Int.J.Curr.Microbiol.App.Sci (2017) *6*(8): 3426-3434

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 8 (2017) pp. 3426-3434**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.608.411

Effect of Dietary Supplementation of Biofloc Meal with Tryptophan on Growth and Survival of GIFT Tilapia

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Keywords

Biofloc meal, Tryptophan, GIFT tilapia, Nutrition, Protein feed, Survival.

Article Info

Accepted:
27 June 2017
Available Online:
10 August 2017

A B S T R A C T

A 60-day indoor growth trial was conducted to study the effect of dietary supplementation of biofloc meal with tryptophan on growth and survival of juvenile GIFT tilapia. Five isonitrogenous and isoenergetic experimental diets (32% protein) were prepared at different enrichment levels of crystalline tryptophan viz., 0.1% (T1), 0.2% (T2), 0.3% (T3), 0.4% (T4) enriched with 20% biofloc meal included diet and control diet (T0) without tryptophan and biofloc meal. 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 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 GIFT tilapia were recorded in T3 (41.84±1.09 g) followed by T4 (39.01±2.37g), T1 (36.77±2.54 g) and T2 (33.37±1.05g). Hence, the present study was assessed that diet with 20% biofloc meal supplemented with 0.3% tryptophan can improve the growth and survival of GIFT tilapia without any adverse effect on the fish performance.

Introduction

Aquaculture production has increased steadily in recent years and is the fastest growing food production sector and has become a valuable component of national development and poverty reduction plans in many areas of the world (Prabu and Santhiya, 2016). 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, 2010). Bioflocs can contain up to 30% crude protein in their composition and about 2% lipids (Azim and Little, 2008; Ballester *et al.,* 2010; Luo *et al.,* 2014; 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; 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.

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 on growth and survival of GIFT tilapia.

Materials and Methods

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.

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 $3/4^{th}$ 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.

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 (AOAC, 1995).

Experimental treatment diets

Five isonitrogenous and isoenergetic experimental diets were formulated viz., 0.1% (T0), 0.2% (T1), 0.3% (T3), 0.4% (T4) tryptophan with 20% biofloc meal and a control diet (T0) without biofloc meal and tryptophan. Control diet was compared against four experimental diets with different levels of tryptophan with 20% biofloc meal in the experimental diets. Commercial feed (T5) was used as an external control. The ingredient composition of experimental diet is presented in Table 1.

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 0.1% (T0), 0.2% (T1), 0.3% (T3), 0.4% (T4) tryptophan enriched diets were estimated proximate analysis following the standard protocols (AOAC, 1995).

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.

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.

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 pHof 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, 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 (APHA, 1998).

Sampling

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

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

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

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 GIFT tilapia and which was assessed by Duncan multiple range test (Duncan, 1995).

Results and Discussion

Proximate composition of biofloc meal and experimental diets

Proximate composition (%) of biofloc meal and experimental diets are presented in Tables 3. The dried biofloc contain 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.

Growth performance

The calculated bio growth parameters of the experimental diets are showed in table 4. The mean body weight gain recorded in T3 was39.90±1.08 g, whereas in T1, T2 and T3 this was recorded as 34.78±2.55g, 31.35±1.02 and 37.13±2.38 g respectively. T3 yielded better body weight gain among all the experimental diets. T3 showed better performance among all the tryptophan enriched diet group. The feed conversion ratio (FCR) of the T1, T2, T3 and T4 were 1.17±0.02%, 1.23±0.02%, 1.03±0.01%, 1.10±0.0%respectively. The feed efficiency ratio (FER) of the diet T1, T2, T3 and T4 were 0.85±0.01%, 0.81±0.01%, 0.97±0.01% and 0.91±0.01 respectively (Table.4).

The protein efficiency ratio (PER) of the diet T1, T2, T3 and T4 were 10.87±0.79%, 9.48±0.59%, 12.05±0.48% and 11.27±1.08% respectively (Table.4). The specific growth rate (SGR) of the diet T1, T2, T3 and T4 were 4.84±0.12%, 4.67±0.02%, 5.12±0.04% and5.05±0.11% respectively (Table.4).Similarly, higher values of growth parameters like weight gain, FCR, SGR, PER, FER among the treatment was registered in the 0.3% tryptophan enriched diet (Table.4).
Few studies have used biofloc meal as an ingredient in aquaculture diets. Inclusion of biofloc meal as a dietary ingredient in shrimp diet found to improve the growth performance of *L. vannamei* (Ju *et al.,* 2008: Kuhn *et al.,* 2009, 2010). The application of biofloc technology improved the feed conversion and protein retention indicating indirectly that the consumption of biofloc contributes to the growth of cultured organisms (Avnimelech, 2009; Hari *et al.,* 2004; Wasieliesky *et al.,* 2006; Xu *et al.,* 2012). According to Xu *et al.,* (2012), bioflocs affect positively the digestive enzyme activity of the farmed aquatic organisms. Anand *et al.,* (2014) concluded that the digestive activity of the animals was improved when the artificial diet was supplemented with bioflocs.

Among the tryptophan enriched diets with biofloc meal highest mean body weight of GIFT tilapia were recorded in T3 (41.84±1.09 g) followed by T4 (39.01±2.37g), T1 (36.77±2.54 g) and T2 (33.37±1.05 g). FCR, SGR and FER showed a highly significant difference among the treatments ($p < 0.05$).Our current studies were supported by Santiago and Lovell (1998) suggested that tryptophan of 0.28% in the diet of juvenile Nile tilapia has improved the growth performance. The present study agreed based on studies conducted upto 2010, NRC (2011) has recommended that tryptophan requirement of 0.28% (as % of dry diet) for *Oreochromis nilotius*.

Nutritional composition of biofloc differs according to environmental condition, carbon source applied, total suspended solid level, salinity, stocking density, light intensity, phytoplankton and bacteria communities and ratio, etc. Weight gain of *Oreochromis niloticus* was improved when 35% protein diet formulated from casein was supplemented with lysine and tryptophan (Teshima *et al.,* 1986).

Fig.1 Average body weight of GIFT tilapia to assess the optimum inclusion level of tryptophan in The biofloc meal incorporated GIFT tilapia diet

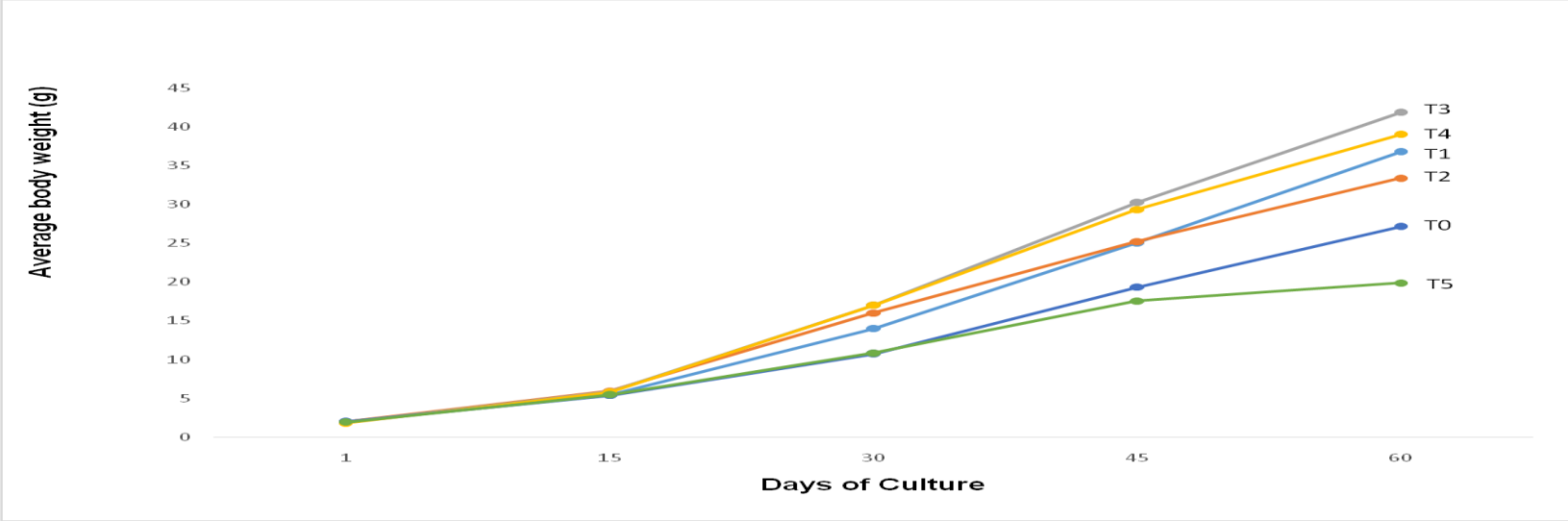

Table.1 Ingredient composition of formulated GIFT tilapia diet with 0.1%, 0.2%, 0.3%, 0.4% tryptophan enriched with added 20% biofloc meal

SL. NO	INGREDIENTS	INCLUSION L EVEL (%)				
		CONTROL (T0)	TRYPTOPHAN 0.1% (T1)	TRYPTOPHAN 0.2% (T2)	TRYPTOPHAN 0.3% (T3)	TRYPTOPHAN 0.4% (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	12.69	12.58	12.49
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	Tryptophan	0	0.22	0.31	0.42	0.51

Table.3 Proximate composition of experimental diets to assess the optimum level of 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	8.29±0.42	32.60±0.08	6.21±0.06	7.81±0.08	15.33±0.06	3543±11.26
T2	8.25±0.04	32.47±0.02	4.47±0.05	6.20±0.01	21.17±0.49	3658±10.08
T3	8.73.06	32.16±0.05	5.21±0.06	7.32±0.06	21.63±0.05	3452±4.91
T4	7.22±0.06	32.56±0.05	5.66±0.04	7.13±0.06	17.33±0.05	3422±6.38
T5	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 in the experiment to assess the optimum level of tryptophan 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^{a}±0.02	27.16^{b}±0.50	25.13^{b}±0.52	9.66^{a}±0.33	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	1.99^{bc}±0.00	36.77^{ab}±2.54	34.78^{ab}±2.55	10^{a}±0.00	347.80^{ab}±25.50	367.73^{ab}±25.46	429.33^{a}±22.04	1.17^{de}±0.02	0.85^{b}±0.02	10.87^{ab}±0.79	4.84^{ab}±0.12
T2	2.01^{c}±0.03	33.37^{a}±1.05	31.35^{a}±1.02	9.66^{a}±0.33	303.64^{a}±19.18	323.15^{a}±20.13	398.00^{a}±19.62	1.23^{e}±0.02	0.81^{a}±0.01	9.48^{a}±0.59	4.67^{a}±0.02
T3	1.94^{abc}±0.04	41.84^{ab}±1.09	39.90^{ab}±1.08	9.66^{a}±0.33	385.58^{ab}±15.44	404.36^{ab}±16.31	417.00^{a}±11.93	1.03^{a}±0.01	0.97^{e}±0.01	12.05^{ab}±0.48	5.12^{bc}±0.04
T4	1.87^{a}±0.00	39.01^{ab}±2.37	37.13^{ab}±2.38	9.66^{a}±0.33	360.54^{ab}±34.60	378.68^{ab}±35.16	416.00^{a}±38.07	1.10^{b}±0.00	0.91^{cd}±0.00	11.27^{ab}±1.08	5.05^{abc}±0.11
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

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

The optimum protein level for tilapia has been determined by several researchers and the results were not consistant. The optimal protein requirement was reported as 30% by Wang *et al.*, (1985), 32% Shaiu, Chuang and Sun (1987), 30-35% by Mazid *et al.*, (1979), 36% by Davis and Stickney (1978), 40% by Jauncey (1982). Studies proved that free amino acids are efficiently used as protein bound amino acids in meeting the essential amino acid requirement of fish (Murai *et al.*, 1987; Kim *et al.*, 1991; Rodehutscord *et al.*, 1995).From the present study it could be stated that 0.3% tryptophan with 20% biofloc meal enriched diet (T3) showed higher values of 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 0.3% tryptophan can improve the growth of GIFT tilapia without any adverse effect on the fish performance.

Acknowledgement

We gratefully acknowledge the funding support to this study by the Tamilnadu Fisheries University, Nagapattinam, Tamilnadu and also like to express our heartfelt thanks to The Dean, Fisheries College and Research Institute, Thoothukudi for his support and generosity. We would further like to express our thanks to Mr. T. Govindaraj, Manager, Hi-Tide sea farms, Mahendrapalli, Nagappattinam district, Tamilnadu, India, for helping in the collection of biofloc.

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How to cite this article:

Prabu, E., C.B.T. Rajagopalsamy, B. Ahilan, I. Jegan Michael Andro Jeevagan and Renuhadevi, M. 2017. Effect of Dietary Supplementation of Biofloc Meal with Tryptophan on Growth and Survival of GIFT Tilapia. *Int.J.Curr.Microbiol.App.Sci.* 6(8): 3426-3434.
doi: https://doi.org/10.20546/ijcmas.2017.608.411