International Journal of Probiotics and Prebiotics Vol. 13, No. 4, pp. 139-142, 2018
ISSN 1555-1431 print,
www.newcenturyhealthpublishers.com

Research Article

COMMERCIAL PROBIOTIC INCREASES SURVIVAL RATE AND WATER QUALITY IN AQUARIUMS WITH HIGH DENSITY OF NILE TILAPIA LARVAE (*OREOCHROMIS NILOTICUS*)

[1]Patrick Hainfellner, [1]Marita Vedovelli Cardozo, [1]Mariana Monezi Borzi, [1]Camila Chioda Almeida, [1]Lucas José Luduvério Pizauro, [1]Ruben Pablo Schocken-Iturrino, [2]Gustavo Nogueira Costa and [1]Fernando Antônio de Ávila

[1]PABX: (16) 3209-7100, São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences, Via de Acesso Prof.Paulo Donato Castellane s/n 14884-900 - Jaboticabal, São Paulo, Brazil, PABX: (16) 3209-7100; and [2]IMEVE – Indústria de Medicamentos Veterinários S/A

[Received July 9, 2018; Accepted August 16, 2018]

[*Communicated by Prof. Chandan Prasad*]

ABSTRACT: ***Aquaculture is one of the fastest growing food production sectors in the world and Brazil was highlighted by a significant evolution in this sector. Larval stage mortality is of concern and the use of probiotics is an alternative to improve animal health. This study aimed to evaluate the effect of commercial probiotic (Bacillus spp. mixture), on the water quality and survival of nile tilapia larvae, during the absorption of the yolk sac period in highly populated aquarium. For this, 1500 tilapia larvae were kept in 15 aquariums and exposed to the following five treatments with different levels of probiotic: no probiotic (C), 0.5gL^{-1} (T1), 1.0 gL^{-1} (T2), 1.5gL^{-1} (T3) and 2.0gL^{-1} (T4). In addition, aquarium's water quality was evaluated (dissolved oxygen, temperature, conductivity, salinity and pH). The survival was higher in larvae treated with 1.5 g.L^{-1} and treated groups presented improvements in water quality in relation to dissolved oxygen and conductivity. These results can be attributed to the ability of this probiotic to promote feed conversion ratio, reduce organic matter and improve fish immunity. Thus, the administration of probiotic constituted significantly increases the larvae survival rate and may prove to be a substitute to the use of antibiotics.***

KEY WORDS: Aquaculture, *Bacillus*, Fish, Larvae, Probiotic, Survival

Corresponding Author: Dr. Marita Vedovelli Cardozo, São Paulo State University (Unesp), School of Agricultural and Veterinarian Sciences. 14884-900 Jaboticabal, São Paulo, Brazil; E-mail: maritavedovelli@yahoo.com.br

INTRODUCTION

Aquaculture is one of the fastest growing food production sector in the world and has grown enormously in recent years, especially in Asia (Ramachandran 2013). Nevertheless, Brazil was also highlighted by a significant evolution in its production, this is related to the development of intensive and semi-intensive aquaculture (Saint-Paul 2017), that is favored by a broad hydrographic system. A system that accommodate 12% of freshwater available from the planet, its aquaculture offers the largest potential to increase fish supplies and thus been one of the few countries that may become one of the world's largest fish producers (Saint-Paul 2017).

In this regard, the nile tilapia, *Oreochromis niloticus*, is one of the most cultivated species in the world, appreciated by producers due to their rusticity, fast growth, high-quality flesh and wide acceptance in the consumer market (Marengoni et al., 2015). In addition, been its larviculture simple, relaying, mainly, on the use of formulated diets (Marengoni et al., 2015). However due to the growing demands and the increase of intensive fish farming systems, the mortality in the larval stage is of concern (Vadstein et al., 2013), especially when density is assed. Different reasons were proposed for the mortality in this stage, including, but not limited to physiological and pathological stresses (Vadstein et al., 2013).

Glimpsing the control of disease and to improve health status, fish producer usually administrate antibiotics and chemotherapeutic drugs, which generate significant risks to public health by promoting the selection, propagation, and persistence of bacterial-resistant strains (Martínez Cruz et al., 2012). Nowadays, an excellent alternative method to improve animal health and control diseases is the use of probiotics. It is a relatively new term which is used to name microorganisms that are associated with the beneficial effects on host (Martínez Cruz et al., 2012). The antibacterial effect exhibited by probiotics is due to a variety of factors, including the competition for space, production of inhibitory substances, competition for chemicals or available energy, nutrients and enzymatic contribution, interference of quorum sensing and immunomodulation (Zorriehzahra et al., 2016).

As studies goes, several microorganisms have been evaluated as probiotics and the lactic acid, gram positive, spore forming *Bacillus* spp. have received increasing interest as probiotics in the fish farming industry (Han et al., 2015; Martínez Cruz et al., 2012). This group is more efficient than the gram-negatives in transforming organic matter to CO_2, and it is suggested that in high levels of probiotic, increases the rate of accumulation of dissolved particle organic carbon (Aly et al., 2016). Also, this genus has several advantages over other non-spore formers, their endospores are resistant to harsh environmental conditions and thus, suitable to survive and act at the acidic and alkaline conditions within the gastrointestinal tracts (Meidong et al., 2017).

In this study, we evaluated the effect of commercial probiotic, formulated from a mixture of *Bacillus subtillis* and *Bacillus cererus*, in the survival of newly hatched Nile tilapia larvae during the period of complete absorption of the yolk sac in highly populated aquarium.

MATERIAL AND METHODS

The experimental protocol was approved by the Committee on Ethics in the Use of Animals at the School of Agrarian Sciences and Veterinary Medicine, São Paulo State University (Unesp), Brazil, under protocol number013964/17. The probiotic tested is composed by *Bacillus subtilis* and *Bacillus cereus* var. Toyoi (IMEVE Ind. Med. Veterinários S/A, Brazil). Each strain was grown for 24 h on agar BHI, the bacterial colonies were further cultured in 100 mL of liquid BHI for 24 h. Then, to increase the volume, they were grown in 1L of liquid BHI for another 24 h. Before to use, the cultures were centrifuged for 7 min (8000 × g) to pellet the strains. The supernatant was discarded, and the cell density was adjusted to with the concentrations of 1010 UFC/mL of Bacillus subtilis and 1010 UFC/mL of Bacillus cereus and then freeze-dried.

TABLE 1. Probiotic inclusion levels in the water for each respective treatment.

Treatments	Probiotic Level
Negative control (C)	Without probiotic
Treatment 1(T1)	$0.5gL^{-1}$
Treatment 2 (T2)	$1.0gL^{-1}$
Treatment 3 (T3)	$1.5gL^{-1}$
Treatment 4 (T4)	$2.0gL^{-1}$

For the experiment, 1500 newly hatched larvae of nile tilapia with weight mean 0.011 g ± 0.001, were allocated in 15 aquariums with 1.5 L capacity and 100 larvae each. Thus, aquarium was used for the following treatments with different levels of probiotic: Control with no probiotic (C), treatment one with 0,5 gL^1 (T1), treatment two with 1,0 gL^1 (T2), treatment three with 1,5 gL^1 (T3) and treatment four with 2,0 gL^1 (T4) (Table 1). Each treatment was performed in triplicate and the probiotic was supplied in the water for all consecutive ten days.

The following aquarium's water quality values evaluated were dissolved oxygen, temperature, conductivity, salinity and pH. These parameters were determined twice a day. In addition, aquariums were cleaned, daily, to remove dead fish and residue through siphoning.

Larval survival was assessed using the formula S = (IL-FL/100), where: S was the survival rate (percentage); IL was the number of initial larvae; FL was the final number of larvae followed by the absorption of the yolk sac. Statistically significant difference between the values of the animals exposed to the different levels of probiotic was detected by ANOVA analysis of variance followed by the Tukey test. Significant difference was considered when $p < 0.05$ (Zar 1999).

RESULTS

Among the physical chemical parameter evaluated of the water from the fish aquarium, conductibility was significant higher in T3 (380 $\mu S.cm^{-1}$) and T4 (369 $\mu S.cm^{-1}$) when compared with C and T1, but did not differ between those groups. The lowest conductibility was observed in T2 (286

TABLE 2. Physical chemical analysis results of the water from the fish tank during yolk sac absorption. *Column denoted by different letters indicate significant difference according to Tukey's test (P <0.05)

Treatment	pH	O_2 (mg.L⁻¹)	T (ºC)	Salinity (mg.L⁻¹)	Conductivity (µS.cm⁻¹)
C	8.16 ±0.04[a]	5.01 ±0.56[a]	28.1 ±0.00[a]	0.10 ±0.0[a]	295±13.9[b]
T1	8.31 ±0.09[a]	5.24 ±0.15[a]	28.1 ±0.06[a]	0.10 ±0. [a]	320±37.4[b]
T2	8.50 ±0.13[a]	5.30 ±0.41[a]	28.1 ±0.06[a]	0.10 ±0.0[a]	286±10.1[b]
T3	8.99 ±0.04[a]	5.84 ±0.1[a]	27.7 ±0.06[a]	0.20 ±0.0[a]	380±12.26[a]
T4	8.97 ±0.05[a]	5.69 ±0. [a]	27.7 ±0.06[a]	0.20 ±0.0[a]	369±2.38[a]

TABLE 3. Larvae of tilapia survival rate (percentage) in fish tank, according to probiotic treatment, during yolk sac absorption period. *Column denoted by different letters indicate significant difference according to Tukey's test (P <0.05)

Treatment	Survival rate (%)
C	85.6[c]
T1	90.6[b]
T2	90.3[b]
T3	100[a]
T4	94.3[b]

µS.cm⁻¹) and do not presented any significance with T1 (320 µS.cm⁻¹) and C (295 µS.cm⁻¹). No significant difference was observed between treatments with the following parameters: pH, oxygen, temperature and salinity (Table 2).

As for the larvae survival, it was higher when probiotic was present at the water, been T3 the highest with 100% survival rate, followed by T4 with 94.3%, T1 with 90.6%, T2 with 90.3% and C with 85.6% (Table 3).

DISCUSSION

In this study, temperature remained within the optimum interval for *O. niloticus* of 22 to 30 °C (Caulton 1982). The effect of temperature on growth relay on the interaction between food consumption and metabolism (Sandblom et al., 2014). In this regard, probiotics administration significantly change the proportion of *Bacillus* spp. bacteria in the gut microbiota, thus increasing both feed conversion ratio and efficiency though digestive enzyme activities modulation (Ibrahem 2013).

In addition, although the larvae survival rate in all experimental groups was more than 85%, the supplementation of probiotic presented slight (T1 and T2) to significant (T3 to T4) increases in survival rate as compared to control. This effect could be attributed to the fact that in addition to the digestive enhancements, probiotics also promotes immune response by both producing chemical components and increase of cellular end humoral parameters (Ibrahem 2013).

Considering that adequate densities can increase production and consequently the profitability of the fish production activity, according to Luz et al., (2012) density of 30 larvae/L nilo tilapia larvae were beneficial to optimize the structure and productivity of larviculture, due to the fact that as fish tank stocking density increases, the level of dissolved oxygen diminishes. However, in this study the use of 100 larvae/1.5L or approximately 66,6 larvae/L did not surpass the 4.9 mg/L of dissolved O_2 that have been prove to be stressful for fingerlings Nile tilapia (Abdel-Tawwab et al., 2015). The addition of probiotic, although not significant, increased the amount of dissolved oxygen, with T3 presented the highest concentration. Martínez Cruz et al., (2012) hypothesize that probiotic could balance the production of phytoplankton and thus improve dissolved $O_{2,}$ this could explain the increase of the dissolved O_2 on probiotic treated aquarium.

The water conductibility was significant affect by the addition of probiotic, this fact can be attributed to the efficiency of the bacillus genus in transforming organic matter into CO2, thus it is beneficial to main a high level of probiotic to minimize the accumulation of dissolved particle organic carbon during the growth phase (Martínez Cruz et al., 2012). Maintaining the water within EC up to 1000 µS.cm⁻¹ is considered suitable for fish culture (Boyd 2012) , thus avoiding the undesirable algal blooms.

CONCLUSION

The use administration of 1.5 gL^{-1} of probiotic constituted of 4,0 x 10^{11} UFC/g of *Bacillus subtilis* and of *Bacillus cereus* significantly increases the larvae survival rate of Nile tilapia (*Oreochromis niloticus*) during the period of yolk sac absorption. In addition, due to the well know capabilities of probiotic B*acillus* spp in transforming organic matter it is advice that this probiotic genus is used in high stocking density larveculture.

ACKNOWLEDGEMENTS

This work was supported by CNPq and FAPESP (2011/15050-8) and IMEVE S/A.

CONFLICT OF INTEREST

The authors reported no potential conflict of interest.

REFERENCES

Abdel-Tawwab, M., Hagras, A. E., Elbaghdady, H. A. M. and Monier, M. N. (2015). Effects of dissolved oxygen and fish size on Nile tilapia, *Oreochromis niloticus* (L.): growth performance, whole-body composition, and innate immunity. *Aquaculture International* **23**:1261–1274.

Aly, H. A., Abdel Rahim, M. M., Lotfy, A. M., Abdelaty, B. S., Sallam, G. R. (2016). The Applicability of Activated Carbon, Natural Zeolites, and Probiotics (EM®) and Its Effects on Ammonia Removal Efficiency and Fry Performance of European Seabass Dicentrarchus labrax. *Journal of Aquaculture Research & Development* **7**:1-8.

Boyd, C. E. (2012). Water quality, Chapter 3. In Lucas, J. S and Southgate, P. C. (eds.). *Aquaculture – Farming aquatic animals and plants*. Oxford: Wiley-Blackwell, UK. 52–83.

Caulton, M. (1982). Feeding, metabolism and growth of tilapias: some quantitative considerations. In Pullin, R. S. V. and Lowe-McConnell, R. H. (eds.). *The Biology and Culture of tilapias*. Manila, Philippines: ICLARM:157–180.

Han, B., Long, W. Q., He, J. Y., Liu, Y. J., Si, Y. Q. and Tian, L. X (2015). Effects of dietary Bacillus licheniformis on growth performance, immunological parameters, intestinal morphology and resistance of juvenile Nile tilapia (*Oreochromis niloticus*) to challenge infections. *Fish and Shellfish Immunology* **46**:225–231.

Ibrahem, M. D. (2013). Evolution of probiotics in aquatic world: Potential effects, the current status in Egypt and recent prospectives. *Journal of Advanced Research* **6**:765–791.

Luz, R. K., Souza e Silva, W., Filho, R. M., Santos, A. E. H., Rodrigues, L. A., Takata, R., Alvarenga, E. R. and Turra, E. M. (2012). Stocking density in the larviculture of nile tilapia in saline water. *Revista Brasileira de Zootecnia* **41**:2385–2389.

Marengoni, N. G., Machado, L. M. C., Oliveira, C. A. L., Yoshida, G. M., Kunita, N. M. and Ribeiro, R. P. (2015). Morphological traits and growth performance of monosex male tilapia GIFT strain and Saint Peter®. *Semina:Ciencias Agrarias* **36:**3399–3410.

Cruz, P. M., Ibánez, A. L., Hermosillo, O. A. M. and Saad, H. C. R. (2012). Use of probiotics in aquaculture. *ISRN Microbiology* **2012**:1-13.

Meidong, R., Doolgindachbaporn, S., Jamjan, W., Sakai, K., Tashiro, Y., Okugawa, Y. and Tongpim, S. (2017). A novel probiotic Bacillus siamensis B44v isolated from Thai pickled vegetables (Phak - dong) for potential use as a feed supplement in aquaculture. *The Journal of General and Applied Microbiology* **63**:246-253.

Ramachandran, K. (2013). Growing Demand for High-Quality Seafood Products and Production Systems in Aquaculture. *Food and Agriculture Organization of the United Nations* **3**:1–2.

Saint-Paul, U. (2017). Native fish species boosting Brazilian's aquaculture development. Acta of Fisheries and Aquatic Resources. *Acta of Fisheries and Aquatic Resources* **5**:1–9.

Sandblom, E., Grans, A., Axelsson, M. and Seth, H. (2014). Temperature acclimation rate of aerobic scope and feeding metabolism in fishes: Implications in a thermally extreme future. *Proceedings of the Royal Society B: Biological Sciences* **281**:1-9.

Vadstein, O., Bergh, Ø., Gatesoupe, F., Galindo-Villegas, J., Mulero, V., Picshieti, S., Scapigliati, G., Makridis, P., Olsen, Y., Dierckens, K., Defoirdt, T., Boon, N., Schryver, P. D. and Bossier, P. (2013). Microbiology and immunology of fish larvae. *Reviews in Aquaculture* 5:S1–S25.

Zar, J. H. (1999). Biostatistical analysis, 4th Edition, Prentice Hall, Upper Saddle River.

Zorriehzahra, M. J., Delshad, S. T., Adel, M., Tiwari, R., Karthik, K., Dhama, K., Lazado, C. C. (2016). Probiotics as beneficial microbes in aquaculture: an update on their multiple modes of action: a review. *Veterinary Quarterly* **36**:228–241.