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Application of biofloc technology in the larval rearing of zebrafish (*Danio rerio*)

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ABSTRACT

The aims of this study were to investigate the different strategies of biofloc addition to zebrafish (*Danio rerio*) larviculture and to evaluate their growth and biochemical parameters. Three treatments were used: addition of 200 mL biofloc once at the start of the assay (O1), addition of 100 mL biofloc every seven days (1W), and addition of 100 mL biofloc every four days (2W). The 1W and 2W treatments also received 200 mL biofloc at the start of the assay. Regarding water quality, the only difference was the total suspended solid concentration, because the 2W treatment had a higher concentration in the final assay (127.6 ± 24.3 mg·L^{-1}). The final weight, survival rate, and juvenile/larval percentile did not show statistical differences among the treatments. However, the O1 treatment exhibited a higher total length (11.93 ± 0.45 mm) than those in the 2W treatment. The juveniles in the 2W treatment exhibited lower nonprotein thiols and higher thiobarbituric acid reactive substance concentrations than those in the other treatments. Thus, the biofloc system can be a viable alternative to zebrafish larviculture without the use of conventional live food, and the addition of biofloc once (O1) at the beginning of larval rearing achieves good growth and survival results.

Keywords: Ammonia; Live food; Water reuse; Nutrient.

Aplicação da tecnologia de bioflocos na larvicultura do zebrafish (*Danio rerio*)

RESUMO

O objetivo deste estudo foi investigar diferentes estratégias de adição de biofloco na larvicultura de *zebrafish* (*Danio rerio*), avaliando o crescimento e os parâmetros bioquímicos. Três tratamentos foram utilizados: adição de 200 mL de biofloco uma vez no início do ensaio (O1), adição de 100 mL de biofloco a cada sete dias (1W) e adição de 100 mL de biofloco a cada quatro dias (2W). Os tratamentos 1W e 2W também receberam 200 mL de biofloco no início do ensaio. Em relação à qualidade da água, a diferença ocorreu na concentração de sólidos suspensos totais, pois o tratamento 2W teve maior concentração no fim do experimento (127,6 ± 24,3 mg·L^{-1}). Ao final do ensaio, o peso final, a sobrevivência e a porcentagem de juvenis e larvas não apresentaram diferença entre os tratamentos. Entretanto, no tratamento O1, os peixes apresentaram maior comprimento total (11,93 ± 0,45 mm) do que no tratamento 2W. Os juvenis possuíram menor concentração de tiois não proteicos e maior substâncias reativas ao ácido tiobarbitúrico no tratamento 2W. Assim, o sistema de bioflocos pode ser uma alternativa à larvicultura de *zebrafish* sem utilizar alimento vivo, e a adição de bioflocos uma vez (O1) no início da larvicultura proporciona bons resultados de crescimento e sobrevivência.

Palavras-chave: Amônia; Alimento vivo; Reaproveitamento de água; Nutriente.

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INTRODUCTION

Over the last two decades, zebrafish (*Danio rerio*) has been widely used as an experimental model in several areas, including neuroscience, toxicology, behavior genetics, developmental biology, metabolism, human diseases, drug discovery, and aquaculture (Farias and Certal, 2016; Teame et al., 2019). The increased interest in zebrafish research has led to its exponential production worldwide. However, studies on novel methods for husbandry and larval rearing that would optimize the intensive production of zebrafish for research with adequate standardization and fish welfare are scarce (Farias and Certal, 2016).

In altricial species, such as zebrafish, the larvae have a rudimentary digestive system at first feeding, which makes the use of formulated diets difficult (Conceição et al., 2010). Several types of formulated diets have been tested for zebrafish larviculture, but the growth and survival of zebrafish larvae are reduced when compared to those fed live feeds (Carvalho et al., 2006; Farias and Certal, 2016). Thus, the protocols for zebrafish larviculture still rely on live feeds, including paramecia (*Paramecium* sp.), rotifers (*Brachionus* sp.), and *Artemia* nauplii (*Artemia* sp.), until weaning at the juvenile stage (30 days post fertilization) (Lawrence, 2020). Despite the benefits of live feeds (high digestibility, encouragement of prey capture behavior, and nutritional customization by enriching live feeds), zebrafish requires a rather labor-intensive culture, making its production in academic laboratories difficult (Conceição et al., 2010).

Recent research has shown that biofloc technology (BFT) could be a promising system for culturing ornamental fish (Cunha et al., 2020), including zebrafish adults (Avilés-López et al., 2017). BFT is a closed system based on the growth of microorganisms in a culture medium, benefiting from minimum or zero water exchange (Avnimelech, 2012). Therefore, all nutrients can be continuously recycled and reused, in turn favoring biofloc growth.

Bioflocs are microbiological aggregates that remain in water suspensions and are composed of bacteria, microalgae, flagellates, ciliates, rotifers, and nematodes, among other microorganisms (Khanjani and Sharifinia, 2020). These organisms maintain the water quality of the system because they facilitate the uptake of nitrogen compounds that are metabolized to other nutrients, particularly proteins. Moreover, bioflocs can be used as a natural food by fish, thereby reducing the feed conversion ratio and feed costs (Khanjani et al., 2023).

The use of a biofloc system during fish larviculture is advantageous, because it provides an easily accessible food source for the larvae outside regular feeding periods, in turn minimizing potential negative social interactions during feeding (Ekasari et al., 2015). Despite the beneficial characteristics of BFT, few studies have investigated the use of this system during fish larviculture. BFT improved the larval quality and performance of tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) (Ekasari et al., 2015; Ekasari et al., 2016). However, no benefits have been observed for pirarucu (*Arapaima gigas*) larvae cultured using BFT when compared to the use of clear water (Dantas, 2018).

Considering that zebrafish larvae feed mainly on food items within the water column at first feeding and the potentially high nutritional value of bioflocs, the BFT system could be an excellent alternative for zebrafish larvae production, increasing survival, improving animal welfare and immune capacity, and reducing labor in hatcheries. Therefore, the present study investigated different strategies for biofloc addition to be used as food during zebrafish larviculture and evaluated the performance and biochemical parameters of zebrafish larvae.

MATERIAL AND METHODS

The experiment was conducted at the Universidade Federal do Pampa (UNIPAMPA), Rio Grande do Sul, Brazil. All experimental procedures were performed in accordance with the guidelines of the Ethics Committee for Animal Use of UNIPAMPA and were approved by the same committee (approval no. 056/2019).

Biofloc inoculum preparation

The biofloc inoculum was produced in a tank (500 L) with the common carp *Cyprinus carpio* (10 fish weighing 105 ± 17 g), which was aerated by constant supply of saturated oxygen. The fish were fed *ad libitum* with commercial feed (Supra Acqua Line, 28% PB) twice a day for 120 days. The water quality parameters verified were: temperature (24.5 ± 5.2°C); total ammonia nitrogen (TAN, < 0.2 $mg \cdot L^{-1}$) and nitrite (NO_2-N, < 0.2 $mg \cdot L^{-1}$); total suspended solids (TSS, 390 ± 32 $mg \cdot L^{-1}$) and pH (8.1). TAN, NO_2-N, and TSS were determined according to the methods described by Verdouw et al. (1978), American Public Health Association (1998), and Strickland and Parsons (1972), respectively, and pH was measured using a digital pH meter (PG1800; GEHAKA, São Paulo, Brazil).

Spawning and embryo collection

Adult zebrafish obtained from a commercial supplier was kept in a closed water circulation system at 28°C and pH of 8.3, under a 14-h light/10-h dark photoperiod. Males and females were maintained separately and fed *ad libitum* with commercial feed (45% crude protein, Tetraline, Blacksburg, VA, United States of America) twice a day. One day prior to

spawning, 30 couples were transferred to an aquarium (40 L) at the beginning of the dark photoperiod. A trap was used to collect embryos after spawning. The adult fish were removed from the aquarium the following day and placed back into their home tank in the recirculating aquaculture system (RAS). The eggs were inspected before use for health and developmental stages using a stereomicroscope. After 48 hours post fertilization, 50 embryos were randomly distributed in each experimental unit, with four replicate tanks being used for each treatment.

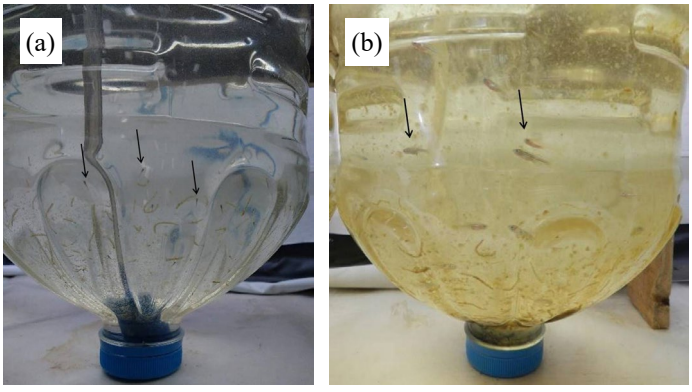
Larvae rearing and experimental treatments

The experimental units (plastic aquariums with conical bottoms, one airstone, and useful volumes of 4 L) were adapted to zebrafish larval requirements. Because of the small size of the aquariums and limited swimming capacity, the aeration flux (low intensity and one airstone in the background) and TSS (initial < 80 mg·L^{-1}) were maintained at a low rate (Fig. 1).

To evaluate BFT performance during larviculture, utilizing three treatments (four replicates; total of 12 units) were tested:

- O1: addition of 19.5-mg biofloc (78 mg) once at the beginning of the experiment;
- 1W: addition of 19.5-mg biofloc (78 mg) at the beginning of the experiment, followed by the addition of 9.75-mg biofloc (39 mg) every seven days for 30 days;
- 2W: addition of 19.5-mg biofloc (78 mg) at the beginning of the experiment, followed by the addition of 9.75-mL biofloc (39 mg) every four days for 30 days.

The water volume in all treatments was adjusted weekly because of evaporation of dechlorinated tap water. During the experiment, zebrafish larvae were fed commercial feed (Alcon Basic, 45% crude protein) twice a day (9 a.m. and 4 p.m.) *ad libitum* until 30 days post fertilization. From four to 16 days post fertilization, zebrafish larvae were fed food with particle sizes of 10–50 µm. Thereafter, the larvae were fed food with particle sizes of 50–150 µm. The experiment was carried out in a climate-controlled room, and the photoperiod was maintained constant (12 h light/12 h dark).



Source: Elaborated by the authors.

Figure 1. Experimental units. (a) Zebrafish near the water surface (four days post fertilization). (b) Zebrafish subjected to O1 treatment (20 days post fertilization) actively swimming and initial pigmentation. The black arrows indicate fish in the water column.

Regarding water quality, temperature and dissolved oxygen were measured twice a day (8:30 a.m. and 4 p.m.) using a handheld dissolved oxygen meter (POL-60; Politerm, São Paulo, Brazil). The pH (measured using a digital pH meter), TAN, NO_2-N, total alkalinity (American Public Health Association, 1998), and total hardness (Adad, 1982) were evaluated twice a week, whereas TSS (Strickland and Parsons, 1972) was measured once a week.

Evaluation of larval performance

All fish larvae were euthanized with eugenol (500 mg/L) at the end of the experiment (30 days post fertilization) and counted to calculate their survival rates. Fifteen individuals from each experimental unit were weighed and photographed using a digital camera. The total length and height of the fish were obtained from each image using the open-source image analysis software ImageJ (National Institutes of Health, Bethesda, MD, United States of America). The juvenile growth rates under each treatment were determined based on external morphological traits (pigment pattern, tail fin, anal fin, and dorsal fin morphology), according to the method proposed by Singleman and Holtzman (2014). The remaining fish from each treatment were stored at -80°C until biochemical analysis.

Analysis of biochemical parameters

Whole-body samples (50 mg) were homogenized on ice in 1-mL Tris–HCl buffer (50 mM, pH 7.4), centrifuged at 3,000 × *g* and 4°C for 10 min, and the supernatants were collected and stored in microtubes at -18°C until further analysis (Leitemperger et al., 2019). Lipid peroxidation was estimated by measuring the production of thiobarbituric acid reactive substance (TBARS). The reaction between malondialdehyde (MDA) and thiobarbituric acid was measured optically according to the protocol described by Buege and Aust (1978). Subsequently, absorbance was measured at 532 nm using a spectrophotometer (UV-M51 Bel Photonics; Servylab, São Leopoldo, Brazil), and the results were expressed as nmol MDA mg·protein^{-1}.

Catalase (CAT) activity was assayed using ultraviolet spectrophotometry (Nelson and Kiesow, 1972). The change in H_2O_2 absorbance at 240 nm over 60 s was measured using spectrophotometry, and CAT activity was expressed as µmol·min^{-1} mg·protein^{-1}. Nonprotein thiols (NPSH) levels were determined, as previously described by Ellman (1959), and the results were expressed as µmol·g^{-1} of tissue. Total protein

content was determined according to Bradford's (1976) method, with bovine serum albumin being used as the standard.

Biofloc composition analysis

Biofloc samples were analyzed in duplicate for crude protein content (N × 6.25 based on the micro-Kjeldahl method), according to the methodology described by Association of Official Analytical Chemists (1995). Fat was extracted and quantified using the cold extraction method described by Bligh and Dyer (1959).

Statistical analysis

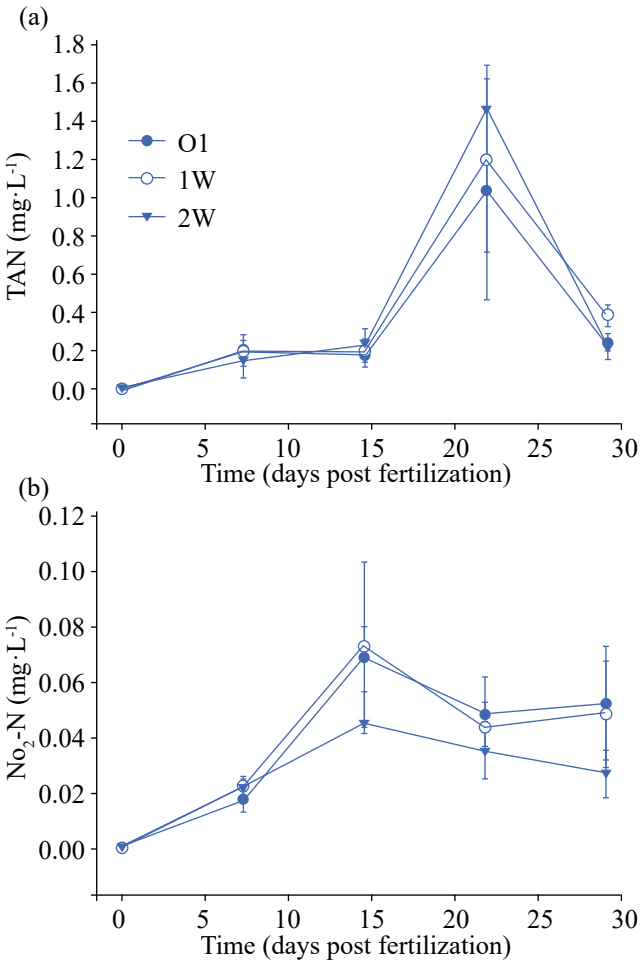
All data were subjected to a one-way analysis of variance. Post hoc comparisons were performed using Fisher's test. Normality and homogeneity tests of variance were previously analyzed, and a significance level of 5% was adopted in every case. Statistical analyses were performed using Statistical Analysis System software (SAS Institute Inc., Cary, NC, United States of America).

RESULTS

Water quality

The water quality results are presented in Table 1. No statistically significant differences were observed in the parameters among the treatments, except for TSS. The TSS concentration increased with an increase in the addition of biofloc to the treatments (2W > 1W > O1).

Regarding BFT evaluation, attention was paid to the total ammonia and nitrite concentrations, and the values obtained over the experimental period are shown in Fig. 2.



O1: only one biofloc addition; 1W: biofloc addition once every seven days; 2W: biofloc addition every four days. Source: Elaborated by the authors.

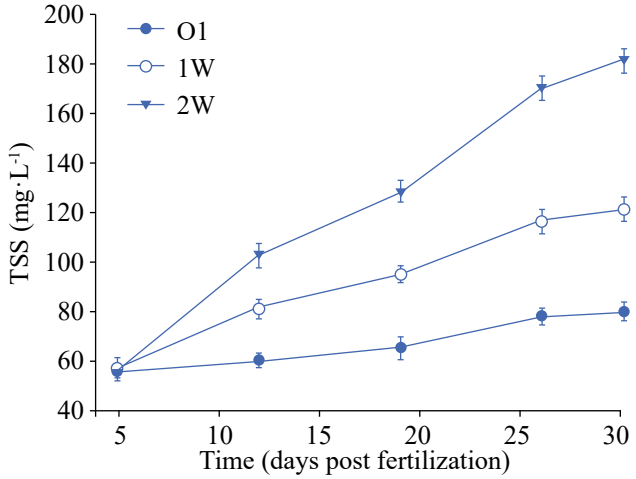
Figure 2. Nitrogen concentration (total ammonia and nitrite) during zebrafish larviculture using biofloc technology. (a) Total ammonia nitrogen (TAN) and (b) nitrite nitrogen (NO_2-N).

Table 1. Water quality parameters during larviculture of zebrafish in biofloc technology.

Parameters	Treatments (biofloc addition)		
	O1	1W	2W
Temperature (°C)	28.42 ± 1.51	28.51 ± 1.50	28.52 ±1.51
O_2 (mg·L^{-1})	6.74 ± 0.65	6.65 ± 0.59	6.67 ± 0.58
TAN (mg·L^{-1})	0.42 ± 0.46	0.49± 0.48	0.52 ± 0.55
NO_2-N (mg·L^{-1})	0.04 ± 0.02	0.04 ± 0.02	0.03 ± 0.011
pH	8.20 ± 0.09	8.19 ± 0.09	8.09 ± 0.09
Total alkalinity (mg $CaCO_3$·L^{-1})	103.33 ± 15.82	104.79 ± 22.30	103.37 ± 25.09
Total hardness (mg $CaCO_3$·L^{-1})	74.35 ± 22.03	82.66 ± 6.59	94.66 ± 10.87
TSS (mg·L^{-1})	57.40 ± 16.5^a	92.0 ± 18.2^b	127.6 ± 24.3^b

O_2: dissolved oxygen; TAN: total ammonia nitrogen; NO_2-N: nitrite nitrogen; TSS: total suspended solids; O1: only one biofloc addition; 1W: biofloc addition once every seven days; 2W: biofloc addition every four days. Values are presented as mean ± standard deviation. Means followed by different letters indicate a difference by Fisher's test ($p < 0.05$). Source: Elaborated by the authors.

As expected, the TSS concentration was higher in the 1W and 2W treatments than in the O1 treatment. An increase in the TSS concentration was observed in the evaluated treatments over the experimental period (Fig. 3).



O1: only one biofloc addition; 1W: biofloc addition once every seven days; 2W: biofloc addition every four days. Source: Elaborated by the authors.

Figure 3. Total suspended solid (TSS) during zebrafish larviculture in biofloc technology.

Performance parameters of zebrafish juveniles

The performance data of zebrafish juveniles are shown in Table 2. Zebrafish juveniles in the O1 treatment exhibited greater heights and total lengths than those reared in water with a high biofloc amount (2W). The final weight of the juveniles did not differ significantly among the treatments. The survival rate ranged from 88 to 94% and did not differ among the treatments. In addition, the percentage of specimens in the juvenile stage was similar among treatments (81.3–82.8%).

Biochemical parameters

The biochemical parameters of zebrafish juveniles evaluated are shown in Table 3. The juveniles in the treatment with a high biofloc amount had lower NPSH concentrations and higher TBARS levels than the juveniles in the treatments with low biofloc amounts (O1 and 1W). CAT activity was not significantly affected in juveniles in the evaluated treatments.

Biofloc composition

The protein and lipid contents (% dry matter) of the biofloc samples were as follows:

- Crude protein: O1 = 27.30 ± 1.91; 1W = 28.38 ± 1.98; 2W = 31.66 ± 0.19;
- Lipid: O1 = 4.5 ± 0.72; 1W = 2.69 ± 0.13; 2W = 2.7 ± 0.04.

No statistically significant differences were observed among the treatments.

Table 3. Biochemical analysis of zebrafish larvae cultured in biofloc technology.

Parameters	Treatments (biofloc addition)		
	O1	1W	2W
NPSH (µmol·g^{-1} tissue)	0.119 ± 0.008^{a}	0.121 ± 0.03^{a}	0.045 ± 0.009^{b}
TBARS (nmol MDA mg·protein^{-1})	0.47 ± 0.09^{ab}	0.40 ± 0.07^{b}	0.61 ± 0.18^{a}
CAT (µmol·min^{-1} mg·protein^{-1})	0.48 ± 0.15	0.40 ± 0.11	0.43 ± 0.11

NPSH: nonprotein thiols; TBARS: thiobarbituric acid reactive substance; CAT: catalase; O1: addition of biofloc only once at the beginning of the experiment; 1W: addition of biofloc once every seven days; 2W: addition of biofloc every four days. Values are presented as means ± standard deviation. Means followed by different letters indicate significant differences according to Fisher's exact test ($p < 0.05$). Source: Elaborated by the authors.

Table 2. Growth, survival, and juvenile rate of zebrafish larviculture in biofloc technology.

Parameters	Treatments (biofloc addition)		
	O1	1W	2W
Initial total length (mm)	3.86 ± 0.30	3.86 ± 0.30	3.86 ± 0.30
Final total length (mm)	11.93 ± 0.45^{a}	11.75 ± 0.80^{ab}	11.40 ± 0.63^{b}
Height (mm)	2.52 ± 0.13^{a}	2.49 ± 0.19^{ab}	2.38 ± 0.14^{b}
Final wet weight (mg)	16.88 ± 1.54	15.04 ± 3.29	14.91 ± 3.56
Survival rate (%)	94.58 ± 4.16	88.33 ± 6.80	92.15 ± 6.07
Juveniles rate (%)	82.86 ± 8.15	81.31 ± 8.36	82.58 ± 3.11

O1: addition of biofloc only once at the beginning of the experiment; 1W: addition of biofloc once every seven days; 2W: addition of biofloc every four days. Values are presented as means ± standard deviation. Means followed by different letters indicate significant differences according to Fisher's exact test ($p < 0.05$). Source: Elaborated by the authors.

DISCUSSION

The water quality parameters remained within the optimal range for zebrafish growth and survival (Hammer, 2020; Lawrence, 2020). The pH and alkalinity remained constant because of groundwater use, which has high pH and alkalinity values (nearly 8.3 and 120 $mg \cdot L^{-1}$ $CaCO_3$, respectively).

TAN increased rapidly during the assay, which is considered normal to the biofloc system, because time is necessary for the stabilization of heterotrophic and autotrophic bacteria (Ahmad et al., 2017). Moreover, the low concentration of solids in the zebrafish units could also contribute to the increase in TAN.

In BFT systems, it is crucial to strike a balance in the amount of TSS to prevent issues related to fish health due to gill clogging while still enabling sufficient microbial biomass to uptake and/or oxidize ammonia (Hargreaves, 2013). However, the highest TAN values were observed for a short period, and nitrite concentration did not exhibit a substantial increase, but it remained significantly below the lethal concentration (242.55 ± 15.79 mg $NO_2 \cdot L^{-1}$ – LC 50–96 h; Doleželová et al., 2011).

TSS is a measurement used to determine appropriate biofloc amounts, and the recommended range for tilapia and shrimps is 200–1,000 $mg \cdot L^{-1}$ (De Schryver et al., 2008; Gaona et al., 2017). However, it is impossible to maintain fish larvae during the initial larviculture phase with such a high concentration of TSS, as it requires intense aeration and water circulation. Therefore, it is imperative to utilize low TSS concentrations and moderate aeration during larviculture. Conversely, larviculture requires a low amount of food, in turn resulting in a gradual TSS increase and low availability of live food, indicating that biofloc/inoculum addition is necessary.

The first feeding of zebrafish starts after they inflate their gas bladder and maintain active swimming, which generally occurs at four–five days post fertilization for zebrafish larvae maintained at 28°C (Lawrence, 2020). Therefore, the nutritional quality, size, and abundance of live food during this phase are critical for successful larviculture and posterior development. Thus, the biofloc present in the first feed was likely associated with the high growth and survival rates observed in this study.

The final length and survival rate of zebrafish in the O1 treatment were 12 mm and 94.5%, respectively, which is one of the highest survival rates reported in the literature. Compared to previous studies, significant survival results for zebrafish larviculture have been reported by Carvalho et al. (2006) (55–86% at 21 days from first feeding), Aoyama et al. (2015) (52.2–91.1% at 30 days post fertilization), Martins et al. (2019) (76.3–79% at 30 dpf), Navarro-Guillén et al. (2021) (51.19 ± 10.38% at 22 days post fertilization), and Printzi et al. (2021) (55–90% at 24 days post fertilization).

In addition to survival rates, the results of growth parameters evaluated in the present study were satisfactory and similar to those observed when live food was added to the larval cultures. For example, total lengths of 14.3 mm (21 days from the first feeding; Carvalho et al., 2006), 8.94 ± 1.34 mm (30 days post fertilization; Martins et al., 2019), and 6–9 mm (24 days post fertilization; Printzi et al., 2021) have been observed when fish are fed live food. Notably, Padeniya et al. (2022) evaluated a multistrain probiotic in comparison to antibiotic (amoxicillin) usage, and the results demonstrated that the use of probiotics improved the morphometric parameters (total length). Therefore, the most balanced water medium can provide good health and nutritional conditions for larval development.

The comparison of different systems and protocols can cause interpretation errors, and for zebrafish larviculture most studies have used RAS to evaluate feeding protocols. In our study, we did not consider the addition of live food, but the constant presence of live food in the bioflocs. The constant availability of live food through biofloc addition during larviculture achieved good results, which is associated with the natural predatory behavior and improved fish welfare (Watts et al., 2016; Martins et al., 2019).

The values of protein and lipid contents of the biofloc samples were similar to those observed in previous studies (Khanjani and Sharifinia, 2020). Rotifers (*Brachionus* spp., 26–60% crude protein and 7–28% crude fat (Lubzens and Zmora, 2003; Evangelista et al., 2005) and artemia, approximately 50% crude protein and 17% crude fat (Evangelista et al., 2005) have been used extensively in larviculture. Enrichment with nutrients, such as microalgae and fatty acids, is a common practice for improving the nutritional value of live food. However, the challenges associated with such practices are labor-intensive, more structures are required (tanks and aeration), culturing microorganisms is time-consuming, and the possibility of contamination. Therefore, analyzing the nutritional content of bioflocs could help improve their nutritional value and enhance their use as alternatives to live food.

Several studies have sought alternatives to live food production, and Kagali et al. (2022) suggested the biofloc system as an alternative system, with the potential to avoid enrichment necessities and microbiological contamination. Therefore, the direct use of bioflocs, such as a larviculture system, is promising because of the stability of water quality parameters.

Unsuitable conditions in aquaculture systems (e.g., water quality parameters, chemical pollutants, diet, diseases, and

other factors) can induce oxidative stress in fish (Dilmi et al., 2021). The analysis of antioxidant parameters is crucial for the evaluation of fish competence against the general stress that causes oxidative stress and immunosuppression. Among the enzymes comprising the cellular antioxidant defense system that act against reactive oxygen species generated are superoxide dismutase (SOD), CAT, glutathione peroxidase (Vasconcelos et al., 2007; Dilmi et al., 2021). Glutathione is a part of the nonenzymatic antioxidant defense system and is the only NPSH present in aerobic cells (Vasconcelos et al., 2007).

In the present study, biochemical analysis of zebrafish larvae revealed a reduction in NPSH concentration and an increase in TBARS concentration in fish treated with the highest biofloc amount. CAT activity was not altered in the fish subjected to the three biofloc treatments. The increase in TBARS concentration revealed lipid peroxidation in zebrafish larvae, whereas the observed reduction in NPSH concentration suggested a compensatory cellular response to lipid peroxidation.

According to Dilmi et al. (2021), the absence of an impact on antioxidant capacity may indicate that the biofloc environment did not alter the health status of fish. The authors observed that SOD and CAT activities in juvenile tilapia reared in a biofloc system with different carbon-to-nitrogen ratios (14:17 or 20:1) were not altered.

The biofloc system used for larviculture should be well adapted with adequate water flow and aeration because of larval behavior and fragility. The structures used in this study are simple and can be considered advantageous over the complex RAS. Biofloc is a system that can be utilized for zebrafish larviculture; however, further research regarding the welfare, physiology, immunology, and engineering of the system is necessary.

CONCLUSION

The present study suggests that bioflocs can be used as a food source for zebrafish larvae. BFT has demonstrated excellent results in zebrafish larviculture, resulting in high survival and growth rates. The addition of biofloc once at the beginning of larval rearing (O1 treatment) was sufficient for the system, which enhanced the stabilization of nitrogen compounds. Finally, a biofloc system has the following advantages: maintains good water quality, high survival and growth rates, without water renewal, which could potentially reduce production costs.

CONFLICT OF INTERESTS

Nothing to declare.

FINANCIAL SUPPORT

Not applicable.

AUTHORS' CONTRIBUTIONS

Conceptualization: Martins GB; **Investigation:** Oliveira B, Ferrigolo FRG; **Data curation:** Martins GB, Lanes CFC; **Formal Analysis:** Martins GB, Oliveira B, Pretto A, Lanes CFC; **Writing – original draft:** Martins GB, Pretto A, Lanes CFC; **Writing – review & editing:** Martins GB, Ferrigolo FRG, Pretto A, Lanes CFC; **Final approval:** Martins GB.

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