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Farming tambaqui (*Colossoma macropomum*) in static clear water versus a biofloc system with or without *Bacillus subtilis* supplementation

Driely Kathriny Monteiro dos Santos[1] · Juliana Tomomi Kojima[2] · Thiago Macedo Santana[1] · Diogo Pereira de Castro[3] · Paula Taquita Serra[4] · Naiara Silva Menezes Dantas[1] · Flávio Augusto Leão da Fonseca[5] · Luís André Morais Mariúba[4] · Ligia Uribe Gonçalves[1,2]

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Abstract
The use of probiotics can beneficially improve the water quality in the biofloc technology (BFT) system consequently enhancing the fish performance. This study focused on the effect of *Bacillus subtilis* on a clear water static system compared to the BFT system on the productive performance, proximate body composition, and diversity of intestinal bacterial communities of tambaqui (*Colossoma macropomum*) juveniles submitted to four treatments: clear water static system with (CW + BS) or without (CW) *B. subtilis* and BFT with (BFT + BS) or without (BFT) *B. subtilis*. For the study, 20 juveniles (five replicates) per treatment were used for 60 days. For the *B. subtilis* treatments, water was inoculated at weekly intervals with a 4×10^8-cell mL^{-1} culture suspension. Results revealed that regardless of *B. subtilis* supplementation, fish reared in BFT displayed better zootechnical performance than those in CW—weight gain (BFT 33.57 ± 4.08 g; CW 19.97 ± 5.42 g), protein efficiency (BFT 0.16 ± 0.02; CW 0.11 ± 0.02), feed conversion ratio (BFT 0.71 ± 0.08; CW 0.84 ± 0.40), and relative growth rate (BFT 1.52 ± 0.12; CW 1.06 ± 0.15)—which suggests that biofloc consumption was 31.9% crude protein. Moreover, fish reared in BFT + BS had a higher condition factor (2.30 ± 0.09). In conclusion, BFT is regarded as a promising system to save water, decrease aquaculture effluents, and promote tambaqui farming. Although *B. subtilis* is a common bacterium found in tambaqui gut, its supplementation in the BFT system improved the fish condition factor.

Keywords Biofloc technology system · *Colossoma macropomum* · Probiotic · Zootechnical performance

✉ Ligia Uribe Gonçalves
ligia.goncalves@inpa.gov.br

Extended author information available on the last page of the article

Introduction

Globally, the growth of the aquaculture industry is continued to meet the rising food demand of the world's growing population (Jones et al. 2020). The biofloc technology (BFT) system recycles nitrogen effluent produced by heterotrophic bacteria, lowers the concentration of dissolved ammonia, and decreases water toxic compounds with low or zero water exchange (Avnimelech 1999; Hargreaves 2006). Besides, the microbial community, which is the basis of this technology, is continually available as a food source for filter-feeding fish (Luo et al. 2014). To date, favorable results have been reported for *Litopenaeus vannamei* and *Oreochromis niloticus* farmed by the BFT system (Wasielesky et al. 2006; Samocha et al. 2007; Azim and Little 2008).

Inoculation of probiotics in BFT-based systems can enhance fish resistance and can therefore contribute to the biosecurity of aquaculture systems (Kathia et al. 2017). The application of probiotics beneficially improves the water quality, reducing the growth of pathogens, stimulating the fish immune responses, and improving the nutrition of fish and crustaceans (Das et al. 2017).

Bacillus subtilis as dietary probiotic is a proven beneficial bacteria because it promotes a favorable balance of host intestinal microbiota by suppressing harmful bacteria in *Oreochromis niloticus* (Carvalho et al. 2011). Moreover, it was found that the addition of *B. subtilis* in the BFT system water does not affect the bacterial composition of biofloc and maintains optimal water quality parameters, especially nitrogen compounds required by *Oreochromis niloticus* (Kathia et al. 2018).

Tambaqui (*Colossoma macropomum* Cuvier 1818) is an Amazonian fish and the main native aquaculture species farmed in Brazil, whose annual production is 287,910 tons (PeixeBr 2019). Tambaqui can tolerate handling and low dissolved oxygen concentrations (Gomes et al. 2003; Aride et al. 2006), and its reproduction can be controlled in fish farming (Vieira et al. 1999; Almeida et al. 2016). Moreover, as tambaqui is an omnivorous fish with a high water filtration capacity (Aride et al. 2006), it is ideally suited to be reared in systems based on the BFT system, particularly for enhancing the aquaculture productivity in the Amazonian region. Therefore, its potential for making a major contribution to the local socio-economic development and for reducing pollution and deforestation is relatively high. This study aimed to evaluate the efficacy of *B. Subtilis* on static aquaculture systems based on either clear water (CW) or BFT on the growth, productivity, and proximate body composition of farmed tambaqui

Material and method

Ethical approval

This study complied with the standards required by the National Council for the Control of Animal Experimentation (CONCEA) and was approved by the Ethics Committee on Animal Experimentation and Research of the National Institute for Research in the Amazon (INPA), Manaus, Amazonas, Brazil (Protocol No. 031/2017).

Fish, rearing conditions and experimental setup

Tambaqui juveniles (average initial body weight = 23.24 ± 0.34 g; length = 8.9 ± 0.06 cm) were stocked in 200-L indoor tanks (20 fish tank^{-1}) in a randomized experimental design (n =

5) and assigned to four treatments based on two static systems with BFT or CW (artesian well), each with or without *B. Subtilis* supplementation: CW: clear water static system with 50% daily water exchange; CW + BS: clear water static system with 50% daily water exchange with *B. Subtilis* inoculation; BFT: biofloc technology static system; BFT + BS: biofloc technology system with *B. Subtilis* inoculation. In the tanks with BFT, water was added when evaporation was observed. The *B. subtilis* concentration in the water of treatments CW + BS and BFT + BS was based on Dias et al. (2018), in which the bacteria of the genus *Bacillus* were added to diets to be inoculated weekly with 75 mL of a 4×10^8-cell mL^{-1} suspension of *B. subtilis*. All the fish were acclimated to systems for 5 days until they normally accepted diets. Fish were fed 2.6 mm extruded commercial feed, Nutripiscis® (45% crude protein; 8% lipid; 4% crude fiber; 15% ash; 12% moisture) until apparent satiety 3 times a day for 60 days. Every month, all the fish in each experimental unit were anesthetized by immersion in 100 mg of benzocaine L^{-1} (Gomes et al. 2001) to take the biometric measurements.

Analysis of water parameters

Water quality parameters, such as dissolved oxygen (6.01 ± 0.03 mg L^{-1}), temperature (25.9 ± 0.2 °C) and pH (6.61 ± 0.28), were monitored daily by a multiparameter meter (ProODO, YSI®). The ammonia N-TAN (1.13 ± 0.86 mg L^{-1}), nitrite N-NO_2 (0.68 ± 0.18 mg L^{-1}), and nitrate N-NO_3 (4.04 ± 1.22 mg L^{-1}) levels were monitored twice weekly, and the concentrations of carbonic gas (16.4 ± 9.2 mg L^{-1}), alkalinity (10.9 ± 4.57 mg L^{-1}), and hardness (61.1 ± 54.53 mg L^{-1}) were determined weekly using colorimetric and titulometric kits (Alfakit AT 101; Alfakit, Florianópolis, SC, Brazil). If pH went below 6 in the BFT experimental units, it was restored to pH 7 by adding sodium bicarbonate ($NaHCO_3$). The biofloc volume was determined weekly using an Imhoff cone as described by Avnimelech and Kochba (2009). The leftovers and feces of the CW and CW + BS experimental units were removed from the bottom of tanks by siphoning with 50% of the total water volume being exchanged.

Zootecnical performance

The fish biometric data were used to calculate the following parameters: weight gain (WG) = final weight − initial weight; feed conversion ratio (FCR) = feed intake / body weight gain; protein efficiency ratio (PER) = body weight gain / protein intake; feed intake (FI) = total feed consumed during the experiment; relative growth rate RGR (% day^{-1}) = $(e^g - 1) \times 100$, where e is the neper number and $g = (\ln(FW) - \ln(IW))/(Ne)$, where FW = total final weight (g), IW = total initial weight (g), and Ne = number of experimental days; condition factor (K) = 100 − (body weight / total body length^3).

Proximate composition of bioflocs

Bioflocs were filtered from 50 L of tank water using a thin mesh (120 μm) for the proximal composition analysis, such as dry matter, moisture, protein, and lipids (AOAC 2010).

Proximate body composition

At the end of the experiment, two fish from each experimental unit were anesthetized by immersion in 300 mg benzocaine L^{-1} until the loss of reflex activity and no reaction to external

stimuli were noted. Afterward, fish were euthanized by spinal medulla rupture according to the rules of the CONCEA (2018). Fish samples were frozen to be subsequently analyzed for proximate composition (AOAC 2010).

Microbiological analysis

Two other fish were transported to the laboratory of the Oswaldo Cruz Foundation, FIOCRUZ, to detect and identify intestinal *B. subtilis*. The contents of fish intestines were collected by scraping and were then transferred to a sterile test tube containing 3 mL of brain heart infusion (BHI) broth to be incubated overnight at 37 °C. The next day, the samples of the resulting culture were used to inoculate MacConkey agar, *Pseudomonas* isolation agar (PIA), and *Shigella* and *Salmonella* agar (SS). The colonies that subsequently grew on plates were quantified, and those that differed morphologically were separated for biochemical identification purposes (Elbing and Brent 2002).

B. subtilis identification

To detect *B. subtilis*, the samples of intestinal contents were incubated overnight at 37 °C in 3 mL of heart infusion broth. Then the samples of the resulting culture were used to inoculate blood agar plates. After colonies had grown, those showing hemolytic characteristics were analyzed. The samples of colonies showing a positive reaction were subsequently used to be inoculated in a MiLi biochemical medium for the motility analysis. For identification purposes, the results were assessed by the ABBIS online platform (http://www.tgw1916.net/bacteria_logare_desktop.html).

To make identifications, the following biochemical tests were run for the enterobacteria-based MiLi, EPM, and citrate gallery: decarboxylation of lysine, motility, indole production, sugar fermentation, urease production, and H_2S and citrate utilization (ANVISA, 2004). The results were assessed using the ABBIS online platform. This platform is available at http://www.tgw1916.net/bacteria_logare_desktop.html.

Statistical analyses

The zootechnical performance, proximate body composition, and water quality data were subjected to normality (Shapiro-Wilk) and homogeneity (Levene) tests. The variables that did not meet homoscedasticity were data-transformed by natural logarithms (Alkalinity and Carbon dioxide). A one-way ANOVA test was performed. When a significant difference was found, treatments were compared by Tukey's test using the TIBCO Statistica 13.3 software. All the significance analyses were performed using $P < 0.05$.

Results

Water quality

The BFT and BFT + BS systems had lower ammonia concentrations (< 0.34 mg L^{-1}; $P <$ 0.001) than the CW and CW + BS systems (< 1.98 mg L^{-1}) (Table 1; Fig. 1a). No significant

Table 1 Water quality parameters (mean ± standard deviation) of the different tambaqui rearing systems for 60 days

Variables	CW	CW + BS	BFT	BFT + BS	ANOVA *P* value
N-TAN (mg L^{-1})	1.98 ± 0.20[a]	1.93 ± 0.16[a]	0.34 ± 0.11[b]	0.28 ± 0.04[b]	< 0.001
N-NO_2 (mg L^{-1})	0.59 ± 0.20	0.86 ± 0.13	0.67 ± 0.10	0.60 ± 0.11	0.067
N-NO_3 (mg L^{-1})	2.60 ± 0.67[d]	3.28 ± 0.25[c]	5.09 ± 0.29[b]	5.19 ± 0.35[a]	< 0.001
pH	6.97 ± 0.07[a]	6.72 ± 0.11[b]	6.32 ± 0.06[c]	6.41 ± 0.18[c]	< 0.001
CO_2 (mg L^{-1})	6.50 ± 1.19[b]	7.93 ± 1.94[b]	25.07 ± 1.89[a]	26.20 ± 4.80[a]	< 0.001*
Alkalinity (mg L^{-1})	6.67 ± 1.31[c]	6.13 ± 0.74[c]	11.13 ± 1.32[b]	16.33 ± 3.32[a]	> 0.001*
Hardness (mg L^{-1})	8.51 ± 1.42[b]	7.31 ± 1.04[b]	113.59 ± 1.06[a]	114.80 ± 0.43[a]	> 0.001
T (°C)	25.97 ± 0.02[b]	26.09 ± 0.04[a]	25.78 ± 0.03[d]	25.86 ± 0.05[c]	> 0.001

Means followed by different letters in row indicate differences between treatments ($P < 0.05$)

CW clear water, *CW + BS* clear water with *B. subtilis* supplementations, *BFT* biofloc technology system, *BFT + BS* biofloc technology system with *B. subtilis* supplementations

*Ln data transformation

difference appeared in nitrite concentrations (< 0.73 mg L^{-1}) in any treatment (Fig. 1b). Higher nitrate levels were found in the water of the BFT system (> 5.09 mg L^{-1}; $P < 0.001$) (Fig. 1c). CO_2 levels were detected in the BFT and BFT + BS treatments (< 26.20 ± 4.80 mg L^{-1}), which were higher than in the CW systems (< 7.93 ± 1.94 mg L^{-1}; $P < 0.001$).

The BFT and BFT + BS systems showed higher concentrations of calcium and magnesium salts, with average hardness values of 113.59 and 114.80 mg L^{-1}, respectively. No significant differences were observed in the dissolved oxygen concentration in water. The mean pH value in water in the CW treatment (pH 6.97) differed from that in the treatment, which included *B. subtilis* (pH 6.72; Fig. 1d). The average temperature was 25.93 °C. It did not vary much (range of 0.3 °C), even though a statistically significant difference appeared among treatments. The flocs volume did not differ among treatments with BFT (8.1 ± 1.53 mL L^{-1}) and BFT + BS (9.3 ± 5.63 mL L^{-1}) (Fig. 2). The treatments composed of the BFT system did not produce

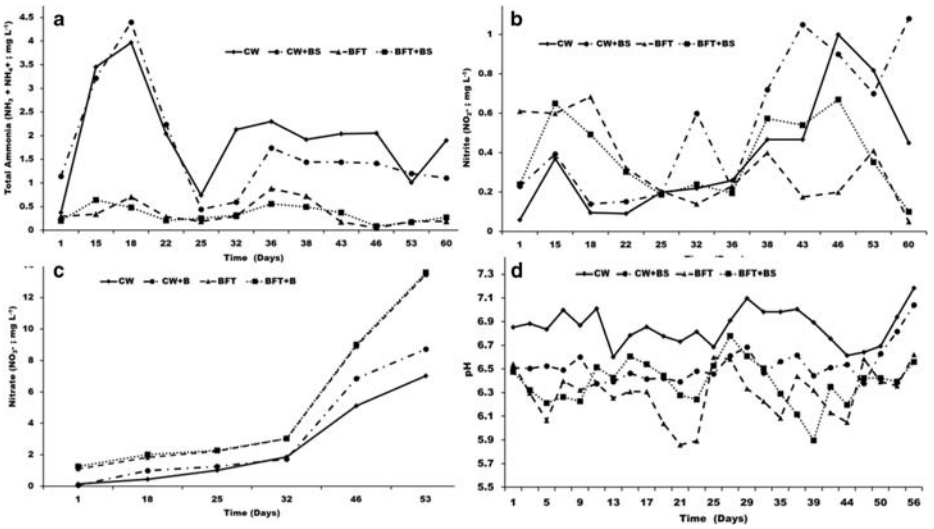

Fig. 1 Water quality parameters of the different tambaqui rearing systems for 60 days. (a) Total ammonia (NH_3 + NH_4^+; mg L^{-1}); (b) nitrite (NO_3^-; mg L^{-1}); (c) nitrate (NO_2^-; mg L^{-1}); (d) pH

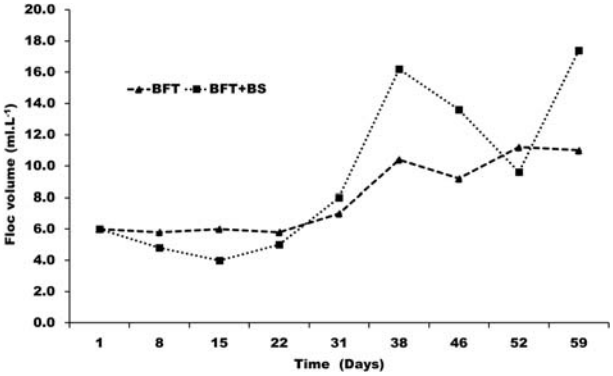

Fig. 2 Floc volume levels in the water of BFT and BFT + BS systems for 60 days

effluents during the 60-day experiment and helped to save 61,850 L of water compared to the CW system with daily changes of 50% water.

Growth performance

The weight gain of the tambaqui juveniles reared in the BFT systems (32.26 ± 1.36 g; $P < 0.001$) was heavier than those of CW (20.10 ± 1.42 g), particularly in the treatment to which no *B. subtilis* was added (Table 2). The treatment with the BFT system had a better feed conversion ratio (0.71 ± 0.08). The fish from the treatments with the BFT system had a better ($P < 0.001$) protein efficiency ratio (0.16 ± 0.02). No significant difference appeared for feed intake among treatments ($P = 0.362$).

The tambaqui juveniles reared in the BFT system obtained the highest averages of relative growth rate (1.52 ± 0.12; $P < 0.001$) and supplementation with *B. subtilis* was the BFT system improved condition factor (2.30 ± 0.09; $P = 0.029$). Survival rates were similar in all the treatments with mean values of 95.5% (Table 2).

Proximate composition

The biofloc composition was not significantly affected by the inclusion of *B. subtilis* and contained 89.99 ± 0.31% dry matter, 31.88 ± 0.53% crude protein, and 0.33 ± 0.11% lipids.

Table 2 Zootechnical performance (mean ± standard deviation) of the tambaqui reared in different aquaculture systems for 60 days

Variables	CW	CW + BS	BFT	BFT + BS	ANOVA *P* value
WG (g)	20.24 ± 4.02^{b}	19.97 ± 5.42^{b}	33.57 ± 4.08^{a}	30.96 ± 4.57^{a}	< 0.001
FI (g)	411.10 ± 26.75	407.53 ± 53.37	471.56 ± 17.64	483.52 ± 22.99	0.362
FCR	0.84 ± 0.40^{a}	1.06 ± 0.18^{a}	0.71 ± 0.08^{b}	0.80 ± 0.10^{ab}	0.002
RGR (% day^{-1})	1.06 ± 0.15^{b}	1.05 ± 0.21^{b}	1.52 ± 0.12^{a}	1.43 ± 0.15^{a}	< 0.001
K	2.10 ± 0.08^{b}	2.20 ± 0.12^{ab}	2.22 ± 0.07^{ab}	2.30 ± 0.09^{a}	0.029
PER	0.11 ± 0.02^{b}	0.11 ± 0.02^{b}	0.16 ± 0.02^{a}	0.14 ± 0.02^{a}	< 0.001
SURV (%)	95.00 ± 7.07	96.00 ± 4.18	93.00 ± 5.70	98.00 ± 2.74	0.509

Means followed by different letters in row indicate differences between treatments ($P < 0.05$)

CW clear water, *CW + BS* clear water with *B. subtilis* supplementations, *BFT* biofloc technology system, *BFT + BS* biofloc technology system with *B. subtilis* supplementations, *WG* weight gain, *FI* feed intake, *FCR* feed conversion ratio, *RGR* relative growth rate, *K* condition factor, *PER* protein efficiency ratio, *SURV* survival

There was only one significant difference in protein levels in the proximal composition of tambaqui juveniles ($P < 0.05$; Table 3).

Microbiological analysis

Fifteen bacterial species, primarily those belonging to Enterobacteriaceae, were identified from their growth on MacConkey agar, *Shigella* and *Salmonella* agar, and *Pseudomonas* isolation agar and based on the results of the EPM, MiLi, citrate, arginine, and ornithine biochemical tests. Four bacteria species were isolated from fish intestines in all treatments: *Citrobacter* sp., *Escherichia coli*, *Proteus* sp., *B. subtilis*. *B. subtilis* was detected in the guts of the tambaqui reared in one of the CW experimental units (Table 4).

Discussion

Water quality

All the water parameters herein measured fell within the appropriate range recommended for tambaqui farming (Cavero et al. 2009; Lima et al. 2019). The higher CO_2 concentrations recorded in the BFT and BFT + BS system treatments were due to both fish and microbial respiration and organic matter decomposition (Van Wyk and Scarpa 1999; Wasielesky et al. 2006). Higher CO_2 concentrations, in turn, resulted in lower pH values, which went below 7 and was the minimum recommended pH value for microbial growth (Boyd et al. 2011). The amplitude in water parameters can cause microbial biomass oscillation and affect floc formation (Minabi et al. 2020). However, the floc volume levels in our study presented a stable concentration (9.3 mL L^{-1}), which did not cause any discomfort to tambaqui.

The recorded low alkalinity values were due to the heterotrophic and nitrifying bacterial consumption of 7.07 g of alkalinity per gram of the total ammoniated nitrogen oxidized to nitrate (Ebeling et al. 2006). Despite adding sodium bicarbonate ($NaHCO_3$), it was not possible to attain the ideal alkalinity level, which should be as high as possible, to strike an optimal balance for the microbial population in biofloc systems (Furtado et al. 2014). However, the lower alkalinity levels in the biofloc system did not affect biofloc formation, survival, and growth of tambaqui, as previously observed in Nile tilapia reared under conditions with 61.75 mg L^{-1} alkalinity (Kubitza 2011).

The BFT system provided higher water hardness values, which still fell within the ideal range (40–120 mg L^{-1}) for rearing juvenile tambaqui (Cavero et al. 2009). The high water hardness levels recorded in treatments BFT and BFT + BS are related to the accumulation of

Table 3 Proximate composition (mean ± standard deviation) of the tambaquis reared in different aquaculture systems for 60 days

Variables	CW	CW + BS	BFT	BFT + BS	ANOVA *P* value
DM	88.97 ± 0.95	89.64 ± 0.49	87.80 ± 1.78	88.29 ± 0.64	0.076
CP	50.95 ± 0.82^{ab}	49.22 ± 1.90^{ab}	47.34 ± 2.82^{b}	51.57 ± 2.88^{a}	0.039
LP	21.96 ± 3.10	24.35 ± 1.66	22.07 ± 1.54	23.72 ± 3.03	0.344

Means followed by different letters in row indicate differences between treatments ($P < 0.05$)

CW clear water, *CW + BS* clear water with *B. subtilis* supplementations, *BFT* biofloc technology system, *BFT + BS*, biofloc technology system with *B. subtilis* supplementations, *DM* dry matter, *CP* crude protein, *LP* lipid

Table 4 Bacterial identification in the guts of the tambaquis reared in different aquaculture systems for 60 days

Bacteria	CW	CW + BS	BFT	BFT + BS
Bacillus subtilis	+	+	+	+
Brenneria nigrifluens	+	−	+	−
Citrobacter sp.	+	+	+	+
Edwarsiella sp.	−	−	−	+
Enterobacter sp.	−	+	+	−
Escherichia coli	+	+	+	+
Escherichia vulneris	−	−	+	−
Klebsiella sp.	+	−	+	+
Morganella sp.	−	+	−	−
Proteus sp.	+	+	+	+
Providencia sp.	−	+	−	−
Pseudomonas sp.	+	−	+	+
Salmonella sp.	+	+	+	−
Shigella sp.	+	−	−	+
Yersinia sp.	−	−	+	−

CW clear water, *CW* + *BS* clear water with *B. subtilis* supplementations, *BFT* biofloc technology system, *BFT* + *BS* biofloc technology system with *B. subtilis* supplementations, + present, − absent

calcium and phosphate, which were derived from the diet and also from adding bicarbonate to correct pH (Poleo et al. 2011). The total water hardness is determined by summing divalent cations, among which Ca^{2+} is that with the highest binding affinity in biofloc (Luo et al. 2013). Thus, an increase in Ca^{2+} levels can have implications for biofloc structure and/or composition by promoting higher density and smaller floc size (Luo et al. 2013). The lower hardness levels recorded in the water of the CW and CW + BS treatments can be explained by the fact that water from artesian wells was used for these treatments.

The lower ammonia levels in the BFT system suggested that floc volume was efficient in metabolizing ammonia, which was recorded at concentrations within the desirable range for rearing juvenile tambaqui juveniles (< 0.5 mg L^{-1}) (Lima et al. 2019). In contrast, higher ammonia concentrations (1.96 ± 0.18 mg L^{-1}) were detected in treatments CW and CW + BS, which can be attributed to the low water exchange rates recorded during experiments. Notwithstanding, there was no interference (0.05) in either fish survival or feed intake as ammonia levels were below the lethal limit for tambaqui (7.84 mg L^{-1}; > 50 h of exposure), and the exposure time was shorter (Souza-Bastos et al. 2017).

The nitrite levels in water remained high in all the treatments compared with the recommended level for tambaqui farming (Cavero et al. 2009), although the mean nitrite values went below the critical values (1.82 ± 0.98 mg L^{-1}) described for this species (Costa et al. 2004). For BFT and BFT + BS, the numbers of nitric bacteria were insufficient (in the genus *Nitrobacter*) to facilitate the oxidation of nitrite to nitrate. High nitrite concentrations are a matter of concern in aquaculture because nitrites are capable of oxidizing blood hemoglobin by converting it into a molecule that is incapable of transporting oxygen (Jia et al. 2015). Despite the nitrate that was derived from nitrite accumulating in the water of the BFT and BFT + BS systems, levels were not high enough to be toxic because nitrate toxicity is comparatively rare and only occurs at high concentrations, e.g., above 1000 mg L^{-1} for freshwater fish (Baldisseroto 2002; Hickey and Martin 2009).

Growth performance and proximal composition

The high survival rates observed in all the treatments are indicative of favorable tambaqui rearing conditions. The tambaqui subjected to the BFT and BFT + BS treatments obtained

heavier weight gains given their ability to filter water (Aride et al. 2006). This characteristic enables fish to exploit continual biofloc availability (Avnimelech 2007) and has been observed in other filtering species reared in BFT systems, such as shrimp (*Farfantepenaeus paulensis* and *Litopenaeus vannamei*) (Wasielesky et al. 2006; Emerenciano et al. 2011), Nile tilapia (Azim and Little 2008), and common carp, *Cyprinus carpio* (Adineh et al. 2019). The best protein efficiency rate of tambaqui was detected in BFT, which can be explained by the presence and availability of bioflocs in water all the time. The bioflocs herein monitored, which contained 31.9% crude protein, represented an additional source of nutrients, as previously described in other aquaculture studies (Wasielesky et al. 2006; Azim and Little 2008; Emerenciano et al. 2011). Our results of relative growth rate were similar to those found in tambaqui reared in recirculation systems using biological aerated filters (Lima et al. 2019), which correspond to experiments performed under laboratory conditions.

The favorable results obtained using BFT in terms of fish weight gain, and their condition factors in BFT + BS are closely related to floc volume that darkens water and may have stimulated increased feed consumption. Such conditions are more likely to lead to tambaqui performance, as they are similar to the features that characterize the animals' natural environment. Indeed, it has been previously shown that tambaqui reared with artificial lighting show clear signs of stress and perform worse than those that remain in complete darkness (Aride et al. 2006).

Microbiological analysis

We found that the fish submitted to the BFT treatment harbored a wider variety of intestinal bacteria as a result of biofloc consumption. Despite the presence of pathogenic bacteria in the guts of the fish reared in the CW and BFT + BS systems, such as *Shigella* sp., it had no detrimental effects on fish performance compared to the fish reared in the BFT and CW + BS systems.

The addition of *B. subtilis* did not promote better zootechnical performance in tambaqui which may be suggested to the possibility of this bacterium being assimilated by fish being hampered when it is added to water. However, higher values for the condition factor can be attributed to bacteria supplementation in the BFT + BS system (Table 2). A condition factor is a tool that evaluates fish welfare in relation to the environment or development stage (Nunes et al. 2019). The microbiota equilibrium probably resulted in isometric growth in the fish reared in BFT + BS system. Likewise, tambaqui fingerlings supplemented with *B. cereus* 3.9×10^6 CFU g^{-1} in the diet for 120 days showed a heavier weight gain and survival when challenged with *Aeromonas hydrophila* (Dias et al. 2018). Experimental time is another factor that can explain why no effect was observed like that in the tambaqui juveniles that were fed diets supplemented with probiotics for 90 days (Paixão et al. 2017). For example, it has been previously demonstrated that positive responses for the zootechnical performance of tilapia that were fed supplements containing *B. subtilis* were observed only after 120 experimentation days (Carvalho et al. 2011). There are also reports about using *B. subtilis* being more effective in improving larval performance in rainbow trout than in juvenile trout (Merrifield et al. 2010), which indicates that this treatment may be more effective in earlier fish life cycle phases. The bacterium herein identified in fish guts forms the majority of natural microbiota in different environments. So it would appear that supplementation with *B. subtilis* in rearing systems has no qualitative effects on tambaqui gut microbiota.

However, further research is needed to better identify the intestinal microbiota of fish because culture media may be deficient in nutritional requirements, and bacteriological culture techniques performed in conjunction with molecular analyses can allow a more comprehensive study of existing aquaculture populations (Pond et al. 2006). In the present day, probiotics research in aquaculture is still very limited. Studies on the use of organic acids and bacterial fermentation products in aquafeed can potentially make an important contribution to enhancing fish farming efficiency.

Conclusion

Rearing tambaqui in a BFT system for 60 days showed higher growth and weight gain values, favored by food availability in the form of the microbial proteins that make up bioflocs. Such rearing improved tambaqui productivity, minimized the use of water, and reduced effluent volumes. *B. subtilis* is a common bacterium found in tambaqui gut, even in the fish of the system to which bacterium was added. However, its supplementation in the BFT system improved the fish condition factor.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

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Affiliations

Driely Kathriny Monteiro dos Santos[1] · Juliana Tomomi Kojima[2] · Thiago Macedo Santana[1] · Diogo Pereira de Castro[3] · Paula Taquita Serra[4] · Naiara Silva Menezes Dantas[1] · Flávio Augusto Leão da Fonseca[5] · Luís André Morais Mariúba[4] · Ligia Uribe Gonçalves[1,2]

[1] Programa de Pós-graduação em Ciência Animal, Universidade Federal do Amazonas, Av. General Rodrigo Octavio Jordão Ramos, 1200 - Coroado I, Manaus, Amazonas 69067-005, Brazil

[2] Instituto Nacional de Pesquisas da Amazônia, Av. André Araújo 2936, Manaus, Amazonas 69060-001, Brazil

[3] Hidrotec da Amazônia, Km 18, Manaus, Amazonas BR-174, Brazil

[4] Programa de Pós-graduação em Biologia Celular e Molecular, Fundação Oswaldo Cruz, Rua Terezina, 476 - Adrianópolis, Manaus, Amazonas 69057-070, Brazil

[5] Instituto Federal de Educação, Ciência e Tecnologia do Amazonas - Campus Zona Leste, Av. Cosme Ferreira, São José Operário, Manaus, Amazonas 69083-000, Brazil