Application of immunostimulants in larviculture: Feasibility and challenges

ZhouJin

Shanghai Fisheries University, E-mail: Zhoujin9346@sina.com)

Immunostimulants are valuable for the control of fish diseases and may be useful in fish culture. Microbial pathogens are one of the main problems in the rearing of larvae. It is therefore important to develop methods for establishing microbial control at all stages of the cultivation progress. One possibility is immunostimulation, which includes methods of enhancing the capacities of the specific and non-specific immune systems. There are many experiments on nonspecific immunostimulation of fish that suggest that the method has considerable potential for reducing losses in aquaculture, both during larval and on-growing stages. So, on one hand, we can say that use of immunostimulation in larviculture is possible. On the other hand, due to the small size and fragility of larvae the development of methods to administer the stimulant, and the adaptation of methods for detecting the response of the immune system is full of challenges. The aim of the paper is to evaluate the status and the potential for immunostimulation as an element in the strategy for solving microbial problems in larviculture. The focus will be on fish, but the ideas are also applicable to other groups of organisms relevant to aquaculture.

The characteristics of the larval immune system

The main function of the immune system is to protect the animal against disease causing microbes. The immune system comprises both nonspecific and specific components, and involves both cellular and humoral factors. It is well known that larvae do not have the ability to develop specific immunity during the early stages of development. In this respect, fish are reliant on passive immunostimulation from maternal antibodies, and this mechanism has been shown in tilapias (Mor et al, 1990; Sin et al 1994). In any given species, size rather than age seems to be the most critical determinant of the development of immunity. For example, in salmonids, memory-dependent immunity has been achieved for sizes > 0.26g (8 weeks of age), whereas in carp it was acquired at

9-10 weeks (Ellis 1988).

The nonspecific immune system is probably the main defence against microorganisms in larvae. Although we understanding of the components of the innate defence system of fish is growing, relatively little is known about the functioning and the ontogeny of the general immune system in larvae (Olafsen et al 1993). In the few fish species that have been studied, the major lymphoid organs are not fully developed at the time of hatching, and the phagocytic activity is mainly associated with gills, skin and gut (Eills,1988). It is therefore possible that during the stages when the lymphoid organs are developing, the main cellular defence is by the phagocyte populations within the integument (Ellis 1988).

The nonspecific or innate immune system is regarded as the first line of defence in animals. Furthermore, it seem that the microbial problems in larviculture are most likely due to opportunistic bacteria rather than specific pathogens (Munro et al,1995).This emphasizes the importance of nonspecific defence system for larvae under intensive hatchery conditions, and the need for more knowledge of the ontogeny and functionality of the nonspecific immune system of larvae.

Immunostimulation of larvae: Possibilities and constraints

Immunotherapy comprises all methods that utilize immunological principles to prevent or treat disease. In human and veterinary medicine, immunotherapy has already been applied but it is still regarded as an area for growth. Methods of transplanting immunologically active cells or transferring immunoglobulins are not relevant to larviculture, and therefore not treated further in this study. Immunomodulation, and in particular immunostimulation, seems to be the most suitable immunotherapeutic method for larviculture in the foreseeable future.

Immunomodulation may be direct at both specific and nonspecific immunity. Vaccination is probably the best-known method of specific immunostimulation, and it entails increased resistance against a specific antigen (pathogen).

Fingerlings of round herring

Nonspecific immunostimulation refers to a condition in which the immune response is changed to a condition with higher response towards a variety of antigens. An example of nonspecific immunostimulation is macrophage activation. An immunostimulant may be defined as an agent that stimulates the nonspecific immune mechanisms when given alone or the specific mechanisms when given with an antigen. It is believed that in eggs and larvae the effects of immunostimulation may be to enhance the "inheritance" of an immune defence, but they last for only a short period. A special case of immunostimulation may be to enhance the immune defence of larvae through immunostimulation of the mother (maternal immunity).

Many different types or groups of substance are known to act as immunostimulants, such as lipopolysaccharride (LPS),b-1,3-glucan, peptidoglycan and so on. It is important to consider the specificity of immunostimulants for two reasons. First, a stimulation of an immune system may be too intense, and may harm or even kill the host. This is well-known in humans, where the activation caused by LPS in connection with infections may cause septic shock and death (Morrison et al,1994). Secondly knowledge of the functions of different immunostimulants may be used to stimulate those parts of the immune system that may be more relevant in certain situations.

Three factors are essential to consider in the design of an immunostimulation strategy. First, it is important to remember that in most cases we do not have a specific microbial problem, but rather a general one involving large numbers of bacteria and a high proportion of opportunistic species. Secondly, the immune system of larvae is poorly developed, consisting mainly of nonspecific defences. Thirdly, the immune defences of maternal origin will be significant only during the earliest developmental stages. Although this period and hence the maternal immune defence may be critical, aquaculturists cannot rely on this part of the immune defence of the larvae alone. These three factors mean that research aimed to develop methods for immunostimulation of larvae should place the highest priority on stimulation of the nonspecific defence system. This work should principally involve stimulation of the nonspecific defence of the larvae itself, and should also include stimulation of nonspecific maternal defences if possible. In cases where specific pathogens are known to cause problems, stimulation of the specific defence may be considered through immunization of the broodstock.

Immunostimulation of larval fish: Nonspecific defences

Newly hatched fish larvae have not acquired specific imune defence, but maternally transferred specific immunity may also be of significance (Mor and Avtalion, 1990). Contrary to earlier conceptions, there are recent observations indicating that larvae start to develop immunocompetence relatively early. Bergh et al (1995) observed presence of lymphoid cells early in the first feeding period of Atlantic halibut. Padros and Crespo (1996) have described development of lymphocytes in lympoid organs of turbot at the time around

Applying oral peptidoglycan, a very important immunostimulant metamorphosis. These findings clearly indicate the importance of nonspecific immune defence in the larvae stages.

A number of studies have been performed on stimulation of the nonspecific immune defence of both fresh and seawater fish, describing a wide range of attractive methods for prophylaxis in aquaculture (Vadstein 1997). Most investigations have so far involved juvenile or adult stages, but development of strategies for nonspecific immunostimulation in culture of the earliest stages of fish may have considerable potential (Vadstein 1997).

During their 4-5 week yolk sac period larvae of Atlantic halibut are exposed to stress from high numbers of opportunistic pathogenic bacteria compared to what they experience in their natural conditions (Hansen 1993). Use of nonspecific immunostimulants has been shown to enhance viability of halibut yolk sac larvae during 4 weeks incubation, from on average 10% survival in control groups to 52% survival in treated groups (Vadstein et al 1993a). The immunostimulant used in the halibut larvae experiment was an alginate rich in mannuronic acid polymers, which is stimulatory towards human monocytes (Espevik et al 1993) and fish. The immunostimulant (termed FMI) was administered to the incubation water of small, stagnant units, and the larvae were thus long term exposed during the whole yolk sac period. The route for uptake was not studied, but stimulation of the nonspecific defence of both the skin and gut surfaces can be suggested. Immersion of marine larvae in immunostimulants at an early pre-feeding stage is therefore believed to be a suitable technique for immunostimulation, preferably in periods with stagnant or low water exchange conditions. The technique must be carefully prepared for the actual organism, regarding immunostimulant, concentration, duration of immersion, developmental stage of larvae and frequency for administration of the stimulant. Immersion has been documented as efficient also for carp (Siwicki et al 1988),even though oral administration or injection seem to be more efficient (Vadstein,1993a).

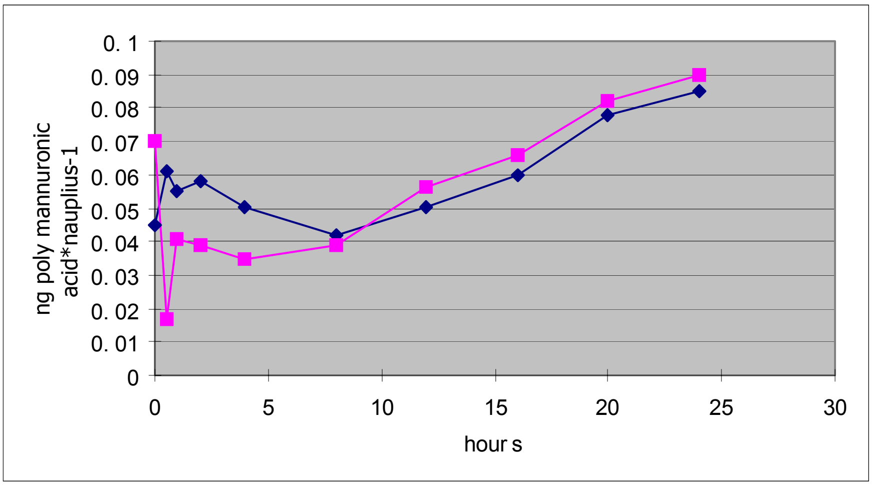
A technique for administration of immunostimulants via live food organisms has been developed. The immunostimulant is immobilized in alginate microcapsules (2-30um) and ingested by live food organisms, such as rotifers or Artemia nauplii. Fig 1 shows the content of polymannuronic acid in Artemia nauplii after grazing on alginate beads, and illustrates the relatively rapid decrease in content after transfer of the nauplii to the larval tanks. The regime for feeding the fish larvae is therefore important for successful use of live food organisms for administration of immunostimulants to larvae. The method is non-stressing and very suitable for prophylactic treatment in the earliest stages in larval first feeding. The efficiency of the method was demonstrated in two experiments where FMI was administered to turbot fry via alginate beds and Artemia two days prior to a challenge with a fish pathogenic *Vibrio anguillarum*. Mortality reductions of 39 and 48% were obtained in stimulated vs non-stimulated groups (Skiermo et al, 1995). Progress in the development of formulated first food for marine larvae, and entrapping immunostimulant in food particles suitable for larvae is therefore a challenge.

An export-oriented farm - mainly turbot and flounder

Challenges in future

As the review above demonstrates, more knowledge is needed before a strategy for specific and nonspecific immunostimulation can be developed for larviculture. The few studies on stimulation of specific maternal immunity and the more numerous studies of stimulation of nonspecific defence of fish suggest that there is a good possibility of developing efficient methods for immunostimulation in

Stability of the non-specific immunostimulant poly mannuronic acid in Artemia nauplii that had been grazing on alginate microcapsules containing the 14C-labelled stimulant, after tranefer to larval tank condition. The experiment was run with two replicate cultures (Skjermo and Vadstein, unpublished results)



to funds comparable to those available to human medicine; therefore research should focus on the immunostimulants that have already been intensively studied in other areas. The knowledge and the spin off from these other areas represent a considerable resource for aquaculture research.

For stimulation of maternal immunity, it is important to clarify whether it is possible to stimulate nonspecific immunity in a way that is relevant to larviculture. Due to the shorter duration of such stimulation, nonspecific stimulation through the maternal pathway may not be feasible, but a clarification of this point is necessary before and final conclusion can be drawn. Stimulation of specific immunity is the most promising method for applying maternal stimulation. As a general method, it is not suitable, but in the cases where specific problem

organisms have been identified, it should be possible to evaluate the potential of the method. Such an evaluation should include optimization of immunization procedures, and evaluations in both challenge experiments and under production conditions. It is important that evaluations are done under both sets of conditions, because even if stimulation of maternal immunity does not have significant positive effects in a challenge test, in which the problem organism occurs at high densities, it may do so under production conditions. The method is not restricted to bacteria (Sin et al 1994).

The challenges related to nonspecific immunostimulation of the larva itself are more diverse than those related to maternal immunity. Reliable larval experiments are expensive, due to the complexity and the poor reproducibility of such experiments. More information is required on the developmental stage in which immunostimulation is possible, and for these additional evaluation criteria other than challenge tests will be needed. The research on the ontogeny of the nonspecific immune system should focus not only on the development and the functioning of relevant organs, but should also include studies of the immune system in the integument. The development of methods of assessing stimulation is a special challenge; due to the small size of larvae. Priority should be given to the establishment of methods for determining parameters that occur early in the cascade of reactions triggered by immunostimulants. The research directly related to the establishment of an immunostimulation strategy should include evaluation of administration procedures, dose-response relationships, and the evaluation of the duration of stimulation.

Prospects

Immunostimulation is one element in a strategy to achieve microbial control. Direct stimulation of nonspecific immunity and stimulation of specific defence mechanism of maternal origin seem to be the most promising methods for larvae. Based on available knowledge, it is concluded that although this technique is still in its infancy, immunostimulation of fish has a considerable potential for reducing losses in aquaculture, during both larval and on-growing stages. The experience with larvae, however, is very limited. More research and developmental work on immunostimulation of relevance for larviculture is needed if immunostimulation would contribute to the development of the aquaculture industry.

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