Winter School on

'RECENT ADVANCES IN DIAGNOSIS AND MANAGEMENT OF DISEASES IN MARICULTURE'

7th to 27th November, 2002

Course Manual

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NUTRITION AND DISEASE RESISTANCE IN FISH

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Outbreak of disease problems is a major concern and exerts heavy economic losses in fish farming as in any other farming practice. Aquaculturists are, therefore, interested in developing cost-effective management strategies that can either prevent the outbreak or reduce the severity of epizootics. Nutritional modification is one of the important management strategies currently under exploration. The influence that dietary factors may have on disease outbreaks in cultured fishes has been recognized since many years. In contrast to extensive type of farming systems where fish obtain all or part of their dietary nutrient needs from naturally available food organisms, fish in intensive culture conditions rely more on the provision of a nutritionally complete diet. Here, diet can have significant effects on disease resistance mechanisms and hence on infectious disease incidence and severity. A number of nutrients, mainly vitamins, minerals and fatty acids have been implicated in this regard.

In nutritional modulation of resistance to infectious diseases, one must consider a proper balance of macro- and micronutrients, including amino acids, fatty acids, vitamins and trace elements, which are essential for the development of immune system starting at the larval stage. Deficiencies in these nutrients may impact several development events including the proper development of lymphoid organs. Marginal deficiencies may negatively affect the immune system at later stages of life. Severe deficiencies will increase susceptibility to disease and may result in the death of the animal. Adequate nutrition is also required for the cells of the immune system to divide and synthesize effector molecules. The diet supplies the immune system with the amino acids, PUFA, enzyme co-factors and energy necessary to support lymphocyte proliferation and the synthesis of effector (e.g., immunoglobulins, lysozyme, complement etc.) and communication molecules (cytokines and eicosanoids). In this paper, the role of different nutritional factors on disease resistance of fish is discussed with emphasis on dietary vitamin C

I. Vitamins

Vitamins are a group of complex organic substances, which are essential for a wide variety of metabolic processes. Fish require vitamins in their diets for growth, health and general body function. Among the vitamins, vitamin C has attracted the attention of fish health researchers, probably owing to its role in disease resistance and immune response. Ascorbic acid deficiencies have been commonly found in intensively cultured fish due to its instability during processing and storage of feeds and its rapid depletion *in vivo* during stress. Besides the essentiality for growth, vitamin C has been demonstrated to play an important role in functioning of the immune system when supplied at levels higher than standard doses in several fish groups.

I.I. Vitamin C (Ascorbic acid)

Ascorbic acid is optically active and the L- form (L-ascorbic acid) is the physiologically active form. L- ascorbic acid is a strong reducing agent. Because ascorbic acid is extremely sensitive to degradation during processing and storage in fish diets, its derivatives with sulphate or phosphate at the C-2 position in the lactone ring have been used to increase resistance to oxidation. In animals, ascorbic acid is synthesized from glucose *via*, the glucuronic acid pathway of metabolism. In this biosynthetic pathway L-gulonolactone oxidase is the terminal enzyme. It converts L-gulono-1, 4-lactone to 2-keto-L-gulonolactone, which is spontaneously converted to ascorbic acid. The involvement of vitamin C in disease resistance and immune response has been established particularly in species lacking the enzyme gulonolactone oxidase. Ability to synthesize ascorbic acid is absent in insects, other invertebrates and many species of marine and fresh water fish, guinea pigs, bats, primates, human beings and some highly evolved birds.

Biochemical functions of Vitamin C

Ascorbic acid functions as a cofactor in collagen synthesis. The stability of collagen depends upon its triple helix structure, which in turn depends on the presence of the unique amino acid, hydroxy proline. Ascorbic acid stimulates collagen synthesis by stimulating hydroxylation of proline, which is catalyzed by prolyl hydroxilase. The hydroxyproline to proline ratio is a useful indicator of vitamin C deficiency in fish at an early stage of development. The mechanical strength of connective tissue is dependent upon the activity of lysyl oxidase, which is also regulated by ascorbate. Because of its role in collagen synthesis, ascorbic acid is essential for the maintenance of an effective epithelial barrier and for normal wound healing in fish.

Another important function of vitamin C is its ability to quench oxygen radicals arising from cellular respiration. Lipid peroxidation and protein degradation are mediated by cytochrome P 450 and specifically prevented by ascorbic acid. Ascorbate also prevents O_2 – initiated free-ion independent protein oxidation.

Ascorbic acid is also known to protect the collagen of the extracellular matrix from oxidative degradation. The extracellular matrix contains numerous macrophages that undergo oxidative burst during phagocytosis and release reactive metabolites such as superoxide anion, hydrogen peroxide, redox proteins and metalloproteinases. These are antimicrobial substances, however they may compromise host responses by causing oxidative damage of collagen. The oxidised collagen subsequently undergoes proteolysis by metalloproteases. As in the case of microsomal proteins, ascorbic acid apparently prevents collagen oxidation. Once collagen oxidaion is prevented, the subsequent proteolytic degradation of collagen is also prevented. This imparts a specific important role to ascorbic acid for the protection of collagen in the extracellular matrix of tissues. Therefore, a potential and apparently specific role of ascorbic acid is to protect tissues against oxidative damage both at the intracellular and extracellular levels.

Vitamin C also functions as a general water-soluble redox reagent, a regulator of steroid synthesis, a modulator of the hexose monophosphate shunt and an activator of hepatic microsomal hydroxyases. Ascorbic acid plays a role in the transformation and utilisation of folic acid and in the absorption of iron. Owing to the involvement of ascorbic acid in the synthesis of steroid hormones, the vitamin also has an effect on reproduction.

Role of vitamin C on disease resistance and immune response

Ascorbic acid can affect disease resistance in fish in numerous ways. It acts as a biological agent for hydrogen transport and hence involved in a variety of enzyme reactions. It acts synergistically with vitamin E and selenium to maintain activity of glutathione peroxidase and superoxide dismutase. Thus it helps in preventing oxidative damage to neutrophils and other phagocytes during respiratory burst activity, thereby helps to enhance motility and phagocytosis by these cells. It is also a necessary factor in the regulation of the synthesis of corticosteroids. During stress conditions production of cortisol is regulated to check its immunosuppressive action. Vitamin C has also shown to enhance complement activity, inflammatory response, iron metabolism, antibody responses and a variety of other immune functions. Specific effects on a variety of non-specific resistance mechanisms and the specific immune response have also been reported in fish. The wound healing response in relation to ascorbate supplementation of the diet indicates the importance of the ascorbate status of fish as a predisposing factor to disease outbreak in intensive aquaculture, where injuries from handling and rearing techniques are common.

In recent years, considerable research has been directed to determine the role of vitamin C on the disease resistance and immune response in fish. High doses of vitamin C intake have shown to increase the resistance to several bacterial, viral and parasitic pathogens in fish (Lovell and Lim, 1978; Li and Lovell, 1985; Navarre and Halver, 1989; Verlhac and Gabaudan, 1994). The proposed mechanisms of disease resistance vary between species. Vitamin C has been shown to stimulate serum hemolytic complement activity, proliferation of immune cells, phagocytosis, release of signal substances, inflammatory responses and antibody production. However, there are conspicuous conflicts that may possibly be explained by differences in total diet composition, actual tissue levels of ascorbic acid achieved under various experimental designs , differences in antigens used and species differences.

1.2. Vitamin E

Vitamin E is an essential fat-soluble vitamin and primarily functions as antioxidant, both in feedstuffs and *in vivo*. It is the major lipid soluble membrane antioxidant of cells and protects unsaturated fatty acids from oxidation. Fish tend to have a high percentage of the long chained, highly unsaturated fatty acids incorporated into cell membranes to maintain membrane fluidity at low body temperatures. Hence vitamin E is of prime importance in fish nutrition. Studies indicate that vitamin E can act as an immunopotentiator when fed at levels above the daily requirement. It has been shown to affect phagocytosis and humoral and cellular immune responses. Vitamin E enhances proliferation, chemotaxis and bactericidal activity of phagocytes at moderate dosages. However, excessive doses may reduce the intracellular killing ability, if the killing depends on peroxidic damage to engulfed organisms. Vitamin E supplementation stimulates cell proliferation in immunopoietic organs, increases the number of antibody-producing plasma cells and stimulates T helper lymphocytes. The modulation of eicosanoids such as prostaglandin, thromboxane and leukotriene biosynthesis by vitamin E is also well documented and probably plays a key role in immunoregulation.

Though several studies have shown the positive role of vitamin E in immunomodulation of fish, the results are not consistent (Bendich, 1990; Blazer and Wolke, 1984; Hardie et al., 1990). Vitamin E requirement of a species depends on factors such as temperature, dietary levels of unsaturated fatty acids, total fat level, presence of other antioxidants in the feed, dietary selenium and ascorbic acid levels More research is necessary before using high levels of dietary vitamin E as a general immunopotentiator in fish. The interactions of vitamin E with dietary components, such as selenium and unsaturated fatty acids, and their effects on disease resistance need to be evaluated.

I.3. Vitamin A

Vitamin A is a fat-soluble vitamin that is important in maintaining the integrity of epithelial and mucosal surfaces, and in the production of mucous secretions. In homeotherms, vitamin A and carotenoids are known to affect a number of disease resistance mechanisms. Vitamin A influences hematopoiesis of phagocytes and lymphocytes, enhance phagocytosis and intracellular killing, increase production of macrophage activating lymphocytes and specific antibodies.

Vitamin A has been implicated in disease resistance in fish, but has yet to be well studied. It has shown to enhance protective responses such as cell-mediated and mucosal immunity and also the activity of Natural cytotoxic cells (NCC) (Graves et al., 1985)

I.4. B Vitamins

The structurally diverse B vitamins act as enzyme activators and play a key role in carbohydrate, protein and lipid metabolism. An extensive study to evaluate the role of folic acid, pyridoxine, riboflavin and pantothenine acid on the immune function and disease resistance in Chinook salmon, suggested that dietary levels of these vitamins recommended by NRC (1981) for optimum growth were also adequate for optimum immune function (Leith and Kaattari, 1989). Obviously, additional studies are required to determine the role of these vitamins in immune response and disease resistance in fish.

II. Minerals

Numerous minerals, including iron, selenium, copper and zinc have shown to be important in disease resistance and immune responses in homeotherms. These elements are required as coenzymes for metalloenzymes and are vital for maintenance of cellular functions in the immune system of higher vertebrates. Mineral effects on disease resistance in fish have not been well studied. Elements, such as calcium and numerous trace minerals, can be absorbed through the gills as well as from the gut. Hence water concentrations may have a major influence on mineral status and hence confound nutritional studies.

In homeotherms, iron and the iron-binding protein, transferrins were shown to have profound effect on resistance to infectious diseases. Iron is an essential nutrient for fish as well as microbes and ability of pathogens to infect host depends on its availability. Maintaining low concentrations of free iron in mucus membranes and in other tissues is one of the primary non-specific host defenses against bacterial infections. Research on terrestrial animals suggests two different views. One proposes that an iron deficiency protect against infection by limiting the amount of iron available to bacteria. In this interpretation iron supplementation would increase susceptibility to infection. The second view implies that an iron deficiency predisposes animals to infection thus iron supplementation would increase disease resistance. Iron deficiency in fish is not a desirable means of controlling infection because iron deficiency predisposes animals to infection, thus iron supplementation would increase disease resistance.

The influence of a variety of dietary trace minerals has been studied in relation to Bacterial Kidney Disease (BKD) infections in Atlantic salmon. A preliminary study of the nutritional status of BKD infected Atlantic salmon, indicated liver vitamin A and serum zinc, iron and copper levels were significantly reduced when compared to non-infected fish (Paterson et al., 1981). A reduction in the prevalence of lesions was noted in fish that were fed diets high in trace elements and low in calcium. Increased levels of iodine and fluoride (1.5 versus 4.5 mg/kg of each mineral) significantly reduced BKD infections under natural hatchery situations, when other minerals (Mg, Zn, Co, Cu and Fe) were at low levels. The same magnitude of decrease in infections was not observed at high levels of these other minerals. Interpretation of these results is difficult since the host response to BKD is still poorly understood, and no one nutrient was varied. Browser et al.(1988) observed that high levels of dietary fluoride (4, 8 and 16 mg/kg) were effective in reducing mortalities of rainbow trout to BKD under more controlled conditions.

III. Macronutrients and disease resistance

III.1. Protein

Very little research has been conducted on effects of protein and carbohydrate on disease resistance factors in fish. Salomoni *et al.* (1987) compared two isocaloric diets for their effects on immunity of common carp. Humoral antibody response to SRBC were significantly lower in fish fed the low protein (15.2% versus 42.8%) and low fat (5.6% versus 82%) diet compared to the fish fed the control diet. Henken et al. (1987) studied the effects of feeding level on antibody production to *Yersinia rukeri* in African catfish (*Clarias gariepinus).* Fish fed above maintenance levels had higher antibody titers than those fed maintenance level or not fed. Although the protein requirements and their relationship to immunologial function in mammals has been widely studied, only a few attempts have been made to study these interactions in fish. Kiron *et al.* (1995) found that lysozyme activity and C-reactive protein values were reduced in protein deficient rainbow trout.

III.2.Carbohydrate

Cold water fish have a limited ability to utilize carbohydrate for energy purposes and excess carbohydrates are known to produce pathological conditions in the liver and detrimentally affect the natural microbial population in the gut. To date reports on the detrimental effects of carbohydrate on fish health and immune function are not conclusive.

III.3. Lipids

Dietary lipids, particularly certain essential fatty aids are important modulators of the immune response in homeotherms. Dietary lipids strongly influence the fatty acid composition of membrane phospholipids. The fatty acid composition of membranes determines physical properties such as fluidity and permeability. Hence the activity of membrane associated enzymes, membrane receptors, binding of mitogens, antigens and soluble mediators can all be affected by fatty acid composition. In addition the cycloxygenase and lipoxygenase products of fatty aids (prostaglandins, thromboxanes and other eicosanoids) have important roles in immune modulation. Macrophages and other immune cells are the main producers of these eicosanoids.

Although, the relationship between dietary lipid type and immunological functions in terrestrial animals has been studied extensively, only a few attempts have been made to investigate these interactions in aquatic animals. Fish tissues contain relatively higher concentrations of polyunsaturated fatty acids (PUFA) than found in mammals. PUFAs are important components of all cell membranes, which makes fish tissue highly vulnerable to lipid peroxidation. Generally, essential fatty acids (EFA) requirements of freshwater fish can be met by the supply of 18:3 n-3 and 18:2 n-6 fatty acids in diets, whereas the EFA requirement of marine fish can only be met by supplying long-chain PUFA, 20:5n-3 and 22:6n-6 (NRC, 1993). Freshwater fish are able to elongate and desaturate 18:3 n-3 to 22:6n-3, whereas marine fish, which lack or have a very low activity of 5-desaturase, require the long chain PUFA, eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA). The quantitative requirements and deficiency signs of EFA in several freshwater and marine fish have been documented (NRC, 1993), however, the functional role of n-3 and n-6 PUFA in non-specific and specific humoral and cellular immunity is not clearly defined.

Twenty-carbon PUFAs derived from EFA are precursors of the eicosanoids, prostaglandins and leucotrienes with diverse pathophysiological actions including immune response and inflammatory processes. Eicosanoids are synthesized from linolenic acid (20:3 n-6), arachidonic acid (20:4, n-6) and eicosapentaenoic acid (20:5,n-3), by the action of two oxygenase enzymes, cycloxygenase and lipoxygenase. Prostaglandins and leucotrienes constitute a group of extracellular mediator molecules that are part of an organisms defense system. They are formed during the inflammatory process, and if the inflammation is caused by invading bacteria, the formation of prostaglandins and leucotrienes will stimulate macrophages and other leucocytes to begin the process of destroying the bacteria. Eicosanoids may be involved in the regulation of the immune system by their direct effects on cells such as macrophages and lymphocytes or their indirect effects *via* cytokines. The nature of dietary lipids and the concentration of essential fatty acids have a direct effect on the eicosanoid metabolism and immune function.

Dietary fat type and amount have been shown to affect prostaglandin synthesis, mitogenic responses to T lymphocytes, humoral immune response and macrophage phagocytosis in homeotherms. Sheldon and Blazer (1991) investigated the influence of dietary lipid on bactericidal activities of channel catfish macrophages. They observed that bactericidal activity was positively correlated with the level of long-chained, highly unsaturated fatty acids in the diet. Despite many conflicting reports on the effects of n-3 PUFAs on the immune response of fish, n-3 enriched diets improve the stability of cell membranes (Erdal et al., 1991; Kligler et al., 1996). This effect is particularly important in aquatic animals, where it is essential to maintain proper membrane function during the wide fluctuations in water temperatures. Channel catfish fed diets with high levels of n-3 fatty acids showed enhanced immune function (especially phagocytic capacity) at low temperatures, while fish fed high dietary levels of n-6 fatty acids enhanced disease resistance factors at high temperatures (Lingenfelser et al., 1995). The physiological effects of water temperatures may be due to the function of n-3 fatty acids in maintaining membrane fluidity, which is considered important during the ingestion stage of phagocytosis.

However, reports on the effect of n-3 and n-6 fatty acids on immune response and eicosanoid production in fish are not conclusive and very often contradictory. More research is needed to clearly establish the role of dietary lipids including marine fish and vegetable oils on immune response and disease resistance in fish. Potential exists to replace marine fish oils (MFO) with alternate lipids of plant and/or animal origin in feed of farmed fish, because the global supply of MFO is becoming increasingly limited. Inorder to replace MFO with alternate lipid sources, it is important to establish that the dietary lipid supply is not only at the correct level with the proper balance of EFA for optimum growth and feed utilization but it can also maintain proper immune function and prevent infectious diseases in farmed fish.

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