Effect of freeze-dried biofloc as A Dietary Supplement on Water Quality and Growth Performance of red Tilapia (Hybrid)

Tarq Binalshikh-Abubkr$^{1,2}$ and Marlia Mohd Hanafiah$^{1,3}$*

$^1$ Department of Earth Sciences and Environment, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia
$^2$ Department of Food Science and Technology, Faculty of Environmental Science and Marine Biology, Hadhramout University, P.O. Box 50512, Mukalla, Yemen
$^3$ Centre for Tropical Climate Change System, Institute of Climate Change, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

*Corresponding E-mail: mhmarlia@ukm.edu.my

Abstract. To investigate the effect of supplementation of dried biofloc produced by freeze-drying method on water quality and growth performance of red hybrid tilapia, two experimental diets (T0 treatment: 0% biofloc; and T1 treatment: 4% freeze-dried biofloc) were examined during 57 days of feeding trial. Diet T0 resulted in higher feed intake than T1 treatment ($P < 0.05$), while no significant differences were found between the two treatments in terms of weight gain, SGR, and FCR ($P > 0.05$). For water quality parameters, values of temperature, EC, TDS, ammonia and nitrate were significantly higher for T1 treatment ($P < 0.05$). While dissolved oxygen level was significantly lower for T1 treatment compared to T0 treatment ($P < 0.05$). Moreover, no significant differences were found for pH and salinity ($P > 0.05$). However, the two treatments showed water quality parameters levels within the range of standard levels of water quality for tilapia culture. In addition, proximate composition of experimental diets showed significantly higher lipid content for T0 treatment than T1 treatment ($P < 0.05$). While no significant differences were found between means of treatments for organic matter, protein, fiber, ash and energy contents ($P > 0.05$). Based on these results, a 4% supplementation of freeze-dried biofloc can be successively included in formulated diets for red hybrid tilapia without any effects on growth in terms of weight gain, specific growth rate, feed conversion ratio and survival, and can result in acceptable water quality levels for red hybrid tilapia.

Keywords: Biofloc technology, red tilapia, water quality, growth performance, proximate composition

Track Name: Land, Water, Forests and Food Security

1. Introduction

In the recent years, feed expenses have become an urgent issue for aquaculture sector because of the high cost of feed ingredients and the continually increasing use of animal or plant origin (such as; fishmeal and soybean meal) as protein sources in aquafeeds to meet nutritional requirements for fish.
feed [1]. About 50% of operating cost comes only from fish feeding [2]. Therefore, to maximize the benefit and to achieve the sustainable goals of this industry, other alternative, more economic protein sources, are needed to be used as a replacement for the partial or even total of traditional ingredients in the formulation of diets [3, 4].

Recently, the use of several microbial biomasses as sources of protein has garnered more attention from researchers [5-9]. Among these, microbial flocs which known also as biofloc (BF) are widely used for culture animals and highly recommended to enhance production through provision of natural food in situ [10]. BF can be collected from biofloc technology systems as a wet meal to culture animals [11, 12] or as a dried meal after drying processes with high protein level [13-15]. BFT systems have many benefits that can increase the profitability of aquaculture industry and also can support the sustainability of this sector [16]. In that sense, BFT can keep land and water resources used for fish farming, and can reduce the environmental impact of aquaculture activities on the external water bodies [17]. The technology as well as the use of biofloc meal as a supplement or replacement of a part of feed ingredients is very promising and it can minimize feed costs [18].

As for the benefits associated with fish nutrition requirements, the nutritional value of biofloc has been investigated by many researchers, and BF showed protein content of 18-49, lipid content of 0.4-7.6, fiber content of 0.2-17.1, nitrogen free extraction (NFE) content of 16.5-36, ash content of 1.4-61, and moisture content of 7.8-10.1 g/100g dry weight [14-28]. For example, Crab et al. [29] reported protein content of 58% in the bioflocs produced using glycerol with Bacillus. Biofloc can be a key to the sustainable aquaculture in future [16].

Biofloc can be successfully consumed by herbivorous and omnivorous fish species [16]. Fish consumption of BF depends on various factors such as the feeding habit of the species and its ability to eat the flakes and the suspended particles density [16]. Due to its omnivorous habit, tilapia can use BF as a food source, and BF can meet up to 50% protein requirements of fish [30]. Red hybrid tilapia is one of potential fish species that can be cultured in freshwater and saline water and can tolerate wide range of water salinity [31-33].

The development of aquaculture sector and the increasing of fish production require information on the effect of feed ingredients, environmental conditions and other factors in the culture tank on the growth of fish [34]. Not only the growth, but also water quality can be affected by those factors [35]. For example, inedible part of the feed, metabolic waste products, and faces of fish will accumulate in culture tank, decompose, and decrease water quality parameters resulting in stressing the fish and production decrease [36]. Therefore, water quality parameters are the key factors which can limit the increase in production of fish farming and need to be managed properly for optimal standards of water quality for cultured animals [37].

Additionally, nutritional value of final product is also an important issue. Consumers prefer high quality, safe and more nutritious food products. Biofloc may bring higher profit of farmed fish if it applied as a replacement of commercial feed or served as a protein source in diets [15]. BF considered a high quality resource of protein, as a result of high level of protein in muscles of fish [29]. These advantages certainly should be more explored [38]. Although, bioflocs show an adequate protein and lipid content for use as an aquaculture feed, more research is required on their amino acid, fatty acid and mineral profile [39, 13].

Growth studies of cultured animals often rely on the proximate composition of experimental diets [12, 21], and fish composition as well. Knowledge of the proximate composition of fish and factors affecting that composition allows us to determine the nutrient transfer efficiency from the feed to the fish body, and also makes it possible to modify composition of the final product [40]. Thus, whole body composition can play an important role in improving the efficiency of formulated diets, reducing overall feed waste, and increase profitability of the culture operation [40]. The changes in body composition of tilapia fish determined by several researchers where carcass of the fish was analyzed at the beginning and end of the experiment [41-43].

Novel methods for integrating biofloc with the aquaculture feeding is required to meet nutritional needs of the cultured animals and maximize production, and also to minimize using traditional protein
sources in feed [17, 28]. Therefore, to investigate the effect of biofloc as a dietary supplementation on growth and water quality of red hybrid tilapia reared in brackish water, supplement of 4 % (in dry weight) freeze-dried biofloc diet was evaluated and compared to a control diet without biofloc (100 % commercial feed).

2. Materials and Methods

2.1. Raw materials

2.1.1. Biofloc sampling

In this study, three indoor red hybrid tilapia biofloc system tanks at a commercial farm located in Sepang, Selangor, Malaysia were used for biofloc sampling. The biofloc was collected by using 40 µm plankton net then transported in a cold-store box with ice to laboratory at Universiti Kebangsaan Malaysia (UKM), Selangor, Malaysia. The collected biofloc was frozen immediately at –80°C for two days then freeze-dried at a vacuum freeze-dryer for three days. After that freeze-dried biofloc was kept in zip-lock plastic bags and stored in a refrigerator at 4°C for later proximate analysis and also for experimental diet.

2.1.2. Tilapia fingerlings

Red hybrid tilapia fingerlings were purchased from a commercial fish farm located in Kuala Selangor, Malaysia. The fish were transported in specific plastic bags with oxygen to the laboratory (UKM). The bags including fish were dived into the aquarium water for 20 minutes to balance the water temperature. Then the fish were allowed to move slowly into the aquarium. The fish were not being fed for 24 h to avoiding any shock.

2.2. Experimental diets

Experimental diets; T0 a control diet (100 % commercial feed) without biofloc and T1 (4 % freeze-dried biofloc, in dry weight) were prepared to be examined for growth and water quality of red tilapia. All the dried ingredients were mixed with warm water to make dough. The dough was pressed through a pelletizer with 2 mm die, formulated in pellet form, and then dried at 60°C until moisture level of below 10 % was reached. The dried diets were crushed to less than 2 mm size, then stored at 4 °C till use.

2.3. Experimental design and feeding trail

The experiment was conducted at the marine laboratory of Earth Sciences and Environment Department, Faculty of Science and Technology, UKM, Bangi, Selangor, Malaysia. An experimental design of 6 glass tanks (64.5×37×38) with proximately capacity of 90.5 L were used to rearing the fingerlings of tilapia. The tanks were provided with oxygen using a large air pump through a net of plastic pipes. Each tank was designed with an individual biology filter (1/6 of the tank size) and a small water pump (power 580 L hr⁻¹).

Before the start-up of the experiment, the fish were acclimated on the new environmental conditions at laboratory for a week. During the acclimation period the fish were fed a commercial feed in a proximate composition of; 12 % moisture, 15 % ash, 34 % protein, 6 % fat, and 7 % fiber as provided by the manufacturing (Red Eagle brand, Sri Edaran Hong Enterprise, Kelantan, Malaysia). After acclimation, 90 fishes were selected with average weight of 4 ± 0.068 g and distributed randomly into experimental tanks with a stocking density of 15 fingerlings per tank for eight weeks (57 days). Fish were fed three times per day at 10 am, 1 pm and 4 pm with a feeding ratio of 10 % of fish biomass weight per tank [44]. The feeding ratio was constantly adjusted throughout the rearing period and reduced to 8 % or 5 % depending on the weekly fish biomass gain [42,43,45]. Uneaten feed was siphoned and filtered for further calculations.

¼ of water was exchanged and the filter wool with fish wastes was removed from the primary stage of biology filter and replaced with a new one every week in each tank. After water exchanging salinity of 10 ppt was maintained to all experimental tanks by adding marine salt (Model: Aquarium Systems Instant Ocean, bucket 25 kg, France). The reason of using 10 ppt salinity is because that is the salinity
of saline water, especially brackish water between 8 ppt [46] and 10 ppt [42], is recommended to improve the growth of tilapia.

2.4. Growth performance
Fish performance was evaluated by body weight gain (WG; g), feed conversion ratio (FCR), feed efficiency ratio (FER), feed intake per fish (FI; g), specific growth rate (SGR %), protein efficiency ratio (PER) and survival. These parameters were determined using methods by Lopez-Betancur et al., [43] and Prabu et al. [47] as following equations:

\[
\text{Weight gain WG (g) = Final Weight (g) – Initial Weight (g)}
\]

Specific growth rate: \(\text{SGR} = \left[\frac{(\text{Ln final weight} - \text{Ln initial weight})}{\text{days}}\right] \times 100\)

Feed conversion ratio: \(\text{FCR} = \left[\frac{\text{total feed fed (g) \div \text{fish biomass increase (g)}}}{\text{}}\right]\)

Feed efficiency ratio: \(\text{FER} = \frac{1}{\text{FCR}}\)

Feed intake: \(\text{FI} = \frac{\text{Total feed consumption per tank \div number of fish per tank}}{}\)

Protein efficiency ratio: \(\text{PER} = \frac{\text{biomass weight gain (g) \div \text{protein intake (g)}}}{\text{}}\)

Survival = \(\frac{\text{(Total number of fish survival \div total number of fish stoked)}}{\times 100}\)

2.5. Water quality parameters
Water quality parameters included; dissolved oxygen (DO), temperature, salinity, conductivity (EC), total dissolved solids (TDS) and pH were measured daily in the tanks of fish. A handheld multi-parameter water quality instrument (EcoSense®-EC300A, YSI, USA) was used for temperature, salinity, EC, and TDS. While DO was monitored continuously by a dissolved oxygen meter (YSI 5000) and a pH meter (LAQUA PH1100) was used for pH. A water sample of 500 ml was collected every week from each tank and filtered through dried and weighed Whatman 1822-047 GF/C filter paper under vacuum pressure and used for total ammonia-nitrogen (TA-N) and nitrate-nitrogen (NO3-N) measurements according to standard methods [48].

2.6. Proximate composition
Proximate compositions of the experimental diets (control diet and 4 % freeze-dried biofloc diet) were analyzed in dry matter according to standard methods of AOAC [49]. The dried samples were ground by mortar and pestle and kept at 4°C for further analysis of moisture, ash, protein, lipid and fiber contents [50]. Samples were analyzed in triplicate for all proximate contents.

2.6.1. Moisture measurement
A known grams of flesh was pre-weighed (W1) on a crucible and place in a vacuum oven at 105°C for 24 hours. The sample was removed from the oven, cooled in a desiccator, and re-weighed (W2). Moisture percentage was calculated according to the formula:

\[
\text{Moisture (%)} = \frac{(\text{W1} – \text{W2})}{\text{W1}} \times 100
\]

2.6.2. Total ash measurement
Porcelain crucible with dry sample from the moisture analysis was used for ash analysis incinerated in a muffle oven at 550°C for 4 hours. The crucible was removed from the muffle oven, cooled in a desiccator, and then weighed. Ash content was calculated according to the following formula:

\[
\text{Ash (%)} = \frac{(\text{ash weight \div sample weight})}{\times 100}
\]

2.6.3. Protein measurement
Crude protein content was determined by kjeldahl method. The kjeldahl procedure basically divided into three parts: (1) Digestion, (2) Distillation and (3) Titration. The procedure used in this work
measures the protein percentage as a nitrogen content of the dried sample. The protein content then was calculated assuming a ratio of protein to nitrogen for the specific food being analyzed (N × 6.25). Calculation:

\[
\% \text{ Nitrogen} = (\text{value of HCl} \times 0.1 \times 0.014 \div \text{weight of sample}) \times 100
\]

\[
\% \text{ of crude protein} = \% \text{ Nitrogen} \times \text{Conversion factor}
\]

(Conversion factors for animal and plant origin are 6.25 & 5.90 respectively)

### 2.6.4. Lipid measurement

Crude lipid was determined by soxhlet extraction technique and was calculated as below:

\[
\% \text{ of crude lipid} = (\text{corrected weight of fat} \div \text{weight of sample}) \times 100
\]

### 2.6.5. Crude fiber

Crude fiber was determined by fiber analyzer. The major components of natural dietary fiber are cellulose, hemicelluloses, lignin, and other non-starch plant polysaccharides such as pectin. Dietary fiber is often determined gravimetrically. In such a procedure, digestible carbohydrates, lipids, and proteins are selectively solubilized by chemicals or removed by enzyme-catalyzed hydrolysis. Then, non-solubilized and/or nondigested materials are collected by filtration, and the fiber residue is recovered, dried, and weighed. It is essential either that all digestible materials be removed from the sample so that only non-digestible polysaccharides remain or that the non-digestible residue be corrected for remaining digestible contaminants. Lipids are removed easily from the sample with organic solvents and generally do not pose analytical problems for the fiber analyst.

### 2.6.6. Total nitrogen free extract

The total nitrogen free extract (NFE) was measured as following:

\[
\text{NFE} \% = 100 - [\text{moisture} \% + \text{total ash} \% + \text{crude protein} \% + \text{crude lipid} \% + \text{crude fiber} \%]
\]

### 2.6.7. Gross energy

The gross energy was calculated by formula:

\[
\text{Gross energy (Kcal/100g)} = [\text{crude protein} \times 5.6] + [\text{crude lipid} \times 9.44] + [\text{crude fiber} \times 4.1] + [\text{NFE} \times 4.1]
\]

### 2.7. Data analysis

Statistical analysis was performed using statistical package SPSS version 22. Kolmogorov–Smirnov test for normality and Levene’s test for homogeneity were used for variances. After the normality and homogeneity were met, significant differences of means among treatments were determined using analysis of variance (ANOVA) at 95% confidence interval. The treatments were considered as independent variables (factors) and parameters of growth, water quality and proximate composition were considered as dependent variables (measured variables. The treatments were compared using one-way ANOVA followed by Tukey test (P < 0.05). Descriptive statistics were done for water quality variables to show maximum and minimum values for each treatment.

### 3. Results

Table 1 shows one-way ANOVA of the effect of experimental diets on the growth indicators of red tilapia. The Kolmogorov-Smirnov test indicated that the data came from a normal distribution. The Levene’s test (Homogeneity of variance test) indicated that the data were homogeneous variance between the two groups of data. Treatments; T0 (0% biofloc) and T1 (4% freeze-dried biofloc) were considered as factors for ANOVA.

Statistically, no significant differences were recorded (P > 0.05) for growth indicators final weight (Wf), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency
ratio (FER), protein efficiency ratio (PER) and survival (SG) between the two treatments. While the feed intake (FI) for T0 treatment (86.86 g) was significantly higher ($P < 0.05$) than T1 treatment (72.72 g) (Table 1).

**Table 1.** The effect of experimental diets on growth performance of red tilapia.

| Growth indicators              | Treatments       |
|-------------------------------|------------------|
|                               | T0               | T1               |
| Initial weight (g fish⁻¹)     | 4.05±0.04A       | 4.04±0.05A       |
| Final weight (g fish⁻¹)       | 20.98±0.24A      | 20.42±0.43A      |
| Weight gain (g fish⁻¹)        | 16.93±0.19A      | 16.48±0.46A      |
| Initial biomass (g tank⁻¹)    | 60.74±0.63A      | 60.60±0.68A      |
| Final biomass (g tank⁻¹)      | 272.99±24.07A    | 255.40±15.55A    |
| Biomass gain (g tank⁻¹)       | 212.25±23.43A    | 196.44±16.02A    |
| SGR (%)                       | 2.63±0.14A       | 2.57±0.12A       |
| FCR (g)                       | 0.43±0.03A       | 0.44±0.03A       |
| FER                           | 2.34±0.14A       | 2.27±0.13A       |
| FI (g)                        | 86.86±5.79A      | 72.72±0.33B      |
| PER                           | 6.89±0.40A       | 6.67±0.37A       |
| Survival (%)                  | 86.67±6.67A      | 83.34±3.34A      |

Values are means of the experimental treatments ± SD, values followed with different capital letters in same row are significantly different ($P < 0.5$). SGR (%): Specific growth rate. FCR: Feed conversion ratio. FER: Feed efficiency ratio. FI: Feed intake (g/tank). PER: Protein efficiency ratio. T0: commercial feed 100 %; T1: freeze-dried biofloc 4 %.

In contrast, water quality parameters showed significant differences ($P < 0.05$) between means of the two treatments for temperature (T), dissolved oxygen (DO), electrical conductivity (EC), total dissolved solids (TDS), total ammonia-nitrogen (TA-N) and nitrate-nitrogen (NO₃-N). While no significant differences were recorded ($P > 0.05$) for pH and salinity (table 2).

In general, levels of T, DO, pH, salinity, EC, and TDS were ranged in 25.6-28.1, 7.46-10.68, 7.51-8.37, 8.98-10.35, 12.22-17.61, and 9.18-11.51, respectively for the two treatments (table 2). T1 treatment showed pH and salinity values (7.87 and 9.93, respectively) closer to T0 treatment (7.90 and 10.06, respectively). However, T1 treatment showed significantly higher ($P < 0.05$) temperature (26.27°C) than T0 treatment (26.22°C), and significantly lower ($P < 0.05$) DO level (9.03 ppm) than T0 treatment (9.15 ppm). Furthermore, EC and TDS levels (16.08 mS/cm and 10.76 ppm, respectively) tended to be significantly higher ($P < 0.05$) in T1 treatment compared to T0 treatment (15.85 mS cm⁻¹ and 10.48 ppm, respectively).

Concentrations of total ammonia-nitrogen (TA-N) and nitrate-nitrogen (NO₃-N) ranged from 0.08-1.95 and 0.83-2.40 ppm, respectively. The concentrations of 1.26 ppm TA-N and 1.70 ppm NO₃-N were significantly higher ($P < 0.05$) for T1 treatment compared to 1.21 ppm TA-N and 1.58 ppm NO₃-N for T0 treatment (Table 2).

**Table 2.** Water quality parameters during the experiment period of 57 days (mean ± SD followed by minimum and maximum values).

| Parameters | Treatments (means & range of values) |
|------------|--------------------------------------|
|            | T0                                  | T1                                  |
| T (°C)     | 26.22±0.03A (25.6-28.1)              | 26.27±0.02B (25.7-28.1)              |
| DO (ppm)   | 9.15±0.09A (7.61-10.68)              | 9.03±0.07B (7.46-10.50)              |
| pH         | 7.90±0.02A (7.60-8.37)               | 7.87±0.02A (7.51-8.30)               |
Uneaten feed could be resulting in poor water quality parameters in culture water [51].

The FI can be calculated as total feed consumption per tank divided by number of fish per tank [46]. The total feed consumption is calculated by subtracting the amount of uneaten feed from the total feed fed in each tank. Uneaten feed could be resulting in poor water quality parameters in culture water [51]. In this study, uneaten feed by fish in each tank was siphoned, filtered, dried, and then weighed to calculate the total consumed feed per tank during the 57 days of tilapia rearing. We found that the FI of T1
treatment was lower than the FI of T0 treatment (control). Therefore, the decrease in FI for the freeze-dried diets may be due to the increase of uneaten feed weight for the same treatment.

On the other hand, the drying method had clear effect on water quality parameter. From the results of this study, there were significant effects of using freeze-drying method on most of the water quality parameters, except for pH and salinity. Moreover, total ammonia and nitrate-nitrogen concentrations were higher for freeze-dried biofloc treatment compared to the control treatment.

In the present study, the values of DO, pH and EC might be influenced by the water temperature inside tanks. T1 treatment showed lower DO levels, but higher temperature compared to T0 treatment. In this respect, it is known that there is an inverse relation between temperature and the level of dissolved oxygen in water [52] Similar to Do levels, pH values can be influenced by temperature since there is also an inverse relationship between temperature and pH in water [53]. Although the pH values between the two treatments in the present study showed no significant differences, slightly increase was noted for T0 treatment, while T1 treatment was slightly decrease in pH. Meanwhile, the higher temperature in T1 treatment might cause increase in the EC values in the present study. Hayashi [54] reported that is a direct relationship between temperature and conductivity values in water. For example, increasing of 1°C in temperature causes increasing of 2% in EC value [54]. Therefore, the decrease in DO level and the increase in EC values for T1 treatment in the present study may be due to the increase in temperature for the same treatment.

Nitrate is the final product of nitrification process of nitrogen cycle inside culture tank and is less toxic compared with other inorganic nitrogen compounds (nitrite). However, high levels of nitrate can negatively affect the growth of tilapia fish [50]. Unlike the nitrate, ammonia is the most toxic nitrogen compound which can cause high mortality for cultured fish [26].

In the present study, the use of freeze-dried biofloc as a supplement in the formulation of the experimental diet resulting in lower quality parameters in terms of T, DO, EC, TDS, total ammonia-nitrogen (TA-N) and nitrate-nitrogen (NO$_3$-N) for cultured water of red tilapia compared to the control tanks. Even though, the levels of these parameters are still within the range of standard levels for the cultured red tilapia [36,45].

Nutritionally, the control diet without biofloc (T0 treatment) showed higher content of crude lipid in the present study. While no differences in contents of organic matter, crude protein, crude fiber and total ash between the two treatments. However, freeze-dried biofloc diet showed better values of moisture, total NFE and gross energy. The increase in lipid value (4.88 g/100 g) for T1 treatment in this study can be explained by the low level of lipid in biofloc composition [18, 47].

5. Conclusions
Based on the growth performance results, freeze-dried biofloc diet provided good growth rates similar to control diet in terms of final weight (Wf), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency ratio (FER), protein efficiency ratio (PER) and survival (SG). However, the feed intake factor was lower for freeze-dried diet compared to the control. Although freeze-dried biofloc diet resulted in higher concentrations of TA-N and NO$_3$-N, these concentrations are still within the range of standard levels for the cultured red tilapia. This conclusion was also supported by the proximate composition of diets, since freeze-dried biofloc showed better values of moisture, total NFE and gross energy, and similar values of organic matter, crude protein, crude fiber and total ash compared to the control.

Overall, a 4% supplementation of freeze-dried can be successively included in the formulated diets for red hybrid tilapia without any effects on growth in terms of weight gain, specific growth rate, feed conversion ratio and survival, and also can keep the water quality parameters within the acceptable range of red hybrid tilapia.

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