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Feed Based Vaccine in Aquaculture

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Abstract

Aquaculture is growing more rapidly than all other food animal-producing sectors; its contribution to global supplies of fish, crustaceans, molluscs and aquatic plants. Now days the capture fishery has declined and wild stocks diminished, the aquaculture industry has become an important source of seafood. Over the past 10 years, the contribution of capture fishery to global fish production has declined from 70.2 % in 2001 to 59.9 % in 2011. Simultaneously, aquaculture has steadily increased its contribution from 27.6 to 40.1 % at an average annual growth rate of approximately 6 % (www.fao.org/fishery/topic/16140/en). This unrivalled growth of global aquaculture has not been free of challenges and aquaculture will continue to face challenges as its expansion continues. In fact, a multitude of challenges, such as disease outbreaks and the rising cost of feed, are now threatening the sustainable growth of aquaculture. Among finfish, carps, barbels and other members of the cyprinids constitute 63 % of world aquaculture production with an estimated value of US $34 billion. These fish species have a relatively low value compared with other types of farmed fish such as salmon, trout and seabass, and are typically raised in simple pond systems where they are a food source for families in developing countries.

Keywords: Vaccine; Fish; Aquaculture

Introduction

Aquaculture is growing more rapidly than all other food animal-producing sectors; its contribution to global supplies of fish, crustaceans, molluscs and aquatic plants. Now days the capture fishery has declined and wild stocks diminished, the aquaculture industry has become an important source of seafood. Over the past 10 years, the contribution of capture fishery to global fish production has declined from 70.2 % in 2001 to 59.9 % in 2011. Simultaneously, aquaculture has steadily increased its contribution from 27.6 to 40.1 % at an average annual growth rate of approximately 6 % (www.fao.org/fishery/topic/16140/en). This unrivalled growth of global aquaculture has not been free of challenges and aquaculture will continue to face challenges as its expansion continues. In fact, a multitude of challenges, such as disease outbreaks and the rising cost of feed, are now threatening the sustainable growth of aquaculture. Among finfish, carps, barbels and other members of the cyprinids constitute 63 % of world aquaculture production with an estimated value of US $34 billion. These fish species have a relatively low value compared with other types of farmed fish such as salmon, trout and seabass, and are typically raised in simple pond systems where they are a food source for families in developing countries. The farming of high prised fish will become more industrialized with the increasing investment from multinational companies. Currently, high value species such as salmon and trout, account for 7% of total volume and 16% of total value of cultivated fish worldwide. Atlantic salmon is one of the most intensively farmed fish in the world, includes Norway, Chile, the UK and Canada being the major producers. Farming of high-value marine species such as European seabass and seabream, flounder, halibut, cod, eel, tuna and amberjack/yellowtail will probably increase as appropriate intensive aquaculture systems are developed. Although, crustaceans (e.g., shrimp and lobster), molluscs (e.g., clams and oysters) and aquatic plants are important in global aquaculture.

In all forms of intensive culture system, where single or multiple species are reared at high densities, infectious disease causing agents are easily transmitted between individuals. Fish such as carp commonly farmed in muddy ponds, appear to be more robust than, for instance, Atlantic salmon that are adapted to spend their early life in clean, running fresh water. Independent of high- or low technology farming, good environmental conditions are important to maintain a healthy fish population. In commercial large scale fish farming, the fish are stocked in high density, pathogens quickly spread within a population due to the effectiveness of pathogen transportation in water. The major causative agents of infectious diseases in finfish aquaculture include bacteria (54.9 %), viruses (22.6 %), parasites (19.4 %) and fungi (3.1 %) [1]. Among the diseases, bacterial diseases are the most prevalent disease challenge in fish farming, viral diseases are more difficult to control due to the lack of anti-viral therapeutics, high susceptibility of fish and shrimp during the early stages of life cycle.

There are various methods to control the diseases in aquaculture system such as use of chemicals, antibiotics and regularly exchange of water. The use of antibiotics and chemicals have invited various problems like; development of antibiotic resistance bacteria, bioaccumulation, changes the physiochemical parameters of water, changes the normal physiology of aquatic animals and loss of millions liters of water/day by regularly exchange of water. In case, when fish are no longer eating, treatment options become much more limited and treatment may no longer be effective. The vaccination is an alternative approach have been used successfully in some aquaculture industries to disease prevention, and should be considered fish health management options.

To date vaccines are available for most aquaculture fish species; most are targeting bacterial pathogens and only a few are raised against viruses. Depending on the age and size of the fish, commercial vaccines are administered either orally (by mixing with the feed), by immersion (dip or bath) or by injection through the intraperitoneal (i.p.) or intramuscular (i.m.) route. Intraperitoneal injection is conventionally used to deliver water-in-oil (w/o)-based injectable vaccines whereas intramuscular injection is most often used to deliver DNA plasmids [2].

It is well-known that lymphoid structures associated with the gut of fish are different from those found in mammals. Fish do not have lymph nodes but instead that they have less organized, diffuse gut-associated lymphoid tissue (GALT), which is functionally different from that of mammals [3]. Nevertheless, the fish GALT is capable of local immune responses. Indeed, oral administration of antigens result in the up-regulation of genes related to recruitment of immune cells and local antibody production. As primary vaccines, orally delivered vaccines especially, inactivated whole antigens have traditionally not featured well, often resulting in suboptimal protection against several pathogens. When used for boosting, the oral vaccines have been shown to be capable of enhancing or extending protection, although the antibody response is transient, typically lasting about 3 months. Interestingly, varying the dosage schedule, for example, by administering the vaccine 3 days/week for 2 months instead of 5 days/month gave more effective results. There are several other factors that influence efficacy of oral vaccines include the nature of antigens, formulation, and dosage. The concept of oral vaccination would be that, immune responses induced in the gut would induce local and systemic immune responses, while parenteral delivery will not yield protective immune responses on mucosal surfaces. The oral administration of antigens results in stimulation of both systemic and mucosal responses.

During the 1980s, in the Norway, huge amount of losses occurred in the salmon farming due to bacterial diseases (mostly *Vibrio* spp.) and a total crash in the industry was only stopped by the use of vast amounts of antibiotics. Fish immersion vaccines based on formalin-inactivated broth cultures had been developed which was effective against vibriosis in the USA in the 1970s and similar vaccines were quickly developed against the salmonid *Vibrio* diseases [4]. Due to good efficacy of these vaccines immediately resulted in a decline in the use of antibiotics in aquaculture. However, a new bacterial disease, furunculosis (*Aeromonas salmonicida*) appeared and, as immersion vaccines proved ineffective against this pathogen, than injectable vaccines was used that containing adjuvants were developed in the early 1990s.

The different vaccine adjuvant and a range of different antigen combinations were tested in few years and, it soon became evident that all antigens in one oil-adjuvanted vaccine were the product of choice. The excellent efficacy of these vaccines soon resulted in their extensive use in intensive farming and an immediate and permanent reduction in the use of antibiotics and chemicals, concurrent with a threefold increase in fish production. Proper fish management with good hygiene and limited stress are key factors in the prophylaxis of infectious diseases and are also required for the optimal effect of vaccines [5-7]. Now days, the vaccination is an integral part of most salmon farms and the use of antibiotics is very limited, at least in Northern Europe and North America. During the 1990s and to date, five multinational animal health companies have acquired, or formed, joint venture companies with the smaller companies specializing in the field of aquaculture vaccines. The major producers of fish vaccines are now:

- ✓ Intervet International (The Netherlands)
- ✓ Novartis Animal Health (Switzerland)
- ✓ Schering-Plough Animal Health (USA)
- ✓ Pharmaq (Norway; was part of Alpharma Animal Health until 2004) and
- ✓ Bayer Animal Health (Bayotek)/Microtek, Inc. (Germany/Canada)

The major commercial markets for these companies are currently, the salmon and trout industries in Northern Europe, Chile, Canada and the USA. Commercial vaccines are also available for the catfish industry in the USA and, on a smaller scale, for European seabream, seabass and tilapia. Some limited-use, locally developed vaccines are also available in countries such as China, Russia, Spain and Germany.

Vaccine

Vaccine is a biological preparation that is used for recovers immunity to a particular disease. A vaccine typically contains an agents that is resembles a disease causing microorganism, or it prepare from weakened or killed forms the microbe. The agents that stimulates of body immune system to recognize the agent as foreign, destroy it, and remember it, so that the immune system can more easily recognize and destroy any of these microorganisms that it later affect in future.

Type of Vaccines

Live, attenuated vaccines: Contain a version of the living microbe that has been weakened in the lab so it can't cause any disease. It is the closest thing to a natural infection, due to this region these vaccines are good "teachers" of the immune system. Example: Vaccines against measles, mumps, and chickenpox.

Inactivated vaccines: Produced by killing the disease-causing microbe with the use of chemicals, heat and radiation. It is more stable and safer than live vaccines because dead microbes can't mutate back to their disease-causing state. Example: Vaccines against polio, influenza, rabies and hepatitis A.

Subunit vaccines: Instead of the entire microbe, subunit vaccines include only the antigens that best stimulate the immune system. Sometimes, these vaccines use epitopes the very specific parts of the antigen that recognize the antibodies or T and bind to them. Example: Plague immunization.

Toxoid vaccines: The bacteria are secreted various toxins or harmful chemicals used as a toxoid vaccine. It is used, when a bacterial toxin is the main cause of illness. The used toxins are inactivate by treating them with formalin. Example: Crotalus atrox toxoid is used against rattlesnake bites to vaccinate dogs.

Conjugate vaccines: The certain bacteria have polysaccharide outer coats that are poorly immunogenic. By attaching these outer coats to proteins (e.g., toxins), the immune system can be led to recognize the polysaccharide. Example: Haemophilus influenzae type B vaccine

DNA vaccines: The genes of microbes antigens are introduced into the, some cells will take up that DNA, instructs those cells to make antigen molecules. Example: Influenza vaccine.

Recombinant vector vaccines: Recombinant vector vaccines are produced by isolate gene from bacteria or virus, inserted into plasmid DNA and ligated. This engineered plasmid transformed into another bacterium and allow the bacterium culture to grow and produce the antigenic protein. These purified protein are recovered from bacteria or virus. Example: DPT. Among the vaccines, the most commonly used antigen in fish vaccines to date are inactivated or killed bacterial and viral.

Properties of the ideal vaccine

- It should be safe for the fish, the person vaccinating the fish and the consumers
- It should be protects against a broad strain or pathogen type and gives 100% protection
- It provides long-lasting protection, at least as long as the production cycle
- It is easily applied
- Will not interfere with diagnosis
- It should be cost effective
- It should be readily available, licensed and registered.

Important considerations for fish vaccination

- Species (Salmon, Cod, Sea bass)
- Status of the immune system

- Production cycle and life history
- Which diseases do you want to control?
- When do these diseases occur?
- Farming technology (Handling, mechanisation)
- Environment (temperature, salinity)
- Stress factors, nutrition and cost benefit

Vaccine Delivery Methods

Vaccines are administered to aquatic species has three major routes of delivery; (1) by injection [intraperitoneal (IP) and intramuscular, (2) by immersion [Dip and bath vaccination] and oral vaccination [mixed with feed, coated on top of the feed and bio-encapsulated. Each has its advantages and disadvantages. Among these methods the oral route is most attractive approach of immunization of fish.

Delivery by injection method: In this method the small volume of antigen directly deliver into animal muscle (intramuscular (IM) injection) or into the body cavity (intracoelomic [ICe= intraperitoneal or IP] injection), which allowing the animal to direct stimulation of a systemic immune response. Injection vaccines normally include oil-based or water-based compounds which work as an adjuvant that serves to further stimulate the immune system. Injection is effective for many pathogens that cause systemic disease and protect the animal more than 6 months. This method only used for larger size fishes.

Advantages

- ✓ Every fish in the population is injected, giving more assurance to the producer.
- ✓ The multiple antigens (for different diseases) can be delivered at the same time.
- ✓ Fish stress minimized by anesthetized.

Disadvantages

- ✓ Injection requires more time, labor and skilled personnel.
- ✓ The correct needle size is important.
- ✓ The vaccine may incite a more severe reaction if it is injected into the wrong portion of the fish.
- ✓ Chances of significant handling stress and risk of post vaccination fungal infection
- ✓ The smaller-sized fish (under 10 g) may not respond well to this method.

Delivery by immersion method: In this method, the fish in a solution containing the vaccine is proved to be a gentle and safe way of vaccine delivery. The vaccine is delivered both to the skin and all mucosal surfaces accessible to the surrounding liquid, which contains the antigen (vaccine), which permits immune cells located in the fish skin and gills to become directly exposed to antigens. These immune cells may then produce huge amount of antibody, which protecting the fish from future infection. The delivery of vaccine by immersion method occurs by dip or by bath. Dips are short period, typically 30 seconds, in a high concentration of vaccine Baths are of longer duration, an hour or more and in a much lower concentration of vaccine. In practice, the dips are logistically more practical for large numbers of small size (1-5-g) fish.

Advantages

- ✓ Suitable for mass vaccination of all sizes of fish
- ✓ Less stress
- ✓ Lower labour costs
- ✓ Less risk to vaccination team

Disadvantages

- ✓ Large amount of vaccine required and small duration of immunity
- ✓ Protection may not last long and a second vaccination may required

Delivery by Oral method: In oral vaccination, the vaccine is either mixed with feed, coated on top of feed (top dressed) or bio encapsulated. It is the easiest method because feeding is a normal, ongoing part of the fish production schedule. The disadvantages associated with oral vaccines such as inclusion of high amount of antigen in feed, leaching and the need to protect antigens as they pass through the stomach as well as the formulation of vaccines to improve the stimulation of protective immunity. The following disadvantages of oral vaccination in fish are solved as by;

➢ **Antigen production**

A prerequisite to the production of any vaccine is the ability to scale-up antigens easily and at a low production cost. The bacteria and bacteria-based products, such as subunit antigens, are quite easily propagated by fermentation, scaling up for virus antigens can be challenging.

➢ **Encapsulation techniques**

Encapsulation refers to incorporation of materials such as food ingredients, cells, or others, into small capsules and is accomplished by several different techniques. Encapsulated materials like, vaccine antigens can then be mixed with food for oral administration. Bio-encapsulation is used for small size fish or shrimp (e.g., 1-5 g or less), may be a preferred method of oral delivery. There are three methods used to mix the vaccine in artificial feed such as (1) finished feed is top-dressed with vaccine powder by using adhesive agents such as edible oil or gelatin, (2) finished feed is sprayed with the vaccine if the latter is in liquid form and (3) mixing the antigen with the feed in the production process [8]. The first two methods (top dressing) are quite simple to apply but they have disadvantages like, uneven distribution in the feed and also the threat that the antigens are directly exposed to hostile stomach environment upon feeding, leading to degradation of antigen. In third method, the mixing the antigen with the feed gives the advantage of uniform distribution of the antigens in the feed. Now days most of fish feed produced through an extrusion process at high temperature and pressure antigens would have to be added to the pellet at later stages, either in a vacuum infusion coating process. The incorporated antigens are protecting against the hostile stomach environment, several encapsulations techniques have been developed and tried as discussed below.

- **Use of live feed**

The live food such as *Artemia nuaplii*, *copepods* or *rotifer* are incubated in a vaccine suspension. This live food organisms are non-selective filter feeders, they will accumulate the vaccine/antigen in their digestive tract and, as such, transform themselves into living microcapsules, is then fed to fry or small fingerlings.

- **Biofilms**

Biofilms are defined as communities of microbes adherent on a surface and usually held together by a polymetric extracellular matrix. Recently biofilm of bacterial pathogen has been evolved successfully for oral vaccination of fish and shrimp with high antibody tire and protection. The glycocalyx of biofilm helps to resist the vaccine destruction in the foregut/stomach favouring better antigen delivery to immune responsive sites in the mid gut.

- **Nanoparticles**

In recent years, different forms of nanoparticles are used in oral vaccination of fish. Rajesh and co-worker [9] examined the use of chitosan nanoparticles for orally delivering a DNA vaccine against *Vibrio anguillarum* in sea bass. The results showed that fish took up the antigens, fish were, however, not protected and a relative relative percent survival (RPS) rate of 46% was recorded. A better protection against *V. parahaemolyticus* was recorded in black seabream (*Acanthropagus schelegelii* Bleeker) also vaccinated with a DNA vaccine loaded in nanoparticles, resulting in 72.3% RPS after 3 weeks of post vaccinatio. In case of viral diseases, the encapsulating DNA vaccines against infectious hematopoietic necrosis virus with Poly (D,L-lactic-co- glycolic acid) (PLGA) nanoparticles added to feed pellet in the diet of rainbow trout. The vaccine reached in the lower intestine within 96 h of feeding and also induced low levels of gene expression and specific antibodies but this was not sufficient to protect the fish against lethal challenge [10]. When a DNA vaccine loaded in PLGA against lymphocystis, used in feed of Japanese flounder. The results showed that the several innate immune parameters were induced suggesting that the system could be used as a carrier for plasmid DNA vaccines [11]. Recently, Rivas-Aravena and co-workers [12] reported enhanced protection of Atlantic salmon fed with chitosan nanoparticles-based oral vaccines loaded with a DNA coding an alphavirus replicase (as an adjuvant) while the target antigen was ISAV. The authors reported 77% protection.

- **Alginate Particles**

Alginates are another carrier that promises to revolutionize oral vaccine development Alginate is a natural polysaccharide found in brown algae and have been employed as a matrix for the entrapment of drugs, macromolecules and biological cells. In the aquaculture industry, they have been tested quite extensively. Alginates seem to work well with DNA plasmids, giving RPS values of 67% or more in all species tested. Whether this technology can be transferred to other viral diseases of fish would be interesting.

Advantages

- ✓ Easy to administer and causes no stress to the fish.
- ✓ Lower labour costs

Disadvantages

- ✓ Large quantities of antigen are required
- ✓ Require all fish to be feeding
- ✓ Protection generally weak and of short duration

Potential benefits of feed based vaccines

- ➢ Increased appetite and growth in vaccinated fish as compared to non-vaccinated fish is more because of the better feed conversion rates in vaccinated fish.
- ➢ Immunization of brood male and female fish may have potential as a means of protecting fish against pathogens which affect the early life stages, such as *Flavabacterium psychrophilum* and *Edwardisiella ictaluri.*
- ➢ Reduction of antibiotics and chemical use which produce the drug resistant bacteria.
- ➢ Improvement of industry image for the sanitary quality of the fish produced, as well as from the environment safety stand point of view.

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