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Incorporation of garlic meal (*Allium sativum*) as natural additive to enhance performance, immunity, gonad and larval survival of Nile tilapia (*Oreochromis niloticus*) broodstock

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ABSTRACT

A 60 dyes feeding trial was performed to detect the growth and production indices of broodstock Nile tilapia (*Oreochromis niloticus*), which fed 4 diets comprised different levels of garlic meal (0 , 1, 2 , and 3%). Four iso-protenic and iso-lipidic diets were formulated to be containing 30.25% CP and 19.25MJ/Kg diet and each diet was assimilated in three replicates. Sixteen broodstock fish with an (initial body weight=221.0±1.31g) were indiscriminately selected from the stock acclimatized fish and distributed through 12 circular cement pound $2m^3$ in equal number (n=4) as 3 female (♀):1 male (♂). The water quality values were in the optimal recommended ranges for this species, where dissolved oxygen (5.5±1.2 mg dL^{-1}), temperature (26 ±1.5°C) and pH (7.8±0.5. The current results showed significant differences ($p<0.05$) in growth performance parameters, with the group fed 2% garlic powder diet, followed with 3%, 1% and control groups, respectively. Improved in feed using in terms of (FCR, PER and NPU%) were obtained with 2% garlic meal diet compared to the rest of diets. The fish immunological parameters represented a significance differences in total protein, glucose and lysozyme activity between tested diets. However, insignificance results wereevident in albumin values. The highest significance values ($p<0.05$) in total protein, glucose and lysozyme activity between tested diets were obtained with the group of fish fed 2% garlic powder. In the same manner, the highest productive performance from Relative fecundity, Absolute fecundity and Hatchability rate were recorded in 2% diet. The proximate composition of fish include Dry matter, Crude protein, Crude lipid and Ash % showed no significance different (*P>0 .05*) between different dietary levels of garlic powder. The present results indicated that dietary implication of 2% garlic powder improve growth performance, nutrient using, immune activity, productivity indices and larval rearing rate of Nile tilapia (*O. niloticus*) broodstock.

Key words: Nile tilapia (*Oreochromis niloticus*),garlic powder, Growth performance, Gonad maturation, hatchability rate, larval survival, immune activity.

INTRODUCTION

From the cultivated culture species in the world Nile tilapia has raised dramatically in recent years as a results of these advantages: rapid growth, tolerant for a wide ambit of environmental status, more resistance to stress and disease, and its ability to reared in captivity (El-Sayed *et al.*, 2007; Ng and Romano,2013). However, researches on reproductive biology of this species, through feeding effective on reproductive parameters, eggs quality and survival of larvae are still scarce. Diet contents can influence reproductive physiology, such as ovarian follicle maturity, improve egg quality, hatchability, survival and larval formation (Furuita *et al.*,2009). Moreover, it's evident that the transfer of nutrients through gametes is necessary for normal growth of fish embryos (Navarro *et al.*,2010).

Several studies concluded that the eggs proximate composition in fish is related to nutrients stored in the egg, where it must

be contains sufficient nutrients for embryonic development (Sandnes *et al.*,1984 ; Craik, 1985). Good-quality eggs are show low levels of mortality during fertilization, hatch, and first feeding and those consequently produce the fastest-healthiest growing fry and older fish (Bromage *et al.*,1992).

Insufficient Food showed a decrease in total fecundity with these species: tilapia *Tilapia mossambica* and *Tilapia zillii* (Coward and Bromage,1999), rainbow trout, *Oncorhynchus mykiss* (Springate *et al.*,1985).

The feeding status of broodstock is well known to have direct effects on reproductive performance and off-spring quality (Izquierdo *et al.*,2001; Lupatsch *et al.*,2010). The nutrition of broodstock and larval are amongst most scares areas of fish nutrition researches even though its importance to apply more trials to develop it. Recently their is some studies on the effect of micronutrients, such as vitamin E, vitamin C, carotenoids and certain trace elements and other feed additives on brood-stock performance, but the most-studied area seems to be in dietary lipids and fatty acids (Holt, 2011,Tocher and Glencross,2015 and NRC, 2011).

Garlic was used as a condiment in traditional medicine due to its highly nutritive value, plentiful in phosphorus, calcium and carbohydrates. Additionally its include many important compounds such as silicate, iodine salts, and sulfur salts, which have a positive effects on the function of circulatory system, skeletal system and mange liver diseases. Moreover, it contains many vitamins comprise vitamins A, B complex and C likewise linoleic acid (Dra˘gan *et al.*,2008).

Immuno-stimulants are naturally occurring substances that regulate the immunity system by instigation the host's impedance against diseases that in most circumstances are caused by pathogens, and also they have widely applying to a large degree in aquaculture(Ai *et al.*,2007; Ringo *et al.*, 2010). Garlic was also used as a growth stimulant in tilapia (Diab *et al.*,2002; Shalaby *et al.*,2006; Mesalhy *et al.*,2008, Soltan and El-Laithy, 2008 ; Metwally, 2009; Abdel-Hakim *et al.*,2010). Moreover, garlic can generate antifungal, antioxidant, antiviral, antimicrobial and antiparasitic effects, also is able to boost the immune system (Harris *et al.*,2001).

The present study aimed to evaluate the effect of different dietary ratios of galiric meal as natural feed additive on growth performance, nutrient efficiency, body composition, reproductive performance and larval survival of broodstock Nile tilapia (*O. niloticus*).

MATERIALS AND METHODS

Experimental conditions

Broodstock of Nile tilapia (*O. niloticus*) were obtained from the private hatchery located in Fayoum Governorate, Egypt. Fish were transported to Fayoum Aquatic Research Station Labs. Fish were stocked in the Lab ponds and fed with the tested diets twice daily for 15daysto acclimatize fish for the diets before the study. After 24 hour of starvation, 16 (♀+♂) broodstock fish with mean initial body weight=221.0±1.31g were distributed through 12 circular cement pound $2m^3$ in equal number (n=4) as 3 female (♀):1 male (♂). Fish were hand-fed the experimental diets to apparent satiation twice daily (9:00 a.m. and 3:00 p.m.) and weighting each two weeks to modify the amount of feed consumption. The water system includes two pumps and upstream sandy filter units at a point between the water source (Earthen pond) and tanks. The pumps were drowning the water to the storage tanks and forced it through polyvinyl chloride (PVC) tubes into the rearing cement ponds in open system.

Water quality criteria were in the optimal ranges for *O. niloticus*, where dissolved oxygen (5.5±1.2 mg dL^{-1}), temperature (26 ±1.5°C) and pH (7.8±0.6) as recorded by (Boyd, 1979).

Experimental diets and feeding management

Feeding treatments consisted of 4 iso-proteinc and iso-energetic diets are shown in (Table 1). Garlic was used in the diets in the form of Allicorn meal, which producing in China. The premix is use as: 0,1,2 and 3%.The ingredients were milled, weighed, homogenized and ground into fine powder through a 150-µmmesh before pelleting. The dough was pelleted by California pelleting unit with a size of 2mm diameter. Biometric measurements were taken every 15 days in order to evaluate growth rate. The experimental was done in May and June, 2017 for a duration period of 60 days.

Table 1. Feed ingredient and proximate analysis of the experimental diets (% DM basis).

Ingredients	Garlic level			
	0%	1%	2%	3%
Fish meal (70% CP)	10	10	10	10
Soybean meal (48%CP)	20	20	20	20
Gluten meal (36%CP)	25	25	25	25
Yellow corn	15	15	15	15
Wheat bran	20	20	20	20
Garlic powder	0	1	2	3
Microcrystalline cellulose	3	2	1	0
Fish Oil	5	5	5	5
Vitamin. mineral mix[1]	2	2	2	2
Proximate analysis (DM,basis)				
Dry matter	92.1	92.1	92.1	92.1
Crude protein	30.25	30.25	30.25	30.25
Crude lipid	10.28	10.28	10.28	10.28
Nitrogen free extract	46.79	46.79	46.79	46.79
Crude fiber	3.68	3.68	3.68	3.68
Ash	8.82	8.82	8.82	8.82
Gross energy(MJ kg^{-1} diet)[2]	19.54	19.54	19.54	19.54
ME (MJ kg^{-1}diet)[3]	16.24	16.24	16.24	16.24

1-Vitamin, mineralpremix (vitamin IUkg^{-1} diet and mineral mg/ Kg^{-1} mixture):L-ascorbic acid monophosphate, 120.0; L-α-tocopheryl acetate,20.0, thiaminydrochloride, 4.0,riboflavin, 9.0, pyridoxine hydrochloride, 4.0, niacin, 36.0,D-pantothenic acid hemicalcium salt, 16.6; myoinositol, 42.0; D-biotin, 0.4, folicacid,0.6, menadione,0.1, retinylacetate,1.2, cholecalciferol, 0.06, cyanocobalamin,0.01, $MgSO4{\cdot}7H_2O$, 80.0,$NaH_2PO_42H_2O$,370.0,KCl,130.0, $FeSO_47H_2O$,40.0,$ZnSO_47H_2O$,20.0,Ca-actate, 356.5,$CuSO_4$, 0.2,$AlC_{l3}{\cdot}$ $6H_2O$, 0.15, $Na_2Se_2O_3$, 0.01, $MnSO_4H_2O$, 2.0, $CoC_{l2}6H_2O$,1.0.

2-Gross energy (MJ Kg^{-1} diet) was estimated by using the following calorific values: 23.9, 39.8 and 17.6 KJ g^{-1} diet with protein, crude lipid and nitrogen free extract, respectively (NRC,2011).

3-The metabolizable energy(MJ Kg^{-1} diet) of the experimental diets were calculated as 18.9, 35.7 and 14.7 KJ g^{-1} diet with protein, crude lipid and nitrogen free extract, respectively(NRC,2011).

Growth evaluation

Growth performance and diets efficiency were assessed by using these equations:

- Body gain=[Final body mass-initial body mass].
- Specific growth rate (SGR%)=100×(Ln final weight-Ln initial weight)/time.

- Condition factor (CF g/cm^{-3}) = (wet weight)/(total length^{-3})×100.
- Feed conversion (FCR) = (feed given per fish)/(weight gain per fish).
- Protein efficiency ratio (PER)=(weight gain per fish)/(protein intake per fish).
-Net protein Utilization (NPU%)=100 (Final body protein-initial body protein/protein intake).
- Hepatosomatic index (HSI %) = [liver weight (g)]/[fish weight (g)]×100.

Reproductive management

Reproduction was happen over the course of 4 weeks, with all females being investigated every week. Three females (♀) with 1 male (♂) were stocked in each tank and received the tested diet. After five days, females with eggs detected in the mouth were removed. The eggs were separated through counter flow of the oropharynx and placed in formerly identified pail. Spawned females were examined and weighed before toreturnin the maintenance tank, whilethe eggs were counting. The eggs were incubated into sieves and kept in circular white buckets with a volume of 6 L. Each spawn was conditioned in an individually identified tank maintained in a thermostatic bath system, with constant water at 28.0 ± 0.2 °C, and aeration provided by a porous stone, which kept the oxygen above5mg/L. For determining egg diameter, 15 eggs from each spawn were collected, fixed in Bouin's solution and assay under a stereo microscope. As the oval shape of tilapia eggs, all of them were measured. After hatching, larval were identified to determine hatching rate. Samples of 20 larvae were fixed in Bouin's solution to measure weight, total length (TL), standard length (SL). Remainder larvae were keep until the end of the lecithotrophic period (120h), when it's individually counted and measured as previously described.

Different reproductive criteria were assessed using the following parameters:

Relative fecundity= Eggs No./female weight (g).

Total fecundity= Eggs Number in the spawn.

Average egg production per female =Eggs No.per batch/ Number of spawned females. Hatching rate (%)=Number of hatched larvae/Total number of eggs×100.

Chemical analysis

The contents of each fish body and experimental diets were analysis according to(AOAC,2006). Three fish were randomly chosen from each treatment and immolate before further analysis. Dry matter of diets and body composition were analyzed by drying to constant weight at 105°C for 24h. Crude protein was assay with a Kjeltec™ 2300 Unit (FOSS, Denmark) using the Kjeldahl method. Crude lipid was analyzed through a Soxtec System HT1047 Hydrolyzing Unit (FOSS, Denmark), followed by Soxhlet extraction by using a Soxtec System1043 (FOSS, Denmark). Ash was analyzed by combustion in a CF1100 muffle furnace (Carbolite, UK) at 550°C for 6 h.

Blood immunity analysis

The blood sample was collected from six females in each treatment during the reproductive stage. Fish were restrictive using wet cloth, and then 500 μL of blood was collected by cardiac puncture using sodium heparin (0.1-0.2% mg/mL blood) as anticoagulant. Blood serum was separated by centrifuging blood in 4600 rpm for 10min. Total protein, glucose and albumin were determined via the method of Olesen and Jorgensen (1986). The lysozyme activity level was detected by the turbidimetric assay illustrated by Ellis (1990), in which lyophilized hen egg white lysozyme (Sigma, St. Louis, MO, USA) was used to constitute standard curve. To determine the lysozyme level, a solution of 20 mg of *Micrococcus lysodeikticus* (Sigma, St. Louis, MO, USA) in 100 mL sodium phosphate buffer (0.05 M, pH 6.2) was used. The assay was initiated in a microplate at a dilution of 1:1

(50 lL of phosphate buffer:50 lL of serum), and two fold serial serum dilutions were done by adding 50 lL of diluted serum into the remaining wells filled with 50 lL of PBS. A volume of 125 lL of *M. lysodeikticus* was added to each well. The reaction was performed at 25°C, and the absorbance was measured at 450 nm after 0.5and 5.0 min in a microplate reader (Benchmark, Bio-Rad, USA). The results were assayed in units of lysozyme per mL of serum. One unit is defined as the amount of sample required to reduce absorbance of 0.001/min at 450 nm compared to the control (*M .lysodeikticus* suspension without serum).

Gonads and histomorphometric evaluation

Gonads and liver were extracted and weighted to determine the gonadosomatic index (GSI) and hepatosomatic index (HIS) by these equations: GS =100(WG/W), where W represents the total mass of fish , WG represents gonad mass and HSI=100(WL/W), in which W represents the total mass of the fish and WL represents liver mass.

All the treated fish were however dissected to show the phenotypic character of the gonads. Each male and female were given in the presence of clearly observable testis and egg sac (characterized by thread-like appearance or irregular shapes), respectively. Fish were killed by immersion in anesthetic baths (same procedure as aforementioned) and the middle portion of the intestine was collected. The samples were fixed in Bouin solution at 10% for24h, after which were transferred to70% ethanol for the clothing of the histological slides. For the preparation of the slides all gonads were cut into 0.5-cm segments, dehydrated in increasing concentrations of alcohol, diaphanized in xylol, and embedded in paraffin, to be sectioned to the 5µ thickness and stained by hematoxylin-eosin (HE) Genten *et al.*(2009). The measurements were performed under light microscopy, AX10 Zeiss, Axio Cam MRC camera, with the aid of the ZEN 2012 software.

Larvae evaluation

For initial measurements, 20 larvae were collected from the same batch. On the 7th day, a 20 larvae was collected from each replicate for biometric analysis. The larvae in each treatment were measured every week during 1 month and all survival were counted and weighed.

Statistical analyses

The statistical analyses were carrying out using SPSS version 20, (2016) SPSS Institute, Cary, NC, USA). Fish performance data, gonad and larval parameters were tested for treatment effect using one –way analysis of variance (ANOVA). Significant differences ($p<0.05$) between means were revealed using Duncan test.

RESULTS

The analysis data presented in this experimental cleared that garlic powder enhanced growth performance, eggs and larval survival and immunity index in *O. niloticus* broodstock.

In the present triala highly survival rate was obtained in different broodstock groups, which recorded 100% between tested diets. The mean initial weight was similar between all groups, but after 60 days, the highest final weights were recorded in groups fed 2 and 3% garlic meal diet, respectively. The growth evaluation of the broodstock tilapia include gain, specific growth rate and condition factors were significant increased with increasing dietary level of garlic powder, reaching a highest performance at the dietary level of 2% garlic

(Table 2). However, further increase by using 3% garlic meal induces a slight enhancement in growth indices, without significant difference with 2% garlic level. Moreover, insignificance differences were obtained between fish fed diets (0 and 1% garlic).

Similarly, feed intake resort toincreasewith 2% garlic level, then decreased over this ratio. As detected in (Table 2), the beast values of feed utilization parameters such as (feed conversion, protein efficiency ratio and net protein utilization) were obtained with 2 and 3% garlic levels compared with the rest of treatments. All dietary levels of garlic not affected on hepatosomatic index ratios. As illustrated in (Table 3 and Figs. 1,2), the immunological indicesin fish represented a significance differences in total protein, glucose and lysozyme activity between tested groups. However, no significance difference was obtained in albumin value.

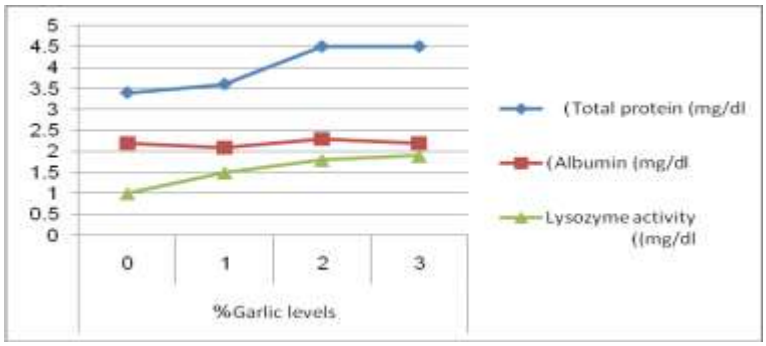

Fig (1) . Total protein, Albumin and Lysozyme activity of Nile tilapia (*Orecromis niloticus*) fed different levels of garlic powder.

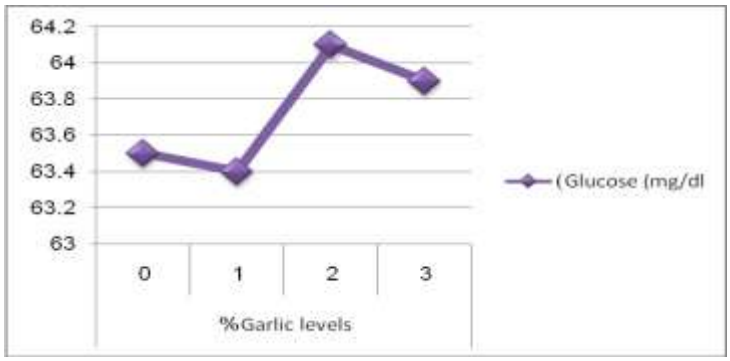

Fig (2). Glucose value in Nile tilapia (*Orecromis niloticus*) fed varying levels of garlic meal.

The GSI values in different treatments were shown in (Table 5). The values obtained were similar between treatments in theincipience of reproductive trial, where by endingthe study the GSI values were significantly different among garlic levels. Moreover, each absolute and relative fecundity were differ between broodstocks, where fish fed 2% garlic level presented the highest values, followed by 0,1 and 3%,respectively. In the same view, the hatchability and larval survival rate were significantly affected by garlic levels.

Table 2. Growth performance and feed utilization of tilapia after fed on different levels of garlic powder diets (Mean±SD n=3).

Parameters	Garlic levels%			
	0	1	2	3
Initial weight (g) ♀	222.6±1.31	219.4±1.31	220.8±1.31	221.2±1.31
Initial weight (g) ♂	260.22	262.18	265.12	266.32
Final weight (g) ♀	261.7^{c}±3.8	259.5^{c}±3.4	284.4^{a}±3.6	280.8^{ab}±3.5
Final weight (g) ♂	311.17	315.12	330.20	328.44
Gain (g) ♀	39.1^{c}±1.1	40.1^{c}±1.2	63.6^{a}±1.1	59.6^{ab}±1.3
Gain (g) ♂	50.59	52.94	65.08	62.12
Specific growth rate ♀	0.26^{c}±0.04	0.26^{c}±0.02	0.43^{a}±0.01	0.40^{ab}±0.02
Specific growth rate♂	0.3	0.3	0.37	0.35
Condition factor♀	1.39^{c}±0.1	1.41^{c}±0.4	1.44^{a}±0.2	1.42^{ab}±0.1
Condition factor♂	1.56	1.58	1.68	1.64
Feed consumed ♀	60.0	63.0	85.0	82.0
Feed consumed♂	75.0	79.0	90.0	92.0
Feed conversion ratio ♀	1.53^{c}±0.2	1.57^{c}±0.4	1.33^{a}±0.2	1.37^{ab}±0.1
Feed conversion ratio♂	1.48	1.49	1.38	1.48
Protein efficiency ratio♀	2.14^{c}±0.4	2.10^{c}±0.2	2.47^{a}±0.5	2.40^{ab}±0.2
Protein efficiency ratio♂	2.23	2.21	2.39	2.23
Net protein utilization♀	33.64^{c}±1.8	33.38^{c}±1.4	38.79^{a}±1.9	38.70^{ab}±1.5
Net protein utilization♂	36.90±1.6	34.98±1.9	37.54±1.2	36.33±1.4
HSI♀	1.30±0.2	1.35±0.1	1.40±0.2	1.41±0.1
HSI♂	1.60±0.4	1.55±0.2	1.58±0.4	1.62±0.1

Means sign by different superscript letters are significant (*P<0.05*).

Table 3. Immunological parameters in fish after fed with different levels of garlic powder diets (Mean±SD n=3).

Parameters	Garlic levels%			
	0	1	2	3
Total protein (mg/dl)	3.4^{b}±0.5	3.6^{b}±0.2	4.5^{a}±0.6	4.5^{a}±0.4
Glucose (mg/dl)	63.5^{b}±1.2	63.4^{b}±1.4	64.1^{a}±1.4	63.9^{a}±1.2
Albumin (mg/dl)	2.2^{a}±0.4	2.1^{a}±0.3	2.3^{a}±0.2	2.2^{a}±0.4
Lysozyme activity (mg/dl)	1.0^{c}±0.1	1.5^{b}±0.3	1.8^{a}±0.2	1.9^{a}±0.1

Means sign by different superscript letters are significant (*P<0.05*).

Table 4. Tilapia whole body analysis after fed on different garlic powder diets, %w/w basis (Mean±SD n=3).

Parameters	Garlic levels%			
	0	1	2	3
Dry matter	27.±1.4	27.3±1.5	27.1±1.4	27.2±1.3
Crude Protein	15.4±1.1	15.3±1.0	15.3±1.4	15.4±1.1
Crude Lipid	5.4±1.2	5.5±1.1	5.2±1.2	5.3±1.0
Ash	6.3±1.1	6.5±1.2	6.6±1.2	6.5±1.1

Initial whole body analysis: 25.8±1.2 dry matter, 15.2±1.1 crude protein, 4.8±1.0 crude lipid and 5.8±1.2 ash.

Table 5. Reproductive performance of broodstok fed with different garlic powder diets (Mean ±SD n=3).

Parameters	Garlic levels%			
	0	1	2	3
Final weight (g)	261.7^{c}±3.8	259.5^{c}±3.4	284.4^{a}±3.6	280.8^{ab}±3.5
G.S.I	1.46^{b}±0.2	1.46^{b}±0.1	1.51^{a}±0.2	1.50^{a}±0.3
Absolute fecundity	1685^{b}±95	1670^{b}±86	1886^{a}±92	1665^{b}±101
Relative fecundity	6.42^{b}±1.4	6.43^{b}±1.2	6.61^{a}±1.6	5.9^{c}±1.8
Hatchability (%)	59	61	66	61
Inter spawning intervals (ISI)	17	16	14	18
Survival rate%	100	100	100	100

Means sign by different superscript letters are significant (*P<0.05*).

Table 6. Growth performance of Nile tilapia fry fed with different levels of garlic powder for 60 days (Mean±SD n=3).

Parameters	Garlic levels%			
	0	1	2	3
Initial weight (g)	0.0087	0.0088	0.0089	0.0088
Final weight (g)	0.8^{c}±0.04	0.9^{b}±0.05	0.96^{a}±0.04	0.85^{c}±0.02
Gain (g)	0.7913^{c}±0.06	0.8912^{b}±0.05	0.9511^{a}±0.04	0.8412^{c}±0.05
Initial length (cm)	0.7	0.7	0.7	0.7
Final length (cm)	1.7^{c}±0.1	2.1^{b}±0.2	2.3^{a}±0.1	2.0^{b}±0.2

Means sign by different superscript letters are significant (*P<0.05*).

The histological examinations of *O. niloticus* tested were presented in Figs 1-4, (T0-T3). The results showed a significance visible effect for garlic powder in gonad structure. Normal structure of testicular wall (TW), seminiferous lobules (SL), germ cells (GC) and spermtozoa (SZ) were observed on control diet (T0). Also, normal shape from seminiferous lobules (SL), lobule boundary cells (LBC), sperm mother cell (SMC), spermtozoa (SZ), and testicular wall (TW) were shown in fish fed 1% garlic meal (T1). In the same trend, fish fed 2 and 3 % (T2&3) garlic powder shown well-defined of testicular wall (TW), lobule boundary cells (LBC), spermatogonia (SG) and spermatozoa (SZ) compared with the other groups. However, the Magnified portion of transverse sections in the ovaries of *O. sniloticu*s were presented in Figures (5-7, G0-G3). Its evident normal stage of oocyte structure in all groups, where the primary yolk oocyte, theca layer, the nucleus of the secondary yolk oocyte (SYO), oil vesicles (OV) and primary oocyte were clear in the ovary. In the same vein, fish fed 2 and 3% (G2 & G3) garlic powder showed well evolution in the yolk globules and secondary yolk oocyte compared with fish fed on the control and 1% garlic meal diets.

Whole body ranges of dry matter (26.9–27.3%), crude protein (15.3-15.4%), lipids (5.3-5.6%) and ash (6.1-6.57%) contents of tilapia fed on the tested diets are illustrated in (Table 4). These ratios were not significantly (*P>0 .05*) by the garlic level in the diets, where the proximate composition in fish not affected. The larvae of tilapia revealed an enhancement in final weight and length through a period of 30 days of feeding at 2% garlic diet compared with other diets as shown in (Table 6).

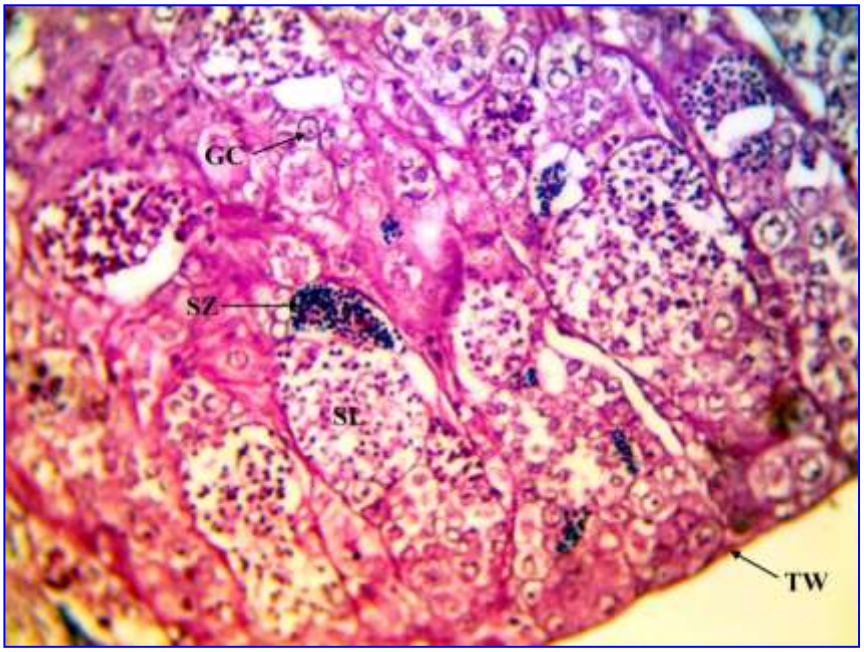

Fig.3.(T0):Photomicrograph of cross section in the testis of Nile tilapia *Oreochromis niloticus*, stained by hematoxylin and eosin, showing the testicular wall (TW), seminiferous lobules (SL) , germ cells (GC) and spermtozoa (SZ) (X 400).

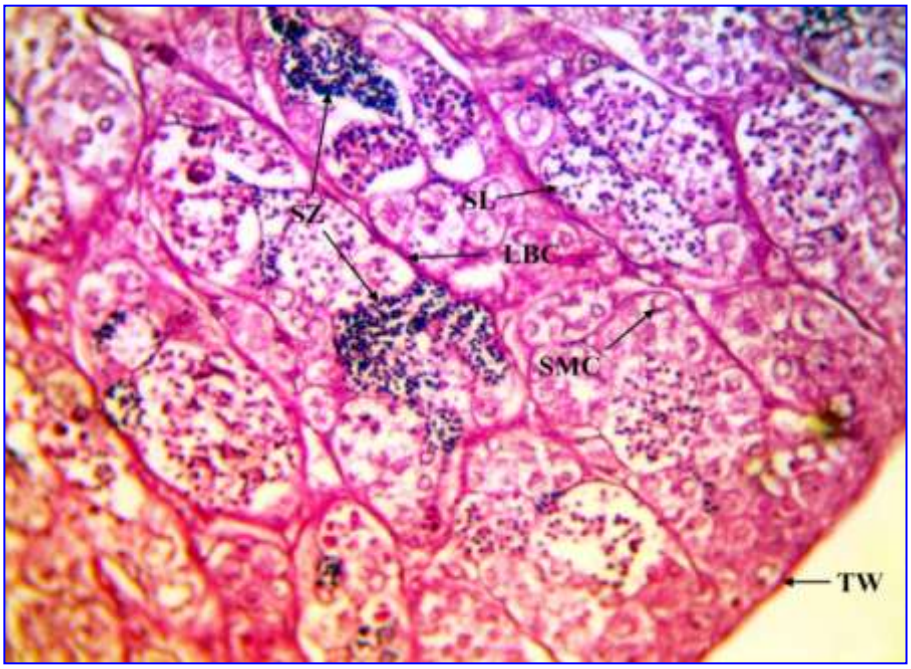

Fig.4.(T1): Photomicrograph of cross section in the testis of broodstock Nile tilapia *Oreochromis niloticus*, stained by hematoxylin and eosin, showing the seminiferous lobules (SL), lobule boundary cells (LBC), sperm mother cell (SMC), spermtozoa (SZ), and testicular wall (TW) (H & E, X 400).

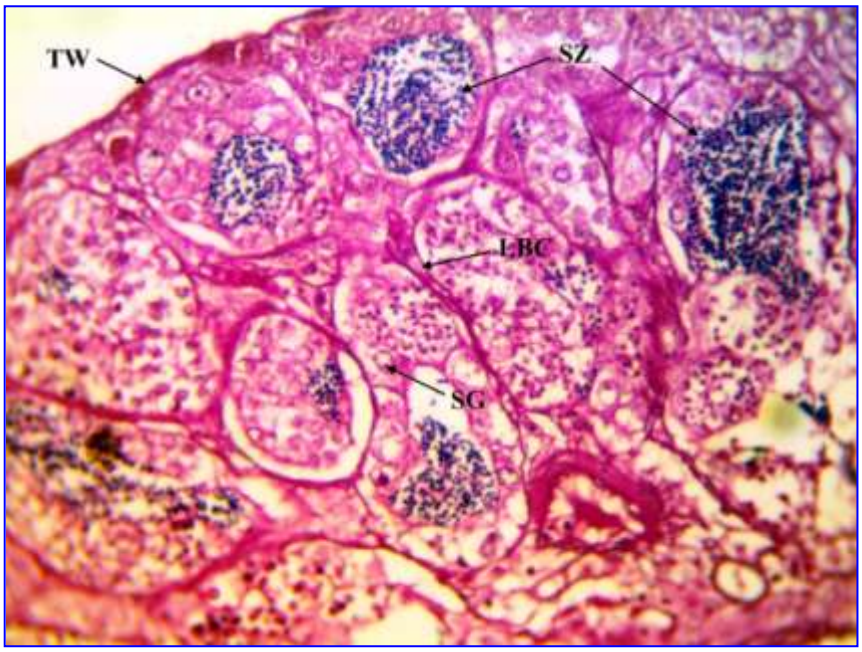

Fig. 5 (T2):Photomicrograph of cross section in the testis of broodstock Nile tilapia *Oreochromis niloticus*, stained by hematoxylin and eosin, showing the testicular wall (TW), lobule boundary cells (LBC), spermatogonia (SG) and spermtozoa (SZ) (X 400).

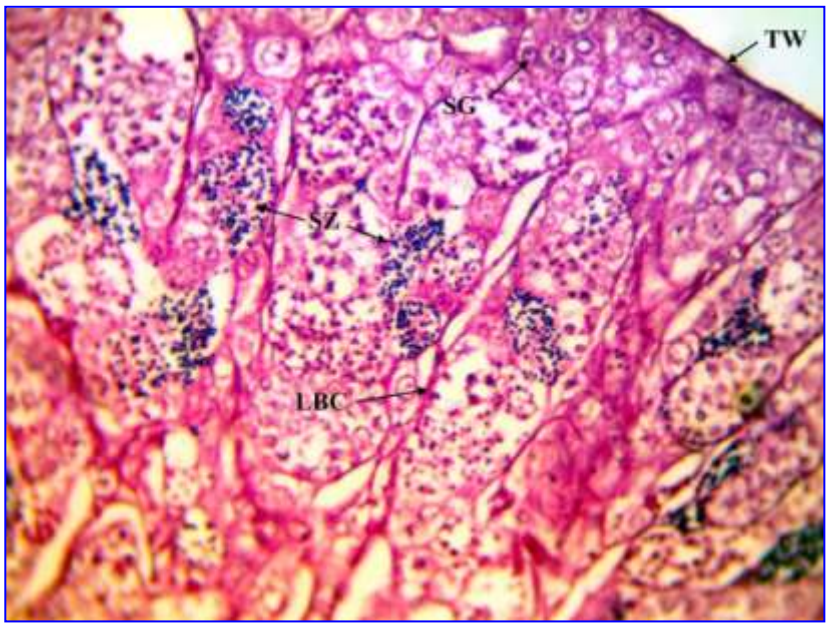

Fig.6 (T3):Photomicrograph of cross section in the testis of broodstock Nile tilapia *Oreochromis niloticus*, stained by hematoxylin and eosin, showing the testicular wall (TW), lobule boundary cells (LBC), spermatogonia (SG) and spermtozoa (SZ) (X 400).

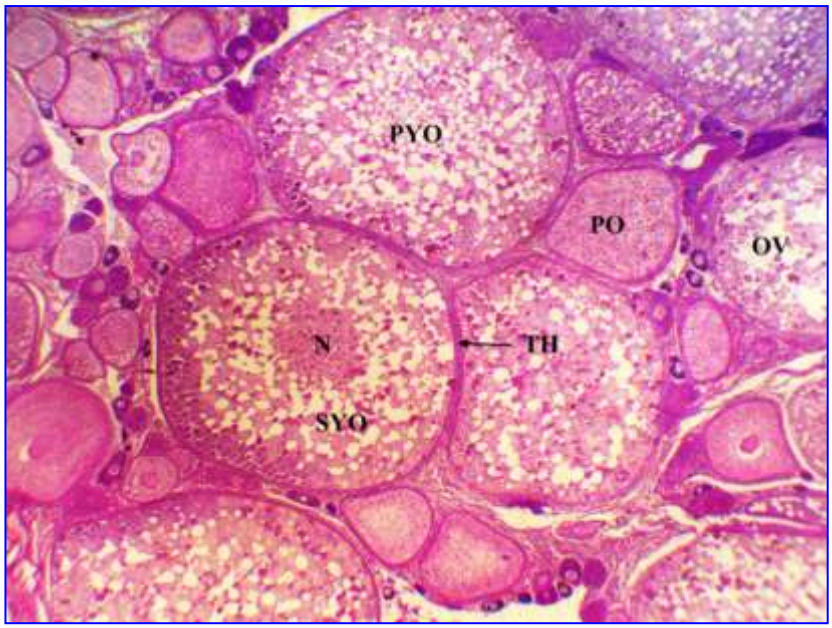

Fig.7 (G0): Magnified portion of cross section in the ovary of broodstock Nile tilapia *Oreochromis niloticus*, stained by hematoxylin and eosin, showing the primary yolk oocyte (PYO), theca layer (TH), the nucleus (N) of the secondary yolk oocyte (SYO), oil vesicles (OV), and primary oocyte (PO) (X 400).

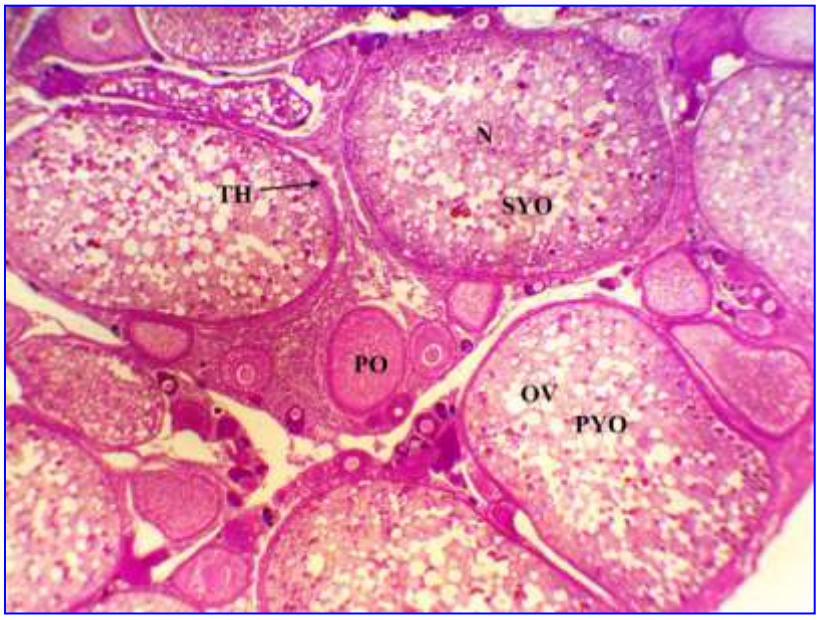

Fig. 8 (G1):Magnified portion of cross section in the ovary of broodstock Nile tilapia *Oreochromis niloticus*, stained by hematoxylin and eosin, showing the primary yolk oocyte (PYO), theca layer (TH), the nucleus (N) of the secondary yolk oocyte (SYO), oil vesicles (OV), and primary oocyte (PO) (X 400).

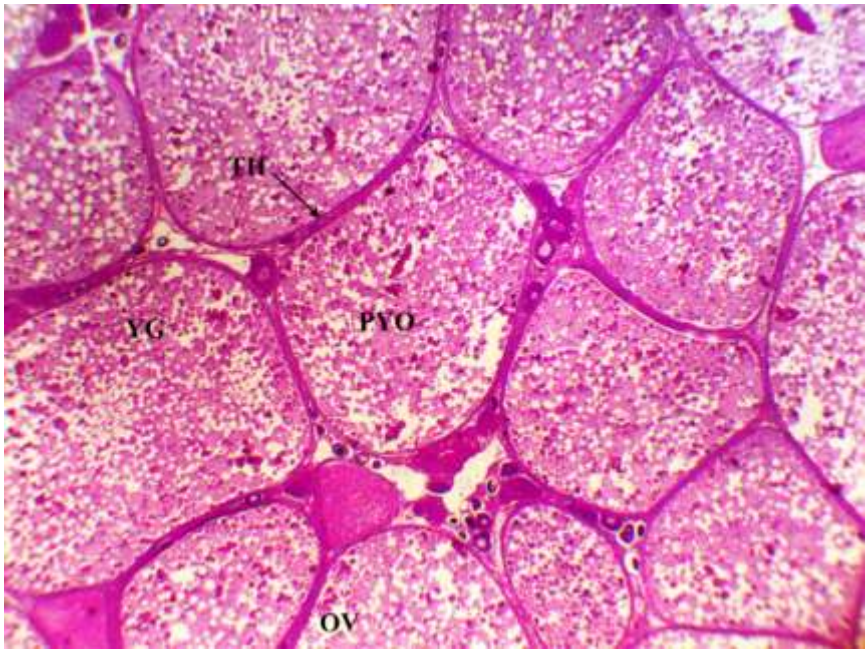

Fig..9 (G2):Magnified portion of cross section in the ovary of broodstock Nile tilapia *Oreochromis niloticus*, stained by hematoxylin and eosin, showing the primary yolk oocyte (PYO), theca layer (TH), oil vesicles (OV), and yolk globules (YG) (X 400).

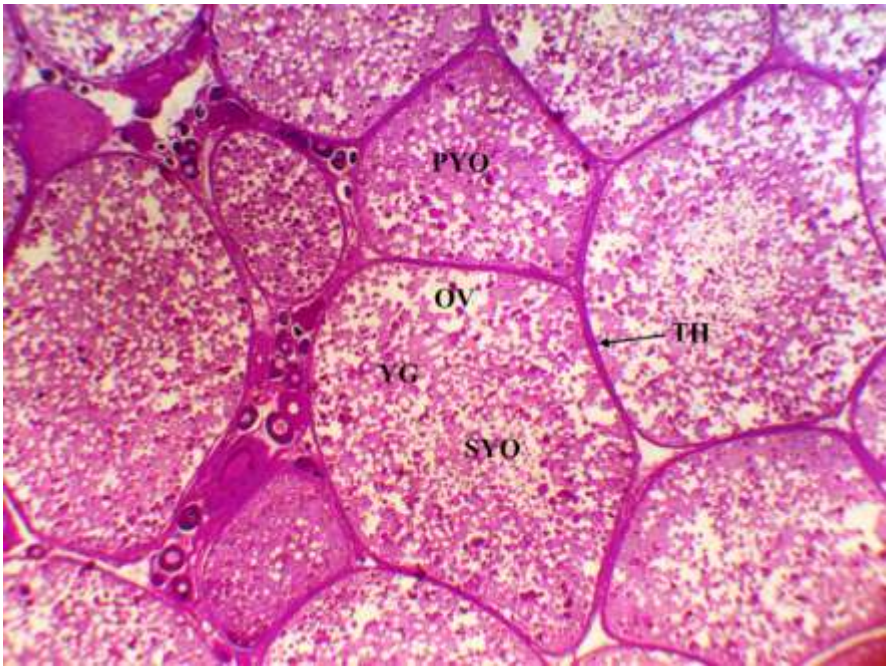

Fig.10 (G3): Magnified portion of cross section in the ovary of broodstock Nile tilapia *Oreochromis niloticus*, stained by hematoxylin and eosin, showing the primary yolk oocyte (PYO), theca layer (TH), oil vesicles (OV), yolk globules (YG) and secondary yolk oocyte (SYO) (X 400).

DISCUSSION

In the current study, the supplementation of garlic meal in tilapia diet was evaluated. The obtained results in growth performance and feed efficiency indicated that the addition of 2% garlic to the diet resulted higher values than in the control (0 garlic) and each 3 and 1% garlic diets. This finding was related to the allicin compound in garlic, which increased both of the growth and feed efficiency by stimulating the digestive enzyme and balancing the enteric microbial flora. Comparable results for the bioactive componentin garlic was obtained with other studies (Talpur and Ikhwanuddin, 2012 ; Khalil *et al.*,2001). Also other works showed that dietary garlic had a positive effect on FBW and SGR (Diab *et al.*, 2002, Shalaby *et al.*,2006, Nya and Austin, 2010; Farahi *et al.*,2010). Additionally, fed garlic diet to Asian sea bass (*Lates calcarifer*) resulted an increase of growth and survival rate (Talpur and Ikhwanuddin, 2012). In this study 100% survival rate was shown in all treatments. The garlic supplementation in tilapia diets recorded positive effects in their performance (Megbowon, 2013). In contrast, the increased levels of dietary garlic in the diet have an opposite effect due to pungent smell in garlic (Platel and Srinivasan, 2004 ; Aly *et al.*,2010). The use of 30g/kg of garlic in the current study had lower growth than that in 20 g/kg garlic, which could be due to high garlic's pungent smell. The present results were in accordance with the ranges of 1-3%, which reported with the previous results in Nile tilapia (Shalaby *et al.*,2006; Diab *et al.*,2002; Metwally, 2009). However, less levels were recommended in Nile tilapia as 0.5% (Abdel-Hakim *et al.*,2010) and 1% Soltan and El-Laithy (2008). Other researchers revealed that 3% incorporation of garlic meal in diets of rainbow trout (*Oncorhynchus mykiss*) and sturgeon (*Acipen serruthenus*) had a positive effect on growth rate and protein efficiency (Farahi *et al.*,2010 ; Lee *et al.*,2014).

It's evident in the present study that the whole body composition remained unchanged with different levels of garlic meal. Fish body fat contents was not affected between treatments, but less decreased was revealed by 2 and 3% garlic addition. The current results are in line with the previous reported results for Nile tilapia (Khattab *et al.*,2005; Shalaby *et al.*,2006 ; Maniat *et al.*,2014). The present of Allicin in garlic prevents the accumulation of fat in fish body due to its effect in bile acid, which increases fat digestion (Elkayam *et al.*, 2003).

The contradiction between these results and some of the earlier studies for the effects of dietary garlic on fish growth performance, feed utilization or body composition can be refer to the differences in fish species or fish size, environmental conditions include water temperature and salinity, type or level of additives ingredients through diet preparation, or garlic source added to the feeds, fish physiology or a combination of these factors together.

Glucose and albumin were represented lower values with fish fed on garlic diet compared to the fish fed with the control diet. These results were agreement to the previous results of (Shalaby *et al.*,2006, Sahu *et al.*, 2007; Talpur and Ikhwanuddin, 2012) , in which glucose and albumin values were decreased when garlic added to the diet. The increasing in total protein value may be due to antiprotease activity induced by garlic, which enhancing protein production.

Lysozyme is a cationic enzyme that breaks β-1,4glycosidic bonds between N-acetylmuramic acid and N-acetyl glucosamine in the peptidoglycan of bacterial cell walls and its known to attack mainly Gram-positive bacteria as well as some Gram-negative bacteria (Alexandar and Ingram,1992).

In the present result the lysozyme activity cleared that the immune system was improved by garlic using and this could be explained by the role of lysozyme in humoral immunity. The same results were recorded with Asian sea bass (*L. calcarifer*) where, lysozyme activity was increased by the inclusion of 10, 15, and 20 g/kg garlic to the diet (Talpur and Ikhwanudd, 2012). Moreover, 5 or 10 g/kg of garlic addition to the diet showed increase in the lysozyme activity of rainbow trout (Nya *et al.*,2010). Lysozyme restrains infection by preventing pathogen connectivity and reproduction (Mirsa *et al.*,2004 ;and Mirsa*et al.*,2006). Rise of lysozyme activity indicates antibacterial property of garlic. Also, using garlic in Asian sea bass diets had positive effects against *Vibrio harveyi* infection (Talpur and Ikhwanuddin, 2012).

In addition to nutrition, reproduction is a fundamental biological process of the organisms, considering that survival and perpetuation of species depend on its life cycle. So, the possibility to controlling the reproductive cycle in fish is one of the most important factors to ensure the success of fish production (Romagosa *et al.*,2013).

Increasing gonado somatic index by using 2 and 3% garlic powder was agree with the previous results of supplemented vitamin E in common carp (*Cyprinus carpio*) diets Gupta *et al.,* 1987; Watanabe and Takashima 1977; Kanazawa, 1985).

The histological characteristics of gonads can be applied to evaluate the current development stage during their reproductive cycle (Bucholtz *et al.,* 2008). The enhancement in structure deformation of testes and ovary by addition 2% garlic powder were cleared. This findings were agree with using other plants as feed carica papaya seed meal in Nile tilapiaAbdelhak*et al*., 2013 and Solomon and Okomoda,2012).

The reproductive performance of fish in these study were enhancing by using 2% garlic powder and this can be indices of that broodstok enter their spawning period with high immunity conduction. This finding was similar to other work that reveled the nutrition of broodstock may influence the quality of the offspring because the nutrients in females diets are deposited into the eggs during vitellogenesis and will be reflected in the quality of the post-larvae. However, little knowledge is known about the effect of maternal diet regarding the performance of the progeny after the end of the vitelline reserves period [53],. In this trial, it's revealed a significance difference in weight, gain and total length of post-larvae between treatments. The highest performance was obtained with 2% garlic level. These results are in line to those reported in Nile tilapia (*O. niloticus*), Ng and Wang (2011).

Conclusion

Fish nutritionists generally evaluate the end results, as fecundity, egg formation and larval survival, but nutrition effects on the biological processes to produce and deposited nutrients on gametes must be deserve more attention. Based on the present results, fish fed in 2% garlic diet showed the best performance of reproduction and high immunity indices. Also it's cleared that garlic had a stimulant effect on the immune system in fish and increasing the lysozyme activity in broodstock of Nile tilapia. Further investigation must be required to detect the effective of garlic on reproductive performance.

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Incorporation of garlic meal (*Allium sativum*) as natural additive to enhance performance, immunity, gonad and larval survival of Nile tilapia (*Oreochromis niloticus*) broodstock

إدخال مسحوق الثوم كإضافات طبيعية لتحسين الأداء ,المناعة , المناسل ونسبة بقاء اليرقات لإمهات البلطى النيلى

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إجريت التجربة لمدة 60 يوم لتحديد النمو و مؤشرات الإ نتاج لإمهات البلطى النيلى التى غذيت على أربع علائق تحتوى 4 مستويات مختلفة من مسحوق الثوم وهى (0, 1 ,2 و3%). كونت 4 علائق متماثلة فى نسبة البروتين والطاقة لتحتوى على30،25 بروتين خام و 19،25 ميجا جول/ كجم عليقة ومثلت كل عليقة بثلاث مكررات. تم إختيار 16 ام بوزن أولى (5 ،221 ± 31 ،1 جم) من الأسماك المؤقلمة وزعت على 12 حوض دائرى أسمنتى سعة 2 متر مكعب بنسبة 3 إمهات : ا ذكر . جودة مياة الاحواض كانت مثلى حيث كانت نسبة الأوكسجين 5 ، 5 ± 1،2 جم/لتر , الحرارة 26 ± 5 ،1درجة و الأس الهيدروجينى 5 ،7±5، 0وأظهرت النتائج معنوية عند مستوى (5 0،0) لكل من قياسات النمو للمجموعة المغذاة عل 2% مسحوق ثوم تبعها كل من 3 , 1 و مجموعة الكنترول التى لاتحتوى مسحوق الثوم وسجل أعلى عائد منإستخدام العليقة ممثلا فى (معامل التحويل الغذائى , الكفاءة البروتينية و صافى إستخدام البروتين) مع نسبة 2% مسحوق ثوم مقارنة ببقية العلائق . أشارت دراسات مناعة الأ سماك معنوية لكل من البروتين الكلى , الجلوكوز و نشاط انزيم Lysozyme بين العلائق المختبرة بينما لم تظهر قيم الالبيومين اختلافات معنوية فى حين ان أعلى نتائج معنوية من البروتين الكلى , الجلوكوز و نشاط انزيم Lysozyme بين العلائق المختبرة حدث مع المجموعة المغذاة على 2% مسحوق ثوم وبنفس الاتجاة سجلت أعلى معدلات انتاج من الخصوبة النسبية , الخصوبة المطلقة ونسبة الفقس ولم تظهر إختلافات تركيب جسم الأسماك من المادة الجافة , البروتين الخام , الدهن الخام والرماد إختلافات معنوية بين المعاملات المحتوية على نسب مختلفة من مسحوق الثوم. كما أشارت الدراسة الحالية الى أن إستخدام 2% من مسحوق الثوم فى علائق إمهات البلطى النيلى يحسن من أداء النمو , إستخدام الغذاء, النشاط المناعى , مؤشرات الإنتاجية ومعدل بقاء اليرقات لإمهات البلطى النيلى.