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Comparing the Suitable Different Protein Level and Economically Viable Feed for Tilapia (*Oreochromis niloticus*) Reared in Glass Tank at Bangladesh

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Authors' contributions

This work was carried out in collaboration among all authors. Author MSH did the Laboratory work, data collection and writing the draft. Author SZC did the draft editing and writing. Author JB did the research design, supervision, writing and editing the draft. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Nile tilapia is one of the most consumable and economical fish species in Bangladesh, traditionally co-cultured by sharing with prawn, shrimp and Indian major carp. In tilapia monoculture, suitable protein level in the diet is crucial for better growth of the fish as well as being economically viable. Addressing this aspect, here in this experiment we tried to adopt suitable and economically sustainable feed for better tilapia growth and production.

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Place and Duration of Study: The experiment was carried out for 75 days in the wet laboratory of FMRT discipline of Khulna University.

Methodology: Three available commercial feed with protein percentages of 32.5% (T_1), 35% (T_2) and 28% (T_3) were fed to the juvenile tilapia (mean weight 0.31g) under the laboratory condition. Fish were nursed in rectangular glass aquaria tank (volume 25L) with each three replications for 75 days at the stocking density of 25fry/tank to determine the effect of these feed on growth of tilapia fry.

Results: After 75 days culture period the final weight of tilapia under treatment T_1 (4.68±0.28⁾ were significantly (p<0.05) higher than treatment T_2 (4.01±0.19) and T_3 (3.26±0.39). Similarly, the highest SGR was found in T_1 followed by T_2 and T_3 , however no significant difference observed among the treatments. FCR was significantly different among the treatment, where the lowest FCR was found in T_1 and higher at T_3 . In contrary, PER was opposite to FCR values, higher in T_1 and lower in T_3 . Again, significant different were observed in protein, lipid and ash content of tilapia among the three treatments. Water quality parameters were found at suitable farming ranges throughout the experimental period.

Conclusion: The finding of this experiment suggested that 32.5% protein enriched feed can be suitable for the better growth of tilapia.

Keywords: Nile tilapia; feed; growth; protein; FCR.

1. INTRODUCTION

Nile tilapia (NT), Oreochromis niloticus is the most commercially important freshwater fish species, immensely forming in many Asian and African countries situated in tropical and subtropical regions [1-5]. Currently NT is being cultured in more than 100 countries in the World extensively in Bangladesh, India, Indonesia, Ethiopia, and most tropical countries [4, 6, 7, 8]. Bangladesh has huge potentiality of NT aquaculture because of favorable agro-climatic condition, and availability of resources including fry, feed and economical labor and huge market demands [7, 9, 10]. Tilapia was first introduced in Bangladesh from Thailand in 1954 and since then, tilapia culture mostly practiced traditionally with extensive and semi-intensive polyculture farm of prawn, shrimp, catfish and Indian major carp. and recently towards to monoculture [10: 11]. Tilapia aquaculture plays an important role in gross fish production, distribution, consumption, marketing, and ensuring livelihood and the food supply to thousands of peoples in Bangladesh [10, 12]. Tilapia production is increased day by day and currently the global production of tilapia attained nearly 6 million in 2020 [5]. Following the trend of last few decades, tilapia aquaculture has significantly increased (ranked third among the top eight tilapia producing countries, [6] and now it is considered as one of the most productive and internationally traded fish food in Bangladesh [7, 13, 14]. In Bangladesh, tilapia is known as aquatic chicken due to its significant contribution in fulfilling the demand of animal protein with low market price [3, 10, 12, 15]. Thus, tilapia culture in freshwater ponds in Bangladesh is getting popular due to its higher market demand and its faster growth, higher survival, and culture possibility in both recurrent and seasonal ponds [7, 9, 16].

Nile tilapia is euryhaline with versatile feeding behavior. characterized by opportunistic omnivorous feeding behavior, and the feeds includes algae, organic particles on the pond bottom, other plant materials as well detritus of lower and upper trophic levels [10, 17, 18, 19]. In addition, like catfish tilapia has high tolerance to environmental variables fluctuations, even capable to survive at low oxygen (even as below 2.3 mg/L) and lower availability of feed on wide range of foods. Thus, make it as one of the best candidate species for the adaptation against the climate change [10, 20, 21].

Although there is considerable potential for tilapia farming in Bangladesh, the production is still not satisfactory. There are many problems raised in tilapia farming includes outbreak of diseases and scientifically sound farming practices, quality fry production, high feed prices and the depletion of cultivable fresh water farm and environmental degradation [10, 12, 14]. Furthermore, Bangladeshi tilapia aquaculture faced some emerging problem: availability of high quality fry, poor management and high quality feed. Feed quality can significantly affect the fish growth rate, environmental parameters and production cost thus eventually contributes to profitability of the aquaculture farm [22, 23]. In addition, due to the use of different types of chemicals includes heavy metals and antibiotics in fish feed deflating the nutritious quality, effects on fish growth and diseases resistance capacities, thus raised potential risk on human health by consumption [10, 23]. In aquaculture, feeding cost is responsible for over 70 percent of the production cost thus inefficient feed management increase the fish production cost [20, 24, 25]. Balanced feed is essential for better fish growth, reproduction, and other physiological functions [4, 25, 26]. The technical knowledge on fish feed and feeding habits is important for the profitable management of both capture fisheries and aquaculture [25, 26].

However, well-balanced low cost formulated feed is essential to ensure fast growth and high yield and cost-effective tilapia culture. Good quality with balanced protein and other nutrients enriched feeds are the important component for the fisheries sustainability, aquaculture development and profitable fish production. Addressing all these issues, this study we are trying to identify a suitable protein levels with economically sustainable feed for better tilapia growth and production.

2. MATERIALS AND METHODS

2.1 Experimental Design and Tank Set Up

This study was conducted at the wet laboratory of Fisheries and Marine Resources Technology (FMRT) discipline of Khulna Universitv Bangladesh. Laboratory trailed was conducted within the rectangular glass aquaria tank at the volume of 25L water. In this experiment, we investigate the effect of different protein level feed application on growth and proximate composition of O. niloticus. Three commercial supplementary feed containing protein percentages of 32.5% (T₁) 35% (T₂) and 28% (T_3) were selected from the local fish feed Nutrient composition of three market. experimental feed shown in Table 1.

2.2 Experimental Animal and Maintenance of Fish in Tank

Tilapia fry (0.31±0.01g) were collected from a local tilapia hatchery and transported carefully to the wet laboratory following the approval of animal ethics regulation by Khulna University and kept in acclimatization tank for 48h at room temperature. During the acclimation period, fish were fed commercial pellet feed (pellet size 0.5

mm) twice a day according to their body weight. After 48h acclimatization period, fish were stocked into the experimental tank at stocking density of 25 fry/25 L aquaria tank ($30 \times 20 \times 20 \text{ cm}^3$). Adequate oxygen supply was ensured by installing aerators machine in each tank.

2.3 Feed and Feeding Management

Before applying to the fish, the proximate composition of selected three commercial feed were analyzed to ensure the exact level of protein, lipid, ash and moisture content in order to compare with the percentages labeled in the feed bag. The fish were fed at the rate of 10% of the total body weight twice a day, such as 50% (6.00) and 50% (17.00). Unused food, dirty residual and dead individuals were removed from the glass tanks daily by siphoning and water was compensated daily with sterilized tap water.

2.4 Fish Sampling and Growth Monitoring

Fish were sampled at every 15 days interval and growth parameter measured by recording the weight of fish using a digital balance. During sampling 15 fish were measured randomly from each treatment. At the end of the experiment, all fish were caught and growth parameter and survival of fish was determined. Following formula were used to calculate the growth parameters –

- (i) Survival rate (%) = $\frac{Nt}{N0} \times 100\%$,
- (ii) Average weight gain (WG)= Wt W0,
- (iii) Daily weight gain (DWG) (g/day)= $\frac{(Wt-W0)}{t}$,
- (iv) Specific growth rate $(SGR) = \frac{\ln(Wt) \ln(W0)}{t} \times 100,$

Where,

- N_t , = total number of live fish at the end, and N_0 =initial stocked fish number, W_t denotes final body weight and W_0 initial body weight of tilapia, and t is the culture period.
- (v) Feed conversion ratio (FCR) = $\frac{\text{Total amount of feed given (g)}}{\text{Weight gain (g)}}$
- (vi) Protein Efficiency ratio (PER) Wet weight gain (g) Protein consumed (g)

	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)	CHO (%)	Fiber (%)	Calcium (%)
Feed1 (T ₁)	32.5	7.52	17.28	12.39	22	4	2
Feed 2 (T ₂)	35.0	7.39	16.99	14.43	22	4.5	2
Feed 3 (T ₃)	28.0	8.0	10	10	20	3.5	2.1

 Table 1. Nutritional composition of three-selected experimental feed applied to the tilapia fish during the experiment

2.5 Measurement of Water Quality Parameters

Physiochemical parameters of the water quality were measured once a week in the water chemistry laboratory of FMRT Discipline, Khulna University. Salinity was measured by a hand refractometer (ATAGO Co. Ltd, Japan, range 0–100 g/L), and the temperature was measured using a digital thermometer (China, model no WT-2, Temperature range 20 to 80°C). Other parameters: pH, dissolved oxygen (DO), alkalinity, nitrite nitrogen and ammonia were measured by HACH kit, (HACH, USA, Model FF-2).

2.6 Whole Body Proximate Composition Analysis

Proximate composition includes of protein, lipid, ash and moisture were analysed from five fish, which randomly caught from each treatment at the end of the feeding period. Protein was measured by using micro Kjeldahl method by measuring the percentage of nitrogen (%N) into the sample multiplies with conversion factor (6.25). The percentage of gross portentous nitrogen (%N) was calculated by the following formula:

- (i) Percentage of nitrogen (%N) = $\left[\left(\frac{Volume \ of \ HCl \ x \ Normality \ of \ HCl \ x \ 0.014}{Sample \ weight \ (g)}\right) x \ 100\right]$
- % Protein = %N x 6.25, where %N = Percentage of nitrogen, 6.25=conversion factor

(ii) % Lipid =
$$\frac{(Weight of lipid x 100)}{Sample weight}$$
 [27]

- (iii) % Moisture= (Weight of wet sample-weight of dried sample)x100 Weight of wet sample, [28]
- (iv) % Ash = weight of Ash x 100/Weight of sample [29]

2.7 Statistical Analysis

All statistical analyses were performed with the SPSS statistical package (Version 28, SPSS Inc., Redmond, USA) and Microsoft office application (Microsoft Corporation, USA). Normal distribution of data was assessed with Shapiro-Wilk test (P=.05). Significant differences between treatments were calculated by T- Student test. In all cases, significant differences were established by one way ANOVA at P=.05.

3. RESULTS AND DISCUSSION

3.1 Growth and Feed Efficiency Parameters

Fish growth parameters includes the mean initial weight, final weight, total weight gained, daily weight gain (DWG), % weight gain, specific growth rate (SGR), survival rate and feed utilization efficiency factors feed conversion ratio (FCR), the protein efficiency ratio (PER), of Nile tilapia fed to different protein labeled feed were shown in Table 2. There was no significant difference in average initial weight (g) among the treatments, while significant difference observed (P=.05) in the mean final weight of tilapia (Fig. 1). The highest mean body weight was found at T_3 $(4.68\pm0.28g)$ followed by T₂ $(4.01\pm0.19g)$ and T₃ (3.26±0.39g). Specific growth rate (SGR) between T_1 and T_2 were not significant, but significantly varies with T₃. Similarly, the highest total weight gain and percentage of weight gain was found higher in T_1 which was significant (P=.05) than T₂ and T₃. On the other hand, feed efficiency parameter, FCR and PER was insignificant between T_2 and T_3 while significant (P=.05) compared to T₁. In addition, the survival rate was not significantly differed among the three treatments.

The nutritive balance of feed controlling influences feed utilization and growth of fish. Thus, It is very important to know the nutritional requirements predominantly for protein, carbohydrate and lipid for ensuring optimum growth of a fish species as well as in formulating a balanced diet [5, 21, 22]. Researcher are trying to establish suitable composition for tilapia feed still there is no evaluation of the stated nutritive value of fish feed produced by different feed companies in our country. Therefore, the farmers have to depend only on the prevailing information about the feed composition and growth performance that is given by the industry [10, 22, 30]. Even there is a possibility to use unauthorized feed ingredients and they do not maintain proper quality in manufacturing feed. Therefore, it is an urgent need to assess the actual nutritive value of the commercial fish feeds available in the market. Protein is considered as the prime growth-boosting component in fishes feed and the fish obtained faster growth if more protein enriched feed provided to the fish [5, 22, 23]. Many studies has been evaluated the growth parameter of nile tilapia at different feeding level [2, 10, 21, 31] and the results more or less similar to the current research. In contrary, feeds with less protein content may lead to lower weight gain and poor immunity that negatively affecting the protein requirements of human [22, 23, 31]. Thus, suitable feed protein is important for successful and profitable tilapia culture although most of the studied raveled fish needed a minimum of 25-50% crude protein in their diet [22, 32]. In this study, we observed higher body weight in T_1 where fish fed with 32.5% protein enriched feed compare to T_2 (35%) and T_3 (28%) protein). This result supported by many previously studies [9, 14, 33, 34].

In addition, the lower food conversion ratio (FCR) in T_1 also is an indication of higher feed

efficiency in 32.5% protein enriched feed. At the same time, important consideration should be given on the feed price, in general, high protein containing feed price is higher comparatively to lower protein feed [10, 14, 22, 34].

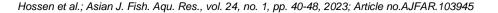
3.2 Body proximate composition

The body proximate composition of most fish consisting of proteins, carbohydrate, water, and lipids and these components make up around 98% of the total mass. Along with this some minor constituents comprise vitamins and minerals and ash. The taste of the fishes depends on all these nutritional ingredients [34,35]. Comparative to other finfish, tilapia is testy and maintained well nutritional balanced within the affordable price, therefore this fish getting popularity among the people in many tropical and subtropical countries [2, 4, 5, 9]. Here in this study, three different types of feed were applied, and the result revealed that there was not significant difference in nutritional composition among the treatments. The proximate compositions of tilapia fed with different types of feed were shown in Table 3. The whole body protein and lipid content was different (P=.05) among statisticallv the treatments. The protein percentage was higher in T_1 (12.04 ± 0.14) and lower at T_3 (10.81 ± 0.25). On the other hand, lipid content of the tilapia was found to be higher in T_3 and lower in T_1 that the values differ significantly (P=.05). Similar finding of our results was also reported in many previously studies [23, 33, 35].

Table 2. Growth parameter (mean ± SD) of nile tilapia (O. niloticus) fed with different types offeed

Growth parameters	Experimental groups				
	T ₁	T ₂	T ₃	-	
Initial Weight (g)	0.31±0.02	0.31±0.01	0.31±0.01	0.609	
Final Weight (g)	4.68±0.28 ^a	4.01±0.19 ^b	3.26±0.39 ^c	0.003	
Weight Gain (g)	4.39±0.29 ^a	3.7±0.16 ^b	2.95±0.38 ^c	0.004	
DWG (g/day)	0.058±0.01 ^a	0.049±0.01 ^a	0.039±0.01 ^b	0.032	
Percentage weight gain (%)	1409.6±11.2 ^a	1193.5±14.5 ^b	951.6±11.8 [°]	0.006	
SGR(% day-1)	3.62±0.02 ^a	3.41±0.09 ^a	3.14±0.03 ^b	0.017	
FCR	1.60 ± 0.008 ^a	1.76 ± 0.003 ^b	1.90 ± 0.012 ^b	0.020	
PER	2.75±0.04 ^a	2.45±0.06 ^b	2.32±0.06 ^b	0.042	
Survival rate (%)	96	98	96		

DWG= daily weight gain, SGR= specific growth rate, FCR=feed conversion ratio, PER= protein efficiency ratio. Different superscript letters in the same parameters indicate significant differences between the treatments (One-way ANOVA, P=.05)



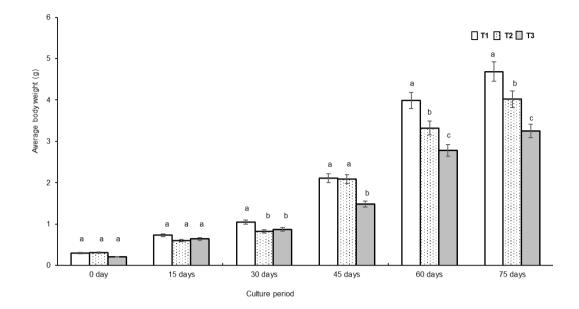


Fig. 1. Growth performance of tilapia (*O. niloticus*) at different treatments Error bars represent the standard error of samples. Letter in the top of the bar indicate the significance

	Treatment			P value
	T ₁	T ₂	T ₃	
Protein (%)	12.04 ± 0.14 ^a	11.64 ± 0.11 ^a	10.81 ± 0.25 ^b	0.004
Lipid (%)	1.86 ± 0.06^{a}	2.04 ± 0.07^{a}	2.75 ± 0.33 ^b	0.042
Moisture (%)	79.94 ± 0.35	79.92 ± 0.79	81.85 ± 0.08	0.372
Ash (%)	4.26 ± 0.31^{a}	3.83 ± 0.15 ^b	3.67 ± 0.12^{b}	0.023

Different subscript letter specify the statistical significance between the treatments (One way ANOVA, p<0.05)

Table 4. Water quality parameters (mean±SD) of experimental tank during the salinity and
temperature exposed period

Water parameter		Treatment	
	T ₁	T ₂	T ₃
pH	7.9±0.02	7.8±0.05	7.8±0.04
Temperature (°C)	24.6±0.5	24.5±0.5	25.25±1.0
Salinity (ppt)	0.5±0.01	0.4±0.01	0.5±0.01
DO (mg/l)	5.65±0.75	5.91±0.70	5.52±0.5
Alkalinity (CaCO ₃ mg/l)	130.5±4.5	135.85±6.7	131.46±5.5
Nitrite nitrogen (mg/l)	0.11±0.01	0.09±0.01	0.16±0.03
Total ammonia (mg/l)	0.36±0.05	0.34±0.04	0.45±0.07

Different letter indicate the statistical significance between the treatments (One way ANOVA, P=.05)

3.3 Water quality parameter

Water quality parameters play significant role for successful growth and aquaculture production of the desired fishes. The optimal water quality is essential for survival, growth, immune activities, metabolism and production of commercial aquaculture species [14, 36]. Although tilapia can withstand a wide range of environmental fluctuations, but controlling optimum water quality is prerequisites for better growth and production [7, 36, 37]. Here in this study important water parameters for aquaculture were measured regularly throughout the experimental period showing in Table 4.

In this study most of the water parameters in the experiment tank were in the acceptable range for tilapia aquaculture which is more or less similar to some previously studied [14, 20, 33, 38]. In the

current study, water temperature was ranged from 24.5±0.5°C to 25.25±1.0°C throughout the experiment which is more or less similar to some previously studied [14, 33, 39]. Dissolved oxygen and *pH* play vital role on growth and production through its direct influence on feed consumption and maintaining oxidation state of fishes [36, 37]. pH and dissolved oxygen (DO) level were in suitable ranges in for tilapia farming [14, 39, 38]. Likewise other parameter, nitrite nitrogen and ammonia value were in suitable ranges and no significant variation observed among the treatment and maintained healthv tank environment, similarly as reported in other studies [7, 10, 14 36].

This study was conducted in the pre-established laboratory condition therefore, the fixed cost including tank set up, aeration and other facilities were not significantly varied throughout the experiment. The variable cost in this experiment were fish frv and feed. We stocked same number of fish frv in each experimental tank, so the frv cost had no difference among the three treatment. Thus, the only variable cost was feed which price depends on the protein percentages and the quality. We applied same amount of feed in each experiment thus the feed cost was varied because of it market price. In the current research, three different protein level (32.5%, 35% and 28%) feed were applied and the unit price were 65tk/kg, 72tk/kg and 62tk/kg (December 2020) respectively. Usually feed with higher protein percentages have higher market price. In this study, feed (T_1) with 32.5% protein having more growth and considered as more economical rate compared to other feed protein level feed.

4. CONCLUSION

Suitable feed selection is very crucial for economically sustainable aquaculture. Although, the optimum dietary requirement of protein and lipids for fish varies species to species and the feed habits, availability of natural foods and physiological state of the fishes. The current study documents that feed with 32.5% protein shown better growth and feed utilization in Nile tilapia, ultimately effective to reduce the feed cost. This study recommends 32.5% protein (T_1) enriched feed, which could be applied for better production and profitable tilapia culture.

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CONSENT

It's not applicable.

ETHICAL APPROVAL

All procedures including fish harvesting and killing performed were completely compliant with the recommendations and regulations of the "Biosafety and animal Ethics Committee," Khulna University, Bangladesh.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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