IMMUNOPREVENTION IN INTENSIVE FISH CULTURE

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IMUNOPREVENCIJA U INTEZIVNOM SISTEMU GAJENJA RIBA

Hemoterapeutici, posebno antibiotici, su dosta korišćeni u intentzivnom sistemu gajenja riba, ali se danas smatra da njihovu potrošnju treba svesti na minimum. Intenzivno korišćenje antibiotika je indukovalo rezistenciju mnogih ne patogenih, ali i patogenih bakterija. Ovakva situacija se smatraju velikom pretnjom za ljudsko i životinjsko zdravlje. Sa razvojem novih imunopreventivnih metoda u akvakulturi, upotreba antibiotika i drugih hemoterapeutika u gajenju riba je značajno redukovana tokom proteklih godina.

Imunoprevencija je novi koncept kontrole zdravlja i bolesti riba koji obuhvata klasičnu specifičnu imunoprofilaksu (vakcine) i nespecifične imunomodulacije ili imunostimulacije. Takođe, aplikacija pro- i prebiotika u ishrani su veoma važan deo efektivne nespecifične imunoprevencije u intezivnom sistemu gajenja riba.

Specifična imunoprofilaksa (vakcine)

Zaštita riba od bolesti primenom imunizacije je godinama predstavljao važan koncept u akvakulturi. Međutim, odbrambeni mehanizmi nižih vertebrata su u neku ruku drugačiji od onih kod sisara, i neke tehnike vakcinacije, kada se primene u uslovima mrestilišta, nisu tako efikasne kao što se očekuje. Jedna od najčešćih nedoumica koje se odnose na upotrebu vakcina je koliko će zaštita da traje. Stoga, istraživanje je usmereno u pravcu poboljšavanja potencije i efikasnosti vakcina kao i kako optimalno aktivirati nespecifične odbrambene mehanizme, ćelijski imnitet kao i specifični imuni odgovor. Vakcinacija riba ima dugu istoriju, i komercijalne vakcine su dostupne za više od polovine glavnih bakterijskih i viralnih oboljenja u akvakuturi. Vakcine za ribe koje se apliciraju injekcijama, imerzijom ili se daju kroz hranu, su postale rutina u specifičnoj zaštiti protiv bakterijskih i virusnih infekcija. Međutim, do sada nisu napravljene komercijalne vakcine protiv parazitskih invazija. Uglavnom su to antibakterijske vakcije (protiv *yersinioze*, *Furunculoze* ili *vibrioze*), bazirane na inaktiviranim bakterijskim suspenzijama. Takođe su značajno razvijene auto-vakcine bazirane na lokalnim patogenim bakterijama. Ove auto-vakcine pružaju dobru ili adekvatnu zaštitu i mogu da se koriste kao imerzione vakcine, korisne za mlade ribe. Žive ili atenuirane bakterije ili virusi su takođe bili uspešni u pripremanju vakcina, na primer, protiv virusa koi herpesa (KHV) kod šarana. Povezani rizici od reverzije na virulentne forme će limitirati opštu upotrebu takvih vakcina u akvakulturi. Vakcine za ribe bazirane na molekularnoj tehnologiji, uključujući i DNK vakcine i upotreba rekombinantnih proteinskih antigena daju dobre rezultate. Međutim, praktični problemi su do sada ometali opštu upotrebu molekularne tehnologije u proizvodnji anti-viralnih vakcina u akvakulturi. Ova vrsta vakcina koristi rekombinantne proteine, uglavnom površinske viralne glikoproteine produkovane u bakterijama ili intramuskularne injekcije plazmida DNK koji kodiraju odgovarajući antigen. Mnoge od ovih ekperimentalnih DNK vakcina su relativno brzo dale visokospecifičnu i dugotrajnu zaštitu u veštačkim infekcijama (challenge tests). Negativna strana je više etičko pitanje koje se odnosi na sudbinu bakterijskih plazmida DNK u organizmu riba. Eksperimentalne studije su pokazale da se oni nepotpuno razgrađuju na mestu injektiranja i mogu dospeti u druga tkiva ili čak biti izlučena iz tela. Ovo dovodi do opasnosti od introdukcije plazmida u druge slučajne domaćine sa nesagledivim posledicama po životnu sredinu.

Uticaj vakcina na zaštitu od bolesti, odnosno efikasnost vakcine, zavisi od vrste ribe, starosti, kondicije i kvaliteta vode, naročito temperature u vreme vakcinacije. Specifična antitela su primarni imuni odgovor, i jedinstvena u fiziologiji riba, jer ovi molekuli imunoglobulina su specifično usmereni protiv individualnih antigena. Uobičajeni Ig riba je tetramerna forma antitela uglavnom označena kao IgM. Suprotno tome, dimerna forma je česta (IgG) i efikasna kod sisara, a pentamerna forma (IgM) je molekul koji se produkuje rano tokom infekcije ili imunizacije vakcinom. Specifično antitelo predstavlja molekul koji ima sledeće funkcije:

- može da stvara komplekse sa antigenima i da inicira druge serumske proteina da započnu komplementarnu kaskadu u liziranju invazivnih patogena.,
- može biti stacionaran ili pričvršćen za ćeliju ili može da predstavlja kominikator koji upravlja cirkuisanjem citotoksičih ćelija u prepoznavanju ili napadu na patogeni agens.

Iniciranje specifičnog imunog odgovora je kontrolisano antigenima koji predstavljaju ćelije (APC, uglavnom makrofagi) koje učestvuju u preuzimanju čestica ili antigena. Informacija o tome kako da se napravi antitelo protiv antigena se prenosi na B ćelije. Kompleksni receptori na ćelijama, kao što su veliki histokompatibilni kompleksi, koji nadgledaju selekciju antitela, uz pomoće informativnih hemijskih molekula, citokinina i interleukina, su uključeni u ekspresiju i kontrolu sekrecije antitela. Specifičan imuni odgovor, može da pamti, i kad se ribe susretnu sa patogenima po drugi put, specifična antitela se mnogo brže i u većeoj količini proizvode.

Starost riba i temperatura okruženja su kritični faktori za uspeh vakcina u akvakulturi. Potrebno je da larve i mlađ riba imaju potpuno razvijene adaptivne sisteme pre nego što se vakcinišu. Mnoge studije su pokazale da je vakcinaciona kupka kalifornijske pastrmke protiv enterične bolesti crvenih usta jedno uspešna na 15 °C ali ne i na temperaturama ispod ili iznad ove. Takođe, naši eksperimenti su pokazali da optimalna temperatura za različite vrste riba determiniše efikasnost vakcina protiv furunkuloze ili jersinioze. Variranje temeprature vode takođe utiče na stepen i dužinu adaptivnog imunog odgovora koji sledi nakon vakcinacije.

Zapravo, ustraživanja su usmerena ka potenciju i efikasnosti antigena kao i kako optimalno aktivirati ne samo specifične humoralne imune odgovore, već i nespecifične odbrambene mehanizme sa celularnim i humoranim imunim odgovorom. Kada se riba inicijalno susretne sa patogenom, nespecifični odbrambeni mehanizam je važniji nego specifični imuni odgovor, jer ovaj zahteva puno vremena za stvaranje antitela i specifičnu celularnu aktivaciju. Generalno, ribe imaju kratak životni vek i većina živi u hladnom okruženju koje usporava razvoj specifičnog imunog odgovora.

Nespecifična imunostimulacija i imunomodulacija

Barijere nespecifične odbrane koje su već prisutne indukuju epitelijalni štit od krljušti, kože i mukusa. Ukoliko je patogeni agent zarobljen mukopolisaharidnim kompleksima mukusa, može biti odstranjen sa ribe ili razgrađen litičkim enzimima. U većini slučajeva, patogeni mikroorganizmi bivaju uništeni digestivnim ili litičkim enzimima. Infekcije se mogu desiti na mestu oštećenog tkiva, što rezultuje u migraciji leukocita u oblast rane i porasta koncentracije serumskih komponenata, uključujući lizozome, transferin, C-reaktivni proteina, ceruoloplazmin i komplementarne komponente.

Fagocitoza i fagociti su glavno oruđe nespecifičnog imunog odgovora na patogene mikroorganizme kao što su virusi, bakterije, gljive i paraziti. Funkcije fagocita efektora su značajne, ali je najvažnije njihova sposobnost da ubijaju patogene. Viralni agensi mogu indukovati produkciju interferona pomoću viralno inficiranih ćelija, koje stimulišu druge ćelije da aktiviraju intracelularne endonukleaze koje će uništiti viralne DNK i RNK i zatvoriti fiziološke puteve koje virusi koriste za replikaciju. Ovaj fenomen se dešava posredstvom jedne populacije nespecifičnih citotoksičnih ćelija (NC). Slično natural kiler (NK) ćelijama, NC ćelije su ne-T, ne-B ćelije koje nemaju sposobnost fagocitiranja. Ove ćelije produkuju vrlo visok nivo citotoksičnih substanci protiv velikog broja ciljanih ćelija.

Upotreba imunomodulatora ili imunostimulatora u intenzivnom sistemu gajenju riba pruža veliki izbor atraktivnih metoda za indukovanje i povećavanje zaštite protiv infektivnih oboljenja. Uopšteno, imunostimulanti i/ili imunomodulatori čine grupu sintetičkih i bioloških jedinjenja koja pojačavaju ćelijski i humoralni imunitet u ljudima, životinjama kao i u ribama. Neki od njih stimulišu samo odbrambene mehanizme, drugi su sposobni da oporave ili moduliraju imunitet nakon supresije indukovane ksenobioticima. Stimulacija odbrambenog mehanizma može biti naročito važna za ribe koje su gajene u ili puštene u okruženja gde su vrste ili serotipovi patogena nepoznati pa imunizacija specifičnim vakcinama može biti beskorisna. Levamisole je bio jedan od prvih imunostimulanata koji je korišćena na ribama radi podizanja nespecifičnih celularnih i humoralnih odbrambenih mehanizama. Drugi imunostimulanti i modifikatori biološkog odgovora koji su korišćeni u istraživanjima na ribama uključuju dimerizovane lizozime (KLP-602), hitozan, bakterijski lipopolisaharid, HMB, metisoprinol i glukani. Upotreba probiotika značajno prisutna u gajenju životinja a od skoro je počela da se koristi u gajenju riba i dala generalno dobre rezultate. Kako funkcionišu probiotske bakterije u različitim životinjama još uvek nije poznato. Smatra se da žive probiotske bakterije utiču na intestinalnu floru svojom antagonističkom aktivnosti i kompeticijom za nutrijente i prostor, redukujući tako broj patogenih bakterija. Probiotici takođe, smatra se, poboljšavaju apetit i rast. Osim toga, stimulišu prirodnu odbranu putem TLR-a, drugim celularnim receptorima, ali da bi do ovog došlo, probiotske bakterije ili njihovi produkti moraju da prođu kroz mukoznu i epitelijalnu barijeru alimentarnog trakta i pripreme normani odbrambeni mehanizam riba. Blagotvoran uticaj može biti dugotrajan, minimalno mesec dana nakon aplikacije u hranu.

Uticaj imunomodulatora i/ ili imunostimulatora na celularni mehanizam riba se može utvrditi uzimanjem uzoraka neletalne krvi i hematopoetskih organa i praćenjem promena u brojnosti leukocita, aktivnosti i funkcije. Makrofage, neutrofili i druge fagocitarne ćelije povećavaju mobilizaciju enzima, oksidativnu radikalnu produkciju i fagocitarnu aktivnost. Subpopulacija T limfocita povećava proliferacioni odgovor i citotoksičnu aktivnost, a povećava i proliferativni odgovor B limfocita kao i nivo antitela sekretornih ćelija. Uticaj imunostimulatora na humoralni odbrambeni mehanizam riba se može pratiti u uzorcima krvi, analizom promena Ig u serumu ili plazmi i nivoa specifičnih antitela, lizozima ili ceruplazminske aktivnost i nivoa citokinina.

Imunostimulanti se mogu koristiti na sličan način kao hemoterapeutici ili hemijske materije ili u kombinaciji sa vakcinama. Ribe se mogu pripremiti za ovakve buduće tretmane sezonskim izlaganjem patogenima ili stresom rukovanja. Mnogi parametri okruženja i fiziološki parametri utiču na ekperimente i protokole za upotrebu imnostimulanata za ribe, kao što je vreme, oprema za doziranje, temperatura okruženja, stabilnost svake komponente, karakteristika vakcine i vrsta ribe. Prvo razmatranje je da li će se substanca koristiti samostalno, kao jedan tretman ili će biti davana zajedno sa programom za imnuizaciju.

Nekoliko proizvođača riblje hrane sada nude obroke sa dodatnim prirodnim i sintetičkim imunomodulatorima za upotrebu u akvakuturi. Trenutno je preporučeni način davanja imunostimulanata u hrani svaki dan tokom jedne do dve nedelje. Mnoga pitanja ostaju otvorena koja se odnose na potrebe i korišćenje imunomodulatora, posebno da bi se odredio načina davanja, odgovarajuća veličina doze, vreme i dužina apliciranja, kao i uticaj na okruženje.

IMMUNOPREVENTION IN INTENSIVE FISH CULTURE

Chemotherapeutics, but especially antibiotics have been used extensively in intensive fish culture in may years but the present consensus is that should be kept at minimum. Excessive used of antibiotics induced resistance of many non-pathogenic but also pathogenic bacteria. These situations are considered a major threat to human and animal health. With the development of new immunoprevention methods in aquaculture, the use of antibiotics and other chemotherapeutics in fish culture has been greatly reduced in the past years.

Immunoprevention is a new concept of control fish health and diseases that involves classical specific immunoprophylactic (vaccine) and nonspecific immunomodulation or immunostimulation. Also the application of pro- and prebiotic in diets are very important part of effective nonspecific immunoprevention in intensive fish culture.

Specific immunoprophylactic (vaccines)

The protection of fish against diseases by immunization has been an important concept for many years in aquaculture. However, defence mechanisms in the lower vertebrates are somewhat different from those in mammals, and some vaccination techniques when actually applied to hatchery conditions are not as effective as they should be. One of the most frequent uncertainties regarding the use of vaccines is how long the protection will last. Therefore, research is concentrating on how to improve the potency and efficacy of the vaccines and how to optimally activate the nonspecific defence mechanisms, cell-mediated immunity and the specific immune response. Fish vaccination has a long history but commercial vaccines are available for more than half of the major bacterial and viral diseases in aquaculture. Fish vaccines administrated by injection, immersion or in the feed are now start to by routinely used in specific protection against bacterial or viral diseases. However, no commercial vaccines have so far been produced against parasitic invasions. Generally anti-bacterial vaccines (against *Yersiniosis*, *Furunculosis* or *Vibriosis*) are based on inactivated bacterial suspension. Also, auto-vaccines based on the local pathogenic bacteria are very intensively are developed. These auto-vaccines have given good or adequate protection and can be used as immersion vaccines, useful for younger fish. Also live attenuated bacteria or virus have also been used successfully in vaccine preparation, for example, against koi herpes virus (KHV) infection in common carp. However, associated risks of reversion to virulent forms will limit the general use of such vaccines in aquaculture. Fish vaccines based on molecular technology, including DNA vaccines and the use of recombinant protein antigens given promising results. But practical considerations have so far hindered the general use of molecular technology for production anti-viral vaccines in aquaculture. This type of vaccine, make use of recombinant proteins, mainly surface viral glycoproteins, produced in bacteria or of intra muscular injection of plasmid DNA encoding the antigen of interest. Many of these experimental DNA vaccines have given relatively fast, high specific and long time protection in challenge test. But the negative side is the ethical question of what happens to the bacterial plasmid DNA in the fish organism. Experimental study showed that this is not completely degraded at the injection side and may reach other tissues or even be secreted. This introduces the danger of the plasmid being introduced to another unrelated host with unforeseeable consequences for the environment.

The influence of vaccine on the protection against disease – vaccine effectiveness is depending on the fish species, the age of fish, their condition and quality of water, especially the temperature in vaccination time. Specific antibody is the primary result of the specific immune response, and unique in the fish physiology because these immunoglobulin molecules are specifically directed against individual antigens. The common Ig in fish is a tetrameric form of antibody and is usually designated as IgM. By contrast, the dimeric form is common (IgG) and efficient in mammals with the pentameric form (IgM) being a molecule produced early in infection or immunisation by vaccine. The specific antibody is a recognition molecule and as such has several function:

- it can complex with antigens and initiate other serum proteins to begin a complement cascade in lysing invasive pathogen,
- it can be stationary or attached to cells or act as a communicator to direct circulating cytotoxic cells to recognise and attack pathogenic agents.

The initiation of the specific immune response is controlled by antigen presenting cells (APC, mostly macrophages) involved in particle or antigen uptake. The information concerning how to make antibody against is transferred to the B cells. Complex receptors on the cells, such as the major histocompatability complexes, that guide the selection of the antibody, assisted by messenger chemical molecules, cytokines and interleukins, that are involved in the expression and control antibody secretion. The specific immune response can hold memory, and when the fish meet the pathogen for a second time, specific antibody is more rapidly produced and in a greater quantity.

The age of the fish and environmental temperature are a critical factors for successful of vaccination in aquaculture. Fish larvae and fry need to have fully developed adaptive system before vaccination is attempted. Many studies showed that bath vaccination of rainbow trout against enteric red mouth disease is only successful at 15°C but not at temperature below or above this. Also our experimental study showed that optimal temperature for fish species determined the effectiveness of vaccines against *Furunculosis* or *Yersiniosis*. Variations in water temperature can also affect the magnitude and duration of adaptive immune response following vaccination.

Actually research is concerned on the potency and efficacy of the antigens and how to optimally activate not only specific humoral immune response but also nonspecific defence mechanisms with cellular and humoral immune responses. When a fish initially encounters a pathogen, the nonspecific defence mechanisms are more important than the specific immune response, as the latter requires a long time for antibody build-up and specific cellular activation. In general, fish have short life spans and most live in cool water environments which slow development of the specific immune response.

Nonspecific immunostimulation or immunomodulation

The nonspecific defence barriers already in place induce physical epithelial shield of the scales, skin and the mucus. If an pathogenic agent is entrapped by the mucopolysaccharide complexes of the mucus, it may be scuffed from the fish or held to be digested by the mucus lytic enzymes. In most cases, the pathogenic microorganisms are destroyed by digestive and lytic enzymes. Inflammation may happen at a tissue-damaged site, resulting in the migration of leukocytes to the wound areas and the elevation of serum component concentrations, including lysozyme, transferrin, C-reactive protein, ceruloplasmine and complement components.

Phagocytosis and phagocytes are the main tools of the nonspecific immune response to pathogenic microorganisms such as viruses, bacteria, fungi and parasites. The effectors functions of phagocytes are considerable, but most important is their ability to kill pathogens. Viral agents may induce the production of interferon's by viral-infected cells, stimulate other cells to activate intracellular endonucleases that will destroy viral DNA or RNA and will shut down physiological pathways that viruses use for replication. This phenomenon is mediated by a population of nonspecific cytotoxic (NC) cells. Similar to natural killer (NK) cells, NC cells are non-T, non-B cells which are not phagocytic. These cells produce very high levels of cytotoxicity substance against a wide variety of target cells.

The use of immunomodulators or immunostimulators in intensive fish culture offers a wide range of attractive methods for inducing or increasing protection against infectious diseases. In general, immunostimulants or/and immunomodulators comprise a group of synthetic and biological compounds that enhance the cell-mediated and humoral mediated immunity in human, animals and also in fish. Some of them only stimulate the defence mechanisms, others are able to restore or modulate immunity after suppression induced by xenobiotics. The stimulation of the defence mechanism may be particularly important for fish that are raised in or released to environments where the species or serotypes of pathogens are unknown and immunization by specific vaccines may be futile. Levamisole was one of the first immunostimulants used in fish to elevate the nonspecific cellular and humoral defence mechanisms. Other immunostimulants and biological response modifiers that have been used in fish research include dimerized lysozyme (KLP-602), chitosan, bacterial lipopolisaccharides, HMB, methisoprinol and glucans. The use of probiotics is well established in animals farming and has in recent years also been used in fish culture with generally good results. How probiotic bacteria work is not always fully understood in different animals. Live probiotic bacteria are believed to affect the intestinal flora by their antagonistic activity and competition for nutrients and space reducing the number of pathogenic bacteria. Probiotics also thought to improve appetite and hence increase growth. They may also stimulate innate defence through TLRs, other cellular receptors but for this to take place the probiotic bacteria or its products would have to pass the mucosal and epithelial barrier of the alimentary tract and prime the normal defence mechanism of the fish. The beneficial influence can be long lasting, minimum after one month of application in feed.

The influence of immunomodulators or/and immunostimulators on the fish cellular mechanisms can be followed by taking samples of nonlethal blood and haematopoietic organs and observing changes in leukocytes numbers, activity and functions. Macrophages, neutrophils and other phagocytic cells increase the enzyme mobilisation, oxidative radical production and phagocytic activities. The subpopulation of T lymphocytes increase the proliferative responses and cytotoxic activity, as well as increase the proliferative response of B lymphocytes and level of antibody secreting cells. The influence of immunostimulators on fish humoral defence mechanisms can be followed in blood samples analysed for changes serum or plasma total Ig and specific antibody levels, lysozyme and ceruloplasmine activity and cytokines levels.

Immunostimulants may be used in patterns similar to those of chemotherapeutics or chemicals and in combination with vaccines. The fish could be prepared for a predicted event, such as seasonal exposure to pathogens or handling stress, by a treatment prior to the event. Many environmental and physiological variables will influence experiments and protocol for the use of immunostimulants in fish, including timing, dosage requirement, environmental temperature, stability of each component, the characteristic of the vaccine and species of fish. The first consideration is whether the substance is to be used alone as a single treatment or whether it will be used in conjugation with an immunization program.

Several fish food manufactures now offer diets supplemented with natural and synthetic immunomodulators for use in aquaculture. Currently recommended schedules for feeding some immunostimulators all for administration every day for 1 to 2 weeks. Many question remain concerning the regiments and use of immunomodulators, particularly as to routes of administration, appropriate dosages, time and length of application, and influence on the environment.