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# Manipulating diet composition to develop and maintain the zooplankton for Nile tilapia under biofloc condition

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## ABSTRACT

This study examined the effects of feeding Nile tilapia guar-meal diets on the composition of zooplankton community generated within rearing tanks under the biofloc conditions. The experiment lasted for 70 days for rearing Nile tilapia in biofloc tanks with zero water exchange. Five practical diets were formulated to be isonitrogenous (30% CP) and isocaloric (20 KJ/g diet). Diets were assigned as: diet 1, CTRL or control 0- guar meal, diet 2: SBM50 where 50% of the soybean meal protein was replaced by guar meal, diet 3: SBM100 where 100% of soybean meal protein was replaced by guar meal, diet 4: FM50 where 50% of fish meal protein was replaced by guar meal, and diet 5: FM100 where 100% of fish meal protein was replaced by guar meal. Both control and FM100 treatments recorded the significantly highest ($P > 0.05$) total zooplankton count. *Lecane*, *Philodina* and *Vorticella* were the most dominant species identified within the zooplankton community of all dietary groups. The total zooplankton count decreased with the increase of guar meal inclusion level in the diet and the opposite trend was shown for *Lecane sp*. These results indicated that feeding Nile tilapia guar meal-diets significantly affect the zooplankton composition generated within the biofloc rearing tanks. It was concluded that biofloc zooplankton community, as a secondary natural food source, is the best when 50% of the SBM is replaced by guar meal in Nile tilapia feeds.

**Key words**: Biofloc, Guar meal, zooplankton profile, fish meal, soybean meal.

## INTRODUCTION

Zooplankton is consider as an important link in aquatic food chain and therefore, contributes to the production in fresh and marine water ecosystems (Sharma, 1998). Zooplankton consume the primary producers (phytoplankton) and form a major food source for tertiary producers. Therefore, zooplankton is one of the basic principles of natural food for fish production in nature. Studies on planktonic composition and morphometric, physical and chemical characteristics of water bodies are necessary to obtain the basic information on the biodiversity within rearing ponds under different production system (Rajagopal *et al.*, 2010).

The biofloc system technology combines the nutrients removed from water body with the production of microbial biomass. The microbial biomass produced can be used as an additional nutritional source for cultured fish and/or shrimp (McIntosh, 2000; Moss *et al.*, 2001; Samocha *et al.*, 2001; Weirich *et al.*, 2002; Schneider *et al.*, 2006; Schryver *et al.*, 2008). The bioflocs are composed of phytoplankton, zooplankton, bacteria, and detritus in the form of suspended and aggregated particles (Schryver *et al.*, 2008). Delivering carbon source by fertilization in the system optimizes the growth of heterotrophic bacteria and subsequently zooplankton, while bacteria convert inorganic nitrogen into bacterial

protein, enhance the water quality and allow the fish or shrimp to grow at high stocking densities (Avnimelech,1999; Bratvold and Browdy, 2001; Boyd and Clay, 2002; Hari *et al.*, 2004; Avnimelech, 2006 and Zidan *et al*., 2017).

The present study is a preliminary trial initiated to grow Nile tilapia in tanks under the biofloc system, i.e zero water exchange rate and describe their rearing conditions when fed guar meal diets as a plant source for protein. Previous researches showed that guar meal can partially replace either soybean meal or fish meal protein in Nile tilapia diets. Therefore, the objective of this study is to investigate the effect of guar meal incorporation, as an alternate protein source, within Nile tilapia diets on zooplankton composition produced under biofloc system conditions.

## MATERIALS AND METHODS

This experiment is conducted in Fish Nutrition Lab National Institute of Oceanography and Fisheries (Qanater Khaireya, Branch) Egypt, and lasted for 70 days.

### Experimental fish

Nile Tilapia, *Oreochromis niloticus* are purchased from commercial hatchery (Mohamed Goda, Fayom), with initial body weight ranged from 6.8 to 7.5g. Fish were randomly distributed into 15 plastic tanks (12 fish per tank). Five dietary groups were established in triplicate. Fish were acclimated to the experimental conditions for two weeks before initiation of the feeding trial.

### Diets and feeding protocol

Five isonitrogenous (30% crude protein) and isocaloric (20 KJ/g diet), practical diets were formulated and produced in the laboratory. The experimental diets were: (1) control diet, 0 guar meal; (2) 50% of dietary soybean meal protein was replaced by guar meal (SBM50), (3) 100% of soybean meal protein was replaced by guar meal (SBM100) , (4) 50% of fish meal protein was replaced by guar meal (FM50), (5) 100% of fish meal protein was replaced by guar meal (FM100).

The fish are fed 4% of their body weight, two times daily (at 9:00 am, 15:00pm) for six days a week. Fish of each tank were bulk weighed bi-weekly after 24 h of last meal. Starch was used, as a source of organic carbon (Avnimelech, 2007), and added to Tank waters as a liquid, and its amount wa calculated according to the equation of Hargreaves (2013) (total starch = Total Nitrogen diet × 0.75×10 / carbon % at source, , one time daily for six days a week.

### Installation of rearing tanks and measurements of water quality parameters

This study was conducted by using 50 Lindoors plastic tanks supplied with fresh well waters. Tanks were managed by the zero-water exchange system. Each tank was aerated by air pump**s to** maintain the appropriate dissolved oxygen level for Nile tilapia. During the experiment, water temperature ranged between 28 & 30°C. Other water quality parameters (Dissolved oxygen, pH and Total ammonia nitrogen) were observed daily to ensure the suitability of tank waters for the biofloc production as well as for growing Nile tilapia The Floc volume was weekly measured by Imhoff cone according to Avnimelech (2009) method. The Biofloc sample was collected by siphoning, then filtered through a zooplankton net (20µ) and gradually sun dried for 12 h.

### Taxonomy and total count of zooplankton

Zooplankton were collected from experimental tanks10 liters of water, and filtered through plankton net (55 µ mesh size, 25 cm diameter and 80 cm length). Each collected sample was transferred to a labeled clean bottle and immediately fixed with 4 % formaldehyde. In the laboratory, three subsamples (one ml each)

of the homogenized plankton samples were transferred into a counting cell and zooplankton species were identified. The subsamples were examined under a binocular research microscope with magnification varied from 100X to 400X. Zooplankton community density was calculated as the number of individuals per cubic meter from the equation given by APHA(1995) as follows:

No.per $m^3$ = (CxV′) / (V″ x V‴) x 1000;

Where, C= number of organisms counted;

V′= volume of concentrated sample, ml

V″ = volume counted, ml.

v‴= volume of the grab sample, liters.

Zooplankton species were identified according to Wallace and Snell (1991), Foissner and Berger (1996).

**Biofloc proximate analysis**

Biofloc samples were analyzed, to determine its major nutrients composition, according to the standard method of AOAC (1995). Dry matter (DM) was measured by oven drying at 105$^{o}$C, crude protein (N x 6.25) by the Kjeldahl method using a Kjeltech auto-analyzer (Model 1030, Tecator, Hoganas, Sweden), crude fat by the method of Bligh and Dyer(1959), and ash according to the standard method of AOAC (1995).

**Statistical analysis**

At the end of experiment, data were subjected to one-way analysis of variance (ANOVA) using the statistical software (SPSS 18). Duncan multiple range test was used to detect individual differences between treatment means (Duncan 1955). Data were presented as means ±standard deviation (S.D) and a rejection level of P>0.05 was used for significant differences.

## RESULTS AND DISCUSSION

**Nutritional value of biofloc**

Biochemical nutritional composition of the biofloc meal, generated within the tanks of the five dietary groups, was presented in Table (1). No significant differences (P > 0.05) were observed for either protein or lipids content of the floc meal among dietary groups. However, the floc meal from the SBM100 tanks has the highest ash content among all dietary groups. The nitrogen free extract/carbohydrates content of the floc meal were the highest for both SBM50 and FM50 dietary groups among all treatments.

**Table 1.Biochemical composition (mean ± SE, n=3) of biofloc meal (%DM) generated from the tanks of biofloc**.

| **Dietary groups** | Crude protein | Lipids | Ash | Nitrogen Free Extract* |
|---|---|---|---|---|
| **Control, CTRL** | 35.93±1.63 | 1.12±0.30 | 29.65±0.38$^{b}$ | 35.31±1.24$^{ab}$ |
| **SBM 50** | 31.65±0.10 | 0.80±0.20 | 29.87±0.49$^{b}$ | 38.60±0.89$^{a}$ |
| **SBM 100** | 30.43±1.43 | 0.73±0.12 | 38.44±2.77$^{a}$ | 30.39±2.06$^{b}$ |
| **FM 50** | 29.10±4.00 | 1.08±0.21 | 31.33±0.18$^{b}$ | 38.49±2.27$^{a}$ |
| **FM 100** | 34.55±0.55 | 0.78±0.11 | 33.49±0.27$^{b}$ | 31.18±0.49$^{b}$ |

*Calculated by difference

Means in the same column with different letters are significantly different (P <0.05).

The nutritional quality of biofloc to cultured fish was good but rather variable. The dry-weight protein content of biofloc ranged from 25 to 50 %, with most estimates between 30 and 45% (Maicá *et*

*al*., 2012; Martínez-CÓrdova *et al*., 2015). In the present study, the protein content of biofloc meal (29.1 to 35.9 %) was higher than the values earlier reported by Azim and Little (2008), Ekasari *et al*. (2010), Becerra-Dorame *et al.* (2011) and Emerenciano *et al*. (2012), but lower than those (43.1% or 49%) obtained by Maicá *et al*. (2012) or Martínez-CÓrdova *et al*. (2015), respectively. Likewise, the estimated lipids content of the biofloc meal in the present study were close to that (1.13%) given by Martínez-CÓrdova *et al*. (2015), but lower than (2.1-3.6%) reported by Maicá *et al*. (2012). Also, the floc meal carbohydrate content, in the present research, was similar to that previously obtained by Becerra-Dorame *et al*. (2011); Emeren´ciano *et al*. (2012) and Martínez-CÓrdova *et al*. 2015). In this connection, Hepher *et al*. (1983); Henderson and Clark (1990); Watanabe *et al*. (1990) claimed that protein requirement for Nile tilapia (25-30% CP) can be met by the biofloc meal.

**Zooplankton community composition**

Zooplankton community composition in Nile tilapia rearing tanks of each dietary group was given in Table (2). Control and FM100 treatments recorded the highest (P > 0.05) total count of zooplankton among all dietary groups. *Vorticella, Philodina* and *Lecane spp.* were the predominant species observed within the zooplankton community. *Vorticella spp*. recorded the highest count (23-118, 1000/L) for all dietary groups, followed by *Philodina* (4-9, 1000/L) then *Lecane spp.*(2-5, 1000/L).

**Table 2. Estimated count (mean ±SD, n=3) of major zooplankton species (1000 organism/ L) found in waterof Nile tilapia rearing tanks for the 5 dietary groups.**

| dietary group | *Lecanesp.* | *Philodina sp.* | *Vorticella sp.* | Total zooplankton count |
|---|---|---|---|---|
| Control,CTRL | **2.**67±0.17$^{b}$ | **9.**17±8.33$^{a}$ | **118.**33±10.14$^{a}$ | **130.**17±10.14$^{a}$ |
| SBM 50 | **4.**17±0.83$^{ab}$ | **9.**17±833.33$^{a}$ | **66.**67±8.33$^{b}$ | **80.**00±7.50$^{b}$ |
| SBM 100 | **4.**17±0.83$^{ab}$ | **5.**00±1.44$^{b}$ | **23.**33±13.33$^{c}$ | **32.**50±12.50$^{c}$ |
| FM 50 | **5.**83±0.83$^{a}$ | **4.**17±0.83$^{b}$ | **41.**67±8.33$^{bc}$ | **51**.67±7.12$^{bc}$ |
| FM 100 | **3.**33±0.83$^{ab}$ | **5.**00±1.44$^{b}$ | **110.**00±10.00$^{a}$ | **118.**33±9.61$^{a}$ |

Means in the same columns with different letters are significantly different (P <0.05).

Substituting half or all soybean meal (SBM) with guar meal has led to a significant (P < 0.05) decrease in the total zooplankton count (in SMB50 & SBM100 groups) as compared to the control group. Likewise, substituting half of the fish meal (FM) with guar meal has resulted in a significant reduction in zooplankton count, in comparison to that of control group. Unexpectedly, when all dietary FM amount was replaced by guar meal, the resultant zooplankton amount in the rearing tanks is comparable (P>0.05) to that of CTRL group (Table 2). The amount of *Vorticella spp.* in floc-zooplankton followed the same trend of variation for the different dietary groups as that of the total zooplankton count. However, a significant decrease in *Philodina sp.* amount was observed when SBM or FM was replaced by guar meal at both levels, except for SBM50 group which was comparable to that of CTRL group. On the contrary, the amount of *Lecane sp.* showed an increasing trend for all dietary groups, particularly in FM50 group, as compared to the controls (Table 2).

These variations indicated that the dietary protein source (either plant or animal source) affects the composition of zooplankton community of floc initiated in the rearing tanks of Nile tilapia. Moreover,

the decrease of zooplankton communities in the treatments of the present study may suggest their consumption by fish.

It is well established that Nile tilapia, *Oreochromis niloticus*, is a planktonic feeder omnivorous species (Dantas and Attayde, 2007). This fish is a filter feeder that also uses visual predation (Huchette *et al*., 2000). According to Attayde *et al*. (2006), omnivorism allows the species to survive in rich environment containing high diversity of plankton. A basic factor in designing a biofloc system is the species to be cultured. Species such as tilapia (and shrimp) have the physiological adaptations that allow them to consume biofloc and digest microbial protein, thereby taking advantage of biofloc as food source. The present research confirms earlier studies that Nile tilapia is an appropriate species for production under the biofloc system.

The water rich of biofloc organisms is a good source for natural food for Nile tilapia therefore, low protein diets can be provided. The externally grown flocs can be redirected to the rearing tank as food for fish, and lower the demand for feed protein (Tacon *et al*., 2002; Burford & Lorenzen, 2004; Avnimelech *et al*., 2008). These results are in the line with those of Divakaran and Kim (2001) who showed that shrimp might have consumed a portion of the zooplankton community under biofloc system.

Previous studies have indicated that the organic carbon can be supplied either as additional organic carbon source (e.g. glucose, starch,etc ….) or by changing the feed composition, thus increasing its organic carbon content (Avnimelech, 1999). Also, the costs of the different organic carbon sources will be a determining choice factor. Carbohydrate addition can result in the production and accumulation of bioflocs (Avnimelech, 2007; Emerenciano *et al*., 2011), which could serve as an important food source for the zooplankton therefore, increase the growth of fish or shrimp. The previous studies also reported that the carbon source plays a vital role in the biofloc formation, composition and its nutritive value, and accordingly can lead to increase in protein utilization and supply of essential lipids and vitamins for the growth of fish and shrimp (Crab *et al*. 2012).

## Conclusion

Biofloc system contains high nutritive components such as zooplankton and phytoplankton and therefore can be consider as a promising sustainable system for Nile tilapia production in Egypt. The present study suggested that zooplankton community profile within the floc produced in the rearing units can be controlled by modifying the additional organic carbon source from the diet.

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**تغيير مكونات العليقة لتطوير تركيب بنائي من الهائمات الحيوانية مناسب لتغذية البلطي تحت نظام البيوفلوك**

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**المستخلص**

لدراسة مدى تأثير مكونات عليقة البلطي على تركيب الهائمات الحيوانية تحت نظام البيوفلوك تم إستخدام 5 علائق متساوية في محتوى البروتين (30% بروتين خام) و الطاقة (20 كيلو جول/ جرام) لكل المعاملات ولكن تختلف في محتواها من مصادر البروتين النباتي . وكانت المعاملات التجريبية 1- عليقة الكنترول خالية من كسب الجوار 2- عليقة تم فيها إستبدال 50% من بروتين كسب فول الصويا بكسب الجوار 3- عليقة تم فيها إستبدال كامل لكل بروتين فول الصويا بكسب الجوار 4- عليقة تم فيها إستبدال 50% من بروتين مسحوق السمك بكسب الجوار 5- عليقة تم فيها إستبدال كامل لكل بروتين مسحوق السمك بكسب الجوار. وتم تغذية الأسماك على العلائق لدراسة تأثيرها على تركيب الهائمات الحيوانية و تراوحت قيم الأنواع ليكان و فيلودينا و فورتيسلا و عدد الهائمات الحيوانية الكلية بين 2666.67:5833.33& 4166.67 : 9166.67 & 66666.67 : 110000 على التوالي. قيم فيلودينا و فورتيسلا وعدد الهئمات الحيوانية الكلية إنخفضت مع زيادة مساهمة مسحوق الجوار بالعلائق وهذا يدل على أن تكوين العليقة لة دور في تركيب الهائمات الحيوانية تحت نظام البيوفلوك.