Development and Efficacy of Fish Vaccine Used Against Some Bacterial Diseases in Farmed Tilapia

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Abstract: Tilapia aquaculture is one of the fastest-growing type of fish production in Egypt. Nile tilapia (*Oreochromis niloticus*) is largely cultivated in many localities, where septicaemic bacteria are the most common pathogens of cultured warm water fish and cause major losses to the freshwater aquaculture in Egypt and elsewhere. The use of vaccines, combined with good health management techniques, can result in substantial disease prevention and production becomes more predictable. Vaccines are a preventative measure as opposed to antibiotic treatment which is used after a disease outbreak. Most bacterial vaccines used in aquaculture to date have been inactivated vaccines obtained from a broth culture of a specific strain(s) subjected to subsequent formalin inactivation. The best results are obtained with those bacterins that include both bacterial cells and extracellular products. Whereas with some vaccines acceptable levels of protection are achieved with aqueous formulations administered by injection or immersion, for other bacterins, an acceptable level of protection can only be achieved by immunization with oil-adjuvanted bacterins delivered by injection. Each has its advantages and disadvantages. The most effective method will depend upon the pathogen and its natural route of infection, the life stage of the fish, production techniques, and other logistical considerations.

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Introduction:

Nile tilapia *Oreochromis niloticus*, is the most important species on account of its fast growth rate, adaptability to a wide range of culture conditions and high consumer acceptability. For these reasons, over the past 40 years, it has been transferred throughout the world to over 100 countries to become the mainstay of tilapia farming in many different culture systems, at all levels of intensification, from subsistence production to highly intensive farming.

Tilapia can tolerate water temperatures as low as 12 °C and can survive in water temperatures below 10 °C for prolonged periods of time. Some species are also known to survive and grow in salt water. Being real omnivores, tilapia will eat almost anything and are therefore often called 'aquatic chickens'. Because of the favourable culture characteristics mentioned above, tilapia is considered the most ideal species for small-scale fish farming. **(Hepher and Pruginin, 1990).**

In intensive large scale aquacultures where tilapia are reared at high densities, tilapia is prone to various health problems since pathogens can be so easily transmitted between individuals. The risk is elevated if the keeper of the aquaculture fails to provide the tilapia with optimal conditions, e.g. when it comes to water quality, temperature and salinity.

For most warm water aquaculture facilities, disease prevention consists primarily of good husbandry techniques. When disease outbreaks occur, diagnostics are conducted to determine the cause, and then the fish are given an oral treatment, an immersion (a dip or a bath), or, in rare cases, an injection treatment. Costs incurred from delayed production and growth, treatment chemicals, mortalities, and labor can be significant. In many prevention of disease is preferable to disease treatment **(Grisez and Tan 2005).** Two approaches to disease prevention cases, when fish are no longer eating, treatment options become much more limited and treatment may no longer be effective.

Vaccination is becoming an increasingly important part of aquaculture, since it is considered a cost effective method of controlling different threatening diseases. The term vaccination strategy has been defined to include the decision as to which diseases to vaccinate against, as well as the vaccine type, vaccination method, the timing of vaccination and the use of revaccination. One important consideration for development and commercialization of vaccines includes the application methods and procedures that can be integrated into the normal production protocols of the target fish species that are relevant to the typical ecology and epidemiology of the disease (i.e. seasonal occurrence, fish size, host and geographic range of the disease). **(Toranzo et al., 2009).**

Problem description

Fish is considered as one of the main principal sources of the national income, stimulating local

market economies. Aquaculture represents one of the most clear and effective solution for nutritive problems and the promising hope to cover the gap in food consumption **(Sadek, 2000).** Bacteria are important pathogens for both cultivated and wild fish, and are responsible for mass losses in fish production which is an important economic limiting factor in intensive aquaculture. **(Claudia and Jeffrey, 2009).**

While the intensification of aquaculture has led to remarkable improvements in productivity, it is also associated with disease epidemics, involving bacterial, fungal, viral and parasitic pathogens. Disease is undoubtedly one of the biggest constraints on production, development and expansion of the aquaculture industry. Diseases can be controlled in a number of ways, for example, introduction of specific-pathogen-free (SPF) broodstock, optimization of feed, improvement of husbandry techniques and good sanitation. In conjunction with good health management, prophylactic immunization (vaccination) is an indispensable tool for disease control in aquaculture **(Gudding et. al., 1999; Evelyn, 2002).**

Vaccination has become an increasingly important aspect of aquaculture. Several bacterial and viral vaccines, either mono- or multivalent, have been successfully developed and commercialized **(Bostock, 2002; Evelyn, 2002).** They have proved to be cost effective. In salmonid farming, the use of vaccines is now so widespread that basically all fish stocked in sea cages have been vaccinated. Taking Norwegian salmon farming as an example, the use of antibiotics has dropped to virtually zero and production has increased tremendously **(Markestad and Grave, 1996; Bostock, 2002).**

Vaccines are administered to fish in one of three ways: by mouth, by immersion, or by injection. Each has its advantages and disadvantages. The most effective method will depend upon the pathogen and its natural route of infection, the life stage of the fish, production techniques, and other logistical considerations. A specific route of administration or even multiple applications using different methods may be necessary for adequate protection. **(Komar et al., 2004).**

Scientific background:

Commercial vaccines are available against different bacteria (*Yersinia ruckeri*, *Aeromonas salmonicida*, *Vibrio anguillarum*, *V. ordalii*, *V. salmonicida*, *Edwarsiella ictaluri*). These consists of formalin-killed broth cultured (bacterines), and they frequently include an adjuvant and therefore have to be administered by injection. But some vaccines can also be delivered by other routes, such as immersion, spray, and oral. Administration strategy depends on the size and species of fish, the bacterial species, and the type of vaccine available **(Benmansour and de Kinkelin, 1997).**

The vaccination of six month old carp and rainbow trout orally against vibriosis using a supernatant fraction of the V. anguillarum bacterin encapsulated in alginate microparticles was discussed by **Joosten et al. (1997).** This method attempted to avoid premature digestive degradation of the vaccine. Fish were administered with a diet supplemented with either alginate microparticles with antigen, microparticles without antigens or non encapsulated antigens. Uptake of the antigen in the hind gut was more rapid in case of encapsulated antigens.

A commercial vaccines against enteric red mouth disease (ERM), furunculosis and vibriosis either as single component or combination vaccines for immersion , injection or oral vaccines was prepared by **Larsen and Pedersen (1997).** However the recent results suggest that with the regime of vaccines available, fish should be vaccinated with an ERM immersion vaccine in the hatchery approximately four weeks before transfer to the ponds. To cover the growth period in freshwater an oral booster should be given two to three months later.

Four challenge methods were used by **Nordmo and Ramstad (1997)**: intraperitoneal (i.p) and intramuscular (i.m) inoculation, bath and cohabitation exposure, were evaluated as methods for testing the efficacy of furunculosis vaccines in Atlantic salmon. Groups of fish vaccinated i.p. with 1 of 2 different vaccines containing aluminum phosphate as adjuvant were challenged with A. salmonicida 6 and 12 weeks after vaccination. Relative percent survival (RPS) was calculated daily during a 3-week observation period after challenge. A large variation in protection measured by RPS, both between methods and between different time points for each method was found. RPS was similar in the i.p. and cohabitation challenge. The i.m. challenge produced very low RPS. The bath exposure resulted in RPS value intermediate to the cohabitation and injection methods. After i.p. and i.m. challenges, the initial peak mortality caused by the inoculation was followed by a secondary increase in morality, probably because of shedding of bacteria into the water during the first mortality phase and hence contributing to a superinfection state.

The specific humoral response of teleost fish to extracellular bacteria using rainbow trout Vibrio anguillarum (Listonella anguillarum) model was evaluated by **Palm et al. (1998).** Treatment groups of 200 fish were immunized by oral, immersion and injection routes. All 3 delivery methods conferred full protection in controlled laboratory challenges. Before boosting, serum antibody titres did not correlate with protection in the orally and immersion vaccinated groups. Yet, titres measured 10 and 17 days after

boosting correlated positively with protection in all 3 vaccinated groups. The route of vaccination administration strongly affected the size of the antibody response when measured by ELISA.

Three species of carp; catla, rohu and common carp were inoculated by **Azad et al. (1999)** with a biofilm vaccine against A. hydrophila by mouth at 107, 1010, 1013 cfu/fish for 10, 15 and 20 days. The highest dose level given for 15 days induced the highest serum antibody titres and protection in all 3 species. Responses were higher in the 15 and 20 days groups than in the 10 days groups. Increases in the antibody titres and protective response during 60 days after inoculation.

55 common carp were administered with one of the 3 antigenic preparations of A. hydrophila: formalin- killed cells 1%, heat killed cells (60oC, 4h) and live cells by the oral route (109Cells /ml), immersion (107Cells /ml) or i/p injection (109 cells/ fish) on days 0 and 20 of the experiment. Three groups of 5 fish were administered with PBS and used as controls. Serum samples were collected 45 and 60 days after immunization and humoral immunity was measured using ELISA. Formalin- killed cells induced the highest immune response. Oral administration of antigens induced higher immune response than the other routes of administrations **(Akhlaghi, 2000).**

Common carp fingerlings were vaccinated by **Azad et al. (2000)** orally using biofilm (BF) and free cells (FC) of A. hydrophila, incorporated in diets for 15 or 20 days. The antigen dose was 1010cfu/fish /day. Serum agglutination titres of BF-fed carp were significantly higher than those of the FC- fed group. Peak titres (4.5 + 0.29) were recorded in carp vaccinated with BF for 20 days at 60 days after vaccination. Carp vaccinated with BF for 15 day were protected against both injection and immersion challenge at 60 days after vaccination. However protection was lower in carp challenged by injection.

In assessing the most important bacteria, for Egyptian aquaculture, consideration have been given to the organisms that cause the most commercial damage and the organisms that are the most difficult to treat or are the most persistent **(Ahmed and Shoreit, 2001).** There are a wide variety of pathogenic bacteria that can infect your pond. By far the most common are *Aeromonas, Pseudomonas, Vibrio, Edwardsiella and Yersinia spp.*

The influence of monovalent A. hydrophila and A. sobria vaccines on the induction of protective immunity against heterogenous strains of Aeromonas in carp was evaluated by **Kozinska and Antychowicz (2001).** The level of immunity in carp after single and double vaccination was also compared. Separate groups of carp were immunized with 1S-95 (A. hydrophila) or 4R- 96 (A. sobria) antigens by i/p injection or by immersion. The immunization efficacy was evaluated by using challenge tests with various heterologus strains of Aeromonas. Fish immunized by immersion demonstrated a particularly high level of immunity.

Protection against atypical furunculosis in spotted wolfish vaccinated with monovalent or multivalent vaccines containing different strains of atypical A. salmonicida was comparatively compared by **Vera et al. (2002).** In addition, these vaccines were compared to a vaccine containing an atypical A. salmonicida isolated from Atlantic halibut. Vaccinated fish were challenged with three different A. salmonicida strains. Significant protection was obtained against both homologous challenge. The best protection, regardless of the strain used for challenge was obtained with the multivalent vaccine composed of three different bacterial strains. Also, some of the monovalent vaccines resulted in significant protection.

The preparation and characterization of alginate microspheres encapsulating inactive Aeromonas hydrophila cells for Nile tilapia oral immunization was studied by **Rodrigues et al. (2003).** The microspheres were prepared by different emulsion techniques, varying the ration between the oil in water and the gelification agent, alginate concentration, and the stirring rate. This will protect the vaccine from destruction in the digestive system and possible interaction with the feed components. This eliminated the stress caused by parental administration and quickly vaccinating large numbers of fish with reduced costs.

A vaccine against Vibrio vulnificus was prepared by **Esteve et al. (2004-a)** which protects eels against vibriosis after vaccination by triple prolonged immersion. Protection lasts for at last 6 moths, but later, protection decreases and eels can suffer stress-related vibriosis. They designed an oral vaccine that can be used for reimmunization at any developmental eel stage. With this aim, the efficacy of vaccine mixed with food as an oral booster was tested in an eel farm. The protection and the immune response in serum and mucus and bile were evaluated in reimmunized and control fish for a 60 day period. Reimmunization significantly increased protection (RPS) and antibody titres. This was performed after bath infection challenges with the pathogen.

The efficacy of a bivalent vaccine against eel diseases caused by Vibrio vulnificus was discussed by **Esteve et al. (2004-b)** after its administration by four different routes. Vulni vaccine, a vaccine against vibriosis caused by *Vibrio vulnificus* serovar E, confers acceptable level of protection to eels after its administration by prolonged immersion in three doses. Recently, a new pathogenic serovar, named serovar A, has been isolated from vaccinated eels in a Spanish

freshwater eel farm. The main objective of this work has to design a bivalent vaccine, and to study its effectiveness against the two pathogenic serovars. With this aim, eels weighing around 20 g were immunized with bivalent vaccine by oral and anal intubation, intraperitoneal injection (i.p.) and prolonged immersion. The overall results indicated that; (I) the new vaccine delivered by oral and anal intubation induced protection levels higher than 80%, to that achieved after i.p. vaccination (ii) oral and anal vaccination induced a significant systemic and mucosal immune response (iii) the protection after vaccination by whichever routes was related to antibody titres in plasma. The oral delivery system is a promising way which may be used in intension culture facilities during the whole growth period of eels.

The oral immunization using alginate microparticles as a useful strategy for booster vaccination against fish lactococcosis was introduced by **Jesus et al. (2004).** Fish were orally immunized with a variety of different *Lactococcus garvieae* vaccines including encapsulated and non-encapsulated bacterial cells. An aqueous-based bacterin administered by intraperitoneal injection (i.p.) was employed as positive control. The best protective rates by oral immunization were obtained with the alginate-encapsulated vaccine (RPS of 50 %); this, however, does not warrant the use of this formulation as a primary immunization method. They also evaluated the efficacy of this vaccine as booster immunization strategy. Fish were primary i.p. vaccinated with aqueous- based vaccine and 3 months later were boostered with the oral vaccine. Four weeks after revaccination, protection reached RPS values of 87%, which indicated the value of this encapsulated vaccine to increase the duration of the protection of rainbow trout against lactococcosis.

With oral vaccination, the vaccine is either mixed with the feed, coated on top of the feed (topdressed) or bio-encapsulated. When antigens are to be incorporated in feed, the heat sensitivity of the antigen has to be considered. When vaccines are used as top dressing in feed, a coating agent is usually applied, either to prevent leaching of the antigen from the pellets or to prevent breakdown of the antigen in the acidic environment of the fish stomach. For sensitive antigens, various microencapsulation methods are being evaluated and tested. Bio-encapsulation is used where fish fry are to be vaccinated. In this case, live feed, such as Artemia nauplii, copepods or rotifers, are incubated in a vaccine suspension after which they are fed to the fry. Since these live organisms are non-selective filter feeders, they will accumulate the antigen in their digestive tract and as such, transform themselves into living microcapsules. Oral vaccination has the advantage that it is a very easy vaccine administration method with no stress to the fish. However, oral vaccines have a very short term stability once mixed with the feed. In most cases, only limited protection can be obtained and the duration of protection can be rather short. Moreover, although oral vaccination is the preferred method from a fish farmer's perspective, at present there are few, if any, effective oral vaccines in the market.

Immersion vaccination works on the ability of mucosal surfaces to recognize pathogens they had been in contact with. When fish are immersed in water containing the diluted vaccine, the suspended antigens from the vaccine may be adsorbed by the skin and gills. Then, specialised cells, such as antibody-secreting cells, present in the skin and gill epithelium will be activated and will protect the fish when fish are exposed to the live pathogen at a later stage. Other cells located in the epithelium of skin and gills, such as antigen presenting cells (macrophages), also absorb vaccine antigens and transport them to specialised tissues where the systemic immune response builds up. In immersion vaccination, there are two application methods: dip and bath. In dip vaccination, fish are immersed for a very short duration, usually 30 seconds, in a highly concentrated vaccine solution, usually 1 part vaccine product to 9 parts water. With bath vaccination, fish are exposed for a longer period, usually one to several hours, in a lower concentration of vaccine. Of the two alternatives, dip vaccination is more widely used since it facilitates fast vaccination of large numbers of fish (up to 100kg of fish per litre of vaccine). Immersion vaccination is widely used for vaccination of fry from 1 to 5 g. It is an effective method that results in relatively good protection. The limitations of immersion vaccination are that the duration of immunity is not very long and a booster vaccination is required when the disease prevails over longer periods. Also, the method is impractical for larger size fish due to cost-effectiveness and the stress that could be induced by vaccination **(Komar et al., 2004).**

A biofilm of *Aeromonas hydrophila* for oral vaccination of Clarias batrachus was evaluated by **Nayak et al. (2004).** Fish were fed with fish paste incorporating biofilm (BF) or free cells (FC) of *A. hydrophila* for 20 days and monitored for serum antibody production up to 60 days post- vaccination. Serum agglutinating antibody titre and relative percent survival (RPS) following challenge were found to be significantly higher in Catfish fed with BF vaccine compared to that with FC.

The oral vaccination of African Catfish with *Vibrio anguillarum* O2 on antigen uptake and immune response by absorption enhancers in lag time coated pellets was studied by **Stefaan et al. (2004).** The lag

time coat prevents premature release of the encapsulated vaccine in the tank before ingestion of the pellets by the fish. To monitor the antigen uptake, a competitive ELISA was used. The antibody response was measured using an indirect ELLSA. Feeding of bacterin- layered pellets without absorption enhancers resulted in a rather low antigen uptake and antibody levels. Skin mucus antibody levels were higher after oral vaccination compared to i.p. and control group.

Formalized inactivated bacterin, outer membrane protein and lipopolysaccharide (LPS) vaccines were prepared from this isolate and injected intraperitoneally (IP) in three groups of *Clarias gariepinus* fish. The quality control analysis proved that the prepared vaccines were free from any contaminant. The safety tests illustrated that the prepared vaccines did not show any abnormalities or adverse reactions among the injected fish during the observation days. Antibody titers to *Edwardsiella tarda* vaccines were estimated using microagglutination and ELISA methods. The agglutinating and ELISA antibody titers of fish vaccinated with outer membrane protein were 2560 and 2570 at 4 weeks post vaccination, respectively, followed by LPS (1280 and 2132) and formalin-inactivated vaccine (1040 and 1382), respectively **(El-Jakee et al., 2008).**

Three types of formalized whole culture *Aeromonas hydrophila* vaccine (FWC) were prepared, FWC vaccine alone, FWC vaccine mixed with Freund's complete adjuvant (FCA), and FWC vaccine mixed with Freund's incomplete adjuvant (FIA), tested for sterility and administered to female Nile tilapia (*Oreochromis niloticus*) using two methods of delivery. Micro-agglutination and the double immuno-diffusion tests were performed on serum, mucus and eggs to evaluate maternal immunity. The relative level of protection (RLP) was calculated after challenge infection **(Mai et al., 2008).**

Monovalent, killed and live attenuated vaccines of *Aeromonas hydrophila* and *Pseudomonas putida* were used in the immunization of red tilapia against Motile Aeromonad and Pseudomonad septicemias. There were 4 treatments and a 5th control group with 3 replicates per each. A 4th replicate was kept for replacement of natural mortality among the experimented fish. The 4 treatments included, Heat-killed vaccine of *A. hydrophila*, Live-attenuated vaccine of *A. hydrophila* (using herbs), Heat-killed vaccine of *P. putida* and Live-attenuated vaccine of *P. putida*. A total of 160 brood stocks of *O. niloticus* with 250 g average body weight were used for all treatments (8 fish per each glass aquarium). Vaccination was conducted via the Intra Peritoneal route (I/P) as an initial dose followed by 2 booster doses every 2 weeks. The last dose was applied via the immersion route. The evaluation of vaccination was carried out through periodical antibody titration of the serum of the examined fish (every 2 weeks) using direct agglutination method as well as by the experimental challenge 3 months after the initial immunization. Results revealed that there were a significant difference between the vaccinated and non vaccinated fish of the control group regarding antibody titers and Relative Percent Survival (RPS) of the challenge test. Differences in immunity levels within the vaccinated groups themselves were demonstrated. **(Abdel-Hady et al., 2009).**

Different vaccine preparations and formulations for vaccination of Tilapia species were tried by adding formalin to the bacterial culture (bacterin) and used by immersion and oral routes. Fish were vaccinated by using monovalent, bivalent and polyvalent vaccines and the efficacy of these vaccines were tested by using

the challenge test with the detection of RPS (Relative Percent Survival) and by using indirect ELISA for estimation of the immune response of fish during and after vaccination. The results of fish vaccination showed that the polyvalent vaccine when used in Tilapia fish through the immersion route was of easier administration and of higher efficacy (RPS) and it was effective against more than one type of bacteria **(Kamelia et al., 2009).**

Silva et al. (2009) studied The efficacy of a polyvalent bacterin vaccine against *Aeromonas hydrophila*, *Pseudomonas aeroginosa* and *Enterococcus durans* administered by different routes in Nile tilapia was assessed by analyzing hematological and immunological parameters 7 and 21 days after vaccination. Treatments consisted of: non-vaccinated tilapia; tilapia vaccinated by intraperitoneal injection with 2x108 formalin-inactivated bacteria·mL-1; tilapia vaccinated orally with 2x107 formalin-inactivated bacteria·g-1, feed for 5 days; tilapia vaccinated by immersion bath in 2x107 formalin-inactivated bacteria·mL-1, for 20 minutes. Vaccinated fish groups presented higher hematocrit, number of erythrocytes and leukocytes than the non-vaccinated group. Serum agglutination titer of intraperitoneally vaccinated fish was higher on both evaluation periods for the three bacteria strains. Only on day 21 post-vaccination fish from the oral and immersion vaccination groups presented higher serum agglutination titer than the non-vaccinated fish for *A. hidrophila* and *E. durans*. Serum antimicrobial activity in vaccinated fish was higher for *P. aeroginosa* and *E. coli* than in non-vaccinated fish on both evaluation periods. The different vaccine administration routes stimulated hematological and immunological responses in Nile tilapia 21 days post-vaccination, but intraperitoneal vaccination presented higher total

number of leukocytes, lymphocytes and serum agglutination titer.

Most bacterial vaccines used in aquaculture to date have been inactivated vaccines obtained from a broth culture of a specific strain(s) subjected to subsequent formalin inactivation. The best results are obtained with those bacterins that include both bacterial cells and extracellular products. Whereas with some vaccines acceptable levels of protection are achieved with aqueous formulations administered by injection or immersion, for other bacterins, such as those devised for salmonids against *Aeromonas salmonicida* subsp. salmonicida, an acceptable level of protection can only be achieved by immunization with oil-adjuvanted bacterins delivered by injection. The ideal vaccine formulation is a polyvalent vaccine which protects simultaneously against the majority of the diseases to which a particular fish species is susceptible. In addition, these polyvalent vaccines must cover all the main serotypes of each pathogen existing in a particular geographical area. Examples of the efficacy of polyvalent vaccines are those used in salmonids and turbot in which polyvalent vaccines give similar or superior protection than the respective monovalent vaccines. However, care must be taken in the formulation of polyvalent vaccines because the problem of antigen competition can occur, especially when these vaccines are administered by injection **(Toranzo et al., 2009).**

Lucienne et al (2010) evaluate an inactivated *S. agalactiae* vaccine in tilapia for the control of streptococcal disease outbreaks. Tilapia, weighing approximately 20 g each, were intraperitoneally (i.p.) inoculated with 0.1 mL of the vaccine at a dose of 2.0 × 108 colony-forming unit (CFU) mL−1. One group of tilapia (treatment 1) received one vaccine dose, and the other group of tilapia (treatment 2) received two doses, with an interval of 21 days. The control group was i.p. inoculated with 0.1 mL tryptic soy broth fish−1. Immunized and control tilapia were i.p. challenged with 0.1 mL of 3.0 × 107 CFU mL−1 at 30 days post vaccination. The fish were monitored daily for disease signs and for mortality for 16 days post challenge. A statistically significant difference (P=0.0045) was found between the mortality of treatments 1 and 2. The value of relative per cent of survival of 83.6% and 96.4%, respectively, indicate that this vaccine was efficient in Nile tilapia.

Humoral response in red tilapia against formalin-killed *Aeromonas hydrophila* and Streptococcus sp. vaccine administered by intraperitoneal injection was evaluated by **Prasad and Areechon (2010).** The result indicated that *A. hydrophila* vaccine induced significantly differed (P<0.05) high mean peak antibody titers of 925.87±467.92 and 4983.47±1832.74 in both primary and secondary immune response, respectively. However specific antibody produced by red tilapia in response to administration of Streptococcus sp. vaccine revealed only weak secondary response of 101.33±45.38. In separate experiment, relative protection in red tilapia immunized with *A. hydrophila* and Streptococcus sp. vaccine was conducted. Immunization were done by direct immersion for 1 hr in vaccine suspension and then challenged 2 weeks after by immersing fingerlings for 6 hr with virulent *A. hydrophila* and Streptococcus sp. Percent cumulative mortality in vaccinated and unvaccinated groups was compared after 14 days of post challenge. Red tilapia immunized by *A. hydrophila* vaccine demonstrated a particularly high level of immunity (76.67%) compared with unvaccinated (43.33%). Streptococcus sp. vaccine greatly reduced the mortality in vaccinated (31.67%) compared with unvaccinated fish (55%) but these differences in mortality were insignificant (P>0.05).

To prevent streptococcosis caused by *S. iniae*, a formalin killed vaccine was applied in red tilapia *Oreochromis niloticus* x *O. mossambicus* by injection, immersion and oral vaccination. At 1 week post vaccination, levels of antibody titer and some blood parameters response to different routes of administration were significantly different. The best disease resistance was found in the group injected with vaccine plus B-(1,3/1,6)– glucan with the relative percent survival (RPS) of 95.12% followed by pure vaccine injection (RPS = 80.49%), immersion (RPS = 41.46%) and oral vaccination (RPS = 9.75%). No difference in blood parameters of tilapia after vaccination for 4 weeks was observed. However, antibody titer of the group received vaccine plus B-(1,3/1,6)–glucan and vaccine alone were significantly higher than the other groups. RPS of fish at week 4 post vaccination showed the same trend as the highest disease resistance recorded in the group injected with vaccine plus B-(1,3/1,6)–glucan (RPS=76.00%) which significantly differ from vaccine alone (RPS=54.00%). Immersion and oral vaccination showed less effect on disease protection at week 4 post vaccination. The result from the present study indicated that formalin-killed *S. iniae* vaccine provided excellent efficacy against *S. iniae* infection in tilapia by intrapertoneal injection and B-(1,3/1,6)–glucan increased the effectiveness of vaccine produced from *S. iniae* **(Naraid and Akkarawit, 2011).**

Atia et al. (2012) studied four different prepared *Ps. fluorescens* antigens to develop the best adequate strategy to control such infection in cultured Nile tilapia. One thousand and fifty Nile tilapia (*Oreochromis niloticus*) were divided to 5 equal groups and used for vaccination trial. Fish in groups 1-5 were injected intraperitonial with 0.2 ml from each

of sterilized saline, Formalin killed bacterin, Extracellular product (ECP) suspension, Sonicated cells (SC) suspension and mixture of ECP & SC suspension; respectively. At 1, 2, 4, 6, and 8 weeks post vaccination, ten fish from each group were randomly used for the collection of whole blood and tested for nitro blue tetrazolium (NBT), neutrophil adherence tests, lysozyme activity and the serum bactericidal test. The NBT, Neutrophil adherence and lysozyme activity of vaccinated fish showed significant increases in all immunized groups in comparison with control at 1, 2 and 4 weeks post vaccination. Serum bactericidal activity and Antibody titer were significantly increased in all immunized groups at all periods of experiment. Mixture of Sonicated and extracellular product vaccine showed the best serum bactericidal activity and antibody titer against *Ps. fluorescens*. The relative percent of survival (RPS) after challenge with *Ps. fluorescens* at 4, 6 and 8 weeks post vaccination was significantly increased in all immunized groups in comparison with control. There are significant increases in RPS among group immunized with a mixture of sonicated and extracellular product antigen than other three immunized groups at 4 weeks only. The higher values of the relative percent of survival was seen in the mixture of sonicated and extracellular product antigen followed by formalin killed antigen, sonicated cell antigen then extracellular product antigen. It could be concluded that all prepared vaccines are efficient against *Ps. fluorescens* infection, however a mixture of sonicated and extracellular product antigen seemed superior to other vaccines especially in bactericidal activity, antibody titer and RPS against *Ps. fluorescens*.

Craig et al. (2012) tested the ability of a killed bivalent *S. iniae* and *V. vulnificus* vaccine delivered by IP injection at protecting sex reversed hybrid tilapia (*Oreochromis* niloticus × *Oreochromis* aureus) against challenge with each bacterium, independently. In two independent trials, vaccination of tilapia with the bivalent vaccine conferred protective immunity against *V. vulnificus* and *S. iniae* as demonstrated by significant differences (Pb0.05) in survival curves between the sham-vaccinated and vaccinated groups. Relative percent survival values ranged from 79 to 89% for *V. vulnificus* and 69 to 100% for *S. iniae* following challenge of bivalent vaccinated fish. Use of this bivalent formulation may be a cost-effective strategy to reduce losses in tilapia coinfected with these two important bacterial pathogens.

Formalin-killed, heat-killed and lipopolysaccharide vaccines against *Aeromonas hydrophila* and a bivalent formalin-killed vaccine against *A. hydrophila* and *A. veronei* bv. sobria were tested in rainbow trout (*Oncorhynchus mykiss*). The evaluation of trout fish immune response after vaccination with Aeromonads bacterins by immersion and bath challenge route was undertaken using an indirect enzyme-linked immunosorbent assay (ELISA).To test the strength of protection, the challenge process was examined using 10 cells of the live bacteria/ml of *A. hydrophila*. The results showed that the relative 5 percentage of survival in the trout fish groups vaccinated by heat-killed type of vaccine were significantly higher (P<0.05) than that the other types of vaccines (84%). In addition, the Fish vaccinated with the bivalent vaccine of *A. hydrophila* and *A. veronii* and formalin-killed vaccine showed a high percentage of RPS (67%), while it was measured as 34% for the LPS vaccine. Thus, the bivalent and formalin-killed types of vaccines have higher RPS values compared to the LPS group **(Sajjad et al., 2012).**

Conclusion:

- Prophylactic using of vaccines for increasing the immune defense of fish is of great value for prevention of bacterial diseases in fish farms, while antibiotics are used during and after the disease.
- Avoidance the use of antibiotics due to their expense, the short period of protection they offered, the need for repeated treatments in extended outbreaks of disease, the difficulties caused by resistant bacterial strains and increased control on residues in carcasses which reveals the threats to consumers.
- There are three common methods for vaccinating fish: immersion, injection and oral routes. These methods vary in terms of ease of administration, cost, stress on the fish, survival rates, dosage control, the amount of labor involved and the duration of protection.
- Immersion vaccination is an easy and effective immunization method. Fish are immersed in a dilute vaccine for a short period of time, thirty seconds to two minutes, and released into the culture unit. This method is limited to operations where fish will not be moved after stocking as this procedure can only be used during stocking time. Immersion vaccination is more costly for large sized fish**.**
- Oral vaccination is the most convenient way to immunize fish because the vaccine can be administered on the fish, anytime during the culture cycle and in all types of culture systems. The vaccine is either incorporated or adhered to the feed and then fed to the fish. It is the least stressful method because handling is not required. Oral vaccination is not cost effective when immunizing larger fish. Oral vaccination provides

efficacy compared to the other two methods. The main problem appears to be the destruction and absorption of antigens by the fish digestive system.

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