




Growth performance and hematological parameters of banded cichlid *Heros severus* fed at different feeding rates and feeding frequencies

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Summary

Knowledge of the ideal feeding rate and feeding frequency for native species is important to improve the feed efficiency and reduce production costs. Thus, the present study evaluated feeding rate and feeding frequency effect for growth performance and health of *Heros severus* fingerlings. An experiment was carried out in completely randomized design with two feeding frequencies (2 and 4 times a day), three feeding rates (3%, 6% and 9% of body weight.day⁻¹) and three replicates for 90 days. No differences were observed ($p > 0.05$) in the growth performance for fingerlings between two and four feeding frequencies, but lower feeding frequency (two times a day) caused lower uniformity for weight at the end of 90 days. Furthermore, the increase in the feeding rate from 3% to 6% or 9% (biomass.day⁻¹) promoted higher weight and length gain, specific growth and length ratio (SGR_L, SGR_w) and biomass gain ($p < 0.05$). Lower feeding rate also caused blood change such as reduced values for glucose, triglyceride, erythrocyte, thrombocytes and leukocytes. Thus, *H. severus* fingerlings can be reared using feeding rate 6% (Biomass.day⁻¹) with two meals a day for better utilization of the feed without affecting its hematological parameters.

KEYWORDS

acara severo, feeding management, growth performance, hematology, ornamental fish

1 | INTRODUCTION

Currently, in the national and international ornamental fish market, amazon fish species have high values for aquarist community due to their different colors, patterns, size and specific behavior (Abe et al., 2015; Anjos et al., 2009; Prang, 2008).

Nonetheless, the Brazilian fish farming of ornamental fish still are focused on exotic fish, and is not appreciating native fish species (Abe et al., 2015; Abe, Dias, Reis, Sousa, et al., 2016; Cardoso et al., 2018). The lack of public policy for native ornamental fish rearing

as well as technology packs in captivity production stand out as the main problem (Zuanon Salaro et al., 2011).

Particularly, the amazon fish species Banded Cichlid *Heros severus* have specific characteristics as ornamental fish such as behavior of Neotropical cichlid, making couple for reproduction with parental care. They present moderate aggressiveness during reproductive cycle and vibrant colors range of olive green on body until red in the eyes (Abe, Dias, Reis, Sousa, et al., 2016; Lowe-McConnell, 1969; Mérigoux et al., 1998). This fish has a high value as ornamental fish (USD\$ 20.00) for international market being

only captured and not reared (Abe, Dias, Reis, Sousa, et al., 2016; Aquarium, 2021).

Knowledge in basic management for captivity rearing of native fish species can improve the market making it a source of income to farmers, protect wild fish and provide fish species with greater sanitary quality for aquarists (Abe et al., 2015; Anjos et al., 2009; Rossoni et al., 2014).

For these reasons, rate and feeding frequency are important tools for growth performance and production costs (Kodama et al., 2011). When using low levels of feeding rate, the lower nutrient amount can affect the growth performance and immunological system (Santos et al., 2014).

The nutritional efficiency of this feeding management can be determined through the blood changes evaluating the improvement or reduction of immunological defense system (Abdel-tawwab Ahmad et al., 2010; Habte-tzion et al., 2013). Therefore, this study evaluated the growth performance and health of *H. severus* submitted to different rates and feeding frequencies.

2 | MATERIAL AND METHOD

Ethical committee for animal care of the Pio Décimo College (Protocol 16/2018) approved this study. Juveniles of *H. severus* (180 fish) (33.40 ± 1.43 mm and 0.80 ± 0.13 g) were distributed in 18 aquariums (75 L) plugged in water recirculation system containing physical and biological filter. The experiment occurred in factorial design (2×3) with two feeding frequencies (2 and 4 meals day^{-1}), three feeding rates (3%, 6% and 9% of biomass. day^{-1}) and three replaces lasting 90 days. All fish received specific commercial ration for ornamental cichlid with composition: moisture 15%; crude protein 39%; lipid 6.5%; crude fiber 2.5%; mineral material 8.5%; calcium 2.8% and phosphor 5.3% (Poytara, 2021).

For each 30 days of experiment (30, 60 and 90 days) juveniles of *H. severus* undergone anesthetic procedure using eugenol solution (65 mg L^{-1} Roubach et al., 2005). Afterwards, they were measured (total weight and total length) to calculate feeding rate and determine growth performance: total length (TL), total weight (TW), length gain ($\text{LG} = \text{final length} - \text{initial length}$), Weight gain ($\text{WG} = \text{final weight} - \text{initial weight}$), specific growth rate for length ($\text{SDR} = (\ln[\text{final length}] - \ln[\text{initial length}]) / \text{days} \times 100$), specific growth rate for weight ($\text{SGR} = (\ln[\text{final weight}] - \ln[\text{initial weight}]) / \text{days} \times 100$), uniformity (U) for Length (UL) or Uniformity for Weight (UW) ($U = (X/X_1) \cdot 100$), where: X, total number of fish with final unit; X_1 , number of fish into experimental weight (FW) or total length (TL) $\pm 20\%$ inside of the final weight mean or total length mean into experimental unit (Furuya et al., 1998). biomass (BM) biomass gain ($\text{BG} = \text{final biomass} - \text{initial biomass}$), feed consumption ($\text{FC} = \text{final weight of ration} - \text{initial weight of ration}$), apparently feeding conversion ($\text{AFC} = \text{BG} - \text{FC}$) relative condition factor according to Le Cren (1951) ($\text{Kr} = \text{observed weight} / \text{estimated weight}$), and survival ($S = \text{final fish number} - \text{initial fish number}$).

At the end of experiment, after anesthetic procedure using eugenol solution (65 mg L^{-1} Roubach et al., 2005) nine fish per treatment undergone blood collect in the caudal puncture vein with the aid of syringe (insulin syringes) containing EDTA (5%). Afterwards, $10 \mu\text{l}$ aliquot of each blood sample was used to determine glycemic values (Accu-Check® Active Roche), triglyceride and cholesterol (Accu-Trend® Plus Roche). With the aid of refractometer Lumen RHC-200ATC, it determined total plasmatic protein (TPP).

Total count of erythrocyte ($10^6 \mu\text{l}^{-1}$) was carried out adding $10 \mu\text{l}$ from blood sample in eppendorf containing 1 ml of sterile saline solution (NaCl 0.65%), homogenized and then counted in Neubauer chamber (Tavares-dias & Moraes, 2004; Vallada, 1999). Hematocrit concentration was determined according to Goldenfarb et al. (1971), and hemoglobin concentration with $10 \mu\text{l}$ from blood sample using LABTES cianometahemoglobin in biochemical analyzer Thermo plate (Collier, 1994). With these data, it was calculated hematimetric index: mean corpuscular volume ($\text{MCV} = \text{Htx}10/\text{er}$), mean corpuscular hemoglobin (MCH: $\text{Hbx}10/\text{Er}$) and mean corpuscular hemoglobin concentration (MCHC: $\text{Hbx}100/\text{Ht}$) (Vallada, 1999).

For white blood cell count, was carried out blood smears stained with May-Grunwald-Giemsa according to Rosenfeld (1947) and indirectly quantified according to Ranzani-paiva et al. (2013). All collected data were submitted to normality and homoscedasticity tests from Shapiro Wilk and Bartlett. Survival data, without normal distribution, passed by transformation of arc sine root before statistical analysis. Afterwards, all data were conducted to analysis of variance (ANOVA) followed by Tukey test ($p < 0.05$) using the statistical program Past3.

3 | RESULTS

Over the experimental time, water quality parameters remained stable ($p > 0.05$) showing mean values of dissolved oxygen $7.23 \pm 0.3 \text{ mg L}^{-1}$, temperature $28.5 \pm 0.2^\circ\text{C}$, pH 6.7 ± 0.3 electric conductivity $341.34 \pm 24.35 \mu\text{S.cm}^{-1}$ and total ammonia $0.1 \pm 0.04 \text{ mg L}^{-1}$.

Rate and feeding frequency have no statistical difference ($p > 0.05$) for any experimental time (30, 60 and 90 days) (Table 1). Results of growth performance for the three experimental time are present in Tables 2–4. With regard to feeding frequencies, only uniformity for weight showed statistical difference at the 90 days, remaining similar throughout the 30 and 60 days.

However, growth performance showed better results with the increase of feeding rate (3%, 6% and 9% Biomass. day^{-1}), observing higher length gain, weight gain, specific development rate and specific growth rate for 6% and 9%. The highest values of growth performance occurred after 60 days for 9%. Survival and relative condition factor have no statistical difference ($p > 0.05$) among the feeding rate, but did elevate uniformity for weight, consumption ration and apparently feeding conversion.

TABLE 1 Mean values (\pm standard deviation) for performance of *Heros severus* juveniles Submitted to different rate and feeding frequencies after 30 days

Variables	Feeding frequency (Per day)		Feeding rate (%.BM.day ⁻¹)		
	2	4	3	6	9
TL (mm)	33.39 \pm 1.25 a	33.48 \pm 1.97 a	31.37 \pm 0.56 b	34.26 \pm 0.31 a	34.67 \pm 0.65 a
TW (g)	0.79 \pm 0.13 a	0.82 \pm 0.15 a	0.63 \pm 0.02 c	0.88 \pm 0.02 b	0.91 \pm 0.05 a
LG (mm)	8.93 \pm 1.1 a	9.372 \pm 2.1 a	7.13 \pm 0.8 b	10.02 \pm 0.64 a	10.30 \pm 0.97 a
WG (g)	0.5 \pm 0.12 a	0.53 \pm 0.14 a	0.34 \pm 0.02 b	0.59 \pm 0.02 a	0.61 \pm 0.05 a
SDR(l) (%)	3.28 \pm 0.53 a	3.43 \pm 0.63 a	2.63 \pm 0.18 b	3.71 \pm 0.2 a	3.74 \pm 0.26 a
SDR(w)(%)	1.03 \pm 0.11 a	1.09 \pm 0.22 a	0.86 \pm 0.1 b	1.15 \pm 0.08 a	1.17 \pm 0.11 a
UL (%)	98.88 \pm 3.33 a	100 \pm 0 a	98.33 \pm 4.08 a	100 \pm 0 a	100 \pm 0 a
UW (%)	40 \pm 7.07 a	50 \pm 20.62 a	53.33 \pm 13.66 a	48.33 \pm 17.22 ab	33.33 \pm 10.33 b
BM (g)	7.934 \pm 1.3 a	8.18 \pm 1.4 a	6.27 \pm 0.2 b	8.79 \pm 0.2 a	9.11 \pm 0.48 a
BG (g)	7.43 \pm 1.8 a	7.92 \pm 1.5 a	5.66 \pm 1.1 b	8.49 \pm 0.19 a	8.87 \pm 0.5 a
FC (g)	7.62 \pm 1.18 a	8.19 \pm 1.4 a	5.23 \pm 0.16 b	8.62 \pm 0.48 a	8.97 \pm 0.53 a
AFC	1.00 \pm 0.13 a	1.01 \pm 0.07 a	1.00 \pm 0.17 a	1.00 \pm 0.05 a	1.01 \pm 0.05 a
Kr	1.00 \pm 0.03 a	0.99 \pm 0.03 a	0.97 \pm 0.04 a	1.00 \pm 0.01 a	0.99 \pm 0.03 a
S (%)	100 \pm 0 a	100 \pm 0 a	100 \pm 0 a	100 \pm 0 a	100 \pm 0 a

Same letters in line do not differ statistically by Tukey test ($p < 0.05$).

Abbreviations: AFC, apparently feeding conversion; BG, biomass gain; BM, biomass; FC, feed consumption; Kr, relative condition factor; LG, length gain; S, survival; SGR(l), specific growth rate for length; SGR(w), specific growth rate for weight; TL, total length; TW, total weight; UL, uniformity for length; UW, uniformity for weight; WG, weight gain.

TABLE 2 Mean values (\pm standard deviation) for performance of *Heros severus* juveniles submitted to different rate and feeding frequencies after 60 days

Variables	Feeding frequency (Per day)		Feeding rate (%.BM.dia ⁻¹)		
	2	4	3	6	9
TL (mm)	43.77 \pm 3.59 a	43.96 \pm 5.06 a	38.42 \pm 1.70 b	45.31 \pm 0.49 a	47.87 \pm 1.07 a
TW (g)	1.71 \pm 0.44 a	1.83 \pm 0.59 a	1.13 \pm 0.08 c	1.89 \pm 0.1 b	2.28 \pm 0.22 a
LG (mm)	10.38 \pm 2.47 a	10.49 \pm 3.21 a	7.06 \pm 1.45 c	11.04 \pm 0.56 b	13.20 \pm 0.75 a
WG (g)	0.92 \pm 0.32 a	1.01 \pm 0.45 a	0.51 \pm 0.08 c	1.01 \pm 0.09 b	1.37 \pm 0.18 a
SDR(l) (%)	2.50 \pm 0.43 a	2.55 \pm 0.57 a	1.97 \pm 0.26 c	2.55 \pm 0.14 b	3.05 \pm 0.19 a
SDR(w)(%)	0.89 \pm 0.17 a	0.89 \pm 0.22 a	0.67 \pm 0.13 c	0.93 \pm 0.04 b	1.07 \pm 0.05 a
UL (%)	98.89 \pm 3.33 a	92.22 \pm 9.72 a	95 \pm 8.37 a	95 \pm 8.37 a	96.66 \pm 8.16 a
UW (%)	46.66 \pm 16.58 a	57.77 \pm 17.16 a	65 \pm 5.49 a	50 \pm 10 ab	41.66 \pm 13.29 b
BM (g)	17.11 \pm 4.39 a	18.28 \pm 5.93 a	11.35 \pm 0.79 c	18.91 \pm 0.99 b	22.83 \pm 2.24 a
BG (g)	10.38 \pm 2.47 a	10.49 \pm 3.21 a	7.06 \pm 1.45 c	11.04 \pm 0.56 b	13.21 \pm 0.75 a
FC (g)	10.71 \pm 3.4 a	11.46 \pm 4.27 a	7.45 \pm 0.13 c	11.57 \pm 0.35 b	13.24 \pm 1.14 a
AFC	1.10 \pm 0.16 a	1.05 \pm 0.19 a	1.01 \pm 0.15 a	1.00 \pm 0.03 a	1.00 \pm 0.06 a
Kr	1.00 \pm 0.03 a	1.00 \pm 0.02 a	1.00 \pm 0.04 a	1.00 \pm 0.02 a	1.00 \pm 0.02 a
S (%)	100 \pm 0 a	100 \pm 0 a	100 \pm 0 a	100 \pm 0 a	100 \pm 0 a

Same letters in line do not differ statistically by Tukey test ($p < 0.05$).

Abbreviations: AFC, apparently feeding conversion; BG, biomass gain; BM, biomass; FC, feed consumption; Kr, relative condition factor LG, length gain; S, survival SGR(l), specific growth rate for length; SGR(w), specific growth rate for weight; TL, total length; TW, total weight; UL, uniformity for length; UW, uniformity for weight; WG, weight gain.

TABLE 3 Mean values (\pm standard deviation) for performance of *Heros severus* juveniles submitted to different rate and feeding frequencies after 90 days

Variables	Feeding frequency (per day)		Feeding rate (%BM.dia ⁻¹)		
	2	4	3	6	9
TL (mm)	53.65 \pm 5.25 a	54.72 \pm 6.15 a	46.98 \pm 0.78 c	55.93 \pm 0.45 b	59.65 \pm 1.54 a
TW (g)	3.37 \pm 1.04 a	3.66 \pm 1.30 a	2.07 \pm 0.13 c	3.75 \pm 0.21 b	4.73 \pm 0.36 a
LG (mm)	9.88 \pm 1.94 a	10.75 \pm 1.41 a	8.56 \pm 1.57 b	10.62 \pm 0.69 a	11.78 \pm 0.75 a
WG (g)	1.66 \pm 0.62 a	1.84 \pm 0.72 a	0.94 \pm 0.13 c	1.86 \pm 0.13 b	2.44 \pm 0.22 a
SDR(l) (%)	2.21 \pm 0.29 a	2.28 \pm 0.22 a	2.01 \pm 0.25 b	2.28 \pm 0.1 b	2.43 \pm 0.18 a
SDR(w)(%)	0.67 \pm 0.09 a	0.73 \pm 0.07 a	0.67 \pm 0.04 b	0.70 \pm 0.04 a	0.73 \pm 0.04 a
UL (%)	100 \pm 0 a	97.78 \pm 6.67 a	100 \pm 0	100 \pm 0	96.67 \pm 8.16
UW (%)	44.44 \pm 19.44 a	65.56 \pm 13.33 a	68.33 \pm 11.69 a	55 \pm 19.75 ab	41.67 \pm 18.35 b
BM (g)	33.71 \pm 10.44 a	36.65 \pm 12.96 a	20.74 \pm 1.28 c	37.52 \pm 2.09 b	47.27 \pm 3.57 a
BG (g)	9.88 \pm 1.94 a	10.75 \pm 1.41 a	8.56 \pm 1.57 b	10.62 \pm 0.69 a	11.78 \pm 0.75 a
FC (g)	16.88 \pm 8.14 a	16.89 \pm 7.05 a	8.32 \pm 0.24 c	16.58 \pm 1.11 b	25.75 \pm 1.38 a
AFC	1.64 \pm 0.56 a	1.53 \pm 0.51 a	1.00 \pm 0.20 c	1.56 \pm 0.10 b	2.19 \pm 0.18 a
Kr	0.98 \pm 0.03 a	1.00 \pm 0.02 a	1.00 \pm 0.01 a	1.00 \pm 0.01 a	0.98 \pm 0.04 a
S (%)	100 \pm 0 a	100 \pm 0 a	100 \pm 0 a	100 \pm 0 a	100 \pm 0 a

Same letters in line do not differ statistically by Tukey test ($p < 0.05$).

Abbreviations: AFC, apparently feeding conversion; BG, biomass gain; BM, biomass; FC, feed consumption; Kr, relative condition factor; LG, length gain; S, survival; SGR(l), specific growth rate for length; SGR(w), specific growth rate for weight; TL, total length; TW, total weight; UL, uniformity for length; UW, uniformity for weight; WG, weight gain.

TABLE 4 p value for variables performance of *Heros severus* juveniles submitted to different rate and feeding frequencies in different times

Variables	p value								
	30 days			60 days			90 days		
	FF	FR	FR \times FR	FF	FR	FR \times FR	FF	FR	FR \times FR
TL (mm)	0.0001	0.0658	0.0660	0.0001	0.0746	0.0785	0.0001	0.6584	0.0812
TW (g)	0.0001	0.0647	0.0598	0.0001	0.0646	0.0744	0.0001	0.7451	0.0845
LG (mm)	0.0001	0.0646	0.6584	0.0001	0.0426	0.0636	0.0001	0.0846	0.9845
WG (g)	0.0001	0.0549	0.6583	0.0001	0.0456	0.0626	0.0001	0.0876	0.0546
SDR(l) (%)	0.0001	0.0596	0.0985	0.0001	0.0780	0.0850	0.0001	0.0660	0.0624
SDR(w)(%)	0.0001	0.0625	0.8465	0.0001	0.0544	0.0754	0.0854	0.0894	0.0694
UL (%)	0.5412	0.0607	0.6589	0.8547	0.0627	0.8457	0.0001	0.0547	0.7549
UC (%)	0.0485	0.0535	0.6845	0.0548	0.0796	0.0825	0.0012	0.0654	0.0541
BM (g)	0.0231	0.0905	0.0754	0.0001	0.0626	0.0786	0.0001	0.0846	0.0985
BG (g)	0.0132	0.0606	0.0845	0.0001	0.0612	0.0626	0.0001	0.0845	0.0584
FC (g)	0.0354	0.0631	0.0565	0.0001	0.0665	0.0744	0.0001	0.0654	0.4558
AFC	0.6545	0.0659	0.3544	0.0001	0.0601	0.0890	0.0001	0.0598	0.0985
Kr	0.9845	0.0686	0.6548	0.6584	0.8754	0.8455	0.5157	0.4845	0.8545
S	—	—	—	—	—	—	—	—	—

Abbreviations: AFC, apparently feeding conversion; BG, biomass gain; BM, biomass; FC, feed consumption; Kr, relative condition factor; LG, length gain; S, survival; SGR(l), specific growth rate for length; SGR(w), specific growth rate for weight; TL, total length; TW, total weight; UL, uniformity for length; UW, uniformity for weight; WG, weight gain.

Despite the greater growth performance, the increase of feeding rate promoted reduction of uniformity for weight increasing apparent feeding conversion ($p < 0.05$). Nonetheless, relative condition factor, survival and uniformity for length have no statistical difference ($p > 0.05$).

Hematological parameters such as glucose, plasmatic protein, cholesterol and triglycerides did not interact between rate and feeding frequency ($p > 0.05$). However, glucose and triglycerides values reduced with the lower feeding rate (Table 5).

For red blood cells, feeding frequency did not provoked statistical difference among the treatments, but feeding rate of 6 and 3% provided reduction in erythrocytes, hemoglobin and VCM (Table 6).

For white blood cell, feeding frequency provided reduction in lymphocyte and neutrophil number. Furthermore, feeding rate reduction caused lower values of total leukocytes, thrombocytes, lymphocytes, monocytes and neutrophil (Table 7).

4 | DISCUSSION

Growth performance of *H. severus* submitted to feeding frequency two and four times a day have no statistical difference ($p > 0.05$) as observed by Paixão et al. (2019), juveniles of Jundiá *Rhamdia quelen* reared in excavated tank (Canton Weingartner et al., 2007), production of robalo-peva *Centropomus parallelus* in fresh water (Corrêa Gervazio et al., 2010) and rearing of tilapia *Oreochromis niloticus* (Santos et al., 2014).

For other side, juveniles of pacu *Piaractus mesopotamicus* obtained better results with feeding frequency three times a day (Dieterich et al., 2013; Pouey et al., 2012) if compared to the present study. Nonetheless, for juveniles of grass carp *Ctenopharyngodon idella* and yellow tail lambari *Astyanax bimaculatus*, they needed feeding frequency four times a day to demonstrate a better growth (Hayashi et al., 2004; Marques et al., 2008). These different results

TABLE 5 Mean values (\pm standard deviation) of biochemical assays of blood in juveniles of *Heros severus* at the end of experiment

Feed Frequency	Glucose (mg dl ⁻¹)	Protein (mg dl ⁻¹)	Cholesterol (mg dl ⁻¹)	Triglycerides (mg dl ⁻¹)
2	42.97 \pm 10.4 A	5.34 \pm 0.37 A	202.8 \pm 28.1 A	223.1 \pm 39.1 A
4	44.38 \pm 7.9 A	5.67 \pm 0.17 A	183.1 \pm 31.6 A	186.4 \pm 46.1 A
Feed Rate (%)				
3	34.3 \pm 6.13 B	5.33 \pm 0.42 A	173 \pm 14.6 A	170.38 \pm 28.9 B
6	49.2 \pm 8.9 A	5.55 \pm 0.33 A	213 \pm 22.5 A	213.54 \pm 36.3 AB
9	47.5 \pm 5.9 A	5.65 \pm 0.13 A	192 \pm 26.5 A	230.01 \pm 51.1 A
<i>p</i> value				
FF	0.7052	0.0791	0.3111	0.0656
FR	0.0011	0.0807	0.2623	0.049
FF \times FR	0.7317	0.0232	0.2307	0.9133

Note: Same letters in line do not differ statistically by Tukey test ($p < 0.05$).

TABLE 6 Mean values (\pm standard deviation) of red blood cell in *Heros severus* at the end of experimental time

Frequency	Erythrocyte ($\times 10^6$)	Hematocrit (%)	Hemoglobin (mg dl ⁻¹)	MCV (fL-1)	MCHC (g dl-1)	MCH (pg)
2	2.26 \pm 0.5 A	27.95 \pm 1.7 A	6.92 \pm 0.6 A	453.5 \pm 0.18 A	24.7 \pm 2.24 A	0.16 \pm 0.04 A
4	2.47 \pm 0.4 A	29.66 \pm 2.1 A	7.17 \pm 1.6 A	493.8 \pm 0.16 A	24.4 \pm 6.68 A	0.14 \pm 0.02 A
Rate						
3	1.87 \pm 0.4 B	28.64 \pm 2.8 A	6.1 \pm 1.1 B	374.9 \pm 0.19 B	21.6 \pm 5 A	0.17 \pm 0.05 A
6	2.47 \pm 0.3 AB	29.63 \pm 1.9 A	7.6 \pm 0.7 A	494.9 \pm 0.05 AB	25.9 \pm 2.8 A	0.15 \pm 0.02 A
9	2.75 \pm 0.4 A	28.15 \pm 1.2 A	7.3 \pm 1.2 A	551.1 \pm 0.07 A	26.3 \pm 5.41 A	0.16 \pm 0.03 A
<i>p</i> value						
FF	0.2945	0.3254	0.4732	0.2945	0.2793	0.2793
FR	0.0062	0.4125	0.0068	0.0062	0.2021	0.2021
FF \times FR	0.9251	0.4654	0.3111	0.9251	0.0768	0.0521

Note: Same letters in line do not differ statistically by Tukey test ($p < 0.05$).

TABLE 7 Mean values (\pm standard deviation) of red blood cell in *Heros severus* at the end of experimental time

Frequency	Thrombocytes ($10^3 \mu\text{l}^{-1}$)	Leucocytes ($10^3 \mu\text{l}^{-1}$)	Lymphocytes ($10^3 \mu\text{l}^{-1}$)	Monocyte ($10^3 \mu\text{l}^{-1}$)	Neutrophil ($10^3 \mu\text{l}^{-1}$)
2	23.4 \pm 7.02 A	87.3 \pm 22.8 A	62.8 \pm 15.1 B	62.8 \pm 4.0 A	13.18 \pm 4.3 B
4	22.9 \pm 6.3 A	95.6 \pm 16.6 A	67.18 \pm 10.7 A	67.18 \pm 3.4 A	15.89 \pm 4 A
Rate					
3	17.7 \pm 1.1 B	70.8 \pm 13.5 B	52.5 \pm 9.8 B	52.5 \pm 2.6 B	9.83 \pm 2.1 B
6	26.3 \pm 5.8 A	101.5 \pm 6.1 A	70.8 \pm 6.4 A	70.8 \pm 1.6 A	17.64 \pm 1.6 A
9	25.4 \pm 7.3 A	102.1 \pm 8.5 A	71.6 \pm 12.1 A	71.6 \pm 3.7 A	16.12 \pm 3.7 A
p value					
FF	0.8522	0.1818	0.3069	0.3903	0.0326
FR	0.0188	0.0011	0.004	0.0092	0.0001
FF \times FR	0.0636	0.1426	0.087	0.4654	0.6486

Note: Same letters in line do not differ statistically by Tukey test ($p < 0.05$).

demonstrated that some factors as fish species, life stage or production system can affect the relationship between feeding frequency and performance.

Paixão et al. (2019) reported that the uniformity of length and weight of *H. severus* did not show significant differences at the end of 30 days regardless of feeding frequency, however the results of present study, uniformity of the weight of banded cichlid was decreased after 90 days, which is caused by the lower availability of food and competition within of trial unit. According to Wang et al. (1998), smaller feeding frequency can favor a smaller number of fish into rearing system, making it less uniform. However, when submitted to more feeding frequencies, they have opportunity to feeding.

According to Abe, Dias, Reis, Couta, et al. (2016), they also observed uniformity for weight 50% in larvae culture of ornamental fish *Pyrrhulina brevis* submitted to feeding rate four times a day. They claim that in specific stages of development greater aggressiveness and social behavior into shoal can influence its uniformity values. Therefore, despite the smaller mean of uniformity for weight, that value would be normal for ornamental fish rearing with two feeding frequencies (Ribeiro et al., 2008) considering aggressiveness and social behavior factors observed in *H. severus*.

During the larviculture of *H. severus* over different rate and feeding frequencies, according to Abe, Dias, Reis, Couta, et al. (2016), the reduction of feeding frequency can cause the worst growth performance. They also not observed statistical difference of uniformity for weight or length showing different results when compared to the present study. Different stages of development can influence in social behavior and consequently uniformity. For these reasons, make it necessary to determinates for each stage of development its ideal feeding handling.

With regard to the feeding rate, it caused reduced uniformity for weight and increased food consumption. This increase of food consumption allowed higher growth performance, but without uniformity. Thus, the increase of feeding rate provides a better performance, but reduced uniformity with larger and more individual aggressiveness, promoting uneven growth inside of treatments. Ribeiro et al. (2008) also observed

this territorialism behavior for Angel fish *Pterophyllum scalare* reared in aquarium, resulting in reduced uniformity next to the 50%.

According to Marques et al. (2004) the increases of feeding rate (1%, 3%, 5% and 7%) for grass carp promotes statistical difference in performance, but not resulting in smaller uniformity for weight, opposite of the present study.

Rate and feeding frequency can influence in the uniformity for fish, but some other factors also are important as the fish species, stocking density, stage of development, rearing system and water quality parameters (Gonçalves Junior et al., 2014; Takahashi et al., 2010). These parameters must be used for ornamental fish rearing because the commercialization commonly carried out per unit and not for weight. The smaller unit (small fish) can influence in production scale.

In captivity, where the species need have provided food, the feeding rate have an important paper for development of fish and production costs. Insufficient amount of food provides a worst growth, while ideal amount of food completes all physiologic necessities and promotes better growth as well as nutrient exploitation improving the production (Kodama et al., 2011).

The increases of feeding rate 3% to 9% a day, improves the performance for Banded cichlid *H. severus*. Marques et al. (2004) found similar results for juvenile of grass carp, determining 6% a day as the best feeding rate with higher apparently feeding conversion and growth performance. For juvenile of *Trichogaster trichopterus*, increased feeding rate of 6 to 9% a day promotes significant increases weight, length and final biomass, but reduced apparently feeding conversion (Zuanon et al., 2004) such as observed to the juveniles of *H. severus*.

Thus, the higher feeding rate of the present study would be satisfactory for a daily diet. The reduction of feeding rate provides reduced performance, resulting in larger consumption of ration and worst feeding efficiency reflecting in higher production costs. Beyond of performance, survival and condition factor are important factors for production systems of ornamental fish, directly related to the health fish amount. In the present study, these

parameters (rate and feeding frequency) did not present statistical difference indicating an adequate management and ideal captivity conditions. Nonetheless, hematological values showed problems for this management impossible to identify only with performance values.

Particularly, despite the red blood cell do not differ statistically among the *H. severus* submitted to the different feeding frequencies, it demonstrated that feeding frequency does not spoil the red blood cell production. According to Bittencourt et al., (2013) reduced feeding frequency does not influence in the number of erythrocytes, hemoglobin and hematocrit despite the nutritional deficient. However, they observed an increase of lymphocyte and neutrophil number, indicating immunological changes caused by poor nutrient absorption and smaller feeding frequency.

In juveniles of *Megalobrama amblycephala* reduced feeding frequency (4 to 3 times a day) cause oxidative stress with reduction of leukocytes, promoting immunosuppression and reducing the resistance against pathogen (Li et al., 2014). For the present study, it was observed immunosuppression for *H. severus* submitted to feeding frequency two times a day.

The reduced glucose caused by reduced feeding rate (3% a day) would be a mechanism of emergency when the fish move energetic source from the liver to the muscle (Adamante, 2005). Thus, reduced values of glucose and triglycerides would be a body strategy to avoid the nutritional insufficiency.

The lowest used feeding rate did not cause lower growth, but is not sufficient to keep the homeostasis, promoting reduction in erythrocyte, hemoglobin and MCV. Thus, lower amount of the food caused lower nutrient amount available to the fish, promoting anemic process with possible deficit to the oxygen transport for tissues (Kondera et al., 2017).

The reduction of feeding rate (6% to 3% a day) caused problems to the immunological system of the fish with reduced thrombocyte and leukocyte. According to Lim and Klesius (2003) with juvenile of bagre *Ictalurus punctatus*, were observed similar results, demonstrating nutritional insufficiency due to the short cycles of feeding deprivation caused by immunosuppression reducing leukocytes making it more susceptible to fish pathogen.

5 | CONCLUSION

Juveniles of *H. severus* must be submitted to feeding rate 6% with two feeding frequency a day for optimization of the work, without reductions in performance or blood changes. However, also can be reared at feeding rate 6% to obtain better uniformity and feeding utilization having reduction in performance, but without affect their health.

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CONFLICT OF INTEREST

The authors have no any conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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