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Original Article

Growth and survival of *Betta splendens* fed microbial aggregates from *ex-situ* biofloc technology (BFT)

Crescimento e sobrevivência de *Betta splendens* alimentados com agregados microbianos provenientes da tecnologia de bioflocos *ex-situ* (BFT)

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Abstract

The betta (*Betta splendens*) is a carnivorous ornamental species that requires live food. In this study, we evaluated the growth and survival of *Betta splendens* fingerlings fed *ex-situ* biofloc (BFT). A total of 375 fingerlings (8.3 ± 4.1 mg and 0.8 ± 0.14 cm) were distributed into 15-L plastic tanks (n=25), with five replicates for each of the following treatments: (i) exclusive live food (LF100), (ii) exclusive biofloc (BFT100), (iii) live food supplemented with biofloc (LF100-BFT100), and a reduction of 15% (iv) and 30% (v) in live food with biofloc supplementation (LF85-BFT100 and LF70-BFT100, respectively). The fish were fed newly hatched brine shrimp (*Artemia* spp. nauplii) and/or fresh biofloc, twice daily, over a 16-day experimental period. Dietary supplementation of *Beta splendens* with *ex-situ* BFT (LF100-BFT100, LF85-BFT100, and LF70-BFT100) resulted in a similar final weight (P>0.05) compared to exclusive brine shrimp (LF100). Regarding total length, the fish in the LF100-BFT100 group were superior to those in the LF100 group (P<0.05). The LF100 and LF100-BFT100 groups showed survival rates of 100% and 98.4%, respectively, which were not statistically different (P>0.05). For all analyzed variables, fish exclusively fed biofloc (BFT100) exhibited unfavorable performance. These results indicate that dietary *ex-situ* biofloc supplementation, without reducing the supply of live food, can be an interesting alternative in the rearing of *Betta splendens* since biofloc improves growth performance and maintains a good survival rate.

Keywords: ornamental fish, alternative foods, *Artemia* spp. nauplii, macroaggregates.

Resumo

O beta (*Betta splendens*) é uma espécie ornamental carnívora que necessita de alimentos vivos. Neste estudo, avaliamos o crescimento e a sobrevivência de alevinos de beta alimentados com bioflocos produzidos *ex-situ* (BFT). Um total de 375 alevinos (8,3 ± 4,1 mg e 0,8 ± 0,14 cm) foi distribuído em tanques plásticos de 15 L (n=25), com cinco repetições para cada um dos seguintes tratamentos: (i) alimentação viva exclusiva (LF100), (ii) biofloco exclusivo (BFT100), (iii) alimentação viva suplementada com biofloco (LF100-BFT100), e uma redução de 15% (iv) e 30% (v) na alimentação viva com suplementação de biofloco (LF85-BFT100 e LF70-BFT100, respectivamente). Os peixes foram alimentados duas vezes por dia com artemia recém-eclodida (*Artemia* spp. nauplii) e/ou bioflocos frescos durante um período experimental de 16 dias. A suplementação de BFT (LF100-BFT100, LF85-BFT100 e LF70-BFT100) na dieta de *Beta splendens* resultou em peso final semelhante à dieta contendo exclusivamente artemia (LF100). Em relação ao comprimento total, os peixes do grupo LF100-BFT100 foram superiores aos do grupo LF100 (P<0,05). Os grupos LF100 e LF100-BFT100 apresentaram taxas de sobrevivência de 100% e 98,4%, respectivamente, as quais não diferiram estatisticamente entre si (P>0,05). Para todas as variáveis analisadas, os peixes alimentados exclusivamente com bioflocos (BFT100) apresentaram desempenho desfavorável. Esses resultados indicam que a suplementação dietética com bioflocos *ex-situ*, sem redução na oferta de alimento vivo, pode ser uma alternativa interessante no cultivo de *Betta splendens*, uma vez que o bioflocos melhora o desempenho em crescimento e mantém boa taxa de sobrevivência.

Palavras-chave: peixe ornamental, alimentação alternativa, náuplios de *Artemia* spp, macroagregados.

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1. Introduction

The Siamese fighting fish, also known as betta (*Betta splendens*) is an ornamental carnivorous species native to Southeast Asia. It inhabits regions characterized by low water columns, such as swamps, ponds, and rice fields. It tolerates a wide range of water temperatures (Faria et al., 2006). As a member of the suborder Anabantoidei, bettas are air-breathing fish that have adapted to survive in oxygen-deprived waters (Apriliani et al., 2019).

Live food plays a crucial role in promoting the health and development of bettas, particularly during their first three months of life. Post-larvae and fry can be nourished with various forms of zooplankton, including *Daphnia* spp., *Artemia* spp., and *Moina* spp. (Daugs, 1967; Monvises et al., 2009). In practice, ornamental fish farmers often employ "green water" (i.e., fertilized water) to provide live feed like zooplankton to larvae and fingerlings, reducing their reliance on artificial feed (Zuanon et al., 2011). Additionally, brine shrimp (*Artemia* spp.) is commonly incorporated into betta diets due to its rich protein content and because it allows for the inclusion of essential macro and micronutrients through an enrichment process (Radhakrishnan et al., 2020). Nevertheless, the high market prices of brine shrimp cysts, which can account for 50% or more of total fish farming expenses, are primarily attributed to the costly protein sources used in their production (Daniel, 2017; Faizullah et al., 2019).

One potential substitute or supplementary source of nutrition for bettas is microbial aggregates derived from biofloc production. Biofloc technology (BFT) represents a sustainable form of aquaculture that involves minimal or zero water exchange since nitrogen compounds are recycled into microbial protein (Emerenciano et al., 2013). Biofloc is composed of bacteria, zooplankton, microalgae, organic matter, and other components that can serve as food for other aquatic organisms, such as fish and shrimp (Faizullah et al., 2019). Biofloc can be introduced to fish in three different ways: *in-situ*, in which fish consume the floc freely in the cultivation tank (Diatin et al., 2019); *ex-situ*, in which the harvested aggregates from a BFT cultivation tank are offered to fish as fresh food (Martínez-Córdova et al., 2017); or by incorporation into aquafeeds as a feed ingredient, e.g., biofloc meal (Bauer et al., 2012). Considering that bettas typically inhabit still waters and that BFT systems require constant aeration and circulation of the water to avoid particle sedimentation and anoxic zones (Emerenciano et al., 2017), rearing bettas in this environment may not be ideal. To address this issue, biofloc can be utilized as an additional fresh food source in the diet of bettas through *ex-situ* BFT methods. *Betta splendens* requires a diet containing 35% protein for optimal growth and reproductive performance (James and Sampath, 2003), while BFT can contain up to 49% protein in its composition (Khanjani and Sharifinia, 2020).

Furthermore, ornamental fish production is characterized by higher unit costs compared to fish production for human consumption (Satam et al., 2018). Thus, efficient control of mortality throughout the production cycle is essential to increase profitability. Creating an optimal environment for the species and providing cost-effective nutrition are key factors in achieving better growth and survival rates (Pleeging and Moons, 2017). Therefore, the present study aims to assess the growth and survival of *Betta splendens* fingerlings fed *ex-situ* biofloc.

2. Material and Methods

2.1. Location and fish acquisition

The experiment was carried out at the Experimental Fish Farming Station of the Federal University of Mato Grosso do Sul (UFMS), Campo Grande, Mato Grosso do Sul, Brazil. This research study was approved by the Ethics and Animal Welfare Committee at UFMS (approval nº 1.164/2021).

To obtain larvae for the experiment, two pairs consisting of two male and two female *Betta splendens* (betta fish) were bred. The adult bettas were procured commercially and housed in the laboratory, each placed in individual 6-L aquaria filled with clean freshwater. The aquariums underwent regular maintenance, which included siphoning and 80% of water exchange every two days, using dechlorinated tap water, until the time of breeding.

For the breeding process, two 10-L glass aquaria, each with a useful volume of 5 L, were employed. A piece of foam measuring 8 × 15 × 1 cm was placed in each breeding aquarium to facilitate the construction of bubble nests by the male bettas. After 48 h, the bubble nests were already established beneath the foam pieces, and one female betta was introduced into each breeding aquarium to initiate courtship and the nuptial embrace. Courtship activities were closely monitored, and as soon as the male betta concluded the courtship, the female was promptly removed from the aquarium.

Following the birth of all the larvae, they remained in the breeding aquariums for three days after hatching to receive parental care. Subsequently, the larvae were randomly counted and equally distributed into four 30-L plastic tanks for their maintenance until the start of the experiment. During this phase, the fingerlings were fed *Artemia* nauplii three times a day to apparent satiety. To maintain water quality, 50% of the water volume was replaced with clean freshwater, and waste was siphoned every three days to prevent the accumulation of nitrogen compounds.

2.2. Experimental design

A total of 375 betta fingerlings, aged 13 days, with an average weight of 8.3 ± 4.1 mg and a mean total length of 0.8 ± 0.14 cm (mean ± standard deviation), were randomly distributed into five experimental units, each with a usable volume of 15 L, for each treatment (n=5), resulting in a total of 25 experimental units (with partial water changes). The treatments consisted of five distinct experimental diets: LF100 (100% brine shrimp, referred to as the 'positive control'), BFT100 (100% BFT, considered the 'negative control'), LF100-BFT100 (100% brine shrimp + 100% BFT), LF85-BFT100 (85% brine shrimp + 100% BFT), and LF70-BFT100 (70% brine shrimp + 100% BFT).

Throughout the 16-day experimental period, the fish were fed twice daily, at 08h00 and 16h00, following the

recommendations outlined by Santos et al. (2014). From the ninth day until the 16th day, the quantities of the experimental diets were increased by 25% to ensure that all fish remained satiated as they grew, based on daily intake observations (Table 1). This feeding methodology was adapted from Mandal et al. (2010). The photoperiod was maintained at 12 h of light and 12 h of darkness during the experiment, following the protocol described by Sales et al. (2016).

At the end of the experimental period, 15 betta fingerlings were selected from each of the 25 experimental units to determine their final weight (g) and length (cm). Additionally, the survival rate (%) was determined by calculating the difference between the initial and final numbers of fish at the end of the experimental period.

2.3. Water quality monitoring

Daily, prior to the morning feeding, all 25 experimental units underwent siphoning to remove waste. The waste from each tank was siphoned into a bucket containing acrylic wool, effectively removing all fish residues and functioning as a mechanical filter. The filtered water was then returned to the respective unit. Subsequently, the water pump was operated for 10 min to activate the circulating system, allowing any remaining waste from the experimental units to be captured by a mop of acrylic wool placed at the end of the draining pipe (mechanical filter). An additional 10-min operation of the water pump occurred one hour before the afternoon feeding, ensuring the units were clean before introducing fresh food to the fish. To maintain optimal water quality for the betta fingerlings, half of the total volume of the experimental units was replaced with freshwater every four days, following the methodology outlined by Emerenciano et al. (2007, 2011).

The temperature (°C), dissolved oxygen (mg L^{-1}), and pH were evaluated daily in each experimental unit using a multi-parameter instrument (YSI Professional Plus, Yellow Springs, USA). Total ammonia nitrogen (TAN, mg L^{-1}), ammonia (mg L^{-1}), nitrite (mg L^{-1}), and alkalinity (mg $CaCO_3$ L^{-1}) were measured three times a week using colorimetric test kits (Labcon Test - Alcon®, Camburiú, Santa Catarina, Brazil). Table 2 describes the water quality parameters.

2.4. Acquisition and characterization of BFT and Artemia spp. nauplii

The biofloc utilized in the experiment was generated through the cultivation of male Nile tilapia (*Oreochromis niloticus*) at a stocking density of 10 kg m^{-3}. Six weeks before the introduction of bettas into the experimental units, these tilapia were placed in two 500-L plastic tanks (B1 and B2), each with a useful volume of 410 L and a salinity level of 3‰. They were continuously maintained in these tanks throughout the experiment to serve as a constant source of *ex-situ* biofloc for the betta experiment. The tilapia were fed twice daily, with their feed intake being carefully recorded. To control the C:N ratio (20:1), molasses was added daily until the biofloc in both tanks reached full maturation (Emerenciano et al., 2017). The average water quality parameters in both tanks (B1 and B2) remained within the appropriate range for BFT (T °C: 27.7; DO: 6.5 mg L^{-1}; pH: 7.5; NO_2: 0.25 mg L^{-1}; total TAN: 0.4 mg L^{-1}; settling solids (SS): 24.7 mL L^{-1}; and alkalinity: 78.5 mg $CaCO_3$ L^{-1}). Daily measurements of settling solids (SS) were obtained from both BFT units using 1-L water samples allowed to settle for 30 min in Imhoff cones.

Table 1. Composition of the treatments (experimental diets) for *Betta splendens*.

Treatment*	Days 1-8	Days 9-16
LF100	60 nauplii	75 nauplii
BFT100	10 mL BFT	12.5 mL BFT
LF100-BFT100	60 nauplii + 10 mL BFT	75 nauplii + 12.5 mL BFT
LF85-BFT100	51 nauplii + 10 mL BFT	64 nauplii + 12.5 mL BFT
LF70-BFT100	42 nauplii + 10 mL BFT	53 nauplii + 12.5 mL BFT

*LF100: 100% brine shrimp; BFT100: 100% BFT; LF100-BFT100: 100% brine shrimp + 100% BFT; LF85-BFT100: 85% brine shrimp + 100% BFT; and LF70-BFT100: 70% brine shrimp + 100% BFT. The percentages in each experimental diet refers to the maximum values established in specific period (1st to 8th and 9th to 16th experimental days).

Table 2. Means ± Standard deviation of water quality parameters in the experimental units over 16 days of experiment.

Treat[1]	Parameter[2]						
	Temp (°C)	DO (mg L^{-1})	pH	TAN (mg L^{-1})	Amm (mg L^{-1})	Nitrite (mg L^{-1})	Alka (mg $CaCO_3$ L^{-1})
LF100	27.8 ± 0.5	4.39 ± 0.44	7.05 ± 0.08	0	0	0	28.13 ± 9.57
BFT100	27.8 ± 0.47	4.31 ± 0.33	7.09 ± 0.12	0	0	0.11 ± 0.13	28.13 ± 9.57
LF100-BFT100	27.9 ± 0.44	4.14 ± 0.33	7.07 ± 0.11	0	0	0.25 ± 0.14	28.13 ± 9.57
LF85-BFT100	27.9 ± 0.49	4.12 ± 0.43	7.10 ± 0.12	0	0	0.07 ± 0.12	30.69 ± 8.73
LF70-BFT100	27.8 ± 0.5	4.10 ± 0.40	7.09 ± 0.12	0	0	0.07 ± 0.12	28.13 ± 9.57

[1]Treatment: LF100: 100% brine shrimp; BFT100: 100% BFT; LF100-BFT100: 100% brine shrimp + 100% BFT; LF85-BFT100: 85% brine shrimp + 100% BFT; and LF70-BFT100: 70% brine shrimp + 100% BFT; [2]Temp: Temperature; DO: Dissolved oxygen; TAN: Total ammonia nitrogen; Amm: Ammonia; Alka: Alkalinity.

To feed the bettas, 6 L of water from each BFT unit were combined, and the microbial aggregates were separated by decanting them into 2-L conical plastic bottles before transferring them to a beaker. Observations conducted prior to the experiment determined that 10 mL was an appropriate portion size, providing sufficient quantities to be consumed without excess or limitation. This observation aided in estimating the daily doses of biofloc (measured using Imhoff cones) for each experimental unit. There was a 25% increase in the volume of biofloc offered to the betta fingerlings from the ninth day until the 16th day, mirroring the increase in supply of brine shrimp nauplii, as described in Table 1. A schematic representation of the formation and use of BFT in this experiment is depicted in Figure 1.

Brine shrimp nauplii (*Artemia Salina* RN®) were hatched daily in a brine shrimp incubator with a volume of 380 mL. The incubator contained saltwater with a salinity level of 30‰, 1 g of *Artemia* spp. cysts, and a constant supply of aeration, along with a 24/0-h photoperiod. The hatched nauplii were collected and transferred to a beaker, and then a 100 µL aliquot was placed on a slide under a magnifying glass for counting. This counting process was repeated in triplicate after each new hatch, with the average of the three counts used to predict the number of nauplii mL^{-1}. Following the decanting of the BFT and the counting of the nauplii, specific volumes of both were sampled to be offered to each treatment.

At the end of the experiment, 50 L of water from both BFT tanks were mixed, decanted, filtered using a paper filter, and then stored at -20 °C. *Artemia* spp. nauplii were hatched in saltwater with a salinity level of 30% in 2-L conical plastic bottles, equipped with constant aeration, for 24 h. They were subsequently netted and also stored at -20 °C. The proximate composition of both the BFT and brine shrimp was analyzed in accordance with Association of Official Analytical Chemists (George Junior, 2016) standards at the Laboratory of Applied Nutrition, Faculty of Veterinary and Animal Science, Federal University of Mato Grosso do Sul, Brazil. The samples were dried in a forced-air oven (55 °C) until constant weight. Duplicate analyses were conducted for ash content (muffle furnace at 600 °C), crude protein (Kjeldahl method), and crude lipid content (ANKOM XT15 Lipid Extractor, ANKOM Tecnology, Inc., Macedon, NY, USA). Table 3 shows the results of these analyses.

2.5. Statistical analysis

All dependent variables underwent the Shapiro-Wilk and Levene tests to evaluate the normality and homogeneity of variances assumptions, respectively. Since these assumptions were met in all cases, the variables were analyzed using a model with one independent variable (ANOVA - One-way), followed by Tukey's multiple comparison test. The angular transformation was used for the statistical analysis of the 'survival rate' variable. All statistical analyses were conducted following the recommendations of Zar (2010) and employed the Statistical Analysis System (SAS 2002). A significance level of 0.05 was applied to all tests.

3. Results

Table 4 displays the comprehensive results of the growth performance and survival rate of *Betta splendens* fed exclusive brine shrimp, exclusive *ex-situ* biofloc, and brine shrimp supplemented with *ex-situ* biofloc.

Regarding final weight, the groups supplemented with biofloc (LF100-BFT100, LF85-BFT100, and LF70-BFT100) showed similar results to the group fed only live food

Table 3. Proximate composition (%) of the microbial aggregates (BFT) and *Artemia* spp. nauplii.

Variable	BFT	*Artemia* spp.
Moisture	99.51	98.93
Ash	18.86	8.20
Crude protein	41.94	54.69
Crude lipid	0.22	15.38

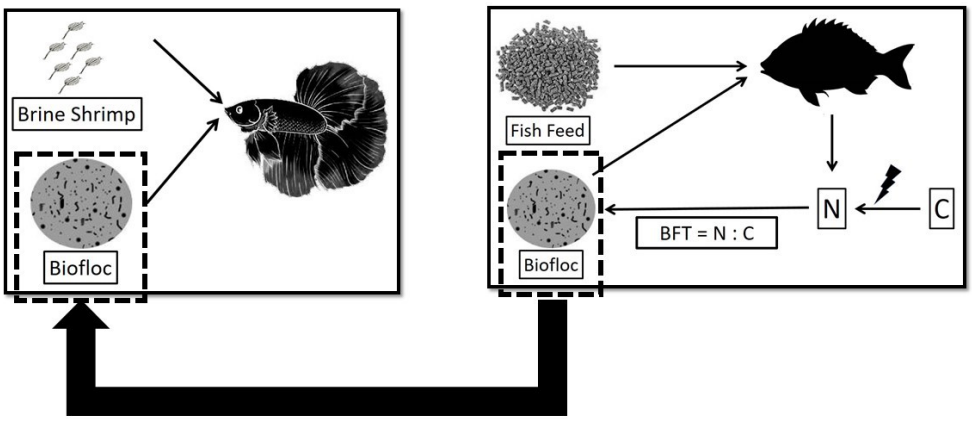

Figure 1. Schematic representation of formation and use of biofloc to feed *Betta splendens*. The macroagregates were produced in 500-L tanks with tilapia (*Oreochromis niloticus*) fed commercial fish feed. A source of carbon (molasses) was used in the tank to provide energy for bacteria and other microorganisms present in the water. After complete formation, the aggregates were harvested and offered *as is* for the betta fish in the experimental units.

Table 4. Mean final weight, total length, and survival of *Betta splendens* fed live food only (LF), biofloc only (BFT), and live food supplemented with biofloc (LF-BFT) during 16 days.

Treatment[1]	Final weight (mg)	Total length (cm)	Survival (%)[2]
LF100	16.820 ab	1.064 bc	100 a
BFT100	10.980 c	1.036 c	30.05 c
LF100-BFT100	18.000 ab	1.150 a	98.4 ab
LF85-BFT100	18.720 a	1.116 ab	91.17 b
LF70-BFT100	16.440 b	1.104 abc	89.85 b
CV[3] (%)	7.080	3.820	9.350
P-value[4]	<.0001	0.003	<.0001

[1]LF100: 100% brine shrimp; BFT100: 100% BFT; LF100-BFT100: 100% brine shrimp + 100% BFT; LF85-BFT100: 85% brine shrimp + 100% BFT; and LF70-BFT100: 70% brine shrimp + 100% BFT; [2]Survival rate presented as % after reverse transformation; [3]CV: Coefficient of variation; [4]*P-value* of ANOVA. Mean values followed by the same letter in the row do not differ statistically by Tukey's Test at the 5% significance level. At the beginning of the experiment, *Betta splendens* had an average weight of 8.3 ± 4.1 mg and an average total length of 0.8 ± 0.14 cm.

(LF100) (P<0.05), while dietary BFT supplemented with reduction of 15% of live food (LF85-BFT100) resulted in greater final weight (P<0.05) than the 30% of live food reduction (LF70-BFT100). On the other hand, the BFT treatment showed the lowest final weight among all treatments (P<0.05). The length of the fish in the LF100-BFT100 group was greater (P<0.05) compared to those of the LF100 and BFT100 treatments, but did not differ significantly (P>0.05) from those of the LF-BFT85 and LF-BFT70 groups. Survival rate was higher (P<0.05) in the LF100 group (100%) compared to LF-BFT85 (91.17%) and LF-BFT70 (89.85%), but did not differ (P>0.05) from the LF100-BFT100 group (98.4%). The lowest survival rate (30.05%) was observed in the BFT group.

4. Discussion

Bettas are carnivorous fish. Despite being able to adapt to extruded feeds from an early age, live food is essential during the fry stage for the development of the digestive system, stimulating the capture of inert food during co-feeding period (Fosse et al., 2013), as well as the development of hunting skills (Zuanon et al., 2011). The absence of live food during the fry stage is related to impaired growth and reduced survival in several fish species, including betta (Fosse et al., 2013). Accordingly, *Betta splendens* fed solely biofloc exhibited the least favorable outcomes for all evaluated parameters, even though biofloc contains 41.94% crude protein level, which is greater than the 35% required for optimal growth and improved reproduction parameters in the species (James and Sampath, 2003).

This can be attributed to the fact that biofloc alone may not meet all the nutritional requirements due to large variations in its nutritional profile, influenced by factors such as light exposure, water salinity, carbon and protein sources introduced into the water, and the established microbial community (Emerenciano et al., 2015). Another important factor is bettas' predatory nature, driven by movement stimuli, often capturing their prey from beneath or while the food is sinking (Pleeging and Moons, 2017). During our experiment, after supplying *ex-situ* biofloc, the particles slowly sunk to the bottom of the tanks, and the bettas' response to this food was not immediate. After a while, the fish slowly "grazed" the bottom, capturing the aggregates of interest. Grazing is one of the feeding behaviors exhibited by bettas, in addition to searching and capturing prey/food (Cardoso et al., 2020). Therefore, the inferior productive performance of the betta fish fed *ex-situ* biofloc exclusively may be explained by the fact that the exclusive biofloc diet does not meet their nutritional requirements or stimulate consumption, thereby not promoting gastrointestinal development.

On the other hand, reducing brine shrimp nauplii by 15 or 30% did not significantly affect the weight of the bettas when they also received *ex-situ* BFT as a food source, whereas the diet without reduction in live food associated with biofloc supplementation improved the growth of the fish. Similarly, goldfish (*Carassius auratus*) also showed enhanced growth with only 10% dietary biofloc supplementation (Wang et al., 2015). Although the biofloc aggregate was offered fresh to the bettas, a study involving dried *ex-situ* BFT with a 5% reduction in commercial feed reported comparable growth in Pacific white shrimp *Litopenaeus vannamei* (Uawisetwathana et al., 2021). These results evidence that dietary *ex-situ* biofloc supplementation can be an interesting nutritional strategy to maintain or improve the growth parameters of *Betta splendens* compared to a diet containing exclusively live food.

Although biofloc technology has been extensively studied for tilapia (Monroy-Dosta et al., 2013; Brol et al., 2017; Laice et al., 2021) and penaeid shrimp (Ray and Lotz, 2012; Emerenciano et al., 2015; Martins et al., 2020) in the last decade, very little information exists on the potential benefits of BFT for ornamental fish. Apart from its high protein concentrations (Khanjani and Sharifinia, 2020) and intermediate levels of long-chain polyunsaturated fatty acids (Emerenciano et al., 2013), biofloc can contain a diverse range of microorganisms that can serve as live prey, probiotics, or even immunostimulants (Ahmad et al., 2017), all of which are beneficial for fish nutrition and health (Durigon et al., 2019). Therefore, the improvement

observed in the growth of *Betta splendens* when fed a maximum amount of live food supplemented with biofloc compared with live food exclusively may be related to the role of biofloc compounds in stimulating growth when nutritional requirements are met.

In this context, some studies have demonstrated improved growth parameters (Faizullah et al., 2015, 2018; Castro et al., 2016) and skin pigmentation (da Cunha et al., 2020) in *Carassius auratus* reared using biofloc technology. Honorato et al. (2021) also reported higher growth performance in a biofloc system compared to a clear water system for *Geophagus brasiliensis* juveniles (4.76 ± 1.67 g) over 40 days. Other studies have shown satisfactory growth results for *Corydoras aeneus* (Diatin et al., 2019) and *Cyprinus carpio* (Najdegerami et al., 2016; Castro-Mejía et al., 2018) in BFT culture tanks. Nevertheless, the information regarding the effects of the biofloc on performance of several ornamental species, including bettas is scarce, which makes this study necessary and innovative.

In addition to improving the *Betta splendens*' growth, dietary *ex-situ* biofloc supplementation without reducing the supply of live food maintained their survival rate comparable to that of the live-food group (98.4% and 100%, respectively), which may be related to the improved health and development of the betta fingerlings provided by biofloc. In commercial operations, higher survival rates translate into increased profitability per unit sold (Satam et al., 2018). Mandal et al. (2010) achieved a 93% survival rate for betta fry (0.19 ± 0.01 g) by replacing 75% of live food (tubifex) with a formulated diet over a 105-day period. Similarly, a survival rate of 91.8% was reported for *Carassius auratus* fingerlings (1.48 ± 0.08 g) reared in biofloc supplemented with commercial diets containing 32% crude protein (Faizullah et al., 2015). Fosse et al. (2013) emphasized that offering live food for an extended period results in higher survival rates, even when supplementing with formulated feed.

In conclusion, our study indicates that dietary *ex-situ* biofloc supplementation without reducing the supply of live food can be an interesting alternative for rearing *Betta splendens* since biofloc improves growth performance and maintains a good survival rate.

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