

## Effect of Probiotic Additive On Growth of the Prawn *Macrobrachium Rosenbergii* (De Man 1879) Cultivated in A Recirculating Aquaculture System

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### Abstract

The search for healthy and sustainable food increases every year, and the aquaculture of freshwater prawns could meet these demands if new production technologies are adopted. Therefore, a probiotic mixture was incorporated into commercial feed to evaluate the effect on the growth and development of *Macrobrachium rosenbergii* cultivated in a recirculating aquaculture system (RAS). The experiment was carried out inside a greenhouse at the São Paulo Agribusiness Technology Agency (APTA), Brazil. 150 prawns weighing  $0.92 \pm 0.05$  g were equally distributed into 10 circular 500-liter tanks. Two diets were provided: with and without probiotic additive. The probiotic additive consisted of the following microorganisms: *Bacillus licheniformis*, *Bacillus subtilis*, *Enterococcus faecium*, *Lactobacillus plantarum*, and *Saccharomyces cerevisiae*. The experimental design was completely randomized with two treatments, as noted, four cultivation periods (0, 25, 50 and 75 days) and five repetitions (tanks). Water quality parameters, zootechnical parameters and Gompertz growth curves were evaluated to compare the growth rates. At 75 days of cultivation, the probiotic mixture proved to be beneficial, as determined by the higher final weight of prawns ( $p < 0.05$ ) and better absolute and relative growth rate. The probiotic mixture improved the weight and growth of *Macrobrachium rosenbergii* in RAS, indicating its advantage for small- to large-scale farmers by producing healthier animals with faster growth, thus reaching commercial size in a shorter period of time.

**Keywords:** Absolute Growth Rate, Crustacean, Bacillus, Prawn Farming, Recirculating Aquaculture System

## Introduction

The rearing of aquatic organisms, or aquaculture, has shown considerable expansion in the last three decades [1, 2, 3]. It has more than tripled in live-weight volume from 34 Mt in 1997 to 112 Mt in 2017 [2, 3]. Crustaceans represent 7.5% (8.44 Mt) of global aquaculture production with 30 species reported in 2017. The global production of crustaceans grew at an annual rate of 9.9% between 2000 and 2017, which is higher than that of farmed fish (5.7%) [2].

Freshwater crustacean production was 2.53 Mt in 2017. Giant tiger prawn, giant river prawn and oriental river prawn were the most cultivated species [2, 4], representing 50%. Freshwater prawn farming is one of the fastest growing sectors in aquaculture in the world, and species of the *Macrobrachium* genus are the most cultivated [5]. *M. rosenbergii* is one of the main species commercially produced in Brazil [6] owing to its biological characteristics, omnivorous feeding, fast growth and rusticity (Santos, 2013), yet little is known about its cultivation in Brazil. Worldwide, *M. rosenbergii* is popularly known as giant river prawn, and in Brazil, it is known as Malaysian shrimp.

Prawn farming could result in an increase in demand for these animals, promoting improvement in the production chain [7] in addition to meeting the demand for sustainable aquaculture with a low environmental impact [8]. The implementation of more sustainable technologies adds value to the commercialization of freshwater shrimp. Examples include low water renewal in cultivation, reduction of artificial feed in food, and integrated cultivation with fish [7].

The role of freshwater systems has gained attention in part because of advances in feed technology and breeding, as well as destructive habitat conversion, particularly from shrimp farming in mangrove ecosystems. Pathogens, parasites, and pests are a chronic risk for the aquaculture sector, a risk that has been amplified by intensification of production and increased trade and supply chain integration since 2000 [9]. The implementation of RAS could solve part of this problem. Given the high disease and water quality risks in many farming areas, RAS is also a cost-effective cultivation alternative (Boopathy, 2018). New feed additives and probiotics have increased considerably in recent years. Probiotic additives in feed are extremely important to obtain better performance and greater growth, resulting in less expenditure on inputs and leading to a satisfactory economic return to producers of saltwater shrimp [10-14] and freshwater prawns [15-21].

According to Dantas [22], the use of probiotics in water or feed can improve an animal's performance by the maintenance of feeding activity, considering an increase in the animal's immunological resistance. The use of probiotics contributes to an improvement in animal performance, and it can reduce the number of pathogens [23]. According to [24], the addition of two or more probiotics can result in increased immunity, growth and survival of prawns typically afflicted with pathogens. In the cultivation of *M. rosenbergii*, the use of the probiotic *Lactobacillus plantarum* improved the immune response of animals [18]. The same was observed in the diet of *M. rosenbergii* using *Bacillus subtilis* [15].

Therefore, this study aimed to determine the effect of a feed-based probiotic mixture on the growth and zootechnical performance of the prawn *Macrobrachium rosenbergii* cultivated in a recirculating aquaculture system (RAS).

## Material and Methods

The experiment was carried out in a RAS inside a greenhouse with environmental control located at the Agribusiness Technology Agency of São Paulo (APTA) in Presidente Prudente, state of São Paulo, Brazil, for a period of 12 weeks.

Initially, 150 prawns weighing  $0.92 \pm 0.05$  g were equally distributed into 10 circular 500-liter tanks with pipe-shaped shelters. The cultivation system was provided with a water circulation of  $10 \text{ m}^3 \text{ h}^{-1}$  and automatic temperature control. Water temperature was maintained at 28°C. Dissolved oxygen, which was supplied with radial mechanical aerators, was measured daily at an average of 5.6

mg L<sup>-1</sup>. The pH, ammonia, nitrite and nitrate were analyzed weekly with maximum mean values and standard deviation (SD) of 7.9 (1.31), 0.25 (0.06), 0.08 (0.02) and 15.5 (1.98), respectively.

The prawns were fed three times a day with extruded high-density feed, containing 38% crude protein and 2 mm particle size, as shown in Tables 1 and 2.

The amount of feed was initially offered as 7% of tank biomass. As prawns developed, this amount decreased to 5% of the biomass of each tank.

Description	Use (%)	1,000.00 kg
Ground corn	15.23	152.30
Soybean 60%pb (spc)	21.45	214.51
Wheat bran 16%.	8.73	87.28
Wheat flour	7.00	70.00
Meat meal	4.29	42.91
Marine fish flour 55%	20.00	200.00
Squid flour	2.00	20.00
Tilapia flour	8.00	80.00
Actipro hemoglobin	4.00	40.00
Nutribinder	0.70	7.00
Salt	0.50	5.00
Limestone 38% calcium	2.00	20.00
Dicalcium phosphate	0.20	2.00
Fish oil (marine)	2.00	20.00
Aquagest omf	0.20	2.00
Vitamin c 35%	0.10	1.00
Raguife antioxidant <sup>1</sup>	0.10	1.00
Mycotoxin adsorbent	0.30	3.00
Antifungal	1.00	10.00
Emulsifier <sup>2</sup>	0.20	2.00
Aquabite <sup>3</sup>	1.00	10.00
SP1 (Alltech) <sup>4</sup>	0.50	5.00
Premix	0.50	5.00
	100.00	1000.00

<sup>1</sup>Binder = Soluble fish = palatable. <sup>2</sup>Natural hepatoprotector and digestibility enhancer. The product has an emulsifying action, breaking the mycelium of fat into smaller sizes for better assimilation by prawns, as well as helping to release enzymes for better food digestion. Product composed of organic acids, plant extracts and natural emulsifier. <sup>3</sup>Fish soluble = palatable. <sup>4</sup>Source of polyunsaturated fatty acids

**Table 1:** Values in % of ingredients used in the formulation of freshwater prawn feed

The probiotic additive supplied by Biomart Animal Nutrition Import and Export LTDA was used. It is comprised of the following guaranteed levels of microorganisms: *Bacillus licheniformis* 4.5x10<sup>9</sup> UFC/g, *Bacillus subtilis* 4.5x10<sup>9</sup> UFC/g, *Enterococcus faecium* 3.0x10<sup>9</sup> UFC/g, *Lactobacillus plantarum* 3.0x10<sup>9</sup> UFC/g, and *Saccharomyces cerevisiae* 5.0x10<sup>8</sup> UFC/g.

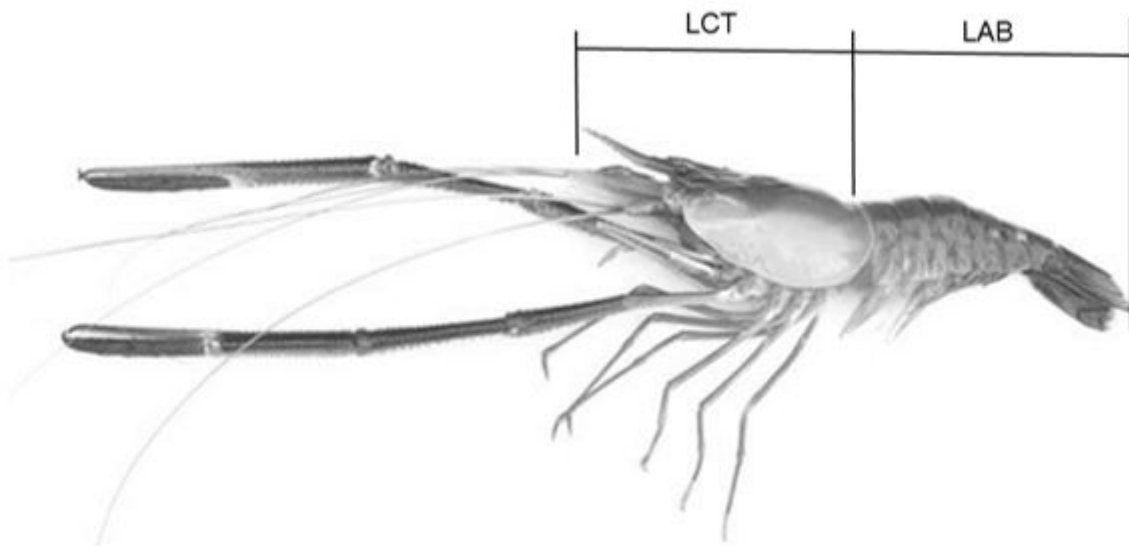
During feed formulation, the probiotic additive was added and homogenized in a concentration of 2% soybean oil and then sprinkled on the feed. The concentration used during the formulation was as follows: 10 kg of feed was mixed with 0.2 kg (2%) of vegetable oil containing 10 g (0.1%) of the probiotic additive. In the control treatment, the prawns received no probiotic additive at any time. Only commercial feed was sprinkled with 2% soybean oil.

Description	Unit	Use
DE (digestible energy)	Kcal / Kg	3321.4
DP (digestible protein)	%	34.2
Crude protein	%	38.5
Fat	%	8.0
Raw fiber	%	2.5
Ash	%	12.3
Calcium	%	4.0
Total Phosphorus	%	1.8
Starch	%	18.9
Phosphorus Dis	%	0.8
Arginine	%	2.4
Lysine	%	3.1
Threonine	%	1.8
Tryptophan	%	0.4
Methionine	%	0.7
Vitamin	mg / kg	650.0
Mix	mg / kg	100.0

**Table 2:** Nutritional composition of freshwater shrimp ration

## Biometry

Prawns were measured in length, divided into cephalothorax and abdomen, as shown in Figure 1.



**Figure 1:** Representation of the division in the length measurement between cephalothorax (LCT) and abdomen (LAB). Adapted from Mahalder, B. [www.bdfish.org](http://www.bdfish.org)

At the end of each period (0, 25, 50, 75 days of cultivation), the prawns were weighed, and both feed conversion and gain in biomass were calculated. At the end of the experiment, the total weight gain, feed conversion, amount of feed provided, specific growth rate, and overall mortality and survival rate were also determined.

Apparent feed conversion (FC) was determined as

$FC = B_f - B_i / F_i$ , where  $B_f$  = final biomass,  $B_i$  = initial biomass /  $F_i$  = feed intake in the period.

Biomass (B) was determined as

$B = A_w \times N$ , where  $A_w$  = average weight and  $N$  = number of prawns/tank.

Total weight gain was determined as

$WG = WG_f - WG_i$ , where  $WG_f$  = final weight gain and  $WG_i$  = initial weight gain.

The gain in biomass (GB) was determined as

$GB = GB_f - GB_i$ , where  $GB_f$  = final biomass gain, and  $GB_i$  = previous biomass gain.

The specific growth rate (SGR) was determined as

$SGR = (\ln W_f - \ln W_i) \times 100 / ND$ , where  $W_f$  = final weight,  $W_i$  = initial weight and  $ND$  = number of days of cultivation.

Mortality (M) rate was determined as

$M = N_i - N_f$ , where  $N_i$  = initial number, and  $N_f$  = final number of prawns.

Survival rate (S) was determined as

$S (\%) = N_t / N_i \times 100$ , where  $N_t$  = total number of live animals, and  $N_i$  = total number of prawns at the beginning.

## Data analysis

The experimental design was completely randomized with 2 treatments evaluated in 4 periods (0, 25, 50, 75) with 5 replications (tanks). Analysis of variance and growth model fit were performed using SAS software (SAS OnDemand for Academics, Copyright © 2020 SAS Institute Inc.).

The Gompertz growth model given by  $y = A e^{-B e^{-Kx}}$  was fitted. In this model,  $A$  is the asymptotic value for weight or size at maturity,  $B$  is an integration constant, and  $K$  is a function of the maximum growth rate, or maturation index. Additionally, the absolute (AGR) and relative (RGR) growth rates were determined, respectively  $K \ln(u-1)$ ,  $K \ln(u-1)$  and  $u = y / A$ . The weight (WI) and age (AI) at the inflection point are  $A/e$  and  $(\ln B) / K$ , respectively [25].

The curve parameters for each treatment were compared by their confidence intervals at 95% probability. Equations and adjusted coefficient of determination ( $R^2_{Adj}$ ) were provided. Estimates were obtained by weighted least squares, considering autoregressive errors [25-27]. The inverse of weight variance was used as a weighting factor; therefore, the F tests and confidence intervals were valid.

## Results

Abdomen and cephalothorax measurements showed a statistical difference at day 25 of rearing. Prawns in the control treatment were larger than those in the probiotic treatment. The abdomen and cephalothorax relationship (AB/CT) did not present any statistical difference during this period of cultivation (Table 3).

Days	Probiotic	AB (cm)	CT (cm)	AB/CT
0	With	5.13 (0.52) A	2.21 (0.24) A	2.33 (0.20) A
	Without	5.06 (0.54) A	2.10 (0.46) A	2.52 (0.60) A
25	With	6.38 (0.95) B	2.69 (0.33) B	2.37 (0.26) A
	Without	7.34 (0.60) A	3.06 (0.42) A	2.43 (0.32) A
50	With	7.60 (0.51) A	3.32 (0.42) A	2.31 (0.24) A
	Without	8.06 (0.75) A	3.49 (0.33) A	2.31 (0.15) A
75	With	9.23 (1.04) A	4.01 (0.39) A	2.30 (0.19) A
	Without	9.65 (0.88) A	4.04 (0.49) A	2.40 (0.24) A

\*Values followed by the same letter in the column do show no statistical differences ( $p>0.05$ )

**Table 3:** Mean length of the abdomen (AB) and cephalothorax (CT) and the AB/CT ratio of Malaysian prawns fed with probiotics in different periods

After 75 days of cultivation in a recirculating aquaculture system, the prawns showed no statistical differences in zootechnical parameters between the probiotic and control treatments. Weight gain was measured as 4.38 (0.21) g and 3.80 (0.61) g, the feed conversion rate was measured as 6.36 (0.72) and 6.31 (0.57), total feed provided was 506.98 (43.30) g and 455.24 (64.14) g, and specific growth rate was measured as 2.31 (0.06) and 2.19 (0.17) for probiotic and control treatments, data representing mean and standard deviation respectively.

In addition, the survival rate showed no statistical difference during the total cultivation period between the treatments. The survival rate was high following final mean values of 91.8% (10.6) for the probiotic treatment and 89.2% (13.4) for the control. These values can be considered high for the production of freshwater shrimp in RAS.

At the end of 75 days of cultivation, prawns fed with feed containing the probiotic mix had higher final weight than those in the control treatment ( $p<0.05$ ). Biomass, on the other hand, did not show a statistical difference in any cultivation period (Table 4).

Days	Probiotic	Final weight (g)		Biomass (g)	
0	Without	0.90	(0.03) A	25.20	(0.75) A
	With	0.94	(0.08) A	26.39	(1.98) A
25	Without	1.94	(0.08) A	48.64	(2.21) A
	With	1.91	(0.18) A	49.54	(5.40) A
50	Without	3.36	(0.34) A	82.75	(10.40) A
	With	3.54	(0.20) A	90.18	(6.39) A
75	Without	4.70	(0.61) B	85.29	(30.72) A
	With	5.32	(0.27) A	107.18	(13.11) A

\*Values followed by the same letter in the column do show no statistical differences ( $p>0.05$ )

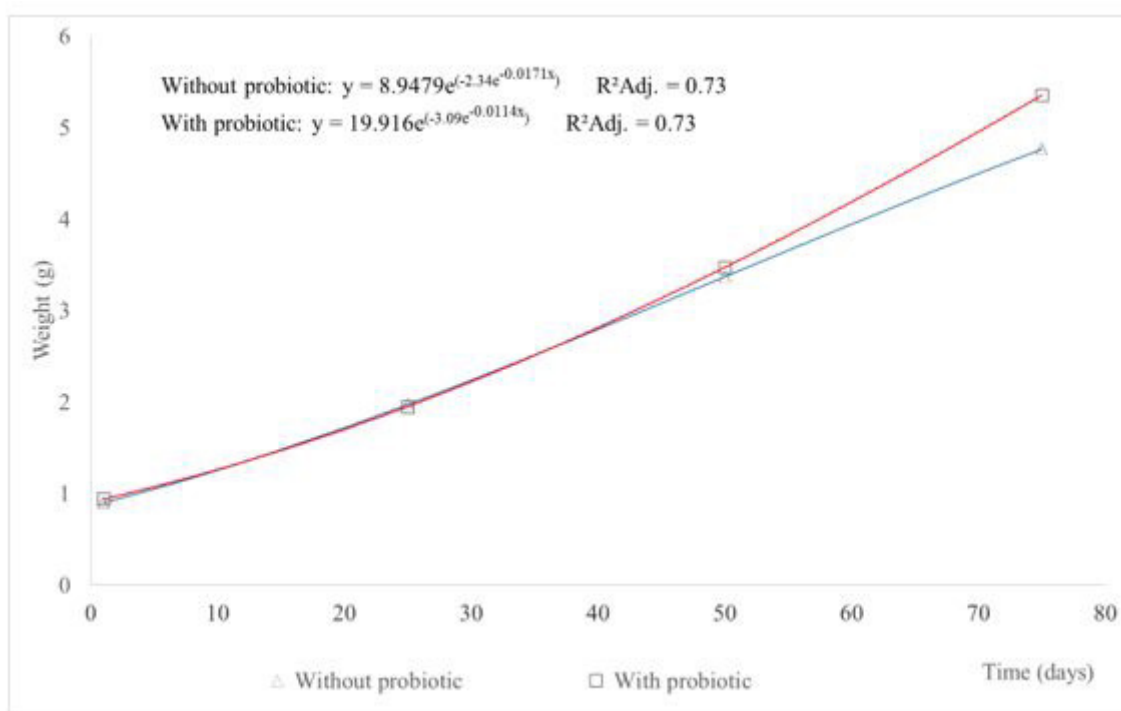
**Table 4:** Mean and standard deviation of final weight and biomass of shrimp treated with probiotics in different periods

Table 5 presents the parameter estimates for the Gompertz model. No differences were noted in estimates of “A” and “K” between treatments. The model fit is shown in Figure 2.

Probiotic	Estimates		Confidence Interval			
	A (g)	K (%)	A (g)		K (%)	
			Lower	Upper	Lower	Upper
Without	8.9479 a	0.0171 a	6.5481	14.6499	0.0124	0.0217
With	19.9159 a	0.0114 a	11.2109	56.9944	0.0072	0.0155

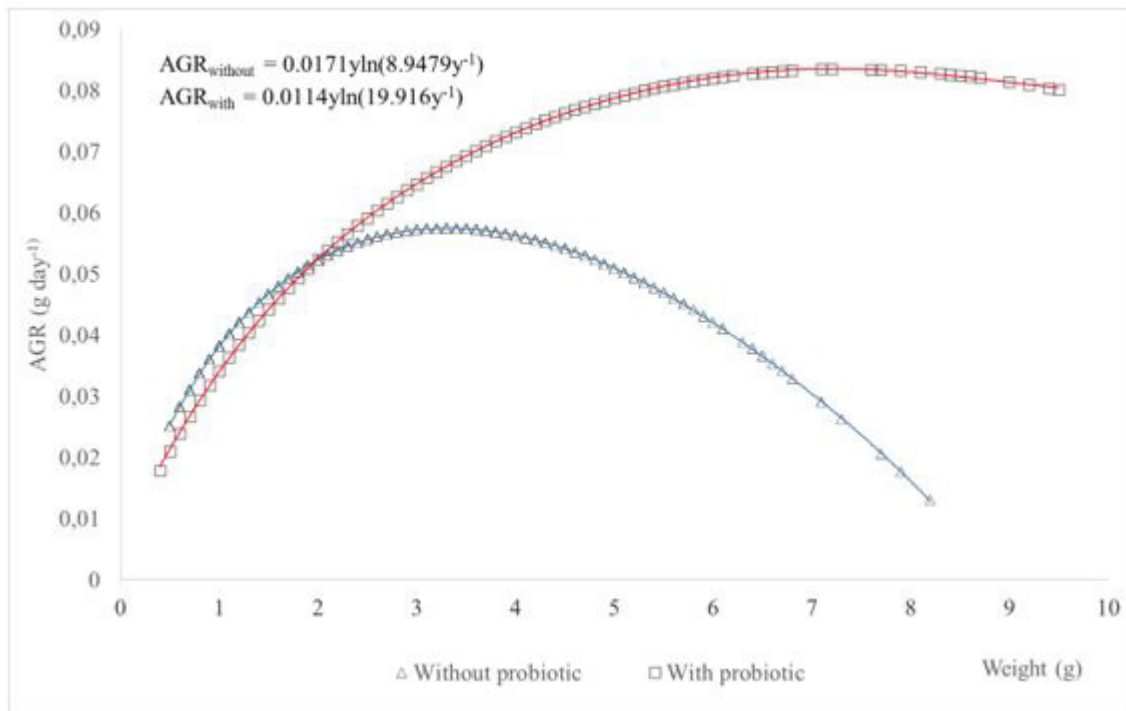
\*Values followed by the same letter in the column do show no statistical differences (p>0.05)

**Table 5:** Estimated parameters "A" and "K" and confidence intervals of the Gompertz growth model of Malaysian shrimp cultivated in a recirculating system fed with feed containing probiotics

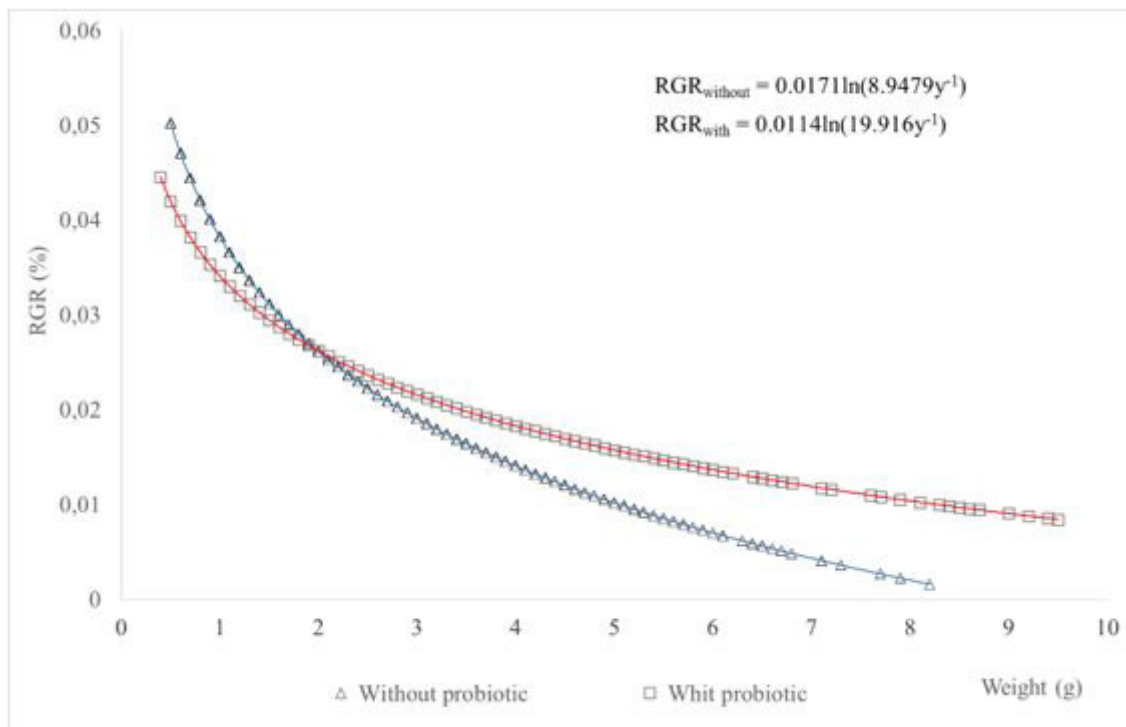


**Figure 2:** Gompertz growth model of Malaysian prawns cultivated in a recirculating aquaculture system fed with feed containing probiotics (n = 1,016 observations)

Figures 3 and 4 show the absolute (AGR g/day) and relative (RGR %) growth rates of prawns fed with feed containing probiotics, as well as changes in the values of these variables with the growth of organisms



**Figure 3:** Absolute growth rate (AGR) of Malaysian shrimp cultivated in a recirculating aquaculture system fed with feed containing probiotics. Each point represents the estimate of each observation (498 and 518 observations in treatments without and with probiotics, respectively)



**Figure 4:** Relative growth rate (RGR) of Malaysian shrimp cultivated in a recirculating aquaculture system fed with feed containing probiotics. Each point represents the estimate of each observation (498 and 518 observations in treatments without and with probiotics, respectively)



The weight and age at the inflection point and the maximum AGR are shown in Table 6. Prawns fed with probiotics had higher weight (7.33 g), age (99 days) and AGR (0.084) at inflection when compared to those that received feed without probiotics (3.29 g, 49.63 days and 0.056 g/day, respectively).

Probiotic	Weight (g)	Age (days)	AGR (g/day)
Without	3.29	49.63	0.056
With	7.33	98.92	0.084

**Table 6:** Values of weight (g), age (days) and absolute growth rate (AGR g/day) at the inflection point of Malaysian prawns cultivated in a recirculation aquaculture system fed with feed containing probiotics

## Discussion

Probiotics in aquaculture have become a prophylactic alternative to the use of antibiotics. Competition for nutrients and areas of adhesion does occur between probiotic microbiota and existing pathogens. However, probiotic microbiota can still produce enzymes that improve the health of cultured organisms, as well as promote stimuli for the immune system [28]. According to [29], the use of probiotics increases the performance and appetite of cultivated species, decreasing the use of antibiotics. [30] reported that the addition of probiotics in treatment tanks ensures a greater use of the feed from microorganisms that influence the digestion and absorption of food.

[31] cultivated *Macrobrachium amazonicum* at different levels of storage and found differences in the average length of prawns at 60 days, ranging from 6.35 cm (0.19) to 6.73 cm (0.17), in an open-air recirculation system. Biometrics of the freshwater prawn *M. rosenbergii* in the present study showed statistical difference only on the 25 days of cultivation. Prawns in the control treatment were larger in cephalothorax 3.06 (0.42) cm and abdomen 7.34 (0.60) cm than those in the probiotic treatment measuring 2.69 (0.33) cm and 6.38 (0.95), respectively. Even with this difference between treatments, the mean weight and shrimp biomass did not show a statistical difference in the same period.

After 25 days of cultivation, prawns from the control treatment were longer and had less body thickness compared to prawns from the probiotic treatment (Table 3). No statistical difference was noted between abdomen and cephalothorax in any period of cultivation. These data suggest that any differences between treatments were minimized after the initial period of activation and positive responses of probiotic microorganisms.

[19] noted that the use of probiotics improved feed conversion in Amazon prawn farming compared to treatment without probiotics. [32] also observed an improvement in feed conversion of post-larvae of *M. rosenbergii* in different crop cycles. [11] reported that cultivation of *Litopenaeus vannamei* fed with probiotics in the ration had better feed conversion, higher survival rate and greater tolerance to stress.

The final mean values of the survival rate did not show statistical differences between the probiotic treatment 91.8% (10.6) and the control 89.2% (13.4), but the two treatments showed a high survival rate in *M. rosenbergii* cultivation. Good water quality and proper management throughout the cultivation may have contributed to this high rate in both treatments. [10] reported that the use of probiotics in the cultivation of *Peneaus monodon* proved to be effective, obtaining a maximum and minimum survival rate of 95.2% and 75.1%, respectively, in the rearing system. In an experiment by [20], the use of probiotics showed better efficiency when compared with that of the control treatment in *M. rosenbergii* cultivated in a BFT (Biofloc Technology) system. However, during a period of 60 days of cultivation using *B. licheniformis* as probiotic treatment, [16] reported results similar to those of control treatment in our *M. rosenbergii* cultivation.

We showed that the combination of several bacteria and a yeast to formulate a probiotic mixture was beneficial in the cultivation of *M. rosenbergii* in RAS under a controlled environment. This sets a precedent for the possibility of greater efficiency in large-scale

production. The gain in biomass was not statistically different, but this may have occurred because of the high values of standard deviation between treatments, a fact attributed to the heterogeneity of animals in each treatment.

In the study by [12], a statistical difference can be observed in the body weight of *Peneaus monodon* prawns cultivated in ponds containing 37.67 g (1.15) of probiotic compared to prawns cultivated in control ponds at 27.33 g (0.58). The same split in data was reported by [13], cultivating the marine shrimp *Litopenaeus vannamei*. They also obtained better results for the use of probiotics added to the water compared to the control treatment. [14] found no statistical difference in the final weight of *Litopenaeus vannamei* among control, prebiotic (inulin), probiotic (*Lactobacillus plantarum*) and symbiotic (*Lactobacillus plantarum* + inulin) treatments, using only one bacterium as a probiotic.

The Gompertz model adjustment for weight x age data of Malaysian prawns cultivated in RAS fed with feed with or without probiotics was good (high  $R^2$ Adj), showing applicable estimates and reliability. Prawns that received the probiotic had the highest AGR. Moreover, prawns above 4.0 g had increasing TCA, while prawns that did not receive a probiotic had a decreasing AGR from 3.0 g onwards. The same can be observed in the RGR, in which the decreasing curve was more accentuated in prawns that received feed without probiotic, when compared to the RGR of prawns that received feed with probiotic.

Figure 4 shows that prawns receiving the probiotic additive reached the inflection point at 7.33 g and daily growth at of 0.084 g/day and that this growth rate could be maintained for up to 99 days. In contrast, the control treatment was inferior, demonstrating a decrease in growth when prawns reached their inflection point at 3.39 g and a growth rate at 0.056 g / day, maintaining this growth only up to 49 days. It is noteworthy that the AGR was 50% higher in prawns that received feed containing probiotics when compared to the control group without probiotics.

[21] reported that the growth in weight and length of *M. rosenbergii* prawn using the probiotic RABAL at different doses was statistically superior to the control treatment, indicating that the use of probiotic was beneficial in that production system. After the first week of cultivation of *M. rosenbergii* larvae, [14] observed that the weight was 26.13 mg (0.986), representing a daily gain of 3.34 mg (1.237) for the probiotic treatment, while prawns in the control treatment weighed 25.76 mg (0.838) and daily gain of 2.76 mg (0.0547). [33] cultivated *M. rosenbergii* PL at different densities, 4, 8, 12, 16, 20, for a period of six months. Growth curves were determined, and they observed that increasing density caused a decline in the growth curve. These data show that the use of probiotics directly influences the final weight and growth in prawns, and, as shown in the present study, a shorter cultivation time could be achieved.

## Conclusion

From our results, it can be concluded that the incorporation of a probiotic mixture in commercial feed was beneficial in the rearing of the freshwater prawn *M. rosenbergii* at the end of 75 days of cultivation. The probiotic improved weight and growth in RAS, implying an advantage for small- to large-scale farmers in cultivating healthier animals with a faster rate of growth, attaining commercial size in a shorter period of time.

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## **Author Contributions**

Vinicius Vasconcelos Silva did the acquisition, interpretation of data and written; he has approved the submitted version and agrees to be personally accountable for the author's own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work

Rondinelle A. S. Salomão did substantial contributions to the acquisition of data and written, he has approved the submitted version agrees to be personally accountable for the author's own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work

Vander Bruno dos Santos did the design of the work, analysis, interpretation of data and final written; he has approved the submitted version and agrees to be personally accountable for the author's own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work

## **Conflicts of Interest**

The authors declare no conflict of interest.

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