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Optimum Dietary Biofloc Meal Inclusion Level in Relation to Growth Performance, Feed Utilization, Body Composition and Digestibility of Rohu (*Labeo rohita*) (Hamilton, 1822)

Bhenkatesh Padhan* and S. Athithan

Department of Aquaculture, TNJFU-Fisheries College and Research Institute, Thoothukudi 628008, Tamil Nadu, India

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BP conducted the feeding trial, analyzed the data and drafted the manuscript. SA conceptualized and designed the study, formulated the experimental diets and corrected the manuscript.

Key words
Biofloc meal, *Labeo rohita*, Growth performance, Body composition, Digestibility

ABSTRACT

Biofloc meal being a source of nutrients and bioactive compounds is a sustainable protein source in aquafeed. A 60-day feeding experiment was performed to study the effects of dietary biofloc meal inclusion on growth performance, feed utilization, body composition and digestibility of rohu (*Labeo rohita*) fingerlings. A total of 630 rohu fingerlings were randomly distributed evenly as 30 fish per tank into 21 tanks and three tanks per group. The rohu fingerlings of 4.30 ± 1.21 g initial weight were fed seven diets containing biofloc meal inclusion levels of 0, 5, 10, 15, 20, 25 and 30% (referred to as B0, B5, B10, B15, B20, B25 and B30, respectively). Results indicated that 20% biofloc meal inclusion showed the highest growth, feed utilization, body composition and digestibility of rohu fingerlings. The third-order polynomial regression analysis indicated that 20.50% dietary biofloc meal inclusion could perform higher growth performance. However, the higher dietary inclusion level of biofloc meal could retard growth and digestibility performance. In conclusion, biofloc meal may be added as a supplementary feed ingredient rohu fingerlings diet at a 20% inclusion rate with improved growth performance, feed utilization, body composition and digestibility.

INTRODUCTION

In aquaculture, fish nutrition plays an important role as feed cost represents over 50% of the production costs (Rana *et al*., 2009). Manufacturers primarily rely on fish meal as a gold standard protein source for aquafeed because of its palatability and balanced nutrition (Richard *et al*., 2011). However, in recent years the price of fish meal skyrocketed sharply as a result of stagnating capture fisheries (Yan *et al*., 2014).

To support the sustainability of aquaculture, considerable research has been conducted on the replacement of fish meal using alternative feed ingredients such as plant derived protein, animal-based protein and microbial protein (Gatlin *et al*., 2007). However, many of these are associated with limitations like low palatability and digestibility, presence of anti-nutritional, deficiency of some essential amino acids and bioactive molecules (Wang *et al*., 2016).

The biofloc technology being cost-effective and environment-friendly adheres to the principle of sustainability in aquaculture (Naylor *et al*., 2000). Bioflocs are associated with aggregates of suspended particles and microbes such as bacteria, fungi, invertebrates and detritus (Krummenauer *et al*., 2011). In aquaculture, the biofloc culture system has been extensively studied for various commercially important fishes and shrimps (Emerenciano *et al*., 2011; Xu and Pan, 2012; Da Silva *et al*., 2013; de Souza *et al*., 2014; Kumar *et al*., 2014). The concentrated and dried biofloc mass is called biofloc meal which is a good source of additional nutrients such as protein, lipid, vitamin and mineral (Avnimelech, 2006; Crab *et al*., 2010; Decamp *et al*., 2002; Tacon *et al*., 2002; Xu *et al*., 2012), along with many bioactive compounds (Crab *et al*., 2012; Ferreira *et al*., 2015; Ju *et al*., 2008). Some researchers have studied biofloc meal as alternative protein source for aquafeed industry (Bauer *et al*., 2012; Dantas *et al*., 2016; Kuhn *et al*., 2010).

Earlier reports suggest biofloc meal could be regarded as beneficial in terms of growth performance and

* Corresponding author: bhenkateshpadhan@gmail.com
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digestibility in fish (Long *et al*., 2015) and shrimp (Kuhn *et al*., 2009; Xu and Pan, 2012). Study conducted by Himaja *et al*. (2016) and Prabu *et al*. (2018) on growth performance of *Catla* and GIFT tilapia, respectively by replacing fish meal in the diet, showed higher growth performance with 20% biofloc meal inclusion. *Litopenaeus vannamei*, growth parameters were unaffected by a diet including biofloc meal at inclusion levels ranging from 10% to 30% (Bauer *et al*., 2012; Dantas *et al*., 2016; Kuhn *et al*., 2010). Related to the cost perspective of biofloc meal there were scarcity of information available in literature. A study done by Kuhn *et al*. (2009) during 2008-2009 estimated cost for production of biofloc meal as approximately $0.4 to $1000 per metric ton. During the same time frame the cost of fishmeal was approximately from $1000 to $1225 per metric ton, which suggests feasibility of inclusion of biofloc meal replacing fish meal in the fish feed.

Rohu, primarily being herbivorous to omnivorous species, readily feeds on plant materials (Talwar and Jhingran, 1991). Therefore, biofloc could be considered as an alternative protein source in *Labeo rohita* diet according to its feeding habit. Mahanand *et al*. (2013) experimented to find optimum feed mix for the growth of rohu with biofloc as a component and he obtained the optimum growth parameters of rohu at a feed mix containing 50% fish feed and 50% wet floc. But no earlier studies has been conducted on biofloc meal as a supplementary feed ingredients in formulated feed of rohu. Thus, an attempt was made to investigate the effects of dietary biofloc meal inclusion on growth performance, feed utilization, carcass composition and digestibility of rohu (*L. rohita*) providing valuable information for the sustainable aquaculture of rohu.

MATERIALS AND METHODS

Diet preparation

Seven diets were prepared having iso-nitrogenous and iso-lipidic in nature and an average crude protein level of 30.02% and crude lipids of 10.40% (Tables I and II). The experimental diets contained biofloc meal inclusion levels of 0, 5, 10, 15, 20, 25 and 30% (referred to as B0, B5, B10, B15, B20, B25 and B30, respectively).

Biofloc meal was obtained by collecting wet biofloc from a commercial biofloc-based tilapia fish farm in Tiruppur, Tamil Nadu, India. The suspended biofloc mass was collected by decanting approach (Johnson and Chen, 2006) and then passed through sequential filtration with nylon bags (250 and 50 µm meshes) and cellulose filter (10 µm mesh) as described by Dantas *et al*. (2016). For drying, the wet concentrated biofloc mass was dried in a well-ventilated area protected from direct sunlight and after that it was oven dried at 50 °C for 48 h. The biofloc meal contained 25.23% crude protein, 2.11% crude fat, 3.61% crude fibre, 23.5% total ash, 33% carbohydrate and 12.55% moisture.

Table I. Ingredients composition and proximate composition of the formulated feed.

Ingredients/ Components	Different experimental diets						
	B0	**B5**	**B10**	**B15**	**B20**	**B25**	**B30**
Feed ingredient							
Biofloc meal	0	5	10	15	20	25	30
Fish meal	17	16	15	14	13	12	11
GNOC	30	30	30	30	30	30	30
Rice bran	27	25	23	21	19	17	15
Wheat flour	17	15	12	11	9	9	7
Corn flour	4	4	5	4	4	2	2
Vitamin mineral mixture	5	5	5	5	5	5	5
Total	100	100	100	100	100	100	100
Proximate composition							
Moisture (%)	2.55	3.21	2.84	2.94	3.55	4.02	2.8
Crude fat (%)	11.23	9.9	10.64	10.15	10.28	10.08	10.54
Ash content (%)	21.16	22.12	23.1	23.18	24.09	24.15	24.18
Crude protein (%)	30.07	29.96	30.03	30.04	30.12	30.09	29.86
Crude fibre (%)	14.45	13.45	14.22	12.04	10.06	10.68	11.26
CHO (%)	20.54	21.36	19.17	21.65	21.9	20.98	21.36
Gross energy (Kcal/ 100 g)	358.62	348.81	347.21	352.81	355.51	349.68	354.29

Table II. Growth performance of rohu fed with different experimental diets.

Diet treatment	Parameters				
	Survival rate (%)	**WG (g)**	**SGR (%)**	**FCR**	**PER**
B0	96.66±3.33^{a}	5.85±0.18^{ab}	1.53±0.04^{ab}	2.07±0.08^{cd}	1.61±0.07^{ab}
B5	98.89±1.92^{a}	5.77±0.15^{a}	1.50±0.02^{a}	2.07±0.05^{cd}	1.60±0.04^{ab}
B10	95.55±1.92^{a}	6.27±0.09^{bc}	1.60±0.01^{bc}	1.94±0.08^{bc}	1.71±0.07^{bc}
B15	94.44±3.85^{a}	6.41±0.25^{c}	1.61±0.04^{bc}	1.87±0.07^{ab}	1.78±0.07^{c}
B20	96.66±3.33^{a}	7.04±0.10^{d}	1.69±0.03^{c}	1.70±0.02^{a}	1.95±0.02^{d}
B25	95.55±1.92^{a}	6.50±0.24^{c}	1.63±0.05^{c}	1.84±0.06^{ab}	1.80±0.06^{cd}
B30	95.55±1.92^{a}	5.61±0.07^{a}	1.47±0.01^{a}	2.13±0.02^{d}	1.56±0.01^{a}

Values (mean ± SD, n = 3) in the same column with different superscripts differ significantly ($p < 0.05$). WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio. For details of diet treatments see Table I.

As an inert marker, 0.5% chromic oxide (Cr_2O_3) was added to the diets to analyze digestibility (Gomes *et al*., 1995). All the feed ingredients were thoroughly ground to a particle size of lesser than 300 µm before diet preparation. Distilled water was then poured into the dry ingredients and properly mixed. The mixers were then first steamed for 20 minutes and then pelletized to form pelleted diets of size 2.0 mm in diameter. The pellets were then dried in a hot air oven for 24 h at 60 °C, ground into desired particle sizes, and stored in air-tight containers at - 20 °C until use.

Growth trial

The feeding trial was performed at the fish farm complex, Fisheries College and Research Institute, Thoothukudi, Tamil Nadu, India. Rohu fingerlings (4.303 ± 1.211 g) were acclimatised for 7 days and then randomly stocked into 21 indoor cement tanks (length 1.25 m/ width 0.65 m/ height 0.9 m) at a stocking density of 30 fingerlings per tank. A sufficient aeration facility was also fitted to each tank. An eight-week growth trial was carried out from April to May 2022. The experimental design consists of six treatment diets (B5, B10, B15, B20, B25 and B30) and one control diet (B0), which were assigned to 21 experimental tanks in triplicate design. Feeding was offered at 3 - 5% body weight twice (10:00 and 17:00) a day. The Physico-chemical parameters were determined following the guidelines provided in the standard for water and wastewater quality assessments (APHA, 2005). Throughout the growth trial, measurements of water temperature (28.5-32.6 °C), pH (7.98-8.60), and dissolved oxygen (5.6-8.16 mg L^{-1}) were made daily, whereas total alkalinity (125-175 mg L^{-1}), total hardness (40-70 mg L^{-1}), ammonia-nitrogen (0.01-0.05 mg L^{-1}), nitrite-nitrogen (0-0.01 mg L^{-1}) and nitrate-nitrogen (0-0.03 mg L^{-1}) were made on weekly. The rohu fingerlings starved for 24 h after the feeding experiment ended in order to remove intestinal content before sampling. Rohu fingerlings were counted and weighted to analyse growth performance parameters such as survival rate (SR), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER).

Parameters of growth performance were calculated as follows:

Survival rate (%) = (Total number of live fishes on the final day of the experiment / Total number of fishes on the initial day of the experiment) × 100

Weight gain (g) = Final weight (g) – Initial weight (g)

Specific Growth Rate (SGR) (%) = [ln (final body weight) – ln (initial body weight)] / Number of days of culture × 100

Feed Conversion Ratio (FCR) = Dry feed fed to fish / Wet weight gain of fish

Protein Efficiency Ratio (PER) = Wet weight gain of fish / Dry protein fed to fish

Feed, faeces and fish body composition analysis

Standard procedures were used to determine the proximate content of the samples (AOAC, 2005). By drying the materials to a constant weight at 105 °C, the dry matter was calculated. The Kjeldahl technique (Kjeltec TM8400, FOSS, Sweden) was used to calculate the amount of crude protein, and it was calculated by multiplying nitrogen by 6.25. By the ether-extraction method, the crude lipid was measured. After being burned at 550 °C for 16 h in a muffle furnace, the ash was inspected. Carbohydrate content was calculated as nitrogen free extract using the difference method of Hastings (1976). Gross energy was calculated according to NRC (2011).

Apparent digestibility analysis

The indirect technique was adopted in order to assess the apparent protein digestibility coefficients by employing an inert marker, chromic oxide (Cr_2O_3) in the diets (Gomes *et al*., 1995). To prevent contaminating the faeces, the remaining feed was carried away promptly after feeding. After day 6 of feeding with the experimental diets, faeces were collected daily and kept in airtight plastic pouches at -20 °C for subsequent examination. The chromic oxide (Cr_2O_3) content of diets and faeces sample was evaluated by adopting the acid digestion technique (Furukawa and Tsukahara, 1966) and comparing the absorbance from a standard curve (at 370 nm absorbance) of chromic oxide. According to Cho and Slinger (1979), the apparent digestibility coefficient of nutrients and energy for experimental diets was computed which is given as follows:

Apparent digestibility coefficients (ADC) of dry matter (%) = [1-(a/a')] ×100

Apparent digestibility coefficients (ADC) of nutrients or energy (%) = [1-(a/a'× b'/b)] ×100

Where, a is Cr_2O_3concentration in feed. a' is Cr_2O_3 concentration in faeces. b is nutrients or energy content in feed. b' is nutrients or energy content in faeces.

Statistical analysis

One-way ANOVA with Duncan's test for multiple comparisons was used to compare the growth, survival, feed utilization, fish body composition and apparent digestibility coefficients of rohu between the experimental diets at a significance level of 0.05. Third-order polynomial regression model (Robbins *et al*., 1979) was conducted to determine the optimal dietary inclusion level of biofloc meal for *L. rohita* on the basis of SGR and FCR. Prior to analysis, raw data were diagnosed for normality of distribution and homogeneity of variance with Kolmogorov-Smirnov test and Levene's test, respectively (Zar, 1999). All the statistical analysis were performed with the software SPSS for windows release 22.0 (SPSS Inc, 2013).

RESULTS

Growth performance and feed utilization

The results of the growth performance of *L. rohita* fed with three diets were shown in Table III. No significant difference was observed in the survival rate of *L. rohita* between the experiment diets at the end of the experiment ($p > 0.05$) (Table III). All the rohu fingerlings showed significant growth during the 60-day experiment, and individuals on diet B20 had significantly increased their weight gain compared to others ($p < 0.05$). The least weight gain was found in individuals on diet B30. But insignificant difference was seen between B5, B30 and B0.

The SGR was observed significantly highest in the rohu fingerlings fed with the B20 diet. But B20 and B25 diets showed no significant difference in SGR. SGR showed cubic rather than a linear response to dietary biofloc level, with the highest values of 1.69 % d^{-1} in diet B20. Based on the third-order polynomial regression model of SGR, the optimal dietary inclusion level of biofloc meal was estimated to be 20.50% of the diet (y = -6E-05x3 + 0.0022x2 - 0.0108x + 1, R^2 = 0.9365) (Fig. 1).

Table III. Body composition of *Labeo rohita* fingerling fed experimental diets.

Treat-ment	Moisture	Crude protein	Crude fat	Ash
B0	71.56 ± 0.01	17.33 ± 0.00	4.11 ± 0.00	3.86 ± 0.24
B5	71.59 ± 0.01	17.26 ± 0.05	4.11 ± 0.00	3.56 ± 0.01
B10	71.53 ± 0.02	17.23 ± 0.00	4.12 ± 0.01	3.56 ± 0.01
B15	71.58 ± 0.01	17.26 ± 0.05	4.12 ± 0.00	3.72 ± 0.24
B20	71.57 ± 0.02	17.29 ± 0.05	4.11 ± 0.01	3.72 ± 0.24
B25	71.59 ± 0.01	17.23 ± 0.00	4.11 ± 0.00	3.57 ± 0.01
B30	71.60 ± 0.01	17.26 ± 0.05	4.12 ± 0.00	3.56 ± 0.01

Results are mean of triplicate estimations ± SE. Means in the same column without superscripts are insignificantly ($P > 0.05$) different.
For details of diet treatments see Table I.

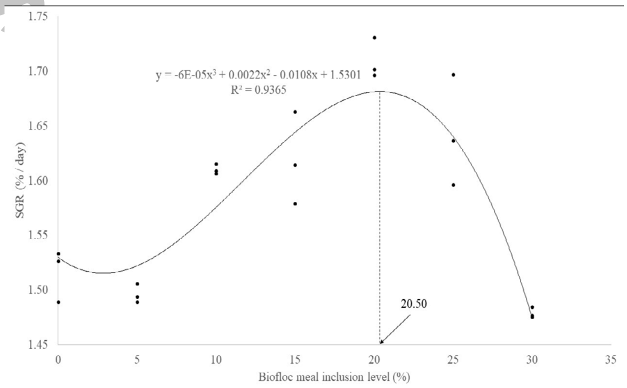

Fig. 1. Relationship between dietary biofloc inclusion level and specific growth rate (SGR) of *Labeo rohita* fed with the experimental diets.

In terms of feed utilization parameters such as the PER and FCR, better performance was observed in B20 diets and Between B20 and B25, no statistical difference ($P > 0.05$) was found. However, the minimum FCR was seen at 20.5% biofloc meal inclusion level according to third-order polynomial regression analysis of FCR (y = $0.0001x^3 - 0.0042x^2 + 0.0192x + 2.0705$, R^2 = 0.9628) (Fig. 2). In addition, it was observed that with increase in biofloc meal inclusion level from the diet B5 to B20, yielded higher weight gain, SGR and PER, and higher biofloc meal inclusion level from the diet B25 to B30 yielded lower value. Also, lower FCR was shown when biofloc meal inclusion level from the diet B5 to B20, and thereafter (B25 and B30), FCR increased. Control group, B0 diet is statistically comparable with B5 and B30 in parameters such as weight gain, SGR, FCR and PER.

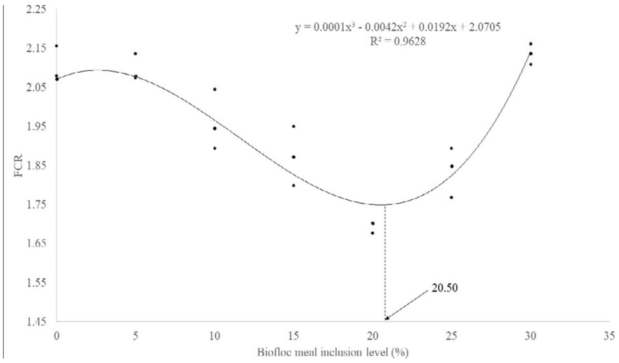

Fig. 2. Relationship between dietary biofloc inclusion level and feed conversion ratio (FCR) of *Labeo rohita* fed with the experimental diets.

Body composition

Body composition analysis of rohu fingerlings fed with different experimental diets is presented in Table III. Moisture, crude protein, crude fat and ash contents in Rohu body composition did not show any statistical differences among all dietary treatments ($P > 0.05$).

Digestibility

The apparent digestibility coefficients (ADCs) for dry matter (ADCDM), protein (ADCP), lipid (ADCL), carbohydrate (ADCC) and gross energy (ADCE) of *L. rohita* fed with the experimental diets are presented in Table IV and graphically depicted in Figure 3.

Dietary inclusion level of biofloc meal had a significant effect on apparent digestibility coefficient values such as ADCDM, ADCP, ADCL, ADCC and ADCE ($p < 0.05$). The highest values of ADCDM, ADCP, ADCL, ADCC and ADCE were observed in diet B20. The lower ADCDM was observed in B30 diet and which does not differ statistically from B0 and B5 diets. The Rohu fed 20% biofloc level showed significantly higher ADCP (87.90%) than other treatments ($p < 0.05$). Although B20 showed higher ADCL, but no significant difference was found among the diets B10, B15, B20 and B25 ($p > 0.05$). In terms of ADCC, B20 and B25 showed significantly higher values as compared to others ($p < 0.05$). The ADCE values in diets B0 and B30 were significantly least than other diets ($p < 0.05$).

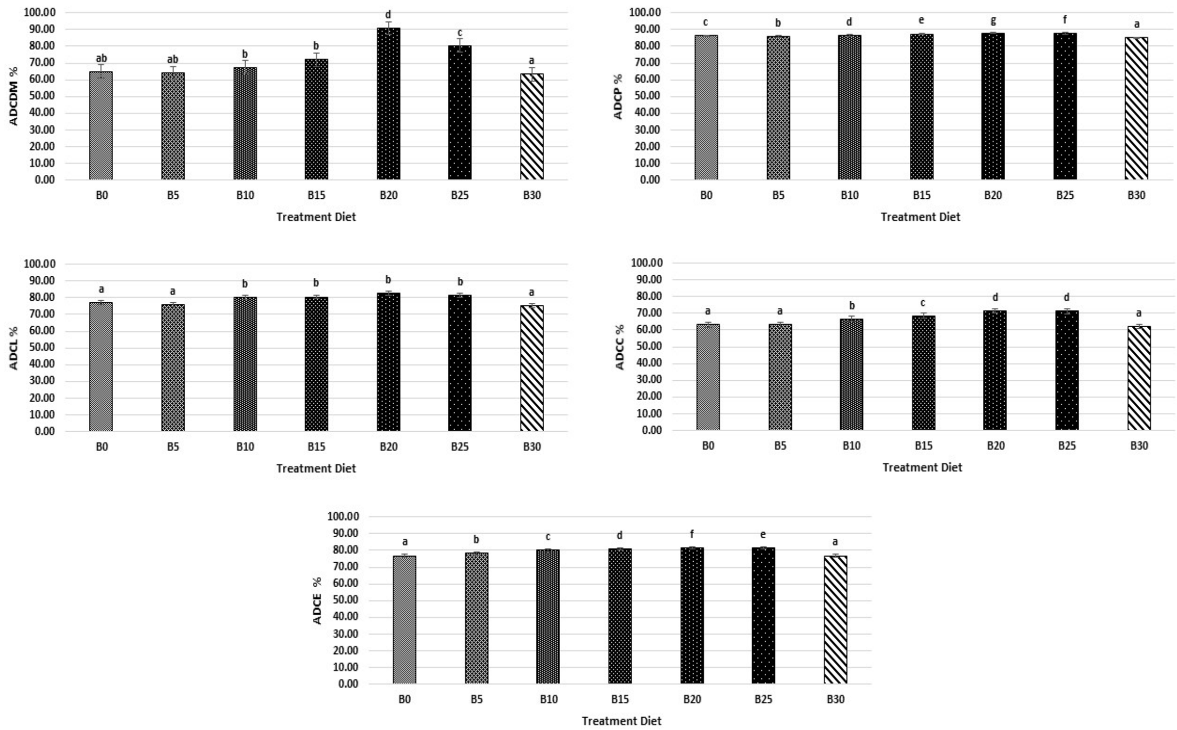

Fig. 3. Apparent digestibility coefficient for dry matter (ADCDM), for protein (ADCP), for lipids (ADCL), for carbohydrates (ADCC) and gross energy (ADCE) of *L. rohita*. Different alphabets on bars in the same graph indicate statistically significant differences.

Table IV. Apparent digestibility coefficients (ADCs) for dry matter (ADCDM), protein (ADCP), lipid (ADCL), carbohydrate (ADCC) and gross energy (ADCE) of *L. rohita* fed with the experimental diets.

Treatment diet	ADCDM %	ADCP %	ADCL %	ADCC %	ADCE %
B0	64.92 ± 5.61^{ab}	86.22 ± 0.00^{c}	77.19 ± 3.13^{a}	63.13 ± 0.15^{a}	76.81 ± 0.60^{a}
B5	63.99 ± 2.34^{ab}	85.91 ± 0.08^{b}	75.73 ± 1.60^{a}	63.09 ± 0.62^{a}	78.15 ± 0.38^{b}
B10	67.39 ± 5.43^{b}	86.44 ± 0.03^{d}	80.20 ± 0.00^{b}	66.63 ± 0.57^{b}	80.01 ± 0.09^{c}
B15	72.01 ± 3.44^{b}	87.33 ± 0.03^{e}	80.48 ± 1.52^{b}	68.44 ± 0.95^{c}	80.79 ± 0.42^{d}
B20	90.67 ± 4.62^{d}	87.90 ± 0.03^{g}	82.66 ± 1.19^{b}	71.39 ± 0.48^{d}	81.53 ± 0.14^{f}
B25	80.47 ± 3.40^{c}	87.70 ± 0.06^{f}	81.51 ± 0.00^{b}	71.28 ± 0.47^{d}	81.38 ± 0.08^{e}
B30	63.31 ± 3.60^{a}	85.01 ± 0.03^{a}	75.17 ± 0.00^{a}	62.09 ± 0.64^{a}	76.75 ± 0.10^{a}

Values (mean ± SD, n = 3) in the same column with different superscripts differ significantly ($p < 0.05$). For details of diet treatments see Table I.

In overall apparent digestibility coefficient values increased firstly and then decreased as dietary biofloc level increased from diets B0 to B30, with highest values of 87.90% ADCP, 82.66% ADCL, 71.39% ADCC and 81.53% ADCE in diet B20.

DISCUSSION

In the present study, the biofloc meal used was having crude protein 25.23%, crude fat 2.11%, 3.61% crude fibre, 23.5% total ash, 33% carbohydrate and 12.55% moisture. The biofloc meal has been reported of having 20−60% crude protein and 0.5−5% crude lipid contents in range (Azim and Little, 2008; Ju *et al*., 2008). The high ash concentration in the biofloc meal may be related to the excess amount of acid-insoluble oxides and mixed silicates as observed by Tacon *et al*. (2002). But also, in contrary biofloc meal may be regarded as an excellent supply of necessary minerals and trace elements that can aid in fish growth (Tacon *et al*., 2002; Ju *et al*., 2008; Avnimelech, 2006). Previous studies showed that growth performance, feed utilization and digestibility could be improved by dietary biofloc meal inclusion in fish (Long *et al*., 2015) and shrimp (Kuhn *et al*., 2009; Xu and Pan, 2012).

Dietary inclusion levels of 20% biofloc meal significantly enhanced weight gain, SGR, FCR and PER of *Labeo rohita*, implying that additional nutrients, trace minerals and bioactive compounds in biofloc might have result in better growth performance and higher feed utilization of rohu. Study conducted by Himaja *et al*. (2016) and Prabu *et al*. (2018) on growth performance of Catla and GIFT tilapia respectively by replacing fish meal in the diet, showed higher growth performance with 20% biofloc meal inclusion. Experiment performed on *L. vannamei*, showed growth parameters were unaffected by a diet including biofloc meal at inclusion levels ranging from 10% to 30% (Bauer *et al*., 2012; Dantas *et al*., 2016; Kuhn *et al*., 2010). Comparable rohu fingerlings body composition indicated no negative impacts on biofloc meal inclusion in the formulated diet significantly.

The results also found that ADCs for nutrients and energy increased remarkably in the B20 group. It has been documented that various bioactive component in biofloc contributed to the production of endogenous enzymes of organisms (Anand *et al*., 2014; Zhang *et al*., 2016; Ziaei-Nejad *et al*., 2006).

However, weight gain, SGR, FCR and PER of sea cucumber all showed remarkable downward trends as dietary biofloc level increased from 20% to 30%, along with the deceasing ADCs of nutrients and energy. Previous studies also indicated that high dietary biofloc meal inclusion could reduce the acceptance of diet (Ajiboye *et al*., 2012; Gamboa-Delgado *et al*., 2017; Himaja *et al*., 2016; Kiessling and Askbrandt, 1993; Prabu *et al*., 2018) and digestibility performance due to higher microbial protein, and further influence growth performance of aquatic animals (Kuhn *et al*., 2010; Anand *et al*., 2014). Based on the third-order polynomial regression models of SGR and FCR, it was concluded that 20.5% were optimal dietary replacement levels of biofloc.

CONCLUSION

The present study demonstrated that appropriate dietary biofloc meal inclusion level could improve growth performance of rohu (*Labeo rohita*) fingerling, by enhancing feeding utilization and digestibility of rohu. 20% biofloc meal inclusion resulted in the highest growth performance and digestibility of rohu fingerlings. The third-order polynomial regression indicated that 20.50% dietary inclusion level of biofloc meal could performed higher growth performance of rohu fingerlings. On the other hand, higher dietary inclusion level of biofloc meal (B30 diet) could retard growth and digestibility performance. Future studies are needed to focus on the performance of biofloc meal inclusion in the diet of rohu by outdoor application.

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Ethical statement and project approval

The experiment was conducted following the procedures of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment and Forests (Animal Welfare Division), Govt. of India on care and use of animals in scientific research. This study was approved by the ethical committee of Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Nagapattinam, Tamil Nadu, India.

IRB approval

Approval have given for the article by the Advisory Committee Members of TNJFU-Fisheries College and Research Institute, Thoothukudi-628008, Tamil Nadu, India.

Statement of conflict of interest

The authors have declared no conflict of interest.

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