Influence of moderate hypoxia on vaccine efficacy in Nile tilapia Oreochromis niloticus against Vibrio anguillarum and Streptococcus agalactiae

Summary

専　攻 Major	APPLIED MARINE BIOSCIENCES	氏　名 Name	AHANGAMA GALLAGE Sanchala Shyama Darshani
論文題目 Title	Influence of moderate hypoxia on vaccine efficacy in Nile tilapia *Oreochromis niloticus* against *Vibrio anguillarum* and *Streptococcus agalactiae*. （中等度の低酸素がティラピアの *Vibrio anguillarum* 及び *Streptococcus agalactiae* に対するワクチン効果に及ぼす影響）		

Whether it is terrestrial or aquatic, oxygen is the most vital element for all most all form of life. Oxygen is the final electron acceptor in cellular respiratory process in which energy generates in the form of ATP. On the other hand, oxygen is essential for various cellular reactions mediated with oxidases, peroxidases etc. In view of those, adequate amount of oxygen in water as well as in body is important for wellbeing of the aquatic animal including fish. In an aquatic environment, dissolved oxygen is the major limiting factor due to water contains low oxygen amount compared to the air. Today, many intensive aquaculture systems are having lower oxygen than naturally available amount due to high stocking density, excess feeding, accumulation and decomposition of fish waste, higher bacteria load, nutrient enrichment mediated algal blooms, low water flow rate etc. Limitation of oxygen in water or hypoxia known to creates the similar internal environment in fish hence all most all functions including growth, reproduction, behavior locomotion, metabolisms and immunity may adversely affect Hypoxia is known as immune modulator in fish which promotes disease outbreaks. Several scientific studies have revealed hypoxia-mediated immune compromising and increased susceptibility to infectious diseases in fish. However, until now, no studies have addressed the effects of hypoxia or moderate hypoxia on vaccine efficacy in fish. This could likely be an important limiting factor in our ability to predict vaccine efficacy. Therefore, this study carried out to reveal the influence of moderate hypoxia on vaccine efficacy in Nile tilapia. To accomplish this goal, immunological basis of

protection offered via vaccination and influence of moderate hypoxia (55 ±5% dissolved oxygen saturation) on those mechanisms were examined in Nile tilapia following vaccination with formalin inactivated *Vibrio anguillarum* and *Streptococcus agalactiae.*

In the first part of this research, vaccine efficacy against *V. anguillarum* was examined using serum antibody titer as surrogate marker. In addition, several haematological and immunological parameters were also examined. The fish were acclimatized either to moderate hypoxic or normoxic (85±5%DO saturation) conditions for 2 weeks and immunized with formalin inactivated *V. anguillarum* ($5x10^9$ CFU/ml). When Nile tilapia raised and vaccinated under normoxic condition, serum antibody titer was significantly higher than that of moderate hypoxic fish at all the detected time points. In addition, absolute lymphocyte count in blood was also significantly lower in moderate hypoxic group compared to normoxic group at 14^{th} days post vaccination (dpv). Serum bactericidal activities were measured and it was found to be significantly higher in normoxic group compared with moderate hypoxic group at 7^{th} and 14^{th} dpv. Serum killing of *V. anguillarum* appear to be mainly via antibody dependent classical complement pathway. Following vaccination, fish were transferred between normoxic and moderate hypoxic groups at 0 hour or 7^{th} dpv in order to examine the importance of first week of vaccination on antibody production in fish and results revealed that even though continuous supply of higher oxygen is necessary to gain maximum antibody response, first week following vaccination can be considered as critical where important immune regulatory pathways are activated. This view was further supported by results obtained from gene expression experiments where transcription level of all the detected immune related genes in spleen (IgM, IL-1β, TCR-β, MHC-IIβ) except B cell activating factor were significantly lowered following exposure to moderate hypoxia during first week post vaccination. Plasma chemistry analytes and electrolytes were shown non-significant variation between normoxic and moderate hypoxic groups

throughout the study. In contrast, packed cell volume exhibited significant alteration attributed to hypoxia indicating that Nile tilapia demonstrating an adaptive response toward moderate hypoxia. Overall this study explained that moderate hypoxia negatively affects on vaccine efficacy in vaccinated Nile tilapia by lowering antibody production, serum killing and immune related gene expression. Furthermore, studies carried out to find the possible counter measure to enhance the vaccine efficacy in moderate hypoxic fish revealed that booster vaccination might be useful where no difference was found in antibody titer and serum bactericidal activities between normoxic and moderate hypoxic fish. However, it should be noteworthy that antibody mediated other defensive mechanisms that we didn't study here may not be recovered as antibody titer hence those should be subjected for further studies.

Streptococcosis caused by *S. agalactiae* considered as one of the deadly bacterial disease in tilapia aquaculture around the world which causes mass mortalities even up to 100% in some farms.

This ubiquitous bacterium evolved several advance strategies to avoid many immune mechanisms. Even though this bacterium has several strategies to overcome immune response, specific antibodies proved to be effective to inhibit pathogen survival and distribution in the host hence vaccine appear to be promising prophylactic measure against this pathogen. Therefore, second part of this study was conducted in order to understand the pathogen clearance mechanisms in vaccinated Nile tilapia following experimental challenge with *S. agalactiae* and influence of moderate hypoxia on those mechanisms. At first, fish were acclimated to moderate hypoxic or normoxic conditions and vaccinated with formalin inactivated *S. agalactiae* pellet ($5x10^{10}$ CFU/ml) via intra-peritoneal (IP) injection. Antibody titer was measured at 0, 7^{th}, 15^{th} and 30^{th} dpv and serum bactericidal activities and serum lysozyme activities were also detected. At 30^{th} dpv, fish were challenge with live *S. agalactiae* ($1.3x10^{7}$ CFU/fish) via IP injection and

mortality were recorded daily. The tissue samples and blood collected at 1st ,3rd ,5th ,7th and 15th day post challenge were analyzed for viable bacteria count. In addition, several *In vitro* studies carried out with head kidney leukocytes (HKLs) to reveal dissolved oxygen (DO) dependency and antibody dependency of their cellular functions. Serum antibody titer was significantly higher in normoxic vaccinated group compared to the moderate hypoxic vaccinated group at 15th and 30th dpv. *S. agalactiae* appear to be resistance for serum killing even when presence of specific antibodies. The cumulative mortality in vaccinated normoxic fish were significantly lower (5.5%) compared to the moderate hypoxic vaccinated fish (20%) and control groups reflecting pre challenge antibody titer is correspondence with protection against *S. agalactiae*. Lowest cumulative mortality among control groups was found in normoxic control fish (45.5%) while highest was in moderate hypoxic fish (74.5%) indicating DO dependency of the pathogen clearance in these fish. Highest pathogen burden was in moderate hypoxic control fish and lowest was detected in normoxic vaccinated fish at all the detected time points in brain, head kidney and blood. Furthermore, blood of normoxic vaccinated fish was free from *S. agalactiae* at all detected time point while moderate hypoxic fish took more than 5 days for total clearance of pathogen in their blood. Pathogen burden in tissues and blood appear to be directly correlate with survival and rapid clearance of bacteria in blood seems to be important for the survival of the fish. Highest bacteria burden observed in moderate hypoxic control group might reflecting the compromised innate immune clearance mechanisms under moderate hypoxic condition. This idea was confirmed by the results obtained in an *in vitro* study where phagocytosis and intracellular reactive oxygen species (ROS) production exhibited oxygen dependent variations. Furthermore, presence of specific antibodies in the opsonising serum and the amount of antibodies increased the phagocytosis, ROS production and lowered intracellular survival of *S. agalactiae* in the head kidney leukocytes (HKLs). Therefore, it is clear that higher cumulative mortality in moderate

hypoxic fish even after vaccination not only linked to lower antibody production but also to the lowered phagocytes function under moderate hypoxic conditions. Overall this study highlighted that mechanisms of vaccine protection against *S. agalactiae* mainly via antibody dependent phagocytic pathway and efficacy of this mechanism depends upon optimum DO and amount of specific antibodies presence in the serum.

In this study, for the first time, I revealed that moderate hypoxia negatively affects on vaccine efficacy in Nile tilapia hence research outcome of this study will broaden our knowledge on manipulation of vaccine immunology under moderate hypoxic condition. Furthermore, data presented here will be important to optimize future vaccination programs in aquaculture industry in order to gain maximum benefits from vaccination and to become a viable and sustainable industry by limiting opportunities for infectious diseases.