



## Effect Of Protein Levels On Growth Performance And Survival Rate Of Mono Sex Nile Tilapia Under Biofloc System

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### Abstract

The current study was conducted to evaluate the "effect of protein levels on growth performance and survival rate of monosex Nile Tilapia under biofloc system". This study was done to assess the optimum level of biofloc meal inclusion in the diet of mono-sex Nile tilapia. The experiment was conducted over a period of 60 days. Growth sampling was done at every fortnight with all the stocked animals from each tank by taking total length and body weight. For this t experiment, four isonitrogenous and isoenergetic experimental diets were formulated viz., C, T1, T2 and T3. A control diet (C), without biofloc was compared against three prepared diets formulated with different level of biofloc at 15% (T1), 30% (T2) and 45% (T3) by manipulating fish meal and soybean meal levels. Commercial diet (T4) was also used to compare the experimental diets. The growth performance such as of feed conversion ratio (FCR), feed efficiency ratio (FER), protein efficiency ratio (PER), specific growth rate (SGR), average weight gain and survival were assessed and statistically analysed. The results showed that among the biofloc meal-containing meals, T1 (15% Biofloc) produced the best results in terms of average body weight growth, FCR, SGR, PER, and FER.

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**Keyword: Mono sex Nile Tilapa, growth performance, survival rate, BFT.**

## 1. INTRODUCTION

The Nile tilapia, *Oreochromis niloticus*, is regarded one of the most significant fish species in tropical and subtropical aquaculture. (FAO, 2012). It provides key sources of animal protein and income around the world (Sosa et al., 2005). Tilapia can grow and reproduce in a variety of environmental circumstances and can withstand stress caused by handling (Sidik et al., 2014). The mono-sex male tilapia population is widely known for its high production potential and low management requirements (El-Sayed, 2006). Today, tilapia has become the shining star of aquaculture, sometimes known as 'aquatic chicken', and consumption has expanded around the world (Fitzsimmons, 2005). Fish feed accounts for more than half of the overall cost of fish production (Craig and Helfrich, 2002), hence aquaculture sustainability is dependent on feed source and

management. As a result, feeding solutions such as bioflocs and periphyton-based culture are being developed to maximize the contribution of natural food, which will help to increase aquaculture production. In the intensive aquaculture business, the highest production cost is spent on feeding the organisms, and approximately 60% is wasted by the organisms, causing phosphorus, carbon, and nitrogen to accumulate in water as suspended matter or dissolved chemicals and be expelled from the system via gasification or water change, polluting other natural water bodies and nearby soils, resulting in economic losses to producers (Gutierrez-Wing & Malone, 2006). To reduce the environmental damage caused by aquaculture, an alternative technology available is to optimize production using the "Biofloc" Technology (BFT), which was developed in the 1970s and is based on microbial communities that help to minimize or avoid water exchange while also producing microbial protein that can be used as food for growing organisms (Avnimelech, 2009). Biofloc contains up to 30% crude protein and around 2% lipids (Luo et al., 2014). Biofloc contains acceptable quantities of protein, fat, carbohydrate, and ash, making it ideal for use in aquaculture feed (Crab et al. 2010). In this regard, the current study was planned to evaluate inclusion of biofloc meal at different levels in the diet of mono sex Nile tilapia.

## 2. MATERIALS AND METHODS

The present study was carried out at the experimental fish laboratory of Aquaculture Division, Department of Zoology and Applied Aquaculture, Barkatullah Vishwavidyalaya, Bhopal, experiments were designed to illustrate effect of biofloc and protein levels on growth response and survival rate of Mono Sex Nile Tilapia

### Experimental fish and Culture techniques

Mono Sex Nile Tilapia seeds were obtained from M.M. Fish Seed Cultivation Private Limited in Raipur, Chhattisgarh. All fish seeds were appropriately acclimated in FRP tanks and reared for 15 days on a commercial diet. The fish were weighed before the experiment and were ranked accordingly. The experiment used an average of 150 and 240 Mono sex tilapias weighing 2 grammes each.

### Experimental setup and fish feeding ingredients

The experimental setup consists of 15 plastic troughs (40 litre capacity) with 5 treatments (3 biofloc meal-incorporated meals, 1 control diet, and 1 commercial diet) in triplicate.

The main materials employed in the fish feed experiment were dried biofloc meal, fishmeal, cassava starch, soybean meal and rice bran. The biofloc was obtained from the Biofloc Ponds from Raipur, Chhattisgarh. 23 kg of wet biofloc was collected and dried in the sun for 8 hours. The total weight of dry biofloc meal was 2.1 kilogrammes. The dried flocs were pulverised into fine particles and stored in an airtight container. Soybean meal, fish oil, fish hydrolysate, monocalcium phosphate, vitamin, mineral, cassava starch, rice bran, and common salt were purchased at the local market in Bhopal.

### Experimental treatment diet categories and preparation

The experimental diets were formulated and prepared from the above procured ingredients. Four iso-nitrogenous and iso-energetic experimental diets were formulated viz., C, T1, T2 and T3. A control diet (C), without biofloc was compared against three prepared diets formulated with different level of biofloc at 15% (T1), 30% (T2) and 45% (T3) by manipulating fish meal and soybean meal levels. Commercial diet (T4) was also used to compare the experimental diets. The ingredient composition of experimental feeds is presented in Table 1.

All the ingredients were mixed with water to make dough. The dough will be steam cooked for 20 min in a pressure cooker at 15 psi. After cooking, dough will be pressed through a pelletizer with 2 mm die and then dried at 60 °C till the desired moisture level (10%). The prepared feeds were stored in air tight containers. The proximate analysis of biofloc and all the experimental diets such as control diet, commercial diet and 15%, 30%, 45% biofloc meal were estimated for proximate analysis following the standard protocols (AOAC, 1995).

### Stocking

The mean weight of tilapia seeds were ranged between 2 – 2.5 g, stocked at 10 numbers per plastic trough. The selected fishes were properly acclimatized and released during stocking in experimental troughs. After the stocking the experimental troughs were covered with plastic net on top in order to prevent the jumping of Mono sex tilapia.

### Feeding

Feeding were done thrice a day (9:00, 12:00 and 16:00 H) at ad libitum. Each of the experimental diets were fed by hand slowly to avoid wastage. Feed was given until apparent satiation. Feeding were in crease or decreased based on their apparent satiation.

### Sampling

Growth sampling was done at every fortnight with all the stocked fishes from each tank by taking total length and body weight.

### Growth performance

The growth performance were 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 using the following formulae;

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

$$\text{Feed Efficiency Ratio (FER)} = \frac{1}{\text{FCR}}$$

$$\text{Protein Efficiency Ratio (FER)} = \frac{\text{Total wet-weight (g)}}{\text{Dry Weight of Protein fed}}$$

$$\text{Specific Growth Rate (SGR\%/Day)} = \frac{\text{Ln Final Weight} - \text{Ln initial Weight}}{\text{Experimental in days}}$$

$$\text{Mean Weight Gain (g)} = \text{Final Weight (g)} - \text{Initial Weight (g)}$$

$$\text{Mean Weight Gain (g)} = \frac{\text{Final Weight (g)} - \text{Initial Weight (g)}}{\text{Experimental Duration}}$$

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

### Water Quality

Water quality maintenance was done by daily replacement of about 20%. To monitor the water quality, the water quality parameters measurements was carried out at the initial and final day of the experiment including Water temperature, pH, Alkalinity, Turbidity, Dissolved oxygen, Total hardness, Nitrite, Nitrate and Ammonia (Table-1) Water quality parameters were analyzed following the procedures described by APHA (2005).

**Table-1:** Optimum water quality parameters to be maintained in Mono sex Tilapia culture

S.NO	Water parameters	Unit	Optimum range
1	Water temperature	°C	28-30
2	pH	-	7.5-8.5
3	Alkalinity	mg/l	155 - 170
4	Turbidity	mg/l	100
5	Dissolved oxygen	mg/l	4-6
6	Total hardness	mg/l	610 - 650
7	Nitrite	mg/l	0.05 – 0.1
8	Nitrate	mg/l	10 - 12
9	Ammonia	mg/l	0.01 – 0.05

### Statistical Analysis

All observations were analyzed and tabulated. The data were analyzed using one-way ANOVA using the statistical program SPSS (version 21) to examine the assessment of optimum in the biofloc meal-included diet of monosex Nile tilapia. The ANOVA was followed by the Duncan test (1955) at a significance level of  $P < 0.05$  when needed.

**Table 2:** Ingredient composition of formulated Mono Sex tilapia feed with 0%, 15%, 30%, and 45% Biofloc meal

S.NO	INGREDIENTS	Inclusion Level ( % )			
		CONTROL (C)	15% (T1)	30% (T2)	45% (T3)
1	Biofloc meal	0	15	30	45
2	Fish meal	22	29	27	24
3	Cassava starch	18	15	13	10
4	Soybean meal	26	17	10	3
5	Rice bran	22	12	8	6
6	Fish oil	5	5	5	5
7	Fish hydrolysate	3	3	3	3
8	Monocalcium phosphate	2	2	2	2
9	Vitamin premix	0.5	0.5	0.5	0.5
10	Mineral premix	0.5	0.5	0.5	0.5
11	Common salt	1	1	1	1

Feed Formulation designe dasper methods mentionin Nates(2015).Aquafeed formulation, Acadmic Press, USA.

### 3. RESULT AND DISCUSSION

#### Proximate body and biofloc composition

During the present study proximate composition of biofloc and all experimental diets (control diet, commercial diet, and 15%, 30%, 45% biofloc meal included meals) was assessed using standard protocol (AOAC, 1995). The moisture content in the experimental diets was maximum in commercial diet and minimum moisture content was observed in 45% biofloc meal (Table-3). The crude protein level in the experimental diets was maximum in control and the minimum crude protein level was noticed in commercial diet (Table-3) which had been earlier reported by Hargreaves, 2013 and Faizulla, 2014. Izquierdo *et al.* (2006) stated that protein contributions of bioflocs are most important. The crude fibre content was maximum in the commercial diet and the minimum was perceived in 45% biofloc meal (Table-3). The ether extract level in the experimental diets was maximum in 30% biofloc meal. The total ash content was maximum 45% biofloc meal and the minimum was remarked in Control (Table-3), these results are well supported by (Azimet *et al.* 2008 and Bauer *et al.* 2012). The maximum Gross energy was noticed in Control and the minimum was noticed in commercial diet (Table-3), which had been previously reported by Faizulla, 2014, Valle *et al.*, 2014, Hargreaves, 2013, Emerenciano *et al.*, 2012).

**Table 3 :** Proximate composition of Biofloc meal and experimental diets used in the experiment to assess the optimum level of biofloc meal inclusion in the mono sex Nile tilapia diet.

Diet	Moisture (%)	Crude Protein (%)	Crude Fibre (%)	Ether Extract (%)	Total Ash (%)	Gross Energy (Kcal/kg)
<b>Biofloc meal</b>	10.9±0.18	18.54±0.14	0.91±0.15	0.82±0.11	53.34±0.16	1865±3.76
<b>C</b>	8.17±1.13	33.84±0.35	7.82±0.25	5.52±0.26	23.54±0.25	4041±6.34
<b>T1</b>	8.37±0.27	33.55±0.17	5.25±0.04	8.71±0.05	23.57±0.15	3771±3.45
<b>T2</b>	9.49±0.07	32.84±0.03	3.81±0.07	8.97±0.08	28.57±0.09	3639±5.77
<b>T3</b>	7.88±0.19	32.81±0.08	3.35±0.07	8.59±0.05	31.09±0.6	3442±1.20
<b>T4</b>	12±0.7	28.6±2.2	8.05±0.27	4.00±0.2	29.2±1.45	2961±7.8

#### Growth performance of Mono sex Nile Tilapia

In the present study, maximum mean body weight of Mono sex tilapia was recorded in control (27.98±0.78 g) followed by T1 (25.97±0.51), T2 (22.23±0.23), T3 (20.54±1.22) and T4 (19.87±0.79). However, the mean body weight of the T1 (20% biofloc meal) did not differ significantly with control. Hence, the present study could draw conclusion may be that 20% may be the optimum inclusion level of biofloc meal in the diet of mono sex Nile tilapia. The 20% level of biofloc meal was chosen to achieve the better growth and survival of Mono sex Nile Tilapia. Similar results were reported by Valle *et al.* (2014) suggested replacement of fish meal with 20% biofloc meal showed better result in the diet of *Catlacatla*. Dantaset *et al.* (2016) suggested that the regression analysis indicated that fish meal replacement by biofloc meal at level over 20% improves the growth of *L. vannamei* which is covenant with the findings of our present study. Bauer *et al.* (2012) said that 14% biofloc meal replacement with wheat flour performed good growth in *L. vannamei* FCR, SGR, PER and FER showed a highly significant difference among the treatments ( $p < 0.05$ ). Similarly, significantly higher growth parameters like weight gain, FCR, SGR, PER, FER among the treatments was registered in the control diet. The maximum and minimum weight gain was noticed in control (27.98±0.78 g) and T4 (19.87±0.79) respectively. In the present research trial, increasing the biofloc meal in the diet of Mono sex Nile Tilapia did not resulted in significant increment with a growth rate or FCR as compared with control.

The present finding agree in general with those of Wang (2007) and Anand et al. (2013) who reported that increase in dietary supplementation of probiotic or periphytic algae in shrimp diet do not increase proportionately the digestive enzyme activities and there by the growth of shrimp. In the present study, 20% biofloc meal inclusion level showed better weight gain and FCR than the 40% biofloc meal inclusion level which agreed Ajiboye et al., 2012 suggested reduction in growth rate of fishes was recorded at higher level of microbial supplementation as microbial products at higher level tend to reduce the feed palatability and digestibility. In the present study, the FCR was highest in control (1.94±0.05) followed by T1 (1.88±0.00), T2 (1.79±0.32), T3 (1.75±0.21), T4 (1.52±0.14). The FCR values obtained in the study were in the line with Zhao *et al.* (2012), Xu et al. (2012) and Dantaset al. (2016) whose FCR values ranged from 1.10 – 1.50%. These FCR trends are in agreement with that obtained by Khattab et al. (2010) who suggested that the feed conversion ratio (FCR) increased with increasing weight of fish and decreased with increasing dietary protein level and it ranged from 1.50-1.81 for fry (0.5 g), from 1.98 to 2.21 for fingerlings (20.4 g) and from 2.30 to 2.76 for adult fish (40.5 g) of Nile tilapia. Protein Efficiency Ratio (PER) used as indicators of protein quantity and quality in the fish diet and amino acids balance. So, these parameters are used to assess protein utilization and turnover, where they are related to dietary protein intake and its conversion into fish gain and protein gain. The PER values of the present study was ranging from 4.87±0.53 to 7.43±0.32. Ahamed et al. (2004) suggested that, PER significantly affected by protein level and PER ranged from 1.58-2.35 for fry, from 1.19 to 1.92 for fingerlings and from 0.99 to 1.53 for adult fish of Nile tilapia.

**Table :** Growth performance of mono sex tilapia fed in the experiment to assess the optimum level of biofloc meal inclusion in the mono sex tilapia diet.

Diets	IBW (g)	FBW (g)	WG (g)	Survival	Biomass gain (g)	Total Biomass (g)	Total feed intake (g)	FCR	FER	PER	SGR (%)
C	2.25±0.04	27.98±0.78	25.73±0.54	9.98±0.45	242.78±3.45	262.17±4.37	292.12±2.02	1.94±0.05	0.98±0.13	7.43±0.32	4.58±0.13
T1	2.04±0.21	25.97±0.51	23.93±0.52	9.35±0.22	224.57±2.43	243.78±2.42	290.25±3.24	1.88±0.00	0.79±0.01	6.87±0.13	4.32±0.24
T2	2.09±0.05	22.23±0.23	20.14±0.43	9.47±0.35	194.34±5.89	213.47±5.45	266.34±5.25	1.79±0.32	0.83±0.02	5.87±0.23	3.8±0.21
T3	1.94±0.05	20.54±1.22	18.6±1.21	9.87±9.43	177.58±9.32	194.1±5.53	254.021±7.53	1.75±0.21	0.87±0.00	5.47±0.37	3.87±0.27
T4	1.97±0.01	19.87±0.79	17.9±0.59	8.85±0.57	156.34±9.21	174.25±9.41	238.74±8.34	1.52±0.14	0.74±0.14	4.87±0.53	3.57±0.75
<b>One Way ANOVA (p &lt; 0.05)</b>											
Diet	0.52	0.01	0.01	0.57	0.01	0.01	0.19	0.01	0.01	0.01	0.01

#### 4. CONCLUSIONS

The current study found that all biofloc treatments improved fish growth performance (length gain, weight gain, and specific growth rate) compared to the control group. T1 (15% Biofloc) outperformed the other biofloc meals in terms of average body weight increase, FCR, SGR, PER, and FER.

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