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PREVENTIVE AND PROPHYLACTIC MEASURES IN INTENSIVE SALMONID FISH BREEDING – A REVIEW

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Abstract

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Aquaculture represents the fastest growing animal husbandry in many countries word-wide. Intensive fish production has increased the risk of infectious diseases all over the world. Bacterial pathogens probably cause most disease problems in aquaculture. Most infectious bacterial diseases in commercial fish species continue to be controlled by treatment with chemotherapeutic agents although they represent a potential environmental hazard and could also have negative effects on the quality of the final product. The most important problem of antibiotic treatment is the increase in resistant bacteria, the presence of their residues in aquaculture products and the resulting threat to human health. Therefore, it is most effective to prevent the introduction or onset of the disease. Beside preventing the introduction of pathogens, good water quality, reduction of environmental stressors, adequate nutrition and immunization, many substances improving the health status of fish, such as immunostimulants, probiotics, prebiotics, symbiotics and so on, are used in fish breeding. The present review gives an overview of preventive and prophylactic substances which can be used in salmonid fish breeding to improve the health status of fish.

Keywords: vaccination, immunostimulants, probiotics, prebiotics

INTRODUCTION

Aquaculture represents the fastest growing animal husbandry in many countries world-wide. The demand for fish and shellfish continues to rise. Losses incurred by fish farmers are related to disease, floods, oxygen depletions, predation, chemical poisoning, theft, and miscellaneous causes. Disease is by far the most significant factor (Meyer, 1991). Many of the pathogens that cause disease in fish and shellfish are facultative forms that are ubiquitous in aquatic systems. In nature, a high percentage of apparently normal and healthy animals harbour potential pathogens without evidence of clinical signs or overt disease (Wedemeyer, 1970). The development of disease in aquaculture systems usually occurs as the end result of a disruption of normal environment in which the animals are being reared. Unfavourable conditions, such as crowding, temperature fluctuations, inadequate dissolved oxygen, excessive handling, physical abuse, inadequate diets, or toxic substances may stress the animals (Wedemeyer *et al.*, 1976). Stress is considered to be an important predisposing factor in most bacterial diseases of fish and shellfish and is often followed by clinical disease. In many cases, prompt reduction of stressful conditions may lead to self-cure without the need to resort to chemotherapy (Meyer, 1991).

Intensive fish production world-wide has increased the risk of infectious diseases. However, before any infection can be established, pathogens must penetrate the primary barrier. In fish, the three major routes of infection are the skin, gills, and gastrointestinal (GI) tract. The GI tract is essentially a muscular tube lined by a mucous membrane of columnar epithelial cells that exhibit a regional variation in structure and function (Ringø *et al.*, 2010a).

Wild fish are common hosts to a wide array of parasitic forms, but they seldom affect the survival of populations. In culture situations, conditions are favourable only to relatively few parasite species, but their impact is far greater than it would be in natural waters. Fry, fingerling, or larval stages are exceedingly vulnerable to adverse effects of parasitism. In addition to mortality, parasites may cause cessation of feeding, reduced growth, susceptibility to bacterial or fungal pathogens, and physical deformities. In salmonid culture, serious problems can be caused mainly by *Ichthyophthirius, Ichthyobodo, Chilodonella, Trichodina,* monogenea, and sporozoans (Meyer, 1991). Moreover, other parasite species can cause serious problems, when their full developmental cycle takes place in a fish breeding (Palíková *et al.*, 2014).

Viral diseases cause serious problems in every aspect of aquaculture. If precautions are not taken to prevent the introduction of a viral agent, severe economic losses can occur. Infectious haematopoietic necrosis, infectious pancreatic necrosis and viral haemorrhagic septicaemia are the major viral diseases of trout and salmon (Wolf, 1988) in our conditions. Infectious haematopoietic necrosis and viral haemorrhagic septicaemia are dangerous diseases subjected to regular monitoring according law.

Bacterial pathogens overall probably cause more disease problems than all other causes combined. In virtually every type of aquaculture, bacterial diseases rank number one among etiological agents. Septicaemias and cutaneous lesions are among the manifestations of bacterial infections (Meyer, 1991). The major bacterial diseases of trout and salmon in our conditions include furunculosis, enteric redmouth disease, and flavobacterial diseases.

Most infectious bacterial diseases in commercial fish species continue to be controlled by treatment with chemotherapeutic agents. Chemical therapies are widely used in aquaculture although they represent a potential environmental hazard and could also have negative effects on the quality of the final product (Bagni *et al.*, 2000). Moreover, when antibiotics are used in aquaculture, the drugs typically remain in the open environment and may flow out of production facilities into open waterways or sewage systems, where they may also interact with other environmental contaminants (Benbrook, 2002). The most important problem of antibiotic treatment is the increase in resistant bacteria, presence of their residues in aquaculture products and the resulting threat to human health. Smidth *et al.* (2000) found that many bacteria in and around four trout farms were resistant to most antibiotic agents presently available for use in Danish aquaculture.

Chemotherapy should be considered as an emergency or last resort measure. Although chemicals may reduce the incidence of pathogens or control the abundance of facultative organisms, they may also have negative effects on the flora of biological filters (Meyer, 1991).

Unfortunately, aquaculture is driven by commercial forces, and stocking densities and rearing conditions are adjusted to maximize returns within the limits of acceptable risk. Within this scheme antibacterial agents are used widely (Inglis, 2000).

In any animal husbandry, measures to prevent the introduction or onset of disease are always the most effective, cost-efficient, and long-lasting. Successful preventive measures in aquaculture center on 1) preventing the introduction of pathogens, 2) maintenance of good water quality, 3) avoidance or reduction of environmental stressors, 4) adequate nutrition, 5) isolation of cultured animals from feral stocks, and 6) immunization (Meyer, 1991).

Also many substances improving the health status of fish sich as immunostimulants, probiotics, prebiotics, symbiotics and so on, are used in fish breeding.

VACCINATION

Vaccination is an important disease management strategy used to maintain good health in humans and animals worldwide as an ideal method of preventing infectious diseases. Vaccines developed for aquaculture have reduced the use of antibiotics in fish production. Original fish vaccines were bacterins (formalin-killed bacteria) delivered through immersion or injection that induced humoral (antibody) immunity. Next generation vaccines relied on multiple killed antigens delivered with an adjuvant to enhance vaccine effectiveness. Following work showed the use of various strategies to develop modified live vaccines for use in fish. A modified live vaccine is a live pathogen that has been rendered non-pathogenic or avirulent by physical, chemical, or genetic engineering methods. Modified live vaccines are advantageous in that they can be easily delivered (i.e., by immersion to young fish) and stimulate both humoral and cellular immunity of long duration. Disadvantages include issues with modified live vaccine safety to the host and environment (Shoemaker and Klesius, 2009). Another possibility of disease control is the development of DNA vaccines (Sommerset *et al.*, 2005).

There are many commercial vaccines available on the market. Most of these vaccines are for the control of bacterial diseases in aquacultural breeding of salmonid fish, especially in the breeding of Atlantic salmon and rainbow trout. Vaccination in the conditions of the Czech Republic is on a low level. Vaccination of salmonid fish against listed exotic and non exotic viral diseases is not possible according to legislative rules. Vaccines against parasitic diseases are not available. Vaccination is possible only against bacterial diseases. The situation is complicated by the requirement that the vaccines must be registered in the Czech Republic. The following commercial vaccines are registered in the Czech Republic: AquaVac ERM oral (oral emulsion for rainbow trout against yersiniosis – ERM), AquaVac ERM (concentrate for soaking suspension for rainbow trout against yersiniosis), AquaVac FNM PLUS (inject emulsion for fish against furunculosis), and AquaVac relera (concentrate for emulsion for bath or for emulsion for injection against yersiniosis – ERM). It follows from this listing that there is no immersion vaccine available against furunculosis. This situation can be dealt by preparing a specific vaccine with formalin-killed bacteria strains isolated from fish on the given farm.

Although mineral oil-adjuvanted injection vaccines are by far the most efficient in protection against diseases, the use of these vaccines often results in adverse side effects including extensive adhesions and pigmentation of the peritoneum (Utoloki *et al.*, 2006). Therefore, alternative eco-friendly treatments must be considered. Treatments that may have a less significant environmental impact include the strategic use of immune-stimulants, probiotics and prebiotics.

IMMUNOSTIMULANTS

Immunostimulants represent a modern and promising tool in aquaculture, enhancing the resistance of cultured fish to disease and stress. The objectives of immunostimulation in fish include not only promoting a greater and more effective immune response to infectious agents, but also overcoming the immunosuppressive effects of stress (Anderson, 1992). Many groups of substances are known to have an immunostimulant effect in terrestrial animals, but few of them have been shown to be effective in fish. These include glucans and nutritional factors such as ascorbic acid and α-tocopherol (Bagni, 2000). Beta-glucans are the most commonly used term for a heterogeneous group of glucose polysaccharides consisting of a backbone of β-(1.3)-linked β-D-glucopyranosyl units with β-(1.6)-linked side chains of varying length and distribution (Rodríguez *et al.*, 2009). They are a major structural component of fungi cell walls and are also found in some bacteria, plants, algae, yeast, and mushrooms. One of the most common sources of β-glucans is derived from the cell wall of baker's yeast *Saccharidomyces cerevisiae* (Das *et al.*, 2009). Currently, β-1.3/1.6 glucans from baker's yeast *Saccharidomyces cerevisiae* with high purity and activity are considered the most effective immunomodulators and are produced commercially e.g by Biorigin as MacroGard. Beta-glucans bind to specific cell surface receptors of macrophages and neutrophilic granulocytes (Brown and Gordon, 2003). Beta-glucans are capable of enhancing the innate immunity by increasing plasma lysozyme and complement and stimulate the phagocytic activity of macrophages in several cultured fish species (Yano *et al.*, 1991; Chen and Ainsworth, 1992; Jorgensen *et al.*, 1993; Galeotti, 1998; Jeney and Anderson, 1993). Beta-glucans enhance fish resistance against bacterial and viral infections by means of effective stimulation of non-specific cellular and humoral immune functions and have a proven protective effect against fish bacterial pathogens including *A. salmonicida* (Nikl *et al.*, 1993). In addition to individual administration, β-glucans have also been used with bacterial vaccines as adjuvants (Figueras *et al.*, 1998) or with lipopolysacharide as synergiens (Cook *et al.*, 2001) so as to increase the immune response and to protect fish against pathogens. The use of β-glucans in prospering, clinically healthy aquaculture is questionable, and does not have to result in a specific effect on the immune system response, nevertheless, their usefulness in a breeding endangered by stress stimuli, infectious diseases or adverse environmental factors is indisputable (Dobsikova *et al.*, 2012). Similarly, ascorbic acid can stimulate the natural immune response through macrophage and complement activities; dietary concentrations significantly influence resistance to diseases (Waagboo, 1994; Dunier *et al.*, 1995; Verlhac *et al.*, 1996). Dietary levels of α-tocopherol are also relevant to immune responsiveness in farmed fish, since suboptimal levels may impair both humoral and cell-mediated immunity, in particular the phagocytic activity of macrophages (Blazer and Wolke, 1984). It is worth considering that the immune response is often diminished with a long-term use of immunostimulants which often leads to the immune status reverting back to control levels or in extreme cases to immunosuppression (Sakai, 1999; Bricknell and Dalmo, 2005).

PROBIOTICS

The definition of a probiotic differs greatly depending on the source. Recent definitions describe the probiotic as any microbial cell provided via the diet or rearing water that benefits the host fish, fish farmer or fish consumer, which is achieved, at least in part, by improving the microbial balance of the fish. In this context, direct benefits to the host such as immune-stimulation, improved disease resistance, reduced stress response, improved GI morphology etc., and benefits to the fish farmer or consumer such as improved fish appetite, growth performance, feed utilization, improvements of skeleton quality, flesh quality and reduced malformations, are regarded (Merrifield *et al.*, 2010). The population of endogenous microbiota may depend on genetic, nutritional, and environmental factors. However, microorganisms present in the immediate environment of aquatic species have a much greater influence on the health status than is the case with terrestrial animals or humans. The gut microbiota of aquatic animals probably comprise indigenous microbiota together with artificially high levels of allochthonous bacteria maintained by their constant ingestion from the surrounding water (Ringø and Birkbeck, 1999).

A wide range of microalgae (*Tetraselmis*), yeasts (*Debaryomyces*, *Phaffia* and *Saccharomyces*) and Gram-positive (*Bacillus*, *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Micrococcus*, *Streptococcus* a *Weissella*) and Gram-negative bacteria (*Aeromonas*, *Alteromonas*, *Photorhodobacterium*, *Pseudomonas* a *Vibrio*) has been evaluated. Probiotics actively inhibit the colonization of potential pathogens in the digestive tract by antibiosis or by competition for nutrients and/or space, alteration of microbial metabolism, and/or by stimulation of the host's immunity. Probiotics may improve nutrition by the production of vitamins, detoxification of compounds in the diet, and breakdown of indigestible components (Irianto and Austin, 2002a). The bacteria do not permanently colonize the intestinal system of fish but need to be continuously introduced with the feed (Autin, 2010). The potential of probiotic administrations to improve resistance against viral infections of fish is presently unknown. If probiotics can enhance the immune status, it is reasonable to speculate that this may reduce susceptibility to viral infections (Merrifield *et al*., 2010). Probiotics, such as *Lactobacillus rhamnosus*, have been demonstrated to reduce rotavirus and poliovirus infections in humans (Marteau *et al*., 2001; De Vrese *et al*., 2005). On the other hand, many studies have focused on probiotics improving resistance against bacterial diseases and reducing mortality of fish including salmonids, and reviews have been published of the use of probiotics in aquaculture and in salmonids (Balcázar *et al*., 2006; Merrifield *et al*., 2010). Moreover, when the GI tract is involved as an infection route, mucosal adhesion is considered to be a critical early phase in all infections caused by pathogenic bacteria (Knudsen *et al*., 1999; Namba *et al*., 2007). When the bacteria are able to colonize the intestinal mucus, they can cross the GI tract lining by transcellular or intracellular routes. Probiotic strains of *Carnobacterium* sp. have been demonstrated to augment immune responses in rainbow trout, upon challenge with fish pathogens *A. salmonicida* and *Yersinia ruckeri,* increasing phagocytic activity, respiratory burst as well as serum and gut mucosal lysozyme activity and the expression of IL-1β and TNFα in head kidney leukocytes (Kim and Austin, 2006a, 2006b). Similarly, Panigrahi *et al*. (2007) describe augmentation of innate immunity using three freeze-dried probionts (*Lactococcus rhamnosus, Enterococcus faecium, Bacillus subtilis*) fed to rainbow trout. Likewise other studies present the positive effects of various probiotics (*Lactococcus lactis, L. sakei, Leuconostoc mesenteroides*) on head kidney phagocytic activity and serum alternative complement activity, and lower proliferation of the pathogen *A. salmonicida* in the intestine (Balcázar *et al*., 2007a; Balcázar *et al*., 2009). Balcázar *et al*. (2009) also have demonstrated that *A. salmonicida* is located on the intestinal surfaces of fish with asymptomatic infections and probiotic administration may provide a viable alternative to antibiotics by interfering with pathogen colonization and modulation of the immune response. Pieters *et al*. (2008) demonstrated that dietary probiotics *A. sobria* strain GC2 can protect rainbow trout against skin infections caused by *A. bestiarum* and the eukaryotic pathogen *I. multifiliis*. In addition, fermentation products of probiotics, such as short-chain fatty acids effectively act as adopted regulators of both innate and adaptive immune mechanisms in human cells (Van Nuenen *et al*., 2005). Through the combined administration of multiple favourable probiotic candidates it may be possible to produce greater benefits compared to administration of individual probionts. However, the probiotics must not be pathogenic, not only with regard to the host species but also with regard to aquatic animals in general and human consumers. They must also be free of plasmid-encoded antibiotic resistance and be resistant to bile salts and low pH (Merrifield *et al*., 2010).

In 2003, Ringø *et al*. wrote that if we are going to defeat our enemies, the pathogens, we have to be at the same place and time as they are. If probiotic bacteria mostly colonize the pyloric caeca, the probionts will have no effect if the pathogens mostly colonizes the mid or hindgut regions and translocate in these regions. The intraperitoneal (IP) method of disease challenge overrides one of the possible methods of probiotic protection against pathogens by masking the potential effect of probiotic competitive exclusion within the GI tract. Gastric probiotics may reduce or even prevent gastric infections. The IP challenges do not reflect the effect of probiotics on resistance to infection; rather, they demonstrate the effect of probiotics on infected fish. Therefore, it is recommended that immersion or cohabitation studies are conducted in future challenge experiments in order to truly assess the full potential of candidate probionts (Merrifield *et al*., 2010). Probiotic administration methods vary greatly and are not always practical for production level of fish farming. The specific probiont will likely be dependent on the fish species, rearing conditions, and desired outcome of supplementation (Merrifield *et al*., 2010). In salmonid studies, live-cultures are most commonly sprayed or top-dressed onto basal diets. But lyophilised cells, dead cells, disrupted cells, cell-free supernatants and spores have all showed some degree of success (Merrifield *et al*., 2010). Most commonly used probiotics have been administered via the feed; however, some information regarding administration of probiotics to salmonids via rearing water is available (Irianto and Austin, 2002b; Austin *et al*., 1995). These methods may be more applicable in re-circulation facilities or for bathing treatments applied regularly or during times of disease.

Dose dependent studies are currently limited and somewhat contradictory. Appropriate levels are likely to vary depending on the probiont species, host fish species, host physiological status, rearing conditions and the specific goal of the feeding application (Merrifield *et al*., 2010). Supplementation has proved to provide short-term benefits but generally probionts have not been detected within the GI tract for periods beyond one to three weeks after reverting to non-supplemented diets (Robertson *et al*., 2000; Balcázar *et al*., 2007b; Panigrahi *et al*., 2005; Kim and Austin, 2006a) and presumably probiotic benefits are lost after the probiont is removed from the host. Therefore, there appear to be 3 distinct options for administrative strategy: 1) short-term administration limited to times of need, which is effective for gastric colonization, stimulating the immune system, and providing protection against disease when fed prior to pathogenic infection (Irianto and Austin, 2002b); 2) constant feed supplementation incorporated into the diet, however, we must consider the possibility that it may not be appropriate to use constant probiotic supplementation for extended periods, similarly as with long-term use of immunostimulants (Sakai, 1999; Bricknell and Dalmo, 2005); or 3) cyclic feeding of supplemented diets for short periods which may provide direct benefits of short-term administration during the supplemental feeding phase, and during the unsupplemented stage where gastric probiotic populations persist for a number of weeks (Balcázar *et al*., 2007a; Kim and Austin, 2006a) it may provide certain protection against transient pathogens and continue to induce some degree of immunostimulation (Nikoskelainen *et al*., 2003; Balcázar *et al*., 2007a). Currently, there are no data supporting this hypothesis.

On the basis of the OFIMER program with BioMar and several research institutions, BioMar developed an innovative dietary probiotic concept which resulted in the introduction of the first approved industrial trout and salmon feed containing probiotics in 2010 (DETZ, 2010).

PREBIOTICS

A prebiotic is defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or the activity of specific health promoting bacteria that can improve the host's health (Gibson and Roberfroid, 1995). According to Gibson *et al*. (2004), only three oligosaccharides were classified as prebiotics: inulin, transgalactooligosaccharide (TOS), and lactulose. A more recent study includes fructooligosaccharides (FOS) in the list of prebiotics (Roberfroid, 2007). Prebiotics have demonstrated some benefits in fish (Ringø *et al*., 2010b) but the use of prebiotics in salmonid studies remains relatively limited. For instance, mannanoligisaccharide supplementation induced significant improvements of immunity indicators compared to control groups (Staykov *et al*., 2007). Mannanoligisaccharides (MOS) are glucomannoprotein-complexes derived from the cell wall of yeast (*S. cerevisiae*) (Sohn *et al*., 2000). The commercial product GroBiotic®-A is a mixture of partially autolyzed brewer's yeast, dairy ingredient components, and dried fermentation products (Li and Gatlin, 2005). Sealey *et al*. (2007) described the effect of dietary partially autolysed yeast and GroBiotic®-A on rainbow trout. Fingerlings were fed experimental diets containing 2% prebiotic for 9 weeks. The results showed that the growth performance, immune response, and TNF-α mRNA expression level remained unaffected throughout the experimental period. On the contrary, the whole body energy content and survival from infectious haematopoietic necrosis virus (IHNV) challenge was significantly increased in fish fed either partially autolysed yeast or GroBiotic®-A.

SYMBIOTICS

A symbiotic, the combined administration of probiotics and prebiotics, is based on the principle of providing a probiont with a competitive advantage (a fermentable energy source) over competing endogenous populations, thus effectively improving the survival and implantation of the live microbial dietary supplement in the gastrointestinal tract of the host (Gibson and Roberfroid, 1995). One symbiotic study has been conducted in salmonids (Rodriguez-Estrada *et al*., 2009). In this study the individual application of the dietary *E. faecalis* and MOS provided a wide range of benefits with regards to immune response and survival in a challenge study with *Vibrio anguillarum*.

CONCLUSION

Each intensive system has its own specifics including health problems. Based on a regular monitoring of the health status of the fish stock, the main problems in a given breeding can be estimated. By maintaining good water quality, reducing environmental stressors, providing adequate nutrition and immunization, and with the use of selected substances improving the health status of fish, such as immunostimulants, probiotics, prebiotics, symbiotics and so on, losses can be reduced to a minimum.

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