## **ORIGINAL ARTICLE**

# Aquaculture Research WILEY

# Effects of an Enterococcus faecium-based probiotic on growth performance and health of Pirarucu, Arapaima gigas

Natalino da Costa Sousa<sup>1</sup> Márcia Valéria Silva do Couto<sup>1</sup> Higo Andrade Abe<sup>1</sup> Peterson Emmanuel Guimarães Paixão<sup>2</sup> I Carlos Alberto Martins Cordeiro<sup>3</sup> Emilly Monteiro Lopes<sup>3</sup> Jonathan Stuart Ready<sup>4</sup> Gabriel Fernandes Alves Jesus<sup>5</sup> Mauricio Laterça Martins<sup>5</sup> José Luiz Pereira Mouriño<sup>5</sup> I Paulo César Falanghe Carneiro<sup>6</sup> Alexandre Nizio Maria<sup>6</sup> Rodrigo Yudi Fujimoto<sup>6</sup>

<sup>1</sup>Graduate Program in Animal Science, Federal University of Pará (UFPA), Belém, Brazil

<sup>2</sup>Graduate Program in Health and Environmental, Tiradentes University (UNIT), Aracaju, Brazil

<sup>3</sup>Federal University of Pará (UFPA), Bragança, Brazil

<sup>4</sup>Laboratory for Integrated Ichthyology, Centre for Advanced Studies of Biodiversity, Federal University of Pará (UFPA), Belém, Brazil

<sup>5</sup>Aquaculture Department, AQUOS – Aquatic Organisms Health Laboratory, Federal University of Santa Catarina (UFSC), Florianópolis, Brazil

<sup>6</sup>Brazilian Agricultural Research Corporation (EMBRAPA), Aracaju, Brazil

#### Correspondence

Rodrigo Yudi Fujimoto, EMBRAPA-Brazilian Agricultural Research Corporation Tabuleiros Costeiros, Av Beira Mar 3250, 49025-040 Aracaju, SE, Brazil. Email: ryfujim@hotmail.com

**Funding information** FAPESPA-Amazon Foundation for Supporting Studies and Research

#### Abstract

Intensive fish farming has resulted in an increased concern for disease outbreaks. Probiotic use is one of the strategies being developed to improve fish health and productivity. Measures of probiotic colonization, growth performance, haematological characteristics and parasite load were used to evaluate the effect of diets supplemented with Enterococcus faecium on growth and health of Arapaima gigas juveniles. A completely randomized design with four treatments (diet with E. faecium at  $1 \times 10^{6}$  CFU/g and  $1 \times 10^{8}$  CFU/g, control diet and diet with the culture medium MRS) and three replicates was used. Ninety-six Arapaima juveniles were distributed in 12 cages fed with the specified diet for 68 days. Colonization of the intestinal tract by lactic acid bacteria reduced the total number of heterotrophic bacteria in fish fed with probiotics compared to controls. Fish fed a supplemented diet containing  $1 \times 10^8$  CFU/g presented higher values of weight gain, survival and fish growth uniformity, and lower values of feed conversion ratio. The prevalence of Trichodina sp. could have affected the survival of fish in the control group. Reduction in parasite load and an increase in haematocrit, the number of erythrocytes, thrombocytes, neutrophils and monocytes were also observed in fish fed the diet containing  $1 \times 10^8$  CFU/g. Enterococcus faecium presented a probiotic effect in A. gigas juveniles and can be recommended for use at a concentration of  $1 \times 10^8$  CFU/g to modify the gut microbiota, improve growth performance and haematology and reduce parasitic load.

#### KEYWORDS

Amazonian fish, pirarucu, probiotic, supplementation

# 1 | INTRODUCTION

Arapaima gigas is a species endemic to the Amazon region, known locally as 'pirarucu', and with a production of 8.6 tons in 2016. Its

production has expanded because it presents rapid growth, reaching 10 kg in just one year, breaths air and accepts artificial feed despite being a carnivorous fish (Cavero et al., 2003; IBGE, 2016; Oliveira et al., 2012). The production of native species has grown by 43.7% WILEY-

in Brazil (Brasil, 2013; Peixe Br, 2018), and this increase is a result of the use of new production strategies, including net cages and feeds to improve growth performance and immune responses (Brum et al., 2017; Inoue, Maciel, Affonso, Boijink, & Tavares-Dias, 2016; Merrifield et al., 2010).

The current intensification of aquaculture has led to an increase in the incidence of bacterial diseases, causing outbreaks in 82% of fish (Dong et al., 2015; Loch et al., 2017; Silva et al., 2012; Tavares et al., 2018). Therefore, antibiotics are commonly used to control these diseases. However, the widespread and inappropriate use of antibiotics may result in selection of resistant pathogenic strains as well as cause damage to the environment or humans (Martinez, 2009; Mo, Chen, Leung, & Leung, 2017).

In this context, reports are available indicating the use of probiotics to reduce disease outbreaks by enhancing immunological system (Dias et al., 2018; Soltani, Ghosh, et al., 2019; Soltani, Lymbery, Song, & Hosseini-Shekarabi, 2018). The use of probiotics is a strategy to increase production of fish, modulating the intestinal microbiota (Mouriño et al., 2015; Standen et al., 2015), improving the immune response (Giri, Sukumaran, & Oviya, 2013; Jatobá Moraes, Rodrigues, Vieira, & Pereira, 2018; Moraes et al., 2018; Mouriño et al., 2012) and improving growth performance (Dias et al., 2018; Giri et al., 2013; Hamdan, El-Sayed, & Mahmoud, 2016), during diverse stages of growth (Hamdan et al., 2016; Jha, Bhujel, & Anal, 2015; Nakandakare et al., 2013; Ridha & Azad, 2016).

Lactic acid bacteria have shown to be promising probiotics with positive results in various aquatic species including *Dicentrarchus labrax* (Piccolo et al., 2015), *Oreochromis niloticus* (Jatobá et al., 2018, 2011), *Labeo rohita* (Giri et al., 2013) and *Astyanax bimaculatus* (Moraes et al., 2018). The positive effect is due to their capacity to colonize the gastrointestinal tract, modifying the microbiota of the intestine and inhibiting the growth of other microorganisms, and improving the immune system (Giri et al., 2013; Jatobá et al., 2018; Mouriño et al., 2015; Yamashita et al., 2017).

However, little is known on the effect of lactic acid in open aquaculture systems such as cage systems with species such as *A. gigas*. The use of probiotics in *Arapaima* culture may be a useful tool to reduce mortality in critical phases and to improve the growth performance of the species in the cage systems.

Thus, this study was carried out to evaluate the effect of dietary supplementation with *Enterococcus faecium* on the growth performance and health of A. *gigas* juveniles.

#### 2 | MATERIAL AND METHODS

#### 2.1 | Enterococcus faecium strain

The bacteria *E. faecium* was isolated from healthy A. *gigas*, selected by in vitro assays by our research group according to Mouriño et al. (2016), identified by MALDI-TOF mass spectrometry (Microflex) (Seuylemezian et al., 2018), with identification confirmed using a molecular approach by Neoprospecta microbiome technologies (Santa Catarina State, Brazil). This study was approved by an Ethics Committee for Animal Experimentation (CEUA/172008FAPESPA01).

### 2.2 | Diet preparation

Commercial feed (crude protein 45%, fat 8%, moisture 12%, fibre 4%, mineral 14%, phosphorus 0.60% and calcium 2.5%, Nutripiscis-Purina) was sprinkled with *E. faecium* grown in Man Rogoza Sharpe (MRS: Acumedia<sup>®</sup>) culture at the concentrations  $1 \times 10^9$  CFU/ml and  $1 \times 10^7$  CFU/ml at ratio of 100 ml/kg of commercial feed. The feed containing *E. faecium* was homogenized in hermetically sealed containers and incubated at 35°C for 24 hr, after that growth time, the feed was dried at 35°C for a further 24 hr in aerobic conditions. This process was also performed for both the control feed using sterile MRS (no probiotic) and the control feed without either MRS or probiotic.

A test was made to verify the concentration of lactic acid bacteria in the processed feed. After drying, the different feed types were stored for 15 days at 20°C. Bacterial quantification was made daily by macerating 1 g of each feed type in 1 ml of 0.65% sterile saline solution and performing eight serial dilutions (1:10). Dilutions  $10^{-6}$  and  $10^{-8}$  were plated on agar MRS culture medium and incubated for 48 hr at 35°C to count the number of colony forming units (CFU/g).

#### 2.3 | Fish and experimental design

A total of 120 Arapaima juveniles were acclimatized for 10 days in tanks of 5,000 L. Then, 96 individuals (11.49  $\pm$  1.70 g and 12.55  $\pm$  1.42 cm) were selected by similar size and distributed in 12 net cages of 1 m<sup>3</sup> at stocking density of eight fish per cage. This stocking density and biomass corresponded to the smallest number of individuals required per replicate for statistical analysis and the minimum stocking density recommended by Cavero et al. (2003) for culture of the species in cages. During the following 68 days, fish were fed at 10% of live weight (adjusted based on biometric measures) three times per day (9:00, 14:00 and 17:00 hr).

The experiment followed a complete randomized design with three replicates and four treatments: control diet (CD); diet with bacterial culture medium MRS (DMRS); diet supplemented with *E. faecium* at the concentration of  $1 \times 10^6$  CFU/g (D10<sup>6</sup>); and diet supplemented with *E. faecium* at the concentration of  $1 \times 10^8$  CFU/g (D10<sup>8</sup>).

#### 2.4 | Growth and blood analysis

Biometric measures were taken twice (at 32 and 68 days) to determine the growth performance, determining biomass, weight gain, survival, feed conversion ratio (Cavero et al., 2003; Dias et al., 2018), specific growth rate (SGR(%): In final weight – In initial weight/days of experiment), relative condition factor (Kn: observed weight/expected weight) and fish uniformity growth following Ribeiro, Preto, and Kochenborger (2008). Blood samples were also taken from individuals by caudal puncture using 3% EDTA. Fish were anaesthetized by eugenol (60 mg/L) applied via gill absorption (Honczaryk & Inoue, 2009).

A blood aliquot was used to determine glucose levels using an AccuCheck Active<sup>®</sup> testing device, and another aliquot was used for preparing blood smears to obtain cell counts. The remaining blood was used to determine the following: total erythrocyte number (Er), haematocrit % (Ht) according to Goldenfarb, Bowyer, Hall, and Brosious (1971), total plasmatic protein (TPP) using a refractometer (Quimis<sup>®</sup>) and haemoglobin (Hb) by cyanmethemoglobin method (Collier, 1944). The following haematological parameters were also calculated: mean corpuscular volume (MCV: Ht × 10/Er); mean corpuscular haemoglobin (MCH: Hb × 10/Er); and mean concentration of corpuscular haemoglobin (MCCH: Hb × 100/Ht) (Vallada, 1999).

#### 2.5 | Intestinal microbiota

At the end of the experiment, samples of 20% of the juveniles from each treatment were kept in fasting for 24 hr, anesthetized and then euthanized. One gram of midgut was sampled and homogenized in 0.65% sterile saline solution, diluted serially (1:10) and the dilutions  $10^{-4}$ and  $10^{-6}$  seeded on tryptone soya agar (TSA) and MRS agar plates. The plates were incubated at 30°C for 24 hr for total heterotrophic bacterial counts and for 48 hr for counts of Lactobacilli following Mouriño et al. (2012). Gram staining was performed to observe strains with the characteristics of *E. faecium* (Jatobá et al., 2011; Mouriño et al., 2012), and identification was carried out using MALDI-TOF mass spectrometry.

#### 2.6 | Parasitological analysis

An analysis of endo- and ectoparasites was made for all previously sampled fish. Fragments of muscle, gut, eyes, liver, spleen, swim bladder and mucus were observed under a microscope. The mucus sample was preserved in 10% formalin in order to quantify parasites in a Sedgwick-Rafter chamber. Parasites were identified according to Thatcher (2006). Prevalence and mean intensity were calculated according to Bush, Lafferty, Lotz, and Shostak (1997).

#### 2.7 | Statistical analysis

Data were tested for normality (Shapiro–Wilk) and homoscedasticity (Levene's). Subsequent analysis of variance tests were performed applying post hoc Tukey tests for separation of means (p < .05) (Zar, 2009). Microbiological counts showed non-parametric characteristics and were therefore log-transformed [logX10 (x + 1)].

#### 3 | RESULTS

#### 3.1 | Probiotic in the diet

After 9 days, the  $D10^6$  supplemented feed presented 6.76 ± 0.056 log CFU/g and the  $D10^8$  supplemented feed presented

8.79 ± 0.028 log CFU/g, maintaining their experimental concentrations. By the twelfth day, there was a significant reduction (p = .0057) in the probiotics cultured from the feeds (D10<sup>6</sup> 4.7 ± 0.36 log CFU/g and 6.10 ± 0.32 log CFU/g for D10<sup>8</sup> treatment). Therefore, new feed was made each nine days for the fish during subsequent experiments.

Aquaculture Research—WILF

#### 3.2 | Heterotrophic and probiotic bacterial count

A reduction in gut heterotrophic bacteria (Figure 1) concentration was observed in fish supplemented with D10<sup>8</sup> and D10<sup>6</sup> (4.32 ± 0.07 log CFU/g and 4.64 ± 0.37 log CFU/g, respectively) compared to control treatments (CD and DMRS) (p = .0062). Furthermore, a significant increase (p = .0021) in gut lactic acid bacteria concentration was found in fish supplemented with *E. faecium* (Figure 1), presenting colonization values of 5.78 ± 0.64 log CFU/g and 7.50 ± 0.52 log CFU/g for the D10<sup>6</sup> and D10<sup>8</sup> feed treatments, respectively, compared to 3.5 ± 0.50 log CFU/g in the DMRS feed treatment.

# 3.3 | Water quality, growth and haematological parameters

No differences in water quality were observed between treatments: temperature =  $31 \pm 1.1^{\circ}$ C; dissolved oxygen =  $6.9 \pm 0.8$  mg/L; total ammonia =  $0.32 \pm 0.2$  mg/L; and pH =  $7.6 \pm 0.6$ .

After 32 days of biomass, weight gain, survival and growth uniformity were higher and the feed conversion ratio lower for fish fed with the D10<sup>8</sup> treatment, compared to other treatments (Table 1). Most of these measures showed the same tendency after 68 days, though both the D10<sup>6</sup> and D10<sup>8</sup> treatments showed increased uniformity compared to control groups (Table 1). Furthermore, fish mortality significantly increased (p = .00039) in the not-supplemented



**FIGURE 1** Bacterial counts from the intestines of *Arapaima* gigas fed not-supplemented control diet (CD), not-supplemented control diet with MRS (DMRS) and supplemented diets with different concentrations of *Enterococcus faecium* (D106 and D108). Different letters indicate significant difference identified by Tukey test (5%)

TABLE 1 Growth performance of juveniles of Arapaima gigas fed with control diets and diets supplemented with Enterococcus faecium

	B (g)	WG (g)	S (%)	FCR	SGR (%)	U	Kn
Growth performance of Arapaima gigas juveniles after 32 days							
CD	$58.3 \pm 3.89^{b}$	$17.0 \pm 0.99^{b}$	$79.1 \pm 4.16^{b}$	$2.5 \pm 0.16^{b}$	$2.1\pm0.20^{a}$	$24.4 \pm 13.4^{b}$	$1.0 \pm 0.1^{a}$
DMRS	$58.7 \pm 3.91^{b}$	$16.9 \pm 1.45^{b}$	$79.1 \pm 4.18^{b}$	$2.5\pm0.08^{b}$	$2.4\pm0.10^{\text{a}}$	$27.7 \pm 34.6^{b}$	$1.0\pm0.10^{a}$
D10 <sup>6</sup>	$77.9 \pm 5.02^{b}$	$18.5 \pm 1.83^{ab}$	$86.6 \pm 4.83^{ab}$	$2.3\pm0.15^{b}$	$2.6 \pm 0.13^{a}$	$42.8 \pm 20.7^{b}$	$0.9 \pm 0.2^{a}$
D10 <sup>8</sup>	$129.2 \pm 4.81^{a}$	$20.8 \pm 1.04^{a}$	$95.8 \pm 7.1^{a}$	$2.0\pm0.09^{\text{a}}$	$2.6 \pm 0.51^{a}$	$78.5 \pm 16.2^{a}$	$0.9 \pm 0.2^{a}$
Growth performance of Arapaima gigas juveniles after 68 days							
CD	$363.2 \pm 14.3^{b}$	$76.5 \pm 18.8^{a}$	$66.6 \pm 7.4^{b}$	$2.6\pm0.32^{b}$	$2.9 \pm 0.12^{a}$	$33.3 \pm 18.0^{b}$	$0.9 \pm 0.1^{a}$
DMRS	$316.8 \pm 42.3^{b}$	67.7 ± 12.8 <sup>a</sup>	66.6 ± 7.1 <sup>b</sup>	$2.6\pm0.19^{b}$	$2.8 \pm 0.29^{a}$	$44.4 \pm 13.8^{b}$	$0.9 \pm 0.1^{a}$
D10 <sup>6</sup>	$346.4 \pm 51.8^{b}$	$67.2 \pm 12.5^{a}$	$70.8 \pm 6.9^{b}$	$2.4\pm0.25^{b}$	$2.8 \pm 0.15^{a}$	71.1 ± 7.70 <sup>a</sup>	$0.9 \pm 0.1^{a}$
D10 <sup>8</sup>	583.2 ± 38.7ª	$80.4 \pm 4.48^{a}$	95.8 ± 7.1 <sup>a</sup>	$2.0\pm0.04^{a}$	$3.0\pm0.17^{\text{a}}$	$73.8 \pm 7.10^{a}$	$0.9 \pm 0.1^{a}$

*Note*: Values are mean  $\pm$  *SD*. Different letters in the same column indicate significant differences identified by Tukey test (5%). Control diet (CD), control diet with MRS (DMRS) and diets supplemented with different concentrations of *E. faecium* (D10<sup>6</sup> and D10<sup>8</sup>).

Abbreviations: B, Biomass; FCR, feed conversion ratio; Kn, relative condition factor; S, survival; SGR, specific growth rate; U, growth uniformity; WG, weight gain.

treatments. The lactic acid bacteria count was slightly higher in the fish fed DMRS, but this was not associated with changes in growth performance.

Increased lactic acid bacteria in the intestines of fish in the D10<sup>8</sup> treatment resulted in an increase in the erythrocyte count (1.9  $\pm$  0.07  $\times$  10<sup>6</sup>  $\mu$ l<sup>-1</sup>) and haematocrit (44.5  $\pm$  1.6%) (*p* = .0011) compared to other treatments (Table 2).

Regarding the immune cells, higher values (p = .0020) of thrombocytes (11.6 ± 0.38 × 10<sup>3</sup> µl<sup>-1</sup>, D10<sup>8</sup> and 10.4 ± 0.22 × 10<sup>3</sup> µl<sup>-1</sup>, D10<sup>6</sup>) and total leucocytes (75.9 ± 0.62 × 10<sup>3</sup> µl<sup>-1</sup>, D10<sup>8</sup> and 70.2 ± 2.2 × 10<sup>3</sup> µl<sup>-1</sup>, D10<sup>6</sup>) were observer in both D10<sup>8</sup> and D10<sup>6</sup> treatments. More specifically, there was an increase in the number of neutrophils (21.7 ± 2.81 × 10<sup>3</sup> µl<sup>-1</sup>) and monocytes (14.2 ± 1.2 × 10<sup>3</sup> µl<sup>-1</sup>) under the D10<sup>8</sup> treatment, but only an increase on monocytes (12.4 ± 1.7 × 10<sup>3</sup> µl<sup>-1</sup>) under the D10<sup>6</sup> treatment compared to the CD and DMRS control treatments (Table 3).

#### 3.4 | Parasitological indices

A significant reduction (p = .0057) was observed in the prevalence and mean intensity of *Trichodina* sp. in mucus and in the mean intensity of the same parasite in the gills for fish in the D10<sup>8</sup> treatment. No significant differences were found for the prevalence and mean intensity of *Lernaea* sp. between the treatments evaluated (Table 4).

# 4 | DISCUSSION

The use of lactic acid bacteria as probiotics in fish diets has been reported as an animal benefit promoter, improving growth and disease resistance (Dawood et al., 2016; Giri et al., 2013; Jatobá et al., 2018; Mohammadian et al., 2019; Mouriño et al., 2015). However, the present study is the first to report on the potential use of autochthonous *E. faecium* to modulate intestinal microbiota

	CD	DMRS	D10 <sup>6</sup>	D10 <sup>8</sup>
Erythrocytes (×10 <sup>6</sup> μl <sup>-1</sup> )	$1.3 \pm 0.04^{b}$	$1.5 \pm 0.09^{b}$	1.7 ± 0.11 <sup>ab</sup>	$1.9 \pm 0.07^{a}$
Glucose (mg/dl)	$59.9 \pm 4.2^{a}$	$41.4 \pm 6.7^{a}$	44.9 ± 5.1ª	$52.5 \pm 4.4^{a}$
Haemoglobin (g/dl)	$12.4\pm1.03^{\text{a}}$	$13.5\pm0.94^{\text{a}}$	$13.4 \pm 1.03^{a}$	$13.4\pm0.99^{\text{a}}$
Protein (g/dl)	$4.65 \pm 0.63^{a}$	$4.82 \pm 0.89^{a}$	$4.48 \pm 0.63^{a}$	$4.54 \pm 0.72^{a}$
Haematocrit (%)	$37.96 \pm 2.1^{b}$	$41.09 \pm 1.9^{b}$	$40.85 \pm 1.9^{b}$	$44.5 \pm 1.6^{a}$
MCV (fL)	$271.27 \pm 18^{a}$	$264.12 \pm 30^{a}$	222.91 ± 35.9ª	$212.35 \pm 42.8^{a}$
MCH (g/dl)	$90.48 \pm 13^{a}$	$88.01 \pm 16.4^{a}$	73.24 ± 12.2ª	$72.84 \pm 15.9^{a}$
MCCH (g/dl)	$34.45 \pm 1.9^{a}$	$33.11 \pm 2.1^{a}$	34.27 ± 1.5 <sup>ª</sup>	$35.40 \pm 1.8^{a}$

**TABLE 2** Haematological parameters for red blood cells in *Arapaima gigas* juveniles fed with Control diet (CD), control diet with MRS (DMRS) and diets supplements with *Enterococcus faecium* (D10<sup>6</sup> and D10<sup>8</sup>)

*Note*: Values are mean  $\pm$  *SD*. Different letters in the same row indicate a significant difference identified by Tukey test (5%).

Abbreviations: MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume.

**TABLE 3** Total thrombocytes, total leucocytes and differentiated leucocytes (×  $10^3 \mu l^{-1}$ ) in Arapaima gigas juveniles fed with control diet (CD), control diet with MRS (DMRS) and diets supplemented with Enterococcus faecium (D10<sup>6</sup> and D10<sup>8</sup>)

	CD	DMRS	D10 <sup>6</sup>	D10 <sup>8</sup>
Thrombocytes	$5.3 \pm 0.52^{c}$	$5.7 \pm 0.1.52^{\circ}$	$10.4 \pm 0.58^{b}$	$11.6 \pm 0.62^{a}$
Leucocytes	$63.3 \pm 1.2^{b}$	$63.7 \pm 1.1^{b}$	$70.2 \pm 2.2^{ab}$	$75.9 \pm 1.62^{a}$
Lymphocytes	$50.4 \pm 1.02^{a}$	$51.3 \pm 0.99^{a}$	$47.4 \pm 1.8^{a}$	$49.9 \pm 1.4^{a}$
Monocytes	$4.8 \pm 2.1^{b}$	$3.7 \pm 3.2^{b}$	$12.4 \pm 1.7^{a}$	$14.2 \pm 1.2^{a}$
Neutrophils	$10.2 \pm 2.32^{b}$	$8.4 \pm 2.89^{b}$	$13.6 \pm 3.32^{ab}$	$21.7 \pm 1.11^{a}$

Aquaculture Research

*Note*: Values are mean  $\pm$  *SD*. Different letters in the same row indicate a significant difference identified by Tukey test (5%).

**TABLE 4** Prevalence (%) and mean intensity (MI  $\pm$  *SD*) of parasites in the mucus and gills of *Arapaima gigas* juveniles fed with not-supplemented diets (CD and DMRS) and diets supplemented with *Enterococcus faecium* (D10<sup>6</sup> and D10<sup>8</sup>)

	Trichodina sp.				Lernaea sp.	
	P (%)		МІ		P (%)	МІ
Treatment	Mucus	Gill	Mucus	Gill	Mucus	Mucus
CD	100 <sup>b</sup>	83.33ª	$102 \pm 10.2^{b}$	$32.3 \pm 3.5^{b}$	50 <sup>a</sup>	$1.33 \pm 0.02^{a}$
DMRS	100 <sup>b</sup>	83.33ª	$103 \pm 10.8^{b}$	39.5 ± 3.0 <sup>b</sup>	33.33ª	$0.66 \pm 0.01^{a}$
D10 <sup>6</sup>	66.66 <sup>ab</sup>	50.00 <sup>a</sup>	$66 \pm 7.8^{ab}$	$13.0 \pm 2.4^{ab}$	33.33ª	$0.66\pm0.01^{\text{a}}$
D10 <sup>8</sup>	50 <sup>a</sup>	33.33ª	$40 \pm 6.1^{a}$	$8.0 \pm 1.9^{a}$	33.33ª	$1.00 \pm 0.01^{a}$

*Note:* Different letters in the same column indicate a significant difference identified by Tukey test (5%).

to improve growth as well as haematological and immunological responses for A. gigas.

In order to obtain the benefits of probiotic supplementation, the colonization of the host's intestinal tract is primordial (Pérez-Sánchez, Ruiz-Zarzuela, Blas, & Balcázar, 2014; Verschuere, Rombaut, Sorgeloos, & Verstraete, 2000). Modifying the intestinal microbiota reduces the growth of pathogenic bacteria through the release of bacterins and reuterins as well as through competition for space and nutrients (Balcázar et al., 2008; Gatesoupe, 2008; Ghanbari, Jami, Kneifel, & Domig, 2013). In the present study, there is a beneficial effect from this probiotic supplementation for *Arapaima* juveniles.

Successful colonization of the A. *gigas* intestinal tract was observed both by the reduction in total heterotrophic bacteria counts in addition to the increase in *E. faecium* count in the gut of fish fed with supplemented feed. Lactic acid bacteria are reported to adhere to the intestinal mucosa, probably forming barriers against other bacteria and stimulating the immune system by occupying binding sites (He et al., 2017; Li et al., 2018). So, change in the intestinal microbiota of A. *gigas* promoted by feed with  $1 \times 10^8$  CFU/g (D10<sup>8</sup>) *E. faecium* is expected to have been the cause of the improvement in the growth and physiological performance.

Probiotic supplementation in fish can stimulate the hematopoietic organs, increasing the amount of red blood cells, increasing the capacity for oxygen transport, and even influencing the transport of metabolites and the humoral defence of the host (Ganguly & Prasad, 2012; Gobi et al., 2018) as observed in *O. niloticus* (Telli et al., 2014), *Colossoma macropomum* (Dias et al., 2018), *Cyprinus carpio* (Valiallahi, Pourabasali, Janalizadeh, & Bucio, 2018) and *Oncorchynchus mykiss* (Soltani, Kane, Taheri-Mirghaed, Pakzad, & Hosseini, 2019). Regarding growth performance, there was an increase of 8.6% in weight gain observed in fish fed with probiotic compared to the control treatments. This is within the range of 4%–39.2% observed in other fish fed with probiotic-supplemented diets (Dias et al., 2018; Giri et al., 2013; Jatobá et al., 2011; Soltani, Kane, et al., 2019; Standen et al., 2016). Improved fish growth with probiotic supplementation can be attributed to improved digestion as well an increase in intestinal villi, increasing the contact surface for nutrient uptake and production of digestive enzymes, improving digestibility and nutrient retention (Dawood et al., 2016; Dias et al., 2018; Gupta, Gupta, & Dhawan, 2014; Jatobá & Mouriño, 2015; Sankar, Philip, Philip, & Singh, 2017; Soltani, Kane, et al., 2019).

In the present study, although juveniles of A. gigas fed diets containing *E. faecium* at a concentration of  $1 \times 10^8$  CFU/g (D10<sup>8</sup>) had increased weight compared to control treatments during the first 32 days, there was no difference in weight gain after 68 days. This is attributed to lower survival of fish in the control treatments (66.6 ± 7.4%), which resulted in lower density in that cage, allowing for faster growth and weight gain, increasing its standard deviation. The other growth performance metrics continued to show improved results for the D10<sup>8</sup> treatment after 68 days.

These beneficial effects on growth performance also have been reported in *O. niloticus* (Abumourad, Kenwy, Ibrahim, Hanna, & Soliman, 2014; Lara-Flores & Olvera-Novoa, 2013; Wang, Tian, Yao, & Li, 2008), *Epinephelus coioide* (Sun, Yang, Ma, Song, & Li, 2012), *C. carpio* (Dehaghani, Baboli, Moghadam, Ziaei-Nejad, & Pourfarhadi, 2015) and *O. mykiss* (Abedian Amiri, Azari Takami, Afsharnasab, & Razavilar, 2017), improving weight gain, specific growth rate, feed conversion, protein efficiency and survival. -WILEY-

#### Aquaculture Research

The health and survival of fish may reflect the improvement in the immune system stimulated by the probiotic bacteria supplied in the diets (Falaye, Emikpe, Ogundipe, & Oyebanji, 2017; Jesus et al., 2016). In the present study, probiotic supplementation resulted in an increase in total leucocytes, thrombocytes, neutrophils and monocytes, indicating an immunostimulatory effect, resulting in a higher protection against adverse conditions and pathogens during cultivation as seen in other species (Dias et al., 2018; Falaye et al., 2017; Mouriño et al., 2015; Telli et al., 2014; Venkatalakshmi & Ebanasar, 2015). Thrombocytes are cells that act directly in the immune defence of fish participating in coagulation and phagocytic processes (Haugland, Jordal, & Wergeland, 2012; Li et al., 2016; Tavares-Dias, Ono, Pilarski, & Moraes, 2007). Neutrophils and monocytes are cells involved in the innate response, which are also extremely important because they recognize and migrate to inflamed tissues, performing phagocytosis of foreign bodies (Arango Duque & Descoteaux, 2014; Farias et al., 2016; Navegantes et al., 2017).

An increased resistance to pathogenic infections resulting from increased numbers and activity of these immune defence cells has been verified in *Piaractus mesopotamicus* (feed supplemented with a mix of *Bacillus cereus* and *B. subtilis*; Farias et al., 2016), *O. niloticus* (feed supplemented with *Lactobacillus plantarum*; Van Doan et al., 2019) and *C. carpio* (feed supplemented with *E. faecium*; Gopalakannan & Arul, 2011). These previous studies indicate generalized benefits of the use of probiotics and support their use in pirarucu rearing.

Fish fed the supplemented diet (D10<sup>8</sup> treatment) were also found to have lower infestation of *Trichodina* sp. in their mucus and gills compared to other treatments. Trichodinids are recognized as one of the main fish parasites that are responsible for economic losses due to high mortality rates in fish farming (Maciel, Garcia, Chagas, Fujimoto, & Tavares-Dias, 2018; Martins, Cardoso, Marchiori, & Pádua, 2015) and especially in mortality of pirarucu farming in conjunction with monogeneans, branchiura, protozoans and nematodes (Araujo et al., 2009; Marinho et al., 2013). The high mean intensity of trichodinids observed here in the experimental fish that did not receive feed that was supplemented with probiotics could have been the cause of higher mortality in these treatments.

The fish integument is directly in contact with the environment, with the mucus acting as the first physical barrier against parasite infestation (Guardiola, Cuesta, Abellán, Meseguer, & Esteban, 2014; Raj et al., 2011). The parasite resistance promoted by *E. faecium* may be associated with the colonization of these bacteria in the gut, resulting in stimulation of skin-associated lymphoid tissue (SALT), increasing the number of mucus-producing cells, total proteins (such as immunoglobulin) and enzymatic activities (lysozymes) (Das, Nakhro, Chowdhury, & Kamilya, 2013; Lazado & Caipang, 2014; Van Doan et al., 2019). These studies have been shown to increase resistance to parasitic infections in fish in association with an increase in leucocytes (Pieters, Brunt, Austin, & Lyndon, 2008). Again, this supports the use of probiotic-supplemented diets to reduce parasitic load as a viable strategy for pirarucu rearing.

In conclusion, the use of *E. faecium* as a probiotic dietary supplement for juveniles of *A. gigas* modulates the intestinal microbiota, improving the immune system, the growth performance and resistance to parasitic infections. Supplementation at concentration of  $1 \times 10^8$  CFU/g in the feed is recommended as a new strategy for the aquaculture of this fish species. The results also suggest an important role for these supplements in new studies that aim to understand modulation of the mucosal immune responses of fish integumentary systems.

#### ACKNOWLEDGMENTS

The authors thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for a doctoral scholarship to N.C. Sousa; the National Council of Scientific and Technological Development (CNPq) for financial support to M.L. Martins (305869/2014-0), J.L.P. Mouriño (308292/2014-6) and R.Y. Fujimoto (305195/2016-6); and FAPESPA-Amazon Foundation for Supporting Studies and Research for financial support.

#### CONFLICT OF INTEREST

The authors have no conflicts 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.

#### ORCID

Natalino Costa Sousa b https://orcid.org/0000-0002-7580-5650 Higo Andrade Abe b https://orcid.org/0000-0002-5717-9641 Peterson Emmanuel Guimarães Paixão b https://orcid. org/0000-0002-8949-4232 Mauricio Laterça Martins b https://orcid. org/0000-0002-0862-6927 José Luiz Pereira Mouriño b https://orcid. org/0000-0002-8619-0882

#### REFERENCES

- Abedian Amiri, A., Azari Takami, G., Afsharnasab, M., & Razavilar, V. (2017). The comparative effects of dietary supplementation with *Pediococcus acidilactici* and *Enterococcus faecium* on feed utilization, various health-related characteristics and yersiniosis in rainbow trout (Oncorhynchus mykiss Walbaum, 1792). Iranian Journal of Fisheries Sciences, 16, 753–773.
- Abumourad, I. M. K., Kenwy, A. M., Ibrahim, T. B., Hanna, M. I., & Soliman, W. S. (2014). Enterococcus faecium probiotic as a growth promoter and its impact on the expression of the host innate immune in cultured Oreochromis niloticus. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 5, 1747–1761.
- Arango Duque, G., & Descoteaux, A. (2014). Macrophage cytokines: Involvement in immunity and infectious diseases. *Frontiers in Immunology*, 5, 591. https://doi.org/10.3389/fimmu.2014.00491

Aquaculture Research

- Araujo, C. S. O., Gomes, A. L., Tavares-Dias, M., Andrade, S. M. S., Belem-Costa, A., Borges, J. T., ... Barbosa, M. N. (2009). Parasitic infections in pirarucu fry, *Arapaima gigas* Schinz, 1822 (Arapaimatidae) kept in a semi-intensive fish farm in Central Amazon, Brazil. *Veterinarski Arhiv*, 79, 499–507.
- Balcázar, J. L., Vendrell, D., de Blas, I., Ruiz-Zarzuela, I., Muzquiz, J. L., & Girones, O. (2008). Characterization of probiotic properties of lactic acid bacteria isolated from intestinal microbiota of fish. Aquaculture, 278, 188–191. https://doi.org/10.1016/j.aquac ulture.2008.03.014
- Brasil Ministério de Pesca e Aquicultura (MPA) (2013). Boletim estatístico da pesca e aquicultura, Brasilia-Brasil (128p.).
- Brum, A., Pereira, S. A., Owatari, M. S., Chagas, E. C., Chaves, F. C. M., Mouriño, J. L. P., & Martins, M. L. (2017). Effect of dietary essential oils of clove basil and ginger on Nile tilapia (*Oreochromis niloticus*) following challenge with *Streptococcus agalactiae*. *Aquaculture*, 468, 235–243. https://doi.org/10.1016/j.aquaculture.2016.10.020
- Bush, A. O., Lafferty, K. D., Lotz, M. J., & Shostak, A. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology*, 83, 575–583. https://doi.org/10.2307/3284227
- Cavero, B. A. S., Ituassú, D. R., Pereira-Filho, M., Roubach, R., Bordinhon, A. M., Fonseca, F. L. A., & Ono, E. A. (2003). Biomassa sustentável de juvenis de pirarucu em tanques-rede de pequeno volume. *Pesquisa Agropecuária Brasileira*, 38, 723–728. https://doi.org/10.1590/ S0100-204X2003000600008
- Collier, H. B. (1944). The standardizations of blood haemoglobin determinations. *Canadian Medical Association Journal*, 50, 550–552.
- Das, A., Nakhro, K., Chowdhury, S., & Kamilya, D. (2013). Effects of potential probiotic *Bacillus amyloliquifaciens* FPTB16 on systemic and cutaneous mucosal immune responses and disease resistance of catla (*Catla catla*). Fish & Shellfish Immunology, 35, 1547–1553. https ://doi.org/10.1016/j.fsi.2013.08.022
- Dawood, M. A. O., Koshio, S., Ishikawa, M., Yokoyama, S., El Basuini, M. F., Hossain, M. S., ... Moss, A. S. (2016). Effects of dietary supplementation of *Lactobacillus rhamnosus* or/and *Lactococcus lactis* on the growth, gut microbiota and immune responses of red sea bream, *Pagrus major. Fish & Shellfish Immunology*, 49, 275–285. https://doi. org/10.1016/j.fsi.2015.12.047
- Dehaghani, P. G., Baboli, M. J., Moghadam, A. T., Ziaei-Nejad, S., & Pourfarhadi, M. (2015). Effect of synbiotic dietary supplementation on survival, growth performance, and digestive enzyme activities of common carp (Cyprinus carpio) fingerlings. Czech Journal of Animal Science, 60, 224–232. https://doi.org/10.17221/8172-CJAS
- Dias, J. A., Abe, H. A., Sousa, N. C., Couto, M. V., Cordeiro, C. A., Meneses, J. O., ... Fujimoto, R. Y. (2018). Dietary supplementation with autochthonous *Bacillus cereus* improves growth performance and survival in tambaqui *Colossoma macropomum*. *Aquaculture Research*, 49, 3063– 3070. https://doi.org/10.1111/are.13767
- Dong, H. T., Nguyen, V. V., Le, H. D., Sangsuriya, P., Jitrakorn, S., Saksmerprome, V., ... Rodkhum, C. (2015). Naturally concurrent infections of bacterial and viral pathogens in disease outbreaks in cultured Nile tilapia (*Oreochromis niloticus*) farms. *Aquaculture*, 448, 427-435. https://doi.org/10.1016/j.aquac ulture.2015.06.027
- Falaye, A. E., Emikpe, B. O., Ogundipe, E. T., & Oyebanji, V. O. (2017). Effects of dietary supplementation of *Lactobacillus plantarum* on survival, hematological profile and histopathology of selected organs of *Clarias gariepinus* fingerlings. *Comparative Clinical Pathology*, 26, 61–69. https://doi.org/10.1007/s00580-016-2346-6
- Farias, T. H. V., Levy-Pereira, N., de Oliveira Alves, L., de Carla Dias, D., Tachibana, L., Pilarski, F., ... Ranzani-Paiva, M. J. T. (2016). Probiotic feeding improves the immunity of pacus, *Piaractus mesopotamicus*, during Aeromonas hydrophila infection. Animal Feed Science and Technology, 211, 137-144. https://doi.org/10.1016/j.anife edsci.2015.11.004

- Ganguly, S., & Prasad, A. (2012). Microflora in fish digestive tract plays significant role in digestion and metabolism. *Reviews in Fish Biology* and Fisheries, 22, 11–16. https://doi.org/10.1007/s11160-011-9214-x
- Gatesoupe, F. J. (2008). Updating the importance of lactic acid bacteria in fish farming: Natural occurrence and probiotic treatments. *Journal Molecular Microbiology Biotechnology*, 14, 107–114. https:// doi.org/10.1159/000106089
- Ghanbari, M., Jami, M., Kneifel, W., & Domig, K. J. (2013). Antimicrobial activity and partial characterization of bacteriocins produced by lactobacilli isolated from Sturgeon fish. *Food Control*, 32, 379–385. https ://doi.org/10.1016/j.foodcont.2012.12.024
- Giri, S. S., Sukumaran, V., & Oviya, M. (2013). Potential probiotic Lactobacillus plantarum VSG3 improves the growth, immunity, and disease resistance of tropical freshwater fish, Labeo rohita. Fish & Shellfish Immunology, 34, 660-666. https://doi.org/10.1016/j. fsi.2012.12.008
- Gobi, N., Vaseeharan, B., Chen, J. C., Rekha, R., Vijayakumar, S., Anjugam, M., & Iswarya, A. (2018). Dietary supplementation of probiotic *Bacillus licheniformis* Dahb1 improves growth performance, mucus and serum immune parameters, antioxidant enzyme activity as well as resistance against *Aeromonas hydrophila* in tilapia *Oreochromis mossambicus*. Fish & Shellfish Immunology, 74, 501–508. https://doi. org/10.1016/j.fsi.2017.12.066
- Goldenfarb, P. B., Bowyer, F. P., Hall, T., & Brosious, E. (1971). Reproducibility in the hematology laboratory: The microhematocrit determination. *American Journal of Clinical Pathology*, 56, 35–39. https://doi.org/10.1093/ajcp/56.1.35
- Gopalakannan, A., & Arul, V. (2011). Inhibitory activity of probiotic Enterococcus faecium MC13 against Aeromonas hydrophila confers protection against hemorrhagic septicemia in common carp Cyprinus carpio. Aquaculture International, 19, 973–985. https://doi. org/10.1007/s10499-011-9415-2
- Guardiola, F. A., Cuesta, A., Abellán, E., Meseguer, J., & Esteban, M. A. (2014). Comparative analysis of the humoral immunity of skin mucus from several marine teleost fish. *Fish & Shellfish Immunology*, 40, 24– 31. https://doi.org/10.1016/j.fsi.2014.06.018
- Gupta, A., Gupta, P., & Dhawan, A. (2014). Dietary supplementation of probiotics affects growth, immune response and disease resistance of Cyprinus carpio fry. Fish & Shellfish Immunology, 41, 113–119. https ://doi.org/10.1016/j.fsi.2014.08.023
- Hamdan, A. M., El-Sayed, A. F. M., & Mahmoud, M. M. (2016). Effects of a novel marine probiotic, *Lactobacillus plantarum* AH 78, on growth performance and immune response of Nile tilapia (*Oreochromis niloticus*). *Journal of Applied Microbiology*, 120, 1061–1073. https://doi. org/10.1111/jam.13081
- Haugland, G. T., Jordal, A. E. O., & Wergeland, H. I. (2012). Characterization of small, mononuclear blood cells from salmon having high phagocytic capacity and ability to differentiate into dendritic like cells. *PLoS ONE*, 7, e49260. https://doi.org/10.1371/journ al.pone.0049260
- He, S., Ran, C., Qin, C., Li, S., Zhang, H., de Vos, W. M., ... Zhou, Z. (2017). Anti-infective effect of adhesive probiotic Lactobacillus in fish is correlated with their spatial distribution in the intestinal tissue. *Scientific Reports*, 7, 13195. https://doi.org/10.1038/s41598-017-13466-1
- Honczaryk, A., & Inoue, L. A. K. (2009). Anestesia do pirarucu por aspersão direta nas brânquias do eugenol em solução aquosa. *Ciencia Rural*, 39, 577-579. https://doi.org/10.1590/S0103-8478200800 5000084
- Inoue, L. A. K. A., Maciel, P. O., Affonso, E. G., Boijink, C. L., & Tavares-Dias, M. (2016). Growth, parasitic infection and hematology in *Colossoma macropomum* Cuvier, 1818 fed diets containing *Allium sativum. Journal of Applied Ichthyology*, 32, 901–905. https://doi. org/10.1111/jai.13086
- Instituto Brasileiro de Geografia e Estatística IBGE (2016). Produção da Pecuária Municipal. Rio de Janeiro (Vol. 44, 51p.).

Aquaculture Research

Jatobá, A., Moraes, K. N., Rodrigues, E. F., Vieira, L. M., & Pereira, M. O. (2018). Frequency in the supply of Lactobacillus influence its probiotic effect for yellow tail lambari. *Ciência Rural*, 48, e20180042. https ://doi.org/10.1590/0103-8478cr20180042

WILEY-

- Jatobá, A., & Mouriño, J. L. P. (2015). Lactobacillus plantarum effect on intestinal tract of Oreochromis niloticus fingerlings. Ciência Animal Brasileira, 16, 45-53. https://doi.org/10.1590/1089-68916i127789
- Jatobá, A. B., Vieira, F. N., Neto, C. C. B., Mouriño, J. L. P., Silva, B. C., Seiffter, W. Q., & Andreatta, E. (2011). Diet supplemented with probiotic for Nile tilapia in polyculture system with marine shrimp. *Fish Physiology Biochemistry*, 37, 725–732. https://doi.org/10.1007/ s10695-011-9472-5
- Jesus, G., Vieira, F., Silva, B. C., Junior, M., Ushizima, T. T., Schmidt, E. C., ... Mouriño, J. (2016). Probiotic bacteria may prevent haemorrhagic septicaemia by maturing intestinal host defences in Brazilian native surubins. *Aquaculture Nutrition*, 23, 484–491. https://doi.org/10.1111/anu.12416
- Jha, D. K., Bhujel, R. C., & Anal, A. K. (2015). Dietary supplementation of probiotics improves survival and growth of Rohu (*Labeo rohita* Ham.) hatchlings and fry in outdoor tanks. *Aquaculture*, 435, 475–479. https ://doi.org/10.1016/j.aquaculture.2014.10.026
- Lara-Flores, M., & Olvera-Novoa, M. A. (2013). The use of lactic acid bacteria isolated from intestinal tract of Nile tilapia (Oreochromis niloticus), as growth promoters in fish fed low protein diets. Latin American Journal of Aquatic Research, 41, 490–497. http://dx.doi.org/103856/ vol41-issue3-fulltext-12
- Lazado, C. C., & Caipang, C. M. A. (2014). Mucosal immunity and probiotics in fish. Fish & Shellfish Immunology, 39, 78–89. https://doi. org/10.1016/j.fsi.2014.04.015
- Li, L., Dang, Y., Shen, Y., Xu, X., Huang, W., & Li, J. (2016). Hematological and immunological plasma assays for grass carp (*Ctenopharyngodon idella*) infected with Aeromonas hydrophila as an immune model in carp aquaculture. Fish & Shellfish Immunology, 55, 647–653. https:// doi.org/10.1016/j.fsi.2014.04.015
- Li, X., Ringø, E., Hoseinifar, S. H., Lauzon, H. L., Birkbeck, H., & Yang, D. (2018). The adherence and colonization of microorganisms in fish gastrointestinal tract. *Reviews in Aquaculture*, 11(3), 603–618. https ://doi.org/10.1111/raq.12248
- Loch, T. P., Hawke, J. P., Reichley, S. R., Faisal, M., Del Piero, F., & Griffin, M. J. (2017). Outbreaks of edwardsiellosis caused by Edwardsiella piscicida and Edwardsiella tarda in farmed barramundi (Lates calcarifer). Aquaculture, 481, 202–210. https://doi.org/10.1016/j.aquac ulture.2017.09.005
- Maciel, P. O., Garcia, F., Chagas, E. C., Fujimoto, R. Y., & Tavares-Dias, M. (2018). Trichodinidae in commercial fish in South America. *Reviews* in Fish Biology and Fisheries, 28, 33–56. https://doi.org/10.1007/ s11160-017-9490-1
- Marinho, R. G. B., Tavares-Dias, M., Dias-Grigório, M. K. R., Neves, L. R., Yoshioka, E. T. O., Boijink, C. D. L., & Takemoto, R. M. (2013). Helminthes and protozoan of farmed pirarucu (*Arapaima gigas*) in eastern Amazon and host-parasite relationship. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 65, 1192–1202. https://doi.org/10.1590/S0102-09352013000400035
- Martinez, J. L. (2009). Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environmental Pollution*, 157, 2893–2902. https://doi.org/10.1016/j.envpol.2009.05.051
- Martins, M. L., Cardoso, L., Marchiori, N., & Pádua, S. B. (2015). Protozoan infections in farmed fish from Brazil: Diagnosis and pathogenesis. *Revista Brasileira de Parasitologia Veterinária*, 24, 1–20. https://doi. org/10.1590/S1984-29612015013
- Merrifield, D. L., Dimitroglou, A., Foey, A., Davies, S. J., Baker, R. T. M., Bogwald, J., ... Ringo, E. (2010). The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture*, 302, 1–18. https://doi.org/10.1016/j.aquaculture.2010.02.007

- Mo, W. Y., Chen, Z., Leung, H. M., & Leung, A. O. W. (2017). Application of veterinary antibiotics in China's aquaculture industry and their potential human health risks. *Environmental Science and Pollution Research*, 24, 8978–8989. https://doi.org/10.1007/s11356-015-5607-z
- Mohammadian, T., Alishahi, M., Tabandeh, M. R., Nejad, A. J., Karami, E., & Zarea, M. (2019). Effects of autochthonous probiotics, isolated from *Tor grypus* (Karaman, 1971) intestine and *Lactobacillus casei* (PTCC 1608) on expression of immune-related genes. *Aquaculture International*, *27*, 239–260. https://doi.org/10.1007/ s10499-018-0320-9
- Moraes, A. V. D., Pereira, M. D. O., Moraes, K. N., Rodrigues-Soares, J. P., Jesus, G. F. A., & Jatobá, A. (2018). Autochthonous probiotic as growth promoter and immunomodulator for Astyanax bimaculatus cultured in water recirculation system. Aquaculture Research, 49, 2808–2814. https://doi.org/10.1111/are.13743
- Mouriño, J. L. P., Pereira, G. V., Vieira, F. N., Jatobá, A. B., Ushizima, T. T., da Silva, B. C., ... Martins, M. L. (2016). Isolation of probiotic bacteria from the hybrid South American catfish *Pseudoplatystoma reticulatum× Pseudoplatystoma corruscans* (Siluriformes: Pimelodidae): A haematological approach. *Aquaculture Reports*, *3*, 166–171. https:// doi.org/10.1016/j.aqrep.2016.03.001
- Mouriño, J. L. P., Vieira, F. D., Jatobá, A. B., Silva, B. C., Jesus, G. F. A., Seiffert, W. Q., & Martins, M. L. (2012). Effect of dietary supplementation of inulin and W. cibaria on haemato-immunological parameters of hybrid surubim (*Pseudoplatystoma* sp). Aquaculture Nutrition, 18, 73–80. https://doi.org/10.1111/j.1365-2095.2011.00879.x
- Mouriño, J. L. P., Vieira, F. N., Jatobá, A., Silva, B. C., Pereira, G. V., Jesus, G. F. A., & Martins, M. L. (2015). Symbiotic supplementation on the hemato-immunological parameters and survival of the hybrid surubim after challenge with Aeromonas hydrophila. Aquaculture Nutrition, 23, 276–284. https://doi.org/10.1111/anu.12390
- Nakandakare, I. B., Iwashita, M. K. P., Dias, D. C., Tachibana, L., Ranzani-Paiva, M. J. T., & Romagosa, E. (2013). Incorporação de probiótico na dieta para juvenis de tilápia do Nilo: Parâmetros hematológicos, imunológicos e microbiológicos. *Boletim Instituto de Pesca*, 32, 121-135.
- Navegantes, K. C., Gomes, R. S., Pereira, P. A. T., Czaikoski, P. G., Azevedo, C. H. M., & Monteiro, M. C. (2017). Immune modulation of some autoimmune diseases: The critical role of macrophages and neutrophils in the innate and adaptive immunity. *Journal of Translational Medicine*, 15, 36. https://doi.org/10.1186/s12967-017-1141-8
- Oliveira, E. G., Pinheiro, A. B., de Oliveira, V. Q., da Silva Júnior, A. R. M., de Moraes, M. G., Rocha, Í. R. C. B., ... Costa, F. H. F. (2012). Effects of stocking density on the performance of juvenile pirarucu (*Arapaima* gigas) in cages. *Aquaculture*, 370, 96–101. https://doi.org/10.1016/j. aquaculture.2012.09.027
- Peixe BR (2018). Anuário Peixe Br da piscicultura (71p).
- Pérez-Sánchez, T., Ruiz-Zarzuela, I., Blas, I., & Balcázar, J. L. (2014). Probiotics in aquaculture: A current assessment. *Reviews in Aquaculture*, 6, 133–146. https://doi.org/10.1111/raq.12033
- Piccolo, G., Bovera, F., Lombardi, P., Mastellone, V., Nizza, S., Di Meo, C., ... Nizza, A. (2015). Effect of *Lactobacillus plantarum* on growth performance and hematological traits of European sea bass (*Dicentrarchus labrax*). Aquaculture International, 23, 1025–1032. https://doi.org/10.1007/s10499-014-9861-8
- Pieters, N., Brunt, J., Austin, B., & Lyndon, A. R. (2008). Efficacy of infeed probiotics against Aeromonas bestiarum and Ichthyophthirius multifiliis skin infections in rainbow trout (Oncorhynchus mykiss, Walbaum). Journal of Applied Microbiology, 105, 723–732. https://doi. org/10.1111/j.1365-2672.2008.03817.x
- Raj, V., Fournier, G., Rakus, K., Ronsmans, M., Ouyang, P., Michel, B., ... Vanderplasschen, A. (2011). Skin mucus of *Cyprinus carpio* inhibits cyprinid herpesvirus 3 binding to epidermal cells. *Veterinary Research*, 42, 92. https://doi.org/10.1186/1297-9716-42-92

- Ribeiro, F. A. S., Preto, B. L., & Kochenborger, F. (2008). Culture systems for freshwater angelfish (*Pterophyllum scalare*). Acta Scientiarum Animal Scienc, 30, 459–466.
- Ridha, M. T., & Azad, I. S. (2016). Effect of autochthonous and commercial probiotic bacteria on growth, persistence, immunity and disease resistance in juvenile and adult Nile tilapia Oreochromis niloticus. Aquaculture Research, 47, 2757–2767. https://doi.org/10.1111/ are.12726
- Sankar, H., Philip, B., Philip, R., & Singh, I. S. B. (2017). Effect of probiotics on digestive enzyme activities and growth of cichlids, *Etroplus suratensis* (Pearl spot) and Oreochromis mossambicus (Tilapia). Aquaculture Nutrition, 23, 852–864. https://doi.org/10.1111/anu.12452
- Seuylemezian, A., Aronson, H. S., Tan, J., Lin, M., Schubert, W., & Vaishampayan, P. (2018). Development of a custom MALDI-TOF MS database for species-level identification of bacterial isolates collected from spacecraft and associated surfaces. *Frontiers in Microbiology*, 9, 780. https://doi.org/10.3389/fmicb.2018.00780
- Silva, B. C., Mouriño, J. L. P., Vieira, F. N., Jatobá, A., Seiffert, W. Q., & Martins, M. L. (2012). Haemorrhagic septicaemia in the hybrid surubim (*Pseudoplatystoma corruscans* and *Pseudoplatystoma fasciatum*) caused by Aeromonas hydrophila. Aquaculture Research, 43, 908–916. https://doi.org/10.1111/j.1365-2109.2011.02905.x
- Soltani, M., Ghosh, K., Hoseinifar, S. H., Kumar, V., Lymbery, A. J., Roy, S., & Ringo, E. (2019). Genus *Bacillus*, promising probiotics in aquaculture: Aquatic animal origin, bio-active components, bioremediation and efficacy in fish and shellfish. *Reviews in Fisheries Science* & Aquaculture, 27, 331–379. https://doi.org/10.1080/23308 249.2019.159010
- Soltani, M., Kane, A., Taheri-Mirghaed, A., Pakzad, K., & Hosseini, P. S. (2019). Effect of the probiotic, *Lactobacillus plantarum* on growth performance and haematological indices of rainbow trout (*Oncorhynchus mykiss*) immunized with bivalent *streptococcosis/lactococcosis* vaccine. *Iranian Journal of Fisheries Sciences*, 18, 283–295. https://doi. org/10.22092/ijfs.2018.117757
- Soltani, M., Lymbery, A., Song, S. K., & Hosseini-Shekarabi, P. (2018). Adjuvant effects of medicinal herbs and probiotics for fish vaccines. *Reviews in Aquaculture*, 1–17. https://doi.org/10.1111/raq.12295
- Standen, B. T., Peggs, D. L., Rawling, M. D., Foey, A., Davies, S. J., Santos, G. A., & Merrifield, D. L. (2016). Dietary administration of a commercial mixed-species probiotic improves growth performance and modulates the intestinal immunity of tilapia, *Oreochromis niloticus*. *Fish & Shellfish Immunology*, 49, 427–435. https://doi.org/10.1016/j. fsi.2015.11.037
- Standen, B. T., Rodiles, A., Peggs, D. L., Davies, S. J., Santos, G. A., & Merrifield, D. L. (2015). Modulation of the intestinal microbiota and morphology of tilapia, Oreochromis niloticus, following the application of a multi-species probiotic. Applied Microbiology and Biotechnology, 99, 8403–8417. https://doi.org/10.1007/s00253-015-6702-2
- Sun, Y. Z., Yang, H. L., Ma, R. L., Song, K., & Li, J. S. (2012). Effect of Lactococcus lactis and Enterococcus faecium on growth performance, digestive enzymes and immune response of grouper Epinephelus coioides. Aquaculture Nutrition, 18, 281–289. https://doi. org/10.1111/j.1365-2095.2011.00894.x
- Tavares, G. C., de Queiroz, G. A., Assis, G. B. N., Leibowitz, M. P., Teixeira, J. P., Figueiredo, H. C. P., & Leal, C. A. G. (2018). Disease outbreaks in farmed Amazon catfish (*Leiarius marmoratus × Pseudoplatystoma corruscans*) caused by *Streptococcus agalactiae*, *S. iniae*, *and S.*

*dysgalactiae*. *Aquaculture*, 495, 384–392. https://doi.org/10.1016/j. aquaculture.2018.06.027

Aquaculture Research

- Tavares-Dias, M., Ono, E. A., Pilarski, F., & Moraes, F. R. (2007). Can thrombocytes participate in the removal of cellular debris in the blood circulation of teleost fish? A cytochemical study and ultrastructural analysis. *Journal of Applied Ichthyology*, 23, 709–712. https ://doi.org/10.1111/j.1439-0426.2007.00850.x
- Telli, G. S., Ranzani-Paiva, M. J. T., de Carla Dias, D., Sussel, F. R., Ishikawa, C. M., & Tachibana, L. (2014). Dietary administration of *Bacillus subtilis* on hematology and non-specific immunity of Nile tilapia Oreochromis niloticus raised at different stocking densities. *Fish & Shellfish Immunology*, 39, 305–311. https://doi.org/10.1016/j. fsi.2014.05.025
- Thatcher, V. E. (2006). Amazon fish parasites (Vol. 1, 496p). Sofia, Bulgaria: Pensoft Publishers.
- Valiallahi, J., Pourabasali, M., Janalizadeh, E., & Bucio, A. (2018). Use of *Lactobacillus* for improved growth and enhanced biochemical, hematological, and digestive enzyme activity in common carp at Mazandaran, Iran. North American Journal of Aquaculture, 80, 206– 215. https://doi.org/10.1002/naaq.10027
- Vallada, E. P. (1999). Manual de Técnicas Hematológicas (p. 104p). São Paulo, Brazil: Editora Atheneu.
- Van Doan, H., Hoseinifar, S. H., Tapingkae, W., Seel-audom, M., Jaturasitha, S., Dawood, M. A., ... Esteban, M. Á. (2019). Boosted growth performance, mucosal and serum immunity, and disease resistance Nile Tilapia (Oreochromis niloticus) fingerlings using corncob-derived Xylooligosaccharide and Lactobacillus plantarum CR1T5. Probiotics and Antimicrobial Proteins, 22, 1–12, https://doi. org/10.1007/s12602-019-09554-5
- Venkatalakshmi, S., & Ebanasar, J. (2015). Immunostimulatory effect of Lactobacillus sporogenes on the nonspecific defense mechanisms of Oreochromis mossambicus (Peters). International Journal of Fisheries and Aquatic Studies, 2, 362–369.
- Verschuere, L., Rombaut, G., Sorgeloos, P., & Verstraete, W. (2000). Probiotic bacteria as biological control agents in aquaculture. *Microbiology and Molecular Biology Reviews*, 64, 655–671. https://doi. org/10.1128/MMBR.64.4.655-671.2000
- Wang, Y. B., Tian, Z. Q., Yao, J. T., & Li, W. F. (2008). Effect of probiotics, *Enteroccus faecium*, on tilapia (*Oreochromis niloticus*) growth performance and immune response. *Aquaculture*, 277, 203–207. https://doi. org/10.1016/j.aquaculture.2008.03.007
- Yamashita, M. M., Pereira, S. A., Cardoso, L., de Araujo, A. P., Oda, C. E., Schmidt, É. C., ... Mouriño, J. L. P. (2017). Probiotic dietary supplementation in Nile tilapia as prophylaxis against streptococcosis. *Aquaculture Nutrition*, 23(6), 1235–1243. https://doi.org/10.1111/ anu.12498
- Zar, J. H. (2009). *Biostatistical analysis* (5th ed., p. 576p). Upper Saddle River, NJ, USA: Prentice-Hall Inc.

How to cite this article: da Costa Sousa N, do Couto MVS, Abe HA, et al. Effects of an *Enterococcus faecium*-based probiotic on growth performance and health of Pirarucu, *Arapaima gigas. Aquac Res.* 2019;00:1–9. <u>https://doi.</u> org/10.1111/are.14332