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Effects of garlic and onion oil extracts as a natural growth promoters on growth performance, nutrient utilization, whole body composition and hematological parameters of Nile Tilapia (*Oreochromis niloticus*) fingerlings

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ABSTRACT

The use of dietary additives in fish farms is one of the methods commonly used to improve weight gain, feed efficiency, and/or disease resistance in cultured fish. *Allium sativum* (garlic) and *A. cepa* (onion) are herbs or herbal derivatives that can replace antibiotics and other harmful growth promoters. The present study deals with the influence of different levels (0.5 or 1.0%) of each of *A. sativum* and *A. cepa* extract in fish diet as a natural growth promoter on growth performance, nutrient utilization and hematological parameters of mono-sex all male Nile Tilapia (*Oreochromis niloticus*) fingerlings.

Five treatments were triplicated and a total number of 1500 fish (14.0±0.19 g) were stocked in 15 cages (2×4×1 m) in earthen pond. At the end of the experiment (after 120 days), the highest ($P<0.05$) weight gain (WG) and specific growth rate (SGR) were observed in fish groups fed the diets supplemented with 1% garlic or onion extracts. Also, the different diets showed no significant different on feed conversion ratio (FCR). After 120 days, the fish groups fed the diets supplemented with garlic and onion extracts showed a significantly ($P<0.05$) increase in the number of erythrocytes (RBC), leucocyte (WBC) compared to the control group. Albumin (g/dl), total proteins g/dl and haemoglobin (g/dl) levels had significant change ($P>0.05$) in treatment groups and control. The results of this study demonstrated that dietary1.0% garlic or onion extracts could improve growth, hematological parameters and immune function of mono-sex all male Nile Tilapia fingerlings reared in cages in earthen pond system .

Key words: Garlic, onion, *Allium sativum, Allium cepa,* Nile Tilapia fingerlings, *Oreochromis niloticus,* mono-sex male, growth performance, feed utilization, body composition, hematological parameters

INTRODUCTION

Nowadays antibiotics are largely used for treatment and control or to reduce harmful bacterial contamination, but there is also a need to replace them with natural substances to avoid their negative effects (Farahi *et al.*, 2010). Phytoadditives are fodder additives obtained from medicinal plants or plant extracts. Many plant products are used by humans and animals, including fish (Gabor *et al*., 2010). As they are safe and eco-friendly, herbs are directly taken by humans as food or medicine. Herbal products are gaining importance in aquaculture (Kim *et al*., 2012). The main advantage of using these phytoadditives is that they are natural substances that don't pose any threat to fish health, human health or to the environment. Research is still in progress to determine their way of action and the possible side effects that can appear as a result of their use, and to determine the possibility of using other plants as phytoadditives. Recently, phytoadditives and probiotics have been reported as potential alternatives, among other feed additives, to antibiotics in aquaculture diets. Much of this interest arises from increased public awareness and banned to the use of antibiotics as growth promoters in aquaculture. In the last decade, some studies show the positive effects of dietary phytoadditives and feed additives on growth and feed utilization in fish (Farahi *et al*., 2010).

The use of garlic and onion extract as natural phytoadditives in poultry and fish diets enhance the positive efficiency and safety immune systems. The use of herbs, garlic, due to its structure and composition play a beneficial role in human and animal nutrition (Hamail 1992). Dietary garlic as a growth promoter in Nile tilapia (*O. niloticus*) improved body weight gain, feed intake and feed efficiency (Shalaby *et al.*, 2006). Garlic is a main vegetable extensively cultivated in many countries. It is used as food for humans as well as some animals and as remedy for several diseases, as reported in folk medicine. Garlic has been a subject of considerable interest for centuries as a flavouring agent, traditional medicine, and a functional food to enhance physical and mental health. Garlic was studied in different forms of extracts: aqueous, ethanol and dried powder (Shin and Kim, 2004). It contains a variety of organosulfur compounds such as allicin, ajoene, diallyl disulfide, S-methylcysteine sulfoxide and S-allylcysteine (Chi *et al*., 1982). A wide array of beneficial effects of garlic such as antihypertensive, antihyperlipidemic, antimicrobial, hypoglycaemic, anticancer, antidote (for heavy metal poisoning), anticarcinogenic, hepatoprotective and immunomodulation have been reported by several researchers (Agusti, 1996;Yeh and Liu, 2001).

Onion (*A. cepa* L.) has a high content of free and glycosidically bonded quercetin and oxidized quercetin derivatives (Chi *et al*., 1982; Farahi *et al.*, 2010). Onion has been known to have antibacterial, antioxidant, and/or anticancer effects (Francis *et al.*, 2005; Gabor *et al.*, 2010) and it reduces endogenous lipogenesis and increases catabolism of lipids (Hamail, 1992). Onion contains a wide variety of microconstituents such as trace elements, vitamins, flavonoids and sulfur compounds (Hussein *et al*., 2001).

The main objective of this study was to investigate the effect of using garlic and onion extracts as phytoadditives on the growth, feed utilization, body composition and some hematological indices in the diet of all male Nile Tilapia (*O. niloticus*) fingerlings reared in cages in earthen pond system.

MATERIALS AND METHODS

1. Experimental conditions:

The study was conducted in the fish farm belonging to General Authority of Fish Resources Development (GAFRD) located at El–Hamul city (El- Zawya fish farms), Kufr El-Sheikh Governorate, Egypt. The main water source of the farm is the drainage canal called Hamdy canal , during the rearing period from 29th June to 18th October 2014 (120 days) .

The feeding trial was conducted in five groups of healthy mono sex (all-male) fingerlings Nile Tilapia (*O. niloticus*) fish in fifteen 9 m^3 (3×3×1 m) net cages (in triplicate) each stocked with 50 fish into two earthen pond with a total area of 525 m^2 (15 × 35 m.) for each, with a depth of 1.5 m. The treatments were used in this study as following:

Group1 T1: (control) fish fed the basal diet without garlic or onion oil extracts.
Group2 T2: fish fed the basal diet supplemented with 0.5 % Garlic oil extracts.
Group3 T3: fish fed the basal diet supplemented with 1.0 % Garlic oil extracts.
Group4 T4: fish fed the basal diet supplemented with 0.5 % onion oil extracts.
Group5 T5: fish fed the basal diet supplemented with 1.0% onion oil extracts.

2. Ponds preparation

Prior to the experiment, ponds were drained and aquatic vegetation cleaned. The dikes of ponds were repaired. Ponds were filled (on day-2) with water. Quicklime (on day-3) was applied to the ponds by spreading methods at the rate of 10 kg/pond. After one week of lime application the ponds were fertilized with both of urea and TSP each of 1 kg/pond. The required TSP and urea were mixed together and dissolved in plastic bucket for 10-12 hours then applied to the pond. After 5 days of fertilizer application, all-male Nile Tilapia (*O. niloticus*) fingerlings were stocked in cages. The cages were suspended on the pond surface waters by direct attachment to wooden piers. The nets were cleaned regularly during feeding time. Dead fish were removed to keep the environment good for fish. The cages were lifted from water every 3 days to check the any damage of the net.

3. Stocking of Nile Tilapia fingerlings:

All male Nile Tilapia (*O. niloticus*) fingerlings were purchased from a private fish hatchery at El- Hamoul. The experimental fish were transported at early morning to the experimental site in containers. The fish were kept for acclimatization at the experimental conditions for seven days before the trial began. After the groups were allocated and fish acclimatized on the control diet (without garlic or onion oil extracts), samples of fish were weighed using a digital scale and measured using a measuring board to obtain the initial samples of the groups. A total of 7500 fish (50 fish/cage × triplicate treatment group ×5 alternative dietary source for tilapia) with a mean initial body weight of 14±0.3 g/fish were randomly stocked into fifteen 9 m^3 net cages. A completely randomized design was adopted, where five diets were fed to triplicate groups.

4. Preparation of garlic, onion oil extracts and the experimental diets:

To prepare the diets, a commercial pellet fish diet was crushed, mixed with the appropriate garlic and onion oil extracts concentration and water, and then made again into pellets, which were allowed to dry for 18 h at 45^{O}C by air circulation and stored in a dried room during the whole experimental period. Control diet was prepared by adding only water. All male Nile Tilapia (*O. niloticus*) fingerlings were fed the experimental diet for 16 weeks in rate of 3% of the body weight per day, spread across two feeding times (09:00 am and 15:00 pm) for 7 days a week, for 120 days and fish were weighed biweekly. The composition and chemical analysis of the basal diet were illustrated in Table (1).

Table 1. Proximate composition of the experimental diets.

Chemical composition (%)	Experimental Fish Groups				
	T1 Control 0.0 %	T2 Garlic (5 g/ kg feed)	T3 Garlic (10 g/kg feed)	T4 Onion (5 g/kg feed)	T5 Onion (10 g/kg feed)
Garlic oil extracts	0	0.5	1.0	0.0	0.0
Onion oil extracts	0	0	0	0.5	1.0
Dry matter	90.8	90.8	90.8	90.8	90.8
Moisture	9.2	9.2	9.2	9.2	9.2
Crude protein	24.97	24.97	24.97	24.97	24.97
Crude fat	3.13	3.13	3.13	3.13	3.13
Ash	15.5	15.5	15.5	15.5	15.5
CF	8.2	8.2	8.2	8.2	8.2
Soluble carbohydrate[1]	39	39	39	39	39
Gross energy (GE)[2]	**3400**	**3400**	**3400**	**3400**	**3400**

1 Nitrogen-free extracts (NFE) = 100 − [% Ash +% lipid +% protein +% Fiber].
2 GE (kJ/g) = (protein content × 23.6) + (Lipid content × 39.5) + (carbohydrate content × 17.2).

5. Sampling of fish and Feeding:

At least twenty five live fish in each cage -in-pond were randomly scooped out of each cage unit for their measurements and immediately returned to their respective cages. The cage nets were inspected and cleaned during each sampling. Feeding rate was adjusted weekly. Daily feed consumption (feed given to fish) and mortalities were recorded. Dead fish were replaced immediately with similar size specimens within the first ten days of culture.The amounts of feed in respective fish type were determined through the sampling that was carried out weekly throughout the culture period to monitor growth performance. At the end of the experiment, water was pumped out of pond and all fish from each cage were harvested, recovered, weighed in kilograms and counted. The individual body weight and total fish weight were taken. The total length of fish was measured as well (Precision = 1 mm).

6. Analytical methods:

Before and at the end of the experimental period of 120 days, five whole fish from each of the treatment and control cages were randomly collected using hand net. The captured fish were degutted and cleaned with tap water and sacrificed by a lethal dose of anesthesia (150 mg/L MS- 222), homogenized in a blender, and stored at–20°C for proximate analysis of the carcass composition. Initial body analyses were performed on a pooled sample of 50 gram fingerlings, which was frozen prior to the study. The proximate composition (moisture, crude protein, crude lipid and ash) of fish body composition have been carried out before and after the experiment according to the methods of **AOAC (1997)**.

The nutritional composition of the experimental diets ratio of test feeds was analyzed prior to diet formulation. All the ingredients were ground to fine powder before being subjected to proximate analysis (Table 1).The analyses involved the following nutrients: dry matter (DM), crude protein (CP), ether extract (EE), ash, nitrogen free extracts (NFE) and crude fiber (CF). CP was estimated from Kjedahl nitrogen, while EE was quantified as the loss in weight after extraction of the sample with petroleum ether. Ash was determined by combustion dry samples in a muffle furnace at 550°C for 12 hours. CF was determined by a consecutive alkaline acid digestion, which was followed by ashing the dry residue at 550°C in a muffle furnace for 12 hours. Carbohydrate as nitrogen-free extract (NFE) was determined by the difference method. Each ingredient was homogeneously ground and passed through a 100 u.m sieve. Before diet formulation, the proximate composition of feed was determined (Table 1).

7. Pond and water quality parameters management:

The experimental pond was fertilized fortnight at a rate of 2.0 kg N and 0.250 kg P/feddan with Urea and Triple Super phosphate (TSP). Physico-chemical parameters of water in the vicinity of the cages were monitored prior to stocking and three times a week thereafter to ensure that they were within the recommended limits for fish growth. Sample collections were made between 8.00 am and 9.00 am on each sampling day and were measured *in situ*. Key water quality parameters, which included water temperature (°C), pH, dissolved oxygen (DO-mg/l), un-ionized ammonia (NH_3-N) (mg/l), Nitrite (NO_2-N) (mg/l) and Nitrate (NO_3-N) (mg/l) were measured three times a week in the cages experiment. For water quality parameters study, three water samples were collected from each of the cages and outside the cages i.e; from pond in which the cages were set up. The water samples were kept in separate clean white plastic bottles and each was marked properly. Water temperature, conductivity dissolved oxygen, and pH were analyzed and recorded on the dike of the pond. Water temperature was measured and monitored daily around 09:00 hours and 13:00 hours GMT using a glass Celsius thermometer. DO was measured using model 57 oxygen meter (YSI industries, Yellow Springs, Ohio, 4387, USA), while a glass electrode pH meter, Hi-9024 microcomputer (Hanna Instruments Ltd., Chicago, IL., USA), was used to take pH measurements), while un-ionized ammonia (NH_3-N) (mg/l), Nitrite (NO_2-N) (mg/l) and Nitrate (NO_3-N) (mg/l) were determined according to **Boyd (1990).**

8. Growth performance and feed utilization parameters:

Growth performance for each treatment group was determined and feed utilization was calculated as described by **Sveier *et al.* (2000)** as follows:

Mean weight gain = *Wf* – *Wi*

Mean daily weight gain = *Wf* – *Wi/* t

Specific growth rate (SGR) = 100 × [(loge *Wf* − log*e Wi*)]/t

Where, log *e* is natural log, *Wf* is the final mean wet weight (g), *Wi* is the initial mean wet weight and t is the time in days (**Ricker, 1975**).

Condition Factor (K) =100 (Wt/L^3),

Where, Wt is fish body weight (g), L is total length (cm).

Feed intake = Total feed consumed/No of fish stocked

Food conversion ratio (FCR)=dry weight of feed consumed (Feed intake g)/wet weight gain(g).

Protein efficiency ratio (PER%) = Wet body weight gain (g)/ Crude protein fed (intake)

Protein productive value (PPV%)=100 [protein gain (g)/protein fed (g)]

Energy utilization (EU;%)=100 [energy gain (g)/energy intake (g)].

Hepatosomatic index (HSI) = 100 [liver weight (g)/ total body weight (g)].

9. Blood sample collection and Hematological assays

At the end of the experiment, three fish were sampled randomly from each cage and were anesthetized with clove solution, and about 4 mL of blood was drawn from the caudal vein, using a syringe. Then, blood samples were introduced to both heparinized and non-heparinized tubes in order to perform haematological and immunological studies, respectively. Blood sera were obtained by centrifuging blood samples at 3000 rpm (15,609 g) for 10 min using a Heraeus Labofuge 400, and the sera were removed with a disposable transfer pipette and stored at -20^{O}C until analysis for biochemical and immunological studies.

The erythrocyte (RBC) and leukocyte (WBC) counts were determined using a Neubaeur hemocytometer according to Martins *et al*. (2002). Hemoglobin levels (Hb) were obtained by the cyanomethemoglobin spectrophotometry method. Biochemical analysis was performed in the sequence: total protein (Biuret method), albumin (Bromocresol Green method).

10. Economic analysis

At the end of the experiment, all fish were sold and the prices of fish were attributed to the Egyptian local fish market price in July 2014. Economic analysis was conducted to determine economic returns. The analysis was based on market prices in Egypt for harvested fishes and all other items, which was expressed in Egypt .The following simple equation was used :

R=I-(FC+VC+Ii)

Where, R=net return, I=income from monosex tilapia sale, FC=fixed/common costs, VC=variable costs and *Ii*=interest on inputs

The Benefit-cost ratio, BCR was calculated as:

BCR = net benefit (Total net return) / total expenditure (Total input cost)

Profit index, PI : PI = ((Value of fish/kg) / (Total cost of production))/ Production (Kg/m3)

Production (Kg/m3) = (Total weight of fish harvest (Kg)) / (volume of cage).

11. Statistical analysis

All the data collected during experiment were recorded and preserved in computer spread sheet. Mean value and standard deviation (S.D.) were calculated from the results. One way analysis of variance (ANOVA) was applied for comparison of the mean values using SPSS (Statistical Package for Social Sciences 2006 version 15.0); and level of significance was based on $p<0.05$ was established . Duncan's Multiple Range Test (DMRT) (Duncan, 1955) was applied to compare means for detection of the level of variation among treatments.

RESULTS AND DISCUSSION

1. Water quality parameters:

The mean (± SE) values of the water quality parameters (Table 2) for the cages and in pond at selected stations were within the suitable range for fish culture. All the water quality parameters analyzed showed no significant difference ($p>0.05$) except the levels of nitrate among culture regimes. It was reported that level of ammonia gas content lower than 1mg/l in pond water was acceptable for pond fish culture (Boyd 1990). The concentration of un-ionized ammonia-nitrogen was within acceptable limits, and there was no significant difference of mean ammonia and nitrite among the treatments except for nitrate but did not negatively affect the growth of fish.

Table (2): Mean value with standard Error (±SE) of water quality parameters as recorded from inside of cages and outside of cages (pond) during the experimental period (120 days).

Water quality parameters	Cages (Experimental Fish Groups)			pond
	Control	Garlic	Onion	
Temperature (°C)	27.8± 0.11 a	28.1± 0.21 a	27.9 ± 0.10 a	27.10 ± 0.3 a
Dissolved Oxygen(mg/l)	7.63± 0.16 a	7.28± 0.11 a	7.43 ± 0.14 a	7. 70 ± 0.0 a
pH (pH unit)	7.51± 0.51 a	7.86± 0.71 a	7.64 ± 0.54 a	7.4 ± 0.0 a
un-ionized ammonia (NH_3-N mg/l)	0.038±0.015a	0.062±0.041a	0.054±0.005a	0.014±0.03a
Nitrite (NO_2-N) (mg/l)	0.07±0.0022a	0.011±0.0033a	0.009±0.0002a	0.07± 0.03a
Nitrate (NO_3-N mg/l)	0.017±0.016a	0.038±0.018a	0.032±0.006a	0.021±0.01a

Means followed by the same letters in each row for each trait are not significantly different ($P>0.05$) .

2. Growth performance

Results of Table (3) revealed that averages of initial weights and lengths of fish in experimental groups ranged between 14.19±0.9 and 14.33±0.06 for weight and 9.43± 0.05 and 9.62±0.94 for length, with insignificant differences among the experimental indicating the complete randomization of individual fish among the experimental treatments at the start of the experiment. At the end of the experimental period (120 days), when compared with the (T1) control group, average of final fish weights indicated that male Nile Tilapia (*O. niloticus*) fingerlings fed on diets containing 1.0% garlic and 1.0% onion extracts as natural phytoadditives reared in cages in earthen pond system showed pronounced positive effects on their growth performances and improvement in their body weight more than the control group (T1).

Results of Table (3) showed that significant ($p < 0.05$) increase in average weight gain, daily weight gain, specific growth rate and condition factor (K) were observed in fingerlings that fed with 0.5%, 1.0 % garlic, 0.5% and 1.0% onion extracts as natural phytoadditives included diet compared to that of T1 (control diet, without garlic and onion extracts). Gradual improvement in growth performance indices was recorded and coinciding with increasing onion extract inclusion level in monosex male Nile Tilapia (*O. niloticus*) fingerlings diets and values of %WG and SGR in fish fed 0.5 and 1.0 % onion extract diets were relatively similar and insignificantly different comparing with each other but significantly different with the T1 (control group) (Table 3).There were improvements in the growth responses of fish fed on dietary feed additives. Incorporation of feed additives in diets of aquaculture fish aims to enhance fish performance, immunity and quality of flesh. Searching for new feed additives is still a very important point for aquaculture researchers. In this study the highest growth performance was observed in fish fed diets containing garlic, specially 1% garlic (258.33 g) or 1% onion extracts (219.89 gm). This agrees with the results of Shalaby *et al*. (2006).

Table (3): Growth performance of monosex (all-male) Nile Tilapia fed on different levels of Garlic and Onion extracts.

Items	Experimental Fish Group1			Group 2	
	T1 Control 0.0%	T2 Garlic 0.5%	T3 Garlic 1.0%	T4 Onion 0.5%	T5 Onion 1.0%
Initial weight (IW) (g)	14.33±0.06	14.28 ±0.28	14.19 ± 0.9	14.22±0.22	14.24±0.24
Final weight (FW)(g)	182.34±0.56 c	237.39±0.36b	258.33±0.39 a	205.13±0.68 b	219.89±0.88 a
Initial length (IL) (cm)	9.62±0.94	9.48 ±0.92	9.54 ±0.93	9.43 ±0.05	9.51 ±0.05
Final length (FL) (cm)	20.12±0.05 c	23.36±0.6 a	24.31±0.06 a	21.16±0.05 b	22.14±0.05 a
Weight gain (WG) (g)	168.01±0.61 c	223.05±0.53b	244.14±0.43 a	190.91±0.86 b	205.64±0.28 a
Average Daily gain	1.50±0.005 c	1.99±0.005 b	2.18±0.003 a	1.70±0.033 b	1.83±0.088 a
Specific growth rate	2.27±0.01 c	2.51±0034 b	2.59±0.017 a	2.38±0.02 b	2.44±0.032 a
Condition Factor (K)	2.24±0.02 a	1.86±0.014 b	1.80±0.17 c	2.16±0.026 b	2.02±.018 c

Means followed by the same letters in each row for each trait are not significantly different (P>0.05).

3. Feed utilization:

The effects of different levels of garlic and onion extracts as natural phyt-oadditives in experimental diets on feed utilization of all male Nile tilapia (*O. niloticus*) fingerlings in all the experimental groups were illustrated in Table (4). There were greater improvements in the feed conversion ratio (FCR) of fish fed on dietary feed additive than the control fish. Feed intake increased with increasing garlic and onion extracts levels. Results illustrated in Table 4 also demonstrate a pronounced decrease in feed conversion ratio (FCR) values with increasing garlic and onion extracts levels. FCR values in T2 and T3 fish groups (1.22 &1.15), respectively relative to T1(control) and T4 groups (1.47 & 1.39, respectively). Values of PER and PPV at all garlic and onion inclusion levels were also elevated and the highest PER and PPV (3.31&73.95, respectively) were recorded in the T3 fish group. In general, growth rates and feed efficiency at T3 were not significantly different than those at T5. There were significant differences in growth performance across the different dietary feed additives. These results were in agreement with those obtained by Khattab *et al*. (2004), Gomes *et al.* (1993) and Degani *et al.* (1997).

Garlic and onion extracts as natural phytoadditives inclusion in fish diets led to mend in all feed utilization indices. Values of FCR and PER of Garlic and onion extracts groups were similar and significantly better when compared with the control group ($P<0.05$). Values of PPV at all Garlic and onion extracts inclusion levels were significantly higher when compared with the T1 group ($P < 0.05$) as presented in Table (4).

The present results suggest that dietary feed additives promoted the growth of Nile tilapia fingerlings where these additives enhance nutrient utilization, which was reflected in improvement of weight gain, FCR, PER, PE and SGR. Similarly, in catfish *C. gariepinus* (Turan and Akyurt, 2005), tilapia *Oreochromis niloticus* (Khattab *et al.*, 2004), olive flounder *Paralichthys olivaceus* (Cho and Lee, 2012) and shrimp *Peneaus indicus* (Olmedo Sanchez *et al*., 2009) feed additives in diets promoted growth and feed efficiency.

Table (4): Feed utilization of monosex (all-male)Nile Tilapia (*O. niloticus*) fingerlings fed on different levels of Garlic and Onion extracts.

Items	Experimental Fish Group 1			Group 2	
	T1 Control 0.0 %	T2 Garlic 0.5%	T3 Garlic 1.0%	T4 Onion 0.5%	T5 Onion 1.0%
Feed Intake (FI) (g)	268.67± 0.33 c	290.19± 0.74 b	294.56± 0.55 a	286.17±0.66b	288.14±0.46 a
Protein Intake (PI) (g)	67.16± 0.08 c	72.54± 0.18 b	73.64±0 .14 a	71.54±0.16 b	72.03±0.11 a
Feed conversion ratio	1.47± 0.07 a	1.22± 0.04 b	1.15± 0.03 c	1.39±0.06 b	1.31±0.04 c
Protein efficiency ratio	2.50± 0.01 c	3.07± 0.01 b	3.31± 0.008 a	2.67±0.07 b	2.85±0.005 a
Protein productive value	68.94± 0.47 c	69.96±0.26 b	73.95± 0.13 a	69.59± 0.35 b	71.63± 0.31b
Protein retained (PR%)	49.67± 0.10 b	50.02± 0.46 b	52.75± 0.16 a	49.78±0.22 a	49.67±0.11 a
Energy retained (ER) (Kc)	76.70± 0.90 a	67.71± 0.38 b	60.34± 1.83 c	71.61±0.46 a	71.66±0.39 a
Energy utilization (EU) (Kc)	0.84± 0.01 a	0.68± 0.03 b	0.59± 0.008 c	0.73±0.03 b	0.74±0.01 b
(HIS %)	3.85± 0.18 a	3.39± 0.19 b	3.34± 0.22 b	3.64± 0.26 b	3.59± 0.21 b

Means followed by the same letters in each row for each trait are not significantly different (P>0.05).

The growth promotion effect of diets supplemented with garlic or onion extracts can be attributed to the improved feed efficiency. This is in agreement with the results on Nile tilapia (**Shalaby *et al*., 2006**; Soltan and El-Laithy, 2008; Metwally, 2009), sterlet sturgeon (Lee *et al.*, 2014), and Seabass fry (Saleh *et al*., 2015), where the incorporation of different levels of garlic increased final weights and specific growth rates of fish. Soltan and El-Laithy (2008) reported that the incorporation of 1% garlic into diets improved survival rate of Nile tilapia. Similarly, better growth effects were found with higher incorporation levels of garlic meal in diets for Nile tilapia by Shalaby *et al*. (2006), who tested garlic incorporation levels from 10 g/kg to 40 g/kg diet, and recommended the incorporation of 3% dietary garlic to increase growth, reduction of total bacteria, and improvement of fish health and welfare. Similarly, Lee *et al*. (2014) suggested that dietary garlic powder incorporation of about 3% could positively affect growth performance and protein retention in fingerling sterlet sturgeon (*Acipenser ruthenus*). Farahi *et al.* (2010) used different levels (1%, 2% and 3%) of galic meal in rainbow trout diets and reported that the body protein was higher in 3% garlic group compared to the other experimental groups and that growth performance and fish health were improved with the addition of galic meal in trout diets. Metwally (2009) used diets containing garlic in three different forms; natural garlic (40g/kg diet, 4%), garlic oil capsules (Strongus®, pure garlic oil capsules; 250 mg/kg diet) and garlic powder tablets (32 g/kg diet, 3.2%), and reported that the dietary addition of garlic in any form can promote growth rate, decrease mortality and increase the antioxidant activity in fish.

In animal nutrition studies, the hepatosomatic index (HSI) is used as an indicator for the energy reserve status of the animal. Since the liver is a target for the metabolism in the fish body, the hepatosomatic index is an effective biomarker for the detection of hazardous effects derived from environmental factors (Pait and Nelson 2003). The highest HSI (3.85%) was found in fish fed T1 (control 0 %) among treatments ($p<0.05$). Although fish fed T3 (1% garlic) showed the lowest HSI of 3.34% among fish groups, it was not significantly different ($p>0.05$) from four other groups (T2,T3, T4, and T5). No mortality was recorded in all fish groups during the experimental period. HSI was lower in fish groups fed diets containing higher garlic and onion extracts (T2 and T5) than that of the group fed 0.0% control which showed the lowest growth performance. Therefore, such observed hepatomegaly in the T1 control group may partially reflect that HSI level deviates from the normal range in fingerling Nile tilapia because of high dietary lipid and that garlic and onion extracts diets administration may lead to a significant decrease in liver lipid. The present results showed that fish groups fed T1(0.0%) containing diets had significantly higher HSI than that of fish groups fed garlic and onion extracts. In contrast, Shalaby *et al.* (2006) showed that incorporation of garlic into Nile tilapia diets (10, 20, 30, and 40 g per kg diet) had no significant effects on HSI. Shalaby *et al.* (2006) found that supplementing garlic meal in Nile tilapia diets at increasing levels from 1 to 4 %, did not affect the HSI in percent. In contrast,. Similarly, Lee *et al.* (2014) also presented significantly lower HSI in sturgeon fed diets containing garlic powder than that of fish group fed diets without garlic inclusion.

Many authors recorded the positive effects of administrating garlic in diets on growth and feed utilization of many fishes including; African catfish, *Clarias gariepinus* (Agbebi *et al.*, 2013); rainbow trout, *Oncorhynchus mykiss* (Gabor *et al.*, 2012); and Nile tilapia, *Oreochromis niloticus* (Shalaby *et al.*, 2006; Metwally, 2009; Aly *et al.*, 2008). In the present study, enhancement in fish growth performance and feed utilization can be interpreted according to Khalil *et al*. (2001) who mentioned that garlic contains allicin, which promotes the performance of the intestinal flora, thereby improving digestion, and enhancing the utilization of energy. This would then lead to improved fish growth.

Parallel to the present results, growth enhancing properties of an onion extract based diet showed an increase in bodyweight gain as recorded by Bello *et al.* (2012) in African catfish (*C. gariepinus*) juveniles and also by Apines-Amar *et al*. (2012) in the brown-marbled grouper, *Epinephelus fuscoguttatus*.

Most of the positive effects of onions can be attributed to the presence of cysteine sulfoxide (CSO) with S-propenyl-CSO as the predominant S compound (Ostrowska *et al.*, 2004). Sulfur-containing compounds such as methyl sulfonate methane (MSM) have immunomodulation properties which are attributed to S being a component of the antioxidant enzyme Glutathione peroxidase. Additionally, the varied components of onion may exert further biological effects on enhancing growth through different mechanisms either separately or synergistically (Apines-Amar *et al*., 2012). Contrary to the previous results, growth performance of Olive Flounder, *Paralichthys olivaceus* was not affected by the use of onion powder **(Cho and Lee, 2012).**

4. Whole body compositions of monosex male Nile Tilapia

The biochemical composition of the whole body of Nile Tilapia (*O. niloticus*) fingerlings at the end of the feeding trial is shown in Table 5. Fish fed T3 showed the highest ash content (15.79 %), while that of fish fed T1 showed the lowest value (14.97 %). NFE content ranged from 10.02 % (T3) to 10.23% (T1 control). The proximate composition was significantly different (p<0.05) among the five experimental fish groups.

Table (5): Proximate composition (%) of the whole body of monosex (all-male) Nile Tilapia

Items	Initial	Experimental Fish Group 1			Group 2	
		T1 Control	T2 0.5% Garlic	T3 1.0% Garlic	T4 0.5% Onion	T5 1.0% Onion
Dry matter	21.54+0.23 c	28.34+0.26 b	29.16+0.18 a	29.2+0.20 a	29.26+0.31 a	29.56+0.14 a
Crude protein	46.52+0.42 c	51.10+0.11b	51.45+0.45 b	54.17+0.17 b	51.18+0.21 a	51.11+0.31 a
Crude fat	18.62+0.21 d	23.79+0.05 a	22.64+0.05 c	20.12+0.06 b	23.16+0.16 b	23.31+0.09 b
Ash	22.16+0.32 a	14.97+0.12 b	15.56+0.16 b	15.79+0.54 b	15.43+0.64 b	15.57+0.19 b
NFE	12.70+0.026a	10.23+0.18 b	10.12+ 0.02 b	10.02+0.05 b	10.13+0.15 b	10.15+0.03 b
Total	**100**	**100**	**100**	**100**	**100**	**100**

Means followed by the same letters in each row for each trait are not significantly different (P>0.05).

Feed additives significantly increased the level of whole-body protein content in Nile tilapia fingerlings fed garlic and onion extracts at all inclusion levels. This increase can be interpreted by that administration of garlic or onion extracts in diets may cause a rise in muscle free amino-acid contents which lead to an enhancement in protein synthesis. Ash content of fish had increasing trend but was not significantly different compared with control group (p > 0.05). Also, the NFE content of fish was significantly different compared with control group (p > 0.05). Increment in crude protein contents was recorded in *O. mykiss's* body when fed with diets that included 3% garlic (Gabor *et al*., 2010). In contrast, Cho and Lee (2012) used onion powder at different inclusion levels in the diet of Olive Flounder, *P. olivaceus* and did not record any significant differences in fish protein content between fish groups.

Farahi *et al*. (2010) showed that inclusion of garlic in the diet increased fish protein content and decreased whole body fat in fish. Banerjee and Maulik (2002) reported that compounds in garlic lower the activity of lipogenic and cholestrogenic enzymes in liver. On the other hand, compounds in garlic increase the excretion of acidic and neutral steroids that cause the excretion of cholesterol from the body content. Water-soluble sulfur compounds such as S-alLyl Sulfur Said cysteine (SAC) and Diallyl-di-sulfide (DADS) of garlic extract inhibit the synthesis of cholesterol (Yeh and Liu, 2001). As well as allicin of garlic causes inhibition of accumulation of fat in body (Elkayam *et al*., 2003).

Growth performance and feed utilization indices in the present work indicate an enhancement in growth and feed utilization for all fish groups fed garlic or onion extracts at all inclusion levels compared to the control fish group.

5. Hematologic and blood chemistry of monosex male Nile Tilapia.

Results Table (6) showed a significant variations (P<0.05) in Albumin, Total proteins, Haemoglobin,WBC and RBCs values among all inclusion levels or in relation to the control group either in fish fed garlic or onion extracts as feed additives. Results in Table (6) also showed a gradual elevation in Albumin (g/dl), total proteins, Hb, WBC and RBC content accompanying the increment in garlic and onion extracts inclusion level. The values were found to be significantly higher in T3 and T5 when compared to the T1 control group. Fish fed of Garlic and Onion extracts at all supplementation levels show a significant (P<0.05) increase in blood serum biochemical and haematological parameters values of Nile Tilapia (*O.*

niloticus) fingerlings comparing with the control group. An elevation in WBC values in all fish groups that had received garlic was apparent when compared with the control but it was significantly higher only in fish fed the T3 diet.

Table (6): Biochemical and Haematological parameters of Nile Tilapia Blood serum

Items	Experimental Fish Group1			Group 2	
	T1 Control 0.0	T2 Garlic 0.5%	T3 Garlic 1.0%	T4 Onion 0.5%	T5 Onion 1.0%
Albumin g/dl	1.81±0.005 c	2.00±0.005 b	2.23±0.005 a	1.91±0.005 b	2.01±0.008 a
Total proteins g/dl	4.10±0.05 c	4.62±0.05 b	5.25±0.05 a	4.57±0.18 b	4.80±0.05 a
Haemoglobin Hb (g/dl)	7.70±0.50 c	8.21±0.06 b	8.80±0.07 a	8.10±0.07 b	8.30±0.08 a
WBC(·103/ll)	36000±0.57c	43000±0.08b	51000±0.06a	41000±0.01b	47000±0.12b
RBC(·106/ll)	2.01±.07 c	2.47±0.20 b	2.56±0.01 a	2.30±0.01 b	2.45±0.35 a

Means followed by the same letters in each row for each trait are not significantly different (P>0.05).

The present study demonstrated that administration of Garlic and Onion extracts induced significant increases in albumin (g/dl), total proteins, hemoglobin, white blood cells and red blood cells content in fish fed diets containing garlic and onion extracts significantly differed from the control, in agreement with Martins *et al*., (2002). These values were highest in fish fed the 1% garlic diet, followed by those fed the 1% onion diet .These results agree with those of Shalaby *et al.* (2006). Total protein of plasma increased in treatments which agrees with the results of Hussein *et al*. (2001) but Shalaby *et al.* (2006) said it was not significantly high in treatments. The results of the study showed that use of garlic can effectively improve growth performance and fish health.

6. Economic efficiency:

The economic efficiency was calculated on the Total return (LE); Net return (LE/Treatment) and the % of the smallest value of net return , thus other costs were equal for the experimental groups. Results of the same table indicate that incorporation of Garlic and Onion extracts as feed additives in diets of Nile Tilapia (*O. niloticus*) fingerlings increased the costs of one kg of diet and the increase was more pronounced at higher incorporation level (1%) compared to the lower one (0.5%). Results of Table (7) reveal that Net return (LE/Treatment) for the control (T1 0.0 %); T2 (0.5% Garlic); T3 (1.0% Garlic) ; T4 (0.5% Onion) and T5 (1.0% Onion) groups were 116 ; 185.5 ; 193 ; 185.5 and 193 , respectively which indicate incorporation of Garlic and Onion extracts to growing Nile tilapia diets increased the % of the smallest value of net return by 159 (T2) ,166(T3) ,107 (T4) and 123 (T5 group) percent compare to the control group. There results indicate that Garlic and Onion extracts supplementation increased the costs of one kg of diet but this was compensated through the better gain in weight which resulted in reduction of gain costs. In general results of Table (7) indicate that incorporation of Garlic and Onion extracts at 1% level to growing Nile tilapia diets resulted in the highest reduction in gain costs due to diet.

Table 7: feed cost (L.E) for one Kg weight gain produced by fish fed Garlic and Onion extracts as feed additives in diets of Nile Tilapia (*O. niloticus*) fingerlings.

Items	T1 Control	T2 0.5% Garlic	T3 1.0% Garlic	T4 0.5% Onion	T5 1.0% Onion
A- Variable costs (LE/Trea)					
a. Nile Tilapia fingerlings	30	30	30	30	30
b. Feeds	322	348	353.5	343	346
c. garlic extract	-	62	106	-	-
c. Onion extract	-	-	-	34	69.5
d. Poutry manure	15	15	15	15	15
e. Triple supper phosphate	5	5	5	5	5
f. Urea	1.5	1.5	1.5	1.5	1.5
Total variable costs (LE)	373.5	461.5	511	428.5	467
B- Fixed costs (LE treat)					
a. Depreciation (materials & others)10%	37.5	45	51	428.5	46.5
b. Transport	20	20	20	20	20
Total fixed costs (LE)	57.5	65	71	62.5	66.5
Total operating costs (variable & fixed)	431	426.5	582	491	533.5
Returns					
Total return (LE) *	547	712	775	615.5	659.5
Net return (LE/Treatment)	116	185.5	193	185.5	193
% of the smallest value of net return	**100**	**% 159**	**% 166**	**107 %**	**123 %**

***** The economical evaluation of results was carried out according to market prices in 2014 in LE.
O. niloticus = 100/1000 fry. Tripe super phosphate = LE 1200/1000 kg.
Urea = LE 1650/1000 kg. Manure = LE 610/ l000 kg.
Fish feed (25% protein) = LE 3900 /1000 Kg.

The nutritional effects of the feed additive level used must be carefully determined and taken into account when selecting an economic inclusion level. Therefore, the recommended level of the inclusion is 1% Garlic and Onion extracts feed and this choice is based on the nutritional parameters and economical analysis.

Conclusion

The present study showed an enhancement in growth, feed utilization, fish protein content, some hematological parameters and enhancing in fish tolerance to environmental stressors when garlic or onion extract was added in Nile Tilapia (*O. niloticus*) fingerlings diets. The recommended levels for supplementation of dietary garlic or onion extracts in fish diets are 1% /kg. Results also showed a slight edge for garlic over onion.

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تاثير مستخلصات الثوم والبصل كمنشطات نمو طبيعية على اداء النمو والاستفادة من الغذاء والتركيب الكيميائى ومقاييس الدم لاصبعيات أسماك البلطى النيلى

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المستخلص

فى هذه الدراسة تم اختبار تأثير اضافة مستويات مختلفة من مستخلص الثوم ومستخلص البصل . وقد تم اختبار مستويين من مستخلص الثوم والبصل وهما 0.5 ، 1% لكل منهما بالأضافة إلى عليقة المقارنه التى لم يضاف لها اى مستخلصات وبالتالى تم تغذية اصبعيات أسماك البلطى فى هذه الدراسه على واحدة من خمس علائق. واستخدمت فى هذه الدراسة 750 من أصبعيات سمك البلطى النيلى وحيد الجنس (ذكور) حيث وزعت الأسماك عشوائيا على 15 قفص بمعدل 50 سمكة لكل قفص (3×3×1 م) وهذه الأقفاص وضعت فى حوضين ترابيين واستمرت تغذية الأسماك على هذه العلائق لمدة 120 يوم وفى نهاية التجربة تم دراسة تاثير العلائق المختبره على على اداء النمو والإستفادة من الغذاء والتركيب الكيميائى ومقاييس الدم لأصبعيات أسماك البلطى النيلى حيث حققت الأسماك التى تغذت على العلائق المحتوية على 1% مستخلص الثوم او البصل قد أعطت أفضل مقاييس للنمو (وزن الجسم النهائى، الزيادة فى وزن الجسم، معدل النمو النوعى) . وقد أوضحت النتائج ان هذه العلائق قد اعطت افضل مقاييس للإستفاده من الغذاء وصفات الدم وكذلك العائد الإقتصادى ولذلك تنصح هذه الدراسة بإستخدام مستخلصات الثوم أو البصل فى علائق اسماك البلطى النيلى كمنشطات نمو طبيعية هامه.