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EVALUATION OF DIETARY ADDITION OF GARLIC (*ALLIUM SATIVUM* L.) LOBES ON GROWTH PERFORMANCE, FEED UTILIZATION, AND PHYSIOLOGICAL RESPONSES OF *OREOCHROMIS NILOTICUS*, FINGERLINGS

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Abstract

Garlic (*Allium sativum*) is one of the earliest known medicinal plants and has been used to improve growth and resistance of a number of livestock and fish. The present study was carried out to evaluate the effects of dietary supplemented by dried garlic (*A. sativum*) lobes (DGL) on growth performance, feed utilization, chemical composition of the whole fish body, and physiological parameters of monosex Nile tilapia, *Oreochromis niloticus* fingerlings for 60 days. Fish with average body weight (17.14 ± 0.35) were randomly distributed into four treatments (0, 1, 2, 3% DGL / kg diet).Results revealed that 1% DGL was the best treatments, which gave the highest significantly ($P \leq 0.05$) values in growth performance, feed utilization, chemical composition of the whole fish body and hematological and biochemical parameters among all treatments. So, it is recommended that the inclusion of 1% DGL / kg diet as feed additives of all male monosex *O. niloticus* are more useful for improving their growth performance, feed utilization, chemical composition of the whole fish body and physiological responses.

Keywords: Nile tilapia, Garlic, Growth performance, Physiological parameters.

INTRODUCTION

Herbal medicinal products are widely used around the world (Jordan *et al*., 2010). A growing interest has emerged in using herbs in animal feeds by both researchers and feed companies (Logambal and

Michael, 2000). Since ancient times, garlic (*Allium sativum*)has been used worldwide as a seasoning, spices, and herbal remedies or folk medicine (Rivlin, 2001).Garlic has been used in ancient Egypt in treating many ailments such as heart diseases, headache, bites worms and tumors (Aly *et al*., 2008), as well as ancient Chinese medicine indications that garlic were used to treat, improve male potency and enhance sexual prowess (Kahn, 1996). It is known to possess a vast variety of biological functions such as antithrombotic (Block *et al*., 1986), anticancer (Mousa, 2001), antioxidant (Wu*et al.*, 2001), improving immune-system (Kang*et al.*, 2001), lower serum lipid and glucose levels (Lawson *et al.*, 2001) and blood pressure (Ali *et al.*, 2000). Also, Agarwal (1996) revealed that garlic has been widely used as foodstuff since antiquity. Dietary garlic has a potential effects as a growth promoter or immune, and physiological enhancer agent for fish, *Clarias gariepinus* (Thanikachalam *et al*., 2010); *Oncorhynchus mykiss* (Nya and Austin, 2011) and *Cyprinus carpio* (Naeiji *et al*., 2013).Partially, dietary garlic not only improved the growth performance (Soltan and El-Laithy, 2008; Mahfouz *et al*., 2009),but also enhancement both of the immune-system (Ahmed *et al*., 2008;Metwally, 2009), and physiological responses (Diab *et al*., 2002; Shalaby *et al*., 2006) of *Oreochromis niloticus*.

Bioactive components of garlic, including several sulfur-containing compounds such as Allicin (responsible for the distinctive odor), diallyl-disulfied, diallyl-trisulfied and others, which are responsible for most of the pharmacological properties of garlic (Amagase *et al*., 2001). Allicin (*dially thiosulfinate*) is the most abundant compound representing about 70% of all thiosulfinates present, or formed in crushed garlic. Also, garlic is a rich source of calcium (Ca), phosphorus (P), zinc (Zn), iron (Fe) ; has a high content of carbohydrates and as a consequence a high nutritive value; contains silica (Si), sulfate (S) salts, B_1, B complex, A and C vitamins (Han *et al*., 1995).Therefore, the objectives of the present work were to evaluate the effects of graded levels (0, 1, 2 and 3% / kg diet) of dried garlic (*A. sativum*) lobes(DGL) on growth performance, feed and nutrients utilization, fish carcass composition, and hematological and biochemical parameters of male monosex Nile tilapia *O. niloticus* for 60 days in an indoor feeding experimental diets.

MATERIALS AND METHODS

Fish and experimental management:

This study was conducted in Fish Research Laboratory, Animal Production Department, Faculty of Agriculture, Mansoura University, Al Dakahlia Governorate, Egypt. Fish were stocked in rearing tank for two weeks as adaptation period during this time they were offered the basal diet. A total of 84 all male monosex *O. niloticus* fingerlings at average initial body weight of 17.14 ± 0.35 g were randomly distributed. The fish were stocked at rates of 7 fish / glass aquarium (90 x 50 x 40 cm), where three aquaria were referred to every dietary treatment. Each aquarium was supplied with 108 L dechlorinated tap water and an air stone connected with electric compressor. The replacement of the aquaria water was done partially every day to re-new the water and to remove the wastes. Light was controlled by a timer to provide a 14h light: 10h dark as a daily photoperiod.

Dietary treatments:

The ingredients and chemical composition of a basal diet (BD) are shown in Table 1.The lobes of garlic were cleaned and shade-dried in a drying oven at 50°C for 72 hours. Dried garlic lobes (DGL) were milled into fine particle size (< 250 µm), and kept in a dry, air-tight transparent plastic container. All ingredients and additive were milled and mixed, then pressed by manufacturing machine (pellets size 1mm). DGL were added in experimental diet at levels 0, 1, 2 and 3% / kg diet. During the experimental period (60 days), the fish were fed a rate of 3% of the live body weight daily, six days a week and twice daily, at 8.0 am and 2.0 pm.

The fish were weighed every two weeks by a digital scale (accurate to ± 0.01 g) to adjust their feed quantity every aquarium according to the actual body weight changes.

Table 1. Ingredients and proximate chemical analysis (% on dry matter basis) of the experimental diet

Ingredients	%
Fish meal (65%)	22
Soybean meal (44%)	27
Yellow corn	21
Wheat bran	20
Corn oil	3
Molasses	5
Vit. & min premix[1]	2
Nutrients composition	
Dry matter (DM, %)	89.89
Crude protein (CP, %)	30.13
Ether extract (EE, %)	4.42
Ash (%)	11.91
Total carbohydrate (%)	53.54
Gross energy (GE) (Kcal/100 g DM)[2]	426.2
Protein/energy (P/E) ratio (mg CP/Kcal GE)[3]	70.69

[1]Vit.& min premix (each 1 Kg premix contains; Vit. A, 12000,000 IU; Vit.D_3, 3000,000 IU; Vit.E, 10,000 mg; Vit.K_3, 3000 mg; Vit.B_1 200 mg; Vit.B_2, 5000 mg; Vit.B_6, 3000 mg; Vit. B_{12}, 15 mg; Biotin, 50 mg; Folic acid 1000 mg; Nicotinic acid 35000 mg; Pantothenic acid 10,000 mg; Mn 80g; Cu 8.8g; Zn 70 g; Fe 35 g; I 1g; Co 0.15g and Se 0.3g).

[2]GE (Kcal/100 g DM) = (CP x 5.64) + (EE x 9.44) + (Total carbohydrate x 4.11) calculated according to **NRC(1993)**.

[3] P / E ratio (mg protein / Kcal gross energy) = CP / GE × 1000

Experimental procedures:

At the end of the experiment, the remained fish were sampled from each aquarium and kept frozen (- 20°C) for chemical analysis. The chemical analysis of the basal diet and whole fish body were carried out according to AOAC (2004). Water quality parameters included temperature (via a thermometer), pH (using Jenway Ltd., Model 350-pH-meter) and dissolved oxygen (using Jenway Ltd., Model 970- dissolved oxygen meter) were measured weekly according to Abdelhamid (1996). All tested water quality was suitable for rearing Nile tilapia (*O. niloticus*) fingerlings as cited by Abd El-Hakim *et al*. (2002). Since, water temperature ranged between 24 and 28^{o}C, pH values 6.55 – 7.60 and dissolved oxygen 5.50 – 8.80 mg/l. Body weight of individual fish was measured biweekly to calculate the growth performance parameters such as final weight (FW, g); total weight gain (TWG, g); average daily gain (ADG, mg/fish/day); relative growth rate (RGR); specific growth rate (SGR, % / day) and survival rate (SR, %), and feed utilization parameters such as feed intake (FI, g / fish); feed conversion ratio (FCR); feed efficiency (FE, %); protein efficiency ratio (PER); protein productive value (PPV, %) and energy utilization (EU, %) according to Abdelhamid (2000).

Blood samples were collected from caudal vein in the caudal peduncle at the end of the experiment. Adequate amounts of whole blood in small plastic vials containing heparin were used for the determination of hemoglobin (Hb) by using commercial kits (Diamond Diagnostic, Egypt). Also, total red blood cells count (RBCs) and total white blood cells count (WBCs) were counted on an A_o Bright -Line Hämocytometer model (Neubauer imroved, Precicolor HBG, Germany), as well as the packed cell volume (PCV %) was measured according to Stoskopf (1993). Other blood samples were collected and transferred for centrifugation at 3500 rpm for 15 min to obtain blood plasma for determination of the total protein according to Gornall *et al*. (1949), albumin according to Weichsebum (1946) and globulin by difference according to Doumas and Biggs (1972).

Statistical analysis:

All data were statistically analyzed using general liner models (GLM) procedure according to SAS (2001) for users guide (SAS version 9.2) to detect the overall effects of treatments. All ratios and percentages were arcsine-transformed prior to statistical analyses. The differences between mean were compared for the significance (P ≤ 0.05) using Duncan's multiple rang test (Duncan, 1955).

RESULTS

Growth performance:

Data in Table 2 showed that fish fed DGL were significantly (P ≤ 0.05) improved all growth performance parameters compared to the control (0 %). Fish fed 1% DGL was the highest significant (P ≤ 0.05) in all growth performance parameters among other treatments. However, increasing levels of DGL up to 1% caused significantly (P ≤ 0.05) decreased of all growth performance parameters. No significant (P ≥ 0.05) differences in SR % among all levels.

Table 2. Growth performance parameters of male monosex *O. niloticus* fed different levels of dried garlic lobes.

DGL Level (% / kg diet)	FW (g)	TWG (g)	ADG (mg/fish/day)	RGR (%)	SGR (% / day)	SR (%)
0	34.15^{c}	17.01^{c}	0.282^{c}	99.20^{c}	1.15^{c}	92.87
1	38.57^{a}	21.42^{a}	0.358^{a}	125.0^{a}	1.35^{a}	100.0
2	35.31^{b}	18.17^{b}	0.303^{b}	106.0^{b}	1.20^{b}	92.30
3	34.45^{c}	17.31^{c}	0.289^{c}	101.0^{c}	1.17^{c}	94.65
± SEM	0.163	0.163	0.002	0.948	0.008	0.345
***P*- value**	0.001	0.001	0.001	0.001	0.001	0.283

Mean in the same column having different small letters are significantly different ($P \leq 0.05$).
FW, Final weight; TWG, total weight gain; ADG, average daily gain; RGR, relative growth rate; SGR, specific growth rate; SR, survival rate, and SEM, standard error of means.

Feed utilization:

Feed utilization parameters (FI, FCR, FE, PER, PPV and EU) of *O. niloticus* illustrated in Table 3. Results revealed that the addition of 1% DGL led to improve significantly (P ≤ 0.05) of FCR, FE, PER, PPV and EU compared to other treatments. While, fish fed 2% DGL gave the highest FI (P ≤ 0.05) among other levels. On the other hand, results indicated that the increasing levels of DGL up to 1% had impaired effects (P ≤ 0.05) on all feed utilization parameters except FI.

Table 3. Feed utilization parameters of male monosex *O. niloticus* fed different levels of dried garlic lobes.

DGL Level (% / kg diet)	FI (g / fish)	FCR	FE (%)	PER	PPV (%)	EU (%)
0	28.51[c]	1.68[b]	59.66[bc]	1.98[bc]	29.25[b]	12.39[b]
1	29.16[b]	1.36[c]	73.47[a]	2.44[a]	35.43[a]	14.01[a]
2	30.14[a]	1.66[b]	60.30[b]	2.00[b]	26.42[c]	11.31[c]
3	29.89[a]	1.73[a]	57.92[c]	1.92[c]	24.79[d]	11.02[c]
± SEM	0.181	0.014	0.593	0.020	0.408	0.144
P- value	0.001	0.001	0.001	0.001	0.001	0.001

Mean in the same column having different small letters are significantly different (*P*≤0.05).
FI, feed intake; FCR, feed conversion ratio; FE, feed efficiency; PER, protein efficiency ratio; PPV, protein productive value; EU, energy utilization, and SEM, standard error of means.

Chemical analysis of the experimental fish:

The chemical analysis of the whole fish body at the start and the end of the experiment was presented in Table 4. Dry matter (DM), crude protein (CP) and Ash at the end were highest than at the beginning of the experiment, while the ether extract (EE) and energy content (EC) decreased at the end than at the beginning of the experiment. Also, results showed that fish fed 1% DGL recorded the lowest significant (P ≤ 0.05) values of EE and EC, and the highest significant value of CP among other levels. On the other hand, the control group was the highest significant (P ≤ 0.05) in DM compared with all levels. However, no significant (P ≥ 0.05) differences in ash contents among all levels of DGL.

Table 4. Chemical composition of the whole fish body of male monosex *O. niloticus* fed different levels of dried garlic lobes.

DGL Level (% / kg diet)	DM (%)	% On dry matter basis			
		EE	Ash	CP	EC (Kcal / 100 g)
At the start of the experiment					
	14.10	22.32	16.71	60.97	554.59
At the end of the experiment					
0	16.60[a]	11.55[b]	18.12	70.34[b]	505.7[a]
1	16.03[b]	7.82[c]	17.95	74.23[a]	492.5[b]
2	15.71[b]	12.09[ab]	18.16	69.75[bc]	507.5[a]
3	15.74[b]	13.43[a]	18.64	67.94[c]	509.9[a]
± SEM	0.093	0.456	0.463	0.681	2.989
***P*- value**	0.005	0.001	0.753	0.001	0.013

Mean in the same column having different small letters are significantly different ($P \leq 0.05$). DM, Dry matter; EE, ether extract; CP, crude protein; EC, energy content and SEM, standard error of means.

Hematological and blood biochemical parameters:

Data of hematological and blood biochemical parameters were illustrated in Figure 1. Results indicated that the addition of 1% DGL gave the highest significant ($P \leq 0.05$) values of Hb concentration, RBCs count, PCV% and WBCs count compared to other levels. While, the control group recorded the highest significant ($P \leq 0.05$) values in blood indices (MCV, MCH and MCHC) among other groups. On the other hand, fish fed 1% DGL gave the highest significant ($P \leq 0.05$) values in total protein, albumin, and globulin among all levels. However, no significant ($P \geq 0.05$) differences of albumin / globulin ratio among all DGL levels.

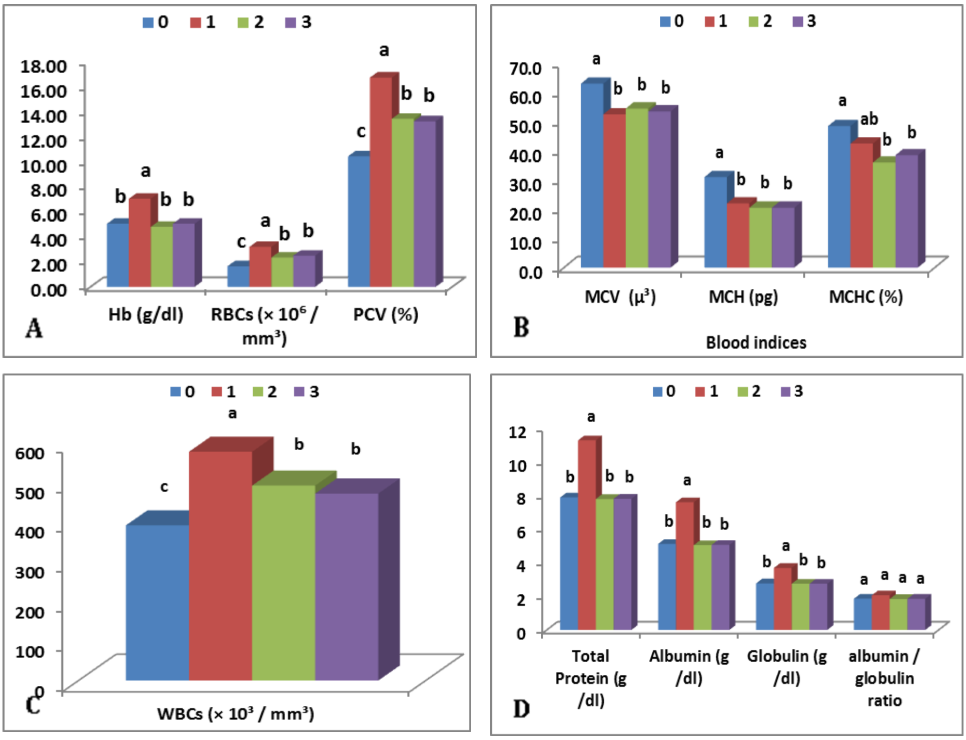

Fig. 1. Hematological and blood biochemical parameters of male monosex *O. niloticus* fed different levels of dried garlic lobes. Mean having different small letters are significantly different ($P \leq 0.05$). Hb, hemoglobin; RBCs, red blood cells; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBCs, white blood cells.

DISCUSSION

Attempts to use the natural materials such as medicinal plants could be widely accepted as feed additives to enhance efficiency of feed utilization and animal productive performance (Mohamed *et al.*, 2003). In the present study, results indicated that addition of dietary DGLled to significantly ($P \leq 0.05$) improve of all growth performance and feed utilization parameters of *O. niloticus* compared to the control group. The present findings are in agreement with those obtained by Khattab *et al.* (2004) and Shalaby *et al.* (2006). In this respect, Soltan and El-Laithy

(2008) reported that the FW, SGR, FI, and FCR of *O. niloticus* increased significantly with increasing levels of *A. sativum*. In addition, Aly *et al.* (2008) and Aly and Mohamed (2010) found the same results of *O. niloticus* after feeding with garlic (10 and 20 g/kg diet fed) for 8 months compared to the control group of fish fed free diet of garlic. Likewise, Abdel-Hakim *et al.* (2010) found that the highest growth performance parameters were recorded with the group fed on the basal diet supplemented with fresh garlic at 3g/kg level, while the diet supplemented with dried garlic (5g/kg) recorded significantly the best feed efficiency parameters compared with the control group. In contrast, negative effects have been reported by Ndong and Fall (2007) who found that hybrid tilapia fed a garlic-supplemented diet at 0.5 and 1% exhibited no improvement in growth compared to those fed a control diet after 2–4 weeks. The differences of results obtained from previous studies and the current study may be due to the difference in the experiments conditions, size and age of fish, addition of garlic…etc.

The positive effects in fish growth performance and feed utilization attributed to the bioactive components of garlic including sulphur containing compounds, such as allin, diallylsulphides, and allicin (Amagase and Milner, 1993).In addition, allicin has an intense garlic flavor with a strong stimulatory effect on olfaction in most aquatic animals, including *Pelodiscus sinensis*, *Ctenopharyngodon idellus*, *Cyprinus carpio*, *Carassius auratus*, and *Oreochromis niloticus* (Lee and Gao, 2012). On the other hand, sulfur compounds in garlic are considered as active antimicrobial agents and improve immunity and therefore stimulate growth (EL-Afify, 1997). Also Sivam (2001) suggested that the improvements due to garlic may due to its antimicrobial, antioxidant, and antihypertensive properties, which reflected to improved growth performance and feed utilization.

In the present study, fish fed 1% of DGL significantly ($P \leq 0.05$) increased the protein content in their whole body; whereas the total lipid and ash contents significantly decreased in the same group compared to the other groups. These results are in agreement with those obtained by Khattab *et al.* (2004); Shalaby *et al.* (2006); Metwally (2009);Mehrim (2009). Likewise, Luo *et al.* (2008) found that garlic could improve the flesh quality of grass carp, *Ctenopharyngodon idellus*. In addition, Aly *et al.* (2008) reported that the post-harvest flesh quality and shelf-life of *O. niloticus* fed a garlic-supplemented diet were improved. On the other hand, there were no significant changes in fish body composition caused by different garlic levels (Diab *et al*., 2002). Also, Abdel-Hakim *et al.* (2010) indicated that supplementation of *O. niloticus* diets with fresh or dried garlic had no significant effects on moisture; dry matter and ash contents of fish whole bodies, while it released significant effects on the whole body protein and fat compared to the control group. The promising findings in the present study on chemical composition of *O. niloticus* may be due to the dietary supplementation with 1% DGL, which was enhanced plasma total protein (Fig. 1d), as well as may be increased the growth performance (Table 2), and improved PER and PPV (Table 3).

Knowledge of the haematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes. Normal ranges for various blood parameters in fish have been established by different investigators in fish physiology and pathology (Xiaoyun *et al.*, 2009). Results in Figure1 showed that fish fed 1% DGL significantly ($P \leq 0.05$) increased Hb, RBCs, PCV, WBCs, total protein, albumin, globulin and albumin / globulin ratio compared to the control group. These results are in agreement with those obtained by Martins *et al.* (2002) and Shalaby *et al.* (2006) who verified that addition of *A. sativum* to fish diets increased erythrocytes number, hemoglobin content, hematocrit value, leucocytes, and thrombocytes. Also, Ndong and Fall (2007) found that hybrid tilapia fed a garlic-supplemented diet at 0.5 enhanced in total leucocyte count, respiratory burst, phagyctic activity, phagocytic index and lysozyme activity. Furthermore, Nwabueze (2012) found that RBC, PCV and Hb were significantly higher in *Clarias gariepinus* fed 0.5% garlic supplemented diets than other concentrations.

These hopeful current results in hematological and blood biochemical parameters led to increase the immune responses and healthy status of fish, which reflected to improve the growth performance, survival rate and feed efficiency. Where, *A. 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 (Jeorg and Lee, 1998).Also, *A. sativum* has been antioxidant properties, which could have a protective nature against gastrointestinal neoplasias, against blood clots (anti-platelet action) due in part to the compounds alliin and ajoene, which have fibrinolytic activity. Ajoene inhibits thromboxane synthesis through the inhibition of the cyclo-oxygenase and lipoxygenase enzymes(Schulz *et al.,* 2004).In addition, S-allyl cysteine, present in the crushed garlic, was found to inhibit tumor metabolism and enhance the immune response (Sumiyoshi, 1997). The allyl sulfides enhance the glutathione s-transferase enzyme system, which through their dependent biochemical pathway enhance the liver's detoxification of carcinogenic substances.The *allium* pecies show immune enhancing activities that include promotion of lymphocyte synthesis, cytokine release, phagocytosis and natural killer cell activity (Kyo *et al.,* 1998).

CONCLUSIONS

From the foregoing results, it could be concluded that dietary supplementation of dried garlic (*A. sativum* L.) lobes is useful at levels 1% / kg diet for enhancing growth performance, feed utilization, chemical composition of the whole fish body, hematological and blood biochemical parameters of Nile tilapia *O. niloticus*.

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تقييم الإضافة الغذائية لفصوص الثوم على أداء النمو والإستفادة الغذائية والإستجابة الفسيولوجية لإصباعيات البلطى النيلى

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الملخص العربى

يعتبر الثوم من أقدم النباتات الطبية المعروفة، وقد تستخدم لتحسين النمو والمقاومة المناعية لعدد من الحيوانات المزرعية والأسماك. وقد أجريت هذه الدراسة لتقييم الإضافة الغذائية لفصوص الثوم المجففة على أداء النمو، والإستفادة الغذائية ، والتركيب الكمياوى، والإستجابة الفسيولوجية لأسماك البلطي النيلي وحيدة الجنس لمدة ثمانية أسابيع. تم إستخدام أصباعيات متوسط وزنها ١٧.١٤ ± ٠.٣٥ والتى وزعت عشوائيا على أربعة معاملات (٠، ١، ٢، ٣ ٪ من فصوص الثوم المجففة / كجم عليقة). وأظهرت النتائج أن إضافة ١٪ من فصوص الثوم المجففة / كجم عليقة كانت أفضل المعاملات، والتي أعطت أعلى القيم معنوياً لأداء النمو، والإستفادة الغذائية، والتركيب الكيميائي لجسم الأسماك كله والمقاييس الهيماتولوجية والبيوكيميائية للدم مقارنة بباقى المعاملات. لذلك، توصى الدراسة بإضافة ١٪ من فصوص الثوم المجففة / كجم عليقة كإضافة غذائية لأسماك البلطي النيلي وحيد الجنس لتحسين أداء النمو، الإستفادة الغذائية، والتركيب الكيميائي لجسم الأسماك، والاستجابات الفسيولوجية.