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Co-feeding using live food and feed as first feeding for the small catfish *Trachelyopterus galeatus* (Linnaeus 1766)

[*Coalimentação utilizando alimento vivo e ração na primeira alimentação do pequeno bagre* Trachelyopterus galeatus *(Linnaeus 1766)*]

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ABSTRACT

This study evaluated the effect of co-feeding with commercial feed and live food (enriched or not with microalgae) on the growth and survival of *Trachelyopterus galeatus* larvae. Five treatments were carried out: commercial feed as a control (F); brine shrimp nauplii (BS); brine shrimp nauplii enriched with *Chaetoceros* sp. Microalgae (BSM); combined feed with brine shrimp nauplii (F+BS) and combined feed with brine shrimp nauplii enriched with microalgae (FBSM). The larvae (5.00±0.02 mg and 5.95±0.33mm) were reared for 30 days. There were no significant differences (P>0.05) in water quality between treatments, but there were significant differences in weight, weight gain and survival. The F+BS and F+BSM treatments, which involved co-feeding, showed the best results in final weight (0.168±0.004g and 0.169±0.007g) and weight gain (0.1278±0.004 g and 0.1294±0.007g), respectively. The treatments with live food, enriched or not, showed high survival rates of over 73%, with no significant differences between them. On the other hand, the exclusive use of feed resulted in low survival (36.7 ± 9.53%), indicating that this may result in low growth and make the production of *T. galeatus* larvae unfeasible.

Keywords: aquaculture, *Artemia franciscana*, *Chaetoceros*, larviculture, native fish

RESUMO

Este estudo avaliou o efeito da coalimentação com ração comercial e alimentos vivos (enriquecidos ou não com microalgas) no crescimento e na sobrevivência das larvas de Trachelyopterus galeatus. *Foram realizados cinco tratamentos: ração comercial como controle (F); náuplios de artêmia (BS); náuplios de artêmia enriquecidos com microalga* Chaetoceros *sp. (BSM); ração combinada com náuplios de artêmia (F+BS) e ração combinada com náuplios de artêmia enriquecidos com microalga (F+BSM). As larvas (5,00±0,02mg e 5,95±0,33mm) foram criadas por 30 dias. Não houve diferenças significativas (P>0,05) na qualidade da água entre os tratamentos, mas houve diferenças significativas no peso, no ganho de peso e na sobrevivência. Os tratamentos F+BS e F+BSM, que envolveram coalimentação, mostraram os melhores resultados em peso final (0,168±0,004g e 0,169±0,007g) e em ganho de peso (0,1278±0,004g e 0,1294±0,007g), respectivamente. Os tratamentos com alimentos vivos, enriquecidos ou não, mostraram altas taxas de sobrevivência acima de 73%, sem diferenças significativas entre eles. Por outro lado, o uso exclusivo de ração resultou em baixa sobrevivência (36,7±9,53%), indicando que isso pode resultar em baixo crescimento e inviabilizar a produção de larvas de* T. galeatus.
Palavras-chave: aquicultura, Artemia franciscana, Chaetoceros, *larviculturas, peixe nativo*

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INTRODUCTION

Even during the severe COVID-19 pandemic during 2020, the Brazil reached an aquaculture production about 550 thousand tons, generating revenues of US$ 1.1 billion, and representing a growth of 4.3% compared to 2019 (Pesquisa... , 2020). Fish farming remains the aquaculture sector with the highest production, being the Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) (Cichliformes: Cichlidae) the main fish produced (about 62% of the total fish produced). Surprisingly, although Brazil has the greatest diversity of freshwater fish in the world, its aquaculture activity is mainly composed of non-native species (Pelicice *et al.,* 2017).

The order Siluriformes has gained importance in recent years, proving to be a good alternative to the fish commonly used in fish farming worldwide (e.g., tilapia and carp) (Dauda *et al*., 2018). The most prominent cultured catfish fish is the African catfish *Clarias gariepinus* (Burchell, 1822) (Siluriformes: Clariidae), but due to the invasive potential of this species, its cultivation can imply risks to natural ecosystems in certain countries (Khan *et al*., 2021). Thus, to make aquaculture a more sustainable activity and due to the great diversity of species present in this order, the search for native catfish with zootechnical potential has emerged in recent years.

In Brazil, the catfish *Trachelyopterus galeatus* (Linnaeus, 1766) (Siluriformes: Auchenipteridae), popularly know as “bagrinho” in Baixada Maranhense, has been attracting interest in the aquaculture sector of the State of Maranhão due to its zootechnical characteristics and for presenting an already established market (from fishing activity). *T. galeatus* can reach up to 30.0 cm (males) and 23.0 cm (females) in total length, and a total weight of 675.00 g, and its selling price varies between US$ 5 and 6.5 per kg (Ferraris, 2007; Sousa *et al*., 2016; Froese and Pauly, 2023). The species has a diversified diet, consisting of small fish, insects, worms, microcrustaceans, shrimp and fruits, being considered carnivorous with a tendency to omnivore (Santin *et al*., 2015; Sousa *et al*., 2017; Froese and Pauly, 2023). In addition, this fish shows nocturnal behavior and inhabit lentic waters, being found in ponds, flooded fields, and river tributaries (Luz *et al*., 2012), being well adapted to inhabit hypoxic environments (Froese and Pauly, 2023).

The production of larvae is one of the main obstacles to the native fish farming in Brazil. According to Hung *et al*. (1989), most fish at the early stage of development have low acceptance for ration, resulting in low growth performance. At the beginning of exogenous feeding, larvae have high nutritional requirements, due to high growth rates, and as they still have a developing digestive system, a food source is required to meet these needs (Yúfera and Darias, 2007; Ferreira *et al*., 2017; Lima *et al*., 2017; Couto *et al*., 2018). However, studies regarding the larviculture of *T. galeatus* are not yet available and, therefore, adequate management techniques for this activity are still lacking.

Diet is the key to successful larval fish production, as the quality and quantity of food offered determines growth performance (Øie *et al*., 2017). Thus, knowledge of the feeding behavior of *T. galeatus* in captivity is important for the sustainable development of its culture. Brine shrimp (*Artemia* spp.) are often used as live food for fish larvae (Diemer *et al*., 2012; Pereira *et al*., 2016; Nyang'ate Onura *et al*., 2018), and among its advantages are the easy storage of the cysts and their high nutritional value (Castro-Mejía *et al*., 2011). However, some studies have shown that brine shrimp nauplii do not meet the nutritional requirements for fish larvae since they may lack some essential nutrients, such as essential amino acids and polyunsaturated fatty acids (Lavens and Sorgeloos, 1996; Nielsen *et al*., 2017). Some microalgae species may contain these nutrients, which can be transferred from microalgae to zooplankton, improving the quality of the brine shrimp, for example (Nath *et al*., 2012; Dhaneesh and Ajith Kumar, 2017).

The understanding of the larviculture of native fish is the first essential step for the consolidation of a productive chain in fish farming. Hence, studies on the feeding of larvae of native species, such as the catfish *T. galeatus*, can provide subsidies to assist in the understanding and improvement of the cultivation conditions of the species. Thus, the objective of the present work was to evaluate the effect of commercial feed, live foods, and their combinations on the water quality and growth performance of *T. galeatus* larvae.

MATERIALS AND METHODS

The study was conducted in the Laboratório de Desenvolvimento Aquícola da Amazônia Maranhense (LAQUAM), of the Universidade Federal do Maranhão (UFMA, Pinheiro *campus*), for 30 days. The research was approved by the Ethics Committee on the Use of Animals (23115.010760/2021-76) of the Federal University of Maranhão (CEUA/UFMA), Brazil.

The catfish *T. galeatus* larvae used for the experimentation at the beginning of exogenous feeding (7 days post-hatching DPH) were obtained by induced spawning from broodstock from a commercial fish farm. The broodstock previously sedated with benzocaine (20mg L^{-1}) received intraperitoneal injections using carp pituitary extract, and spawning followed by fertilization occurred by dry extrusion, according to Santos *et al*. (2013). Fertilized eggs were placed in 200 L funnel-type fiberglass incubators with water flow rate of 2 L min^{-1}. After hatching, the larvae remained in the incubators until mouth opening was observed and then were distributed to the experimental units.

Six hundred seventy-five *T. galeatus* larvae (5.0±0.02mg; 5.95±0.03mm) were randomly distributed in fifteen tanks with a usable volume of 0.015 L and stocked at a density of 3.000 larvae m^{-3} (n = 45 larvae per tank). The experimental design was entirely randomized, with five treatments (diets): commercial feed as a control (F); brine shrimp nauplii (BS); brine shrimp nauplii enriched with *Chaetoceros* sp. microalgae (BSM); combined feed with brine shrimp nauplii (F+BS) and combined feed brine shrimp nauplii enriched with microalgae (F+BSM). Three independent replicates were used for each treatment.

Each experimental unit had an individual aeration system (dissolved oxygen >5.0mg L^{-1}) through a central air blower, with temperature maintained at (≈ 28°C), light intensity of 26 µmol photons m^{-2} s^{-1} with natural photoperiod and pH 7. Once a day the tanks were siphoned to remove leftover food and larval feces, with renewal of 50% of the water volume per day.

The microalga *Chaetoceros* sp. (Chaetocerotaceae) used in the experimental diets belonged to the strain bank of the Laboratório de Biotecnologia de Microalgas Nativas da Amazônia Maranhense (L'ALGAM), of the Fisheries Engineering course (UFMA, Pinheiro *campus*). Cultures were cultivated in flasks containing Conway medium (Walne 1966) with the following composition: $FeCl_3.6H_2O$ 1.30g L^{-1}; $MnCl_2.4H_2O$ 0.36g L^{-1}; H_3BO_3 33.6g L^{-1}; EDTA 45.0g L^{-1}; $NaH_2PO_4.2H_2O$ 20.0g L^{-1}; $NaNO_3$ 100.0g L^{-1}; $ZnCl_2$ 1.1g L^{-1}; $CoCl_2.6H_2O$ 1.0g L^{-1}; $(NH_4)6M_{O7}O_{24}.4H_2O$ 0.45gL^{-1}; CuSO4.5H2O 1.0g L^{-1}; Na_2SiO3.5H2O 2.0g L^{-1}; vitamins B12 0.1g L^{-1} and B1 1.0g L^{-1}.

All the flasks were maintained at a constant temperature of 25 ± 1°C, pH 7.9, salinity of 30 PSU, with continuous aeration at a flow rate of 2 L/min, and under an irradiance of 60 µmol photons/m^2/s (fluorescent lamps) with continuous illumination. Periodic qualitative and quantitative monitoring of the cultures, including cell concentration, viability, and potential contaminations by bacteria or protozoa, was performed using a hemocytometer and an optical microscope (objective × 40). After the microalgae reached the stationary phase (~8 days of culture), cells were offered at a density of 10 x 10^4 cél mL^{-1} for enrichment of artemia (Instar II nauplii).

The hatching of *Artemia franciscana* Kellog, 1906 (Anostraca: Artemiidae) was carried out in transparent plastic incubators with a capacity of 1L, using 0.1g of cysts, sea water (30 PSU), at a temperature of 28ºC and constant illumination. After 24 h, the newly hatched nauplii were separated from the cysts by siphoning and were offered to catfish *T. galeatus* larvae until apparent satiation (the larvae stopped approaching the water surface to ingest the food provided) in the BS treatment and after the feed offer in the F+BS treatment. For the BSM and F+BSM treatments, instar II nauplii were enriched with the microalgae and offered under the same conditions described above for the treatments with artemia.

Larvae were fed four times a day (at 09:00 a.m., 12:00 p.m., 03:00 p.m. and 06:00 p.m.) with the respective experimental diets. For treatments F, F-BS and F-BSM, a commercial fish larvae powdered feed provided *ad libitum*, with warranty levels for with 55% crude protein, 9% ether extract, 4% crude fiber, 20% mineral matter, 13% moisture and 0.7% vitamin C was used.

Water quality variables such as pH, dissolved oxygen (DO) and temperature were measured twice a day (8:00 a.m. and 4:00 p.m.) using an oxygen and temperature probe (model YSI 55). Nitrite (NO_2^-) and nitrate (NO_3^-) concentrations were determined once a week (Standard…, 2012).

At the end of the 30 days of the experiment, fish were fasted for 12 hours and sedated by immersion bath with benzocaine (20 mg. L^{-1}). Subsequently, the following measurements were taken: (1) average final weight (g), through the ratio between final biomass and the number of individuals at the end of the experiment; (2) average weight gain (g), through the difference between the average final and initial weights; and (3) survival (number of final fish/number of initial fish) × 100.

The dataset presented a non-normal distribution by the Shapiro-Wilk normality test, thus, the Kruskal-Wallis test was used followed by Dunn's test (with p-value correction according to Bonferroni) to identify significant differences in water quality and zootechnical performance of larvae between treatments. All statistical analyses were conducted considering a 5% significance level and run in the statistical package SciPy (Virtanen *et al*., 2020).

RESULTS

For the water quality variables, no significant differences were observed (P > 0.05). The mean values for all experimental units were 7.23 for pH, 5.94mg L^{-1} for dissolved oxygen and 28°C for temperature, while the mean concentrations of nitrite and nitrate were 0.013 and 0.014 mgL^{-1}, respectively (Table 1).

Table 1. Water quality parameters in the culture of the catfish *Trachelyopterus galeatus* larvae fed with live food (with or without microalgae) and commercial feed

Treatment	pH	Dissolved oxygen (mg L^{-1})	Nitrite (mg L^{-1})	Nitrate (mg L^{-1})
F	7.29 ± 0.03^a	5.93 ± 0.03^a	0.025 ± 0.03^a	0.007 ± 0.009^a
BS	7.26 ± 0.02^a	5.93 ± 0.02^a	0.000 ± 0.00^a	0.004 ± 0.006^a
BSM	7.24 ± 0.03^a	5.95 ± 0.03^a	0.000 ± 0.00^a	0.000 ± 0.000^a
F+BS	7.20 ± 0.08^b	5.95 ± 0.04^a	0.025 ± 0.03^a	0.022 ± 0.031^a
F+BSM	7.19 ± 0.07^b	5.96 ± 0.07^a	0.015 ± 0.02^a	0.035 ± 0.013^a

Values are represented as mean ± standard deviation. Mean values from the same column with different letters differ significantly (P < 0.05) by Dunn's test. F - commercial feed; BS - brine shrimp nauplii; BSM - brine shrimp nauplii enriched with microalgae; F+BS – combined feed with brine shrimp nauplii; and F+BSM – combined feed brine shrimp nauplii enriched with microalgae.

At the end of the feeding experiment, larval growth performance was significantly different (P < 0.05) between treatments for final mean weight, weight gain and survival (Table 2). The values of weight gain, as well as the final average weight, were significantly higher (P<0.05) for the treatments F+BS and F+BSM. On the other hand, the diets F, BS and BSM did not show significant differences among themselves for these variables. The larvae of treatments that were fed with live food and its combinations BS, BSM, F+BS and F+BSM showed the highest values for survival (76.7±9.44%, 80.0±9.43%, 73.3±4.71% and 80.0±80.0±4.71%), respectively, and showed no significant differences between them (P>0.05). However, the treatment F presented the lowest survival (36.7±9.53%), differing significantly (P<0.05) from the others (Table 2).

Table 2. Growth performance of the catfish *Trachelyopterus galeatus* larvae fed with live food (with or without microalgae) and commercial feed

Diet	Final weight (g)	Weight gain (g)	Survival (%)
F	0.043 ± 0.003^b	0.0038 ± 0.003^b	36.7 ± 9.53^b
BS	0.040 ± 0.002^b	0.0035 ± 0.002^b	76.7 ± 9.44^a
BSM	0.044 ± 0.003^b	0.0045 ± 0.003^b	80.0 ± 9.43^a
F+BS	0.168 ± 0.004^a	0.1278 ± 0.004^a	73.3 ± 4.71^a
F+BSM	0.169 ± 0.007^a	0.1294 ± 0.007^a	80.0 ± 4.71^a

Values are represented as mean ± standard deviation. Mean values from the same column with different letters differ significantly (P < 0.05) by Dunn's test. F - commercial feed; BS - brine shrimp nauplii; BSM - brine shrimp nauplii enriched with microalgae; F+BS – combined feed with brine shrimp nauplii; and F+BSM – combined feed brine shrimp nauplii enriched with microalgae.

DISCUSSION

Regarding the water quality parameters, the average temperature of the experiment was within the range considered optimal (between 26 and 31°C) for tropical species of the order Siluriformes, such as *Rhamdia quelen* (Quoy and Gaimard, 1824) (Siluriformes: Heptapteridae), *Pseudoplatystoma corruscans* (Spix and Agassiz, 1829) (Siluriformes: Pimelodidae), and *Lophiosilurus alexandri* Steindachner, 1876 (Siluriformes: Pseudopimelodidae), indicating that the temperature probably did not affect the productive performance of the larvae (Chippari-Gomes *et al.*, 2000; Lima *et al*., 2006; Takata *et al*., 2014). The dissolved oxygen concentration remained above 5.0 mg L^{-1} throughout the experiment, thus being above the minimum limit suggested for freshwater fish species (Boyd, 1998). Likewise, the average pH throughout the experiment remained within the desirable range, between 6.5 and 9.0 (Boyd, 1998). So, it is possible to state that the conditions of the larviculture system met the ideal parameters of cultivation according to the literature for freshwater tropical Siluriformes fish. We emphasize, however, that no studies were found involving the cultivation of catfish *T. galeatus* to determine the limits of thermal tolerance as well as the levels of dissolved oxygen.

The present study demonstrated that the combination of feed with brine shrimp (enriched or not) during the larval rearing of the catfish *T. galeatus* improved the productive performance of the larvae, with the average weight and survival being higher than those of the other treatments, including larvae fed exclusively on live food with or without enrichment. These results corroborate those obtained for other catfish species (Siluriformes), such as *Rhamdia quelen*, the subject of a study conducted by Carneiro *et al*. (2003). In this study, the authors evaluated the isolated and combined effect of live food (*Artemia* sp.) and commercial feed and obtained better larval performance with the combination of the two diets.

Similarly, Salhi and Bessonart (2013) when evaluating only the commercial diet with co-feeding with brine shrimp on the larval performance and fatty acid composition of *Rhamdia quelen*, concluded that the co-fed larvae presented significantly better productive parameters and that their fatty acid requirements were met. Chepkirui-Boit *et al*. (2011) and Nyang'ate Onura *et al*. (2018) also observed that the combination of diets provided better intestinal morphological development resulting in better larval growth in *Clarias gariepinus*.

Live foods such as phytoplankton and zooplankton are indispensable in larviculture for many fish species due to their high nutritional value. For this reason, they are known as live nutrition capsules (Kandathil Radhakrishnan *et al*., 2020a) and when used in combined diets, they usually have an advantage in terms of protein and vitamin proportions (Awaïss and Kestemont, 1998). In addition, nutritional factors provided by live food tend to stimulate larval pancreatic secretions, stimulating endocrine responses and aiding in the maturation of the digestive process (Koven *et al*., 2001).

Furthermore, chemical stimuli and digestive enzymes released by these organisms facilitate the ingestion and digestion of commercial feed by the larvae, resulting in better use of nutrients and promoting better growth (Cañavate and Fernández-Díaz, 1999; Tesser and Portella, 2006; Aderolu *et al*., 2010; Pereira *et al*., 2016). It is also worth noting that the visual stimulation provided by the swimming of live food increases predatory and locomotor activity in fish larvae (Nascimento *et al*., 2015; Souza *et al*., 2020). In general, carotenoids, flavonoids, and other phenolic compounds are recognized to present antioxidant activity, in addition to inhibiting the growth of several pathogenic bacteria and fungi (Dantas *et al*., 2019; Oliveira *et al*., 2021). Thus, the supplementation of microalgae in initial growth phases can contribute to fish resistance and make it less susceptible for abiotic stress and diseases (Charoonnart *et al*., 2018).

Our results showed that survival at the end of the experiment was significantly influenced, ranging from 36.7% for larvae fed exclusively with commercial feed, to 80% for larvae fed with live food and their combinations (co-feeding), may have influenced the immune system through the development of lymphatic tissue associated with the larval gut, due to the microalgae being immune system promoters (Dantas *et al*., 2019; Oliveira *et al*., 2021). As in the present study only the initial and final biometrics were performed, without a weekly follow-up, it was not possible to observe at what moment the supply of feed was more critical during the growth of the larvae. However, our results also demonstrate that the larvae can gain weight only with the supply of feed, which shows that commercial feed contributes, even if in a very small way, with some kind of assistance for the first stages of growth of the larvae of this species. However, survival must also be considered to ensure greater animal resistance during the larval stage and according to Cahu and Zambonino Infante (2001), the supply of live food has been related to improvements in this parameter, which is probably related to the difficulty of the larvae to feed on dry diets in the first days after hatching.

The development of an economic feasibility analysis can help determine the best food management protocol for *T. galeatus* larvicultures. Until now, the most effective strategy for productive performance, based on growth and survival, has been the use of live food *Artemia* enriched with microalgae plus feed. *Artemia* spp. are commonly used in fish larviculture due to their ability to enhance development and survival at this stage of the life cycle (Kandathil Radhakrishnan *et al*., 2020a). However, they are not nutritionally complete, as they are deficient in the concentrations of n-3 and n-6 polyunsaturated fatty acids (PUFAs), particularly docosahexaenoic (DHA), eicosapentaenoic (EPA) and arachidonic acid (ARA) (Chakraborty *et al*., 2007).

These compounds are related to improved development and survival in freshwater fish larvae (Tocher, 2010; Salhi and Bessonart, 2013; Costa *et al*., 2018), which is why several enrichment techniques have been developed to improve nutritional quality, such as the addition of microalgae (Han *et al*., 2001; Navarro *et al*., 1999; Francis *et al*. ,2019; Kandathil Radhakrishnan *et al*., 2020b). Although we did not perform the biochemical analysis of the microalgae-enriched brine shrimp, several studies have verified the potential of *Chaetoceros* sp. in supplying long-chain PUFAs, EPA, DHA, linoleic acid, as well as proteins and carbohydrates (Costard *et al*., 2012; Jesús-Campos *et al*., 2020). Properties from microalgae can be transmitted to these organisms, making the enrichment of brine shrimp nauplii with microalgae a viable solution to meet the nutritional needs of consumers.

Additionally, influencing survival, growth, metamorphosis success rate and general stock quality, it becomes possible to improve the yields of larviculture (Francis *et al*., 2019; Kandathil Radhakrishnan *et al*., 2020a,b), as it was observed in BSM and F+BSM treatments. Our results showed that the offer of live food and its combination with commercial feed (co-feeding) improves survival in exogenous feeding of *T. galeatus* larvae. The main causes for the low survival of fish larvae that were fed only with commercial feed include low food intake, inappropriate nutritional composition to ensure larval development/metamorphosis and an immature digestive system that require a high efficiency of protein hydrolysis to digest and assimilate the complex nutrients in the feed (Rønnestad *et al*., 2013; Khoa *et al*., 2020). Similar results to those found in the present study were obtained for the black catfish *Rhamdia quelen* (Diemer *et al*., 2012), for the curimatã-pacu *Prochilodus argenteus* Spix and Agassiz, 1829 (Characiformes: Prochilodontidae) (Santos *et al*., 2016; Ferreira *et al*., 2017), for the pacamã *Lophiosilurus alexandri* (Pedreira *et al*., 2008) and for the common carp *Cyprinus carpio* Linnaeus 1758 (Cypriniformes: Cyprinidae) (Fosse *et al*., 2018).

CONCLUSION

The results indicate that co-feeding with feed and live food, both with and without enrichment with the microalgae *Chaetoceros* sp., influenced the weight gain, average final weight, and survival of the *T. galeatus* larvae. Larviculture exclusively with feed did not meet the nutritional needs of the species, resulting in low growth and survival rates, as well as irregular production of high-quality larvae.

To improve our knowledge of nutrition, digestibility, immunity, and composition at this stage of larviculture, future research is essential. They should explore various co-feeds, microalgae, and live foods, enriched or not, to determine optimal feeding methods and drive the sustainable development of *T. galeatus* larviculture. In addition to the factors of weight gain and survival rate, additional variables must be analyzed to fully understand the larval performance of this species.

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