Title

**Systemic and mucosal immune response of Nile tilapia broodstock to monovalent and bivalent vaccines against bacteria *Streptococcus agalactiae* and *Aeromonas veronii*.**

Abstract

Fighting infections from the bacteria *Streptococcus agalactiae* and *Aeromonas veronii* in Nile tilapia (*Oreochromis niloticus*) is a hot-topic research for aquaculture. Both bacteria are cosmopolite and are causing high mortality in infected ponds. Moreover, a diseased fish, if consumed raw can easily become a vector for the transmission to animals and/or humans. Prophylactic measures such as fish vaccination are a specific and very effective way of prevention from diseases. In the present study, we aim to demonstrate that immunization is possible in Tilapia. By using inactivated vaccines prepared from formalin-killed pathogens, we were able to produce two monovalent vaccines from *Streptococcus agalactiae* and from *Aeromonas veronii*, and a bivalent vaccine from the combination of the two pathogens with a ratio of 1:1. Four-hundred juveniles individuals were acclimated and split into 4 groups in separate aquariums (1 control and 3 treatments). Then 8 individuals per group were selected for sampling weekly. Immune response, both systemic and mucosal was characterized for humoral immunity using concentrations in Immunoglobulins M and Immunoglobulins T as immunogenic markers with the help of ELISA assays or agglutination antibody test and by RT-PCR for IgM and IgT gene transcription activity before, during and after immunization, on a weekly basis. It is also possible to discard the effect of the innate immune response by determining expression levels of ROS genes by RT-PCR. Optimal protective dose and vaccination patterns have been determined with the help of booster doses. Finally, the fish have undergone a bacterial challenge with a single lethal dose of *Streptococcus agalactiae* or *Aeromonas veronii* respectively of their vaccinations. The outcomes will determine the LD50 for the different groups. This study was motivated because of the lack of availability of vaccination in Thailand and from the necessity to mitigate antibiotic resistance and reduce global antibiotic use.

Introduction

Tilapia (location, popularity..)

Tilapia diseases

Bacterial diseases

*Streptococcus agalactiae* and *Aeromonas veronii*

History of the disease, treatment, mitigation, effect on the fish. Effects. Prophylaxis.

Material and methods

2.1. Experimental design and fish husbandry

Number of fish, age, size, obtention, stocking, food, water parameters, fish sampling/selection

Detection of diseases in fish (select fish and verify that they have no conditions)

## 2.2. Preparation of bacterial culture and formalin-killed vaccines

Strains of bacteria *Streptococcus agalactiae* and *Aeromonas veronii*

Obtention, collection, culture, amplification, harvesting, inactivation, preparation of vaccine (formulation), stockage. Quality control

*2.3. Fish immunization and sample collection*

*Protocol,* Animal Care and Use Committee (MUSC63-014-522

## 2.4. Serum antibody assays and mucus antibody analysis

## 2.5. Efficacy trial – laboratory aquarium challenge

## 2.6. Data analysis

# 3. Results

## 3.1. Systemic antibody response in the immunized Asian seabass

## 3.2. Mucosal antibody response in the immunized Asian seabass

## 3.3. The efficacy test in the laboratory challenge

# 4. Discussion

# CRediT authorship contribution statement

References

**Titration and isotype determination of antibodies**

Concentrations of immunoglobulin specific for GBS PS or TT were determined by ELISA as

described previously [17]. Total Igs and Ig isotypes were assayed using a commercially

available ELISA kit (Bethyl Labs, Texas, US). All titers were expressed as mean ± standard

error. IgG isotypes were determined using sera from day 31 after two immunizations and from

day 60 after three immunizations, as described in the immunization protocol.