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Evaluation of resistance against *Streptococcus agalactiae* in four farmed strains of Nile tilapia (*Oreochromis niloticus*)

Avaliação da resistência contra *Streptococcus agalactiae* em quatro linhagens cultivadas de tilápia do Nilo (*Oreochromis niloticus*)

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Abstract

Nile tilapia (*Oreochromis niloticus*) has always been considered more resistant to diseases compared to other fish species. However, in recent decades, this fish species has also been susceptible to many bacterial, fungal, parasitic, and nutritional diseases. In Brazil, streptococcosis is one of the main diseases that affect tilapia and has caused many economic losses. A resistance test was conducted with 93 tilapia of four varieties (UFLA strain and three commercial varieties: SC1, SC2, and SC3) to evaluate resistance to experimental inoculation with *Streptococcus agalactiae*. Each variety was distributed in five aquariums, with nine specimens of the same variety. Among the five aquariums, four housed fish were intraperitoneally inoculated with 10^7 CFU/fish of *S. agalactiae* and one aquarium harbored fish from the control group, which received 0.1 mL of sterile BHI (Brain Heart Infusion). Significant differences were observed in the survival rates accumulated at the end of the experiment (day 15), with values of 22, 36, 36, and 15% for SC1, SC2, SC3, and UFLA, respectively. The UFLA strain presented lower survival than other strains, which did not differ among the 15 days of challenge. These results demonstrate that there are animals with a better response to the resistance test than others.
Key words: Disease resistance. Streptococcosis. Challenge test. Nile tilapia.

Resumo

A tilápia do Nilo (*Oreochromis niloticus*) sempre foi considerada mais resistente a doenças em comparação com outras espécies de peixes, porém, nas últimas décadas, essa espécie de peixe também foi suscetível a muitas doenças bacterianas, fúngicas, parasitárias e nutricionais. No Brasil, a estreptococose é uma das principais doenças que afetam a tilápia e causou muitas perdas econômicas. Um teste de resistência foi realizado com 93 tilápias de quatro variedades (linhagem UFLA e três variedades comerciais: SC1, SC2 e SC3) para avaliar a resistência à inoculação experimental com *Streptococcus agalactiae*. Cada variedade foi distribuída em cinco aquários, com nove espécimes da mesma variedade. Entre os cinco aquários, quatro peixes abrigados foram inoculados intraperitonealmente com 10^7 UFC / peixe de *S. agalactiae* e um aquário abrigou peixes do grupo controle, que receberam 0,1 mL de BHI estéril (infusão cardíaca cerebral). Diferenças significativas foram observadas nas taxas de sobrevivência acumuladas

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no final do experimento (dia 15), com valores de 22, 36, 36 e 15% para SC1, SC2, SC3 e UFLA, respectivamente. A linhagem UFLA apresentou menor sobrevivência que as demais, o que não diferiu entre os 15 dias de desafio. Esses resultados demonstram que existem animais com melhor resposta ao teste de resistência do que outros.
Palavras-chave: Resistência a doenças. Estreptococcose. Teste de desafio. Tilápia do Nilo.

Streptococcosis is a bacterial disease responsible for causing outbreaks and high rates of morbidity and mortality in farmed fish. *Streptococcus iniae* and *Streptococcus agalactiae* are considered the main pathogenic species in freshwater and marine fish species causing septicemia and meningoencephalitis, with the potential to cause high losses in several countries (Figueiredo, Nobrega, Leal, Pereira, & Mian, 2012). Nile tilapia (*Oreochromis niloticus*) is mostly affected by strains of S. *agalactiae*, resulting in high mortality and considerable economic losses for the aquaculture industry. From 2009, large scale streptococcal outbreaks have occurred continuously in Asia, with variable mortality rates, ranging from 30 to 90% (Li etal., 2015).

In some Latin American countries, *S. agalactiae* has exclusively been isolated from natural outbreaks in tilapia (Jimenez et al., 2011). Stress conditions, such as high stocking densities, harvesting or handling, poor water quality, including high ammonia or nitrite concentrations, and low levels of dissolved oxygen, are associated with disease outbreaks(Evans et al., 2002; Yanong & Francis-Floid, 2013). In tilapia farms, disease propagation usually occurs during high-temperature seasons, which increases susceptibility streptococcosis (Amal & Saad, 2011). Movement of infected fish among farms is the most common cause of *S. agalactiae* dissemination due to horizontal transmission through fecal spread. Some experimental assays showed that *S. agalactiae* could be pathogenic to several fish species through different routes of infection, such as cohabitation, immersion, and intraperitoneal (IP) and intramuscular (IM) injections (Evans et al., 2002). For example, cohabitation with dead fish resulted in infection of healthy ones (Nguyen, Kanai, & Yoshikoshi, 2002; Iregui, Comas, Vasquez, & Verjan, 2016). Moreover, direct contact with infected individuals allows entrance of pathogens through the gills and injured or non-injured skin (Mian et al., 2009).

Genetic variation for disease resistance exists for several pathogens (53 in different fish species) (Odegard, Baranski, Gjerde, & Gjedrem, 2011; Yáñez & Martinez, 2010; Yáñez, Houston, & Newman, 2014). Recently, the presence of additive genetic variation for *S. iniae* and *S. agalactiae* resistance in farmed tilapia has been determined, with heritability ranging from 0.42± 0.07 to 0.58 ± 0.09 (La Frentzet al., 2016) and 0.38 ± 0.11 (Shoemaker et al., 2017), respectively. Thus, selective breeding for *Streptococcus* spp. resistance is a feasible strategy to generate specific tilapia strains, which can cope with disease outbreaks better. However, little is known about streptococcosis resistance in existing farmed tilapia strains. In this study, we evaluated the resistance against *S. agalactiae* in four farmed Nile tilapia strains using inoculation with bacteria in controlled conditions.

The experiment was conducted at the Laboratory of Aquatic Animal Diseases, Department of Animal Science, Federal University of Lavras (UFLA), Lavras/MG, Brazil. The challenge test was performed using 93 fish of three commercial strains (SC1, SC2, andSC3) and the UFLA strain. The challenge was carried out with 22 to 26 animals per strain. The average weight of the challenged fish was 57.45 ± 15 g. Fish were maintained in 57-L tanks, with a constant flow of dechlorinated water (500 mL/h), a controlled temperature of 26 °C, and fed twice a day (2% of biomass weight) with commercial food (32% of crude protein). Before the challenge, fish were subjected to an adaptation period of 15 days. Animals from each strain were

randomly divided into four tanks, with an average of 5.8 (SD= 1.89) fish per tank. Thus, there were four replicate tanks per strain. A control tank was also included.

A pathogenic strain of *S. agalactiae* (st05/14), isolated from Nile tilapia in Minas Gerais State, Brazil, was used to generate the inoculum. The lethal dose 50 (LD50) value was previously calculated using the Reed-Munch technique. The bacteria were grown on tryptic soy agar (TSA) supplemented with horse blood (5%) and incubated at 28 °C for 18h. After that period, BHI (Brain Heart Infusion agar) broth was inoculated with the bacteria and incubated for 12 to 24 h at 30 °C until reaching an Optical Density of 0.1 (10^7 CFU/mL, 600 nm).

Feeding was suppressed 24 h before inoculation. Animals were anesthetized by immersion in a benzocaine solution (100 mg/L), and challenged fish were each inoculated IP with 1mL of 10^7 CFU/mL of *S. agalactiae*. Animals from control groups were inoculated with 1 mL of sterile BHI. Immediately after inoculation, water temperature was raised to 30 °C. Animals were monitored for 15 days, and mortality was recorded daily. Resistance to *S. agalactiae* was determined by the day of death (DD), ranging from 1 to 15, depending on the day of the event for each fish.

Kaplan-Meier curves (Kaplan & Meier, 1958) and Log-rank tests were plotted and estimated for the test period by strain and indicated significant differences (p-value<0.05) in mortality between strains. Cumulative survival rates at the end of the experiment were 22, 36, 36, and 15% for SC1, SC2, SC3, and UFLA strains, respectively.

The trait DD was analyzed using an ordinary fixed-effect linear model (analysis of covariance, ANCOVA), including *tank* and *strain* as factors and *body weight* as a covariate. *Post hoc* multiple comparisons tests were carried out using Fisher's Least Significant Difference (LSD) Test. The analysis was carried out using Info Stat software. Differences were considered significant when p-values < 0.05.

The results from the ANCOVA for resistance against *S. agalactiae* measured as the day of death (DD) in Nile tilapia are shown in Table 1. Strain and tank effects were highly significant (P < 0.0001) when explaining the total variation for DD. However, body weight did not show a significant effect on *S. agalactiae* resistance. The variables strain and tank explained 24 and 46% of the total sum of squares, respectively. These results indicate a strong tank effect and also a high relative importance in the differential susceptibility to *S. agalactiae* by the different strains evaluated in the present study.

Table 1
Analysis of covariance for resistance against S. agalactiae measured as the day of death (DD) in Nile tilapia (Oreochromisniloticus).

Source of variation	SS[1]	DF[2]	MS[3]	F[4]	P- value	SS%
Model	2283.07	20	114.14	8.78	< 0.0001	71%
Strain	779.09	3	259.70	19.97	< 0.0001	24%
Tank	1477.95	16	92.37	7.10	< 0.0001	46%
Body weight	26.03	1	26.03	2.00	0.1614	
Error	936.09	72	13.00			
Total	3219.16	92				

SS[1]: Sum of squares; DF[2]: Degrees of freedom; MS[3]: Mean squares; F[4]: Variation ratio.

Animals from SC1 and SC2 strains did not show a significant difference in *S. agalactiae* resistance. However, fish from UFLA and SC3 strains were significantly less resistant than the formers, with an average day of death of 5 and 8 days post-inoculation, respectively ($p > 0.05$) (Figure 1). Control groups did not show any mortality.

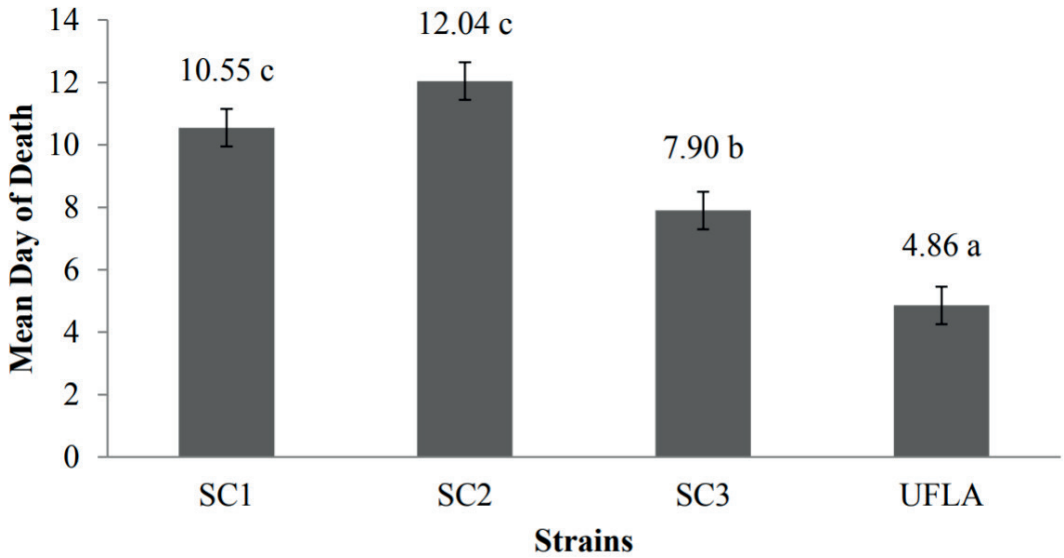

Figure 1. Mean day of death values for four different strains of Nile tilapia after *S. agalactiae* inoculation. Letters indicate statistically significant differences, according to Fisher's Least Significant Difference (LSD) Test (p-value < 0.05).

The results obtained in this study show that different strain of tilapia exhibit differences in mortality rates after experimental challenge against *S. agalactiae*. In a similar study with *S. agalactiae* challenge, Huang et al. (2013) observed differing cumulative mortality rates and immune responses in different tilapia species challenges against *S. agalactiae*. Shoemaker et al. (2017) observed a cumulative mortality rate of 68% at the end of *S. agalactiae* challenge, ranging between 100 and 11% for the most susceptible and resistant family, respectively. The presence of genetic variation for *S. agalactiae* resistance within and between tilapia populations supports the results found in the present study.

In conclusion, there are differences in survival rates between different commercial strains of Nile tilapia challenged with *S. agalactiae*, which may be due to selective processes in certain commercial populations, and may provide fish strains with better response when facing an outbreak. These results suggest that different tilapia strains can display variation for *S. agalactiae* resistance, which can be exploited under farming conditions, where populations are more predisposed to disease outbreaks.

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