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Influence of Biofloc meal and Lysine supplementation on the growth performances of GIFT tilapia

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Abstract
A 60-day indoor growth trial was conducted to study the effect of dietary supplementation of biofloc meal with lysine on growth and survival of juvenile Genetically Improved Farmed Tilapia. Five isonitrogenous and isoenergetic experimental diets (32% protein) were prepared at different enrichment levels of crystalline lysine viz., 1.2% (T1), 1.4% (T2), 1.6%(T3) , 1.8% (T4) enriched with 20% biofloc meal included diet and control diet (T0) without biofloc meal and lysine. A commercial diet (T5) was used to compare the experimental diets. This feeding trial was conducted in 18 numbers of 40 L plastic troughs in triplicates, utilizing GIFT tilapias weighing 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 GIFT tilapia were recorded in T1 (45.24±4.52 g) followed by T3 (42.90±3.26g), T2 (39.12±0.49g) and T4 (37.27±3.08 g). Hence, the present study was assessed that diet with 20% biofloc meal supplemented with 1.2% lysine can improve the growth of GIFT tilapia without any adverse effect on the fish performance.

Key words: Biofloc meal, lysine, GIFT tilapia, protein feed, supplementation, weight gain.

1. Introduction
Tilapia is an aquaculture species that are being produced intensively all over the world. In 2012, the global tilapia production reached 4.5 million metric tons [18] and is expected to increase further exponentially in the future. Asian countries are the major producers as well as the consumers of Tilapia. World aquaculture production of *Oreochromis niloticus* alone presently stands at nearly 3.2 million tons with china being the major producer, contributing over one third of the total global production [40].

Biofloc technology has been widely studied and applied in aquaculture. This system applies the principle of assimilation of excreted dissolved nitrogen by heterotrophic bacteria by managing the C/N ratio in the water [6]. This aggregation of heterotrophic bacterial community comprising microorganisms such as microalgae and zooplankton, as well as trapped organic particles or solids which may fulfil the nutritional requirement of the cultured organisms. [14] The biofloc technology maintaining the water quality in the aquatic system and also this biofloc used as feed for the aquatic organisms [27, 28]. Hence, the use of biofloc as a food source may imply a decrease in the requirement of formulated feed protein [42] and also improve nitrogen utilization efficiency by the cultured animals [7]. In order to evaluate the use of biofloc as a food source, general criteria of aquaculture feed can therefore be applied, i.e. the size of particles, attractiveness and palatability, digestibility, and nutritional content [35].

Lysine is an essential amino acid present in high proportion in fish muscle tissue, involved in the growth and maintenance of positive nitrogen balance, also used in "crosslinking" protein, especially collagen. Moreover, it plays an important role in the synthesis of carnitine, which is important for the transport of long chain fatty acids into the mitochondrion for energy generation [39]. Dietary lysine supplementation is related to advantages on weight gain feed conversion, nitrogen retention and reduction in body lipid contents. 1.43% dietary lysine provided optimum growth in Nile tilapia [34]. Nile tilapia juvenile requires a higher level of lysine 1.56% for proper growth performances [15]. In this context, the present study aims to evaluate the effect of supplementation of biofloc meal with lysine on growth and survival of GIFT tilapia.

2. Materials and methods

2.1 Experimental fish

GIFT tilapia seeds were procured from the State Fisheries Department, Krishnagiri, Tamilnadu, India. All the fish seeds were properly acclimatized in FRP tanks, and were nursed for 15 days with commercial diet. All the fishes were graded according to their weight prior to the experiment. An average of 2 gram sized 180 numbers of GIFT tilapias were selected for the experimental trial.

2.2 Experimental design

The experiment consisted of four treatments, one control and one commercial diet with three replicates, over a 60-day indoor growth trial. Water was filled in the troughs up to 3/4th of its volume. All the troughs were connected with proper aeration facility. The experiment was conducted the indoor biofloc lab at Fisheries College and Research Institute, Thoothukudi, Tamilnadu, India.

2.3 Biofloc as an ingredient for fish feed

Biofloc was collected from Hi-Tide sea farm, Mahendrapally, Nagapattinam district, Tamilnadu, India. Totally 23 kg of wet biofloc were collected and dried under sun light for 8 hrs. The total quantity of dried biofloc meal was 2.1 kg. The dried biofloc were powdered into fine particles and stored in air tight container under refrigeration. The proximate composition of the biofloc meal was analysed following the standard protocols [3].

2.4 Experimental treatment diets

Five isonitrogenous and isoenergetic experimental diets were formulated viz., 1.2% (T1), 1.4% (T2), 1.6% (T3), 1.8% (T4) lysine and control diet (T0) without biofloc meal and lysine. Commercial feed (T5) was used as an external control. The ingredient composition of experimental diet is presented in Table 1.

2.5 Proximate composition of biofloc and experimental diets

The proximate analysis of biofloc and all the experimental diets such as control diet (T0), commercial diet (T5) and 1.2% (T1), 1.4% (T2), 1.6% (T3), 1.8% (T4) lysine enriched diets were estimated proximate analysis following the standard protocols [3].

2.6 Stocking

The mean weight of 2 g ranged fishes were 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.

2.7 Feeding

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

Table 1: Ingredient composition of formulated GIFT tilapia diet with 1.2%, 1.4%, 1.6%, 1.8% lysine enriched with added 20% biofloc meal

S. No	Ingredients	Inclusion l evel (%)				
		Control (t0)	Lysine 1.2% (t1)	Lysine 1.4% (t2)	Lysine 1.6% (t3)	Lysine 1.8% (t4)
1	Biofloc meal	0	20	20	20	20
2	Fish meal	22	25	25	25	25
3	Cassava starch	18	15	15	15	15
4	Soybean meal	26	15	15	15	15
5	Rice bran	22	12.78	12.04	11.90	11.76
6	Fish oil	5	5	5	5	5
7	Fish hydrolysate	3	3	3	3	3
8	Monocalcium phosphate	2	2	2	2	2
9	Vitamin premix	0.5	0.5	0.5	0.5	0.5
10	Mineral premix	0.5	0.5	0.5	0.5	0.5
11	Common salt	1	1	1	1	1
12	Lysine	0	0	0.96	1.10	1.24

2.8 Water quality parameters

During the experimental period, water quality parameters such as Temperature, Dissolved oxygen, pH, and Total alkalinity were measured and recorded daily (Table.2). Water temperature was measured using a thermometer with an accuracy of 0.1ºC. The pH of water was measured using the laboratory model Elico pH meter. Modified /Winkler's titration method [4] was adopted to estimate the dissolved oxygen. Total alkalinity was determined as per the method described in APHA. Total ammonia-N, nitrite-N, nitrate-N, water hardness, and turbidity were assessed twice a week. Ammonia –N, nitrite-N, nitrate-N, water hardness were determined as per the standard methods [4].

Table 2: The range of water quality parameters recorded in the experimental rearing of GIFT tilapia

S. No	Parameters	Range
1	Temperature	28-29 °C
2	Dissolved Oxygen	5-6 ppm
3	pH	8-8.2
4	Salinity	4-5 ppt
5	Ammonia	0.01 – 0.05 ppm
6	Nitrite	0.05 – 0.1 ppm
7	Nitrate	10 - 12 ppm
8	Hardness	610 - 650 ppm
9	Alkalinity	155 - 170 ppm

2.9 Sampling
Growth sampling was done at every fortnight with all the stocked animals from each tanks by taking total length and body weight.

2.10 Growth performance
The 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 using the following formulae;
Feed Conversion Ratio (FCR) = Total feed fed (g) / Total fish weight gained (g)
Feed Efficiency Ratio (FER) = Total feed fed (g) / Total fish weight gained (g)
Protein Efficiency Ratio (FER) = Total Wet-Weight gain / Dry Weight of Protein Fed

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

Mean Weight Gain (g) = Final Weight (g) – Initial Weight (g)
Mean Weight Gain (g) = Final Weight (g) – Initial Weight (g) / Experimental Duration
Survival = Total number of fishes survived / Total number of fishes stocked X 100

2.11 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 lysine in the biofloc meal included diet of GIFT tilapia and which was assessed by Duncan multiple range test.

3. Result
3.1 Proximate composition of biofloc meal and experimental diets
Proximate composition (%) of biofloc meal and experimental diets are presented in Table 3. The 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 gross energy.

3.2 Growth performance
The calculated bio growth parameters of the experimental diets are showed in table 4. Among the lysine enriched diets highest mean body weight of GIFT tilapia were recorded in T1 (45.24±4.52 g) followed by T3 (42.90±3.26g), T2 (39.12±0.49g) and T4 (37.27±3.08 g). T1 yielded better body weight gain among all the experimental diets. The feed conversion ratio (FCR) of the T1, T2, T3 and T4 were 1.06±0.00, 1.15±0.01, 1.15±0.03, 1.19±0.01 respectively (Table.4). The feed efficiency ratio (FER) of the diet T1, T2, T3 and T4 were 0.94±0.01%, 0.86±0.01%, 0.87±0.02% and 0.83±0.01 respectively (Table.4). The protein efficiency ratio (PER) of the diet T1, T2, T3 and T4 were 13.13±1.67%, 11.24±0.47%, 12.31±0.54% and 10.63±0.84% respectively (Table.4). The specific growth rate (SGR) of the diet T1, T2, T3 and T4 were 5.24±0.18%, 5.02±0.04%, 5.16±0.12% and 4.86±0.14% respectively (Table.4). Similarly higher values of growth parameters like weight gain, FCR, SGR, PER, FER among the treatments was registered in the 1.2% lysine enriched diet.

Table 3: Proximate composition of experimental diets to assess the optimum level of lysine inclusion in GIFT Tilapia diet enriched with biofloc meal

Diet	Moisture (%)	Crude Protein (%)	Crude Fibre (%)	Ether Extract (%)	Total Ash (%)	Gross Energy (Kcal/kg)
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	7.82±0.05	32.75±0.05	6.12±0.06	7.00±0.08	19.33±0.05	3477±1.15
T2	9.12±0.06	32.64±0.05	4.34±0.06	5.16±0.06	24.56±0.07	3511±5.77
T3	8.15±0.03	32.90±0.02	5.21±0.06	6.54±0.04	14.43±0.06	3492±4.14
T4	8.10±0.03	32.73±0.05	6.22±0.05	7.43±0.06	22.35±0.06	3605±2.08

Table 4: Growth performance of GIFT tilapia in the experiment to assess the optimum level of lysine inclusion in GIFT 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
T0	2.03±0.02	27.16±0.50	25.13±0.52	9.66±0.33	242.57±3.49	262.07±4.29	292.00±3.05	1.11±0.01	0.90±0.01	7.41±0.10	4.32±0.04
T1	1.92±0.02	45.24±4.52	43.32±4.55	9.66±0.33	420.36±53.47	438.98±53.66	466.00±53.67	1.06±0.00	0.94±0.01	13.13±1.67	5.24±0.18
T2	1.92±0.03	39.12±0.49	37.20±0.49	9.66±0.33	359.82±15.18	378.40±16.01	436.67±14.14	1.15±0.01	0.86±0.01	11.24±0.47	5.02±0.04
T3	1.92±0.01	42.90±3.26	40.97±3.26	9.66±0.33	393.98±17.56	412.61±16.94	474.00±6.80	1.15±0.03	0.87±0.02	12.31±0.54	5.16±0.12
T4	1.99±0.00	37.27±3.08	35.28±3.08	9.66±0.33	340.07±27.05	382.91±11.00	430.33±35.61	1.19±0.01	0.83±0.00	10.63±0.84	4.86±0.14
T5	1.98±0.00	19.88±0.99	17.89±0.98	8.66±0.88	156.82±9.35	174.07±9.73	238.67±8.37	1.37±0.01	0.73±0.01	4.90±0.74	3.84±0.07
One Way ANOVA ($p < 0.05$)											
Diet	0.03	0.11	0.10	0.99	0.21	0.23	0.62	0.01	0.01	0.21	0.04

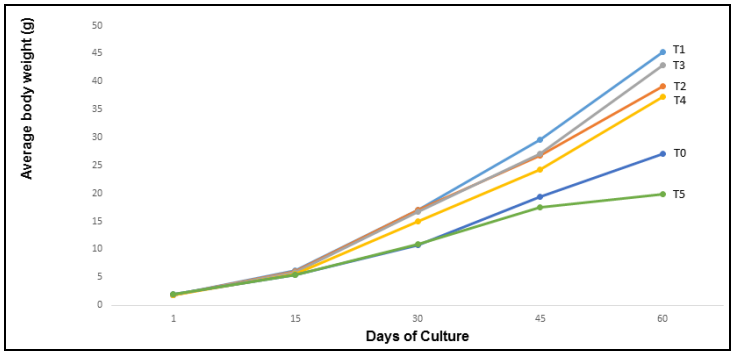

Fig 1: Average body weight of GIFT tilapia to assess the optimum inclusion level of lysine in the biofloc meal incorporated GIFT tilapia diet

4. Discussion

Fish and shrimp diets supplemented with biofloc can improve growth [2]. The concentration of protein of the artificial diet may be reduced if there is abundant natural food in the farming system, such as the biofloc [26]. Bioflocs can contain up to 30% crude protein in their composition and about 2% lipids [8, 9, 29, 42]. Due to its omnivorous habit, Nile tilapia uses well bioflocs as a food source, and bioflocs can meet up to 50% protein requirements [5, 8]. Protein content of biofloc in the present study was 17.92 % which is in covenant with earlier findings of [23, 31]. The value of crude lipid of biofloc in the present study was well within the range which had been previously reported by [24, 17, 22, 41].

The growth response curve indicates that 1.43% dietary lysine provided optimum growth in Nile tilapia [34]. Nile tilapia juvenile requires a higher level of lysine 1.56% for proper growth performances [15]. Lysine is one of the most limiting amino acids in fish nutrition, not only related to fish growth, but to increases fillet yield [19].

Results of the fish performance in this study demonstrated that the synthetic amino acids efficiently used and improves growth without any adverse effect in GIFT tilapia. Rainbow trout totally absorbed the crystalline lysine supplemented in the diet [32]. Among the lysine enriched diets highest mean body weight of GIFT tilapia were recorded in T5 (45.24±4.52 g) followed by T7 (42.90±3.26g), T6 (39.12±0.49g) and T8 (37.27±3.08 g). Hence, the present study was assessed that diet with 20% biofloc meal supplemented with 1.2% lysine was chosen to satisfy for the better growth and survival of GIFT tilapia. FCR, SGR, PER and FER showed a highly significant difference among the treatments ($p < 0.05$). Similarly, significant higher growth parameters like weight gain, FCR, SGR, PER, FER among the treatments was registered in the 1.2% lysine enriched diet. Growth performance of market size Senegalese sole fed on plant protein-based diet supplemented with lysine was comparable with an animal protein-based diet [37]. Diet with 55% soybean meal supplemented with 1.5% lysine can totally replace fish meal in the diet of Nile tilapia fingerlings, without adverse effect on fish performance [16].

Fish growth is determined by the first limiting amino acid and reducing the protein content of fish feeds is one strategy to increase the sustainability of tilapia production via reducing feed costs as well as reducing the environmental impact if economical growth can be maintained with less nitrogen input. The possibility of reducing the dietary protein was demonstrated in diets for carp [38], Nile tilapia [20, 10], rainbow trout [11, 21]. The ideal protein concept should be used to balance amino acids of fish diets [20].

In the present study the FCR was ranged from 1.06 ±0.01 to 1.37 ±0.01. The FCR values obtained in the study were in the line with [30, 33, 36, 43, 42, 25, 13] which ranged from 1.10 – 1.50%. Protein Efficiency Ratio (PER) used as indicators of protein quantity and quality in the fish diet and amino acids balance. These growth parameters are used to assess protein utilization, where they are related to dietary protein intake and its conversion into fish weight gain and their protein gain. Protein efficiency ratio 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 [1]. However the PER values of the present study was ranging from 0.73 ±0.01 to 0.94 ±0.01.

6. Conclusion

From the present study it could be stated that 1.2% lysine with 20% biofloc meal enriched diet (T1) showed higher growth performances than that of all other treated diets as well as control diet and commercial diet. Hence, the present study was assessed that diet containing 20% biofloc meal supplemented with 1.2% lysine can improve the growth of GIFT tilapia without any adverse effect on the fish performance.

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