

## Use of *Saccharomyces cerevisiae* to control the quantity and quality of water in white shrimp culture

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### Abstract

We evaluated the effect of BlueEnergy<sup>Rent</sup> product based on yeast *Saccharomyces cerevisiae* and molasses, on the cultivation of white shrimp *Litopenaeus vannamei*, determining the impact on water quality parameters, and productive. The experiment was carried out at the Acuilan aquaculture farm, located in La Antigua, Veracruz, Mexico, in 2015. In the shrimp culture, 1.5 g/m<sup>3</sup>/day of the active molasses-yeast mixture was added as the first treatment and a daily dose adjusted based on the total ammoniacal nitrogen in the second treatment. Both were compared against a treatment with constant water flow. It was observed that BlueEnergy<sup>Rent</sup> did not negatively affect the quality of the water, the productive parameters presented better levels with the treatment based on the total ammoniacal nitrogen, and it was possible to reduce the consumption of water for each kg of shrimp produced to 3.9 m<sup>3</sup> kg<sup>-1</sup>. Healthy organisms with normal growth were harvested with a feed conversion ratio of 1.19. By using BlueEnergy<sup>Rent</sup> the use of water is improved without affecting crop performance.

**Keywords:** aquaculture, nitrogen compounds, yeast.

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## Introduction

The aquaculture industry has received strong criticism due to the negative environmental and economic effects perceived by excessive water consumption and, at the same time, by the subsequent release of wastewater. Which leads to direct this activity towards a concept of sustainable aquaculture. Although the specific definitions of sustainable aquaculture are varied, the limited use of water is key in any of the definitions and there is an increasing demand on the part of consumers of products grown in systems with environmental responsibility.

At present, numerous efforts have been made to develop recirculation systems and tending to zero water exchange, using chemical and biological filters, which lead to maintaining water quality by eliminating toxic metabolites, such as ammonia ( $\text{NH}_3$ ) and nitrite ( $\text{NO}_2^-$ ), in addition to ammonia nitrogen ( $\text{NH}_3^+ \text{NH}_4^+$ ), which is excreted by organisms and produced by the microbial decomposition of uneaten food (De la Mora *et al.*, 2003).

One option that has been effective in maintaining water quality in aquaculture crops has been the use of beneficial microorganisms such as bacteria and algae, which is known as bio-floc. Serrano and Machuca (2013) in studies with controlled temperature conditions, determined the efficiency of yeasts as a water quality control agent, mainly on the concentration of ammoniacal nitrogen.

The objective of this research was to evaluate the effectiveness of a product based on yeast BlueEnergy<sup>®</sup> on the control of the quantity and quality of the culture water, as well as its effect on the production parameters of shrimp *Litopenaeus vannamei*, one of the culture organisms of greater production and importance in international aquaculture (Plascencia and Almada, 2012).

## Material and methods

### Location of the experiment

The research was carried out at the aquifer farm Acuilan SPR de RL, located in the Central Region of the state of Veracruz. With location of 19° 22' north latitude, 96° 22' west longitude, at an elevation of 20 meters above sea level. It presents an AW<sub>2</sub> climate.

### Acclimatization of postlarvae

The postlarvae (pL) of white shrimp (*L. vannamei*) acquired in the laboratory of Camaronera, SA de CV, Alvarado, Veracruz, Mexico was cultivated. The initial size was pL15, with an average weight of 4.6 mg. The postlarvae were transported in two transparent plastic bags with a volume of 28.0 L of water at a salinity of 30 ‰. They were acclimated for 5 days, until obtaining a salt of 2 ‰.

## Experimental unit

It consisted of concrete ponds, ten of them with a capacity of 16 m<sup>3</sup> and two ponds of 9.6 m<sup>3</sup>. Each pond had permanent aeration through a 0.5 HP blower, maintaining dissolved oxygen levels in suitable ranges for the culture of 5-15 mgL<sup>-1</sup>.

## Cultivation period

After acclimatization, 60 pl m<sup>-3</sup> were sown in each of the 12 concrete ponds with a weight of 19.6 mg. The experimentation began the fourth week of June to the third week of September 2015 completing 86 days of experimentation.

## Product to evaluate

The product BlueEnergy<sup>Rent</sup> (BE) was used, formulated with yeast base *Saccharomyces cerevisiae* together with a mixture of dehydrated molasses, vitamins and minerals, in a ratio of 1:10.

## Experimental design

Three experimental treatments were used: the T1 treatment consisted in the application of a daily dose of BE 1.5 mg m<sup>-3</sup> and zero water exchanges, the T2 treatment consisted of a dose of BE in proportion to the estimated amount of the total ammonia nitrogen (NAT) present in the water and zero water exchanges, the T3 treatment consisted in not adding BE and maintaining a water exchange of 1.28 m<sup>3</sup> h<sup>-1</sup>. The distribution of the treatments was completely random, each with four repetitions.

To determine the BE dose in T2, the NAT present in the water was calculated as mentioned by Brunty *et al.* (1997) applying the following formula:

$$\text{NAT} = 0.604 * \text{N-food} + 3.88$$

On the recommendation of the BlueEnergy<sup>Rent</sup> supplier, the NAT was multiplied by a factor of 11, based on the C:N ratio in the culture water; both doses were weighed on a portable Braunker<sup>®</sup> scale, and then hydrated by shaking for 20 minutes in one liter of water.

## Feeding

The feeding was distributed in 3 schedules giving 25% at 6:00 am, 25% at 12:00 pm and 50% of the food at 6:00 pm.

## Measurement of water quality parameters

Daily the physical and chemical parameters of the water were measured twice a day, morning and evening, with a YSI professional plus<sup>®</sup> equipment and a Hanna<sup>®</sup> brand potentiometer. The parameters measured were: temperature (°C), dissolved oxygen (mgL<sup>-1</sup>), salinity (‰), total ammoniacal nitrogen (mgL<sup>-1</sup>) and hydrogen potential.

## Water use and economic indicators

The following indicators were evaluated: volume of water used ( $\text{m}^3$ ), efficiency of water use ( $\text{m}^3 \text{kg}^{-1}$ ). Production cost (\$ MN), utility (\$ MN).

## Biometrics

Every 8 days, 30 shrimp were caught from each stake and weighed with a portable Braunker® scale, while 10 of these shrimps were measured with a ruler graduated in cm.

In the eleventh biometrics, experimentation was terminated. Each of the ponds was emptied and the final number of shrimp was counted. The 50 organisms from each pond were measured and weighed individually to determine average weight and size.

## Productive parameters

The following productive parameters were determined: individual weight gain (GP), final length (cm), daily growth rate (TCD), final biomass (kg), feed conversion ratio (TCA), survival (%).

## Statistic analysis

The values of the physical and chemical variables and the productive parameters were subjected to an analysis of variance (Anova) of one way and Tukey test, using a level of significance of 0.05.

## Results

### Parameters of water quality

The five parameters of water quality evaluated showed a significant difference of the treatments T1 and T2 compared with T3 (Table 1).

**Table 1. Average value of salinity and pH evaluated in the white shrimp culture (*L. vannamei*) at 8:00 a.m. and 5:00 p.m. respectively**

Parameters	Hour	Treatment 1	Treatment 2	Treatment 3
Salinity ‰	8:00 am	1.73 ±0.12 <sup>a</sup>	1.75 ±0.11 <sup>a</sup>	2.05 ±0.03 <sup>b</sup>
	5:00 pm	1.74 ±0.12 <sup>a</sup>	1.76 ±0.12 <sup>a</sup>	2.05 ±0.03 <sup>b</sup>
pH	8:00 am	9.01 ±0.33 <sup>a</sup>	9.06 ±0.37 <sup>a</sup>	8.26 ±0.17 <sup>b</sup>
	5:00 pm	9.28 ±0.23 <sup>a</sup>	9.3 ±0.27 <sup>a</sup>	8.64 ±0.13 <sup>b</sup>

Means with the same letter within columns are significantly equal, according to the Tukey test ( $p \leq 0.05$ ).

Regarding the temperature obtained, it was observed that during the mornings it remained in the optimum range for shrimp culture, in the afternoon the temperature of the treatments T1 and T2 (without water exchange) exceeded the recommended average (Table 1).

The variations in the levels of O<sub>2</sub> were similar in the treatments T1 and T2, remaining in the range of 4.5 mg L<sup>-1</sup> and up to 8.5 mg L<sup>-1</sup>. In the T3 treatment, O<sub>2</sub> presented higher levels than the T1 and T2 treatments (Table 1).

Salinity was found within the ranges of survival of the shrimp, the values remained stable in T3. The lowest values were recorded in treatments T1 and T2 as shown in Table 1.

Regarding NAT, it is observed that there is a significant difference between the treatments T1 and T2 compared with T3.

The pH reached high values in the treatments T1 and T3 (without water exchange) staying above 9 from the second day and during most of the experimentation, in the T3 the pH remained constant.

### Water use and economic indicators

Regarding the use of water, the T1 and T2 treatments presented greater efficiency (Table 2). The T3 uses 100 times more water with similar performance (Table 3).

**Table 2. Water use and economic indicators.**

Characteristics	Treatment 1	Treatment 2	Treatment 3
Water use (m <sup>3</sup> )	90.3 <sup>a</sup>	90.2 <sup>a</sup>	10 011.972 <sup>b</sup>
Water efficiency (m <sup>3</sup> kg <sup>-1</sup> )	4.19 ±1.46 <sup>a</sup>	3.78 ±1.45 <sup>a</sup>	438.74 ±303.76 <sup>b</sup>
Production cost (\$ kg <sup>-1</sup> )	78.52 ±2.24	74.74 ±2.02	96.32 ±6.6
Utility (\$)	1217.87	1439.07	882.68

**Table 3. Production parameters of the white shrimp culture (*L. vannamei*).**

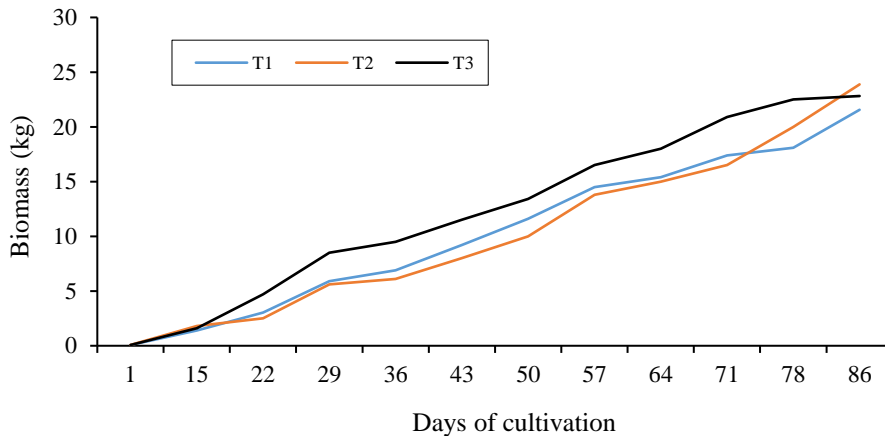
Parameters	Treatment 1	Treatment 2	Treatment 3
Weight gain g	7.17 ±1.26 <sup>a</sup>	7.96 ±1.54 <sup>a</sup>	15.0 ±1.35 <sup>b</sup>
Size cm	10.27 ±0.79 <sup>a</sup>	10.62 ±0.94 <sup>a</sup>	13.31 ±0.31 <sup>b</sup>
Daily growth rate	0.08 ±0.01 <sup>a</sup>	0.09 ±0.01 <sup>a</sup>	0.17±0.01 <sup>b</sup>
Feed conversion rate	1.32 ±0.36 <sup>a</sup>	1.19 ±0.33 <sup>a</sup>	1.42±0.87 <sup>a</sup>
(%) survival	73.7 ±3.03 <sup>a</sup>	66 ±7.91 <sup>ab</sup>	45 ±21.01 <sup>b</sup>

Means with the same letter within columns are significantly equal, according to the Tukey test ( $p \leq 0.05$ ).

Treatment T2 had a lower production cost than T1 and T3 (Table 2), this led to a saving of 22.4% compared to treatment T3. Concerning the efficiency in the use of water, the T3 presents significant difference in relation to T1 and T2 (Table 2).

## Productive parameters

The productive parameters are presented in Table 3, the treatment T2 with the dose according to the presence of TAN in the water was the one that generated the highest biomass 23.8 kg (Figure 1) maintaining the highest feed conversion rate. The T1 maintained the highest survival percentage with 73.7%. In T3 the organisms obtained the highest size and the highest weight gain, which is reflected in a daily growth rate of 0.17 g.



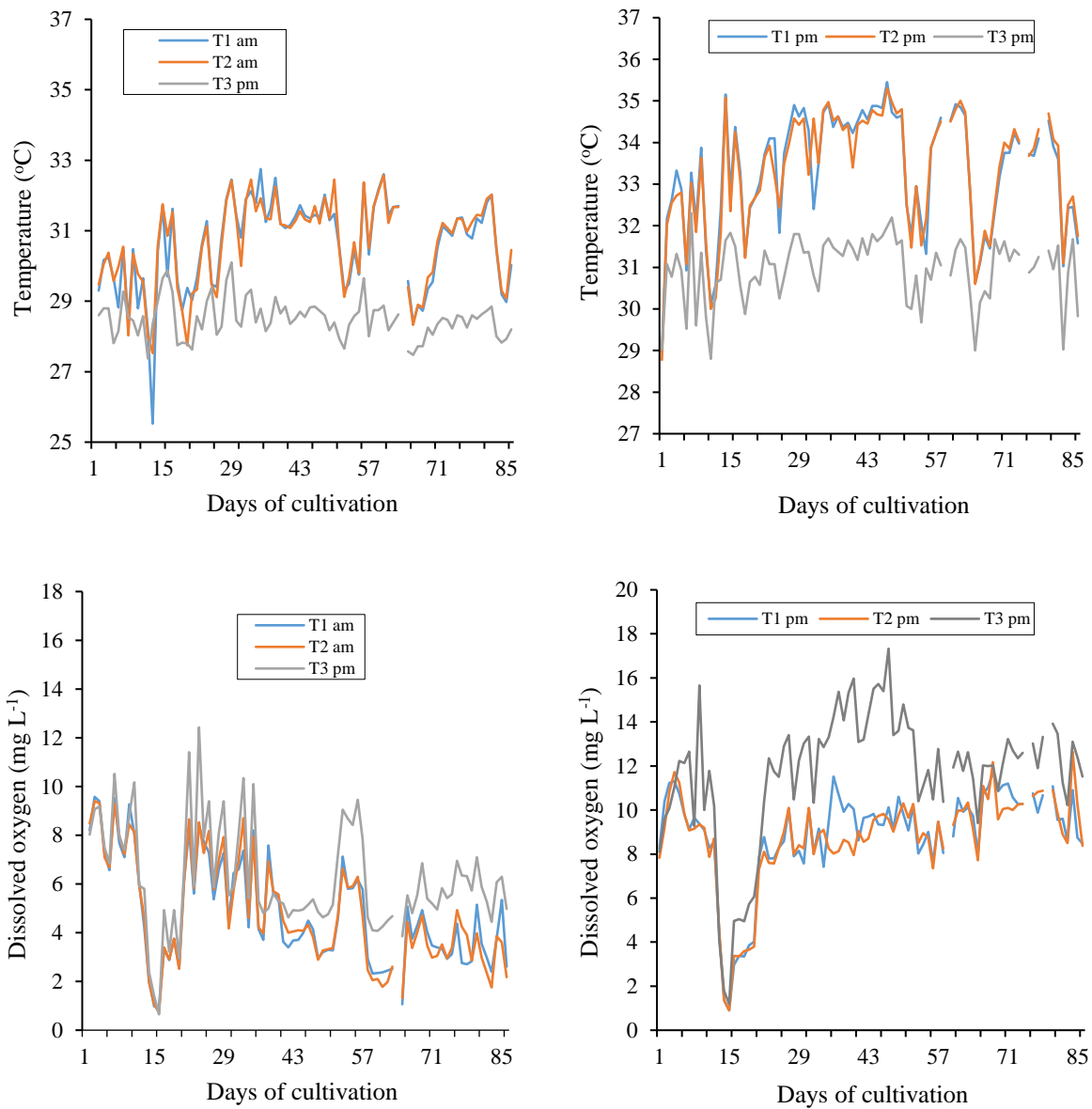
**Figure 1. Increase in shrimp biomass during the experiment.**

## Discussion

The temperatures in the treatments T1 and T2 exceeded the maximum allowable for the shrimp culture, as recommended by Quiñonez *et al.* (2010) that mentions that the shrimp of the species *L. vannamei* maintain their optimum level of cultivation at temperatures of 30 °C because the energy expenditure is not high. The difference between the temperatures of the treatments with zero water exchange and the T3 treatment was due to the fact that the water was stagnant and with direct exposure to the sun, the T3 treatment having a constant flow could maintain its temperature two degrees below (Figure 2).

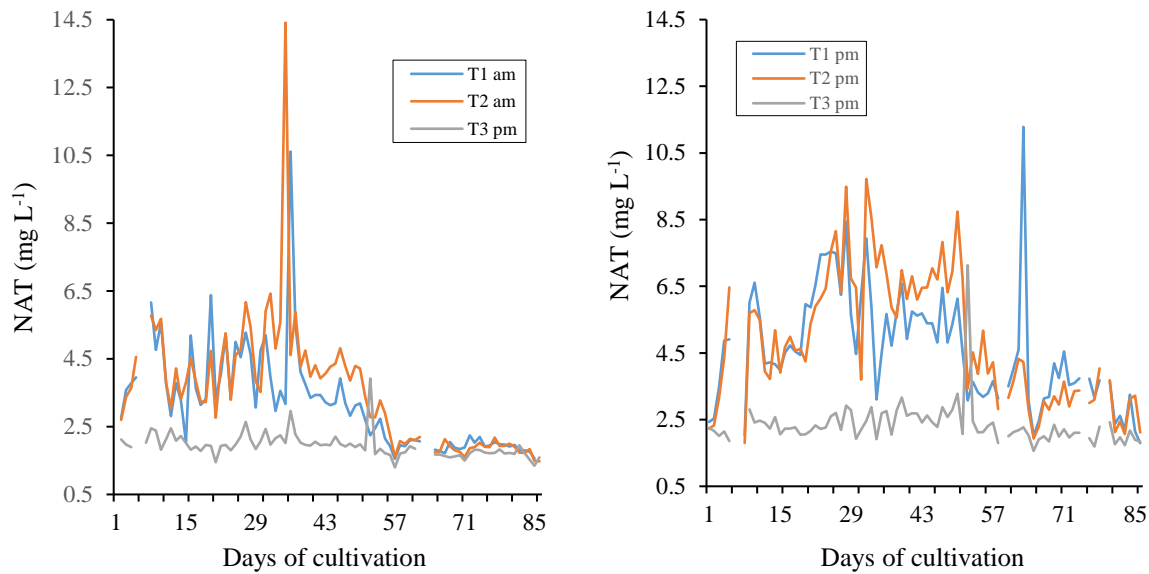
Dissolved oxygen (OD) presented variations between morning and afternoon due to planktonic activity and the presence of filamentous algae of the genus *Cladophora*. The T1 and T2 treatments were within the recommended parameters for the shrimp culture, the T3 treatment showed dissolved oxygen saturations in the afternoons (Figure 2). In works carried out with zero water exchange and planting densities similar to those of the present investigation, average values have been recorded in the OD of 6.08 mg L<sup>-1</sup> to 6.3 mg L<sup>-1</sup> (Audelo-Naranjo *et al.*, 2012; Furtado *et al.*, 2015)

In none of the treatments was below the minimum recommended salinity; however, there was a significant difference between the concentration of salts in T1 and T2 with respect to T3. The amount of dissolved salts in the water was lower in the treatments with zero water exchanges, probably due to the need of the organisms to capture the dissolved minerals and compensate the required salinity. Shrimp require Calcium for the molting process, so this mineral must be taken continuously from the environment (Ceballos *et al.*, 2012).



**Figure 2. Fluctuation of temperature and dissolved oxygen (DO) in the treatments.**

In all three treatments, the recommended concentration of NAT was exceeded, with significant differences of  $\pm 1.5 \text{ mg L}^{-1}$  between morning and afternoon. In the treatments with zero water exchanges the highest concentrations were recorded, the T3 treatment showed the lowest values. The concentration of NAT in the experiment dropped drastically on the seventh day (Figure 3), coinciding with the reports of Luo *et al.* (2013) where the decrease in 5 days of the NAT levels from  $117.285 \text{ mg L}^{-1}$  to  $2.5 \text{ mg L}^{-1}$  was observed when adding a carbon source. The high concentrations of nitrogenous compounds in ponds can be attributed to the decomposition of uneaten food and feces (Campaña-Torres *et al.*, 2009).



**Figure 3. Fluctuation of total ammonia nitrogen (NAT) in the treatments in the morning and afternoon.**

The pH showed a significant difference in value between the treatments with zero replacements and the T3 treatment. In the treatments T1 and T2 pH 9 values higher than the recommended maximum were registered. According to Whetstone *et al.* (2002) with pH values above 9 organisms can survive with a very slow growth, obtaining an optimal growth when the pH value is in ranges of 6-9. When working with *Penaeus monodon* Panjaitan (2010) on zero water exchange, using molasses in different doses and preserving the C:N ratio for the bacterial use of nitrogen compounds, the trend was downward in the pH levels, on the contrary in the present investigation there was a rapid increase in pH levels in the T1 and T2 treatments.

In relation to the efficiency in the use of water, treatments T1 and T2 turned out to be the best alternative to T3 (Table 2). The water expenditure in the T3 treatment was considerably high. Chamberlain (2002) mentions that water consumption for shrimp production on an intensive farm in Texas decreased from  $37.6 \text{ m}^3 \text{ kg}^{-1}$  to  $1.5 \text{ m}^3 \text{ kg}^{-1}$ . In the present work, it was possible to reduce from  $438.74 \text{ m}^3 \text{ kg}^{-1}$  to  $3.78 \text{ m}^3 \text{ kg}^{-1}$ .

Regarding economic efficiency, the production cost of the T1 treatments was lower than that recorded in T3 (Table 2). In the productive parameters a marked difference was registered between the final weight gain of the T1 and T2 treatments compared to the T3 treatment, the treatments with zero water exchange reported a lower growth, the obtained weights coincide with those reported in conditions similar to low salinity and with the use of well water in ranges ranging from 15 g to 8.8 g (Nunes and Velásquez, 2001; Miranda *et al.*, 2010; Quiñonez *et al.*, 2010).

Regarding the obtained height, the treatments T1 and T2 showed the lowest growth with an average size of 10.62 cm. González *et al.* (2008) mention that during its experimentation it was possible to reach lengths of 13 cm in length, coinciding with the sizes reached by the T3 treatment organisms.



The daily growth rate showed a significant difference between the T1 and T2 treatments compared to the T3 treatment, which had the highest daily gain, the growth presented in the treatments with zero water exchange was similar to that reported by González *et al.* (2008) who obtained 0.9 g per week, equivalent to 0.12 g per day. (Table 3).

With respect to TCA, treatment T2 obtained the best rate of 1.19, treatment T3 presented the highest conversion rate 1.42. Sowers *et al.* (2006) reports food conversion rates of up to 2.3. In an investigation with a similar seeding density TCA of up to 1.2 has been reported (Manzo, 2000).

The culture represented a percentage of survival of 73% and 66% for the treatments T1 and T2 respectively, this result can be considered important because other authors obtained survival of 60% and 69% at a salinity of 2 ‰ (Atwood *et al.*, 2003). The T3 treatment presented the lowest survival.

## Conclusions

In relation to the quality of the water, the experimental conditions tending to the zero exchange show increases in temperature and in pH. Regarding nitrogen, there was the presence of plankton and filamentous algae that contribute to the consumption of nitrogen compounds. BlueEnergy<sup>Re<sup>nt</sup></sup> insisted on the control of these compounds, as the dose added to the crop was increased.

The use of BlueEnergy<sup>Re<sup>nt</sup></sup> contributes to reduce the use of water in aquaculture farms, using 110 times less water than in a traditional culture with constant flow.

The low cost of production and significant savings in the use of water that is not only linked to the environmental benefit, but also to the economic benefit in the face of reduced water use.

Obtained visibly healthy organisms, with size and weight appropriate to their stage of life. The use of BlueEnergy<sup>Re<sup>nt</sup></sup> as a water quality control agent has positive effects on the productive response of white shrimp.

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