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Original Article

The effects of dietary supplement with sodium citrate and probiotic on bacterial density in gastrointestinal tract and growth performance of Asian swamp eel (*Monopterus albus*)

Huynh Thanh Toi*, Mashyaka Anicet, and Nguyen Thi Hong Van

College of Aquaculture and Fisheries, Can Tho University, Nink Kieu, Can Tho, Viet Nam

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Abstract

This study aimed to assess the effects of the addition of probiotics and sodium citrate to the diet of Asian swamp eel (*Monopterus albus*) fingerlings, on growth performance and gastrointestinal microbiota. Before the start of the experiment, the fingerlings were acclimatized for two days; 480 fingerlings were distributed equally in 12 tanks containing 50 l of fresh water. Treatments were designed as follows: (T1) control fed with commercial feed, (T2) feed coated with sodium citrate, (T3) feed coated with probiotics and (T4) feed coated with sodium citrate mixed with probiotics. The results of this study showed that both probiotics and the mixture of sodium citrate with probiotics improved growth, stimulated total bacteria, and reduced the number of *Vibrio* in the gastrointestinal tract of *M. albus*.

Keywords: fingerlings, probiotic bacteria, growth rate, prebiotic, synbiotic

1. Introduction

The Asian swamp eel is a freshwater species belonging to the Family Synbrachidae and is native to tropical areas (Turcios & Papenbrock, 2014). It has a high tolerance to a brackish, saline environment and low dissolved oxygen, as 25% of total oxygen in eel respiration is taken through the skin. In addition, *M. albus* is a hermaphrodite: the individual starts life as a female and later changes to a male. Males can also change their sex when females are present in insufficient numbers during reproduction. Each female can produce more than 1,000 eggs per spawning season. Swamp eels have been considered a good choice in traditional cuisines in most Southeast Asian countries due to their tasty, nutritious meat. Recently, eel culture has increased rapidly in most Southeast Asian countries, and eels are commercially reared and accepted by local consumers (United States Fish & Wildlife Service, 2018). However, bacterial diseases have become a bottleneck in Asian swamp eel culture (Haenen *et al*., 2012).

*Corresponding author
Email address: httoi@ctu.edu.vn

Several studies have been conducted on *M. albus*, including work on macro-parasites in the gastrointestinal tract of Asian swamp eels imported from Vietnam to North American live-food markets (Nico, Sharp, & Collins, 2011); an aquaculture overview and studies of ecology (Turcios & Papenbrock, 2014), immunology (Xia *et al*., 2018), maturation, reproduction and growing-out practices (Khanh & Ngan, 2010; Susatyo, Setyaningrum, Winarni, & Chasanah, 2018), hematology (Narejo, Rashid, & Rahmatullah, 2002), and the physicochemical and structural properties of Asian swamp eel skin gelatin (Rosli & Sarbon, 2015). Only a few studies on feed additives have been conducted, including that of Jahangiri and Esteban (2018), which evaluated the use of probiotics in *M. albus*.

Antibiotics and antibiotic growth promoters have recently been used for prophylactic treatments of bacterial diseases. However, the long-term utilization of antibiotics in aquaculture not only causes bacterial resistance but is also harmful to the host animal as well as human consumers, and it causes pollution of the environment (Hai, 2015; Jahangiri & Esteban, 2018). Thus, more research is needed on alternative feed additives in order to resolve health issues and satisfy the nutritional requirements of the animals. It has been highlighted that feed supplemented with some organic salts

and probiotics can influence modification of bacterial density in the gastrointestinal tract and improve growth performance in fish (Corrêa, Dutra-Mouriño, Mouriño, & Cerqueira, 2016; Khaled, 2015; Shah, Afzal, Khan, Hussain, & Habib, 2015). According to Lückstädt and Mellor (2011), organic acids and their salts have great impacts on aquatic organisms in different ways: they act as microbial inhibitors and preservative agents in the diets and minimize the consumption of pathogenic organisms. According to Maroneze, Zepka, Vieira, Queiroz, and Jacob-Lopes (2014), fish fed on feed supplemented with probiotics showed a high survival rate, whereas the addition of organic acids improved growth and feed performance by increasing the phytate hydrolysis, killing pathogens in the gut and enhancing the mineralization and nutrient absorption (Shah *et al*., 2015). Among these, citric acid (CA) and lactic acid (LA) were revealed to be better at raising resistance to bacterial infection than others (Ng, Lim, Romano, & Kua, 2017). Moreover, sodium citrate is normally available at lower cost than other organic acids and is always available in the market. Therefore, this study aimed to assess the effects of feed supplemented with sodium citrate and probiotics on bacteria density in the gastrointestinal tract and growth performance of the Asian swamp eel (*M. albus*).

2. Methodology

This research was conducted at the College of Aquaculture and Fisheries, Can Tho University. The fingerlings, ranging from 7.5 to 9.0 g in weight, were purchased from the hatchery in Can Tho, located 2 km away from Can Tho University. They were then acclimatized for two days to their new conditions before the onset of this experiment and spent 30 days under the different feeding treatment regimes.

2.1 Experimental designs

In this experiment, stocking density was held at 40 eels in each 200-l round water tank (dimensions: height 0.52 m, diameter 0.7 m) containing 50 l of chlorine-free fresh water. During the 30 days of the experiment, the eels were fed with alternative feed diets, corresponding to the following four treatments: T1 (Control) was commercial feed (commonly used feed for rearing swamp eels in Viet Nam) with 40% protein (Tongwei, Tien Giang, Viet Nam); T2 was commercial feed coated with sodium citrate (AR, Xilong Scientific, Guangdong, China, 15 g kg^{-1} of feed), T3 was commercial feed coated with probiotics (0.8 g kg^{-1} of feed), and T4 was commercial feed coated with a mixture of probiotics (0.4 g kg^{-1} of feed) and sodium citrate (7.5 g kg^{-1} of feed). The treatments were performed in triplicates.

The eels were fed twice a day at 08:00 h and 16:00 h at a feeding ratio of 30% of body weight in all treatments. Black plastic filaments were bunched and placed in each tank as shelter for the eels for the entire rearing period.

Probiotics were prepared by the microbiological laboratory of the College of Aquaculture and Fisheries, Can Tho University. Probiotics were screened according to the methodology described by Verschuere, Rombout, Sorgeloos, and Verstraete (2000). The selected probiotics were inoculated for mass production; then bacterial cells were harvested by centrifuge at ± 4,400 x g for 15 min at 4 °C when visible growth appeared, and the cells were sent to the College of Agriculture at Can Tho University for freeze-drying. After freeze-drying, viable cells were determined, after which the probiotics production process was completed. It contained 3.5 x 10^9 CFU g^{-1} of *Bacillus subtilis* isolated from a *Pangasius* culture pond; 1.5 x 10^{12} CFU g^{-1} of *Saccharomyces cerevisiae*; 17,500 UI of amylase; 68,750 UI of protease; 5,000 UI of lipase; 11,250 UI of β-glucans; and 1,250 UI of cellulase and $CaCO_3$.

2.1.1 Feed preparation

Shah *et al.* (2015) indicated that for omnivorous fishes like tilapia (*Tilapia nilotica*), rohu (*Labeo rohita*), and common carp (*Cyprinus carpio*), the dose of citric acid was 2–3% in feed supplement (20–30 g kg^{-1} of feed), while for carnivorous fishes such as seabream, trout, and salmon, it was 1–2%. Swamp eels are also carnivorous, and therefore the dose was chosen at an intermediate level (1.5%). For the probiotics, a dose of 0.8 g kg^{-1} of feed gave the best results in our previous studies (Toi, Van, & Ngan, 2019) and was chosen for testing in this study; a mixture (half probiotics and half citrate) was also added to the feed.

The required concentration of sodium citrate or probiotics for each treatment was dissolved in minimal water and coated onto pellets using a sprayer. Next, feed was incubated at room temperature for 10 min to allow the absorption of sodium citrate, and after that the feed was coated with a binder (Mitavet, Minh Tan Co. Ltd., Viet Nam) at a dose of 6 g kg^{-1} of feed. For the control treatment, the feed was coated solely with the same binder at the same dosage. The feed was then dried and stored at room temperature until given to eels.

2.2 Sample collection and analysis

2.2.1 Physico-chemical parameters

Water temperature was maintained by heaters in the range of 26–27 ˚C, with continuous aeration and with daily water exchange at 75% of total water of each tank. Temperature and pH were measured twice a day at 07:00 h and 14:00 h. Nitrite (NO_2^-) and total ammonia nitrogen (NH_3/NH_4^+) were tested in the morning every three days by a Sera test-kit (Germany).

2.2.2 Biological parameters

Growth performance indicators of experimental fish consisting of weight gain (WG), daily weight gain (DWG), specific growth rate (SGR), and survival were calculated using the following equations:

Weight gain (g) = final weight - initial weight

$$\text{Daily weight gain (DWG; g day}^{-1}) = \frac{\text{final weight - initial weight}}{\text{culured days}}$$

$$\text{Specific growth rate (SGR; \% day}^{-1}) = \frac{100\ (\text{Ln final weight} - \text{Ln initial weight})}{\text{culured days}}$$

$$\text{Survival (\%)} = 100 \text{ X} \frac{\text{final number of eels}}{\text{initial number of eels}}$$

$$\text{FCR} = \frac{\text{feed provided (dry weight) - uneaten feed (dry weight)}}{\text{weight gain (wet weight)}}$$

in which the initial weight was assessed by randomly taking 30 eels from the conditioning tank to weigh individually; meanwhile the final weight was calculated on the entire cohort of eels at the end of the experiment.

The growth gain determination: Ten eels in each tank were taken at random and weighed every 10 days by use of an electronic balance with an accuracy of 0.01g (Ohaus, model: SPX622), after which the eels were returned to their original tanks.

2.2.3 Microbiological parameters

Bacterial cell numbers in the digestive tract were determined by taking samples from five randomly chosen eels in each tank at three rounds (days 10, 20, and 30, of the culture period) and placing them in glass tubes. Before sampling, eels were starved for 24 h, then the sampled eels were immersed in ice-water slurry until unconscious. Thereafter, the digestive tracts were cut, and samples were homogenized. The serial dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} of digestive tract solution were prepared following the protocol of Pepper and Gerba (2004). Then samples from each dilution were taken at 100 µl by micropipette and put in a bacteria-free agar plate (nutrient agar for bacterial total counts and TCBS for *Vibrio* counts). Afterwards it was spread on the agar surface with a sterilized spreader until dry, and each dilution was repeated three times. Bacteria were cultured in an incubator at 28 ˚C for 24 to 48 h until visible colonies appeared. The number of colony-forming units (CFUs) was counted as well as bacterial density (BD), which was calculated by the following formula (Pepper & Gerba, 2004):
BD (CFU /g) = (Average number of colonies) x (dilution level) x 10.

2.3 Statistical analysis

The mean and standard deviation of dataset for each treatment were calculated using Microsoft Excel software. The data were first checked for homogeneity of variance and normality of distribution by Levene's F-test and P-P plot. The data failed to meet these assumptions and were logarithmically transformed. For the same reason, the survival data were square-root transformed. The entire dataset was statistically processed by one-way ANOVA and the Tukey-HSD test with Statistica 7.0.

3. Results

Water quality is essential for growth of aquatic organisms (Bhatnagar & Devi, 2013), and in this study water quality was managed according to Tavares and Santeiro (2013) to a suitable range of quality parameters for aquaculture. In this way, mortality caused by water quality fluctuations was completely avoided throughout the experimental period. During this experiment, the water in all treatment tanks showed an average pH 6.9, dissolved oxygen 4.2 mg l^{-1}, total ammonia 0.41 mg l^{-1}, nitrite 0.05 mg l^{-1} and a temperature of 26 ˚C, and all these values remained within the normal range of Asian swamp eel culture.

3.1 Eel performance

At the end of the experiment, no mortality was observed in any of the treatments. The average weights of fingerlings in those treatments fed with additives, namely sodium citrate, probiotics, and probiotics mixed with sodium citrate, were not significantly different, $p>0.05$, but were significantly higher than for eels in the control group, $p<0.05$. The highest average weight was recorded in fingerlings fed with sodium citrate, and the lowest was found in the control (Table 1).

The DWG and SGR were of a similar tendency to final weight and were also not significantly different for eels fed with additives including sodium citrate, probiotics, and probiotics mixed with sodium citrate, $p>0.05$. However, higher DWG and SGR were recorded in eels fed sodium citrate, and the lowest was in those who received solely artificial feed.

Although between the additive treatments the final weight, DWG, and SGR were not statistically different, the eels fed a diet with sodium citrate (T2) showed better growth than those fed on probiotics alone (T3) or on the mix of citrate and probiotics (T4) (Table 1). The eels' final weight in the citrate treatment was 20% higher than in the control; meanwhile, for other additives the difference was around 14-15%. Furthermore, the GW, SGR, and DWG in fingerlings fed on citrate were 1.2 times higher than in those fed on probiotics alone or on the mixture of citrate and probiotics.

The highest food conversion ratio (FCR, Table 1) was found in the control (6.6), and it was more than two times

Table 1. Growth performance of eels and feed efficiency after 30 days of culture

Treatment	T1	T2	T3	T4
Initial weight (g)	7.8 ± 1.5^{a}	7.9 ± 2.0^{a}	7.9 ± 1.9^{a}	8.0 ± 1.2^{a}
Final weight (g)	9.1 ± 1.0^{a}	10.9 ± 2.2^{b}	10.4 ± 1.9^{b}	10.5 ± 1.8^{b}
Weigh gain (g)	1.3 ± 0.9^{a}	3.0 ± 2.1^{b}	2.5 ± 1.8^{b}	2.5 ± 1.8^{b}
SGR (% day^{-1})	0.509 ± 0.363^{a}	0.997 ± 0.635^{b}	0.868 ± 0.573^{b}	0.879 ± 0.569^{b}
DWG (g/day)	0.044 ± 0.034^{a}	0.099 ± 0.075^{b}	0.083 ± 0.063^{b}	0.085 ± 0.061^{b}
FCR	6.6±1.9	3.1±1.2	3.5±0.8	3.8±1.6

Different superscripts in the same row indicate significant differences ($p<0.05$); SGR: specific growth rate; DWG: daily weight gain; Food conversion ratio: FCR.

higher than with the citrate treatment (3.1) and 1.7–1.9 times that of the others (3.5–3.8). These data indicated that the additive treatments were statistically significant as the experimental group of eels consumed less feed than the control group.

3.2 Bacterial count

Up to day 10 of experiment, the bacterial density in the gut of *M. albus* of T3 was highest among treatments and significantly different from T4, $p<0.05$, as well as from others (Table 2). However, at day 20, the total bacterial count was found highest in T4, significantly different from T1 (control), $p<0.05$, but not from T2 and T3. At day 30, there was no difference in bacterial counts between any treatments, $p>0.05$, even though the highest remained in T4 and the lowest in control (Table 2).

Vibrio counts were not significantly different across the treatments on the first day of the experiment; it had increased in T1, T2 and T3 by day 10, $p>0.05$, while the count decreased in T4 and had a significant difference, $p<0.05$, from the other treatments. Interestingly, from day 20 to day 30, there was a dramatic drop in *Vibrio* appearance in T2 and T3 to very low densities (more than 10 to 15 times lower than on day 10); during the same period, there was no difference from the lowest *Vibrio* spp. density in T4 but significantly ($p<0.05$) T1 recorded its highest *Vibrio* spp. density (Table 3). In addition, the ratio of *Vibrio* per total bacteria was low for eels fed additives compared to the control treatment during the experimental period.

Table 2. Total bacterial density (CFU/g) in the experimental period

Treatment	Total bacterial density (x 10^4 CFU/g)		
	Day 10	Day 20	Day 30
T1	0.62 ± 0.42^{a}	12.06 ± 4.27^{a}	32.67 ± 7.37^{a}
T2	19.27 ± 5.28^{ab}	49.67 ± 14.98^{ab}	37.33 ± 12.74^{a}
T3	53.0 ± 15.72^{c}	34.2 ± 23.16^{ab}	36.33 ± 5.03^{a}
T4	29.53 ± 4.09^{b}	90.0 ± 37.51^{b}	44.33 ± 11.24^{a}

Different superscripts in the same column indicate statistically significant differences ($p<0.05$).

Table 3. *Vibrio* spp density counted in the gastro-intestinal of *M. albus*.

Treatment	*Vibrio* density (x 10 CFU/g)		
	Day 10	Day 20	Day 30
T1	20.70 ± 5.53^{b}	41.0 ± 21.79^{b}	18.3 ± 2.27^{b}
T2	18.10 ± 3.36^{b}	3.0 ± 1.73^{a}	1.23 ± 0.31^{a}
T3	16.50 ± 3.36^{b}	8.0 ± 0.3^{a}	0.77 ± 0.31^{a}
T4	1.70 ± 0.75^{a}	1.33 ± 0.58^{a}	0.37 ± 0.21^{a}

Different superscripts in the same column indicate statistically significant differences ($p<0.05$).

4. Discussion

Investigation of prophylactics in aquaculture to replace the use of antibiotics, both for disease control and growth promotion, has been an issue of interest in many studies; probiotics use began in the 1970s and has been widely applied in hatcheries and grow-out of many aquatic species thereafter (Cruz, Ibáñez, Hermosillo, & Saad, 2012; Hai, 2015). However, the use of such product, whether alone or in a mixture of many bacterial strains, has been questioned due to potential risks to the environmental biota as well as the health and nutrition of the hosts, including human consumers of fish (Raja, Nandhini, Sahana, & Dhanakkodi, 2015). Another promising alternative to antibiotics is organic acids, which are considered safe compounds that have been used for many years in the terrestrial feed industry; they have recently been tried in aquatic animal feed and have shown a number of beneficial effects in growth performance as well as disease resistance (Ng *et al*., 2017). Furthermore, it is hoped that the combination of probiotics and organic acids may introduce a synergistic effect that would benefit the host animals.

In the present study, the addition of citrate (a derivative of citric acid), probiotics, and probiotics mixed with citrate to *M. albus* diets nearly doubled SGR and concurrently decreased FCR by half compared to the control diet. The growth of *M. albus* also depends on feed type, body weight and stocking density. The SGR of small *M. albus* (5.76 g) was recorded at 0.84% in a culture where they fed on golden snail for 60 days (Herawati *et al*., 2018); this was higher than for the commercially-fed *M. albus*, but lower than for those eating the probiotics and probiotics-citrate diets in a recent study. However, Nhan, Tai, Liem, Ut, and Ako (2018) reported that the large size of *M. albus* seeds (16.7 g) had SGR from 1.4 % to 1.7% when stocked at 100 and 180 inds $m^{2\ -1}$ under aquaponic conditions, respectively.

Several studies have shown that organic salts and probiotics are excellent feed additives for many fish species. Goldfish (*Carassius auratus*) fed on feed supplemented with 3% of commercial probiotics showed higher growth performance and survival rate, while the lowest growth and survival were recorded in the control group (Anuar, Omar, Noordiyana, & Sharifah, 2017). In another study, black tiger shrimp (*Penaeus monodon*) fed on feed supplemented with probiotics at 5 mg kg^{-1} showed high growth performance, better FCR, and resistance to pathogenic infections (Vieira *et al*., 2016). Supplementing the feed of silver-grey catfish (*Chrysichthys nigrodigitatus*) fingerlings with 0.02 to 0.06 g kg^{-1} of sodium chloride significantly increased fish growth by improving digestibility and food utilization without any adverse effect (Udoh & Otoh, 2017). In their study on white-leg shrimp (*Litopenaeus vannamei*), Luis-Villaseñor, Macías-Rodríguez, Gómez-Gil, Ascencio-Valle, and Campa-Córdova (2011) showed antagonistic activity of four *Bacillus* strains isolated from the guts of healthy wild adult shrimp using a daily concentration of 10^5 CFU ml^{-1} against pathogenic strains of *Vibrio*. The authors' experimental results confirmed that these probiotics improved the rate of development, reaching a final index of development of 7.00 compared to about 5.76 for the control, with survival reaching 67% compared to about 4.9% for the control.

Regarding the citrate, Baruah *et al*. (2007) formulated 3% acid citric (CA) in the diet for rohu (*Labeo rohita*) fingerlings reared for 60 days, and Khajepour and Hosseini (2012) worked on common carp (*Cyprinus carpio*) fingerlings for 8 weeks with the same CA content in their diet. Both studies confirmed that the addition of CA into rearing diets improved weight gain and SGR while decreasing FCR. A recent study in mrigal carp *Cirrhinus mrigala* fingerlings

(Hussain *et al*., 2017) with different CA content found that the optimum CA additive for this species was 2.5%, since the fish exhibited better growth (32% higher in weight gain) and lower FCR (1.58 vs. 2.09) compared to control as well as to other experimental diets. Therefore, as with most other tropical freshwater species, *M. albus* has excellent growth when its diet is supplemented with sodium citrate and probiotics. However, in our experiment, the mixture of citrate and probiotics did not reproduce the synergistic benefit seen in a previous study with tilapia; the combination resulted in better growth and food utilization than when it was added alone into the diet (Hanan, Abd-Elghany, Elsadek, & Basiony, 2019). This is explained by the difference in feed protein (40% vs. 29%), species behavior, and the life stage of fish used in the two experiments, resulting in differences in bacterial occupation and digestibility in the fish gut. On the other hand, our probiotic mixture consisted of multiple constituents such as enzymes and β-glucan, and it was formulated similar to commercial products but on a tiny scale. Exploitation of probiotics, prebiotics, and enzymes in feed supplementation for livestock and aquaculture have gained attention in recent years (Akhter, Wu, Memon, & Mohsin, 2015; Das, Mondal, & Haque, 2017; Kirkpinar, Açikgöz, Mert, & Işik, 2018; Ohimain & Ofongo, 2013) to seek a synbiotic for heath benefit of the hosts because probiotics are single bacterial strains or mixtures of live bacteria, while the prebiotics (β-glucan, inulin...) are used as food sources for these bacteria in the gut. Therefore, according to Das *et al*. (2017), the combination enhances the beneficial effects of individual probionts. Moreover, it was also mentioned that improving the nutritive value of feed by adding enzymes into dietary probiotics, prebiotic supplementation, and multi-enzyme supplementation may act as growth promoters. In an experiment by Kirkpinar *et al*. (2018) on broilers with probiotics, prebiotics, enzymes alone and their mixtures, the results indicated that probiotics alone, probiotics plus prebiotics, and probiotics supplemented with enzymes significantly increased body weight after 42 days, but that there was no difference between them. Prebiotics alone did not improve growth; in fact, they performed even worse than the control. These prior results may explain why there was no difference between the growth performances of the experimental groups (T2, T3, and T4), although each of these groups significantly differed from the control (Table 1); the results in growth performance from the present study showed a similar trend to that found by Kirkpinar *et al*. (2018).

Concerning the bacterial counts, the results generally agreed with former studies in that citrate and probiotics played a role in controlling the microbiota in fish gut through the pH. The bacteria are affected by fluctuations in pH level and enzymatic activities in the fish gut (Sylvain *et al*., 2016; Wang, Ran, Ringø, & Zhou, 2017). This was clearly seen in days 10 to 20, when bacterial occupation in the intestinal tract reached high densities in all treatments except for the control, due to the presence of citrate or probiotics. At day 30, the similarity in bacterial counts in all treatments was probably due to the microbes that had developed and founded stable colonies in culture tanks that created a balance between the gut and environment. Several studies have confirmed that probiotics stimulate beneficial bacteria and modify gastrointestinal microbiota. Akbar *et al*. (2014) studied post-larval *Brachydanio rerio* with initial pathogenic bacteria of 1.35×10^4 CFU ml^{-1}, supplementing their feed with probiotic bacteria (including *Lactobacillus rhamnosus*, *Bacillus coagulans*, *Bacillus mesentericus*, *Bifidobacterium infantis*, and *Bifidobacterium longum*); after one week, gut analysis showed higher levels of *L. rhamnosus* (2.1×10^3 CFU ml^{-1}) than of *B. longum* (3.25×10^3 CFU ml^{-1}) and *B. mesentericus* (3.15×10^3 CFU ml^{-1}). Analysis of the gut of the post-larvae showed higher *L. rhamnosus* (2.1×10^3 CFU ml^{-1}), when compared to *B. longum* (3.25×10^3 CFU ml^{-1}) and *B. mesentericus* (3.15×10^3 CFU ml^{-1}). Moreover, Bolivar, Legarda, Seiffert, Andreatta, and Vieira (2018) tested the effects of eight different organic salts, including butyrate, propionate, formate, fumarate, glutamate and citrate at two pH levels (6.2 and 7) on *L. plantarum* growth. Sodium citrate and formate inhibited the growth of *L. plantarum*, but sodium glutamate, succinate and fumarate stimulated growth of *L. plantarum*, which shows the feasibility of probiotics to be substituted by other feed additives.

Vibrio act as opportunistic or secondary pathogens that can cause mortality on target aquatic animals (Thompson, Lida, & Swings, 2004). According to Marques (2005), microbes can be categorized into different classes: beneficial (including probionts), pathogens or opportunistic bacteria, and neutral microorganisms; and disease occurs when pathogenic bacteria predominate at high cell counts in the environment. Several studies have shown the efficacy of using probiotics for controlling *Vibrio* spp. in aquaculture. In this work, *Vibrio* density in the intestinal tract of *M. albus* fed with probiotics and probiotics mixed with sodium citrate was significantly lower than in those who received other treatments (sodium citrate and artificial feed). However, probiotics mixed with sodium citrate showed extremely low *Vibrio* density at day 30. Therefore, our results agree with the study of Bolivar *et al*. (2018) in observing that probiotics combined with organic salts in vitro inhibit various aquaculture pathogenic bacteria, including *V. alginolyticus*, *A. hydrophila*, *E. coli*, *P. aeruginosa* and *S. agalactiae*. Silva (2014) observed that feed supplemented with 2% propionate and butyrate (sodium salt) improved growth and modified intestinal microbiota of *L. vannamei*. Therefore, to minimize the incidence of diseases caused by *Vibrio*, probiotics should be used in different forms either alone or mixed with organic salts, and in this study sodium citrate showed good results. However, other studies are needed to evaluate additives that can substitute for sodium citrate and probiotics in aquaculture.

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