# Effect Of Dietary Supplementation Of Biofloc Meal With Tryptophan &Lysine On Growth And Survival Of Mono Sex Tilpa

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**ABSTRACT**
A 60-day indoor growth trial was conducted to study the effect of dietary supplementation of biofloc meal with tryptophan and lysine on growth and survival of mono sex tilapia. Six isonitrogenous and isoenergetic experimental diets wereformulated viz., as 0.2%, 0.3%, 0.4% tryptophan (D1,D2,D3) and 1.2%, 1.4%, 1.8% lysine(D4,D5,D6) enriched with 20%bioflocmeal included diets.This feeding trial was conducted in 18 numbers of 40 L plastic troughs in triplicates, utilizing mono sex tilapias weighing with an average of 2 g size. During the experimental period, water quality parameters were measured and recorded daily. Among the experimental diets highest mean body weight of tilapia were recorded in D4 (46.35±3.95g) followed by D2 (41.37±1.98g), D5 (40.1±0.87 g) and D1 (34.36±0.96 g). Hence, the present study was assessed that diet with 20% biofloc meal supplemented with 1.2 % lysine can improve the growth and survival of mono sex tilapia without any adverse effect on the fish performance.

**Keywords:** Biofloc, growth,lysine,Trypthon,mono sex tilapia.

## Introduction

The Global market for tilapia is growing at the rate of 10-12% per year (FAO, 2020). Fishery professionals referred to tilapia as "aquatic chicken" due to its fast evolution and easy of cultivation, which now has made its farming widespread around the world (Maclean 1984). In India, Mono sex tilapia has become a more poplar species among fish farmers (Bhendarkar*et al*. 2017, Bhendarkar and Brahmane 2021a). The major reasons for its popularity are Suitability for wide range of farming systems, Wide ranges of feed adaptability, Low feed conversion and efficiency, High level of tolerance for changing water quality and extreme events, Tolerance to salinity and disease resistance, Fast Growth Rate in comparison to other fish species, High stocking densities, Production costs are low, White flesh; suitable for a wide variety of value added products ,Harvesting time is shorter, and size more than 200 gm may sell out and Good market demand, easy to be accepted by consumers.

Biofloc is an aggregate formed by bacteria, fungi, invertebrates and small organic and inorganic particles (Avnimelech, 2009), which represents an alternative for protein supplements in fish and shrimp feeds (Kuhn *et al.,* 2009). Bioflocs can contain up to 30% crude protein in their composition and about 2% lipids (Azim and Little, 2008and Xu and Pan, 2012). Nile tilapia uses very well the biofloc as a food source, and biofloc can meet up to 50% protein requirements of tilapia (Avnimelech, 2007 and Azim and Little, 2008).

Building blocks of proteins are amino acids (AA) which play an important role in maintenance, growth, feed intake, nutrient utilization, immunity, behaviour, larval metamorphosis, reproduction as well as resistance to environmental stress and pathogenic organisms in various fishes. Dietary supplementation of L tryptophan at a minimum level of 1.36% reduced the high density group stress and improved growth performance in C. mrigala fingerlings as well (Tejpal*et al.,* 2009). Santiago and Lovell (1988) estimated the dietary requirement of tryptophan as 1g/100g crude protein for Nile tilapia fingerlings. Lysine, an important amino acid, is abundant in fish muscle tissue. It is involved in the establishment and maintenance of a positive nitrogen balance, as well as the "crosslinking" of proteins, most notably collagen. Furthermore, it is essential for the synthesis of carnitine, which is required for the transport of long chain fatty acids into the mitochondria for energy generation (Walton *et al.,* 1984). Dietary lysine supplementation has been linked to increased weight gain, improved feed conversion, nitrogen retention, and decreased body fat content. Lysine is one of the most limiting amino acids for fish nutrition, and it has been related to enhanced fillet yield and fish growth (Furuya*et al.,* 2004).

In commercial biofloc technology, when the concentration of biofloc reaches its maximum capacity, it is necessary to remove the surplus, which is normally discarded. Because biofloc are a source of nutrients, this residue has the potential

to use in aqua feed production. In this context, the present study aims to evaluate the effect of supplementation of biofloc meal with tryptophan and lysine on growth and survival of mono sex tilapia.

**Materials and Methods**

**Experimentalfish**

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 first and second experiments used an average of 150 and 240 Mono sex tilapias weighing 2 grams each.

**Experimental design**

The experiment where arrieed out in 18 plastic troughs with 6 treatments (3 different amounts of tryptophan and lysine) in triplicate. Before beginning the experiment, all troughs were cleaned and disinfected with soap oil, then dried in the sun.The troughs were filled with water up to 3/4 of their contents. All of the troughs were connected to suitable aeration facilities.

**Biofloc as an ingredient for fish feed**

The biofloc was obtained from Biofloc Ponds at 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. Good quality anchovies were sourced at the fish landing centre in Thoothukudi, dried in the sun for two days, powdered, and stored in an airtight container under refrigeration. Soybean meal, fish oil, fish hydrolysate, monocalcium phosphate, tryptophan, lysine, vitamin, mineral, cassava starch, rice bran, and common salt were purchased at the local market in Bhopal.

**Experimental treatment diets**

Six isonitrogenous and isoenergetic experimental diets wereformulated viz., as 0.2%, 0.3%, 0.4% tryptophan (D1,D2,D3) and 1.2%, 1.4%, 1.8% lysine(D4,D5,D6) enriched with 20%bioflocmeal included diets.The ingredientcomposition ofexperimentaldiets is presentedinTable-1.

**Proximate composition of biofloc and experimental diets.**

The proximate analysis of biofloc and all theexperimental diets such as control diet, commercial dietand15%, 30%, 45% bioflocmealincluded diets, 0.2%, 0.3%, 0.4% tryptophan and 1.0%, 1.4%, 1.8% lysine enriched diets were estimated for proximate analysis following thes tandard 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 stockingin experimental troughs. After the stocking the experimental troughs were covered with plasticnetontopinordertopreventthejumpingof Monosextilapia.

**Feeding.**

Feeding were done thrice a day (9:00, 12:00 and 16:00 H) at ad libitum. Each of theexperimental diets were fed by hand slowly to avoid wastage. Feed was given until apparentsatiation.Feedingwereincreasedordecreased basedontheirapparentsatiation.

**Water quality parameters**

During the experimental period, water quality parameters such as Temperature, Dissolved oxygen, pH, and Total alkalinity were measured and recorded daily. Water temperature was measured using a thermometer with an accuracy of 0.1$^{o}$C. The pH of water was measured using the laboratory model Elico pH meter. Modified /Winkler's titration method (APHA, 1998) was adopted to estimate the dissolved oxygen. Total alkalinity was determined as per the method described in APHA, (1998). Total ammonia-N, water hardness, and turbidity were assessed twice a week. Ammonia –N, 21water hardness were determined as per the standard methods (APHA, 1998).

**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;

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

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

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

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

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

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

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

**Statistical analysis**

All the observations were processed and tabulated. Data were analysed by one-way ANOVA using the statistical software SPSS 16.0 for windows (SPSS Inc., Chicago, IL, USA) to test the assessment of optimum enrichment level tryptophan in the biofloc meal included diet of mono sex tilapia and which was assessed by Duncan multiple range test (Duncan, 1995).

**Table-1:** Ingredient composition of formulated mono sex tilapia diet with 0.2%, 0.3%, 0.4% tryptophan and 1.0%, 1.4%, 1.8% lysine enriched with added 20% biofloc meal.

| S.NO | INGREDIENTS | INCLUSION LEVEL (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | TRY* 0.2% (D1) | TRY* 0.3% (D2) | TRY* 0.4% (D3) | LYS# 1.0% (D4) | LYS# 1.4% (D5) | LYS# 1.8% (D6) |
| 1 | Biofloc meal | 20 | 20 | 20 | 20 | 20 | 20 |
| 2 | Fish meal | 24 | 24 | 24 | 24 | 24 | 24 |
| 3 | Cassava starch | 14 | 14 | 14 | 14 | 14 | 14 |
| 4 | Soybean meal | 14 | 14 | 14 | 14 | 14 | 14 |
| 5 | Rice bran | 13.80 | 13.70 | 13.60 | 13 | 12.60 | 12.20 |
| 6 | Fish oil | 5 | 5 | 5 | 5 | 5 | 5 |
| 7 | Fish hydrolysate | 4 | 4 | 4 | 4 | 4 | 4 |
| 8 | Monocalcium phosphate | 3 | 3 | 3 | 3 | 3 | 3 |
| 9 | Vitamin premix | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| 10 | Mineral premix | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| 11 | Common salt | 1 | 1 | 1 | 1 | 1 | 1 |
| 12 | Tryptophan | 0.20 | 0.30 | 0.40 | 0 | 0 | 0 |
| 13 | Lysine | 0 | 0 | 0 | 1 | 1.40 | 1.80 |

Feed Formulation designedasper methods mentionin Nates(2015).Aquafeed formulation, Acadmic Press, USA. **TRY* – Tryptophan,LYS# - Lysine**

**Table-2:** Optimum water quality parameters to be maintained in Mono sex Tilapia culture.

| S.NO | Water parameters | Unit | Optimum range |
|---|---|---|---|
| 1 | Water temperature | ºC | 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 |

**Results and Discussion**

**Proximate composition of biofloc meal and experimental diets.**

The proximate composition of the experimental diets was presented in Table-3.The moisture content in the experimental diets was maximum in D6 (9.98±0.21%) and the minimum was observed in D3 (8.20±0.06%). The crude protein level in the experimental diets was maximum in D4 (34.91±0.06%) and the minimum was noticed in D6 (33.83±0.05%). The crude fibre content was maximum in the D6 (7.37±0.08%) and the minimum was noticed in D5 (5.44±0.02%). The ether extract level in the experimental diets was maximum in D6 (7.98±0.04%) and the minimum was remarked in D5 (6.21±0.05%). The total ash content was maximum in D5 (25.92±0.04%) and the minimum was observed in D3 (19.21±0.13%). The maximum Gross energy was noticed in D1 (3860±9.12Kcal/kg) and the minimum was noticed in D4 (3587±3.21Kcal/kg).

**Tryptophan enriched diets**

Among the tryptophan enriched diets, highest mean body weight of Mono sex Nile Tilapia were recorded in D2 (41.37±1.98 g) followed by D3 (39.47±2.49 g), D1 (34.36±0.96 g) as shown in Table -4 . Hence, the present study was assessed that diet with 20% biofloc meal supplemented with 0.3% tryptophan can improve the growth of Mono sex Nile Tilapia without any adverse effect on the fish performance. The maximum level of 0.3% tryptophan was chosen to satisfy for the better growth and survival of Mono sex Nile Tilapia . FCR, SGR, PER and FER showed a highly significant difference among the treatments ($p < 0.05$). Similarly, significantly higher values of growth parameters like weight gain, FCR, SGR, PER, FER among the treatments was registered in the 0.3% tryptophan enriched diet. Our current studies were supported by the reports of Santiago and Lovell (1998) which suggested that tryptophan of 0.28% in the diet of juvenile Nile tilapia has improved the growth performance. Based on research studies conducted, NRC (2011) has recommended that tryptophan requirement of 0.28% (as % of dry diet) for *Oreochromisnilotius*.

**Lysine enriched diets.**

Among the lysine enriched diets highest mean body weight of Mono sex Nile Tilapia was recorded in D4 (46.35±3.95) followed by D5 (40.1±0.87 g), D6 (39.12±0.49g) and D8 (38.23±2.88 g) as shown in Table -4. Hence, the present study was assessed that diet with 20% biofloc meal supplemented with 1% lysine can improve the growth of Mono sex Nile Tilapia without any adverse effect on the fish performance. The maximum level of 1% lysine was chosen to satisfy for the better growth and survival of Mono sex Nile Tilapia . FCR, SGR, PER and FER showed a highly significant difference among the treatments ($p < 0.05$). Similarly, significantly higher values of growth parameters like weight gain, FCR, SGR, PER, FER among the treatments was registered in the 1% lysine enriched diet. The break point analysis made by Santiago and Lovell (1988) in the growth response curve indicates that 1.43% dietary lysine provided optimum growth in Nile tilapia. Diogenes et al., (2016) found that Nile tilapia juvenile requires a higher level of lysine 1.56% for proper growth performances. Lysine is one of the most limiting amino acids in fish nutrition, not only related to fish growth, but to increases fillet yield (Furuya*et al.,* 2006). Growth and feed efficiency of hybrid tilapia, *O.niloticus*x *O.aureus*, were not improved when lysine were added to a 24% protein diet containing soybean meal as the main protein source (Luo *et al.,* 1986). Such results may be due to different factors such as poor palatability, toxicity of crystalline amino acids or reduced protein synthesis. Poor palatability of crystalline amino acid based diets has been associated with dietary pH, as supplementation with crystalline amino acids reduces the dietary pH (Murai*et al.,* 1987). But in the present study, it was found that good performance in 20% biofloc meal in combination with 1.2% lysine. Growth performance of market size Senegalese sole fed on plant protein-based diet supplemented with lysine was comparable with a animal protein-based diet (Valente et al.2011). El saidy*et al.,* (2002) found that diet with 55% soybean meal supplemented with 0.5% lysine can totally replace fish meal in the diet of Nile tilapia fingerlings, without adverse effect on fish performance.

**Table-3**: Proximate composition of experimental diets to assess the optimum level of tryptophan and lysine inclusion in mono sex Nile tilapia diet enriched with biofloc meal.

| Diet | Moisture (%) | Crude Protein (%) | Crude Fibre (%) | Ether Extract (%) | Total Ash (%) | Gross Energy (Kcal/kg) |
|---|---|---|---|---|---|---|
| **D1** | 9.31±0.07 | 34.40±0.12 | 5.61±0.07 | 6.36±0.06 | 22.21±0.81 | 3860±9.12 |
| **D2** | 9.67±0.16 | 33.95±0.07 | 6.40±0.13 | 6.96±0.02 | 23.14±0.32 | 3590±6.62 |
| **D3** | 8.20±0.06 | 34.86±0.15 | 6.81±0.05 | 7.24±0.05 | 19.21±0.13 | 3631±4.45 |
| **D4** | 8.91±0.05 | 34.91±0.06 | 7.31±0.16 | 6.85±0.07 | 19.86±0.08 | 3587±3.21 |
| **D5** | 9.98±0.21 | 34.74±0.06 | 5.44±0.02 | 6.21±0.05 | 25.92±0.04 | 3671±4.86 |
| **D6** | 9.15±0.06 | 33.83±0.05 | 7.37±0.08 | 7.98±0.04 | 23.54±0.16 | 3767±1.92 |

**Table -4:** Growth performance of mono sex Nile tilapia in the experiment to assess the optimum level of tryptophan and lysine inclusion in mono sex Nile tilapia diet enriched with biofloc meal.

| Diets | IBW (g) | FBW (g) | WG (g) | Survival | Biomass gain (g) | Total Biomass (g) | Total feed intake (g) | FCR | FER | PER | SGR (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| D1 | 2.12±0.06 | 36.48±0.93 | 34.36±0.96 | 9.47±0.42 | 324.75±3.29 | 344.26±18.21 | 419.12±21.48 | 1.73±0.03 | 0.92±0.02 | 10.57±1.04 | 5.78±0.11 |
| D2 | 2.03±0.02 | 43.40±2.04 | 41.37±1.98 | 9.51±0.23 | 406.69±17.51 | 425.47±17.43 | 438.11±14.31 | 1.81±0.02 | 0.99±0.01 | 13.16±0.87 | 6.23±0.07 |
| D3 | 1.96±0.01 | 41.43±1.80 | 39.47±2.49 | 9.48±0.44 | 381.65±25.45 | 397.79±31.52 | 437.32±27.78 | 1.62±0.01 | 0.94±0.01 | 12.38±1.21 | 6.16±0.05 |
| D4 | 1.97±0.04 | 48.32±3.44 | 46.35±3.95 | 9.55±0.53 | 441.47±46.28 | 459.91±37.38 | 487.21±54.77 | 1.63±0.01 | 0.96±0.01 | 14.24±2.01 | 6.35±0.07 |
| D5 | 2.06±0.06 | 42.16±1.21 | 40.1±0.87 | 9.44±0.66 | 380.93±17.24 | 399.51±15.41 | 447.53±12.94 | 1.51±0.03 | 0.89±0.03 | 12.35±0.67 | 6.13±0.01 |
| D6 | 2.01±0.01 | 40.24±2.06 | 38.23±2.88 | 9.56±0.33 | 361.18±30.43 | 403.89±14.71 | 451.65±24.65 | 1.91±0.02 | 0.87±0.01 | 11.74±0.64 | 5.97±0.52 |
| One Way ANOVA ($p < 0.05$) | | | | | | | | | | | |
| Diet | 0.02 | 0.13 | 0.11 | 0.99 | 0.23 | 0.25 | 0.71 | 0.01 | 0.01 | 0.23 | 0.04 |

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