



Effect of dietary supplementation of biofloc meal on growth and survival of GIFT tilapia

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ABSTRACT

A 60 days indoor growth trial was conducted to study the effect of dietary supplementation of biofloc meal on growth and survival of juvenile GIFT tilapia. Four isonitrogenous and isoenergetic experimental diets (32% crude protein) were prepared using biofloc meal at different inclusion levels *viz.*, 0 (T0), 20 (T1), 30 (T2) and 40% (T3). A commercial diet (T4) was used for comparison. The feeding trial was conducted in 15 nos. of 40 l plastic troughs in triplicate, utilising GIFT tilapias weighing an average of 2 g. During the experimental period, water quality parameters were measured and recorded daily. The mean value of water temperature, pH, dissolved oxygen, salinity, ammonia-N, nitrite-N, nitrate-N, hardness and alkalinity recorded in the experimental systems were 28.5°C, 8.1, 5.5 ppm, 4.5 ppt, 0.03 ppm, 0.07 ppm, 11 ppm, 630 ppm and 162.5 ppm respectively. Among the biofloc meal enriched diets, diet T1 with 20% biofloc yielded the best results in terms of average body weight gain, feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER) and feed efficiency ratio (FER). The mean body weight gain recorded in T1 was 25.28±0.81 g. The results demonstrated that biofloc meal is a potential ingredient that can be incorporated in GIFT tilapia diet at 20% level for better growth performance.

Keywords: Biofloc meal, Fish meal, GIFT tilapia, Protein feed, Soybean meal

Introduction

Aquaculture is the fastest growing sector of global fish production (FAO, 2010). Approximately 40% of fish from aquaculture originates from tilapia production (Scorvo-Filho *et al.*, 2010). Today, tilapia has become the shining star of aquaculture, which is also popular as 'aquatic chicken' and the rate of its consumption has increased across the globe (Fitzsimmons, 2005). Annual global production of cultured tilapia has increased consistently in recent years (Ahsan *et al.*, 2013). The GIFT strain was developed by International Centre for Living Aquatic Resources Management (ICLARM; presently World Fish Centre, WFC) through several generations of selection from the base population involving 8 different strains of Nile tilapia *Oreochromis niloticus* (Eknath and Hulata, 2009). Feed costs in aquaculture can be reduced by the adoption of biofloc technology, since the expenses with commercial diets account for more than 50% of the production cost. Biofloc contains up to 30% crude protein and about 2% lipids (Azim and Little, 2008; Ballester *et al.*, 2010; Xu and Pan, 2012; Luo *et al.*, 2014). Biofloc contains adequate levels of protein, lipid, carbohydrate and ash and is suitable for use as an ingredient in aquaculture feed (Crab *et al.*, 2010). In this context, the present study was planned to evaluate inclusion of biofloc meal at different levels in the diet of GIFT tilapia.

Materials and methods

Experimental fish

GIFT tilapia seeds were procured from the State Fisheries Department, Krishnagiri, Tamil Nadu, India. They were acclimatised in FRP tanks, by nursing for 15 days feeding on a commercial diet. They were graded according to weight prior to the experiment. One hundred and fifty numbers of GIFT tilapia having average weight of 2 g were selected for the trial.

Experimental design

The trial was conducted in the indoor biofloc laboratory of Fisheries College and Research Institute, Thoothukudi, Tamil Nadu, India. The 60 days indoor growth experiment carried out in plastic troughs of 40 l, consisted of five dietary treatments with three replicates each. Water was filled in the troughs upto 3/4th of the volume. All the troughs were provided with proper aeration facility.

Biofloc collection and analysis

Biofloc was collected using a plankton net of 100 µm mesh size from Hi-Tide sea farm, Mahendrapally, Nagapattinam District, Tamil Nadu, India. Totally 23 kg

of wet biofloc was collected and dried under sunlight for 8 h. The total quantity of dried biofloc meal obtained was 2.1 kg. It was powdered into fine particles and stored in an air tight container under refrigeration. The proximate composition of the biofloc meal was analysed following standard protocols (AOAC, 1995).

Experimental diets

Four isonitrogenous and isoenergetic experimental diets were formulated *viz.*, T0, T1, T2 and T3. A control diet (T0), without biofloc was compared against the three prepared diets formulated with different levels of biofloc at 20 (T1), 30 (T2) and 40% (T3) by manipulating fish meal and soybean meal level. A commercial diet (T4) was used as an external control. The ingredient composition of the experimental diets is presented in Table 1.

Table 1. Ingredient composition of the formulated feed

Ingredient	Inclusion level (%)			
	Control (T0)	20% Biofloc (T1)	30% Biofloc (T2)	40% Biofloc (T3)
Biofloc meal	0	20	30	40
Fish meal	22	26	27	27
Cassava starch	18	15	13	10
Soybean meal	26	15	10	5
Rice bran	22	12	8	6
Fish oil	5	5	5	5
Fish hydrolysate	3	3	3	3
Monocalcium phosphate	2	2	2	2
Vitamin premix	0.5	0.5	0.5	0.5
Mineral premix	0.5	0.5	0.5	0.5
Common salt	1	1	1	1

The proximate composition of all the experimental diets (T0 - T4) was estimated following standard protocols (AOAC, 1995).

Stocking and feeding

Fish of 2 g mean weight were stocked at 10 nos. per plastic trough. After stocking, the experimental troughs were covered with plastic net on the top in order to prevent fish jumping out of the troughs.

Feeding was done thrice a day (at 9: 00; 12: 00 and 16: 00 hrs) *ad libitum*. Each of the experimental diets was fed by hand slowly to avoid wastage. Feed was given until apparent satiation.

Water quality parameters

During the experimental period, water quality parameters such as temperature, dissolved oxygen, pH, and total alkalinity were measured daily. Water temperature was measured using a thermometer with an accuracy of 0.1°C. pH of water was measured using the laboratory model Elico pH meter. Modified Winkler's titration

method (APHA, 2005) was adopted to estimate dissolved oxygen. Total alkalinity was determined as per the method described in APHA (2005). Total ammonia-N, nitrite-N, nitrate-N, water hardness and turbidity were assessed twice a week, following standard methods (APHA, 2005).

Sampling

Fish sampling was done every fortnight, collecting all the stocked fish from each tank to measure total length and body weight.

Growth performance

Growth performance was assessed in terms of feed conversion ratio (FCR), feed efficiency ratio (FER), protein efficiency ratio (PER), specific growth rate (SGR),

mean weight gain and survival estimated using the following formulae:

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total feed fed (g)}}{\text{Total fish weight gained (g)}}$$

$$\text{Feed efficiency ratio (FER)} = 1 / \text{FCR}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Total wet weight gain}}{\text{Dry weight of protein fed}}$$

$$\text{Specific growth rate (SGR\%/Day)} = \frac{\ln \text{ Final weight} - \ln \text{ Initial weight}}{\text{Experimental duration in days}}$$

$$\text{Mean weight gain (g)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{Mean daily weight gain (g)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Experimental duration}}$$

$$\text{Survival (\%)} = \frac{\text{Total number of fishes survived}}{\text{Total number of fishes stocked}} \times 100$$

Statistical analysis

Data were analysed by one-way ANOVA using the statistical software SPSS 16.0 for windows (SPSS Inc.,

Chicago, IL, USA) and also by Duncan multiple range test (Duncan, 1995).

Results

Water quality parameters

The range of water quality parameters such as temperature, dissolved oxygen, pH and total alkalinity recorded in the experimental systems are presented in Table 2.

Table 2. Range of water quality parameters recorded in the experimental troughs

Parameter	Range
Temperature	28-29°C
Dissolved oxygen	5-6 ppm
pH	8-8.2
Salinity	4-5 ppt
Ammonia	0.01 - 0.05 ppm
Nitrite	0.05 - 0.1 ppm
Nitrate	10 - 12 ppm
Hardness	610 - 650 ppm
Alkalinity	155 - 170 ppm

Proximate composition of experimental diets

Proximate composition of the experimental diets is presented in Table 3. Dried biofloc contained 17.92% crude protein, 0.15% crude fibre, 0.41% ether extract, 51.28% total ash, 10.06% moisture and 1864 kcal kg⁻¹ gross energy. The percentage crude protein content of the prepared experimental diets ranged from 32.54±0.07 to 32.74±0.05.

Table 3. Proximate composition of the experimental diets

Diet	Moisture (%)	Crude protein (%)	Crude fibre (%)	Ether extract (%)	Total ash (%)	Gross energy (kcal kg ⁻¹)
T0	7.66±1.03	32.74±0.05	6.62±0.05	4.42±0.06	22.34±0.05	3942±6.33
T1	8.26±0.07	32.54±0.07	4.15±0.04	8.61±0.08	22.53±0.17	3772±3.46
T2	9.29±0.08	32.74±0.04	2.84±0.05	7.87±0.06	26.55±0.06	3538±5.78
T3	7.26±0.16	32.61±0.04	2.34±0.06	7.57±0.07	30.84±0.06	3444±1.20
T4	11±0.57	27.66±2.02	7.06±0.17	3.00±0.17	28.32±1.45	2856±7.86

Table 4. Growth performance of GIFT tilapia fed experimental diets incorporated with different levels of biofloc meal

Diets	Initial body weight (g)	Final body weight (g)	Weight gain (g)	Survival (%)	Biomass gain (g)	Total biomass (g)	Total feed intake (g)	FCR	FER	PER	SGR
T0	2.03 ^a ±0.02	27.16 ^b ±0.50	25.13 ^b ±0.52	96.6 ^a ±3.32	242.57 ^c ±3.49	262.07 ^c ±4.29	292.00 ^a ±3.05	1.11 ^a ±0.01	0.90 ^d ±0.01	7.41 ^c ±0.10	4.32 ^b ±0.04
T1	2.01 ^a ±0.01	25.28 ^b ±0.81	23.26 ^b ±0.81	96.6 ^a ±3.32	224.40 ^{bc} ±2.62	243.93 ^{bc} ±2.63	290.33 ^a ±3.48	1.19 ^b ±0.00	0.84 ^a ±0.00	6.89 ^{bc} ±0.08	4.21 ^b ±0.05
T2	2.01 ^a ±0.03	22.10 ^a ±0.24	20.09 ^a ±0.23	96.6 ^a ±3.32	194.10 ^{ab} ±4.89	213.53 ^{ab} ±5.33	266.33 ^a ±5.69	1.25 ^c ±0.01	0.80 ^b ±0.00	5.93 ^{ab} ±0.15	3.99 ^a ±0.03
T3	1.97 ^a ±0.02	20.46 ^a ±1.34	18.49 ^a ±1.31	96.6 ^a ±3.32	177.93 ^a ±7.26	194.03 ^a ±5.53	254.00 ^a ±8.62	1.31 ^d ±0.01	0.76 ^a ±0.00	5.45 ^a ±0.22	3.89 ^a ±0.08
T4	1.98 ^a ±0.00	19.88 ^a ±0.99	17.89 ^a ±0.98	86.6 ^a ±4.81	156.82 ^a ±9.35	174.07 ^a ±9.73	238.67 ^a ±8.37	1.37 ^c ±0.01	0.73 ^a ±0.01	4.90 ^a ±0.74	3.84 ^a ±0.07
One way ANOVA (p<0.05)											
Diet	0.51	0.01	0.01	0.54	0.01	0.01	0.18	0.01	0.01	0.01	0.01

Growth performance

The calculated growth parameters for the experimental diets are shown in Table 4. Highest mean body weight of GIFT tilapia was recorded in the control (27.16±0.50 g), followed by T1 (25.28±0.81 g), T2 (22.10±0.24 g), T3 (20.46±1.34 g) and T4 (19.88±0.99 g). However, the mean body weight in T1 (20% biofloc meal) did not differ significantly from the control. FCR, SGR, PER and FER showed significant difference among the treatments (p<0.05). The feed conversion ratio (FCR) of diets T0, T1, T2, T3 and T4 was 1.11±0.01, 1.19±0.01, 1.25±0.01, 1.31±0.01, 1.37±0.01 respectively. The respective feed efficiency ratio (FER) was 0.90±0.01, 0.84±0.01, 0.80±0.01, 0.76±0.01 and 0.73±0.01. Protein efficiency ratio (PER) of diets T0, T1, T2, T3 and T4 was 7.41±0.10, 6.89±0.08, 5.93±0.15, 5.45±0.22 and 4.90±0.74 respectively. Specific growth rate (SGR%) recorded was 4.32±0.04, 4.21±0.05, 3.99±0.03, 3.89±0.08 and 3.84±0.07 respectively for fish fed diets T0, T1, T2, T3 and T4 (Table 4; Fig.1).

Discussion

Nutritional composition of biofloc varies according to the environmental condition, carbon source applied, total suspended solid level, salinity, stocking density, light intensity as well as with changes in phytoplankton and bacterial communities. Protein content of biofloc in the present study was 17.92% which tallies with the earlier findings of Soares (2004), Hende *et al.* (2014) and Neto *et al.* (2015), whereas lesser values of biofloc crude protein was reported by Magondu (2012) and Megahed (2014). The lipid content of biofloc in this study was 0.41%,

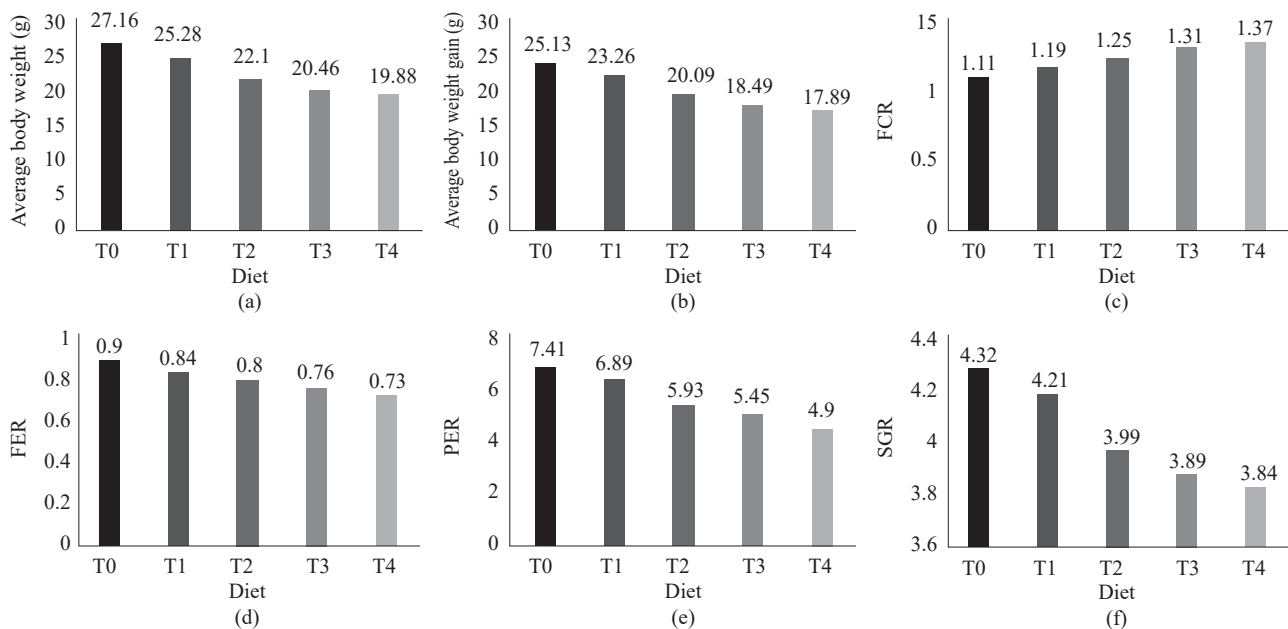


Fig. 1. Growth parameters of GIFT tilapia fed biofloc meal incorporated experimental diets. (a) Average body weight, (b) Average body weight gain, (c) FCR, (d) FER, (e) PER, (f) SGR

being well within the range reported by Emerenciano *et al.* (2013), Wasielesky *et al.* (2006), Faizullah (2015), Hargreaves (2013) and Himaja (2016). Biofloc is usually a poor source of total lipids with concentrations ranging from below 1 to 25 g kg⁻¹ (Azim *et al.*, 2008; Avnimelech 2009; Kuhn *et al.*, 2009; Bauer *et al.*, 2012). The ash content of biofloc in the present investigation was 51.28%, which agreed with earlier reports (Soares, 2004; Maica *et al.*, 2012; Mangodu, 2012; Hende *et al.*, 2014; Neto *et al.*, 2015; Suita *et al.*, 2015). High ash content of biofloc may be related to the abundance of acid-insoluble oxides and mixed silicates (Tacon *et al.*, 2002). Accumulated minerals and metals may also contribute to the high ash content. Biofloc may thus be a good source of essential minerals and trace elements (Tacon *et al.*, 2002; Ju *et al.*, 2012) and it may contribute to animal growth (Avnimelech, 2006).

In the present study, the highest mean body weight of GIFT tilapia was recorded in the control, followed by T1, T2, T3 and T4. However, the mean body weight of fish in T1 group fed 20% biofloc meal did not differ significantly from that of the control. Increase in the dietary biofloc meal level resulted in significant decrease in growth rate and higher FCR when compared with the control. Minimum biofloc meal inclusion (20%) produced better weight gain and lower FCR than the maximum inclusion level (40%) which is in agreement with Ajiboye *et al.* (2012) and Kissling and Askbrandit (1993) who observed reduction in growth rate of fishes at higher levels of microbial supplementation, as microbial products at higher levels

tend to reduce feed palatability and digestibility. In the present study, the survival of fish was 95%, with the exception of T4 (86.6±0.88%). FCR was lowest in the control, followed by T1, T2, T3 and T4 that showed higher values. These values fell in the range from 1.10 to 1.50 as reported by Moss *et al.* (2006), Samocha *et al.* (2007), Taw *et al.* (2009), Zhao *et al.* (2012), Xu *et al.* (2012), Hussain *et al.* (2014) and Dantas *et al.* (2016). PER is used to assess protein utilisation and turnover, where it is related to dietary protein intake and its conversion into fish protein. Ahamed *et al.* (2004) observed that PER is significantly affected by dietary protein level and ranged from 1.58 to 2.35 for fry, 1.19 to 1.92 for fingerlings and 0.99 to 1.53 for adult Nile tilapia. PER values in the present study ranged from 4.90±0.74 to 7.41±0.10.

Earlier studies have reported increase in growth rate, general welfare and survival of fishes reared in microbial floc based systems (Tacon *et al.*, 2002); however, studies that have used floccs as ingredients in aquaculture diets are limited. Kuhn *et al.* (2009) replaced 37% of fishmeal in shrimp feed, achieving similar performance as diets containing lower replacement levels. In another study, Kuhn *et al.* (2010) reported no deleterious effects on growth when *L.vannamei* were fed with diets containing biofloc inclusion from 10 to 30%, regardless of fishmeal substitution of 0 - 67%. The results of the present study suggest that 20% biofloc meal in the diet is optimum for the growth of GIFT tilapia. This finding is supported by the results of Himaja (2016) who obtained higher growth of catla by replacing fish meal in the diet with 20%

biofloc meal. Dantas *et al.* (2016) suggested that fish meal replacement by biofloc meal at 20% improves the growth of *L. vannamei*, whereas Bauer *et al.* (2012) stated that fish meal in *L. vannamei* post-larvae diet could be replaced by biofloc meal up to 30%.

The positive effect of the experimental diets on the growth of GIFT tilapia clearly demonstrates that biofloc meal is a viable alternative ingredient and can be used in the diet up to 20% level. Biofloc meal thus represents an important, alternative ingredient for the development of cost effective feed for fishes, in particular by reducing, dependence on fishmeal as well as environmental impact.

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