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EFFECTS OF GARLIC (*Allium sativum*) AND CHLORAMPHENICOL ON GROWTH PERFORMANCE, PHYSIOLOGICAL PARAMETERS AND SURVIVAL OF NILE TILAPIA *(Oreochromis niloticus*)

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ABSTRACT: We studied and compared the effects of chloramphenicol antibiotic and garlic (*Allium sativum*), used as immunostimulants and growth promoters, on some physiological parameters, growth performance, survival rate, and bacteriological characteristics of Nile tilapia (*Oreochromis niloticus*). Fish (7±1g/fish) were assigned to eight treatments, with three replicates each. Treatment groups had a different level of *Allium sativum* (10, 20, 30, and 40g/kg diet) and chloramphenicol (15, 30, and 45mg/kg diet) added to their diets; the control group diet was free from garlic and antibiotic. Diets also contained 32% crude protein (CP) and were administered at a rate of 3% live body weight twice daily for 90 days. Results showed that the final weight and specific growth rate (SGR) of *O. niloticus* increased significantly with increasing levels of *Allium sativum* and chloramphenicol. The highest growth performance was verified with 30g *Allium sativum* / kg diet and 30mg chloramphenicol / kg diet. The lowest feed conversion ratio (FCR) was observed with 30g *Allium sativum* / kg diet and 30mg chloramphenicol / kg diet. There were significant differences in the protein efficiency ratio (PER) with all treatments, except with 45mg chloramphenicol / kg diet. No changes in the hepatosomatic index and survival rate were observed. Crude protein content in whole fish increased significantly in the group fed on 30g *Allium sativum* / kg diet, while total lipids

decreased significantly in the same group. Ash of whole fish showed significantly high values with 30g *Allium sativum* and 15mg chloramphenicol / kg diet while the lowest value was observed in the control group. Blood parameters, erythrocyte count (RBC), and hemoglobin content in fish fed on diets containing 40g *Allium sativum* and all levels of chloramphenicol were significantly higher than in control. Significantly higher hematocrit values were seen with 30 and 45mg chloramphenicol / kg diet. There were no significant differences in the mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). Plasma glucose decreased significantly with increasing levels of *Allium sativum* but increased significantly with increasing levels of chloramphenicol. Total lipids were significantly reduced with diets containing 40g *Allium sativum* and 30mg chloramphenicol / kg diet, while total plasma protein content was significantly higher in fish fed on diets containing 10, 20, and 30g *Allium sativum*, and 30 and 45mg chloramphenicol / kg diet. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in plasma decreased significantly with increasing levels of *Allium sativum* and chloramphenicol. All *Allium sativum* and chloramphenicol levels decreased total bacteria and coliforms in water, muscles and intestine when compared to the control group. Treated groups had lower mortality rate than the control group during the challenge test. In conclusion, it can be suggested that adding 3% *Allium sativum* to fish diet can promote growth, reduce total bacteria, and improve fish health.

KEY WORDS: *Allium sativum*, chloramphenicol, Nile tilapia, growth parameter, hematology, biochemistry, *Aeromonas hydrophila*.

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INTRODUCTION

Recently, the use of immunostimulants in fish farming has become popular for enhancing the activity of non-specific defense systems and conferring protection against diseases. Many antibiotics are commonly used to promote growth and health in carp, trout, and Nile tilapia (22). Also, artificial feeds supplemented with antibiotics were used to prevent the spread of diseases and improve FCR (54). The use of antibiotics and other chemotherapeutics has several shortcomings including the risk of resistant pathogens, the problem of drug residue in treated fish, and the impact on environmental pollution. Therefore, using immunostimulants seems to be an attractive alternative to control fish diseases (52, 59). *Allium sativum* was used as a growth promoter in *O. niloticus* (18), in which it increased body gain, feed intake, and feed efficiency (1). It was shown to have broad spectrum activities against bacterial agents (Gram-positive and Gram-negative) studied *in vitro* as well as *in vivo* (1) and the greatest antihelminitic activity. Minced garlic together with other biological extraction (Bio-Gamma mix) can be prophylactic against most fish pathogens (30). Moreover, as a herbal remedy, it reduces a multitude of risk factors which play a decisive role in the genesis and progression of arteriosclerosis (61). *Allium sativum* decreases both total cholesterol and low-density lipoprotein (LDL-C) in addition to reducing blood pressure (4). The consumption of its powder may decrease the accumulation of lipids in the liver, increase the excretion of total bile acids in feces, and increase the antioxidant capacity in hamsters (75). Chloramphenicol is a potent antibiotic effective against most bacteria such as rickettsia and the psittacosis-lymphogranuloma group. It suppresses anaerobic bacteria and most Gram-negative bacteria growth (65). Chloramphenicol antibiotic is generally used in fish feed to promote health, but its use for long periods may cause blood dyscrasias such as aplastic anemia, and it has recently been incriminated as carcinogenic. This problem led to the use of other natural products, such as garlic, which has the same effect as chloramphenicol. So this study was carried out to compare the effects of *Allium sativum*, as an antibiotic, and chloramphenicol on growth performance, bacterial growth and hematology of Nile tilapia.

MATERIALS AND METHODS

Experimental diets

Eight experimental diets were formulated to contain different levels of *Allium sativum* powder (10, 20, 30, and 40g/kg diet) and chloramphenicol antibiotic (15, 30, and 45mg/kg diet), which replaced cornstarch. Control diet was free from both *Allium sativum* and antibiotic. Diets were formulated from ingredients commercially available in Egypt, except for fishmeal, which was imported from Denmark. Garlic without skin was purchased from the local market and dried in a Freeze Drier Model (LABCONCO) for 70 hours, then ground to become powder. All diets were isonitrogenous and isocaloric (Table 1). They were transformed into pellet form by Spaghetti Machine, La Parmigiana, Model D45LE, Italy. After being dried, the pellets were transferred to plastic bags and stored in a freezer at -20°C until immediately prior to feeding.

Experimental system and fish

The feeding experiment was carried out in twenty-four glass aquaria. Each aquarium was 75X40X50cm with a total volume of 100l. Experimental aquaria were supplied with well-aerated freshwater using compressed air via an airstone. Fresh tap water was stored in fiberglass tanks for 24h under aeration in order to dechlorinate the water. Aquaria were daily cleaned, and the water exchange rate per day, including fish feces and remaining food, was approximately 25% of the total volume. Then, each aquarium was refilled to a fixed volume also using stored and well-aerated freshwater. The water temperature was adjusted (26-27°C) by a thermostat column heater in each aquarium.

Oreochromis niloticus fingerlings were obtained from the nursery ponds of Central Laboratory for Aquaculture Research, Abbassa, Abu-Hammad, Sharkia. They were acclimated under wet laboratory conditions for two weeks and randomly distributed at a stocking density of 20 fish per aquarium. The initial average fish weight was 7±1g/fish. Each group (represented by three aquaria) was fed on one of the experimental diets. Diets were given at a rate of 3% live body weight two times a day (9 a.m. and 1 p.m.). Fish were biweekly weighted and the amounts of daily given feed were readjusted according to increases in their body weight. At the end of the

experiment, which lasted 90 days, fish in each aquarium were weighted and counted. Different growth parameters were calculated according to Khattab (39).

Growth parameters:

1 - Specific growth rate (SGR, %/day) = ln W_t – ln W_0/ T X 100 (ln = natural logarithm; W_0 = initial weight; W_t = final weight; and T = time in day).

2 - Feed conversion ratio (FCR) = total dry feed consumed (g) / total wet weight gained (g).

3 - Feed efficiency ratio (FER) = live weight gained (g) / dry feed given (g) X 100.

4 - Protein efficiency ratio (PER) = wet weight gained (g) / amount of protein consumed (g).

In another experiment under the same conditions, a part of each diet was mixed with 0.5% chromic oxide and stored until apparent nutrient digestibility measurement. Fish were fed on the same diets once a day in the morning. Six hours after feeding, fish feces were collected by siphoning on a fine mesh net, filtered on Whatman paper No. 1 for 1/2 hour to eliminate water, dried, and stored in a freezer for analysis.

Analytical methods

Chemical analysis of feed ingredients, feces, and fish body composition was performed as recommended by the Association of Official Analytical Chemists (7). Moisture content was determined by oven drying at 105°C for 10h (constant weight). Crude protein was indirectly measured by analysis of total nitrogen (CP = N X 6.25) as per the Kjeldahl method (7). Crude lipid was determined by using Soxhlet apparatus. Ash was detected by weighting samples in a porcelain crucible placed in a furnace at 550°C for 4h. Crude fiber was estimated according to Goering and Van Soest (28). To measure the apparent nutrient digestibility, chromic oxide was determined in feces and diets using the methods of Furukawa and Tsukahara (26).

Table 1: Constituents and proximate chemical composition of experimental diets (dry matter basis).

Ingredients	Control	*Allium sativum* diets (g/kg diet)			
	0	10	20	30	40
Herring fish meal (72%)	10.00	10.00	10.00	10.00	10.00
Poultry byproduct (60%)	15.60	15.60	15.60	15.60	15.60
Soybean meal (44%)	36.00	36.00	36.00	36.00	36.00
Wheat bran (16.4%)	5.00	5.00	5.00	5.00	5.00
Yellow corn (8.5%)	23.34	23.34	23.34	23.34	23.34
Cornstarch	5.00	4.00	3.00	2.00	1.00
Garlic powder	-	1.00	2.00	3.00	4.00
Fish and corn oil (1:1)	2.50	2.50	2.50	2.50	2.50
Vit. & Min. premix*	1.50	1.50	1.50	1.50	1.50
Ascorbic acid	0.06	0.06	0.06	0.06	0.06
Carboxymethyl cellulose	1.00	1.00	1.00	1.00	1.00
Chemical analysis (%)					
Dry matter	92.10	91.60	92.00	91.50	91.80
Crude protein	35.20	34.87	35.01	34.80	33.98
Crude fat	8.33	8.61	8.48	8.76	8.39
NFE**	41.66	41.60	42.40	41.26	43.41
Crude fiber	5.10	5.54	5.30	5.81	5.23
Ash	9.71	9.38	8.81	9.37	8.99
GE†	448.37	448.9	451.76	448.35	449.22

* Vit. & Min. premix (Vitamins and minerals premix): each 2.5kg contains vitamins A, 12 Million International Units (MIU); D_3, 2MIU; E, 10g; K, 2g; B_1, 1g; B_2, 4g; B_6, 1.5g; B_{12}, 10g; pantothenic acid, 10g; nicotinic acid, 20g; folic acid, 1g; biotin, 50g; choline chloride, 500mg; copper, 10g; iodine, 1g; iron, 30g; manganese, 55g; zinc, 55g; and selenium, 0.1g.
** NFE (Nitrogen free extract) = 100 - (protein + lipid + ash + fiber).
†GE (Gross energy) = Calculated as 5.64, 9.44 and 4.11kcal/g for protein, lipid, and NFE, respectively.

Hematological analysis

At the end of the experiment, blood samples were collected from the fish caudal vein by a sterile syringe containing EDTA as an anticoagulant. Blood was used for erythrocyte count (15), hemoglobin content (72) and hematocrit value (11)

determination. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated using the formulae mentioned by Dacie and Lewis (15).

Plasma was obtained by centrifugation at 3000rpm for 15min and the non-hemolyzed plasma was stored in a freezer at -20°C until analysis. Plasma protein content was determined by the Biuret method described by Wootton (74). Glucose concentration was measured according to Trinder (71), using Boehring Mannheium kits. Total lipids were determined colorimetrically using a kit supplied by El Nasr Pharmaceutical Chemical Co., according to Knight *et al*. (43). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically using kits supplied by Diamond Diagnostics, according to Reitman and Frankel (55).

Microbiological analysis

Water samples from the aquaria were monthly collected in sterile glass bottles. Peptone water 0.1% was used for serial dilution; 1ml of water sample was added to 9ml sterile peptone water to 10^{-1} and then diluted to 10^{-4}. Each diluent (1ml) was poured in three Petri dishes; two of them also received plate count agar for total bacterial count using the pure plate count method according to the Standard Methods for the Examination of Water and Wastewater (6); the third Petri dish received MacConky agar for total coliforms count according to Hitchins *et al*. (33). Petri dishes were gently tapped on the sides for a few times. Petri dish of total coliforms count and half of the dishes of total bacterial count were incubated at 35±2°C for 24h, and the other half was incubated at 22±2°C for 48h.

Fish samples (muscles and intestine) were collected at the end of the experimental period for bacteriological examination with thorough asepsis. According to the American Public Health Association (6), one gram of both muscles and intestine was grained with 9ml sterile peptone water in the mortar. One ml of the suspension was diluted by peptone water to 10^{-4}. Each diluent (1ml) was poured in three Petri dishes; from which two received plate count agar and the other received MacConky agar. The incubation periods were 24h at 35±2°C and 48h at 22±2°C. After incubation of water and fish sample dishes, the colonies were counted using colony counter.

At the end of the experiment, 240 *O. niloticus* (30 from each treatment) were challenged by intraperitoneal route with 0.5ml of 10^7 *Aeromonas hydrophila* of 24 hours living.

Statistical analysis

Data were analyzed by analysis of variance using the SAS program (58). Duncan's multiple-range test (19) was used to verify significance of the mean differences among treatments.

RESULTS

Growth performance

Growth performances of the animals after 90 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 chloramphenicol. There were no significant differences in the final weight among fish groups fed on diets with 10, 20, and 40g/kg garlic and among those fed on chloramphenicol levels ($p<0.05$).

The highest amounts of dry feed intake (g/fish/day) were seen in fish groups fed on 30g/kg garlic and 30mg/kg chloramphenicol, respectively.

Results in Table 2 show that FCR decreased significantly to 1.77±0.04 and 1.65±0.25 in *O. niloticus* fed on 30g *Allium sativum* / kg diet and 15mg chloramphenicol / kg diet, respectively, compared to the control group (2.27±0.09). Also, PER increased with increasing *Allium sativum* doses up to 30g / kg diet (1.62±0.03) and slightly increased with doses up to 45 mg chloramphenicol / kg diet (1.34±0.02). Feed efficiency ratio (FER) increased with garlic doses up to 30g/kg, while the opposite trend was found with chloramphenicol diets. There was a significant difference ($p<0.05$) between FER of fish group fed on diet containing 30g garlic / kg diet and that of the other fish groups, and there were no significant differences ($p<0.05$) in the hepatosomatic index between treatments and control.

Table 2: Effects of garlic and antibiotic on growth parameters and survival rate of Nile tilapia (*O. niloticus*) fed on experimental diets during 90 days.

Items	Control	*Allium sativum* levels (g/kg diet)				Chloramphenicol levels (mg/kg diet)		
	0	10	20	30	40	15	30	45
Initial average weight (g/fish)	7.05 ± 0.1[a]	7.07 ± 0.03a	7.07 ± 0.01a	7.06 ± 0.01a	7.06 ± 0.01a	7.07 ± 0.01a	7.04 ± 0.02a	7.08 ± 0.02a
Final average weight (g/fish)	18.17 ± 0.43c	19.47 ± 0.44b	19.53 ± 0.34b	22.11 ± 0.29d	20.42 ± 0.40b	20.15 ± 0.66b	20.7 ± 0.66bd	19.4 ± 0.11b
Average weight gained (g/fish)	11.11 ± 0.44d	12.41 ± 0.46bc	12.64 ± 0.30bc	15.05 ± 0.30a	13.36 ± 0.39bc	13.08 ± 0.67bc	13.66 ± 0.14b	12.32 ± 0.10c
SGR* (%/day)	1.05 ± 0.02d	1.13 ± 0.03bc	1.14 ± 0.02bc	1.23 ± 0.01a	1.18 ± 0.02bc	1.17 ± 0.04bc	1.20 ± 0.01ab	1.12 ± 0.01cd
Dry feed intake (g/fish)	0.28 ± 0.02c	0.28 ± 0.10d	0.28 ± 0.01d	0.30 ± 0.02b	0.29 ± 0.17bc	0.29 ± 0.09d	0.31 ± 0.16[a]	0.29 ± 0.15c
FCR[†]	2.27 ± 0.09a	2.07 ± 0.07ad	2.03 ± 0.05ab	1.77 ± 0.04bc	1.98 ± 0.05ab	1.65 ± 0.25c	2.03 ± 0.01ab	2.12 ± 0.03a
FER[‡]	44.09 ± 1.01d	49.25 ± 2.25cb	50.16 ± 1.107cd	55.74 ± 0.72a	51.19 ± 0.52b	50.29 ± 0.92cb	48.96 ± 0.56cb	47.20 ± 1.03cd
PER[§]	1.26 ± 0.05d	1.39 ± 0.05bc	1.41 ± 0.03bc	1.62 ± 0.03a	1.49 ± 0.03b	1.44 ± 0.07bc	1.40 ± 0.02bc	1.43 ± 0.02cd
HIS[¦] (n=10)	1.80 ± 0.09[a]	1.67 ± 0.01a	1.68 ± 0.01a	1.67 ± 0.01a	1.69 ± 0.03a	1.54 ± 0.19a	1.70 ± 0.03a	1.69 ± 0.05a
Survival rate (%)	96.97 ± 1.92ab	100.0 ± 0.0a	100.0 ± 0.00a	100.0 ± 0.0a	96.67 ± 1.93ab	96.67 ± 1.29ab	100.0 ± 0.0a	94.33 ± 1.0b

Means with the same letters in the same row were not significantly different ($p<0.05$).
*SGR (Specific growth rate) = $\ln W_t - \ln W_o / T \times 100$; W_o = initial weight; W_t = final weight; and T = time.
[†]FCR (Feed conversion ratio) = Total dry feed consumed (g) / Total wet weight gained (g).
[‡]FER (Feed efficiency ratio) = Live weight gained (g) / Dry feed given (g) X 100.
[§]PER (Protein efficiency ratio) = Wet weight gained (g) / Amount of protein fed (g).
[¦] HIS (Hepatosomatic index) = Liver weight / fish body weight.

Table 3 shows that apparent protein digestibility (APD), apparent fat digestibility (AFD), apparent carbohydrate digestibility (ACHOD), and apparent gross energy digestibility (AGED) were higher in treated groups than in control, with significant differences ($p<0.05$) between the group fed on 30g garlic / kg diet and the other groups.

Table 3: Apparent nutrients digestibility (%) for garlic and antibiotic diets administered to Nile tilapia (*O. niloticus*) during the experimental period.

Items	Control	*Allium sativum* levels (g/kg diet)				Chloramphenicol levels (mg/kg diet)		
	(0)	10	20	30	40	15	30	45
APD*	84.01 ± 1.05d	86.59 ± 0.73c	89.53 ± 0.06b	93.70 ± 0.75a	85.68 ± 0.57cd	85.05 ± 0.52cd	89.95 ± 0.63b	86.93 ± 0.44c
AFD**	78.36 ± 0.38e	81.13 ± 0.53cd	84.12 ± 0.82b	87.68 ± 0.59a	81.10 ± 1.21cd	79.97 ± 0.53de	82.99 ± 0.89bc	81.02 ± 0.54cd
ACHOD†	27.89 ± 1.40d	28.27 ± 0.64d	33.95 ± 0.54b	40.31 ± 0.66a	33.69 ± 0.68bc	28.79 ± 0.85d	31.25 ± 0.69c	33.53 ± 0.89 bc
AGED††	61.56 ± 0.68e	63.12 ± 0.53de	67.36 ± 0.74b	72.43 ± 0.87a	64.21 ± 0.70cd	62.64 ± 0.60de	66.29 ± 0.83b	65.47 ± 0.56bc

Means with the same letters in the same row were not significantly different ($p<0.05$);
Data are represented as mean ± SE (n=3).
*APD (Apparent Protein Digestibility, %) = 100 - (100 X %Cr_2O_3 in feed / %Cr_2O_3 in feces X %protein in feces / %protein in feed).
**AFD (Apparent Fat Digestibility).
†ACHOD (Apparent Carbohydrate Digestibility).
††AGED (Apparent Gross Energy Digestibility).

Protein content in fish body was significantly higher in the group fed on diet containing 30g / kg diet of *Allium sativum* (60.92%) than in all other groups of *Allium sativum* and chloramphenicol treatments, and control (Table 4).

Contrarily, total lipids content in fish body decreased significantly (18.35%, $p<0.05$) in fish fed on 30g *Allium sativum*; while in all other groups of *Allium sativum* and chloramphenicol it was similar to control.

Ash content was significantly higher (20.23%; $p<0.05$) in fish fed on 30g *Allium sativum* / kg diet, and the lowest values were obtained with 20g/kg *Allium sativum* and control.

Moisture content in fish body was not significantly affected with treatments.

Table 4: Chemical composition of whole body (% dry matter basis) of Nile tilapia (*O. niloticus*) under different treatments.

Items	Control	*Allium sativum* levels (g/kg diet)				Chloramphenicol levels (mg/kg diet)		
	(0)	10	20	30	40	15	30	45
Moisture	75.39 ± 0.31abc	75.84 ± 0.50abc	76.25 ± 0.18ab	76.71 ± 0.33a	75.19 ± 0.36bc	74.95 ± 0.32c	76.29 ± 0.19ab	75.73 ± 0.35abc
Crude protein	59.15 ± 0.59b	59.45 ± 0.48ab	59.51 ± 0.67ab	60.92 ± 0.28a	58.98 ± 0.24b	59.56 ± 0.41ab	58.14 ± 0.24b	58.32 ± 0.62b
Ether extract	22.76 ± 0.31a	21.18 ± 0.30ab	22.35 ± 0.20ab	18.35 ± 0.36c	21.96 ± 0.16ab	20.54 ± 0.22bc	22.51 ± 0.32ab	21.96 ± 0.52ab
Ash	18.09 ± 0.59b	19.37 ± 0.19ab	18.14 ± 0.25b	20.23 ± 0.35a	19.06 ± 0.25ab	19.90 ± 0.42a	19.32 ± 0.31ab	19.72 ± 0.51ab

Means with the same letters in the same row were not significantly different ($p<0.05$).

Hematological variables

Results of erythrocytes count (RBC), hemoglobin content, and hematocrit percentage are given in Table 5. It shows that diets containing 20, 30, and 40g/kg diet of *Allium sativum* and all levels of chloramphenicol increased all the examined blood parameters, which were significantly different from those of control. Erythrocyte count and hemoglobin content increased significantly in fish fed on diets containing all levels of garlic and chloramphenicol, except in the fish group fed on 10g *Allium*

sativum. Similarly, hematocrit values increased significantly to 19.4±0.040, 21.64±0.509% and 21.24±0.374% in fish fed on 20g *Allium sativum*, 30 and 45 mg chloramphenicol / kg diet, respectively.

Table 5: Erythrocytes count, hemoglobin, and hematocrit values of *O. niloticus* fed on diets containing different levels of *Allium sativum* and chloramphenicol.

Treatments / Parameters	Control	*Allium sativum* levels (g/kg diet)			Chloramphenicol levels (mg/kg diet)			
	(0)	10	20	30	40	15	30	45
Erythrocyte count (c/mm^3)	1.57 ±0.06a	1.52 ±0.05a	1.77 ±0.04c	1.86 ±0.04bc	1.94 ±0.04b	2.18 ±0.09d	2.24 ±0.06d	2.12 ±0.08db
Hemoglobin (g/100ml)	5.32 ±0.22a	5.142 ±0.23a	6.43 ±0.25cb	5.784 ±0.21ac	7.072 ±0.13b	7.165 ±0.20b	8.452 ±0.26e	5.972 ±0.099
Hematocrit values (%)	14.74 ±0.58a	12.4 ±0.74b	12.4 ±0.74b	16.0 ±0.83a	15.0 ±0.44a	16.2 ±0.86a	21.6 ±0.50e	21.2 ±0.37e

Means with the same letters in the same row were not significantly different ($p>0.05$).

Data are represented as mean ± SE; n=5

The blood indices calculated from the mean values of blood parameters are presented in Table 6. It shows that MCH and MCHC were significantly reduced in *O. niloticus* fed on diets containing high levels of chloramphenicol. While, MCV, MCH and MCHC were significantly different among fish fed on diets containing all levels of *Allium sativum*.

Table 6: Changes of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) in the blood of *O. niloticus* fed on diets containing different levels of *Allium sativum* and chloramphenicol.

Treatments / Parameters	Control	*Allium sativum* levels (g/kg diet)				Chloramphenicol levels (mg/kg diet)		
	(0)	10	20	30	40	150	30	45
MCV (μm^3)	93.40 ±5.56abc	81.58 ±4.21a	100.0 ± .106b	85.74 ±4.05b	87.0 ±1.62a	74.17 ± .18a	96.1 ±4.26b	100.78 ±3.58b
MCH (pg)	33.73 ±1.80ac	33.69 ±1.62ac	36.45 ±.732ac	34.46 ±1.65ac	32.67 ±1.42a	32.906 ± .91a	37.64 ±0.85c	27.69 ±0.98b
MCHC (%)	36.11 ± 1.50[a]	41.46 ±0.80b	36.45 ± 1.26[a]	34.45 ±1.35a	32.67 ±1.26a	32.80 ± .14a	37.64 ± .18a	27.69 ±0.66c

Means with the same letters in the same row were not significantly different ($p>0.05$).

Data are represented as mean ± SE; n=5

Biochemical parameters

Table 7 shows significant decreases of plasma glucose in fish fed on diets containing 20, 30, and 40g *Allium sativum* / kg diet. On the other hand, these values were significantly higher in fish administered with higher levels of chloramphenicol (30 and 45mg / kg diet).

Mean values of total plasma protein after incorporation of *Allium sativum* and chloramphenicol antibiotic to *O. niloticus* diets for 90 days are also shown in Table 7. It can be observed that these values increased significantly in all groups when compared to control group, except for those fed on 40g *Allium sativum* and 15mg chloramphenicol / kg diet, in which the increase was not significant.

The mean value of total plasma lipid in control was 7.7±0.16g/l. This level decreased significantly (7.1±0.06, 6.2±0.10, and 5.6±0.11 g/l) in fish fed on diets containing 30 and 40g *Allium sativum* and 30mg chloramphenicol / kg diet, respectively. On the other hand, it increased significantly in fish fed on 45mg chloramphenicol / kg diet.

Table 7: Changes of glucose concentration (mg/l), total protein (g/100), and total lipids (g/l) in the blood of *O. niloticus* fed on diets containing different levels of *Allium sativum* and chloramphenicol.

Treatments / Parameters	Control (0)	*Allium sativum* levels (g/kg diet) 10	20	30	40	Chloramphenicol levels (mg/kg diet) 15	30	45
Glucose	111.51 ±4.18b	106.49 ±1.58a	85.75 ±1.58a	82.56 ±1.49a	61.01 ±0.73c	129.3 ±3.74e	136.13 ±2.33e	133.84 ±4.49e
Total Protein	2.30 ±0.10a	3.59 ±0.55b	3.42 ±0.10b	3.22 ±0.13bc	2.54 ±0.12a	2.63 ±0.16a	2.99 ±0.10c	3.64 ±0.14d
Total Lipids	7.73 ±016b	8.05 ±0.20b	8.89 ±0.19d	7.18 ±006a	6.204 ±0.10bc	7.31 ±0.28ab	5.63 ±0.11e	10.78 ±0.13e

Means with the same letters in the same row were not significantly different ($p>0.05$).

Data are represented as mean ± SE; n=5.

The changes in aspartate aminotransferase (AST) activity in plasma are shown in Table 8. AST activity decreased significantly with increasing levels of *Allium sativum* and chloramphenicol. The highest values were obtained in control group, 10g *Allium sativum* group, and 15mg chloramphenicol group, without significant differences (111.66, 105.83, and 111.33 IU/l, respectively, $p<0.05$); while the lowest ones were obtained with 30g and 40g of *Allium sativum*, 30mg, and 45mg chloramphenicol with significant differences (82.66, 81.87, 64.38, and 75.83 IU/l, respectively, $p<0.05$).

Plasma ALT activity after treatment with *Allium sativum* and chloramphenicol varied significantly between groups, whereas a significant reduction (33.4±2.97, 31.2±1.90, 28.4±0.82, and 26.4±1.64 IU/l) was recorded in fish fed on diets containing 10, 20, 30, and 40g garlic, respectively, $p<0.05$. The same happened with fish fed on 30 and 45mg of chloramphenicol / kg diet.

Table 8: Changes of aspartate amino transferase (AST) and alanine amino transferase (ALT) in the blood of *O. niloticus* fed on diets containing different levels of *Allium sativum* and chloramphenicol.

Treatments / Parameters	Control	*Allium sativum* levels (g/kg diet)				Chloramphenicol levels (mg/kg diet)		
	(0)	10	20	30	40	15	30	45
AST (IU/l)	111.66 ±5.57a	105.83 ±4.50ab	95.83 ±2.63b	82.66 ±1.91c	81.87 ±2.95c	111.33 ±2.78a	64.37 ±2.14d	75.83 ±2.35e
ALT (IU/l)	41.70 ±0.84a	33.4 ±2.97b	31.2 ±1.90b	28.4 ±0.82bc	26.4 ±1.77bc	45.06 ±1.32a	24.48 ±2.091c	25.4 ±1.64c

Means with the same letters in the same row were not significantly different ($p>0.05$).

Data are represented as mean ± SE; n=5.

Bacteriological results

Results of total bacterial count from water of the aquaria where the treatments were applied are illustrated in Table 9. It shows that total bacterial count was higher in the control group ($4.4X10^4$-$8.18X10^4$cfu/ml) than in all groups of *Allium sativum* ($2.8X10^4$-$8.14X10^4$cfu/ml). With chloramphenicol diets, it ranged from $2.4X10^4$-$8.4X10^4$cfu/ml. Total bacterial counts in water samples were $2.8X10^4$-$8.14X10^4$, $2.6X10^4$-$7.9X10^4$, $0.67X10^4$-$4.56X10^4$, and $0.4X10^3$-$2.2X10^4$ cfu/ml with all *Allium sativum* levels (10, 20, 30, and 40 g/kg diet, respectively), and the most effective level was 30g/kg diet.

Results of total bacterial count from muscles and intestine of *O. niloticus* are also shown in Table 9. In muscles, it ranged from 50 to $0.3X10^3$cfu/g with *Allium sativum* treatments and from 0.0 to 90cfu/g with chloramphenicol treatments, which was lower than in control ($1.2X10^3$-$1.3X10^3$cfu/g). The levels 40g *Allium sativum* and 30mg chloramphenicol / kg diet were more effective in reducing total bacteria in muscles (60cfu/g and 25cfu/g, respectively). Total bacterial count in intestine ranged from $0.01X10^6$ to $0.6X10^6$cfu/g among *Allium sativum* levels and from $0.1X10^6$ to $0.8X10^6$cfu/g among chloramphenicol levels, while in the control group it ranged from $2.1X10^6$ to $3X10^6$cfu/g. Also, the highest dose of *Allium sativum* (40g/kg diet) and 30mg/kg diet chloramphenicol were more effective in reducing total bacteria in the intestine ($0.01X10^6$-$0.06X10^6$ and $0.1X10^6$-$0.3X10^6$cfu/g, respectively).

Table 9: Effects of *Allium sativum* and chloramphenicol on total bacteria from muscles, intestine and water among treated *O. niloticus*.

Treatments / Items		Control	*Allium sativum* levels (g/kg diet)				Chloramphenicol (mg/kg diet)		
	TEMP	(0)	10	20	30	40	15	30	45
Muscles	35ºC	$1.2X10^3$	$0.3X10^3$	$0.2X10^3$	60	50	9	0.0	25
	22ºC	$1.3X10^3$	$0.6X10^3$	$0.3\ X10^3$	90	60	90	25	90
Intestine	35ºC	$2.1X10^6$	$0.05X10^6$	$0.03X10^6$	$0.02X10^6$	$0.01X10^6$	$0.4X10^6$	$0.3X10^6$	$0.3X10^6$
	22ºC	$3X10^6$	$0.6X10^6$	$0.24\ X10^6$	$0.2X10^6$	$0.06X10^6$	$0.2X10^6$	$0.1X10^6$	$0.8X10^6$
Water	35ºC	$4.4X10^4$	$2.8X10^4$	$2.6\ X10^4$	$0.67X10^4$	$0.4X10^3$	$2.4X10^4$	$2.4X10^4$	$2.4X10^4$
	22ºC	$8.18X10^4$	$8.14X10^4$	$7.9\ X10^4$	$4.56X10^4$	$2.2X10^4$	$8.18X10^4$	$8.4X10^4$	$8.9X10^4$

Coliforms count from water, intestine, and muscles of *O. niloticus* fed on *Allium sativum* and chloramphenicol diets are illustrated in Table 10. Coliforms from water decreased in all groups fed on *Allium sativum* diets (10, 20, 30, and 40g/kg diet): $0.6X10^3$, $0.12X10^3$, $0.12X10^3$, and $0.1X10^3$cfu/ml, respectively. It also decreased in those groups fed on chloramphenicol diets (15, 30, and 45mg/kg diet): $0.3X10^3$, $0.07X10^3$, and $1.6X10^3$cfu/ml, respectively, compared to control diets ($3.4X10^3$cfu/ml). Coliforms count from muscles was not detected with chloramphenicol diets, while with *Allium sativum* diets it ranged from 0-5cfu/g. In intestine, coliforms count in fish groups fed on 10, 20, 30, and 40g/kg *Allium sativum* was $0.8X10^5$, $0.6X10^5$, $0.45X10^5$ and $0.4X10^5$cfu/g, respectively, and in those fed on 15, 30, and 45mg chloramphenicol, it was $0.03X10^5$, $0.04X10^5$, and $0.28X10^5$cfu/g, respectively, compared to control ($5.9X10^5$cfu/g).

Table 10: Effects of *Allium sativum* and chloramphenicol on total coliforms count in muscles, intestine and water of treated *O. niloticus*.

Treatment / Item	Control	*Allium sativum* levels (g/kg diet)				Chloramphenicol (mg/kg diet)		
	(0)	10	20	30	40	15	30	45
Water	$3.4X10^3$	$0.6X10^3$	$0.12X10^3$	$0.12X10^3$	$0.1X10^3$	$0.3X10^3$	$0.07X10^3$	$1.6X10^3$
Muscles	10	5.0	4.0	1.5	0.0	0.0	0.0	0.0
Intestine	$5.9X10^5$	$0.8X10^5$	$0.6X10^5$	$0.45X10^5$	$0.4X10^5$	$0.04X10^5$	$0.03X10^5$	$0.28X10^5$

Results of the challenge test in Table 11 reveal that mortality rate was 30% with all levels of *Allium sativum* and chloramphenicol, except for the first level of *Allium sativum* (10g/kg diet), which showed a mortality rate of 40%. The mortality rate in control group was 50%. We can conclude that diets with *Allium sativum* and chloramphenicol showed the same effect on the mortality rate of *O. niloticus* challenged with *A. hydrophila*.

Table 11: Challenge test of *A. hydrophila* injected by intraperitoneal route and mortality rate among *O. niloticus* treated with *Allium sativum* and chloramphenicol.

Treatment	Control	*Allium sativum* levels (g/kg diet)				Chloramphenicol levels (mg/kg diet)		
	(0)	10	20	30	40	15	30	45
Number of injected fish	30	30	30	30	30	30	30	30
Mortality (N)	15	12	9	9	9	10	9	9
Mortality (%)	50	40	30	30	30	33	30	30

DISCUSSION

Allium sativum might provide a suitable basis for new anti-*Helicobacter pylori* therapies due to its well-established antimicrobial action (12), chemical complexity and broad-spectrum action, not promoting acquisition of antibiotic resistance. In addition, direct intragastric effects are feasible because *Allium sativum* antimicrobials are not affected by acid environments (45); otherwise the gastric juice enhances the antimicrobial activity of *Allium sativum* constituents (24).

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.

Chloramphenicol supplementation as a feed additive antibiotic has a greater effect than diets without antibiotics in improving live body weight and feed conversion efficiency in broiler chicks.

Antibiotics are largely used for prophylaxis and treatment to eliminate or reduce bacterial contamination to a degree that enhances host defense mechanism (63).

The current experiment aimed to study the effects of *Allium sativum* and chloramphenicol on the health of Nile tilapia. *Allium sativum* was used at the levels 10, 20, 30, and 40g/kg diet and chloramphenicol at 15, 30, and 45mg/kg diet, and their effects are presented in Table 2. This indicates that the final weight, weight gain, and SGR increased significantly with all treatments of both *Allium sativum* and chloramphenicol. The highest growth performance was observed in fish fed on 30g garlic and 30mg chloramphenicol. These results partially agree with those mentioned by Diab *et al*. (18), who obtained the highest growth performance in *O. niloticus* with 2.5% garlic / kg diet. Also, Abou-Zeid (3) showed that *Allium sativum* supplementation positively affected *O. niloticus* biomass and SGR.

Feed intake increased with increasing *Allium sativum* and chloramphenicol levels. Feed conversion ratio decreased with increasing *Allium sativum* levels and increased with increasing chloramphenicol levels. Feed efficiency ratio and PER are used as quality indicators for fish diet and amino acid balance. So, these parameters are used to assess protein utilization and turnover. These results are also in agreement with those obtained by Khattab *et al*. (41), who found that the dietary of Biogen® increased feed intake, FCR, PER, and body composition (crude protein, ether extract, ash, and moisture) in fish. In this study, APD was improved with increasing levels of garlic (from 85.68% to 93.70%); similar results were obtained by Gomes *et al*. (29) in rainbow trout (from 85.02% to 92.43%), Degani *et al*. (16) in hybrid tilapia (from 85.79% to 90.87%), Goddard and Mclean (27) in *Oreochromis aureus* (from 76.8% to 90.8%), and Khattab (40) in Nile tilapia (from 85.65% to 92.25%). Also, AFD improved with garlic and chloramphenicol supplementation to Nile tilapia diets (from 81.02 to 87.68%). A similar improvement (from 78.6% to 83.6%) was obtained by Khattab (40) in Nile tilapia. On the other hand, the low mean (80%) of AFD recorded by Kirchgessner *et al*. (42) in carp (*Cyprinus carpio*) may be due to the fatty acids composition and to the melting point of the fat having a strong influence on digestibility. Addition of *Allium sativum* and chloramphenicol to the diet of Nile tilapia increased AGED from 62.64% to 72.43%. The present results agree with those obtained by Goddard and Mclean (27) in *O. aureus*, which showed an increase from 63.7% to 77.8%.

In this study, results of *O. niloticus* body composition showed that CP and ash increased significantly with diets containing 30g *Allium sativum* and 15mg

chloramphenicol. While total lipid content decreased significantly with the same levels of *Allium sativum* and chloramphenicol. These results agree with those obtained by Abdelhamid *et al*. (2) and Khattab *et al*. (41), who found that inclusion of Biogen® in the diet increased fish protein content and decreased whole body fat in fish. On the other hand, Diab *et al*. (18) reported that there were no significant changes in fish body composition caused by different garlic levels.

The employment of hematological techniques, including evaluation of erythrocytes count, hemoglobin concentration, hematocrit and leucocytes count, has provided valuable knowledge for fishery biologists in the assessment of fish health (9) and in monitoring stress responses (64). These results reflect the health status of fish cultured with all treatments.

The present study revealed that administration of garlic or antibiotic induced significant increases in all blood parameters (erythrocyte count, hemoglobin content, and hematocrit value) in treated fish, which agrees with the results of Martins *et al*. (47), who verified that addition of *Allium sativum* to fish diets increased erythrocytes number, hemoglobin content, hematocrit value, leucocytes, and thrombocytes. *Allium sativum* has some constituents that may play a role in the immune system stimulation and in the function of organs related to blood cell formation such as thymus, spleen, and bone marrow (37). Also, Faisal (23) reported significantly increased values of erythrocyte count, hemoglobin content, and hematocrit in catfish *Clarias garepinus* at the 1st and 3rd days after administration of ciprofloxacin, amoxycillin and ampicillin.

Blood indices (MCV, MCH and MCHC) are particularly important for the diagnosis of anemia in most animals (13). This study showed a significant decrease of MCH and MCHC in fish fed on the highest level of chloramphenicol. So, it is assumed that the decrease or increase of blood indices may be attributed to a defense reaction against chloramphenicol, which occurs by stimulation of erythropoiesis.

Changes in the physiological state often reflect alteration of hematologic and blood biochemical values. Clinical chemical analysis is a fundamental tool used to diagnose and predict the outcome of diseases and to monitor the effects of therapeutic, nutritional and environmental management in human and veterinary medicine. Blood biochemical values are not commonly used as a diagnostic tool in fish medicine, partly because of the lack of reference intervals for various fish species, and also because changes in blood analysis associated with specific diseases and metabolic

disorders are not well characterized with sufficient background data; thus, clinical biochemical analysis could be developed to detect metabolic disorders and sublethal diseases that affect the production efficiency.

In the present study, plasma glucose concentration reduced significantly in fish fed on diets containing the highest levels of *Allium sativum* (30 and 40g/kg diet). These results agree with those of Kumar and Reddy (44), and Thomson (69), who found that feeding mice with 45mg garlic / kg body weight for 28 days induced significant decrease of serum glucose levels. Lower levels of plasma glucose in fish have also been reported in the assessment of physiological effects of *Allium sativum* (60). On the other hand, the significant elevated ($p<0.001$) plasma glucose level in fish administered with all doses of chloramphenicol, compared with control, indicated that this antibiotic affects glucose dynamics in *O. niloticus* in order to obtain more energy to withstand and overcome the existing stress condition. Plasma or serum glucose level is often used as an indicator of non-specific stress (34). Our results agree with those of Tarter (68) and Faisal (23), who verified that plasma glucose concentration in catfish (*Clarias garepinus*) increased significantly after oral administration of ciprofloxacin, amoxycillin and ampicillin. Increased blood glucose levels might have been due to a glucose shift from tissue to blood or to an impairment of glucose mobilization.

Blood serum protein is a fairly labile biochemical system, precisely reflecting the condition of the organism and the changes happening to it under influence of internal and external factors. Booke (10) showed that sex, spawning, food, osmotic pressure, temperature, light, age, hibernation hormones, oxygen depletion, and season are factors that demand total serum protein complement in fish. Significant hyperproteinemia was observed in all fish groups administered with garlic, except in those that received 40g garlic / kg diet and the two highest levels of chloramphenicol. Total plasma protein was not significantly high, which agrees with the results of Hussein *et al*. (36), who showed that serum total protein content was elevated in Male Albino rats after administration of garlic oil. Increase in the serum total protein level in hyperlipidemic rats treated with *Allium sativum* oil could be attributed to the increase in the immunoglobulin level and total globulin concentration (35). Also, Faisal (23) found that serum total protein increased significantly in *Clarias garepinus* after administration of both ciprofloxacin and amoxycillin antibiotics. High serum

protein levels have been reported to be indicative of osmoregulatory dysfunction, hemodilution, or tissue damage surrounding blood vessels (31).

Reduction of total lipid in plasma of *O. niloticus* fed on diets containing high doses of *Allium sativum* (30 and 40g/kg diet) is in agreement with the study by Adler and Holub (4), who verified that serum total lipid and total cholesterol decreased significantly in men treated with garlic and fish oil alone or combined. Also, Hussein *et al*. (36) found that the serum total lipid decreased significantly in albino rats after administration of garlic. On the other hand, the present study showed elevation of plasma lipid in *O. niloticus* after administration of 45mg antibiotic / kg diet. These results agree with those of Abdelhamid *et al*. (2), who reported that fat content in muscles of Nile tilapia increased significantly after administration of 10g flavomycin / kg diet.

Transamination represents one of the main pathways for synthesis and domination of amino acid, thereby allowing interplay between carbohydrate and protein metabolism during the fluctuating energy demands of the organism in various adaptive situations. It is also considered to be important in assessing the state of the liver and some other organs (73). Therefore, attention has been focused on the changes in AST, ALT and alkaline phosphatase (ALP) activities, which promote gluconeogenesis from amino acid, as well as on the changes in aminotransferase activities in the liver (32, 53). Furthermore, AST and ALT activities might be altered by a variety of chemical, biological, and physiological factors or by a disturbance in the Kreb's cycle. Decreased activity of the Kreb's cycle cause a decrease in its intermediates, thereby, ALT and AST compensate by providing a-ketoglutarate (56).

Results of this study showed that serum AST and ALT activities decreased significantly in the fish group fed on all levels of *Allium sativum* and chloramphenicol. These data agree with those reported by El-Shater *et al*. (21) and Augusti *et al*. (8), who found that the lipid parameters and enzyme activities (AST, ALT, and ALP) in serum of rats decreased significantly when they were fed on a diet containing 5% *Allium sativum*. Also, Faisal (23) mentioned reduced AST in serum of catfish after ampicillin administration. These results can be attributed to *Allium sativum*, which may cause stabilized cell membrane and protect the liver against deleterious agents and free radical-mediated toxic damages to the liver cells. This is reflected in the

reduction of liver enzymes. *Allium sativum* helps the liver to maintain its normal function by accelerating the regenerative capacity of its cells.

In the beginning of spring and autumn and with temperature changes, fish are affected by bacteria and fungi, so antibiotics are used for treatment and prevention of diseases. Chloramphenicol is a potent antibiotic that is effective against most bacteria, suppressing the growth of anaerobic and most Gram-negative bacteria (65). It is more effective than other antibiotics against *Aeromonas hydrophila*, which causes most diseases of freshwater fish (20, 57, 66); chloramphenicol is also highly effective in marine organisms (70). This antibiotic is one of the major drugs used in fish farming, orally, in bath or by injection, for controlling bacterial infection (50).

Thus, we studied the effectiveness of garlic as an antibacterial or as a preventive measure for fish diseases in comparison with chloramphenicol, which is considered carcinogenic and teratogenic, showing reproductive toxicity and many problems of antibiotic resistance (38, 46).

Allicin treatment seems to be an enhancing effect for antibody activity (14). Microbiology Safety Standards have not been established for fish culture facilities in the United States nor in any other producing country (48) and total bacteria counts or coliforms counts with antibiotic or garlic treatments have never been detected. Results of total bacterial count revealed that bacterial load in the aquarium water was more affected by *Allium sativum* diets than by chloramphenicol and control diets; however, total bacterial counts with the later diets were lower in number than those found by Nedoluha and Westhoff (51), who reported that aerobic plate counts in fish growing water were $6.3X10^6$cfu/ml in tanks with a stocking density of 11.4g fish / gallon (3g fish/l). Sugita *et al*. (67) found that bacterial count in growing water of pufferfish (*Fugu niphobles*) housed in glass aquaria ranged from 10^4-10^5cfu/ml. Our results were similar to those of Fulford *et al*. (25), who showed that total viable bacterial count in water supplied by the dental unit water systems varied from not detected to 2.16×10^4cfu/ml, and disagreed with those of Al-Harbi and Uddin (5), who reported that counts of total viable bacteria were in the range of $5.5\pm0.8X10^3$ to $2.4\pm1.2X10^3$cfu/ml in pond water without any treatment; these values were lower than total bacterial counts in our control.

Total bacterial counts in muscles and intestine of fish fed on diets supplemented by *Allium sativum* and chloramphenicol were lower than in those of the control group.

Diets with chloramphenicol were more effective in reducing total bacteria in muscles and intestine than diets with garlic. Al-Harbi and Uddin (5) reported that total bacterial count from intestine of tilapia reared in earthen pond was $3.4\pm1.8X10^6$ - $5.8\pm0.4X10^7$cfu/g, which was higher than that of our study.

Coliforms count from water with fish fed on *Allium sativum* and chloramphenicol was lower than that from water with the control group; chloramphenicol diets were more effective in reducing coliforms in water than were garlic diets. Diets with the second level of chloramphenicol (30mg/kg diet) and garlic (20g/kg diet) were more effective than those with the other levels. Total coliforms count in water with fish fed on *Allium sativum* and chloramphenicol diets was lower than that reported by Mckeon *et al*. (48) in prefiltered water of recirculating systems (10^6cfu/100ml), but in filtered water it was $0.16X10^5$cfu/100ml, which agreed with the results in this study.

Also, *Allium sativum* and chloramphenicol groups had lower coliforms count in muscular tissue (0.0-5cfu/g and 0.0cfu/g, respectively) than did the control group (10cfu/g) and the results of Molinari *et al*. (49), who reported coliforms count of 1.710 cells / gram in muscular tissue of *O. niloticus*. Coliforms count from the intestine of fish fed on garlic diets was $0.6X10^5$ - $4.9X10^5$ (cfu/g), and in fish fed on chloramphenicol diets it was $0.03X10^5$ - $2.8X10^5$ (cfu/g), being higher than that in the control group but lower than that in the study by Molinari *et al*. [8,750 cells / gram from the intestine of *O. niloticus*] (49). Coliforms count in water, muscles and intestine showed that chloramphenicol diets were more effective than *Allium sativum* diets; the second level of chloramphenicol and the fourth level of *Allium sativum* were the most effective levels.

Results of the challenge test shown in Table 11 revealed that the mortality rate was 30% with all doses of *Allium sativum* and chloramphenicol, except for the first dose (10g/kg diet) of *Allium sativum*, which showed mortality rate of 40%. On the other hand, the mortality rate of control was 50%. Diets with *Allium sativum* and chloramphenicol showed the same effect on the mortality rate of *O. niloticus* challenged intraperitonealy with *A. hydrophila*. *Allium sativum* had antibacterial activity antagonized by *A. hydrophila* in fresh water as reported by Diab (17) and Diab *et al*. (18).

Finally, 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 stress (62); therefore garlic powder should be added to the diets of freshwater fish.

REFERENCES

1 ABD-ELALLATIF A., EBRAHEEM K. Studies on the effects of *Hibiscus subdariffa*, *Allium sativum* and *Negella sativa* on some bacterial isolates of chickens. *Fac. Vet. Med. Assut. Univ. Egypt*, 1996, 17, 245-51.

2 ABDELHAMID AM., KHALIL FF., EL-BARBERY MI., ZAKI VH., HUSIEN HS. Feeding Nile tilapia on biogen to detoxify aflatoxin diet. In: ANNUAL SCIENTIFIC CONFERENCE OF ANIMAL AND FISH PRODUCTION, 1, Mansoura, 2002. Proceedings... Mansoura: Mansoura University, 2002. p.207-30.

3 ABOU-ZEID SM. The effect of some medical plant on reproductive and productive performance of Nile tilapia fish. Cairo: Cairo University, Faculty of Agriculture, 2002. 212p. [Ph. D. Thesis]

4 ADLER AJ., HOLUB BJ. Effect of garlic and fish-oil supplementation on serum lipid and lipoprotein concentrations in hypercholesterolemia men. *Am. J. Clin. Nutr*., 1997, 65, 445-50.

5 AL-HARBI AH., UDDIN N. Quantitative and qualitative studies on bacterial flora of hybrid tilapia (*Oreochromis niloticus* X *O. aureus*) cultured in earthen ponds in Saudi Arabia. *Aquac. Res*., 2003, 34, 43-8.

6 AMERICAN PUBLIC HEALTH ASSOCIATION. Standard methods for the examination of water and wastewater. 16.ed. Washington: American Public Health Association, American Water Works Association, Water Pollution Central Federation, 1985. 1268p.

7 ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. Official methods of analysis of the Association of Official Analytical Chemists. 14.ed. Arlington: Association of Official Analytical Chemists, 1984. 1141p.

8 AUGUSTI KT., NARAYANAN A., PILLAI LS., EBRAHIM RS., SIVADASAN R., SINDHU KR., SUBHA I., ABDEEN S., NAIR SS. Beneficial effects of garlic (*Allium sativum* Linn) on rats fed with diets containing cholesterol and either of the oil seeds, coconuts or groundnuts. *Indian J. Exp. Biol*., 2001, 39, 660-7.

9 BLAXHALL PC. The hematological assessment of the health of freshwater fish. A review of selected literature. *J. Fish. Biol*., 1972, 4, 593-604.

10 BOOKE HE. A review of variations found in fish serum proteins. *New York Fish Game J*., 1964, 11, 47-57.

11 BRITTON CJ. Disorders of the blood. 9.ed. London: J&A Churchill, 1963. 332p.

12 CAVALLITO CJ., BAILEY JH. Allicin, the antibacterial principle of *Allium sativum* II. Isolation, physical properties and antimicrobial action. *J. Am. Chem. Soc*., 1944, 66, 1950-1.

13 COLES EH. Veterinary clinical pathology. Philadelphia: Saunders, 1986. 615p.

14 COLORNI A., AVTALION R., KNIBB W., BERGER E., COLORNI B., TIMAN B. Histopathology of sea bass (*Dicentrarchus labrax*) experimentally infected with *Mycobacterium marinum* and treated with streptomycin and garlic (*Allium sativum*) extract. *Aquaculture*, 1998, 160, 1-17.

15 DACIE JV., LEWIS SM. Practical hematology. 6.ed. New York: Churchill, 1984. 22p.

16 DEGANI G., VIOLA S., YEHUDA Y. Apparent digestibility of protein and carbohydrate in feed ingredients for adult tilapia (*Oreochromis aureus* X *O. niloticus*). *Isr. J. Aquac.*, 1997, 49, 115-23.

17 DIAB AS. Antibacterial and antifungal effects of *Allium sativum* (garlic), *Hibiscus sabdariffa* (karkade) and *Nigella sativa* (black seeds) extract on some bacterial and fungal isolates from Abbassa hatchery. In: VETERINARY MEDICINE ZAGAZIG CONFERENCE, 6, Hurghada, 2002. Hurghada: Zagazig University, 2002. p. 467-78.

18 DIAB AS., EL-NAGAR GO., ABD-EL-HADY YM. Evaluation of *Nigella sativa* L (black seeds; baraka), *Allium sativum* (garlic) and BIOGEN as feed additives on growth performance and immunostimulants of *O. niloticus* fingerlings. *Suez Canal Vet. Med. J.*, 2002, 745-75.

19 DUNCAN DB. Multiple range and multiple F tests. *Biometrics*, 1955, 11, 1-42.

20 EL-MAAZ AA., HUSIEN MM., YOUNIS AA. Efficacy and pharmacokinetics of chloramphenicol in *Aeromonas hydrophila* infected Clarias lazera. *Beni-Suif Vet. Med. J*., 1999, 9, 251-60.

21 EL-SHATER MA., SOBBHY MH., SHEHAB JG. Some pharmacological and pathological studies on *Allium sativum* (garlic). *Applied. Vet. Med. J*., 1997, 36, 264-83.

22 ESSA AA., HADY AM., MAHA M., MARZOUK MS. Effect of virginiamycin on performance and susceptibility of *O. niloticus* to *A. hydrophila* infection. J. *Egypt Vet. Med. Ass*., 1995, 55, 109-21.

23 FAISAL ASR. Adverse effects of some antimicrobial agents used in fish. Cairo: Cairo University, Faculty of Veterinary Medicine, 2003. 238p. [Ph. D. Thesis]

24 FORTUNATOR MN. On the activity of the phytoncides from garlic in the human organism upon peroral administration. *Farmakal-Toksikal*., 1955, 18, 43-6.

25 FULFORD MR., WALKER JT., MARTIN MV., MARSH PD. Total viable counts, ATP, and endotoxin levels as potential markers of microbial contamination of dental unit water systems. *Br. Dent. J*., 2004, 196, 157-9.

26 FURUKAWA A., TSUKAHARA H. On the acid digestion method for the determination of chromic oxide as an index in the study of digestibility of fish feed. *Bull. Jpn. Soc. Sci. Fish*., 1966, 32, 502-6.

27 GODDARD JS., MCLEAN E. Acid-insoluble ash as an inert reference material for digestibility studies in tilapia, *Oreochromis aureus*. *Aquaculture*, 2001, 194, 93-8.

28 GOERING HK., VAN SOEST PJ. Forage fiber analysis: apparatus, reagents, procedures, and some applications. Washington: USDA/ARS, 1970. 20p.

29 GOMES EF., CORRAZE G., KAUSHIK SJ. Effect of dietary incorporation of a co-extruded plant protein (rapeseed and peas) on growth, nutrient utilization and muscle fatty acid composition of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 1993, 113, 339-53.

30 HEO GJ., KIM BG., YEH JG., KIM JH. Study on efficacy and safety of bio-gamma mix by oral administration to cultured fish. *Korean J. Vet. Research*, 1994, 34, 627-33.

31 HILLE S. A literature review of the blood chemistry of rainbow trout, *Salmo gairdneri* Rich. *J. Fish. Biol*., 1982, 20, 535-69.

32 HILMY AM., SHABANA MB., SAID MM. The role of serum transaminases (SGOT and SGPT) and alkaline phosphates in relation to inorganic phosphorus with respect to mercury poisoning in *Aphanius dispor* Rupp (Teleos) of the red sea. *Com. Biochem. Physiol*., 1981, 68, 69-74.

33 HITCHINS AD., FENG P., WATKINS WD., RIPPEY SR., CHANDLER LA. *Escherichia coli* and the coliform bacteria. In: WATKINS WD. Ed. Food and drug administration bacteriological analytical manual. 8.ed. Arlington: Association of Official Analytical Chemists, 1995: 401-29.

34 HUNN JB., GREER IE. Influence of sampling on the blood chemistry of Atlantic salmon. *Prog. Fish Cult*., 1991, 53, 184-7.

35 HUSSEIN SA. Electrophoretic pattern of serum protein and immunoglobulin level in chickens in relation of age. *Benha. Vet. Med. J*., 1996, 7, 95-107.

36 HUSSEIN SA., ABD-EL-MAKSOUD H., AZAB ME. Certain biochemical effect of garlic oil on normal and experimentally induced hyperlipidemia in male albino rats. In: INTERNATIONAL SCIENTIFIC CONFERENCE, 2, Mansoura, 2001. Mansoura: Mansoura University, 2001. p.103-29.

37 JEORG HG., LEE YW. Protective effect of daily sulfide on N-nitrosodimethylamine- induced immunosuppression in mice. *Cancer Lett.*, 1998, 11, 73-9.

38 JOINT EXPERT COMMITTEE ON FOOD ADDITIVES. Toxicological evaluation of certain veterinary drug residues in food. Prepared by the sixty-second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). February, 2004, 10.

39 KHATTAB YA. Some studies of fish growth. Egypt: Zagazig University, Faculty of Agriculture, 1996. 184p. [Ph. D. Thesis]

40 KHATTAB YA. Effect of substituting black seed cake (*Nigella sativa* L.) for soybean meal in diets of Nile tilapia (*O. niloticus* L.) on growth performance and nutrients utilization. *Egypt J. Aquat. Biol. & Fish*., 2001, 5, 31-46.

41 KHATTAB YA., SHALABY AME., SHARAF SM., EL-MARAKBY HI., RIZKALLA EH. The physiological changes and growth performance of the Nile tilapia *Oerochromis niloticus* after feeding with Biogen® as growth promoter. *Egypt J. Aquat. Biol. & Fish*., 2004, 8, 145-58.

42 KIRCHGESSNER M., KURZINGER H., SCHWARZ FJ. Digestibility of crude nutrients in different feeds and estimation of their energy content for carp (*Cyprinus carpio* L.). *Aquaculture*, 1986, 58, 185-94.

43 KNIGHT JA., ANDERSON S., RAWLE JM. Chemical basis of the sulfo-phospho-vanillin reaction for estimating total serum lipids. *Clin. Chem*., 1972, 18, 199-202.

44 KUMAR GR., REDDY KP. Reduced nociceptive responses in mice with alloxan induced hyperglycemia after garlic (*Allium sativum* Linn.) treatment. *Indian J. Exp. Biol*., 1999, 37, 662-6.

45 LAWSON LD. The composition and chemistry of garlic cloves and processed garlic. In: KOCH HP., LAWSON LD. Eds. Garlic. The science and the therapeutic application of *Allium sativum* and related species. Baltimore: Williams & Wilkins, 1996: 37-107.

46 MARTIN KW., ERNST E. Herbal medicines for treatment of bacterial infections: a review of controlled clinical trials. *J. Antimicrob. Chemother*., 2003, 51, 241-6.

47 MARTINS ML., MORAES FR., MIYAZAKI DM., BRUM CD., ONAKA EM., FENERICK Jr. J., BOZZO FR. Alternative treatment for *Anacanthorus penilabiatus* (Monogenea: Dactylogyridae) infection in cultivated pacu, *Piaractus mesopotamicus* (Osteichthyes: Characidae) in Brazil and its haematological effects. *Parasite*, 2002, 9, 17180.

48 MCKEON C., GLENN E., GERBA CP., FITZSIMMONS K. Microbiological hazards of tilapia culture systems. In: INTERNATIONAL SCIENTIFIC CONFERENCE, 2, Mansoura, 2001. Mansoura: Mansoura University, 2001. p.479-85.

49 MOLINARI LM., BRANDÃO M., ABREU FILHO BA., NAKAMURA CV., FILHO BD. Flora bacteriana de tilápias do Nilo cultivadas em sistema semi-intensivo. In: INTERNATIONAL SCIENTIFIC CONFERENCE, 2, Mansoura, 2001. Mansoura: Mansoura University, 2001. p.499-503.

50 NATIONAL INSTITUTE FOR CLINICAL EXCELLENCE. Drug use in aquaculture. *World Aquac. Soc.*, 2000, 31, 45-6.

51 NEDOLUHA PC., WESTHOFF D. Microbiological analysis of striped bass (*Morone saxatilis*) grown in a recirculating system. *J. Food Prot*., 1997, 60, 948-53.

52 RAA J. The use of immunostimulatory substances in fish and shellfish farming. *Rev. Fish. Sci.*, 1996, 4, 229-88.

53 RASHATUAR SS., ILYAS R. Effect of chronic herbicide intoxication on *in vivo* activities of certain enzymes in the liver of freshwater fish *Nemachelius denisonii* (day). *Toxicol. Lett*., 1983, 16, 249-52.

54 REILLY A., KÄFERSTEIN F. Food safety hazards and the application of the principles of the hazard analysis and critical control point (HACCP) for their control in aquaculture production. *Aquac. Res*., 1997, 28, 735-52.

55 REITMAN S., FRANKEL S. Colorimetric determination of glutamic oxaloacetic and glutamic pyruvic transaminase. *J. Clin. Pathol*., 1975, 28-56.

56 SALAH EL-DEEN M., ROGERS WA. Changes in total protein and transaminase activities of grass carp exposed to diquat. *J. Aquat. Anim. Health*, 1993, 5, 280-6.

57 SALAMA SA. Studies on the bacterial diseases of *O. niloticus* reared in concrete ponds. Egypt: Suez Canal University, Faculty of Veterinary Medicine, 1999. 142p. [Msc. Thesis]

58 SAS INSTITUTE. SAS/SAT User's guide – version 6. Cary, 1989.

59 SECOMBES CJ. Enhancement of fish phagocyte activity. *Fish Shellfish Immunol*., 1994, 4, 421-36.

60 SHEELA CG., AUGUSTI KT. Antidiabetic effects of S-allyl cysteine sulphoxide isolated from garlic, *Allium sativum* Linn. *Indian J. Exp. Biol*., 1992, 30, 523-6.

61 SIEGEL G., WALTER A., ENGEL S., WALPER A., MICHEL F. Pleiotropic effects of garlic. *Wien. Med. Wochenschr*., 1999, 149, 217-24.

62 SIVAM GP. Recent advances on the nutritional effects associated with the use of garlic as supplement. Am. Soc. Nutr. Sci., 2001, 1106 -8.

63 SMITH CM., REYNARD AM. Textbook of pharmacology. Philadelphia: Saunders, 1992. 1231p.

64 SOIVIO A., OIKARI A. Haematological effects of stress on a teleost, Esox lucis L. *J. Fish Biol*., 1976, 8, 397-411.

65 SOMJETLERDCHAROEN A. Chloramphenicol concerns in shrimp culture. *Aquaculture Asia*, 2002, 7, 51-4.

66 SOUSA JA., SILVA-SOUZA AT. Bacterial community associated with fish and water from Congonhas River, Sertaneja, Paraná, Brazil. *Braz. Arch. Biol. Technol*., 2001, 44, 383-94.

67 SUGITA H., IWATA J., MIYAJIMA C., KUBO T., NOGUCHI T., HASHIMOTO K., DEGUCHI Y. Changes in microflora of a puffer fish, *Fugu niphobles*, with different water temperatures. *Mar. Biol*.,1989, 101, 299-304.

68 TARTER FEM. The effect of commonly used chemotherapeutic agent on the blood constituents in chickens. Cairo: Cairo University, Faculty of Veterinary Medicine, 1986. 201p. [Ph. D. Thesis]

69 THOMSON M., ALI M. Garlic (*Allium sativum*): a review of its potential use as an anti-cancer agent. *Curr. Cancer Drug Targets*, 2003, 3, 67-81.

70 TORKILDSEN L., SAMUELSEN OB., LUNESTAD BT., BERGH O. Minimum inhibitory concentrations of chloramphenicol, florfenicol, trimethoprim/sulfadiazine and flumequine in seawater of bacteria associated with scallops (*Pecten maximus*) larvae. *Aquaculture*, 2000, 185, 1-12.

71 TRINDER P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann. Clin. Biochem*., 1969, 6, 24-7.

72 VANKAMPEN EJ. Determination of haemoglobin. *Clin. Chem. Acta*, 1961, 6, 538-44.

73 VERMA SR., RANI S., DELELA RC. Isolated and combined effects of pesticides on serum transaminases in *Mystus vittatus* (African catfish). *Toxicol. Lett*., 1981, 8, 67- 71.

74 WOOTTON LI. Micro-analysis in medical biochemistry in micrometer. 4.ed. London: J&A Churchill, 1964. 264p.

75 YAOLING L., JIUNRONG C., MENGSYH S., MINGLER S., LI YL., CHEN JR., SHIEN MS., SHIEN MJ. The effects of garlic powder on the hypolipidemic function and antioxidative status in hamsters. *Nutr. Sci. J*., 1998, 23, 171-87.