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Role of vitamins C and E in immune response of fishes

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The immune system of animals is a biochemical complex of structure (lymphocytes, T-cells and B-cells, Kupffer's cell of the liver and leukocytes) and processes (formation of antigens and antibodies, phagocytosis, others) to protect against foreign bodies like tumors and toxins.

In the case of fish, the immune responses are influenced by many factors, including stress, hormonal changes, seasonal effects, intercurrent infections, drugs and environmental pollutants. Fishes are protected from infections, transformed cells and other invaders by several interdependent mechanisms.

Nutrition is one of the factors enabling the host to resist diseases by antibody synthesis and cellular immune response. Cells involved in the generation of specific and non-specific immune responses are metabolically activated and are most likely affected by vitamin deficiences. For example, the break down of the first barrier against infection -- the skin -- may be due to deficiencies in protein, zinc and the vitamins A and C.

Nutritional immunology with regard to fishes as a separate discipline is a very new concept although a considerable amount of studies has been done in human cases and land animal models. But it may be the incomplete knowledge about the nutritional requirements of different fishes that had led to the neglect of this field. Vitamins are the most important micronutrients, the deficiencies or excessiveness of which has profound impact on disease development and survival of cultured fishes.

Alterations in immune responses occur when a decrease in micronutritional intake causes a corresponding decrease in body reserves. Immunological abnormalities predict the risk of infection and mortalities. Excessive intake of micronutrients is associated with impaired immune response. Most of the studies on fishes have been carried out for high or pharmacological doses of vitamins, and are restricted only to vitamins C and E. These vitamins are the most important antioxidants in the diet (Lall & Oliver 1991). Tests for immunocompetence are important so safe levels can be established.

Non-specific and specific adoptive elements have shown in-vitro activity associated with resistance against pathogens. But several immune functions have not been characterized.

Vitamins and fish immunity

Research on vitamin nutrition in relation to the immunity in fishes is very limited (Lall & Oliver 1991). Among the vitamins, the antioxidants (vitamins C and E) are found to have an impact on the immune system of fishes (Furanose *et al.* 1992, Verlhac *et al.* 1996).

B-complex vitamins are involved in cellular metabolism by acting either as enzyme activators or coenzymes (thiamin, riboflavin, pantothenic acid, folic acid, niacin, pyridoxine and cyanocobalamine).

Significant modulations in the immune system were found on chinook salmon (*Oncorhynchus tshavyscha*) fed different levels of pyridoxine (Leith *et al.* 1986). However, enhanced levels of vitamins could not elucidate any effect on the immune system of Atlantic salmon (Albrektsen *el al.* 1995). The effect of folic acid on the disease resistance and its interaction with ascorbic acid were proven in channel catfish (*Ictalurus punctatus*). Serum antibody titres on bacterial challenge studies revealed a direct relationship between dietary folic acid content and disease resistance. When enhanced levels of vitamin A were fed to Atlantic salmon, its kidney leukocyte migration and serum bacterial activity were found to be increased, whereas the phagocyte burst activity and eicosanoid production were unaffected (Thompson *et al.* 1994)

Two types of the non-specific defense mechanism are noted in fishes. The first is the complement system consisting of a series of proteins which can be activated as an alternative or a classical pathway. The alternative pathway is non-specific, achieved by various structures possessing repeating units, whereas the classical pathway is the activation of the complement system specifically by the antibodies attached to surface antigens.

The second important non-specific defense mechanism is phagocytosis, a cellular process involving the ingestion of foreign material by specialized cells, such as macrophages and the neutrophils or polymorphonuclear neutrophils.

Basically, specific immunity is characterized by the initial interaction between antigens and lymphocytes. Two populations of lymphocytes are recognized: B-lymphocytes and T-lymphocytes. Both are responsible for humoral and cellular immunity, respectively, whose memory component has been demonstrated in fish.

Humoral immunity is mediated by B-lymphocytes which respond to stimulation with a variety of antigens. B-lymphocytes transform into plasmocytes which produce antibodies specific for the stimulating an-

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tigen. The humoral response is dependent on the nature, dose and route of antigen exposure. Antibodies can also interact with the non-specific immune system by activating the classical pathway of the complement system or by acting as opsonins. Cell-mediated immunity is dependent on the presence of T-lymphocytes and this type of immunity can be transferred passively with T-lymphocytes only and not with serum. It can be activated by using certain adjuvant and intercellular pathogens given as attenuated live vaccines.

Vitamins and immune responses

Panush & Delafuente (1985) reviewed the biological functions of vitamin C in living organisms as a general water soluble redox reagent, cofactor in collagen synthesis, growth activator, regulator of hormone synthesis, modular of hexose monophosphate shunt and indicator of hepaticmicrosomal hydroxylase and as a immunostimulator in fish.

In-vitro studies have demonstrated that vitamin C stimulates the production of interferon and enhances the chemotactic response of neutrophils. The proliferative response of T- and B-lymphocytes including specific antibody production may be also modulated. Several investigations have demonstrated that activities of phagocyte cells, such as random migration, chemotaxis production of hypochlorous acid and modulation of auto-oxidation are all dependent upon ascorbate concentration. Elevated level of ascorbic acid enhances the serum complement activity (Lall & Oliver 1991).

Experimental studies revealed that the effect of vitamin on the immune function of fish is unclear but there is evidence that some non-specific rather than specific immunity may not be directly influenced by dietary vitamin C intake.

Dietary vitamin C exhibits protective effects on pesticide intoxification of both organochlorine and organophospharous compounds. It can antagonize when administered at high dose (Guha *et al.* 1993). Enhancement of phagocytic ability of potential macrophages and circulating neutrophils was observed when vitamin C was fed to channel catfish. There was increased hemolytic activity of complement system when ascorbic acid was supplied at 300 mg per kg of feed (Li & Lovell 1985). When elevated level of vitamin C was fed to channel catfish, there was lower incidence of malformations. Channel catfish were also found to be more resistant to ammonia toxicity and lower levels of dissolved oxygen (Mazik *et al.* 1987). According to Wise *et al.* (1988), an ameliorative effect of ascorbic acid on the nitrate-induced methanoglobenemia in channel catfish was noticed with enhanced feeding levels of vitamin C. Channel catfish showed hundred-fold LC_{50} values than control whereas complement and hemolytic activities were unaffected (Liu *et al.* 1989). Significant increase in antibody levels was obtained with vitamin C supplementation in rainbow trout diet (Navarre & Halver 1989). High levels of antibody production and complement activity in Atlantic salmon could be increased by enhanced levels of vitamin C but failed to produce any effect on the non-specific resistance to vibriosis (Lall *et al.* 1991). So, vitamin C has got beneficial effect on the teleost immune system. The mode of action may be by acting as a break on steriodogenesis through peroxidation of unsaturated lipids, thereby preventing their conversion to cholesterol, an important component of cortisol.

The effect of antioxidant vitamins, i.e. vitamin C, on the immune system is through the tertiary level of stress reactions, with the most marked effect being the elevation of serum complement levels (Hardie *et al.* 1991). The effect may also be apparent at secondary levels of stress reactions. In rainbow trout, increased level vitamin C caused increased complement activity and elevated phagocytic activity by peritoneal macrophages. Higher proliferation of lymphocytes was induced by concavalin A (Verlhac *et al.* 1991). According to Duncan & Lovell (1994), channel catfish show maximum survival and antibody production and minimum blood abnormalities when ascorbic and folic acids were incorporated in the diet.

Verlhac *et al.* (1996) reported that combination of vitamin C with glucan has a stimulatory effect on non-specific parameters except plasma lysozyme.

There are some contradicting reports about vitamin C supplementation and immunomodulation. Elevated ascorbic acid failed to produce any improvement in phagocytic index and bacterial capacities of anterior kidney in channel catfish (Johnson & Ainsworth 1991).

Similarly, vitamin C is required for optimum function of the immune system in homeotherms. It decreases the production of lipid peroxide and reactive oxygen, which are autotoxic and destroy neutrophils and macrophages. Vitamin C functions in the maintenance of membrane integrity in all cells. The phospholipids of mitochondria, the endoplasmic reticulum, and plasma membranes possess special affinities for a-tocopherol.

Blazer & Wolie (1984) reported that the a-tocopherol deficiency suppresses all aspects of humoral and cellular immunity as well as phagocytic index of rainbow trout. Later works revealed that the effect of vitamin e is only on non-specific humoral factors (Hardie *et al.* 1990).

Among blood parameters, only complement activity showed positive correlation with vitamin E consumption.

Epidemiological studies showed that high plasma vitamin E levels can be correlated with the low incidence of infectious diseases but it is very difficult to differentiated the specific action of vitamin E from other dietary antioxidants (Eitenmiller 1997). Furanes *et al.* (1992) reported a reduced mortality of rainbow trout even though the exact mechanism was not fully described. The serum antibody levels were found to be unaffected.

Higher levels of vitamin E had more protective effect on the RBC membrane against peroxidant induced lysis (Wise *et al.* 1993). In salmonids, the higher vitamin E supplementation had an enhancement of phytohaemagglutinin (PHA) and lipopolysaccharide-induced lymphocyte proliferation (Verlhac 1991).

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The safety level of vitamin E has to be determined before administration (Waagbo 1994). According to Pulsford *et al.* (1995), the phagocytic activity of kidney macrophages in flat fishes were enhanced when the fishes were fed higher amounts of vitamin E.

Conclusion

Still no clear conclusions can be drawn with respect to vitamin nutrition and fish immunity. So, attention should be given to improve the earlier recommended levels for different vitamins.

- Testing with different immune functions must include the mechanism by which a single nutrient accelerates different biological functions
- Safety levels of different vitamins must be determined before administration
- For antioxidant vitamins, care should be taken to avoid losses due to atmospheric oxidation and water leaching by using the recommended type such as phosphate esters of ascorbic acid
- More work is needed for vitamins C and E supplementation beyond minimum dietary requirements to clarify the benefits to fish health. So, metabolism of nutritional C and E forms needs further investigative biochemistry

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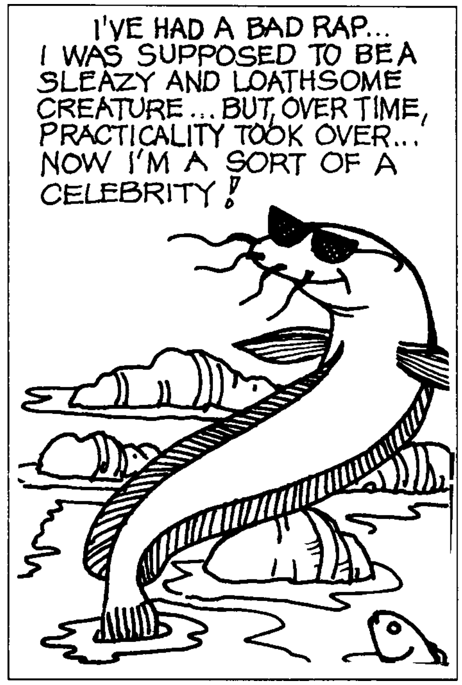

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periods and possibly even during the dry period, as these burrow deep into the soil.

A female GAS can lay 25-320 eggs at one time. Incubation ranges from 10 to 15 days, and, depending on the temperature of the microenvironment, GAS normally lives from two to three years. The GAS do not feed only on rice. They also damage many cultivated and non-cultivated plants such a lotus (*Nelumbo nucifera*), taro (*Colocasia esculenta*), duckweed (*Lenna minor* L.), swamp cabbage (*Ipomoea aquaica*), mat rush (*Juncus decipiens*), water chestnut (*Trapa bicornis*), and water fern (*Azolla* spp.). It has a wide range of possible hosts and food substrate such as commercial livestock feeds, decaying matter, animal flesh, and other important crops. Because of this, GAS is difficult to control. Compounding this problem is the occurrence of heavy rains and the application of pesticides that kill a large number of beneficial organisms, particularly in and around the rice ecosystem.

TABLE 1 Volume of molluscicides purchased, 1980-1998, Philippines

YEAR	VOLUME (kg per ha)
1998	67,340
1997	241,683
1996	130,000
1992	180
1991	159
1990	0
1989	25
1988	64
1987	9
1986	6
1985	3
1984	3
1983	0
1982	0
1981	0
1980	0

Note: Does not include 1993-1995 data
Source: Fertilizer and Pesticide Authority, 1999

Molluscicides have been widely used to control GAS but these can also kill non-pest snails and other beneficial organisms. From 1980 to 1988, the volume of molluscicides purchased increased (Table 1). The biggest volume recorded was in 1997, when about 241,683 kg per ha were purchased. The country has already spent about US$23 million from 1980 to 1998 for molluscicides (Fig. 1).

The Strategic Extension Campaign launched in 1989 by the Food and Agriculture Organization (FAO) of the United Nations, Visayas State College of Agriculture (VISCA), International Rice Research Institute (IRRI) and DA-PhilRice introduced non-chemical methods such as pasturing ducks in rice fields after harvest, handpicking, destroying egg clusters before final harrowing, transplanting older seedlings, and installation of screens in water inlets. These practices however, remain untested in the rainfed, direct-seeded, and

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