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Effects of an *Enterococcus faecium*-based probiotic on growth performance and resistance of Amazon ornamental fish *Heros severus* under short-term fasting

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ABSTRACT

This study aimed to evaluate the deprivation and feeding frequency of H. severus fingerlings fed a diet supplemented with autochthonous probiotic *E. faecium* and challenged with the pathogen *A. hydrophila*. The experiment was carried out in 2 × 5 factorial, consisting of two food frequencies (two and four times a day) and four food deprivations with probiotic (P:7/0, P:6/1, P:5/2 and P:Alt) and one control (C:7/0). The deprivation factor, higher values of growth parameters were observed for fish from the treatments P:7/0 and P:6/1, as well greatest resistance of the *A. hydrophila*. Therefore, the use of probiotic *E. faecium* provided greater growth and resistance for *H. severus*.

Key words: Amazonian ornamental fish; Additive; Prophylaxis; Bacterial challenge.

Efeitos de um probiótico à base de *Enterococcus faecium* no desempenho de crescimento e na resistência do peixe ornamental da Amazônia *Heros severus* em jejum de curta duração

RESUMO

Este estudo teve como objetivo avaliar a privação e a frequência alimentar de alevinos de *H. severus* alimentados com dieta suplementada com probiótico autóctone *E. faecium* e desafiados com o patógeno *A. hydrophila*. O experimento foi realizado em fatorial 2 × 5, sendo constituído por duas frequências alimentares (duas e quatro vezes ao dia) e quatro privações alimentares com probiótico (P:7/0, P:6/1, P:5/2 e P:Alt) e um controle (C:7/0). No fator privação, maiores valores dos parâmetros de crescimento foram observados para os peixes dos tratamentos P:7/0 e P:6/1, bem como maior resistência a *A. hydrophila*. Portanto, o uso do probiótico *E. faecium* proporcionou maior crescimento e resistência para *H. severus*.

Palavras-chave: Peixe ornamental amazônico; Aditivo; Profilaxia; Desafio bacteriano.

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INTRODUCTION

The ornamental fish industry generates billions of dollars annually with the commercialization of various species (Biondo & Burki, 2020; Parappurathu et al., 2021). Cichlids from the Amazon region stand out on the national and international market due to their exuberance of colors, shapes and sizes (Beltrão et al., 2019; Tribuzy-Neto et al., 2020).

Among the species sold, *Acará severo* (*Heros severus*) is native to the Amazon region. It has a yellowish color, with greenish tones in the head region and reddish in the belly, and eight dark brown vertical stripes along the body, which can reach the total length of 20 cm (Kullander, 2003; Staeck & Schindler, 2015). These fish are exported to several countries, such as Germany, the United States of America (USA), and Japan (Novák et al., 2020; Tribuzy-Neto et al., 2020).

Amidst the attractive productive characteristics in *H. severus* breeding, we can highlight its degree of rusticity, management, ease of reproduction, and adaptation to breeding systems, in addition to the commercialization price attributed to size, factors that led to the expansion of this species in ornamental fish farms (Abe et al., 2021; Castro-Castellón et al., 2023; Eiras et al., 2022). Thus, the interest of this species in the ornamental fish industry has stimulated the search for improvements in breeding strategies, such as the use of live food (Eiras et al., 2022), food transition to inert food (Campelo et al., 2020), levels of protein requirements in the diet (Sousa et al., 2021), and the feeding rate and frequency (Abe et al., 2022).

In this sense, food deprivation and frequency are strategies used to optimize fish production. Deprivation, when used correctly, can provide the animal with compensatory growth due to a certain period of food restriction, and frequency stimulates fish to consume food at specific times throughout the day (Abe et al., 2022; Oliveira et al., 2020). These strategies are attractive due to the reduction in the amount of feed offered and better feed conversion and animal growth, in addition to the reduction in operational and input costs (Abe et al., 2022; Fujimoto et al., 2016).

However, there is currently no information on strategies that can promote resistance to bacterial infections and *H. severus* growth in livestock. It is known that diseases of bacterial origin, such as *Aeromonas hydrophila*, can cause high mortality rates in fish due to their rapid dissemination in the aquatic environment, causing injuries to the hosts' integument, which can lead to severe hemorrhage (Abd El-Ghany et al., 2014; do Couto et al., 2022). Thus, for the sustainable development of ornamental fish farming, alternatives for disease prevention and control have been the focus of research with promising results (Ahmadifard et al., 2019; Paixão et al., 2020).

The use of probiotics in fish farming has demonstrated beneficial effects on the health of animals, with improvements in the immune system and resistance to bacterial infections (do Couto et al., 2022; Sousa et al., 2019), in addition to providing modulation of the intestinal microbiota and increased growth of ornamental fish (Dias et al., 2019; Jinendiran et al., 2019). They also improve performance by increasing intestinal villi, allowing more contact with the intestinal epithelium, thus enabling greater absorption of nutrients (El-Saadony et al., 2021; Jahan et al., 2021). Moreover, many probiotic bacteria are transient and need to constantly be added to the feed so that they can colonize and beneficially modulate the intestinal microbiota (Vadassery & Pillai, 2020).

Therefore, the use of probiotics in food frequency and deprivation strategies can improve the effect of these dietary strategies, but there is no information on the effects on growth and resistance to bacterial infections in the strategies used to breed *H. severus* with diets containing autochthonous probiotics. Therefore, the present study aimed to evaluate the deprivation and feeding frequency of *H. severus* fingerlings fed a diet supplemented with *Enterococcus faecium* and challenged with the pathogen *A. hydrophila.*

MATERIAL AND METHODS

All procedures performed in this study were approved by the Animal Use Ethics Committee of the Universidade Federal do Pará (no. 9202300420).

Preparation of diets

The autochthonous probiotic *E. faecium* was selected and isolated from healthy *H. severus* captured in the natural environment and identified by the matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF) technique, using the molecular weight of ribosomal proteins with laser (wavelength of 260–337 nm), by our research group according to Lopes et al. (2022).

The strain was activated and maintained in Man Rogoza Sharpe (MRS) broth at the concentration of 1.2×10^9 colony forming unit (CFU) per milliliter for supplementation in the diets. This experiment used specific commercial food for cichlids (Poytara, with 39% crude protein, 6.5% lipids, 5.3% phosphorus, 8.5% minerals, and 2.5% crude fiber). The feed was exposed to ultraviolet radiation for 15 min to eliminate any bacteria present in the pellets.

To include the probiotic *E. faecium*, the food pellets were crushed and weighed (30 g for each treatment), then sprinkled

on the experimental diets at the rate of 100 mL·kg^{-1} of food and subsequently homogenized in a hermetically sealed container and incubated in an oven for 24 h at 35°C. The control diet did not receive probiotic inclusion. All procedures carried out to prepare the diets were in accordance with Sousa et al. (2019). The final probiotic concentration in the diet was 1 × 10^{8} CFU·g^{-1} of feed. Diet preparation procedures were carried out every 12 days to maintain the probiotic concentration in the feed (Sousa et al. 2019).

Experimental design

The fingerlings were obtained by natural reproduction in the laboratory, and the larviculture phase was carried out for 15 days fed with brine shrimp nauplii and with three days of food transition (Abe et al., 2016; Campelo et al., 2020). For the experiment, 300 *H. severus* fingerlings (11.16 ± 0.689 mm and 25.72 ± 1.4 mg) were used, distributed in 30 tanks with a volume of 20 L in density of 10 fish per tank, and fed at the rate of feeding of 6% per day (Abe et al., 2022).

The experiment with diet supplement probiotic was carried out in a 2 × 5 factorial scheme, in triplicate, with the first factor consisting of two food frequencies: twice a day (Fr 2x) and four times a day (Fr 4x). The second factor, food deprivation, was composed of the following treatments: feeding every day (P:7/0), six days of feeding and one day of deprivation (P:6/1), five days of feeding and two days of deprivation (P:5/2), feeding every other day (P:Alt), and it was also included one treatment control (C:7/0) feeding every day with a diet without *E. faecium* supplementation. The experimental period was 45 days.

A partial water change (40% of the total water volume) was carried out approximately 40 min after the last daily feeding (Dias et al., 2019).

Growth parameters

At the beginning and end of the experiment, weights and lengths were measured to determine the zootechnical performance parameters: total length (TL), total weight (TW), length gain (LG), weight gain (WG), specific growth rate for weight (SGRw), specific growth rate for length (SGRl), uniformity for length (UL) and weight (UW) (Abe et al., 2022), feed conversion rate (FCR) (Sousa et al., 2019), relative condition factor (Kn) (Le Cren, 1951), and survival (S).

Microbiological analysis

For microbiological analysis, three individuals from each replicate were used, totaling 90 fish, which were anesthetized by submersion in benzocaine solution (20 mg/L) (Pramod et al., 2010) and euthanized by spinal section to remove the intestine. Maceration and homogenization of the intestine were performed in 0.65% sterile saline solution (SSS). Subsequently, they were serially diluted (1:10) and dilutions 10^{-4} and 10^{-6} were sown on plates containing tryptone soy agar (TSA) and MRS agar. The plates were incubated in an oven at 30°C to count CFU over 24 h for total viable heterotrophic bacteria and over 48 h for lactic acid bacteria (Mouriño et al., 2017; Sousa et al., 2019).

Bacterial challenge against Aeromonas hydrophila

For the challenge, the bacteria *A. hydrophila* was activated and cultivated in brain heart infusion (BHI) broth for 24 h at 30°C. Subsequently, the pathogen was centrifuged (30 min at 1,800 xg), discarding the supernatant, and the pellet was resuspended in 0.65% SSS, using the concentration of *A. hydrophila* of 1.2 × 10^{8} CFU·mL^{-1}, which is considered a lethal dose (LD50~70) based on the literature (Barros et al., 2023; do Couto et al., 2022).

In this test, the fish were added to containers with a volume of 2 L of water containing the pathogen, then the fish were moved with small nets for 20 seconds, creating a stressor effect (Barros et al., 2023; Meneses et al., 2023). Five fish were used per repetition of each treatment from the previous experiment, which were kept in containers (20-L capacity) in a static system lasting 96 h. During this period, deaths were counted to determine cumulative mortality (Barros et al., 2023; do Couto et al., 2022; Mouriño et al., 2017; Paixão et al., 2020).

The bacterial challenge was carried out in a completely randomized design with three replicates and 12 treatments, as seen in Table 1.

Table 1. Organization of treatments for the bacterial challenge of *Heros severus* against *Aeromonas hydrophila.*

Abbreviation	Treatment
C-	Control without sterile saline solution and *A. hydrophila* immersions
C+ISSS	Control with immersion in sterile saline solution only
F2xC7/0-IAH	The respective treatments two times with immersion in solution containing *A. hydrophila*
F2xP7/0-IAH	
F2xP6/1-IAH	
F2xP5/2-IAH	
F2xPAlt-IAH	
F4xC7/0-IAH	The respective treatments four times with immersion in solution containing *A. hydrophila*
F4xP7/0-IAH	
F4xP6/1-IAH	
F4xP5/2-IAH	
F4xPAlt-IAH	

To confirm the bacterial infection, liver fragments were collected to isolate the pathogen, which were cultured in Petri dishes with TSA and incubated in an oven for 24 h at 30°C, confirming Koch's postulate. The procedures were carried out in accordance with Brum et al. (2017) and do Couto et al. (2022).

Statistical analysis

The data were subjected to normality tests (Shapiro-Wilk's) and homoscedasticity (Levene's) tests. The data were subsequently submitted to analysis of variance (ANOVA), two factors for the performance experiment and one factor for the challenge, followed by Tukey's test for the comparison of means ($p < 0.05$). Microbiology data were log transformed [logX10 (x + 1)].

RESULTS

Water quality

During the experimental period, the physical and chemical parameters of the water remained at: dissolved oxygen of 6.26 ± 0.36 mg·L^{-1}, temperature of 28.7 ± 0.22°C, electrical conductivity of 283.17 ± 26.17 µs·cm^{-1}, pH of 6.7 ± 0.12, and total ammonia of 0.32 ± 0.11 mg·L^{-1}.

Microbiological analysis

There was no interaction between the factors of frequency and food deprivation ($p > 0.05$). Likewise, there was no significant difference for total viable heterotrophic bacteria and lactic acid bacteria for the food frequency factor of 2x and 4x a day (Fig. 1a). However, for the food deprivation factor, a reduction ($p = 0.0084$) of total heterotrophic bacteria was observed in the intestines of fish fed a diet supplemented with *E. faecium* in treatments P:7/0, P:6/1, and P:5/2 compared to groups P:Alt and C:7/0. Furthermore, there was a significant increase ($p = 0.0021$) in lactic acid bacteria in treatments P:7/0, P:6/1, and P:5/2 compared to control fish C:7/0 (Fig. 1b).

Growth parameters

Regarding growth performance, there was no interaction between the factors of frequency and food deprivation ($p = 0.0938$), and the frequencies did not show a significant difference between 2x and 4x. The *H. severus* fingerlings in the deprivation treatments P:7/0 and P:6/1 fed with a diet containing *E. faecium* had the highest values in total length (22.28 ± 1.21 and 22.16 ± 1.08 mm, respectively), total weight (243.02 ± 6.21 and 243.78 ± 5.39 mg), length gain (11.18 ± 0.81 and 11.16 ± 0.73 mm), weight gain (219.77 ± 5.33 and 218.92 ± 5.91 mg), and specific growth rates for length (1.53 ± 0.02% and 1.52 ± 0.05%) and weight (4.98 ± 0.02% and 4.99 ± 0.03%) compared to the other deprivation treatments (P:5/2 and P:Alt) and the control group (C:7/0).

The food deprivation treatments P:7/0, P:6/1, and P:5/2 showed the best apparent feed conversion results (1.19 ± 0.02, 1.21 ± 0.03, and 1.21 ± 0.02, respectively), with 100% fish survival compared to the control group C:7/0 (83.33 ± 5.16%).

In relation to the relative condition factor (Kn) and uniformity in length (UL) and weight (UW), there was no statistical difference between the treatments for the food deprivation factor, just as there was no difference in the growth parameters of the food frequency factor (Table 2).

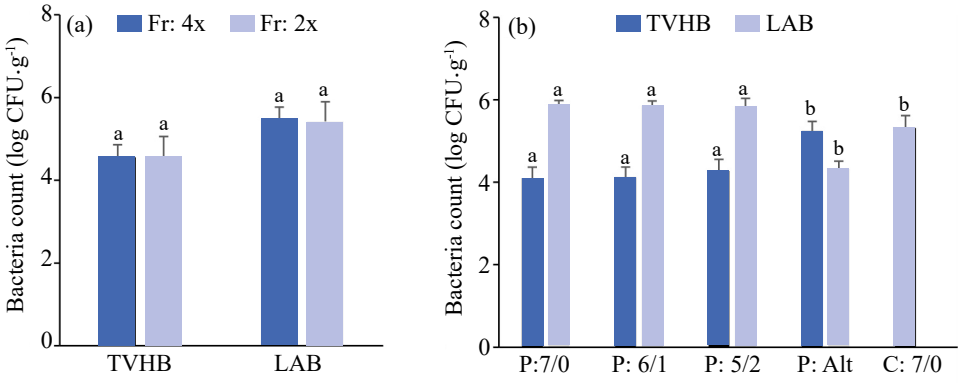

Figure 1. Count of total viable heterotrophic bacteria (TVHB) and lactic acid bacteria (LAB) in the intestine of the Amazonian ornamental fish *Heros severus* fed a diet supplemented with *Enterococcus faecium* offered at (a) two feeding frequencies (Fr: 4x and Fr: 2x) and with (b) food deprivation (P:7/0, P:6/1, P:5/2 and P:Alt), including the control without probiotic supplementation (C:7/0). Values (mean ± standard deviation) with different letters in the bars indicate a significant difference using the Tukey's test (5%).

Table 2. Growth and survival performance of the Amazonian ornamental fish *Heros severus* fed a diet supplemented with *Enterococcus faecium* offered at two feeding frequencies (Fr: 4x and Fr: 2x) and with food deprivation (P:7/0, P:6/1, P:5/2 and P:Alt), including a control diet without *E. faecium* probiotic supplementation (C:7/0)*.

	Feeding frequency (day)			Food deprivation (day)					
	4x	2x	*p*-value	P:7/0	P:6/1	P:5/2	P:Alt	C:7/0	*p*-value
TL (mm)	20.75 ± 1.80a	20.43 ± 1.97a	0.645	22.28 ± 1.21a	22.16 ± 1.08a	21.75 ± 1.34b	18.2 ± 1.29c	18.5 ± 1.32c	0.001
TW (mg)	218.18 ± 7.76a	217.89 ± 8.72a	0.981	243.02 ± 6.21a	243.78 ± 5.39a	239.11 ± 5.09b	182.18 ± 7.75c	183.97 ± 7.45c	0.009
LG (mm)	9.59 ± 1.72a	9.27 ± 1.68a	0.649	11.18 ± 0.81a	11.16 ± 0.73a	10.59 ± 0.92b	7.08 ± 0.87c	7.34 ± 0.85c	0.004
WG (mg)	194.94 ± 6.72a	192.81 ± 7.12a	0.981	219.77 ± 5.33a	218.92 ± 5.91a	213.58 ± 3.98b	155.76 ± 6.96c	157.84 ± 5.39c	0.007
SGRl (%)	1.37 ± 0.19a	1.33 ± 0.21a	0.481	1.53 ± 0.02a	1.52 ± 0.05a	1.48 ± 0.03b	1.09 ± 0.04c	1.12 ± 0.03c	0.002
SGRw (%)	4.76 ± 0.31a	4.77 ± 0.33a	0.712	4.98 ± 0.02a	4.99 ± 0.03a	4.64 ± 0.02b	4.22 ± 0.03c	4.21 ± 0.03c	0.036
UL (%)	94.42 ± 5.41a	95.15 ± 5.69a	0.074	95.33 ± 4.16a	96.66 ± 4.89a	93.33 ± 5.16a	95.05 ± 5.13a	94.18 ± 5.38a	0.767
UW (%)	51.05 ± 6.66a	55.73 ± 5.20a	0.093	53.33 ± 4.40a	56.66 ± 4.72a	55.05 ± 5.47a	49.96 ± 4.96a	54.38 ± 6.12a	0.560
FCR	1.20 ± 0.02a	1.21 ± 0.01a	0.616	1.19 ± 0.02a	1.21 ± 0.03a	1.21 ± 0.02a	1.23 ± 0.02b	1.24 ± 0.03b	0.010
S (%)	94.66 ± 7.43a	92.66 ± 9.61a	0.536	100.00 ± 0.00a	100 ± 0.00a	100 ± 0.00a	85.33 ± 5.47b	83.33 ± 5.16b	0.023
Kn	1.01 ± 0.02a	0.99 ± 0.03a	0.852	1.02 ± 0.02a	0.98 ± 0.03a	1.01 ± 0.02a	0.99 ± 0.03a	0.98 ± 0.03a	0.821

*Values (means ± standard deviation) with different letters in the same line indicate significant differences using Tukey's test (5%); TL: total length; TW: total weight; LG: length gain; WG: weight gain; SGRl: length-specific growth rate; SGRw: weight-specific growth rate; UL: length uniformity; UW: weight uniformity; FCR: feed conversion rate; S: survival; Kn: condition factor.

Bacterial challenge against *Aeromonas hydrophila*

In the acute challenge against the pathogen, fish fed diets supplemented with *E. faecium* in food deprivation of P:7/0 and P:6/1, at a frequency of 2x (42.02 ± 4.2% and 44.22 ± 3.2%, respectively) and 4x (40.00 ± 4.2% and 44.22 ± 3.2%, respectively) per day, had the lowest mortality rates ($p = 0.0034$) after challenge with *A. hydrophila*, compared to the controls (C:7/0) under different frequencies (2x and 4x), which were higher than 73%. When comparing cumulative mortality with the other treatments, these two treatments showed lower mortality. There was no mortality in fish that were immersed in sterile saline solution (C + ISSS) and in the negative control (C-) in which the water did not contain SSE and *A. hydrophila* (Figure 2).

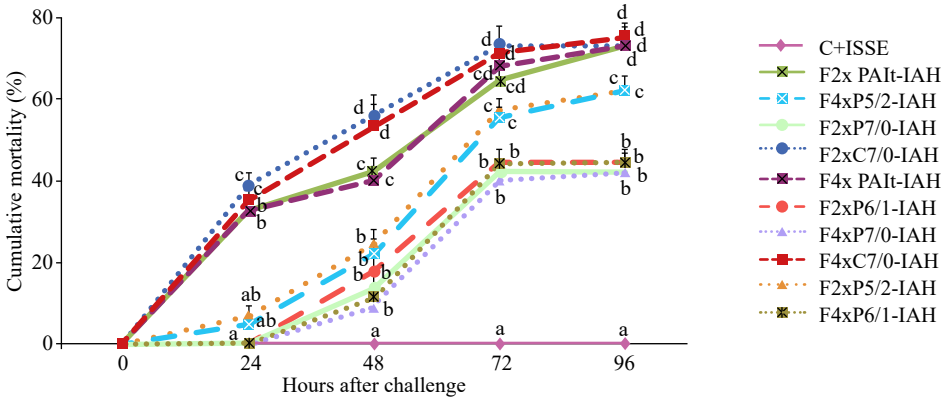

Figure 2. Cumulative mortality (%) of *Heros severus* fingerlings after 96 h challenge with immersion in a solution containing *Aeromonas hydrophila* (IAH) after feeding strategy with diets containing supplementation of the probiotic *Enterococcus faecium* and without supplementation (C:7/0), at two frequencies (F2x and F4x) and with deprivation (P:7/0, P:6/1, P:5/2 and P:ALT), also including a positive control group, were subjected to immersion in saline solution sterile (C + ISSS). Different letters at times after challenge indicate significant differences according to the Tukey's test (5%).

DISCUSSION

Recent studies highlight the beneficial effects of intestinal modulation caused by probiotics, including providing greater health to hosts, increasing the immune system, and providing resistance to diseases in aquaculture (do Couto et al., 2022; Noshair et al., 2023; Paixão et al., 2020). In this study, we emphasize the first report of the resistance of the Amazonian ornamental fish *H. severus* to the pathogen *A. hydrophila* after feeding a diet containing the autochthonous probiotic *E. faecium*, combined with the strategy of frequency and food deprivation.

During the diet digestion process, the probiotic bacteria, after resisting the host's physiological conditions, begin to compete for space and nutrients, in addition to the ability to inhibit pathogens (releasing substances such as bacteriocins), colonizing the animal's intestine (Dias et al., 2022; Lopes et al., 2022; Pereira et al., 2022; Sousa et al., 2019). These mechanisms of action provide modulation in the microbiota, resulting in a healthier intestine and stimulating the immune system of fish (Dias et al., 2022; Hossain et al., 2022; Shekarabi et al., 2022; Zhang et al., 2020).

In the present study, there was modulation of the intestinal microbiota of *H. severus* fed with a diet containing *E. faecium*. However, in the P:Alt food deprivation treatment, a lower amount of lactic acid bacteria and high values in the bacteria count were observed total heterotrophic, similar to the control group (C:7/0). This effect may be related to the alternation of food deprivation, causing oscillations in the concentration of viable probiotics available for adhesion to the intestinal mucosa. Therefore, the concentration and constancy of the supply of probiotics in the diet must be considered as factors that influence the colonization process (Dias et al., 2019; Dias et al., 2022; Opiyo et al., 2019; Sousa et al., 2019). This argument is corroborated by Vadassery and Pillai (2020), who observed a 7.75% reduction in the number of *E. faecium* colonies (CFU/individual) in the *Carassius auratus* intestine when interrupting its supply in the diet for one day and up to a 90% reduction over seven days. Therefore, due to its transitory characteristics, supplementation must be continuous to maintain colonization and allow its beneficial effects.

Modulation in the intestinal microbiota of fish caused by supplementation with the probiotic *E. faecium* has already been reported in *Pterophyllum scalare* (Dias et al., 2019), *Oreochromis niloticus* (Dias et al., 2022), *Arapaima gigas* (Sousa et al., 2019), *Labeo rohita* (Ghori et al., 2018), *Cyprinus carpio* (Gopalakannan & Arul, 2011), and *C. auratus* (Vadassery & Pillai, 2020). Therefore, the adhesion of this bacteria to the intestinal mucosa favored the growth and survival of animals in the breeding environment (Dias et al., 2022; Sousa et al., 2019).

In this study, treatments with food deprivation of P:7/0 and P:6/1 showed the best growth results, regardless of the feeding frequency adopted for management (Fr: 4x and Fr: 2x). The feeding management strategies for *H. severus* fingerlings already reported in the literature show that a feeding rate of 6% in relation to live weight expresses the best zootechnical performance values in relation to the values obtained with a feeding rate of 3% over 30 days (Abe et al., 2022).

The inclusion of the probiotic (*E. faecium*) in the diet used in the deprivation and food frequency strategy for *H. severus* also provided the best apparent feed conversion in treatments P:7/0, P:6/1, and P:5/2. Similar results were observed in the report by Dias et al. (2019), with increased growth and better feed conversion of *Pterophyllum scalare* fed with a diet containing *E. faecium* at the concentration of 10^8 CFU·g^{-1} of feed. This effect on growth performance is related to the adhesion of bacteria in the intestine, providing an increase in intestinal villi with a greater surface contact area, increasing the nutrient absorption capacity (Hossain et al., 2022; Ratvaj et al., 2023), as well as increasing the activities of digestive enzymes (trypsin, lipase, amylase, alkaline phosphatase, and pepsin), which can be produced by probiotics or stimulate production by the host, making more dietary nutrients available to the animal (Arani et al., 2019; Mohammadi et al., 2021; Zare et al., 2021).

Food frequency and deprivation are strategies that can reduce the amount of food offered, as well as feed and labor costs, in addition to improving the use of food and the compensatory growth of fish in farming (Abe et al., 2021; Fujimoto et al., 2016; Oliveira et al., 2020). In the present study, compensatory growth of *H. severus* was observed in the deprivation treatments P:6/1 and P:5/2, since the growth values were higher than those in the control group (C:7/0), with only the P:6/1 treatment having growth values equal to the P:7/0 treatment. In other studies, with ornamental fish *Pyrrhulina brevis* juveniles had compensatory growth only in 6/1 deprivation (six days of feeding and one day of deprivation) (Abe et al., 2022). This growth occurs because during the period of deprivation the fish use energy reserves, such as liver glycogen, to maintain their development, and they try to replenish the levels of reserve energy used during deprivation during the feeding period (Motta et al., 2021).

Better use of the diet, with a compensatory effect on growth, are results arising from strategies for improving the breeding of ornamental fish (Abe et al., 2021). However, the health of the animals is an important factor in order to enable the stimulation

of the immune system and thus increase the resistance of the fish in adverse farming conditions (do Couto et al., 2022). In this study, 100% survival in treatments with deprivation P:7/0, P:6/1, and P:5/2 at any of the food frequencies, in relation to the control group (C:7/0) and the P:Alt, may be a reflection of the improvement in the immune system caused by *E. faecium* supplemented in the diet. This immunomodulatory effect of probiotics in ornamental fish has already been reported in *Aequidens rivulatus* (Neissi et al., 2013), *P. scalare* (Azimirad et al., 2016), *C. auratus* (Mehdinejad et al., 2018), and *Cyprinus carpio haematopterus* (Wu et al., 2023).

The resistance of fish to bacterial diseases is one of the beneficial effects provided by probiotics of great interest in fish farming (do Couto et al., 2022). The results of this study showed an increase in *H. severus* resistance to *A. hydrophila* infection with survival above 73% in P:7/0 and P:6/1 deprivations, regardless feeding frequency. Similar effects have already been reported in *Amphiprion ocellaris* (Paixão et al., 2020), *Poecilia latipinna* (Ahmadifard et al., 2019), and *C. auratus* (Vadassery & Pillai, 2020).

Thus, the adhesion of probiotics in the intestine that bind to the intestine-associated lymphoid tissue (GALT), stimulating the non-specific immune system of fish, with an increase in the production of defense cells (lymphocytes, neutrophils and monocytes), humoral immunity (immunoglobulin), activation of phagocytosis capacity (neutrophils and macrophages) and immunological enzymatic activity (lysozyme and superoxide dismutase), preparing the host to resist and eliminate the infectious pathogen (Abomughaid et al., 2020; do Couto et al., 2022; Gobi et al., 2018; Paixão et al., 2020; Sousa et al., 2019). Therefore, diets with *E. faecium* provide greater resistance for *H. severus* in breeding, providing an alternative for disease prevention.

CONCLUSION

For optimal use of the probiotic strain *E. faecium* in juvenile *H. severus*, it is recommended the P:6/1 treatment (six days of feeding and one day of deprivation) at the frequency of two or four times a day.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

The data will be available upon request.

AUTHORS' CONTRIBUTIONS

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