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Inclusion of biofloc meal in tilapia diets and its effect o the structure of zooplankton community under biofloc system condition

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ABSTRACT

The effect of diets formula on zooplankton community structure and quality subsequently and tilapia fingerling performance under biofloc system condition was examined using three isonitrogenous (30% crud protein) and isocaloric (4500 Kcal kg^{-1}) diets; Control diet (C; Soybean based diet) 25% and 50% of soybean protein that were substituted by floc meal to form FM_{25} and FM_{50} diets, respectively. Protozoa was the most dominant group during the experiments especially ciliated protozoa which was dominated by *Centropyxius* sp. The control treatment showed the highest number for *Centropyxius* sp. (285000 org./L) subsequently the total zooplankton count (325667 org./L) and the best final body weight of tilapia. No significant difference was recorded between control and FM_{25} regarding tilapia final body weight. Despite the lowest different zooplankton group recorded their lowest numbers was under experimental condition of FM_{25} treatment, while the highest percentage of oligochaete worms (5.9%) was noticed for such treatment. Significant positive correlation was found between *Centropyxius* sp. and *Asplanchna* sp. (r= 0.95, P= 0.004) and ciliate protozoa with oligochaete worms (r=0.85, P=0.03). Rotifer population showed a significant correlation with dietary phosphorus and selenium content (r=0.96, p=0.017). Also, the decreasing of rotifers in control (9.11 %) and 25% substitution biofloc experiment (17.3%) was accompanied with the increasing of tilapia final weight to be 31 and 24 g, respectively. This may attributed to the preference and consuming rotifers by fish. So, we conclude that diets formula affect the zooplankton community structure which subsequently showed their reflection on fish performance. More studies are needed to understand the relation among different zooplankton species as a step to control the biofloc meal quality by manipulating diets formula.

Key words: Biofloc system, Floc meal, Zooplankton, Tilapia.

INTRODUCTION

Studying the biofloc system and its condition are recently developed as seeking for economical feed stuffs and avoiding water losses and became an important in modern aquaculture. Biofloc is a biological system depends on activating heterotrophic bacteria to assimilate the excess ammonia in the pond and avoid aquatic organisms' toxicity. Chronic exposure to toxic un-ionized ammonia concentrations as low as 0.06 mgl^{-1} cause gill and kidney damage, reduction in growth, possible brain malfunctioning, and reduction in the oxygen carrying capacity of the fish (Durborow *et al*., 1997). Anytime the un-ionized ammonia is higher than 0.05 mg/L, the fish are being damaged; meanwhile level of 2.0 mgl^{-1} is lethal (Francis-Floyd *et al*., 1990). Maintaining the carbone: nitrogen ratio around 1:10 or above by supplement the culture media with carbon source guarantee the activation of the heterotrophic bacteria and maintaining a good water quality (Avnimelech, 1999).

Floc is the backbone unite of successive biofloc system and consists of other organisms than bacteria such as micro/macro invertebrates, filamentous organisms fungi, ciliates, flagellates, rotifers, nematodes, metazoans and detritus (Manan *et al*., 2016). Besides maintaining the water quality regarding ammonia levels, flocs act as secondary feed source for the aquatic organism (Hargreaves, 2013). Flocs could be grazed directly from pond column by fish or could be used as feedstuff if settled and added into fish diets. Floc meal quality and chemical composition depends largely on the zooplankton structure of the biofloc meal subsequently the nutrient composition of the fish diets. Manipulating the fish diets may result on improving the biofloc meal quality which means better fish feed utilization or high quality settled floc meal. So, in the present study, the effect of different dietary composition where biofloc meal alternated soybean in tialpia diets on the zooplankton community structure was studied. Meanwhile, an attempt was paid to understand the relation between media different nutrient, zooplankton community and relation among zooplankton groups.

MATERIALS AND METHODS

Design of the experiment

Zooplankton in substitution experiment was examined in duplicate, First experiment: control without any Substitution (control), second experiment: 25% Substitution of soyabean (FM_{25}) and Third experiment: 50% Substitution of soyabean (FM_{50}).Tilapia fish fingerlings with average weight of 12.14g were distributed in plastic tanks of 60L. Experimental tanks were supplied with well water and 8L of prepared biofloc to initiate the system. Different tanks were supplied with carbon source to maintain C: N ratio of 1:10 to maintain active biofloc system. Biofloc of different treatments were settled down when total suspended solids reached 300mg/L to maintain the biofloc quality. Three isonitrogenous 30% crud protein and isocaloric 4500 Kcal kg^{-1} diets were formulated; Control diet (C; Soybean meal as main protein source); FM_{25} (25% of soybean meal protein was replaced with floc meal; and 50% of soybean meal was replaced by biofloc meal, FM50) (Table 1). Floc meal was collected and dried during previous trail. Fish final weight and zooplankton count were detected at the end of the experiment which lasted for 56 days.

Collection and analysis of zooplankton samples:

Zooplankton samples were collected from different treatments in biofloc system using zooplankton net (55 µm). Water was agitated well and 5 L of water sample was filtered by zooplankton net. The samples were fixed immediately using formaldehyde solution (5%) and 2ml of Rose Bengal stain (0.5 %) was added after fixation.

The samples were examined under microscope. Three sub-samples (one ml for each) of the homogenized plankton samples were transferred to a counting cell and the different plankters were counted. Zooplankton population was then calculated as the number of individuals per Liter. The organisms were identified with magnification varying from 100X to 400X. Zooplankton were identified according to description and keys constructed by Edmondson (1966); Ruttner-Kolisko (1974); Shehata *et al*. (1998 a & b) and Khalil (2000).

Number of zooplankton was calculated after examination for all the recorded species in each sample and expressed for organisms/litters depending on the following equation according to (APHA, 2005): No of organisms/litter= $(N \times D)/(S \times C)$

Where,

N= Number of organisms; D= volume of sample after filtration; S= Number of subsamples; C= Total volume of the collected sample.

Statistical analysis:

Correlation and regression analysis were established using EXCEL package (version 2007).

Formulation and proximate composition (g kg^{-1} dry weight basis) of experimental diets.

Ingredients (g/kg)	control	FM_{25}	FM_{50}
Fish meal[a]	190	190	190
Soybean meal[b]	360	260	190
Corn[c]	389.4	329.4	239.4
Floc meal	0	160	320
oil[d]	40	40	40
Premix[e]	10	10	10
Carboxy methyl cellulose[f]	10	10	10
Vitamin C[f]	0.5	0.5	0.5
BHT[f]	0.1	0.1	0.1
Total (g)	1000	1000	1000
Proximate composition			
Moisture %	5.74	5.73	5.77
Crude protein %	29.4	29.8	29.3
Lipid %[G]	8.8	8.09	7.41
Ash %	6.39	8.40	10.26
Mineral			
Calcium %	0.88	1.44	2.29
Magnesium %	0.16	0.18	0.29
Sodium %	0.20	0.26	0.73
Phosphor %	0.59	0.66	0.82
Potassium %	0.72	0.34	0.48
Selenium mg/kg	ND	ND	13.8

[a]Local fish meal (70 % CP)
[b]Soy Factory, Food Technology Research Institute, Ministry of Agriculture, Giza, Egypt
[c]Imported yellow corn from Argentina
[d]Commercialfood-grade
[e]Provides per kg of diet: retinyl acetate, 3,000 IU; cholecalciferol, 2,400 IU; all-rac-α-tocopheryl acetate, 60 IU; menadione sodium bisulfite, 1.2 mg; ascorbic acid monophosphate (49 % ascorbic acid), 120 mg; cyanocobalamine, 0.024 mg; d-biotin, 0.168 mg; choline chloride, 1,200 mg; folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamin. HCl, 1.2 mg; sodium chloride (NaCl, 39 % Na, 61 % Cl), 3,077 mg; ferrous sulfate ($FeSO_4 \cdot 7H_2O$, 20 % Fe), 65 mg; manganese sulfate ($MnSO_4$, 36 % Mn), 89 mg; zinc sulfate ($ZnSO_4 \cdot 7H_2O$, 40 % Zn), 150 mg; copper sulfate ($CuSO_4.5H_2O$, 25 % Cu), 28 mg; potassium iodide (KI, 24 % K, 76 % I), 11 mg; Celite AW521 (acid-washed diatomaceous earth silica), 1,000 mg Agri-Vet Co., Cairo, Egypt.
[f]Algomhuria Pharmaceutical Chemical Co., Cairo, Egypt.
[G] Calculated

RESULTS AND DISCUSSION

The highest tilapia final body weight and average number of total zooplankton were observed with the biofloc in control experiment without any substitution (325667 org.l^{-1}), while the lowest value was observed for FM_{25} (Table 2 & Figs. 1&2). More inclusion of floc meal into tilapia diets leads to more accumulation of different minerals. Rotifers population number was noticed to be correlated with dietary content of phosphorus and selenium (r= 0.93, P= 0.017). Rotifera showed the highest variety of species (6 species), however it recorded the lowest density among other zooplankton groups. The highest average number of total rotifer was observed with FM_{50} being 35666 org./L, while the lowest rotifers values was recorded with FM_{25} being 14666 organisms/L. Moderate rotifers number was recorded by the control (29667 organisms/L). In the same context Rotifera species showed different response with the different substitutions. The decreasing of rotifers in control (9.11 %) and 25% substitution (FM_{25}) biofloc experiment (17.3%) was accompanied with the increasing of tilapia final weight being 31 and 24 g, respectively. This may attributed to the preference and consumption of rotifers by fish due to their highest nutritional values. These finding agrees with that of Hegab *et al.* (2017).

The rotifer *Lepadella ovalis* and protozoan *Centropyxius* sp. were observed with a remarkable numbers under the different biofloc condition treatments. *Lecane bulla* and *Lecane Closterocerca* recorded the highest values with control being 21667 and 667 org./L, respectively. *Philodina* spp. numbers increased gradually with substitution being 1333, 2000 and 3666.7 org./L for control, FM_{25} and FM_{50}, respectively. This indicated that, this species has the ability to grow and reproduce under the high suspended matters of biofloc system. Mola and Parveen (2014) stated that the rotifers *Philodina spp*. and *Lecane spp*. and the protozoan *Centropyxius* sp. can survive under stress of hard water conditions. Several significant positive correlations were recorded among different zooplankton species (Table 3). It seems that *Asplanchna* sp. numbers are significantly correlated with the presence of *Centropyxius sp*. (r= 0.95, P= 0.004) (Fig. 3).

Table (2): Zooplankton in different experimental treatments.

Species /Groups	**Control**		**FM_{25}**		**FM_{50}**	
	Average	**%**	**Average**	**%**	**Average**	**%**
<u>Rotifera</u>						
Asplanchna sp.	2000	0.61	0	0.0	0	0.0
Lecane bulla	21667	6.65	7333.3	8.6	29666.67	19.1
L. Closterocerca	667	0.20	333.3	0.4	333.3	0.2
Lecane sp.	0	0.00	3666.7	4.3	333.3	0.2
Lepadella Ovalis	4000	1.23	1333.3	1.6	1666.7	1.1
Philodina sp.	1333	0.41	2000	2.4	3666.7	2.4
Total	29667	9.11	14666.67	17.3	35666.7	22.9
<u>Protozoa</u>						
Vorticella sp.	1667	0.51	6000	7.1	1000	0.6
Centropyxius sp.	285000	87.51	59000	69.4	113666.7	73.0
Total protozoa	286667	88.02	65000	76.5	114666.7	73.7
Oligochete worms	9000	2.76	5000	5.9	5000	3.2
Nematoda	333	0.10	333.3	0.4	333.3	0.2
Total zooplankton	325667	100.00	85000	100	155666.7	100

Table (3): Correlation among different zooplankton groups and between rotifers and dietary phosphorus and selenium under condition of biofloc system.

Species	Regression equation	R^2	Probability
Asplanchna sp. **vs**. *Centropyxius sp.*	y = 0.007x - 455.8	$R^2 = 0.904$	P= 0.004
Oligochete worms **vs**. *L. Closterocerca*	y = 8.700x + 2466.6	$R^2 = 0.690$	P= 0.04
Oligochete worms **vs.** *Centropyxius sp*.	y = 0.028x + 2020.2	$R^2 = 0.730$	P= 0.03
Total rotifers **vs**. phosphorus and selenium	$y = [3.5-16.2 (P)+0.2 (Se)]10^5$	$R^2 = 0.93$	P= 0.017

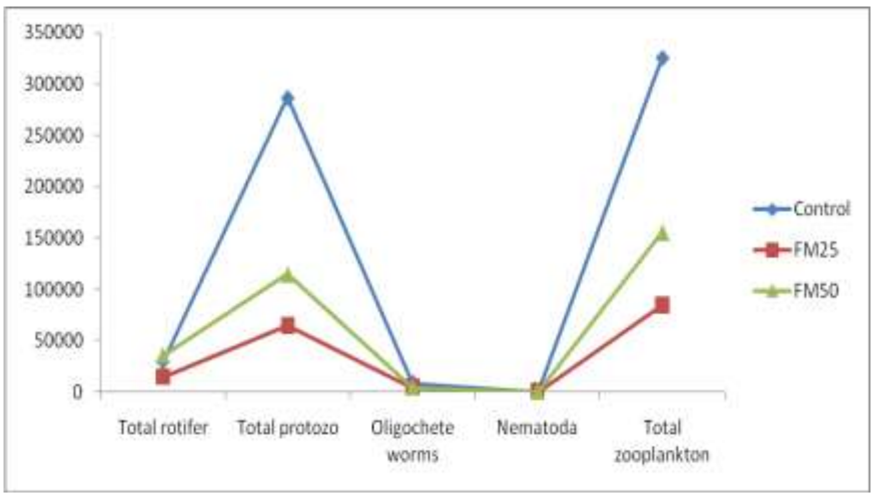

Fig.(1).Zooplankton community structure under different experimental treatment.

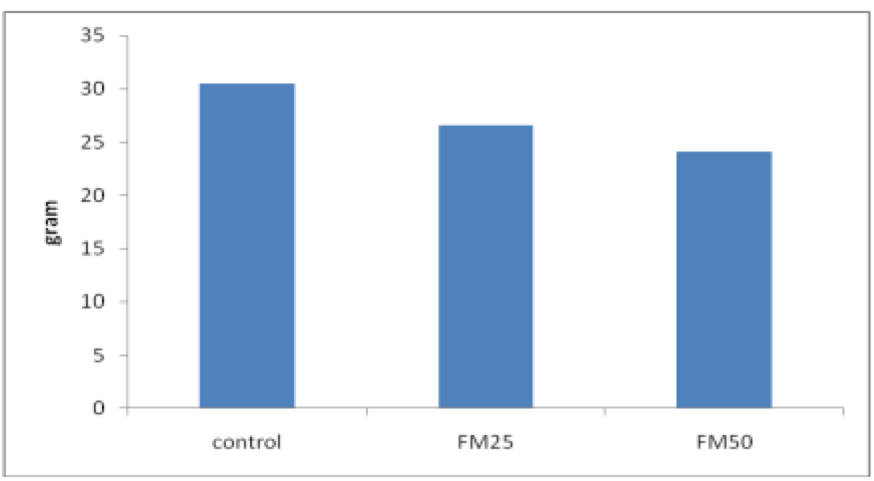

Fig. (2).The effect of different dietary formula on tilapia final body weight .

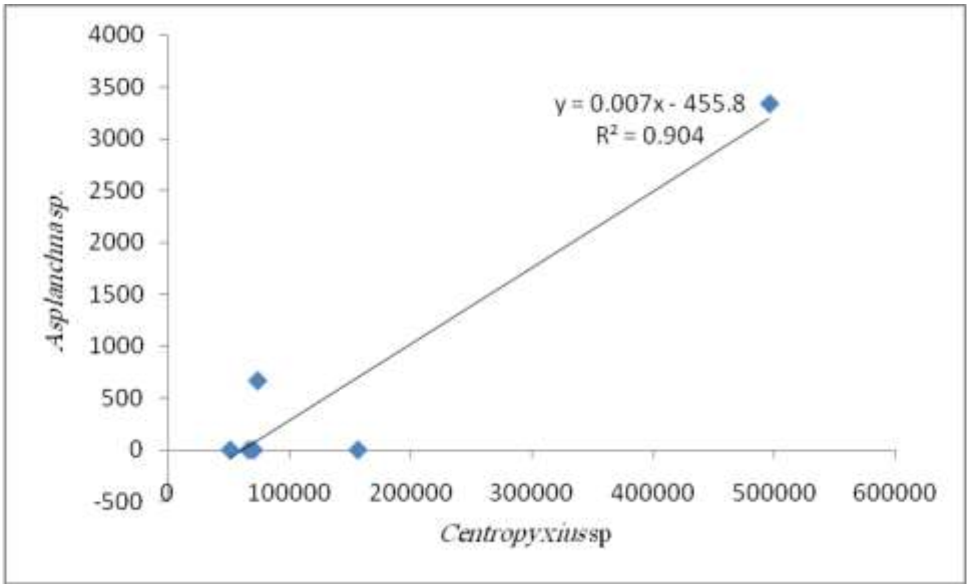

Fig. (3). Relationship between *Asplanchna sp*. and *Centropyxius sp*. population number.

Protozoa was the most dominant group during the experiments especially ciliates which was dominated by *Centropyxius sp*. and *Vorticella sp* (Fig., 6). The protozoan *Vorticella* sp. was observed with low number in most biofloc experiments. Ciliated protozoa recorded 286667 org./Lin the control and 65000 org./Lin FM_{25}. Singh (2009) found that protozoa recorded large numbers in the presence of organic matters. The biofloc conditions stimulated the dominating of ciliated protozoa due to the availability of free bacteria (Madoni, 2017). Also, ciliate protozoa had the ability to consume free bacteria which improve water conditions (Gerardi *et al*., 1995). Meanwhile, Protozoa have effect on the bacterial growth as it release nutrient salts and other stimulatory substances which accelerate the use of carbon by the bacteria (Jurgens and Matz, 2002).Upon the previous, it seems that the increased number of ciliate protozoa have positive effect of biofloc condition subsequently tilapia growth performance where the control showed superiority over all treatments (Fig. 2).

Annelida were observed in the different experimental treatments (Fig. 7). It was represented by Oligochaete worms which recorded its highest numbers (9000 org./L) in control, while it recorded the numbers (5000 org./l) in the other biofloc substitutions of FM_{25} and FM_{50}. Due to its large sizes in comparing to all the other Microinvertebrates, Oligochaete worms (the highest biomass) recorded remarkable percentage in FM_{25} experiment (substitution of 25%) being 5.9%. A positive correlation was recorded between oligochaete worms and the rotifer *L. Closterocerca* (r= 0.83, p=0.04). The Same trend was recorded between oligochaete worms and the *Centropyxius* sp. (r=0.85, P=0.03) (Table 3 & Figs. 4&5). An oligochaete worm seems to have predatory activity against both *L. Closterocerca* and ciliate protozoa which may explain the low number and percentage of total zooplankton in FM_{25} treatment. On the other hand, no significant differences were recorded between control and FM_{25} regarding tilapia final body weight. This may be explained by the high percentage of the oligochaetes in FM_{25} treatment, where it considered as good palatable live feed source for aquatic organisms (Lietz,., 1987). Eels fed on Tubificidae (The most domiat oligochate worms in freshwater) as pre-weaning diet recorded high survival rates (El Hussieny *et al*., 2016). Alanine formed more than 23% and up to 41% of the total free amino acid of oligochete worms e.g. *Limnodrillus spp*. and *Tubifx spp*. (Graney *et al.*, 1986). Saglio *et al.* (1990) mentioned that the combination of amino acids in the oligochate worm (*Tubifex*) recorded similar composition of that present in common carp fish. Pelegrí *et al*. (1995) observed that the presence of oligochaete worms is accompanied with the enhancement of biofloc conditions. Nematoda was the third dominant group after protozoa and oligochata and showed a remarkable number and recorded the highest percentage in FM_{25} treatment being 0.4% of the total zooplankton (Fig. 6).

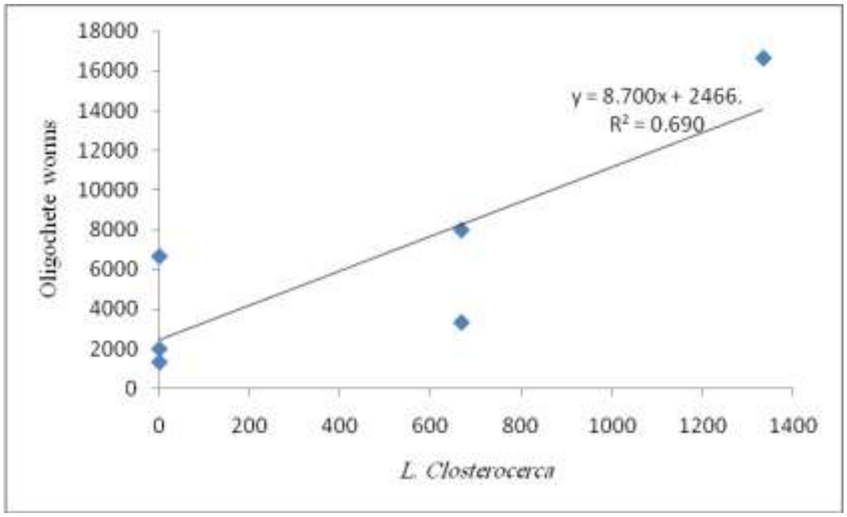

Fig. (4). Relationship between Oligochete worms and *L. Closterocerca* population number.

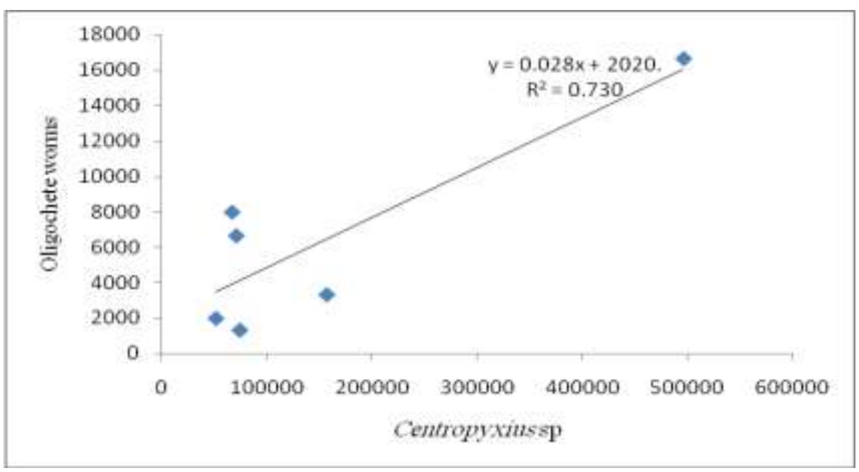

Fig. (5). Relationship between Oligochete worms and *Centropyxius sp*. population number.

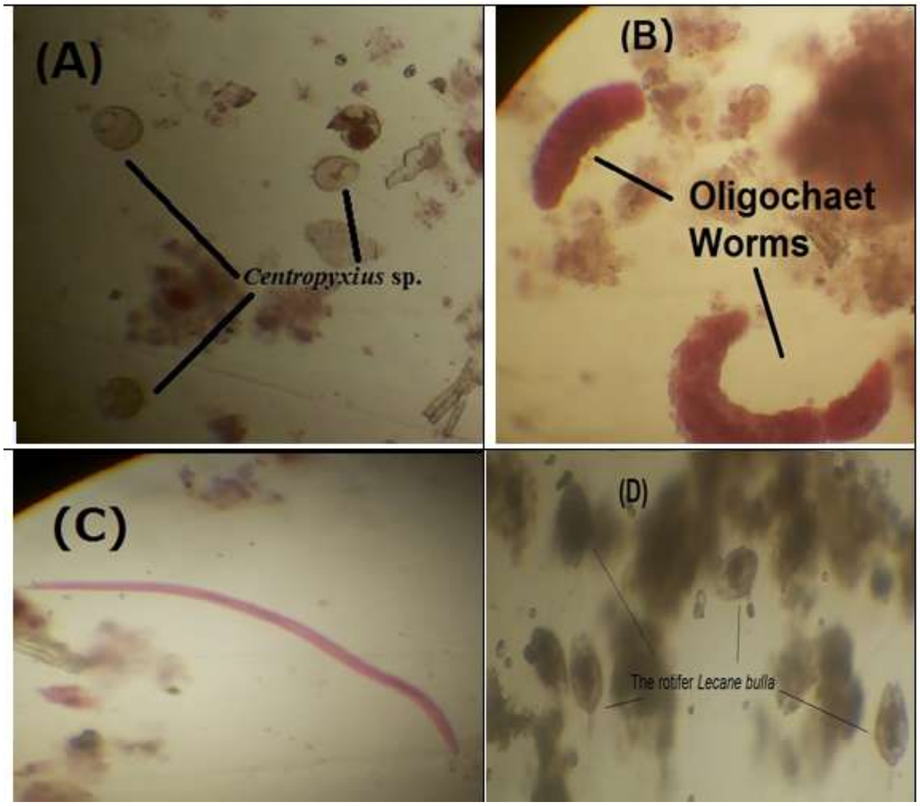

Fig. (6). Protozoan *Centropyxius* sp. (A), Oligochete worms (B), free Nematoda worm (C) and The rotifer *Lecane bulla* (D) which dominate the biofloc system in all tratments.

Conclusion:

It seems that the composition of fish diets affects on the zooplankton community structure. More inclusion of biofloc meal into tilapia diets resulted in more accumulation of different minerals with different balances among each other. Rotifers population number was correlated with dietary content of phosphorus and selenium. Also, the decreasing of rotifers in control (9.11 %) and 25% substitution biofloc experiment (17.3%) is accompanied with the increasing of tilapia final weight being 31 and 24 gm respectively. These relations may also reflect the zooplankton structure generally. The substitute of 25% percent of soybean in biofloc is better than 50% of substitution. So, fine tuning the dietary inputs into biofloc media may enriches the zooplankton community subsequently fish performance. Deep digging in the relation between different fish dietary formula and the zooplankton structure under biofloc condition is needed.

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ادراج مسحوق البروتين الميكروبي في النظام الغذائي للبلطي وتاثيرها علي المحتوي الكلي من الهائمات الحيوانية تحت نظام البيوفلوك

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المستخلص

تم دراسة تأثير تركيب العليقة الغذائية على المحتوي الكلي من الهائمات الحيوانية ونوعيتها، وتم الكشف عن الاداء الانتاجي لاصبعيات البلطي تحت نظام البيوفلوك باستخدام ثلاثة علائق متساوية البروتين (30٪ بروتين خام) و الطاقة (4500 كيلو كالوري) وتشمل الكنترول (اتباع نظام غذائي علي فول الصويا) واستبدال 25% و 50% من بروتين فول الصويا بمسحوق الفلوك لتشكيل الوجبات الغذائية FM25 و FM50 على التوالي. وكانت الاوليات المجموعة الأكثر هيمنة خلال التجارب وخاصة الاوليات المهدبة التي كان يسيطر عليها سينتروبيكسيوس. وأظهرت معاملة الكنترول أعلى رقم لهدبيات من نوع سينتروبيكسيوس (285000 كائن/لتر) وكان المحتوي الكلي من الهائمات الحيوانية (325667 كائن/لتر) للحصول على أفضل وزن جسم نهائي للبلطي. لم تسجل ايۭ اختلافات معنوية بين الكنترول والمجموعة FM25 فيما يتعلق بالوزن النهائي للبلطي. وعلى الرغم من أن أقل مجموعة مختلفة من المحتوي الكلي من الهائمات الحيوانية سجلت أدنى عدد لها في حالة المعاملة FM25 ، لوحظ أعلى نسبة من الديدان قليلة الاشواك (5.9٪) لهذه المعاملة. أظهرت النتائج وجود علاقة ارتباط معنوية بين سينتروبيكسيوس وأسبلانشنا ($r = 0.95$) ، ($P = 0.004$) . وفي نفس السياق كان هناك ارتباط بين الاوليات الحيوانية والديدان قليلة الاشواك ($r = 0.85$) ، ($P = 0.03$). كما اظهرت الدراسة ان هناك علاقة معنوية بين الروتيفرا مع الفوسفور والسيلينيوم ($r = 0.96$)، ($p = 0.017$) . وتم الاعتراف بالنتائج السابقة التي تؤثر على تركيب النظام الغذائي في المحتوي الكلي من الهائمات الحيوانية الذي أظهر فيما بعد انعكاسها على أداء الأسماك. وهناك حاجة إلى مزيد من الدراسات لفهم العلاقة بين انواع العوالق الحيوانية المختلفة كخطوة للسيطرة على جودة مسحوق الفلوك من خلال التغير فى النظام الغذائي.