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AQUICULTURE

Economic feasibility of probiotic use in the diet of Nile tilapia, *Oreochromis niloticus*, during the reproductive period

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ABSTRACT. This work examines the economic advantages of probiotic use in the diet of Nile tilapia broodstock during the reproductive period. For this purpose, *Bacillus subtilis* was applied as a feed additive. The experimental design was completely randomized with three treatment groups: the T_0 control (without probiotic), the T_1 continuous probiotic intake, and the T_2 alternate probiotic intake at a dose of 0.50 g kg^{-1} of feed (10^{10} CFU g^{-1}) with four replicates. For the reproduction assay, 118 females and 48 males of Nile tilapia (proportion 4 males:9 females. hapa^{-1}) (weight 527.65 g ± 185.98 g and length 30.16 cm ± 3.57 cm) were distributed into 12 hapas (3.5 × 2.0 × 1.5 m). Reproductive variables (spawning female percentage, egg production, and fry production) were used to calculate the economic feasibility indexes (total cost of nutrition [TcN], gross revenue [GR], and total operational profit [ToP]). The results show increasing values for spawning female number, collected eggs, and surviving fry in the probiotic groups. We recommend continuous intake of probiotic (feed with addition of probiotic) at a dose of 0.5 g kg^{-1} of feed (10^{10} CFU g^{-1}) during the breeding season of Nile tilapia, due to the suitable reproductive indexes and profitability.

Keywords: *Bacillus subtilis*; fish farming; feed additive; profitability; spawning.

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Introduction

The expansion of aquaculture can be attributed to industrial diet production technologies, where finding ingredients that allow formulation and production of animal feed with high quality and low cost are the primary challenges (Cyrino, Bicudo, Sado, Borghesi, & Dairik, 2010). In addition, the development of feed additives (e.g. phytochemicals, prebiotics, and probiotics) may represent an alternative solution for improving growth performance (Sutili, Gatlin III, Heinzmann, & Baldisserotto, 2018; Wang, Tian, Yao, & Li, 2008), optimizing resistance to infectious diseases (Nayak, 2010; Sutili et al., 2018) and reducing the quantities of antibiotic applied in fish farming (Sutili et al., 2018).

The probiotics are composed by live microorganisms that may provide beneficial effects on the health of the host by modulating the gut microbiota (Fuller, 1989). According to Chantharasophon, Warong, Mapatsa, and Leelavatcharamas (2011) and Newaj-Fyzul and Austin (2015), a wide range of bacterial species have been applied as probiotics including *Bacillus sp.* (e.g. *Bacillus subtilis*, *B. licheniformis,* and *B. cereus*), *Enterococcus sp.* (e.g. *Enterococcus faecium*), and *Lactobacillus sp.* (e.g. *Lactobacillus acidophilus*). *B. subtilis* is frequently used as a probiotic in aquatic feed due to its capacity to form spores and compatibility with lyophilization. This bacterial species supports the adverse conditions encountered in the gastrointestinal tract (e.g. low pH, presence of bile salt and digestive enzymes), remaining viable and promoting beneficial effects related to the improvement of water quality (e.g. oxidation of organic matter), the growth performance (e.g. production of digestive enzymes), the immune system (e.g. innate immunity), and reproduction (e.g. increase of oocyte number and fertilization rate) (Cutting, 2011; Dias et al., 2012a; El-Haroun, Goda, & Chowdhury, 2006; Peredo, Buentello, Gatlin III, & Hume, 2015; Soto, 2017).

The positive effects of probiotic administration on reproductive performance may be related to the availability of key nutrients, such as fatty acids and amino acids that are important components in oocyte formation and maturation (as well as embryo development and fry growth) and may modulate the expression of genes involved in lipid metabolism (Carnevali, Maradonna, & Gioacchini, 2017; Dias et al., 2012a; Rodiles et al., 2018). The pulsed probiotic feeding strategy consisted to supply probiotics (oral via) in alternate periods (seven days) which may provide better effects on immune system modulation such as increased respiratory burst activity in the presence of microbial pathogen (Noffs, Tachibana, Santos, & Ranzani-Paiva, 2015). The cycling short feeding with probiotics may avoid over immune stimulation and maintain the level of protection against bacterial infection (Merrifield, Bradley, Baker, & Davies, 2010).

Among the fish farming species, Nile tilapia is an omnivore fish species, which is resistant to diseases caused by parasites and handling stress and accepts several plant origin feedstuffs (Watanabe, Losordo, Fitzsimmons, & Hanley, 2002). Nile tilapia is widely produced in almost all regions of Brazil, achieving 239.000 tons of production in 2016 and values of 409,230 million dollars (1 dollar = 3.25 real), corresponding to 47.1% of total fish production and 40.9% of total value (IBGE, 2016). However, choice of the most suitable diet for each species broodstock is a major challenge in fish farming, requiring reproductive and work planning strategies. Precise definition of techniques that will provide higher reproductive efficiency is necessary to ensure viable progeny (Merrifield et al., 2010). The objective of this work was to evaluate the economic feasibility of probiotic strain *Bacillus subtilis* addition in Nile tilapia broodstock diet.

Material and methods

The research project was approved by the Ethical and Animal Welfare Committee of the Instituto de Pesca de São Paulo (nº03/2017)

The experiments were carried out at the São Paulo's Agency for Agribusiness Technology (APTA), Pirassununga, SP - Brazil.

Experimental design

The experimental design was completely randomized with three treatments and four replicates. The treatment groups were: T_0 control (feed with no addition of probiotic), T_1 continuous intake of probiotic (feed with addition of probiotic), and T_2 alternate intake of probiotic (7 days of feed with addition of probiotic and 7 days of feed with no addition of probiotic) at a dose of 0.5 g kg^{-1} of feed, during all experimental period.

Experimental diet

The extruded commercial feed utilized contained 32% crude protein (minimum), 6.5% ether extract (minimum), 7% crude fiber (maximum), 10% mineral matter (maximum), 1.2% calcium (maximum) and 0.6% phosphorus (minimum). A lyophilized powder of commercial probiotic *Bacillus subtilis* with 10^{10} CFU g^{-1} (Colony Forming Units) was homogenized in 2% soybean oil, sprayed on the extruded feed, and homogenized. The control diet received 2% soybean oil. The diet was offered twice a day as approximately 1% of the total biomass adjusted every two weeks. The total ration consumption (g) by treatment was calculated using the sum of the four replicates plus the male hapa (only one hapa with males maintained separately from females).

Reproduction assay

The hapas were installed in two bottom earthen ponds with 200 m² area and 1.2 m of depth, with evaporation and infiltration water reposition. An aeration system was installed that dropped a water jet inside each hapa. The four replicates from the control group were placed in one isolated pond.

For the reproduction assay, 118 females (462.99 ± 138.64 g, 28.78 ± 2.75 cm) of Nile tilapia were distributed into 12 hapas (volume of 10.50 m^3). The 48 males (682.84 ± 194.53 g, 33.47 ± 3.14 cm) in each treatment were maintained separately in three hapas (10.50 m^3) until mating and during the rest period.

After a 90-day feeding period for the female and male fish, four males were randomly captured and introduced into the female hapa with females receiving corresponding treatment to start the reproductive period. The eggs were collected from the female mouth after three, five, and seven days. After this, the males were captured again and transferred to the original hapa. After 14 days, the males were transferred to the reproductive hapa again. This management was repeated four times, during 84 days. The fish were weighed at the end of the fourth period. The eggs were transported to the laboratory (in the same locality), passed through a sieve, gently dried with towel paper, and weighed. A sample of 1.0 g eggs was separated for counting and estimates the total number of eggs. The eggs were incubated in plastic conic incubator (5.0 L) and the larvae were maintained after hatching in the same system until 10 mm of total length (called fry) and were counted.

The water quality parameters for dissolved oxygen rate and temperature were monitored once a day, whereas ammonia levels and pH were monitored once a week.

The percentage of spawned females and egg production variable data were submitted to the following statistical analyses: normality and homoscedasticity tests, ANOVA (analysis of variance), and the Tukey test for comparison of means ($p < 0.05$). These analyses considered the following factors: treatments and reproductive periods.

Economic feasibility study

The extra expense related to addition of the probiotic was considered in an economic feasibility evaluation. The price of the feed and the probiotic corresponded to $ 0.56 (US) and $ 60.00 (US) per kilogram, respectively. The economic analysis was performed through adaptation of the variables obtained by Costa, Sabbag, Ayroza, and Martins (2017) applied to the total viable fry number obtained at the end of the experiment.

The following equation was applied to calculate the total cost of nutrition (TcN):

TcN = (Cf+Cp)

where: TcN = total cost of nutrition (US$);

Cf = cost of the feed per treatment (US$)

Cp = cost of the probiotic per treatment (US$).

The additional expenses were compared, by percentage, with the surviving fry rate and fry production. The fry production was calculated from the number of surviving fry, discounting the 20% mortality rate. The cost of fry production through the commercial stage was considered at $12.71 (US) per 1,000 fry, while the selling price of 1,000 fry corresponded to $36.01 (US).

The economic feasibility indexes were calculated with the following equations:

(1) Gross Revenue (GR): the revenue expected from a given production of fry per unit area for a pre-defined selling price, or effectively received, that is:

GR = Pr x Sp

where: Pr = fry production (unit);

Sp = selling price of the product (US$ per one thousand fry).

(2) Operational Profit (OP) or Net Revenue obtained from fry production: the difference between the GR generated by the sale of fry and the total operational cost (TOC) of fry production per area unit.

OP = GR – TOC

where: TOC = cost of fry production (US$ per experiment).

(3) Total operational profit (ToP) or Total Net Revenue: the difference between the GR generated by the sale of fry and the TOC of fry production, including the expenses for nutrition per area unit.

ToP = GR – TOC

where: TOC = cost of fry production and nutrition (US$ per experiment).

Results and discussion

After 174 days, the body weight and total length of the fish were 902.15 g ± 180.34 and 35.53 cm ± 2.32, respectively, for females and 1,226.80 g ± 204.89 and 40.52 cm ± 2.07, respectively, for males. The water quality parameters of the ponds during the experiment were within the ideal range for tilapia culture (Sipaúba-Tavares, Lourenço, & Braga, 2010). The mean values for these parameters were: temperature at 26.9 ± 1.1° C, dissolved oxygen at 5.5 ± 2.7 mg L^{-1}, pH at 7.1 ± 0.27, and total ammonia concentrations below 0.25 mg L^{-1}.

In the second breeding season, the females from the probiotic group spawned more eggs than females that received the control diet. In the fourth period, the temperature was reduced to a mean value of 24.5 ± 1.5° C. However, the spawning percentage remained satisfactory in T_1 (27.7%) and T_2 (25.0%) compared to T_0, which achieved only 5.6% (Figure 1).

The ratio between females and males was the same in the three treatments of this study (four males to nine females). Thus, the use of the probiotic may have reduced the stress of the reproductive phase and optimized the general physiological condition of the animals, providing better spawning performance.

The average egg production was 3,256.89 ± 397.02 per kg of females at T0; 3,188.70 ± 634.40 per kg of females in T1 and 3,367.12 ± 296.25 per kg of females in T2. However, there was no statistical difference between T1 and T2 in relation to the average egg production and total number of fingerlings (Figure 2 and 3).

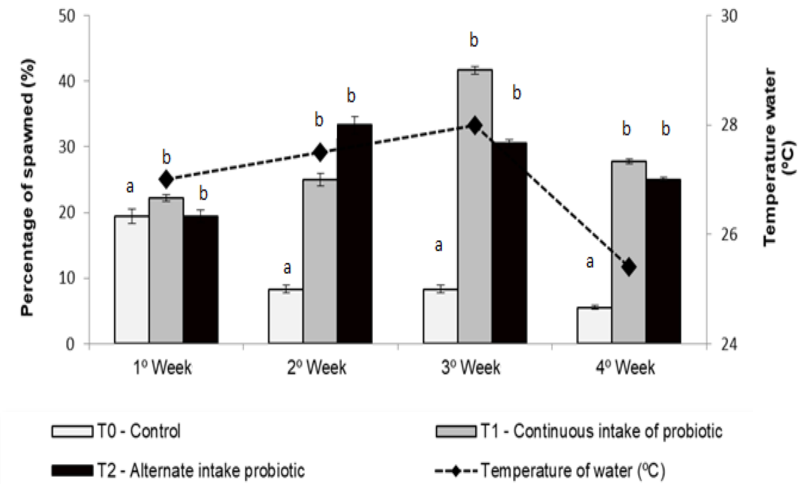

Figure 1. Mean percentage of *O. niloticus* spawning females per treatment in each reproduction period (14 days), obtained in the experiment with use of probiotic. T0- control (feed with no addition of probiotic), T1- continuous intake of probiotic (feed with addition of probiotic) and T2- alternate intake of probiotic (7 days of feed with addition of probiotic and 7 days of feed with no addition of probiotic) at a dose of 0.5 g kg-1 of feed. Different letters in the same week show differences of means values by Tukey test ($p < 0.05$).

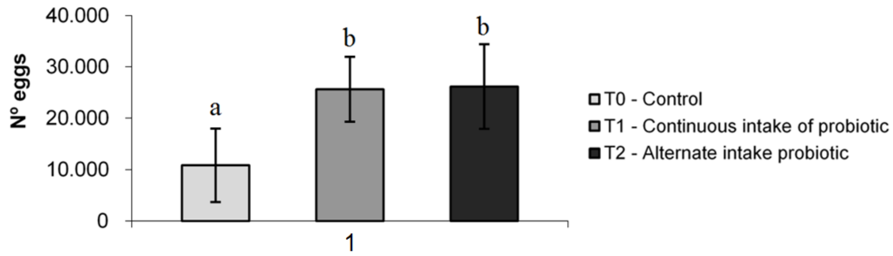

Figure 2. Average number of eggs per treatment, in each breeding season (14 days) of *O. niloticus*, obtained in the experiment with use of probiotic. T_0- control (feed with no addition of probiotic), T_1- continuous intake of probiotic (feed with addition of probiotic) and T_2- alternate intake of probiotic (7 days of feed with addition of probiotic and 7 days of feed with no addition of probiotic) at a dose of 0.5 g kg^{-1} of feed. Different letters in the graphic show differences of mean values by Tukey test ($p < 0.05$).

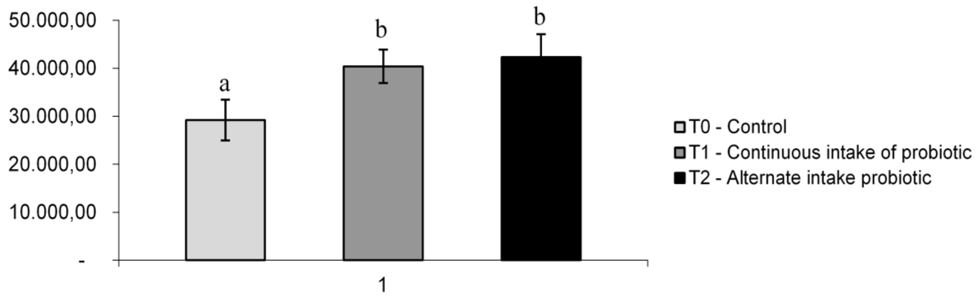

Figure 3. Total number of fry, per treatment, *O. niloticus*, obtained in the experiment with use of probiotic. T_0- control (feed with no addition of probiotic), T_1- continuous intake of probiotic (feed with addition of probiotic) and T_2- alternate intake of probiotic (7 days of feed with addition of probiotic and 7 days of feed with no addition of probiotic) at a dose of 0.5 g kg^{-1} of feed.

The total cost of nutrition (feed + probiotic) in T_2 was 7.44% higher than in T_1 (Table 1). The control group presented higher feed consumption than probiotic groups, which may be related to the lower reproductive performance and lower quantities of eggs incubated in the mouths of females.

The OP generated by sale of the fry was the highest in T_2 (4.5% higher than T_1 and 30.98% higher than T_0).

This treatment corresponds to the alternate probiotic use (7 days of feed with probiotic and 7 days without probiotic) at a dose of 0.5 g kg^{-1} of feed (10^{10} CFU g^{-1}). It is important to emphasize that the ToP was higher in T_2 (4.5% and 31.9% higher than that in T_1 and T_0, respectively).

Table 1. Total feed consumption (TfC), total probiotic consumption (TpC), total cost of feed (TcF), total cost of probiotic (TcP), total cost of nutrition (TcN), total number of surviving fry (TnL), total number of fry produced (TnF), total cost of fry production (TcFr), gross revenue from the sale of fry (GrF), operational profit from the sale of fry (OpF), total operational profit (ToP), obtained in the experiment with *O. niloticus*, with use of probiotic[1].

Item	Un.	T_0	T_1	T_2
Total feed consumption (TfC)	g	39,411.00	35,633.00	38,497.00
Total probiotic consumption (TpC)	g	-	17.82	9.62
Total cost of feed (TcF)	US$	22.07	19.95	21.56
Total cost of probiotic (TcP)	US$	-	1.07	0.58
Total cost of nutrition (TcN)	US$	22.07	21.02	22.14
Total number of surviving fry (TnL)	Un.	29,204.00	40,402.00	42,314.00
Total number of fry produced (TnF)	Un.	23,363.20	32,321.60	33,851.20
Total cost of fry production (TcFr)[2]	US$	296.95	410.81	430.25
Gross revenue from the sale of fry (GrF)[3]	US$	841.31	1,163.90	1,218.98
Operational profit from the sale of fry (Op[4]	US$	544.36	753.09	788.73
Total operational profit (ToP)[5]	US$	522.29	732.07	766.59

[1]T_0- control (feed with no addition of probiotic), T_1- continuous intake of probiotic (feed with addition of probiotic) and T_2- alternate intake of probiotic (7 days of feed with addition of probiotic and 7 days of feed with no addition of probiotic) at a dose of 0.5 g kg^{-1} of feed. [2]The cost of fry production through the commercial stage was considered at $12.71 (US) per 1,000 fry TcFr = Total number of fry produced/1,000*$12,71. [3]The selling price of 1,000 fry corresponded to $36.01 (US). GrF = Total number of fry produced/1000*36.01. [4]Op = GrF-TcFr. [5]Top = Op-TcN

The reproductive scheme used in this experiment followed the findings of Kubitza (2009) by applying four males to nine females. These authors reported that dominant and submissive behaviors among Nile tilapia breeders and increased stock densities reduced the fry production of females. The optimal reproductive indexes occur when one until three females are stocked for each male.

The egg and fry production presented in this work corroborates results obtained by Santos, Oliveira Filho, Santos, Santos Neto, and Lopes (2009). The author demonstrated egg production of 600 units per spawning female in a recirculation system and fry production of 597.05 to 930.6 fry/female/period, depending on the growth pattern of the females.

The temperature registered in the experiment was favorable for the Nile tilapia reproduction, considering temperatures over 24° C cause spawning to become more frequency (Rothbard & Pruginin, 1975), and the results of this work shows a fry production increasing in higher water temperatures.

The beneficial effects of the application of *B. subtilis* as a reproductive enhancer for Nile tilapia may be related to the capacity of this strain to increase feed digestion and nutrient digestibility (Soto, 2017). The absorption of essential amino acids and fatty acids are important to the release of hormones associated with reproduction and metabolism (e.g. lipid metabolism) as well as the formation of oocytes and sperm, increasing both the viability of gametes and the fertilization rates (Carnevali et al., 2017; Dias et al., 2012a).

Nile tilapia breeders fed diet with Hydroyeast Aquaculture® composed by live yeast, probiotic bacteria (*Lactobacillus acidophilus*, *Bifidobacterium longhum*, *B. thermophylu* and *Streptococcus faecium*), enzymes (amylase, protease, cellulase, pectinase, xylanase, phytase and oligosaccharides) presented increased blood sexual hormones levels (serum total testosterone and progesterone), and the sperm quality was better than the control group, due to the direct correlation with testosterone level. In addition, decreased levels of cholesterol in probiotic group also were detected. The authors Mehrim, Khalil, and Hassan (2015) justified the positive results of Hydroyeast Aquaculture® as reproductive enhancer with the capacity to upgrade the nutrient bioavailability and absorption of the fish, and probiotic bacteria may produce substances such as B group vitamins.

According to Avella et al. (2012), *Lactobacillus rhamnosus* accelerated the zebrafish larvae backbone calcification, gonadal differentiation and maturation. The elevated IGF-1 and IGF-1I (related to growth factors), PPARs, VDRα and RAR-γ (related to nutrient metabolism) genes expressions are hypothesized by the authors as the reasons of faster development of the fish.

The inclusion of a probiotic feed additive in animal nutrition may promote several beneficial effects and increase economic value (Lakshmi, Viswanath, & Sai Gopal, 2013). Selection of a bacterial strain involves the following steps: (1) the collection of background information, (2) the acquisition of potential probiotic candidates, (3) the detection of beneficial effects *in vitro* (e.g. inhibition against pathogens) and *in vivo* (e.g. growth promoters, immunostimulants, bioremediators, reproductive enhancers), (4) the biosecurity of strains, and (5) a cost effectiveness analysis (Balcázar et al., 2006; Gomez-Gil, Roque, & Turnbull, 2000).

The evaluation of economic feasibility in aquaculture is important since the focus of fish farming is to produce high quality products at the lowest possible cost. Several studies on the effects of alternative feed ingredients cite economic concerns as one of the important factors to be analyzed (Haygood & Jha, 2018).

In this work, the positive effects of *B. subtilis* on reproductive indexes were observed. Our economic analysis results show that higher fertilization rates, increased egg production, and increased fry production improves cost effectiveness. Similarly, the application of this probiotic species to the freshwater fish *Brycon amazonicus* broodstocks promoted higher fertilization and hatching rates according to (Dias et al., 2012a). Moreover, Dias et al. (2012b) verified the optimum level of *B. subtilis* inclusion at 5×10^9 CFU per kg of feed, which improved the growth performance and was profitable when applied by fish farmers.

Ayyat, Labib, and Mahmoud (2014) tested the combination of three probiotic strains, *Lactobacillus acidophilus*, *Streptococcus thermophilus,* and *Bifidobacterium bifidum,* to the diets of Nile tilapia fingerlings and observed higher growth performance together with economic advantages (return from gain and final margin). The inclusion of probiotics, prebiotics, and symbiotics to Nile tilapia production at low and high density also promoted higher OP due to the improvement of zootechnical performance, such as the final biomass and apparent feed conversion (Azevedo et al., 2015). The reasonable effects of probiotic inclusion may be correlated with fish sex, maturation stage, feeding period and strategy, diet composition, probiotic levels and components (Mehrim et al., 2015).

In this work, a direct relationship between reproductive performance and OP demonstrates higher economic benefits when a probiotic is included in the diet. Furthermore, the continuous administration of *B. subtilis* is shown to be more profitable for Nile tilapia breeders.

Conclusion

The probiotic *Bacillus subtilis* optimizes the reproductive performance of Nile tilapia broodstocks, resulting in economic advantages. We recommend continuous intake of probiotic (feed with addition of probiotic) alternate use of the probiotic *Bacillus subtilis* (7 days of feed with probiotic and 7 days without probiotic) at a dose of 0.5 g kg^{-1} of feed (10^{10} CFU g^{-1}) during the breeding season of Nile tilapia, due to the suitable reproductive indexes and profitability.

Abbreviations

CFU- Colony Forming Unit, GR -Gross Revenue, T_0 - Control group, T_1 - Continuous probiotic intake, T_2 - Alternate probiotic intake, TfC -Total feed Consumption, TcN -Total cost of Nutrition, ToP -Total operational Profit, TcF -Total cost of Feed, TcP -Total cost of Probiotic, TnL -Total number of surviving fry, TnF- Total number of Fry produced, TcFr -Total cost of Fry production.

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