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SHORT COMMUNICATION

Inflammatory response in swim bladder caused by *Aeromonas hydrophila* in tambaqui (*Colossoma macropomum* Cuvier, 1816) supplemented with an autochthonous probiotic (*Bacillus cereus*)

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Worldwide, fish production yields approximately 171 million tonnes per year, of which 54.3 tonnes are from fish farming (FAO, 2020). In Brazil, fish farming produced 758,006 tonnes in 2019, with the *Colossoma macropomum* the native species being the most produced in national territory (Chagas et al., 2019; Peixebr, 2020).

On the other hand, the increased fish production has led to dissemination of infectious agents, such as bacteria, which contribute to elevated mortality rates of farmed fishes (Mouriño et al., 2017; Munir et al., 2019).

In this scenario, fish farmers commonly use antibiotics to control the outbreaks. However, its improper use results in mutation of DNA and selection of resistant bacteria in addition to causing bioaccumulation in the environment, in the musculature of fish and even in humans (Gasser et al., 2019; Van Doan et al., 2019).

For this reason, the employment of safe and prophylactic measures has become recommended for the sustainable aquaculture. Among these methods, the use of probiotics stands out once they act as growth promoter and immunostimulant (Sousa et al., 2019; Van Doan et al., 2019).

Evaluation of the inflammatory response in the swim bladder is a method used to determine the relationship between probiotic and inflammatory processes (Herbert et al., 2000). Increases of inflammatory cells in exudates subjected to different pathogens were observed in different fish species, such as catfish responding to *Edardsiella ictaluri* injection (Herbert et al., 2000) and *Oreochromis niloticus* (Matushima & Mariano, 1996) and *Piaractus mesopotamicus* (2001) in response to carrageenan.

In this context, autochthonous probiotic could aid as a beneficial tool to improve fish performance including host feed efficiency (Dias et al., 2018). However, there are no reports about inflammatory response of *C. macropomum* supplemented with autochthonous probiotic after phlogogen injection.

Finally, the current study aimed to evaluate the inflammatory response induced by *Aeromonas hydrophila* injected into the swim

TABLE 1 The mean and standard deviation values for the blood glucose levels, erythrogram and plasma protein from tambaqui supplemented with a probiotic diet containing *Bacillus cereus* during an inflammatory assay of the swim bladder

		Time (H)				
Parameters	**Treatment**	**12**	**24**	**48**	**72**	**96**
Glucose (mg.100 ml^{-1})	NC	38.00 ± 1.00AB^{a}	36.66 ± 1.52BC^{ab}	36.00 ± 1.73AB^{ab}	34.67 ± 1.15AB^{ab}	32.66 ± 2.30BC^{b}
	PC	34.66 ± 1.52B^{a}	31.66 ± 2.08C^{ab}	22.66 ± 4.2C^{c}	26.00 ± 1.73C^{bc}	28.33 ± 0.57C^{abc}
	10^{4} CFU.g^{-1}	44.33 ± 4.93AB^{ab}	48.33 ± 1.52A^{a}	29.66 ± 3.05BC^{c}	38.33 ± 1.52A^{bc}	43.66 ± 5.13A^{ab}
	10^{6} CFU.g^{-1}	48.33 ± 5.50A^{a}	44.33 ± 3.21AB^{ab}	38.33 ± 2.30A^{b}	39.66 ± 0.57A^{b}	39.66 ± 0.57AB^{b}
	10^{8} CFU.g^{-1}	39.00 ± 5.56AB^{a}	36.33 ± 5.50BC^{a}	29.00 ± 1.00BC^{a}	30.33 ± 4.04BC^{a}	35.66 ± 4.50ABC^{a}
Erythrocyte (x10^{6} μl^{-1})	NC	1.77 ± 0.02A^{a}	1.68 ± 0.09A^{a}	1.66 ± 0.03A^{a}	1.66 ± 0.03A^{a}	1.75 ± 0.20AB^{a}
	PC	1.13 ± 0.04A^{a}	1.22 ± 0.03A^{a}	0.40 ± 0.17B^{b}	0.67 ± 0.06B^{b}	0.96 ± 0.28B^{ab}
	10^{4} CFU.g^{-1}	1.69 ± 0.05A^{a}	1.11 ± 0.05A^{a}	0.66 ± 0.08B^{b}	1.62 ± 0.58A^{a}	1.71 ± 0.21AB^{a}
	10^{6} CFU.g^{-1}	1.83 ± 0.09A^{a}	1.57 ± 0.06A^{a}	0.85 ± 0.11B^{b}	1.63 ± 0.08A^{a}	1.93 ± 0.29AB^{a}
	10^{8} CFU.g^{-1}	2.08 ± 0.23A^{a}	1.61 ± 0.14A^{a}	1.26 ± 0.53B^{a}	1.26 ± 0.04A^{a}	2.72 ± 1.08A^{a}
Haematocrit (%)	NC	25.46 ± 1.04B^{a}	26.46 ± 2.69A^{a}	26.23 ± 1.78A^{a}	26.12 ± 5.96A^{a}	27.10 ± 2.26A^{a}
	PC	25.16 ± 1.60B^{a}	27.16 ± 3.21A^{a}	15.62 ± 4.33B^{b}	11.88 ± 0.32B^{b}	28.33 ± 2.25A^{a}
	10^{4} CFU.g^{-1}	28.00 ± 3.04AB^{a}	26.83 ± 1.75A^{a}	21.16 ± 2.84AB^{a}	15.00 ± 6.06AB^{a}	25.50 ± 8.52A^{a}
	10^{6} CFU.g^{-1}	25.00 ± 1.00B^{ab}	26.00 ± 2.64A^{ab}	19.83 ± 2.08AB^{b}	25.66 ± 5.85A^{ab}	33.16 ± 2.46A^{a}
	10^{8} CFU.g^{-1}	31.66 ± 1.52A^{a}	30.66 ± 2.56A^{a}	25.33 ± 1.60A^{ab}	21.16 ± 2.25AB^{b}	29.33 ± 5.48A^{a}
Total protein (g dl^{-1})	NC	5.27 ± 0.38A^{ab}	5.36 ± 0.28A^{a}	5.49 ± 0.10A^{a}	4.47 ± 0.23AB^{b}	4.42 ± 0.21B^{b}
	PC	4.75 ± 0.13A^{a}	4.58 ± 0.16B^{a}	2.36 ± 0.37C^{c}	2.85 ± 0.08C^{c}	3.56 ± 0.20C^{b}
	10^{4} CFU.g^{-1}	4.91 ± 0.48A^{a}	4.40 ± 0.45B^{ab}	3.68 ± 0.28B^{b}	3.9 ± 0.13B^{b}	3.93 ± 0.17BC^{b}
	10^{6} CFU.g^{-1}	5.18 ± 0.37A^{ab}	5.26 ± 0.27A^{a}	3.48 ± 0.12B^{c}	4.4 ± 0.22AB^{b}	5.40 ± 0.10A^{a}
	10^{8} CFU.g^{-1}	5.58 ± 0.20A^{a}	5.70 ± 0.44A^{a}	4.01 ± 0.12B^{b}	4.83 ± 0.17A^{ab}	5.38 ± 0.12A^{a}
Haemoglobin (g dl^{-1})	NC	10.26 ± 1.02A^{a}	10.16 ± 1.01A^{a}	10.14 ± 1.01A^{a}	10.11 ± 1.00A^{a}	10.08 ± 1.00A^{a}
	PC	9.83 ± 0.94A^{a}	4.81 ± 1.23C^{b}	2.46 ± 0.34D^{c}	4.26 ± 0.50C^{bc}	4.03 ± 0.20B^{bc}
	10^{4} CFU.g^{-1}	10.38 ± 0.96A^{a}	9.17 ± 0.86AB^{a}	5.98 ± 1.65B^{b}	9.40 ± 0.62A^{a}	10.05 ± 1.00A^{a}
	10^{6} CFU.g^{-1}	11.47 ± 0.60A^{a}	8.20 ± 0.70B^{b}	3.65 ± 0.48CD^{c}	8.16 ± 0.69A^{b}	9.87 ± 0.43A^{a}
	10^{8} CFU.g^{-1}	10.60 ± 0.64A^{a}	9.49 ± 0.15AB^{ab}	5.20 ± 0.52BC^{d}	7.41 ± 0.77B^{c}	8.94 ± 0.43A^{b}
MCV (μm^{-3})	NC	143.36 ± 6.08A^{a}	157.38 ± 15.39A^{a}	222.68 ± 15.56A^{a}	157.43 ± 37.61A^{a}	150.25 ± 10.66A^{a}
	PC	140.89 ± 9.53A^{b}	221.11 ± 20.47A^{a}	209.70 ± 24.70A^{a}	178.30 ± 17.88A^{a}	164.98 ± 63.82A^{ab}
	10^{4} CFU.g^{-1}	165.27 ± 15.79A^{b}	217.63 ± 40.73A^{a}	220.30 ± 14.62A^{a}	108.94 ± 40.54A^{b}	147.18 ± 22.44A^{b}
	10^{6} CFU.g^{-1}	136.47 ± 12.43A^{a}	164.49 ± 10.47A^{a}	234.58 ± 22.70A^{a}	157.37 ± 36.31A^{a}	173.84 ± 24.77A^{a}
	10^{8} CFU.g^{-1}	153.56 ± 24.40A^{ab}	191.86 ± 30.83A^{ab}	224.50 ± 89.69A^{a}	166.77 ± 12.95A^{ab}	113.88 ± 24.96A^{ab}

(Continues)

TABLE 1 (Continued)

Parameters	Treatment	Time (H)				
		12	24	48	72	96
MCH (g dl^{-1})	NC	57.79 ± 5.78A^{a}	60.44 ± 5.96A^{a}	60.91 ± 7.16A^{a}	60.85 ± 6.57A^{a}	57.68 ± 4.73A^{a}
	PC	60.27 ± 6.08A^{a}	59.08 ± 9.17A^{b}	69.14 ± 28.85A^{ab}	64.40 ± 12.76A^{ab}	44.22 ± 12.84A^{ab}
	10^4 CFU.g^{-1}	61.33 ± 5.50A^{a}	75.37 ± 20.74A^{a}	92.74 ± 33.55A^{a}	63.88 ± 26.60A^{a}	59.68 ± 13.38A^{a}
	10^6 CFU.g^{-1}	62.44 ± 0.98A^{a}	58.89 ± 2.44A^{ab}	43.77 ± 9.43A^{b}	49.97 ± 2.52A^{ab}	51.81 ± 7.50A^{ab}
	10^8 CFU.g^{-1}	51.02 ± 2.84A^{a}	59.12 ± 4.63A^{ba}	45.00 ± 13.20A^{a}	58.40 ± 4.93A^{a}	35.91 ± 11.86A^{b}
MCHC (g dl^{-1})	NC	40.38 ± 4.84A^{a}	38.43 ± 2.24A^{a}	27.40 ± 1.24A^{a}	40.72 ± 13.81A^{a}	38.38 ± 1.31A^{a}
	PC	38.17 ± 4.35A^{a}	33.51 ± 2.67A^{a}	26.81 ± 5.87A^{b}	35.97 ± 4.99A^{a}	34.33 ± 1.84A^{a}
	10^4 CFU.g^{-1}	37.53 ± 6.79A^{a}	34.36 ± 5.17A^{a}	29.18 ± 11.08A^{a}	37.76 ± 18.97A^{a}	34.25 ± 4.95A^{a}
	10^6 CFU.g^{-1}	46.00 ± 4.21A^{a}	31.60 ± 2.07A^{bc}	28.53 ± 2.65A^{c}	33.24 ± 9.71A^{ab}	28.81 ± 0.88B^{bc}
	10^8 CFU.g^{-1}	33.61 ± 3.72A^{a}	31.10 ± 2.67A^{a}	30.64 ± 2.85A^{b}	35.22 ± 4.85A^{a}	31.00 ± 4.20A^{a}

Note: Different letters indicate significant difference among the treatments ($p < 0.05$). Uppercase letters for comparison between treatments and lowercase letters for comparison of time periods in the same treatment. NC—negative control with NaCl in the swim bladder; PC—positive control injected with inactivated A. hydrophila in the swim bladder.

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

bladder of tambaqui (*C. macropomum*) supplemented with the autochthonous bacterium *Bacillus cereus*.

Previously, 125 juveniles of *C. macropomum* (77.8 ± 4.73 g) were submitted to different diets containing the autochthonous bacterium *B. cereus* (4.2×10^4, 3.9×10^6 and 3.3×10^8 CFU.g^{-1} of ration) and a diet without a probiotic for 120 days (Dias et al., 2018).

For the inflammatory induction process, twenty-five fish (25) from each experimental group (probiotic levels) were anaesthetized by eugenol (60 mg L^{-1} Honczaryk & Inoue, 2009; Souza et al., 2019) sprinkled directly on the gills. Afterwards, they received an injection of 100 µl *A. hydrophila* (2.7×10^6 CFU.g^{-1}), as a phlogogen, into the swim bladder anteromedial region at the end of the operculum and at the height of the lateral line. *A. hydrophila* was previously inactivated at 40°C for 30 min and resuspended in sterile saline solution (0.65% NaCl) (Reque et al., 2010).

Furthermore, fish from the diet without probiotic supplementation were further divided into two groups; the former received injections containing the phlogogen, and the latter received injections of sterile saline solution (0.65% NaCl), thus representing the negative and positive controls respectively.

The fish were placed in twenty-five aquariums (15 L) containing dechlorinated water in a semi-static system with artificial aeration. The water quality parameters were maintained and monitored as follows: temperature of 28.16 ± 2.9°C, pH 6.4 ± 1.2, dissolved oxygen 6.8 ± 1.1 mg L^{-1}, electric conductivity 266.4 ± 41.0 µs cm^{-1} and ammonia 0.65 ± 0.12 mg L^{-1}, Bozzo et al. (2007).

After injection of the inflammatory agent, blood samples were collected from all evaluated specimens at 12, 24, 48, 72 and 96 h. The blood samples were used to determine glucose mg dL^{-1}, total erythrocytes, haematocrit percentage, haemoglobin and total plasmatic protein. Haematimetric indices as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were also determined (Tavares-Dias, 2004; Vallada, 1999).

After each blood sampling, the fish were euthanized by deepening anaesthesia, followed by medullar section and removal of the swim bladder. The organ was internally washed with 0.5 ml of a phosphate buffer and 0.65% NaCl solution, and the exudate was placed into an Eppendorf tube and centrifuged at 1000 rpm for five minutes at 4°C. Afterwards, the supernatant was discarded, and its pellet was resuspended (NaCl 0.65%) for total cell counting in a Neubauer chamber.

Another aliquot was used to make a blood smears and stained (Fontes et al., 2014) to count thrombocytes and differential leukocytes (Bozzo et al., 2007). The inflammatory analysis was determined from the differential counting of cells in the exudate according to Martins et al. (2001) and Raque et al. (2010).

Subsequently, all data were checked for homoscedasticity and normality (Bartlett and Shapiro–Wilk tests, respectively) and then submitted to one-way analysis of variance (ANOVA) with a post hoc Tukey test ($p < 0.05$) for all cases (Castro et al., 2014) using the statistical software BioEstat (Ayres et al., 2007).

TABLE 2 The mean and standard deviation values for the differential leukocyte count and thrombocytes in exudate analysis from tambaqui swim bladder supplemented with a probiotic diet containing *Bacillus cereus* followed by an inflammatory response induced by inactivated *Aeromonas hydrophila*

		Time (h)				
Parameters	**Treatment**	**12**	**24**	**48**	**72**	**96**
Leucocytes (n° μl^{-1})	NC	204.39 ± 26.15C^{a}	218.86 ± 56.11C^{a}	248.86 ± 33.35C^{a}	234.37 ± 80.14C^{a}	230.55 ± 36.08C^{a}
	PC	2616.77 ± 334.85B^{b}	3120.34 ± 341.499B^{ab}	3723.91 ± 333.20B^{a}	2963.59 ± 290.53B^{ab}	2782.51 ± 302.05B^{b}
	10^4 CFU.g^{-1}	2907.52 ± 1434.29B^{b}	3541.12 ± 383.25B^{ab}	4174.72 ± 402.03B^{a}	3329.92 ± 292.11B^{ab}	3128.72 ± 386.10B^{b}
	10^6 CFU.g^{-1}	5108.81 ± 2557.72A^{b}	6222.11 ± 632.14A^{ab}	7335.41 ± 253.34A^{a}	5851.01 ± 452.24A^{ab}	5476.57 ± 649.11A^{b}
	10^8 CFU.g^{-1}	6515.96 ± 3275.51A^{a}	69300.11 ± 383.33A^{ab}	8172.56 ± 843.18A^{a}	6101.81 ± 633.10A^{ab}	5554.32 ± 662.81A^{b}
Thrombocytes (n° μl^{-1})	NC	70.89 ± 9.07C^{b}	83.01 ± 5.61C^{ab}	102.46 ± 8.02C^{a}	83.19 ± 6.05C^{ab}	76.38 ± 9.54C^{b}
	PC	967.12 ± 136.08B^{b}	1151.39 ± 127.37B^{ab}	1325.88 ± 74.60B^{a}	1105.36 ± 32.24B^{ab}	1001.69 ± 90.03B^{b}
	10^4 CFU.g^{-1}	1063.47 ± 136.08B^{b}	1301.89 ± 137.88B^{ab}	1493.64 ± 96.09B^{a}	1251.30 ± 86.21B^{ab}	1137.39 ± 141.40B^{b}
	10^6 CFU.g^{-1}	2265.64 ± 289.91A^{b}	2792.70 ± 338.03A^{ab}	3219.76 ± 245.98A^{a}	2561.46 ± 289.04A^{ab}	2396.89 ± 289.04A^{b}
	10^8 CFU.g^{-1}	2850.73 ± 364.78A^{b}	3438.62 ± 362.72A^{ab}	4126.51 ± 375.81A^{a}	3231.54 ± 418.39A^{ab}	3024.47 ± 418.39A^{b}
Neutrophils (n° μl^{-1})	NC	41.30 ± 5.28D^{b}	51.3 ± 6.75C^{ab}	59.63 ± 4.76C^{a}	47.63 ± 4.76C^{ab}	43.96 ± 5.81D^{b}
	PC	868.61 ± 55.71C^{b}	1025.85 ± 9.61B^{a}	1033.95 ± 35.22B^{a}	889.64 ± 84.63B^{ab}	774.21 ± 71.04C^{b}
	10^4 CFU.g^{-1}	975.97 ± 62.59BC^{b}	1152.63 ± 10.80AB^{a}	1161.73 ± 39.57B^{a}	999.60 ± 95.09AB^{ab}	869.90 ± 79.82C^{b}
	10^6 CFU.g^{-1}	1321.87 ± 203.52AB^{a}	1406.05 ± 342.32AB^{a}	1436.14 ± 259.76B^{a}	1227.14 ± 562.68AB^{a}	1171.56 ± 160.58B^{a}
	10^8 CFU.g^{-1}	1473.03 ± 188.49A^{b}	1546.7 ± 146.24A^{b}	2148.36 ± 237.36A^{a}	1653.70 ± 146.24A^{b}	1546.70 ± 146.24A^{b}
Monocytes (n° μl^{-1})	NC	45.09 ± 6.34D^{b}	55.33 ± 5.81D^{ab}	64.23 ± 7.43 Da	52.69 ± 4.77D^{ab}	49.06 ± 5.28D^{ab}
	PC	684.07 ± 62.36C^{b}	877.71 ± 114.82C^{ab}	941.75 ± 47.18C^{a}	813.46 ± 98.74C^{ab}	794.13 ± 60.28C^{b}
	10^4 CFU.g^{-1}	760.07 ± 69.29C^{b}	975.23 ± 127.58C^{ab}	1046.40 ± 52.42C^{a}	903.85 ± 109.71C^{ab}	714.71 ± 54.25C^{b}
	10^6 CFU.g^{-1}	1213.59 ± 66.26B^{b}	1364.08 ± 117.33B^{b}	1618.57 ± 109.77B^{a}	1460.59 ± 115.67B^{ab}	1296.76 ± 9.92B^{b}
	10^8 CFU.g^{-1}	1620.33 ± 207.34A^{b}	1973.43 ± 207.34A^{ab}	2326.54 ± 207.34A^{a}	1855.74 ± 207.34A^{ab}	1738.04 ± 207.34A^{b}
Lymphocytes (n° μl^{-1})	NC	12.39 ± 1.58C^{b}	17.45 ± 2.12C^{a}	14.42 ± 0.95C^{ab}	13.85 ± 1.73C^{b}	12.95 ± 1.17C^{b}
	PC	38.40 ± 4.91B^{b}	49.77 ± 9.60B^{ab}	58.14 ± 9.60B^{a}	46.98 ± 2.06B^{ab}	40.59 ± 4.12B^{ab}
	10^4 CFU.g^{-1}	42.67 ± 5.46B^{b}	55.31 ± 10.66B^{ab}	64.61 ± 10.66B^{a}	52.21 ± 2.29B^{ab}	45.11 ± 4.58B^{ab}
	10^6 CFU.g^{-1}	84.66 ± 10.83A^{b}	99.78 ± 7.08A^{ab}	118.23 ± 7.08A^{a}	100.29 ± 5.99A^{ab}	90.14 ± 11.90A^{b}
	10^8 CFU.g^{-1}	99.12 ± 12.68A^{b}	114.05 ± 7.68A^{ab}	145.65 ± 17.65A^{a}	110.18 ± 8.75A^{b}	106.65 ± 12.15A^{b}

Note: Different letters indicate significant difference among the treatments ($p < 0.05$). Uppercase letters for comparison between treatments and lowercase letters for comparison of time periods in the same treatment. NC—negative control with NaCl in the swim bladder; PC—positive control injected with inactivated *A. hydrophila* in the swim bladder.

Changes in haematological parameters were observed ($p < 0.05$) among the treatments for glucose, total erythrocytes, haematocrit percentage and total plasmatic protein at different times. These differences were caused by inflammatory processes and influenced by dietary probiotic supplementation (Table 1).

After phlogogen injection, the use of *B. cereus* increased values of glucose, total erythrocyte, total plasmatic protein and haemoglobin on circulating blood, mainly in fish fed with dietary probiotic 3.9×10^6 and 3.3×10^8 CFU.g (Table 1).

The increase in glucose reflects improvements in energetic metabolism and assists the cellular inflammatory response due to insulin action (Lukaski, 2000; Moraes & Garcia-Leme, 1982). In the inflammatory process, insulin provokes vasodilatation caused by a release of nitric oxide, consequently increasing the blood flow and allowing a quick migration of leukocytes to the inflammatory site (Dandona et al., 2000; Prado et al., 2018).

Thus, as the concentrations of erythrocytes and haematocrit in the animals submitted to probiotic supplementation after phlogogen injection, significant interaction in improving between these factors for the synthesis of red blood cells. This result was also reported in aerocystitis induced by *Enterococcus* sp in tilapia, where the animals treated with immunostimulant revealed increase in haematocrit and erythrocyte percentage (Barros et al., 2002; Martins et al., 2008).

In addition to red blood cell alterations, which also showed increased values of total plasmatic protein in the first 48 h after phlogogen injection. This was probably caused by increases in the albumin and globulin concentrations, which enable sanitary and nutritional welfare (Thomas, 2000).

The swim bladder inflammatory process produced a thick, gelatinous and yellowish exudate in the probiotic groups and negative control, differently from what was observed in the exudate of the positive control. The use of probiotic bacterium *B. cereus* in the concentrations of 3.9×10^6 and 3.3×10^8 CFU.g increased the leukocytes and thrombocytes levels in the exudate, demonstrating highest values at 48 h after injection (Table 2). However, the group containing a lower probiotic concentration exhibited similar results to those of the positive control regarding leucocytes levels (Table 2). As far as differential leukocyte count from the exudate, the main cells found were lymphocytes, neutrophils and monocytes, which had their concentrations influenced ($p < 0.05$) over time.

The neutrophil levels were greater in the treatments with the highest probiotic concentrations. The concentrations of monocytes and lymphocytes were significantly ($p < 0.05$) higher for all analysed time intervals when treated with $3.3x10^8$ UFC g^{-1} (Table 2).

Fish fed for 120 days prior to the inflammatory assay exhibited alterations in the haematological parameters in the first 24 h after injection.

The cells in the exudate found in the present study were similar to those found by Bozzo et al. (2007) and Martins et al. (2006, 2009) who applied the same pathogen in the swim bladder of *P. mesopotamicus*. The higher concentrations of probiotic (3.9×10^{-6} and 3.3×10^{-8} CFU.g^{-1}) provoked greater migrations of leukocyte cells to the inflammatory site after the first 12 h. In that case, the addition of probiotics potentialized the migration of defence cells to the injured site due to the interaction between the cellular membrane and intestinal receptors. The immunostimulating effect of probiotics in contact with the intestinal mucosa occurs by the stimulation of the gut-associated lymphoid tissue, which detects the foreign bodies inducing activation of the animal's defence and improves the leukocytes recruitment (Cross et al., 2002; Feria et al., 2017; Nayak, 2010).

Higher concentrations of thrombocytes and lymphocytes ($p < 0.05$) were observed in exudates of groups fed with higher probiotic concentrations (3.9×10^6 and 3.3×10^8 CFU.g) over time. The highest cellular concentration occurred after 48 h ($p < 0.05$), corroborating with Bozzo et al. (2007), who used dietary supplementation containing chromium for *P. mesopotamicus*.

Allied to lymphocyte increases, an increase in neutrophils was also observed regarding the probiotic concentration. Neutrophils have the capacity to migrate to local injuries and potentialize antimicrobial activities (Reite, 2006). These results highlighted the relevance of neutrophils for the innate defence system against pathogens, as well as in the inflammatory process (Ranzani-Paiva et al., 2013; Rodrigues et al., 2018).

The diet with the highest concentration of probiotic, regardless of the inflammatory process, showed the greater concentrations of macrophages in swim bladder exudates; action of probiotics to improve unspecific phagocytic performance is related to the release of mediators that stimulate the immune system. In addition, probiotics of the *Bacillus* genus can synthesize cytokine to improve phagocytic action (Cross, 2002; Maassen et al., 2000).

Thus, dietary supplementation with autochthonous probiotic *B. cereus* for tambaqui at concentrations of 3.9×10^6 and 3.3×10^8 CFU.g^{-1} for 120 days improved the swim bladder inflammatory response caused by *A. hydrophila*.

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CONFLICT OF INTEREST

The authors have no ano conflict of interest to declare.

AUTHOR CONTRIBUTION

Experimental realization: Dias, Couto, Barros, Cordeiro, Paixão, Meneses, Cunha, Pereira and Fujimoto. Data analysis and interpretation: Dias, Diniz, Martins, Mouriño, Maria, Carneiro and Fujimoto. Manuscript preparation and revision: Dias, Alves, and Fujimoto.

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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