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Effectivennes of an autogenous vaccine versus a comercial vaccine for tilapia

Eficácia de uma vacina autógena diante de uma vacina comercial para tilápias

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ABSTRACT

The advancement of Brazilian tilapia farming has brought new health challenges, with Streptococcus agalactiae infections being particularly concerning. To combat this, both commercial vaccines (made with heterologous strains) and autogenous vaccines (made with homologous strains) have been developed. This study analyzed data from three groups: a control group, a group vaccinated with a commercial vaccine (CM), and a group vaccinated with a custom autogenous vaccine (AU). Each group contained 27,500 fish. After rearing, the fish were vaccinated and monitored under identical conditions. The study compared the efficacy of both vaccines, finding that the AU group, while 20% more expensive, resulted in a 2.9% higher survival rate than the CM group and 6.6% higher than the control group. Additionally, the AU group had a 10.6% higher average weight gain than the CM group and 12% higher than the control group. Revenue per tank was 10.49% higher in the AU group compared to the CM group and 14.94% higher than the control group. Moreover, the AU group showed a lower feed conversion rate and higher productivity per cubic meter, indicating the effectiveness of the autogenous vaccine.

Keywords: Aquaculture; Tilapia; Vaccination; Vaccine; Autogenous vaccine

RESUMO

Nos últimos anos, a tilapicultura brasileira enfrentou desafios sanitários crescentes, com a infecção por Streptococcus agalactiae sendo um dos principais problemas. Para combater esse patógeno, vacinas comerciais e autógenas foram desenvolvidas. Este estudo comparou três grupos de peixes: um controle, um vacinado com vacina comercial (CM) e outro com vacina autógena (AU), cada grupo contendo 27.500 peixes. Os peixes, vacinados ao atingir 50,6 gramas, foram monitorados em termos de sobrevivência, ganho de peso e produtividade.Os resultados mostraram que, apesar de o grupo AU exigir um investimento 20% maior que o grupo CM e 100% maior que o controle, ele apresentou uma sobrevivência 2,9% superior ao grupo CM e 6,6% superior ao controle. Além disso, o ganho de peso foi 10,6% maior em comparação com o grupo CM e 12% em relação ao controle. A receita média por tanque no grupo AU foi 10,49% maior que no CM e 14,94% maior que no controle. A conversão alimentar foi melhor no grupo AU, que também apresentou maior produtividade por m³. Esses resultados indicam que, embora mais cara, a vacina autógena oferece vantagens significativas em termos de desempenho e sobrevivência dos peixes.

Palavras-chave: Aquicultura; Tilápia; Vacinação; Vacina; Vacina autógena.

INTRODUCTION

Globally, aquaculture is currently expanding due to the increase of human population, offering a healthier and more affordable protein source (WONG et al., 2024) According to FAO (2022), in 2018 aquaculture was the primary source of fish for human consumption, estimated at 4.5 million tonnes and valuated at 263 billion dollars.

In Brazil, the cultivation of aquatic animals has been growing at an average rate of 30% per year, surpassing the global average of 10% per year. This is a positive sign that Brazil has an affinity for aquaculture activities (SCHULTER; VIEIRA FILHO, 2017). Tilapiculture, the cultivation of tilapia (*Oreochromis niloticus*), has transformed the Brazilian aquaculture landscape. After the 2000s, when the activity was growing discreetly and lacked professionalization, there was an improvement in demand, product quality, and significant investments by large companies in the sector. This professionalization has improved the scenario of tilapia farming, intensifying cultivation, and elevating Brazil to one of the world's largest tilapia producers. It has solidified growth with quality (BARROSO; MUÑOZ; CAI, 2019).

The increased cultivation intensity to meet market demands concurrently imposes stressors on the fish, thereby promoting the persistence and dissemination of potentially pathogenic agents in the cultivation environment (LEIRA et al., 2017). Within this context, bacteria occupy a significant role as potential pathogens in intensive fish farming, due to their capacity for rapid dissemination and opportunistic characteristics.

Immunization is an important strategy to protect aquaculture species from significant diseases, and several recent studies successfully demonstrate the effectiveness of vaccines in providing immunoprotection against bacterial fish pathogens, including *S. agalactiae* (WANG et al., 2020). Vaccination management is an effective method to control *S. agalactiae* infection and prevent mass mortality in tilapia (LIU et al., 2016).

The bacteria with the greatest economic impact on tilapia cultivation in Brazil belong to the *Streptococcus* genus, particularly the species *Streptococcus agalactiae* (WANG et al., 2020), as it is a global agent and the primary pathogen responsible for sepsis and meningoencephalitis in bony fish (EVANS; KLESIUS; SHOEMAKER, 2006). Outbreaks caused by this bacterium lead to high rates of morbidity and mortality in fish populations, resulting in significant economic losses for the farms. Mortality rates in batches can escalate to as much as 90%, especially in the final stage of tilapia cultivation,

a period characterized by elevated levels of feed consumption throughout the rearing process (ABUSELIANA, 2010).

Epidemiological studies have identified the existence of 13 different biotypes of *Streptococcus agalactiae* isolated from tilapia in various parts of the world (OLVIARES-FUSTER et al., 2008). Epithelial streptococci exhibit characteristics such as adherence to epithelial surfaces, invasion of epithelial and endothelial cells, and direct tissue damage, which are some of the consequences of infections contributing to their virulence (NIZET; RUBENS, 2000). The primary mode of transmission is through direct contact with infected fish and/or contaminated food and indirect contact mediated by water in the cultivation system (LIM; WEBSTER, 2008). The bacteria are excreted in the feces of infected fish and can survive in aquatic habitats, increasing the possibility of fecal-oral transmission (NGUYEN; KANAI; YOSHIKOSHI, 2001). Another important transmission route is oral via cannibalism, which leads to the spread of diseases to healthy animals feeding on dead or dying animals (WONGSATHEIN, 2012). Vertical transmission of *Streptococcus agalactiae* in naturally infected tilapia has not been detected in the larvae of infected parent fish (JIMÉREZ et al., 2011).

The main clinical signs include anorexia, skin darkening, erratic swimming, lethargy, body curvature, protruding eyes with corneal opacity and/or unilateral or bilateral intraocular hemorrhage, suffusions on the operculum and base of the fin, epidermal ulcers, and death. Internal lesions are characterized by gill hyperemia, hepatomegaly, and splenomegaly, associated with hyperemia, ascites, and encephalomalacia (SALVADOR et al., 2003). The severity of the disease in tilapia is related to factors such as the strain of *Streptococcus agalactiae*, the infection dose, water temperature, biomass, and animal husbandry practices (CHANG; PLUMB, 2010). High-density conditions, poor water quality, and inadequate management can lead to the release of cortisol, which is an indicator of stress in fish. Animals under stress conditions exhibit anorexia, depletion of glycogen stores, and immunosuppression, reducing their resistance to pathogens (EVANS et al., 2002).

The physiopathology of infections caused by *Streptococcus agalactiae* is not fully understood, but research began with the association of the presence of bacterial colonies with damage to the spleen, liver, kidney, and brain tissue in naturally infected fish (ZAMRI-SAAD; AMAL; SITI-ZAHRAH, 2010). *Streptococcus agalactiae* causes local

necrosis by invading and multiplying within macrophages, which can serve as carriers to enter the bloodstream and spread to various organs, including the brain, crossing the blood-brain barrier, leading to septicemia (MUSA et al., 2009).

In red tilapia *(Oreochromis niloticus)* lesions of focal necrosis, severe hepatic congestion, and infarcted area, petechiae, necrosis and vasculitis associated with bacterial colonies in the spleen, severe congestion of the gills and intestines, hyperemic kidney with significant inflammatory process and thickening of the meninges were observed due to the marked inflammatory infiltrate (ZAMRI-SAAD et al., 2010).

The protective immune responses induced by animal vaccination are considered a vital strategy for the protection of aquaculture species against bacterial infections, which include *Streptococcus agalactiae* (WANG et al., 2020). Inactivated vaccines are the most commonly used due to its simple and economical production, and ecological safety compared to live vaccines (MANUNG'ANDU; MOTOLOKI; EVENSEN, 2014).

Teleost fish possess a well-developed innate immune system, consisting of physical barriers such as the skin and chemical defenses like serum lysozymes and mucus. These defenses cover the skin and mucous membranes and also envelop embryos, forming a protective barrier against environmental pathogens. In addition to lysozyme, the immune system in teleost fish includes molecules such as C-reactive protein, macrophages and other phagocytes, neutrophils, and thrombocytes (WATTS; MUNDAY; BURKE, 1995).

Some vaccines based on formalin-killed cells (FKCs) have been developed previously and have shown high levels of efficacy. FKC vaccines activate the immune system and induce the secretion of immunoglobulin M (IgM) as the first line of defense. Additionally, pro-inflammatory factors can induce an inflammatory response by regulating the expression of other cytokines (WANG et al., 2020).

Conventional physical methods such as heating, ultraviolet (UV) light, sonication, and chemical methods such as the use of formaldehyde, solvents, and detergents are the most commonly used for bacterial inactivation (WATTS; MUNDAY; BURKE, 1995).

However, few commercial vaccines are available for *S. agalactiae*, and these are produced from predefined strains chosen by manufacturers, which may not necessarily match the specific strain found on each individual farm locally (DADAR et al., 2017).

It has been demonstrated through comparative genomics that *Streptococcus agalactiae* has a structured genome with a stable backbone, and differences between many lineages are attributed to other elements. Therefore, small differences between genomes likely occur due to polymorphisms in gene sequences. The genomic characteristics of Brazilian isolates of *Streptococcus agalactiae* showed a number of pseudogenes ranging from 98 to 320 among the isolates, even within each genomic lineage. It is known that serotype B strains are undergoing reductive evolution, and it is common to observe a high percentage of pseudogenes, exceeding 10% of the genome, which is believed to be an adaptive strategy in these hosts (BARONY et al., 2017).

To overcome this obstacle, there are autogenous vaccines, which are prepared from pathogens isolated from infected animals within the same herd that will receive the vaccination. The purpose of autogenous vaccines is to protect those susceptible to the infection and stimulate immunity in the remaining animals of the herd (CARVALHO, 2007).

When evaluating autogenous vaccines for veterinary use, these are classified into two distinct groups. The first group is classified as autovaccines, which are produced by isolating a pathogen from an individual, then vaccinating the same individual with the product. This is commonly seen in companion animals. The second group comprises vaccines manufactured for a herd. These vaccines are produced from pathogens isolated from sick animals within a group or herd, and they are subsequently used in animals that are part of or will be part of the same herd (CARVALHO, 2007). This type of vaccine essentially implements control measures, avoiding the dissemination of specific bacterial strains, since the effectiveness of the S. *agalactiae* vaccine for Nile tilapia seems to be linked to the specificity of the strain (BARONY et al., 2017).

MATERIAL AND METHOD

On a property located in the interior of the state of São Paulo, along the Paranapanema River, data were analyzed from three cultivation groups, each consisting of five batches with an average of 5,500 Nile tilapia (*Oreochromis niloticus*) per batch, totaling 27.500 animals per group. The groups were as follows:

- **CONTROL Group** – 27.500 animals, not vaccinated**.**
- **CM Group** – 27.500 animals, vaccinated with a commercial vaccine**.**

- **AU Group** – 27.500 animals, vaccinated with an autogenous vaccine**.**

Upon their arrival at the property, these animals weighed approximately 1.5 grams and were clinically healthy. They were cultivated until they reached an average weight of 50.6 grams. Throughout this period, the animals were only subjected to the property's feeding management and mortality removal from the tanks. These animals, now referred to as juveniles, underwent the following respective treatments:

- **CONTROL Group** – A single dose of 0.05 mL of placebo saline solution administered via intracoelomic injection.
- **CM**[1] **Group** – A single dose of 0.05 mL of the commercial vaccine administered via intracoelomic injection.
- **AU**[2] **Group -** A single dose of 0.05 mL of the autogenous vaccine administered via intracoelomic injection.

In addition to the vaccination management, the animals from all three groups underwent a classification process. Initially, biometry was conducted with 300 animals from each batch. Afterward, they continued to be cultivated until the second classification, where the batches were biometrically assessed again. This served as evidence for evaluating the final weight. When they reached an average weight of 700 grams, a new biometry was performed to analyze the presence of the vaccine in the coelomic cavity and record the weight of the animals. When they approached 850 grams, the animals were harvested and taken to the refrigeration facility.

For this experiment, the database of the property where the test was conducted was utilized. In this database, a system was updated daily by the property manager with information on feeding, mortality, fish growth, and additional observations. It was possible to acquire data from two distinct categories. The first category encompasses the zootechnical data, which included:

- **Survival rate** (%) = 100 x (*final number of individuals* / *initial number of individuals*).
- **Weight gain**
- **Batch uniformity** = *number of animals outside the upper and lower weight limit of 10% of the biometric mean* / *total animals sampled* x 100.
- **Productivity** = kg/m³ produced = *final number of animals* x *average*

weight of the batch / *area where they were cultured*.

% = percentage

/ = division

X = multiplication

The effectiveness of the vaccine was also evaluated, based on the relative percentage survival (RPS) (AMEND, 1981).

RPS = 1 – (% *mortality in vaccinated animals* / % *mortality in control animals*) x 100

The second category encompasses the economic aspects evaluated from cost research and property reports. This category includes the following factors:

- Vaccination cost per batch = cost of vaccine dose x number of animals to be vaccinated
- Total production cost per batch
- Comparison of revenues per batch
- Difference between revenues

The data were compiled throughout the entire cultivation period, from the batch placement to harvesting, and they were subjected to analysis of variance and mean comparison tests between two groups (Student's t-test).

RESULTS AND DISCUSSION

Table 1 presents the weight gain results, which are related to the initial average weight of the batch and the final average weight obtained through the last biometry in the three treatments over 206 days of the experiment. Through statistical analysis, it was observed that the AU (autogenous) group differs from the other groups in terms of initial weight. It is noteworthy that the AU group, in the first classification before vaccination, had a lower average weight than the other groups. This factor is not a controllable variable, as there are factors from the initial placement to the first biometry that can interfere with the growth of the fish, even though they received the same treatments and management. The table also shows that the percentage of weight gain in the AU group was statistically different and higher than the other two groups.

Table 1: Means and standard deviation of initial weight, final weight, weight gain and percentage of weight gain**.**

	Autogenous	Commercial	Control
Starting weight	35,60±11,99 b	56,60±5,367 a	59,60 ±8,961 a
Final weight	735,7±59,09 a	682,6±99,53 ab	675,8±40,10 b
Weight gain	700,0±53,69 a	625,6±95,65 ab	616,0±33,74 b
% Weight gain	2120±575,20 a	1105±120,90 b	1048±130,20 b

Means followed by the same letter on the same line do not differ from each other (p≤0,05)
Source: created by the author, 2024.

The statistics indicate that the AU group exhibited better performance in terms of the weight of the animals. Despite starting the experiment with a lower average weight, it was able to achieve higher weights and a greater percentage of weight gain in the final biometry.

Table 2, through statistical analysis, indicates that the three groups exhibit significant differences in mortality when comparing the number of animals that died based on collected mortality data to the number of animals initially placed. This highlights the superiority of the two vaccinated groups over the non-vaccinated group and lower mortality in the AU group compared to the CM group.

Table 2: Statistical analysis of the percentage of mortality and animal balance in the experimental groups

	Autogenous	Commercial	Control
Mortality	5,1% a	8% b	11,7% c
Animal balance	26308 a	25425 b	24393 c

Means followed by the same letter on the same line do not differ from each other (p≤0,05)
Source: created by the author, 2024.

The data demonstrate the superiority of the two vaccinated groups over the control group, confirming that the vaccine directly influences the survival of animals on a property. Furthermore, the superiority of the AU group over the CM group indicates that

even vaccines containing the same serotype of *Streptococcus agalactiae*, the vaccine containing the exact genetic specificity of the strain, achieved better survival results.

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Table 3 indicates that the investment made to vaccinate batches with the autogenous vaccine is higher. This is because, due to production specifics, the cost per dose is generally higher when compared to a commercial vaccine, which is produced in larger quantities and with a pre-defined strain. Non-vaccination implies zero cost for vaccine acquisition, making this group the most economical in terms of investment.

Table 3: Statistical analysis of vaccination cost, revenue per tank and the difference between cost and revenue obtained.

	Autogenous	Commercial	Control
Cost per tank	R$660,00 c	R$550,00 b	R$ 0 a
Tank revenue	R$ 29.971,38 a	R$ 26.827,21 a	R$ 25.490,96 b
Revenue - Cost	R$ 29.839,38 a	R$ 26.717,21 a	R$ 25.490,96 b

Means followed by the same letter on the same line do not differ from each other ($p \leq 0,05$)
Source: created by the author, 2024.

The cost per dose used in the experiment was determined based on quotations from vaccine companies at the beginning of the experiment. It amounted to R$0.12 per animal for the autogenous vaccine and R$0.10 per animal for the commercial vaccine. This number was then multiplied by the number of animals placed in each tank at the beginning of the experiment. In general, the cost of acquiring the vaccine accounts for around 1% to 2% of the final unit price of the animal. The overall vaccination cost was not considered for this experiment as there is significant variation and confidentiality regarding the fixed costs of each property.

Bwalya et al. (2020), in a study involving 460 Nile tilapia vaccinated at an average weight of 41.5 grams, demonstrated that an autogenous vaccine made from inactivated whole cells reduced the reisolation of the pathogen *Lactococcus garviae* from 20% in the

non-vaccinated control group to 6% in the group vaccinated with an autogenous vaccine manufactured with a strain homologous to the isolate used for vaccine production. This indicates that there is protection through the autogenous vaccine, reducing the incidence and infection of the analyzed fish post-vaccination, as expected in the present study. The reduced number of dead animals and the higher weight gain suggest that the pathogen for which the vaccine was developed was absent and/or present in fewer isolations.

The production standards of the autogenous vaccine used in this study are of high quality and extremely refined. This may contribute to the higher level of protection observed in the autogenous vaccine group compared to the other two groups studied. The manufacturing process of the autogenous vaccine by the supplying company is likely a key factor in this superior protection. This also justifies the higher cost per dose presented in the study. Despite the high cost presented in the prophylactic method, its cost-effectiveness is considerably more significant.

The quality and selection of initial materials are critical factors in ensuring the safety and efficacy of the vaccine product. The right combination of antigen and adjuvants enhances the prospects of vaccine efficacy. Materials used for the production of autogenous vaccines need to comply with the current regulatory provisions of the country. All materials and suppliers need to be qualified. The isolates used for vaccine production must be pure. The exclusion of foreign agents in the starting material and the final product should preferably be done through strategic testing and risk assessments, including analysis of purification and inactivation steps. Physical examinations should be restricted to foreign agents that cannot be eliminated through risk assessment and ideally should be performed using in vitro tests. The use of autogenous vaccines contributes to current efforts to manage emerging diseases as they can be rapidly updated and produced. They also contribute to reducing the use of antibiotics, especially in food-producing animals, including aquaculture. In the present experiment, the reduction in antibiotic use can be considered, as a higher survival rate in the autogenous vaccine group is expected to result in a reduction in the treatment of sick animals. Autogenous vaccines are an accepted component of a One Health approach, strengthening opportunities in the prevention of infectious diseases (GREIN; JUNGBMÄCK; KUBIAK, 2022).

The incorporation of an adjuvant in a vaccine provides greater protection to vaccinated tilapia compared to tilapia immunized with a vaccine without an adjuvant

(FIRDAUS-NAWI et al., 2012) The adjuvant used in the autogenous vaccine in this study is recommended for intraperitoneal injections in shoals against *Streptococcus agalactiae* infections. The autogenous vaccine incorporated in this adjuvant was found to be non-harmful to the vaccinated groups, and the quantity used did not cause injury at the inoculation site. This is crucial as the vaccine composition should not cause lesions in the site of vaccination, except in the first few days after vaccination due to the expected inflammatory process. After a few days, the inflammation completely disappears. Thus, in the present study, it was observed that the autogenous vaccine is non-toxic and harmless to the animals, demonstrating its safety.

Similar results were obtained by Rivas (2020), who demonstrated that an inactivated bivalent vaccine against A. *sobria* and S. *agalactiae*, produced using strains isolated in the state of Paraná, Brazil, and administered intraperitoneally to Nile tilapia (*Oreochromis niloticus*) in the study, protected and stimulated the immune system against infections caused by the bacteria used in the vaccine. In this study, he achieved an RPS of 91% under experimental conditions, results that are similar to the data analyzed in the present study. Considering an equal disease challenge for all groups, the group vaccinated with the autogenous vaccine would have an RPS of 94.9%.

CONCLUSION

The present study demonstrated, in a field experiment, the effectiveness of the autogenous vaccine in preventing the pathogen isolated from the property for which the vaccine was manufactured. It clearly and field-based highlighted the differences in the benefits of using an autogenous vaccine compared to a commercial vaccine in preventing infections caused by the bacterium *Streptococcus agalactiae* serotype 1b. This provides the productive sector with insights into how economically advantageous it can be to adopt vaccination with an autogenous vaccine manufactured with the homologous strain responsible for the health issue on the property.

Based on the data collected from the property where the test was conducted, it is capable of concluding that for the producer, the autogenous vaccine requires a higher investment in terms of vaccine acquisition cost, which in the experiment in question was 20% higher than the cost of acquiring a commercial vaccine. Conversely, when used, the autogenous vaccine increases the survival rates by 2.9% when compared to the CM group and by 6.6% when compared to the control group. Regarding weight gain, animals

vaccinated with the autogenous vaccine gained 10.6% more in average weight when compared to the CM group and 12% more when compared to the control group. The average revenue per cultivation tank for the AU group was 10.49% higher when compared to the CM group and 14.94% higher when compared to the control group. Considering the same treatment for all groups and repetitions, we can deduce that animals vaccinated with the autogenous vaccine have lower apparent feed conversion than the other two groups, and the productivity per m^3 is higher with the use of the autogenous vaccine.

In terms of economic importance, mortality and weight gain directly impact the revenue and profit of fish farms, as prices are determined per kilogram of fish, and the higher the number of surviving animals, the greater the total live weight in the tanks.

The analysis of tissues using immunohistochemistry, enzyme-linked immunosorbent assay (ELISA), and the reisolation of the pathogen in organs could potentially complement the study with a broader range of laboratory evaluation methods. Additionally, conducting a challenge by inoculating *Streptococcus agalactiae* serotype 1b into fish from all groups under controlled laboratory conditions in a second stage of the study would provide further insights and data on other aspects that complement the obtained zootechnical data.

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