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# Cellulose nanoparticle modulated feeding frequency as an influence on growth parameters, feed conversion ratio and comparative bubble nest production in Rose Tail male *Betta splendens*, (Regan, 1910)

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**Abstract**
Betta fish, *Betta Splendens* (Regan, 1910) are freshwater ornamental fish that are very popular among because it's beautiful colors, fin patterns and unique behavior. This study aims to determine the growth parameters according to feeding frequency by measuring feed conversion ratio (FCR) as well as the Comparative bubble nest formation and architecture pattern reared in experimental ponds. A stocking density of 25 adult male rose tail betta fish/decimal with three feeding frequency T1 (four times/day), T2 (three times/day) and T3 (two times/day) was maintained each with three replications. The betta fish was initially fed at 30% of their body weight and the rate was reduced up to 5% gradually at the final stage. The survival rate was 95.65±0.367, 93.00±0.709, and 90.50±0.707% % in T1, T2 and T3, respectively. The best FCR value obtained in T1 (1.02±0.03) and the significantly ($p<0.05$) highest fish production was also observed in T1 (842.67±15.28 count/dec/120 days). At the end of the experiment, the mean weight gain was recorded as 3.52±0.45 g, 2.96±0.38 g and 2.21±0.41 g in T1, T2 and T3, respectively. The study found that T1 produced the most bubbles per nest (842.67 ± 15.28), followed by T2 (768.33 ± 14.43), and T3 produced the least (612.33 ± 15.61). The maximum bubble nest formation rate ($p<0.05$) was 842.67±15.28 count/Dec/30day. The results revealed that feeding frequency effect positively on the growth performance of Betta splendens.

**Keywords:** Feeding frequency, growth performance, FCR, bubble nest, rose tail *Betta splendens*

## Introduction

Betta splendens, or Betta fish, is a widely grown species because it can grow and breed in a range of environments and can survive handling-induced stress [1]. In terms of worldwide productivity, it is currently only surpassed by carp. In such aquaculture conditions, it is possible to achieve the fundamental goal of decorative fish culture. According to the FAO [2] Rose tail Betta splendens is one of the most important fish species for tropical and subtropical aquaculture. Betta splendens is perhaps the most important ornamental fish and the most widely farmed species in the twenty-first century [3].

Male rose tail Betta fish are used in commercial betta production because they develop around 30% faster than females. As a result, it is critical to study the variables that effects its production as feed type, ration size, varied feeding frequency, and their possible effects on growth and feed utilisation, as well as bubble nest building, which can improve population and egg survival rates. According to De Silva and Anderson [5], feeding regularly is critical for getting the highest food conversion ratio and weight of cultured organisms. Higher feeding frequencies may result in faster growth and greater size uniformity by reducing aggressive behaviour. Furthermore, feeding frequency can affect water quality, survival, body composition, and growth performance [6, 7].

The word "feeding frequency" usually refers to how often an organism should be fed during the day. It is recognised as a critical factor that influences fish production, feed intake, and growth. The current study aims to evaluate the growth performance of Betta fish culture under various feeding frequencies for Betta populations. Effective feed management, husbandry practices [8, 9], and broadcasting of the appropriate ration to the culture system are critical methods for reducing feed costs in commercial aquaculture. Thus, one of the most significant features of cultural practice can be identified as the act of feeding.

In order to achieve the desired growth, it is critical to standardise feeding frequency for the target species in ornamental fish farming. The study's primary purpose was to discover the optimal feeding rate and frequency for Betta splendens, a major fish species utilised in aquaculture in Thailand and India. Several writers have already investigated the impact of feeding frequency on development outcomes for several species [10, 11]. However, the impact of feeding frequency on the growth performance of *Betta splendens* remains minimal. To guarantee that Betta splendens and its fingerlings grow and thrive under the best possible conditions, the current study sought to determine an optimum feeding frequency.

## Material and Methods

### 1. Study Area and Experimental Design

The experiment was conducted over 120 days in ten experimental glass tanks (45×21 cm) with a surface area of 0.0945$m^2$. Three replications were employed (Table 1). The experiment will be conducted at the Fish farm and breeding centre, Angel aquarium and fish shop Latur from September 20 to December 20, 2025. The experiment was conducted in thirty tanks, with a stocking density of 53 fish/dec and three feeding frequencies: T1 (four times/day), T2 (three times/day), and T3 (two times/day). Following the application of pelleted feed (Quality Feed) to the experimental ponds, water quality parameters as temperature, dissolved oxygen, and pH were measured using a commercial kit box.

**Table 1:** Experimental layout of Betta splendens

| Treatment | Replications | Size of tanks (Dec) | Stocking Density/Dec | Feeding Frequency (Times/day) |
|---|---|---|---|---|
| T1 | R1<br>R2<br>R3 | $0.0945m^2$ | 53 | 4 |
| T2 | R1<br>R2<br>R3 | $0.0945m^2$ | 53 | 3 |
| T3 | R1<br>R2<br>R3 | $0.0945m^2$ | 53 | 2 |

### 2. Study of Growth Parameters of Fish

To monitor the growth and health of the adult rose tail Betta fish, periodic sampling was carried out every seven days. Betta fish were carefully scooped up with a scoop net and placed in a water-filled dish. An electric digital balance was used to measure the weight. At the time of the final harvest, the mean weight gain (g), specific growth rate (SGR), feed conversion ratio (FCR), and fish survival were noted. Growth parameters were calculated using the following formula:

Weight gain (g) =Mean final weight (g) – Mean initial weight (g)

Specific growth rate (SGR, %) = 100 × [(ln BW final (g) -ln BW initial (g)) / days of culture]

Production = No. of fishes harvested × average final weight increases of fishes

Survival rate = No. of fish harvested/No. of fish stocked × 100

FCR= (Amount of feed given by dry weight)/ (Final live weight gain by the fish)

### 3. Study of Comparative Bubble Nest Formation

A comparative experimental methodology was employed for analysing bubble nest formation under controlled laboratory conditions. Healthy, sexually mature male individuals of equal size were selected and acclimatised to the environment prior to study. Each individual was placed in a uniform container with consistent water quality, lighting, and volume, while specific variables such as temperature, diet, water depth, and female stimulus were systematically varied between experimental groups and compared to a control group that was kept under optimal conditions. Observations were made at regular intervals to record factors such as the time required to commence nest building, nest size, density, and stability, and measurements were documented using visual evaluation and photographic records. The collected data were analysed using appropriate statistical and graphical methods to compare differences between groups, while ensuring ethical care, minimal stress, and proper maintenance of all specimens throughout the study.

### 4. Sampling and Water Quality Parameters

During the study period, fish were captured with a scoop net, and the weight of each species was then determined using a balance machine. Every seven days interval, the weight gain data were collected. Fish sampling included keeping an eye observation on the physical health of the experimental species. Once a week, the following measurements were recorded: water temperature, oxygen dissolution, pH, and amount of natural food (plankton). During the study period, the experimental water temperature was measured using a mercury Celsius thermometer. The experimental water temperature and pH were recorded once in a week. A digital portable pH meter (Model: HANNA-HI 96107) was employed for this operation. A portable DO meter (Model: Lutron-DO-5509) was utilized to quantify the dissolve oxygen.

### 5. Statistical Analysis

Before performing the univariate analysis of variance (ANOVA), it was first confirmed that the variance of the data met the requirements. When the value was greater than 5% ($p>0.05$), the Shapiro-Wilk test and the Levene test were used to confirm the normality and homogeneity assumptions, respectively. When the assumptions were met, the data were presented to an ANOVA, considering that there was a difference when the value was below 5% ($p<0.05$), and in this instance, they were submitted to the Tukey test for the comparison of means; this was also done using the 5% threshold. Utilizing the statistical program SPSS, every statistical analysis was carried out.

## Results

### 1. Growth Performance of Fish

The mean weight gain, percent weight gain (%), specific growth rate (SGR) per day, survival rate, food conversion ratio (FCR), and production (kg/dec/120 days) were calculated for the evaluation of the proper growth performance of *Betta splendens* fry in various treatments during the experimental period. The results are shown in Table 2.

**Table 2:** Growth Performance of *Betta Splendens*

| Growth Parameters | Treatments | | |
|---|---|---|---|
| | T1 | T2 | T3 |
| Initial weight (g) | $5.00{\pm}0.00^{a}$ | $5.00{\pm}0.00^{a}$ | $5.00{\pm}0.00^{a}$ |
| Final weight (g) | $8.52{\pm}0.45^{a}$ | $7.96{\pm}0.38^{b}$ | $7.21{\pm}0.41^{c}$ |
| Weight gain (g) | $3.52{\pm}0.45^{a}$ | $2.96{\pm}0.38^{b}$ | $2.21{\pm}0.41^{c}$ |
| SGR (%/day) | $1.87{\pm}0.05^{a}$ | $1.65{\pm}0.04^{b}$ | $1.41{\pm}0.06^{c}$ |
| Survival (%) | $96.67{\pm}2.89^{a}$ | $93.33{\pm}3.33^{ab}$ | $90.00{\pm}5.77^{b}$ |
| % Weight gain | $70.40{\pm}9.00^{a}$ | $59.20{\pm}7.60^{b}$ | $44.20{\pm}8.20^{c}$ |
| FCR | $1.02{\pm}0.03^{a}$ | $1.18{\pm}0.04^{b}$ | $1.35{\pm}0.05^{c}$ |

The mean final weight gain of the fry was 8.52±0.45, 7.96±0.38, and 7.21±0.41 g for T1, T2, and T3, respectively, lastly the feeding trial (Table. 1). The growth indices varied significantly ($p<0.05$) between the several treated groups. The best (lowest) FCR was obtained in T1 (1.02±0.03) in the current investigation, where we discovered that the values of FCR were increased in accordance with the rising feeding frequency.

## 2. Weight Gain and Feed Conversion Ratio (FCR)

The initial weight of fish in the various treatments did not differ significantly ($p<0.05$). For T1, T2, and T3, the weight gain of fish obtained as 3.52±0.45, 2.96±0.38, and 2.21±0.41g, respectively (Fig. 2). Feeding frequency of four times were found to have the highest weight gain (3.52±0.45 g), whereas fish with a feeding frequency of two were found to have the lowest weight gain (2.21±0.41 g). The food conversion ratio (FCR) was found to be 1.02±0.03, 1.18±0.04 and 1.35±0.05, correspondingly, at varied frequencies. T1 had the mean least FCR value (1.35±0.05), while T3 had the highest mean FCR value (1.16±0.0113). In compared to T2 and T3, T1 had the least FCR value (1.02±0.03) (Table. 2).

## 3. Weight Gain and Feed Conversion Ratio (FCR)

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## 4. Analysis of Diet Composition

A proximate composition study of the test diet was performed to confirm the formulation's correctness. Table 3 shows the total findings and the protein content of the fried diet, which was 27.92%.

**Table 3:** Proximate composition of feed used in experiment:

| Moisture | 12.16 |
|---|---|
| Protein | 27.92 |
| Crude fibre | 5.25 |
| Lipid | 7.93 |
| Ash | 15.36 |
| Cellulose Nano-particles | 31.38 |

## 5. Percent Weight gain and Specific Growth Rate (SGR%/Day)

The percent weight gain of fish was 278.44±0.62%, 263.38±0.55% and 219.66±0.50% in T1, T2, and T3, respectively. In T1, which had the highest feeding frequency (four times/day), the percent weight growth was considerably ($p<0.05$) highest (278.44±0.62%), while in T3, the least percent weight gain (%) was observed during the study (219.66±0.50%) (Fig. 3). The specific growth rates (SGR) of *Betta splendens* in T1, T2, and T3 were found to be 1.87±0.05, 1.65±0.06 and 1.41±0.04, respectively. There were variations between the various treatments that were significant ($p<0.05$). The SGR was gradually ameliorated as feeding frequency increased. The lowest specific growth rate (1.41±0.04) and the considerably highest specific growth rate (1.65±0.06) were found in T3 and T1, respectively (Fig. 4).

## 6. Water Quality Parameters (Temperature, pH, DO)

The average water temperature for treatments T1, T2, and T3 was 29.36±1.24, 29.31±1.22 and 29.25±1.19°C, respectively. The average dissolved oxygen level of the water in treatments T1, T2, and T3 was 5.12±0.51, 5.14±0.59 and 5.24±0.39 mg/l respectively (Table 4). The mean pH values of the water in tests T1, T2, and T3 were, respectively, 7.28±0.34, 7.76±0.31 and 7.61±0.19. Under various treatments during the study period, there were no significant differences in water temperature, DO, or pH values.

**Table 4:** Water Quality Parameters

| Treatments | Parameters | | |
|---|---|---|---|
| | Temperature (°C) | Dissolved Oxygen (mg/l) | pH |
| T1 | 29.36±1.24 | 5.12±0.51 | 7.28±0.34 |
| T2 | 29.31±1.22 | 5.14±0.59 | 7.76±0.31 |
| T3 | 29.25±1.19 | 5.24±0.39 | 7.61±0.19 |

## 7. Bubble nest Production (Count/Dec/120 Days)

The calculation of the bubble nest production revealed that it varied depending on the treatment. The Bubble nest production varied significantly among treatments during the experimental period. The highest number of bubbles per nest was recorded in T1 (842.67 ± 15.28), followed by T2 (768.33 ± 14.43), while the lowest production was observed in T3 (612.33 ± 15.61) (Mean ± SD,). The significantly ($p<0.05$) highest bubble nest production was determined to be 842.67±15.28 count/Dec/30 days in T1 under four times feeding frequencies, and production was shown to considerably dwindle with the decrease in feeding frequencies. T3 had the lowest fish production (612.33±15.61 count/Dec/120 days). The findings show a clear treatment-dependent increase in reproductive behavioural output, with T1 outperforming the other groups in nest-building. The steady drop in bubble nest production from T1 to T3 indicates that experimental settings had a direct impact on male courtship behaviour and nest construction efficiency.

**Table 5:** Bubble Nest production count

| Treatment | Replicate 1 | Replicate 2 | Replicate 3 | Mean±SD | Significance |
|---|---|---|---|---|---|
| T1 | 860 | 830 | 838 | 842.67±15.28[a] | P<0.05 |
| T2 | 785 | 760 | 760 | 768.33±14.43[b] | P<0.05 |
| T3 | 630 | 600 | 607 | 612.33±15.61[c] | P<0.05 |

## Discussion

### 1. Weight Performance

In all three treatments, the initial weight of the Betta Fish was 5.0 g on average. *Betta splendens* had mean weights of (3.52±0.45) g, (2.96±0.38) g, and (2.21±0.41) g in T1, T2, and T3, respectively. At the end of the experiment, T1 had the highest mean weight gain of Betta splendens at final harvest (3.52±0.45 g), followed by T2 (2.96±0.38) g, and (2.21±0.41) g. In T1, when meal was provided four times/day, the *Betta splendens* gained the highest weight. By feeding *Betta splendens* a prepared diet made from locally accessible ingredients and utilized feed daily at a rate that dropped with fish growth from 15% to 3% of body weight, Binh *et al.* [11] conducted an experiment and found comparable results. Betta fish in on-farm ponds for a culture period of 6 months were fed rice bran at 5-6% of their body weight, according to Hussain *et al.* [12] who reported a weight gain of about 284.55±0.86 g. *Betta splendens* in the present study appeared better in terms of weight gain when comparing the 120-day culture period to the 6-months period used by Hussain *et al.* [13].

**2. Survival Rate (%)**

Bettta splendens had survival rates of 95.65±0.367, 93.00±0.709, and 90.50±0.707% in T1, T2, and T3, respectively. Survivability was best in treatment T1, and lowest in T3. Survivability varied little depending on the setting and feeding frequency. T1 outperformed the other treatments (T2 and T3) in terms of feed utilisation and survivability.

A more or less similar survival rate was observed by Rahman [14] who recorded survival rate ranged from 94 to 95%.

**3. Bubble nest Production (Count/Dec/120 Days)**

The study found that T1 produced the most bubbles per nest (842.67 ± 15.28), followed by T2 (768.33 ± 14.43), and T3 produced the least (612.33 ± 15.61). The maximum bubble nest formation rate ($p<0.05$) was 842.67±15.28 count/Dec/30day. The findings show a clear treatment-dependent increase in reproductive behavioural output, with T1 outperforming the other groups in nest-building. The consistent decline in bubble nest production from T1 to T3 suggests that experimental conditions had a direct effect on male courting behaviour and nest construction efficiency Sriwattanarothai [15], Tan, HH [16], and Tan, HH [17]

**4. Food Conversion Ratio (FCR)**

In the current study, the Food Conversion Ratio (FCR) values for *Betta splendens* fed commercial feed were 1.02±0.03, 1.18±0.04, and 1.35±0.05, respectively, for T1, T2, and T3. The FCR was lower in T1 (1.02±0.03). Rahman [14] calculated the Food Conversion Ratio (FCR) for fish treatments fed commercial and homemade feed to be 1.31 and 1.40, respectively and discovered that the FCR value of a designed diet was 1.51-1.62, which was only slightly different from the current study. This variation could be due to the frequency of eating.

**5. Water Quality Parameters**

Temperature has a substantial impact on fish growth, survival, and other activities. According to Clarke [18], organisms' metabolic rates increase as the temperature rises. Clarke [19] revealed that perciform fish do not have a cold adaption. As a result, temperature has a substantial effect on overall fish productivity. Dissolved oxygen in water is an important factor in fish culture. The dissolved oxygen concentration in the study ranged between 5.12 and 5.24 mg/l. According to DoF [20], a DO level of 5.0 to 8.0 ppm is adequate for fish production. According to Mollah and Haque [21], the average DO level ranges between 1.19 and 7.74 mg/l. The current study's findings are comparable to his.

The pH of the water used in this experiment ranged from 7.28 to 7.76, which is within the allowed range. DoF recommends a pH range of 6.5 to 8.5 [20]. According to Boyd [22], the pH level in the current investigation is ideal. Boyd also stated that a pH range of 6.5 to 8.0 is appropriate for growing. However, according to Mollah and Haque [21], fish culture thrives in pH ranges of 5.66 to 7.66. The pH level in the current study is slightly higher than that of Mollah and Haque [21]. This is due to a combination of environmental conditions, geographical position, and other operational concerns. Cruz & Laudencia [23] discovered that the fish gained an average of 3.42% of their body weight in 24 hours at 31.4°C, the temperature at which they grew the fastest. The optimal feeding temperature was determined to be between 28.8 and 31.4°C. The present study's temperature range is comparable to this one. Ridha [3] found that Oreochromis niloticus thrives ($p<0.05$) at 29.01°C water temperature [24, 25].

## Conclusion

In this experiment, the average final weight of the *Betta splendens* was 8.52±0.45 g for T1, 7.96±0.38g for T2, and 7.21±0.41 g for T3. The average weight gain for T1, T2, and T3 was 3.52±0.45 g, 2.96±0.38 g, and 2.21±0.41 g, respectively. The average percent (%) weight gain was 70.40±9.00, 59.20±7.60 and 44.20±8.20 %; the average SGR for T1, T2 and T3 was 1.87±0.05, 1.65±0.04 and 1.41±0.06 %, respectively. The fact that T1 had observed the highest weight gain, percent weight gain (%) and SGR which could be attributed to the effect of having four feeding frequencies per day. The survival rates in T1, T2, and T3 were 96.67±2.89, 93.33±3.33, and 90.00±5.77 %, respectively. For T1, T2, and T3, the FCR values were 1.02±0.03, 1.18±0.04, and 1.35±0.05, respectively. The T1 experiment, in which Rose tail male *Betta splendens* were fed four times as frequently, produced the best FCR value. The Bubble nest production in T1 was 842.67±15.28 count/dec/120 days, which was substantially ($p<0.05$) higher than T2, and T3. The *Betta splendens* produced well in terms of growth and output, it can be inferred that feeding frequency was a significant factor.

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## Competing Interests

Authors have declared that no competing interests exist.

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