.
43. Raa, J. 1996. The use of immunostimulatory substances in fish and shellfish farming. Rev. Fish Sci., 4, 229–288.
44. Rahman K. 2003. Garlic and aging: A new insights into an old remedy. Ageing Res. Rev., 2, 39–56.
45. Ress, L. P., S. F. Minney, N. J. Plummer, J. H. Slatter and D. A. Skyrme. 1993. A quantitative assessment of the antimicrobial activity of garlic (*Allium sativum*). World Journal of Microbiology and Biotechnology, 9: 303 – 307.
46. Rose, P., M. Whiteman, P. K. Moore and Y. Z. Zhu. 2005. Bioactive Salk (en) yl cysteine sulfoxide metabolites in the genus Allium: the chemistry of potential therapeutic agents. Natural Product Reports, 22, 351-368.
47. Ruangroupan, L., T. Kitao and T. Yoshida. 1986. Protective efficacy of *Aeromonas hydrophila* vaccines in Nile tilapia. Veterinary Immunology and Immunopathology, 12 (1-4): 345-350.
48. Sahu, S. 2004. Antibacterial activity of plant extracts on .fish microbial pathogens. MSc. Diss., CIFA, Kausalyaganga, Bhubaneswar, 237 pp.
49. Sahu, S., B. K. Das, B. K. Mishra, J. Pradhan and N. Sarangi. 2007. Effect of *Allium sativum* on the immunity and survival of Labeo rohita infected with *Aeromonas hydrophila*. J. Appl. Ichthyol., 23:80–86.
50. SAS, 2005. Statistical Analysis System. User`s Guide: SAS Institute Cary, North Carolina.
51. Shewan J. M., R. G. Mac Intosh, C. G. Tucker and A. S. Ehrenberg. 1953. The development of a numerical scoring system for the sensory assessment of the spoilage of wet white fish stored in ice. J Sci.: Food Agriculture, 4: 283-298.

52. Shoemaker, C. A., P. H. Klesius and J. A. Plumb. 1997. Killing of Edwardsiella ictaluri by macrophages from channel catfish immune and susceptible to enteric septicemia of catfish. Vet. Immunol. Immunopathol. 58, 181–190.
53. Sivam, G. P. 2001. Recent advances on the nutritional effects associated with the use of garlic as supplement. Am. Soc. Nutr. Sci., 1106 -8.
54. Sivaram, V., M. M. Babu, G. Immanuel, S. Murugadass, T. Citarasu and M. P. Marian. 2004. Growth and immune response of juvenile greasy groupers (Epinephelus tauvina) fed with herbal antibacterial active principle supplemented diets against Vibrio harveyi infections. Aquaculture, 237, 9–20.
55. Siwicki, A. K., M. Studnicka and B. Ryka. 1985. Phagocytic ability of neutrophils in carp. Bamidgeh, 37: 123-128.
56. Stoskoph, M. 1993. Fish Medicine.W.B. Saunders Company.
57. Subasinghe, R. 1997. Fish health and quarantine, p. 45–49. *In* A review of the State of the World Aquaculture. FAO Fisheries circular no. 886. Food and Agriculture Organization of the United Nations, Rome, Italy.
58. Suetsuna, K. 1998. Isolation and characterization of angiotensin Iconverting enzyme inhibitor dipeptides derived from Allium sativum (garlic). J. Nutr. Biochem., 9, 415–419.
59. Sumiyoshi, H. 1997. New pharmacological activities of garlic and its constituents (Review). Folia Pharmacological Japonica 110 Suppl, 1, 93 – 97.
60. Thatcher, F. and D. Clark. 1975. ICMSF, Microorganisms in Food. Academic Press, New York.
61. Wang, B. H., K. A. Zuel, K. Rahaman and D. Billington. 1998. Protective effects of aged garlic extract against bromobenzene toxicity to precision cut rat liver slices. Toxicology, 126: 213–222.
62. Witte, W., I. Klare and G. Werner. 1999. Selective pressure by antibiotics as feed additives. Infection, 27 (Suppl. 2):35–38.
63. Yang G. C., M. P. Yasaei and S. W. Page. 1993. Garlic as anti-oxidant and free radical scavenger. J. Food Drug Anal., 1: 357–364.
64. Yeh, Y. Y. and L. Liu. 2001. Cholesterol-lowering effect of garlic extracts and organosulfur compounds: human and animal studies. J. Nutr., 131, 989S–993S.
65. Yunxia, Q., S. Jianzhong and W. Guoliang. 2001. A review of principal bacterial diseases of mariculture fish. Transactions of Oceanology and Limnology, 2: 78-87.

تأثير الثوم على إعاشة ونمو ومقاومة وجودة أسماك البلطي النيلي

صلاح الدين مصيلحى على ، نشوه محمود عبدالعاطى ، محمد فتحي محمد

١. *المركز الدولي للأسماك- مركز تدريبي وبحثي لأفريقيا وغرب آسيا- العباسة شرقية- جمهورية مصر العربية*
٢. *قسم المراقبة الصحية على الأغذية -معهد بحوث صحة الحيوان – معمل فرعى الإسماعيلية- جمهورية مصر العربية*

تم تقسيم ألف وستمائة من أسماك البلطي النيلي إلى خمسة مجموعات متساوية كل مجموعة أربعة تكرارات متشابهة وذلك لتقييم كفاءة التغذية المدعمة بالثوم (١٠ ، ٢٠جم/كجم علف) على إنتاج البلطي النيلي. حيث كانت المجموعة الأولى ضابطة (غذيت على عليقه متوازنة) والمجموعة الثانية والثالثة غذيت على عليقه مضاف إليها الثوم (١٠ ، ٢٠ جرام/كجم عليقه) لمدة شهر على التوالي والمجموعتان الرابعة والخامسة تغذت على نفس العليقه المضاف عليها الثوم لمدة شهرين على التوالي ، وبعد تلك الفترات غذيت جميع المجموعات على عليقه متوازنة بعد فترة إضافة الثوم وحتى الشهر الثامن من التجربة ، وقد تم تسجيل وزن الجسم وبعض عناصر الدم ، كما تم إجراء عدوى تجريبية باستخدام ميكروب الايروموناس هيدروفيللا الممرض في نهاية كل إضافة للثوم (شهر وشهرين) وفي نهاية التجربة (ثمانية أشهر)، كما تم إجراء بعض الدراسات على جودة الأسماك المجربة وفترة صلاحيتها. وقد لوحظ زيادة غير معنوية في نسبة خلايا الدم للبلازما بكل المجموعات المعاملة بالثوم ، كما لوحظ زيادة معنوية في العد الكلى لكرات الدم البيضاء بالمجموعة (٢) ، و لوحظ ارتفاع معنوي لقيمة النيتروبلو تنرازلزليم بعد شهرين من المعاملة وخلايا المونوسيت في جميع المعاملات ، ولوحظ زيادة غير معنوية في وزن الجسم للمجموعات من ٢ – ٥ بعد شهر وشهرين من المعاملة بالثوم ولكن لوحظ ارتفاع معنوي في الوزن عند الشهر الثامن لجميع المعاملات ما عدا المجموعة (٢) . وكانت نسبة الإعاشة مرتفعة في جميع المجموعات المعاملة بالثوم بالمقارنة بالمجموعة الضابطة. وكان مستوى الحماية النسبي ضد العدوى التجريبية لجميع المجموعات المعاملة بالثوم على الفترات المختلفة أعلى من المجموعة الضابطة، ولوحظ أن جودة الأسماك وفترة صلاحيتها في جميع المجموعات المعاملة بالثوم أفضل من نظيرتها في المجموعة الضابطة. وقد خلصت التجربة أن جرعتي الثوم لها تأثير مماثل في تنشيط الجهاز المناعي والحالة الصحية ، وعليه يعتبر الثوم محفزاً لإنتاج الأسماك ونموها كما أن له تأثير إيجابي على جودة الأسماك وفترة صلاحيتها ، ومع ذلك يوصى بعمل اختبارات أخرى عديدة تشمل تحليلاً للمردود التجاري والتكلفة قبل إعطاء التوصية باستخدام الثوم في مجال الاستزراع السمكي.