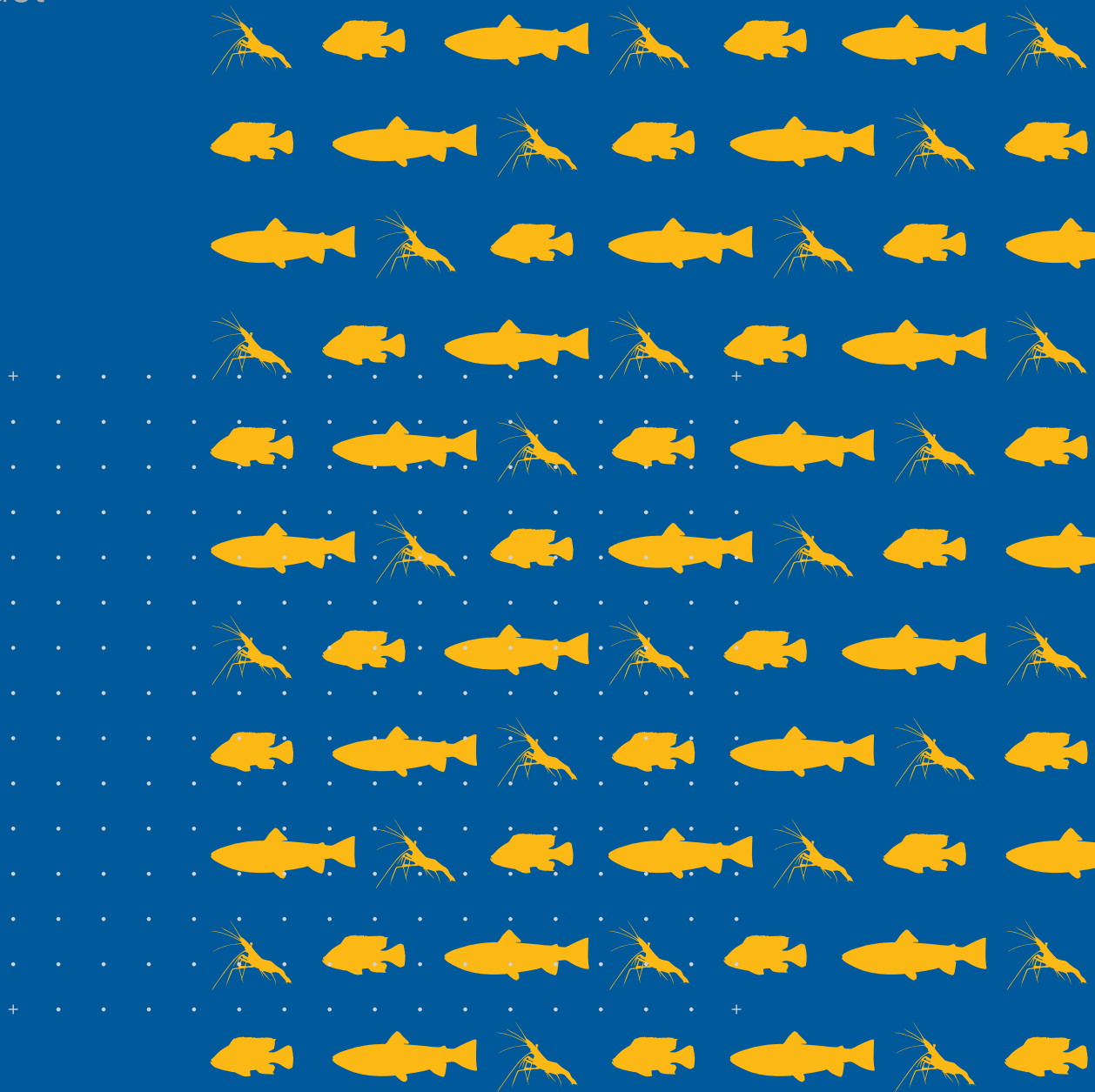


The effect of vitamin C

on fish health

a **DSM** Product





The effect of vitamin C

on fish health



Content

1. Introduction	5
------------------------	----------

2. The immune system of fish	6
2.1 Introduction	6
2.2 The non-specific immune response	7
2.3 The specific immune response	14
2.4 Factors influencing the immune response	15

3. Vitamin C and health status	17
3.1 Vitamin C as a nutritional factor	17
3.2 Vitamin C and the immune response	20
The non-specific immune response	21
The specific immune response	24
3.3 Vitamin C and resistance to disease	27

4. Practical guidelines for vitamin C administration	29
---	-----------

References and bibliography	31
------------------------------------	-----------

1. Introduction

The goals of the aquaculture industry are to optimize growth and to produce high-quality fish. As in all farming, the outbreak of diseases in fish farming can be a major concern. The high susceptibility of fish to stress and the rapid spread of diseases in water have forced aquaculturists to concentrate their efforts on maintaining their fish in good health in order to achieve sustainable economic performances.

Growing healthy fish requires them to be able to develop strong defence mechanisms against pathogen invasion. These are the non-specific and the specific immune response. The non-specific immune response is more important in fish than it is in mammals. Improving the immune response leads to a better vaccination efficiency. Vaccines induce a specific immune response and an increased capacity to kill the pathogens by non-specific defence mechanisms.

Several years ago, during the early development of the salmon industry, antibiotics were commonly used in the treatment of diseases. However, the consumption of drugs has progressively been reduced owing in part to environmental and regulatory concerns and in part to increased resistance of pathogens. Furthermore, the curative effect of oral drugs is minimised by the fact that diseased fish frequently do not feed. The eradication of major diseases by improved husbandry and vaccination

has reduced mortality levels considerably. Although some efficient vaccines against major diseases of finfish are available today, others need to be improved in terms of efficiency and duration of protection. Vaccines against lethal diseases such as VHS and BKD have not yet been developed.

Intensively raised fish may be exposed to stressful situations which often result in a depressed immune status. Good management practices reduce stress and therefore help to maintain healthy animals. However, since not all stressing situations can be avoided, fish with enhanced defence mechanisms will be better prepared to combat the negative effects of stress.

The nutritional quality of the feed is a major factor in sustaining healthy fish. It has been shown that the immune system can be enhanced by the use of immunomodulators such as antioxidant vitamins, carotenoids and other feed additives.

The combination of good management, vaccination and nutritional prophylaxis will insure higher survival rates and improve growth in intensive farming systems (Figure 1).

This publication highlights the importance of vitamin C as an immunomodulator and a key nutritional element in modern fish farming in promoting optimal survival and performance.

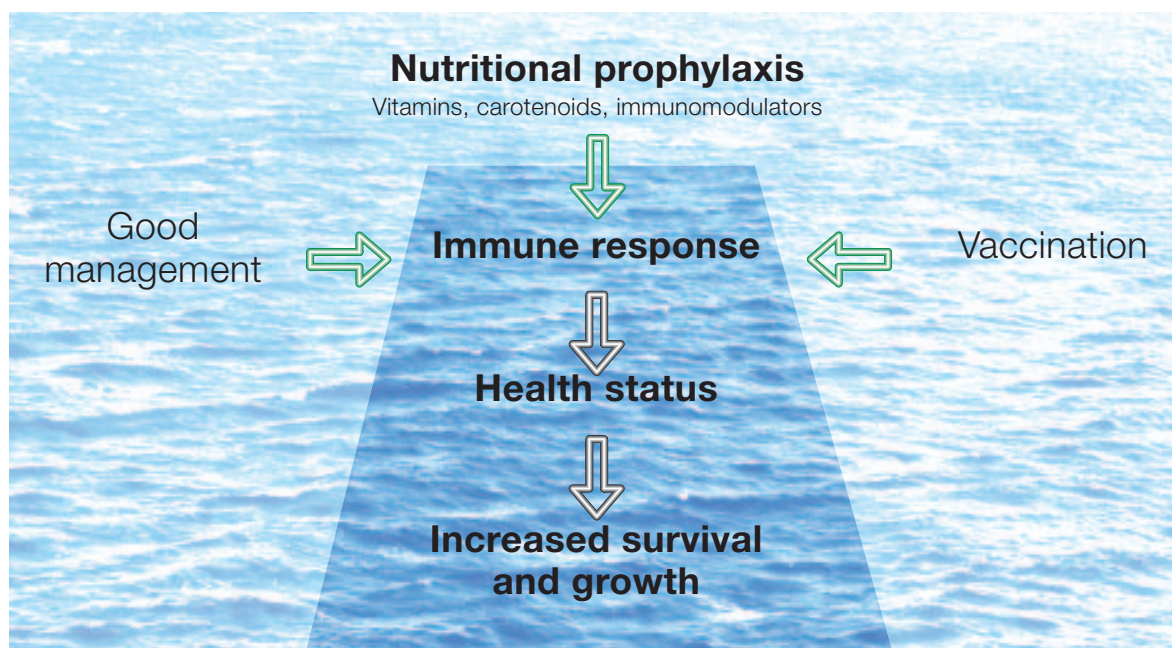


Figure 1:
Benefits of
nutritional prophylaxis
in aquaculture.

2. The immune system of fish

2.1 Introduction

Fish are the most primitive vertebrates and are an important link between invertebrates and higher vertebrates. They possess the non-specific defence mechanisms of the invertebrates such as the phagocytic mechanisms developed by macrophages and granular leukocytes, but were also the first animals to develop both cellular and humoral immune responses mediated by lymphocytes. The main lymphoid organs of fish are the anterior kidney, the thymus and the spleen. In fish, non-specific immunity is considered as the first line of defence and represents a considerable part of the immune response, in contrast to mammals.

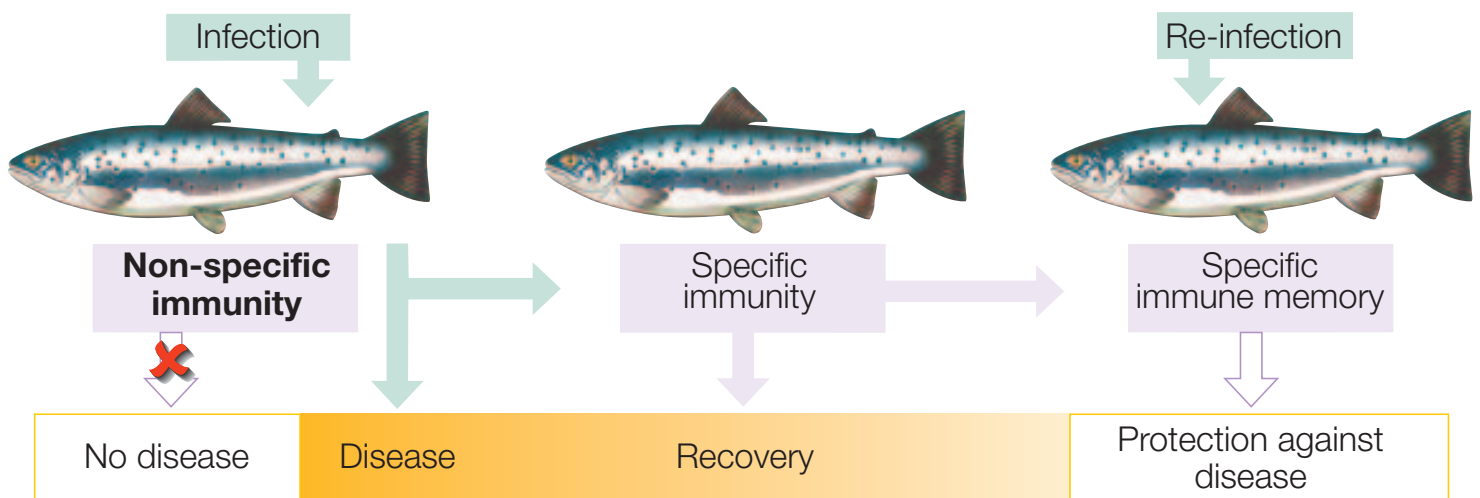
When a pathogen penetrates the body, the non-specific immune mechanisms may be sufficient to stop the infection. If not, the disease will develop and the specific immune mechanisms will also be involved. If the animal

survives, it will be protected against re-infection by the same pathogen, owing to the development of a specific immunological memory. The immune memory in fish is less developed than in mammals (Figure 2).

Non-specific immune mechanisms, defined also as natural or innate immunity, are host-defence mechanisms which do not require a specific recognition of the antigen as occurs in humoral and cell-mediated responses of the specific defence (also called adaptive immunity).

However, some functions of the non-specific response are also involved in the specific response.

Figure 2:
Non-specific and specific immune responses in defence and protection of fish against infection.



Adapted from Roitt et al., 1989



2.2 The non-specific immune response

Table 1 presents the mechanisms involved in the non-specific immune response. The primary line of defence is the skin and mucus. When pathogens enter the body, cellular and humoral non-specific defence mechanisms are involved. The most important cells involved in this defence are the phagocytes. They are helped by several

soluble factors such as complement and lysozyme. There are also minor cell mechanisms (natural killer cells) and a battery of soluble factors that intervene at various levels.

Non-specific immune response

Natural barriers: Skin and mucus

When micro-organisms penetrate the body:

Cellular mechanisms:

- Macrophage and neutrophil activities:
 - Chemotaxis
 - Phagocytosis
 - Pinocytosis
- Killing:
 - oxygen-dependent mechanisms
 - oxygen-independent mechanisms
- Extracellular killing:
 - Natural killer cells
 - Eosinophils

Soluble factors:

- Complement
- Lysozyme
- C-reactive protein
- Transferrin
- Lactoferrin
- Ceruloplasmin
- Lectins, natural agglutinins
- Interferons

Table 1:
The mechanisms involved in the non-specific defence system of fish.

Natural barriers

Skin and mucus

Fish have adapted to their aquatic environment by developing efficient physical and chemical barriers such as skin and mucus as a first line of defence. The skin represents an important non-specific defence mechanism to prevent micro-organisms from entering to the body. The integrity of the skin is of great importance. Wound healing is therefore much faster than in mammals.

Another important barrier is the mucus, which helps in preventing micro-organisms from entering to the body through the skin, gills and gastrointestinal mucosa. The mucus prevents bacteria from adhering to epithelial cells. Furthermore, several components of the non-specific immune response are found in the mucus, emphasizing its importance as a first defence mechanism (i.e. natural antibodies, lysozyme, lysins, complement).

>>

Figure 3:
Rainbow trout infected
with the parasite
Ichthyophthirius multi-
filii.

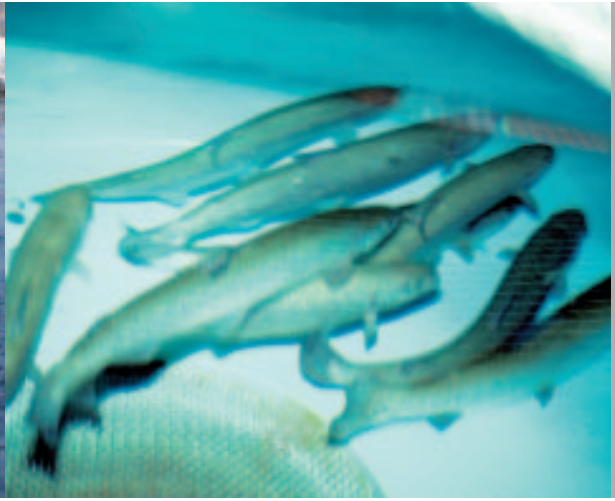
Source: T. Wahli and
W. Meier, Fish Disease
Laboratory, University of
Berne, Switzerland.



<

Figure 4:
Healthy trout.





When micro-organisms penetrate the body

When micro-organisms penetrate the body, the non-specific immune system reacts with cellular mechanisms and soluble factors.

Cellular mechanisms

Cells involved in non-specific defence mechanisms are mainly phagocytes (monocytes/macrophages and granular leukocytes such as neutrophils). Phagocytosis is the most primitive defence mechanism. Originally a nutritive function in lower life forms, phagocytosis has evolved to become solely a protective function in vertebrates. Natural killer cells and eosinophils are also involved and act via an extracellular killing mechanism.

Macrophage and neutrophil activities

Chemotaxis is the process by which phagocytic cells are attracted by various molecules and migrate to the sites of inflammation, tissue damage or immune reactions. Molecules known as chemotactic inducers are

either produced by the bacteria or are components of the immune system, e.g. complement factors. Migration inhibiting factors block the further migration of phagocytes when they are active at a site of infection.

Phagocytosis occurs when bacteria have adhered to the surface of the phagocyte. It involves recognition and attachment of a foreign particle, engulfment and digestion.

A particle attached to the surface membrane initiates the ingestion phase by activating an actin-myosin contractile system which extends pseudopods around it. As adjacent receptors attach to the surface of the microbe, the plasma membrane is pulled around the particle until it is completely enclosed in a vacuole (phagosome). Then, cytoplasmic granules fuse with the phagosome and discharge their contents around the micro-organism, which is subjected to a considerable battery of micro-bicidal mechanisms. An overview of the mechanisms involved in the phagocytosis is given in Figures 5 and 6.

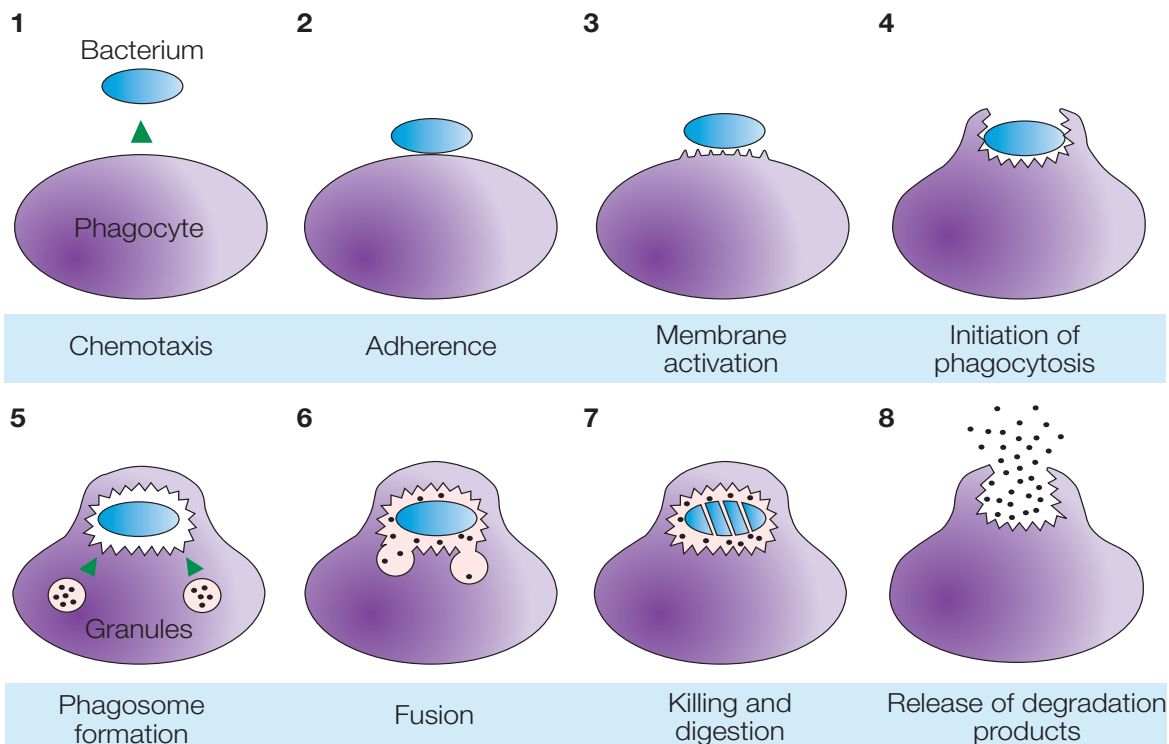
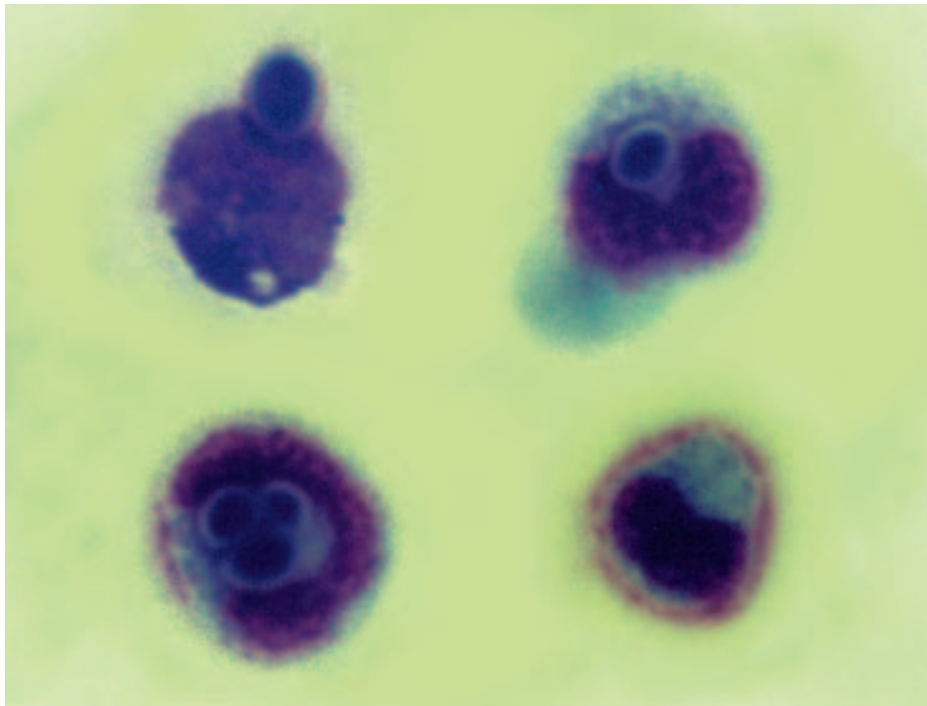


Figure 5: Phagocytosis and killing of a bacterium.

Source: Roitt, 1988

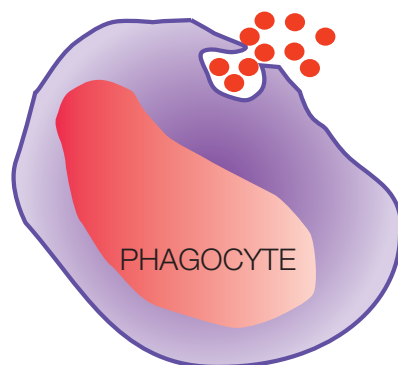
Figure 6:
Macrophages
ingesting yeasts
presented at different
steps of the phagocy-
tosis process.



Pinocytosis is a mechanism comparable to phagocytosis but which is used in the internalisation of particles smaller than for the phagocytosis. This phenomenon is

characterised by the invagination of the membrane to form a small vacuole by endocytosis (Figure 7).

Figure 7:
Vacuole formation by
endocytosis during
pinocytosis of small
particles.





Killing: The killing of bacteria is characterised by oxygen-dependent and oxygen-independent mechanisms.

Oxygen-dependent mechanisms (Figure 8): When phagocytosis is initiated in case of a bacterial invasion, there is a dramatic increase in the molecular oxygen consumption by the phagocytic cells called the oxidative burst. This oxygen is converted via the NADPH oxidase system into superoxide anions, hydrogen peroxides, singlet oxygen and hydroxyl radicals which are powerful microbicidal agents. Furthermore, the combination of peroxides, myeloperoxidase and halide anions constitute a potent halogenating system capable of killing both bacteria and viruses.

Oxygen-independent mechanisms: Low pH, lysozyme and lactoferrin constitute bactericidal and bacteriostatic factors which are oxygen-independent and can function under anaerobic circumstances. Finally, proteolytic enzymes and a variety of other hydrolytic enzymes digest the killed organisms and degradation products are released to the exterior.

Fish granulocytes kill extracellularly through the discharge of their hydrolytic and oxidising enzymes rather than intracellularly via phagosome-lysosome fusion as occurs in mammals. In some fish species, thrombocytes have also been demonstrated to have phagocytic properties.

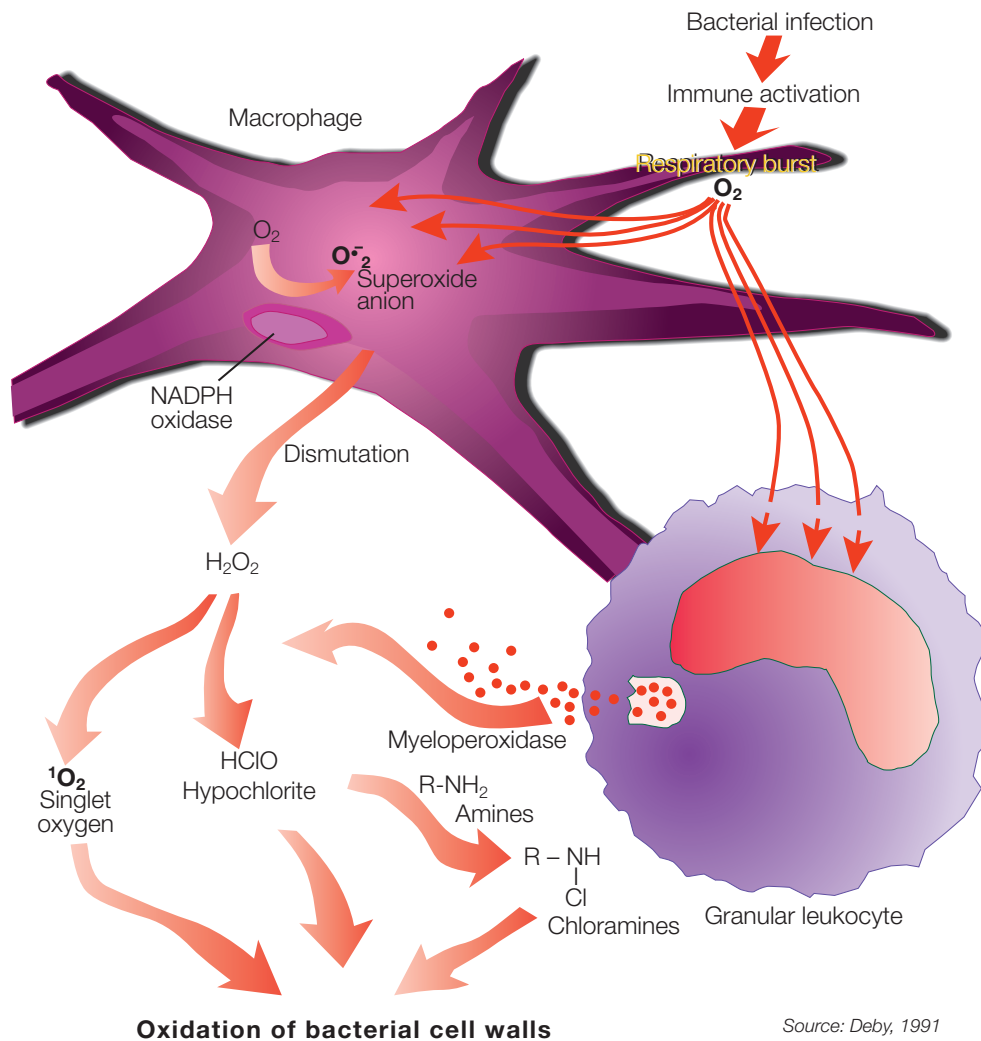


Figure 8: Oxygen-dependent mechanisms leading to bacterial oxidations. The production of reactive oxygen species is one of the weapons that macrophages and granular leukocytes use to fight an infection. The NADPH-oxidase system located in the membranes of these cells is activated in response to the penetration of the pathogen in the organism. This enzymatic system reduces the oxygen in superoxide anion, which produces by dismutation hydrogen peroxide. The release of myeloperoxidase by granular leukocytes transforms hydrogen peroxides into hypochlorite leading to the production of chloramines and singlet oxygen. All these reactive oxygen species derived from hydrogen peroxide are highly oxidant for bacteria and therefore very active at destroying them.

Extracellular killing

Extracellular killing by natural cytotoxic cells and eosinophilic granulocytes are minor cellular mechanisms compared to phagocytosis. Natural cytotoxic cells have antitumoral and antiviral effects while eosinophils are required for the killing of large pathogens.

Natural cytotoxic cells are involved in the killing of virus-infected cells and act through the release of granules which will disrupt the membrane of the infected cells and therefore inhibit the replication of the virus.

Eosinophils are required for the killing of large pathogens such as some parasites which are too large to be eliminated through a phagocytic process.

Soluble factors

Complement: The complement system consists of a group of protein and non-protein components which are involved in both non-specific and specific defence mechanisms.

The complement system can be activated along two different pathways:

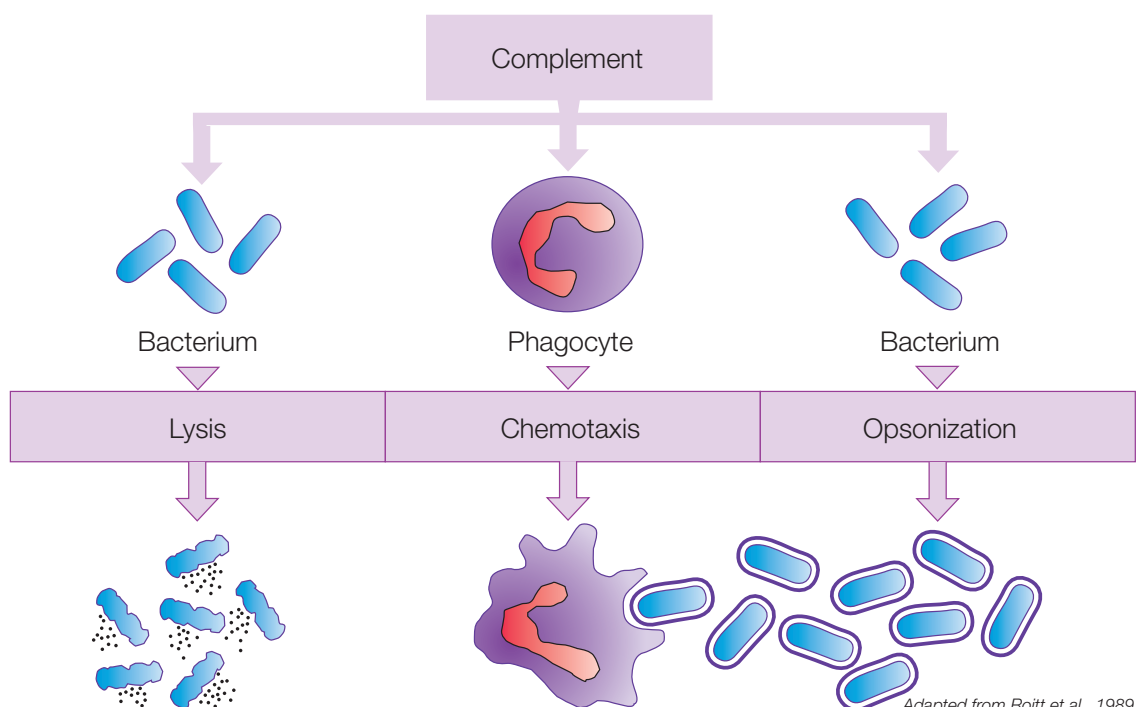
- the alternative pathway which is initiated by contact with certain microbial cell wall polysaccharides. It is related to non-specific immunity.
- the classical pathway related to specific immunity will be detailed in the related section.

Both pathways of complement activation involve a cascade of enzymatic reactions resulting in the opsonisation and/or lysis of foreign cells by disruption of cell membranes.

The alternative pathway allows the elimination of invading pathogens without the presence of antibodies. The functions of the complement system related to non-specific defence mechanisms are presented in Figure 9. The complement allows the lysis of cell membranes from numerous bacterial species. Some components released following activation of the alternative pathway influence the migration of phagocytic cells towards the site of infection. This function is called chemotaxis. Other components cover the bacteria and facilitate their adherence to phagocytes and their subsequent killing by these cells. The phenomenon is called opsonisation.

Figure 9:
Functions of the complement system in the non-specific immune defences:

The complement system is involved in the lysis of bacterial cell membranes. Some components are also able to attract phagocytes towards the infection site (chemotaxis) while others facilitate the adhesion of bacteria to the phagocytic cells (opsonisation). Those functions related to non-specific immunity are also initiated by specific immune mechanisms.





Lysozyme is an enzyme which is able to split mucopolysaccharides from bacterial cell walls and therefore leads to the destruction of pathogens. Lysozyme is mainly produced by phagocytic cells. It is found in the blood stream and is produced mainly in relation to the development of an infection. The destruction of bacterial cell walls by lysozyme facilitates their attack by the complement system.

Other soluble factors include C-reactive protein, transferrin, lactoferrin, ceruloplasmin, lectins, natural agglutinins and interferons. They are involved in non-specific defence mechanisms of fish: C-reactive protein (CRP) is an acute-phase protein found in the serum which increases rapidly upon exposure to bacterial pathogens. CRP reacts with molecules at the cell surface of microorganisms. CRP acts as an opsonisation factor to facilitate phagocytosis or activate the complement system. Transferrin has a protective role in fish. It is an iron-binding protein which limits the amount of free iron in the bloodstream, thus making it unavailable for bacteria during infection. Lactoferrin produced by neutrophils has a similar role to transferrin. Ceruloplasmin is an acute-phase protein in mammal inflammatory processes, also described in fish. Lectins or natural agglutinins are important in neutralising bacterial components

released by pathogens such as exotoxins or in immobilising microorganisms. Hence, they facilitate phagocytosis. Interferons are proteins produced during viral infections and increase the resistance of cells to viral invasion.

Most of the soluble factors are acute-phase proteins. Their concentration could increase up to 100-fold following an infection (Figure 10).

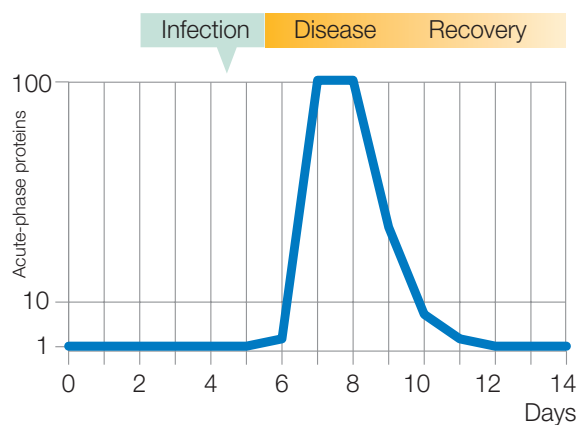


Figure 10: The **acute-phase** proteins are substances found in the plasma whose concentrations increase dramatically following an infection. Adapted from Roitt et al., 1989.



2.3 The specific immune response

When an infectious agent penetrates the organism, non-specific defence mechanisms are stimulated. Their sole activation might be sufficient to stop the infection. If not, the disease will develop, leading to the induction of specific defence mechanisms. These will then lead to the cure of the disease and the set-up of an immunological memory, blocking the development of a new infection caused by the same pathogen.

One example of the development of specific immune response is vaccination. The pathogen is introduced to the organism in an attenuated way or killed in order to avoid the outbreak of the disease but still with the capacity to initiate a specific immune response. This will protect the organism for a certain period of time.

The cells involved in the specific immune response leading to the production of antibodies are macrophages serving as antigen-presenting cells and lymphocytes composed of two populations, T- and B-like lymphocytes. T lymphocytes have a role in cellular cooperation (cell-mediated immunity) and B lymphocytes are the antibody producers.

At the start of the antibody response, there is a certain delay before the first specific antibodies appear in the

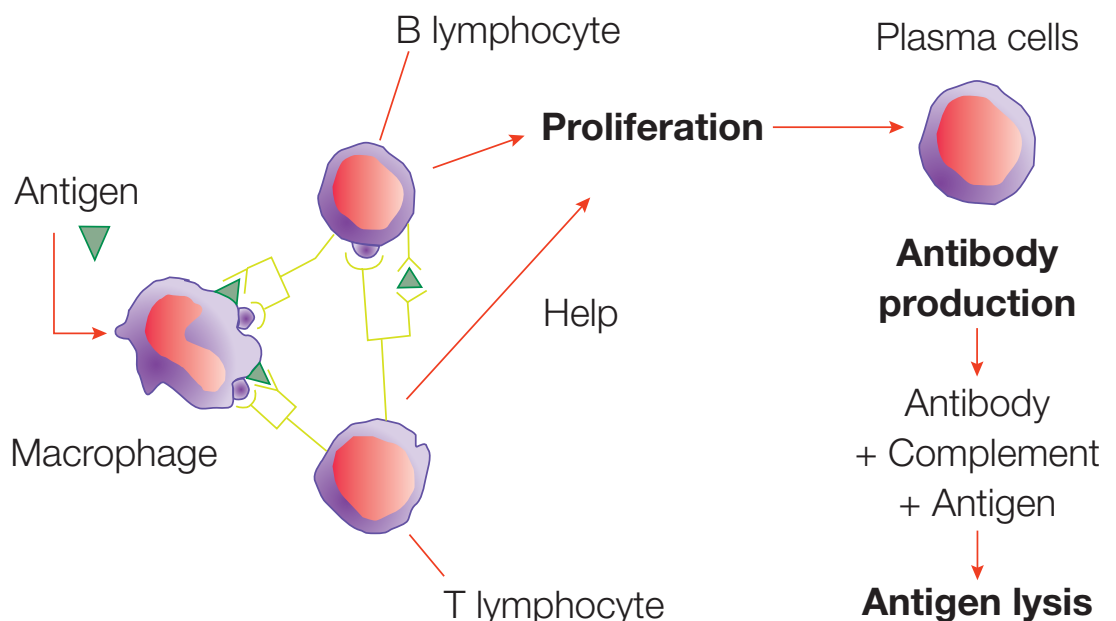
blood stream. During this lag phase, the antigens are processed and cellular co-operation between antigen-presenting cells and lymphocytes occurs.

The macrophages act as antigen-presenting cells in the specific immune response. Their role is to process the antigen and to present the processed antigenic determinants in association with recognition molecules to the lymphocytes.

Subsequently T lymphocytes are activated by interaction with the antigenic determinants and factors secreted by the macrophages (interleukin). The activated T lymphocyte also called helper cells stimulate the differentiation and proliferation of B lymphocytes by secretion of interleukins.

Depending on the circumstances, B lymphocytes will develop into long-lived B memory cells or short-lived plasmocytes. These plasmocytes secrete huge amounts of specific antibodies (immunoglobulins) of type M. These antibodies will bind or kill invading microorganisms presenting the corresponding determinants (Figure 11).

Figure 11:
Specific immune response: cellular co-operation and antibody production.





The complement is involved in the specific immune response through its classical pathway of activation. The antibodies will stick to the membrane of the pathogen. The activation of the complement system is required in order to

process the destruction of the pathogen. This classical pathway of activation of the complement system requires the contact of the antibody with the membrane of the antigen in order to be initiated.

2.4 Factors influencing the immune response

Many factors can influence the immune response of fish. Among them are stressors and environmental factors of natural origin. Nutrients, micronutrients and substances of no nutritional values can also modulate the immune response. Depending on their type, the amount and the duration of exposure, their effect can be either negative

or positive. Substances with immunostimulating properties can compensate the immunodepression caused by other factors, e.g. the immunodepression caused by a stressor can be compensated by an increased intake of vitamin C before a predictable stress event such as grading (Figure 12).

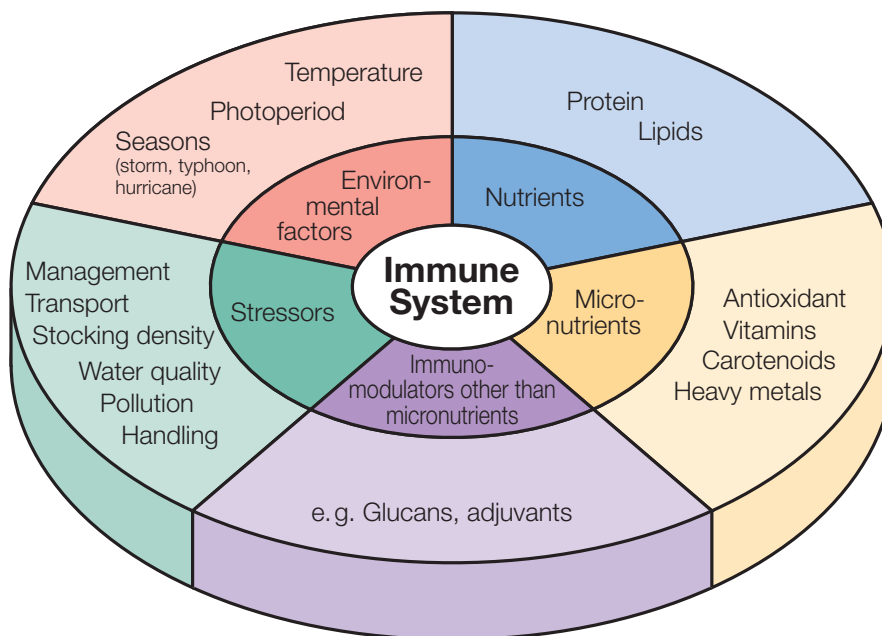


Figure 12:
Factors influencing the immune response.



- **Temperature:** Fish are poikilotherms. Their physiological processes are influenced by temperature. Major defence mechanisms are temperature-dependent and develop faster at the optimal temperature of the fish species concerned. Low temperatures are known to slow down all the metabolic processes including the immune ones. However, high temperatures can also depress the immune functions.
It would appear that antigen processing and cellular co-operation between macrophages and lymphocytes are temperature-sensitive. The normal function of fish lymphocytes is highly dependent on homoviscous adaptation of membrane lipids. Fatty acid composition and environmental temperature are factors determining the fluidity and permeability of membranes as well as the activity of membrane-associated receptors and enzymes.
- **Stress conditions** influence the health status of the fish. Immunodepression is known to be a major secondary effect in the response of an organism to stress. Many situations such as transport, crowding, handling and bad water quality can cause a stress response in fish. The fish will react by secreting high levels of stress hormones (corticosteroids) which are known to be immunosuppressive. The stress response is accompanied by a lymphocyte depletion in blood and in lymphoid organs.
- **Pollutants and heavy metals** are also known to have detrimental effects on the fish immune system causing various effects depending on the nature of the substance. Drugs such as antibiotics can also be immunosuppressive.
- **A well-balanced diet** is essential for adequate host defence mechanisms as well as to optimise growth and the eating quality of fish for human consumption.
- **Micronutrients:** Antioxidant vitamins such as vitamins C and E have been demonstrated to have immunomodulatory properties when fed at elevated doses. The presence of carotenoids in the diet has also been demonstrated to improve the health status of pigmented fish.
- **Immunomodulators other than micronutrients:** Adjuvants are substances that when combined with antigens enhance specific immune response in addition to non-specific defence response. Generally adjuvants slow down the rate of antigen elimination thereby prolonging antigen contact with macrophages and lymphocytes and augmenting the specific immune response. This is the principle of adjuvanted vaccines.
An example of a feed additive with limited nutritional value which is able to improve immune response is glucan from yeasts.

3. Vitamin C and health status

3.1 Vitamin C as a nutritional factor

Essential micronutrient

With the exception of perhaps two or three species, vitamin C biosynthesis does not occur in fish due to the lack of the last enzyme of the biosynthetic pathway: L-gulonolactone oxidase. Vitamin C must therefore be supplied via the feed. Major signs of ascorbate deficiency include reduced growth, scoliosis, lordosis, internal and fin haemorrhage, distorted gill filaments, fin erosion, anorexia and increased mortality (Figures 13 and 14).

Metabolic functions of vitamin C

Because of its modes of actions, vitamin C is involved in several physiological functions including growth, development, reproduction, wound healing, response to stressors and possibly lipid metabolism through its action on carnitine synthesis.

Further, as discussed later, vitamin C plays a significant role in the immune response and resistance to infectious diseases of fish, probably through its antioxidant properties.

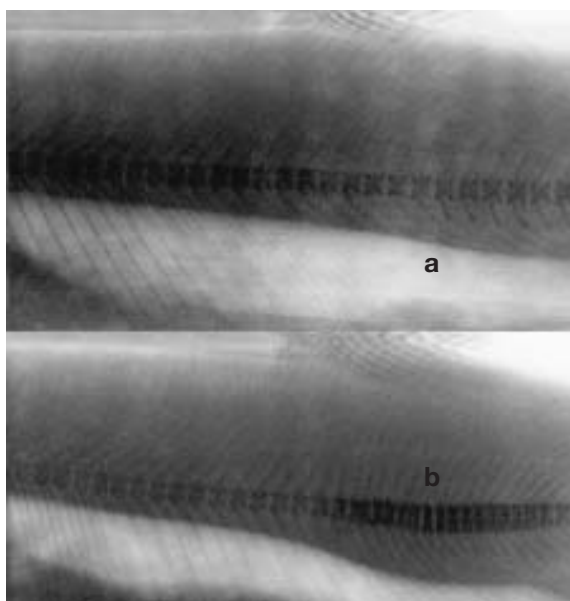
- Vitamin C has no coenzyme functions, unlike other water-soluble vitamins, but acts as a cofactor in many reactions involving hydroxylating enzymes:

– *collagen synthesis*: collagen is an important component of skin, bone, cartilage and endothelium of blood vessels. Therefore, these tissues will be damaged if the formation of collagen is impaired by insufficient vitamin C levels in the body. The hydroxylation of specific prolyl and lysyl residues of procollagen is catalysed by hydroxylases dependent upon ascorbic acid: hydroxyproline residues contribute to the stiffness of the collagen triple helix and bind carbohydrates to form intramolecular cross-links which give the structural integrity of the collagen. Ascorbate deficiency also reduces complement activity (the complement component C1q is rich in hydroxyproline and hydroxylysine).

– *catecholamine biosynthesis*: the stress response is primarily controlled by the endocrine system via cortisol and catecholamines whose synthesis depends upon ascorbic acid-dependent hydroxylases. Ascorbic acid requirement is increased by stressful situations. It can compensate for the stress-induced down regulation of the immune system.



<
Figure 13:
Vitamin C-deficient rainbow trout showing brocken back syndrome.



>
Figure 14:
X-ray of a healthy fish (a) and a vitamin C-deficient fish showing deformed vertebrae (b).



- Vitamin C is also involved in other physiological processes such as:

- *tyrosine metabolism*: the active degradation of tyrosine is made via two oxidases which are vitamin C-dependent. In turbot, vitamin C- deficiency causes hypertyrosinemia and the excretion of tyrosine metabolites.

- *metal ion metabolism*: vitamin C interacts with several metallic elements of nutritional significance (selenium) and reduces the toxicity of metals such as cadmium, nickel, lead (the elements are transformed into their reduced forms, which are absorbed less and excreted more rapidly).

- *protection of cells* from oxidative damage and the regeneration of vitamin E in its metabolically active form.

- *immune reactions*: vitamin C affects immune functions in different ways (protection against free radical-mediated protein inactivation associated with the oxidative burst of macrophages, chemotaxis, stimulation of proliferative response, antibody production and interferon). Vitamin C helps to maintain the integrity of the immune cells through their protection from oxidation and within the cells (high amount of vitamin C stored in the immune cells).

Absorption of vitamin C

The species that cannot synthesise vitamin C absorb ascorbic acid by an active transport mechanism which is Na⁺-dependent. This active uptake of vitamin C seems to be very important at low doses while at high doses, uptake by passive diffusion also occurs.

The uptake of vitamin C in cells such as lymphocytes, neutrophils and mucocytes involves dehydroascorbic acid because ascorbic acid cannot cross their membrane. Once dehydroascorbic acid is taken up by the cells, it is rapidly reduced to ascorbic acid by an intracellular dehydroascorbic acid reductase.

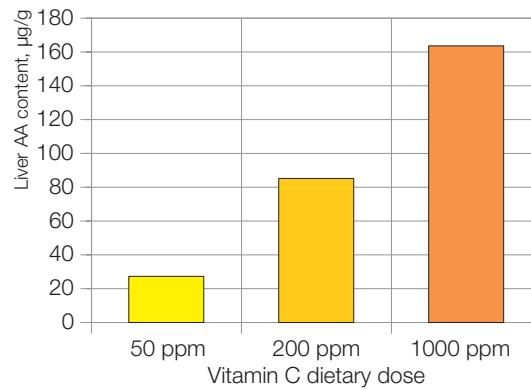
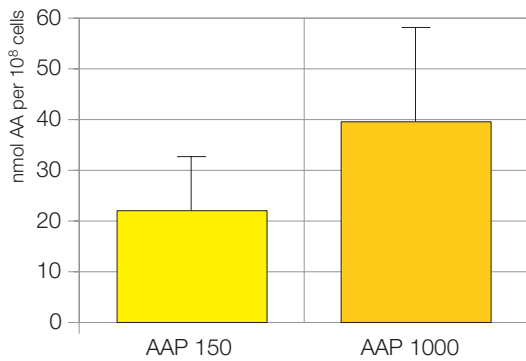
Tissue distribution of vitamin C

Vitamin C is concentrated in many vital organs with active metabolism. The concentration of vitamin C in various tissues is related to the dietary intake of the vitamin (Figures 15 and 16). Moreover, some tissues such as brain, thymus and leukocytes accumulate high concentrations. In these tissues, ascorbic acid levels seem to be retained longer in case of dietary vitamin C depletion compared to storage organs such as liver.

An example of tissue distribution of vitamin C is given in Figure 17. In this experiment, rainbow trout were fed vitamin C as ascorbate phosphate at the dose of 200 mg ascorbic acid equivalents per kg of feed.

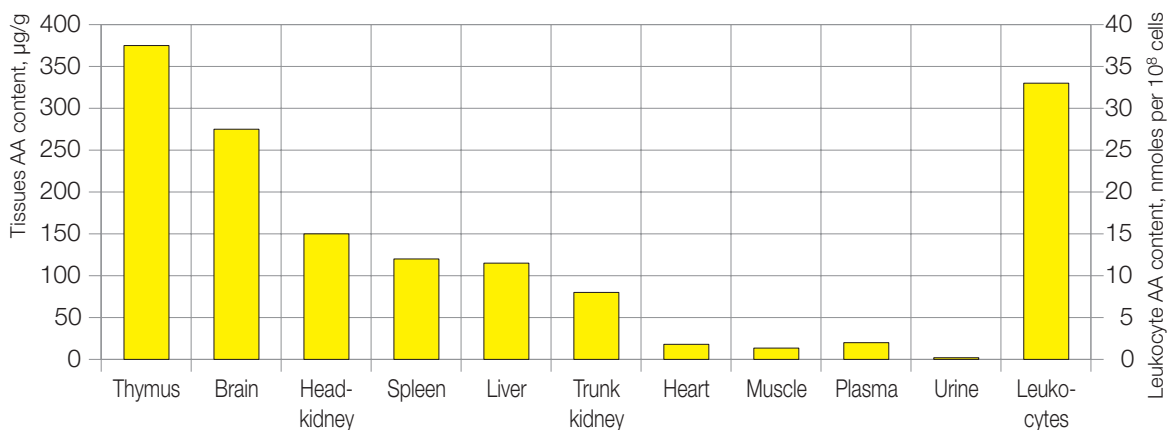
The very high levels found in thymus, brain and leukocytes confirm the hypothesis of the importance of ascorbic acid in preserving vital tissues from oxidation processes.

Liver and headkidney are important storage organs for vitamin C in fish. The high level found in the headkidney is likely to be related to the presence of lymphopoietic tissues. Trunk kidney and spleen are also able to store a large amount of vitamin C. Trunk kidney is the site of chromaffin cells which are responsible for catecholamin biosynthesis. Ascorbic acid is concentrated at the site of catecholamin formation and it is released with newly synthesized corticosteroids in response to stressors.



<<
Figure 15:
Influence of dietary intake of vitamin C on macrophage ascorbic acid (AA) concentration
 in rainbow trout fed an elevated dose of vitamin C for four weeks. (AAP = ascorbate phosphate).
Verlhac et al., in prep..

<
Figure 16:
Liver vitamin C (AA) content in relation to dietary intake of the vitamin in rainbow trout.
Gabaudan et al., 1993.



<
Figure 17:
Ascorbic acid (AA) concentration in various tissues of rainbow trout.
Gabaudan and Verlhac, 1992.

3.2 Vitamin C and the immune response

The following tables are a literature review of the studies concerning the effect of vitamin C on immune response and disease resistance in fish. The order of the tables concerning the immune response follows the organisation of the chapter concerning the description of the fish immune system.

When a positive effect has been demonstrated, the related study has been shadowed. The doses (mg vitamin C/kg of feed) at which the effect has been observed are written in bold characters. Treatments without vitamin C supplementation are normally not quoted in the tabels. The references quoted with * are illustrated with figures.



The non-specific immune response

Natural barriers:

Wound repair	Species	Feeding (weeks)	Vitamin C doses	Reference
	Rainbow trout	24	50-100-200- 400-1000	Halver, 1972
	Rainbow trout	4	20- 150-1000	Wahli et al., 2003
	Coho salmon	24	50-100-200- 400-1000	Halver, 1972
	Channel catfish	16	30- 60	Lim and Lovell, 1978

Cellular mechanisms:

Phagocytosis	Species	Feeding (weeks)	Vitamin C doses	Reference
--------------	---------	-----------------	-----------------	-----------

Ingestion of latex beads	Rainbow trout	12	120- 1200	Blazer, 1982
	Rainbow trout	10	200- 1000	Verlhac et al., 1993
	Rainbow trout	9	0-MRL for A, C and E <i>in combination with carotenoids</i>	Amar ez al., 2001

Ingestion of yeasts	Rainbow trout	4	150- 1000	Verlhac et al., in prep.*
	Turbot	10	400-800-1200	Roberts et al., 1995
	Turbot	18	400- 800 -1200	Roberts et al., 1995

Injection of erythrocytes	Atlantic salmon	26	50-310-2750	Hardie et al., 1991
	Bagrid catfish	9	10- 100	Anbarasu & Chandran, 2001

Ingestion of bacteria	Channel catfish	20	30-60-150-300-3000	Li and Lovell, 1985
-----------------------	-----------------	----	--------------------	---------------------

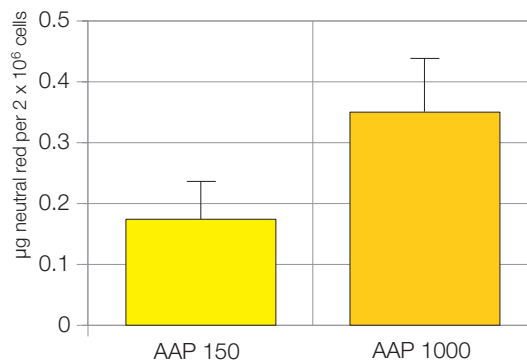
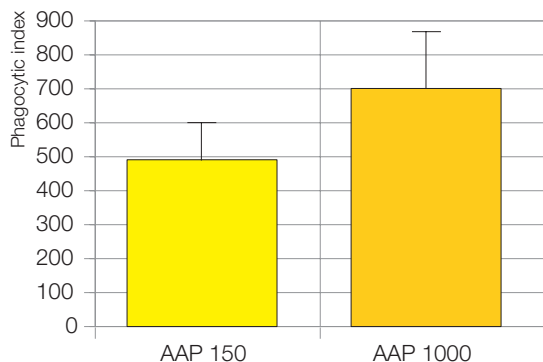
Pinocytosis	Species	Feeding (weeks)	Vitamin C doses	Reference
-------------	---------	-----------------	-----------------	-----------

	Rainbow trout	2	150- 1000	Verlhac et al., 1997
	Rainbow trout	4	150- 1000	Verlhac et al., in prep.*

Macrophage chemotaxis, adherence

Species	Feeding (weeks)	Vitamin C doses	Reference
---------	-----------------	-----------------	-----------

Channel catfish	14	0-50- 3000 with iron at 0 to 300 ppm	Lim et al., 2000
Bagrid catfish	9	10- 100	Anbarasu & Chandran, 2001



<< **Figure 18:** Influence of vitamin C on phagocytosis of yeast cells by macrophages from rainbow trout fed an elevated dose of vitamin C for four weeks. (AAP = ascorbate phosphate). Verlhac et al., non pub.

< **Figure 19:** Influence of vitamin C on pinocytosis of neutral red dye by macrophages from rainbow trout fed an elevated dose of vitamin C for four weeks. (AAP = ascorbate phosphate). Verlhac et al., non pub.

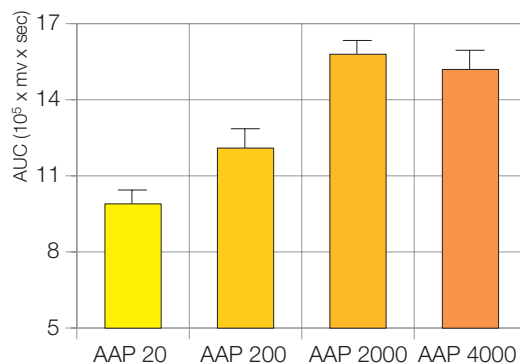


Killing: Oxidative burst	Species	Feeding (weeks)	Vitamin C doses	Reference
O ₂ ⁻ production	Rainbow trout	2	150- 1000	Verlhac et al., 1998
	Rainbow trout	8	60- 1000	Verlhac & Gabaudan, 1994
	Rainbow trout	9	0-MRL-for A, C and E <i>in combination with carotenoids</i>	Amar et al., 2001
	Rainbow trout	16	30-1000	Verlhac & Gabaudan, 1994
	Rainbow trout	20	60-1000	Verlhac & Gabaudan, 1994
	Rainbow trout	32	30- 2000 <i>(combined with vitamin E)</i>	Wahli et al., 1998
	Atlantic salmon	8	60-1000	Verlhac & Gabaudan, 1994
	Atlantic salmon	26	50-310-2750	Hardie et al., 1991
	Bagrid catfish	9	10- 100	Anbarasu & Chandran, 2001

H ₂ O ₂ , ¹ O ₂ , OH ⁻ production	Rainbow trout	2	150- 1000	Verlhac et al., 1998
	Rainbow trout	2	150-1000- 4000 <i>(combined with glucan)</i>	Verlhac et al., 1996
	Rainbow trout	3	20-200- 2000-4000	Verlhac et al., 1995*
	Rainbow trout	4	60- 2000	Dunier et al., 1995
	Rainbow trout	8	60- 1000	Verlhac & Gabaudan, 1994
	Rainbow trout	16	30-1000	Verlhac & Gabaudan, 1994
	Rainbow trout	20	60-1000	Verlhac & Gabaudan, 1994
	Rainbow trout	32	30- 2000	Wahli et al., 1998
	Atlantic salmon	3	150-1000 <i>in combination with lactoferrin</i>	Lygren et al., 1999
	Atlantic salmon	8	60- 1000	Verlhac & Gabaudan, 1994

Natural cytotoxicity	Species	Feeding (weeks)	Vitamin C doses	Reference
Natural killer cell activity	Rainbow trout	9	0-MRL for A, C, and E <i>in combination with carotenoids</i>	Amar et al., 2001

Figure 20:
Influence of vitamin C on oxidative burst of macrophages from rainbow trout fed different doses of vitamin C for three weeks. (AAP = ascorbate phosphate). Verlhac et al., 1995.



Soluble factors

	Species	Feeding (weeks)	Vitamin C doses	Reference
Complement (alternative pathway)	Rainbow trout	2	150- 1000-4000	Verlhac et al., 1996
	Rainbow trout	2	150-1000	Verlhac et al., 1998
	Rainbow trout	4	150-1000	Verlhac et al., not pub.
	Rainbow trout	9	150- MRL for A, C and E <i>in combination with carotenoids</i>	Amar et al., 2001
Lysozyme	Rainbow trout	2	150- 1000	Verlhac et al., 1998
	Rainbow trout	2	150-1000-4000	Verlhac et al., 1996
	Rainbow trout	3	20-200-2000-4000	Verlhac et al., 1995
	Rainbow trout	9	0- MRL for A, C and E <i>in combination with carotenoids</i>	Amar et al., 2001
	Atlantic salmon	3	150-1000 <i>in combination with lactoferrin</i>	Lygren et al., 1999
	Atlantic salmon	36	40-400-2000- 4000 <i>(surviving from infection)</i>	Waagbø et al., 1993*
	Turbot	10	400-800- 1200	Roberts et al., 1995
	Turbot	18	400-800-1200	Roberts et al., 1995

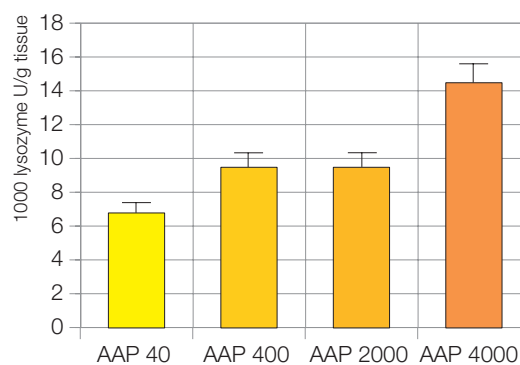


Figure 21:
Influence of vitamin C on headkidney lysozyme activity in Atlantic salmon fed elevated doses of vitamin C for thirty six weeks. (AAP = ascorbate phosphate).
 Waagbø et al., 1993.

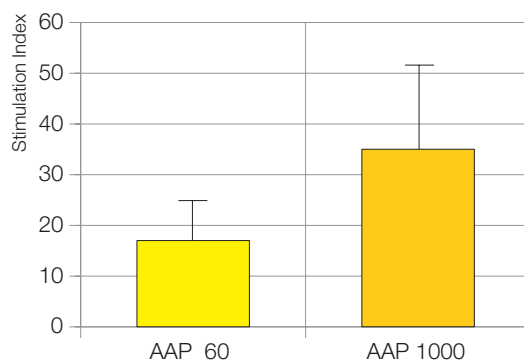


The specific immune response

Lymphocyte proliferation

	Species	Feeding (weeks)	Vitamin C doses	Reference	
T lymphocytes	Rainbow trout	2	150-1000	Verlhac et al., 1998	
	Rainbow trout	3	20-200-2000- 4000	Verlhac et al., 1995	
	Rainbow trout	10	200- 1000	Verlhac et al., 1993	
	Rainbow trout	16	30- 1000	Verlhac & Gabaudan, 1994	
	Rainbow trout	20	60- 1000	Verlhac & Gabaudan, 1994	
	Rainbow trout	32	30- 2000	Wahli et al., 1998	
				(combined with vitamin E)	
	Rainbow trout	in vitro	increase	Hardie et al., 1993	
	Rainbow trout	parenteral	increase	Hardie et al., 1993	
	Atlantic salmon	8	60- 1000	Verlhac & Gabaudan, 1994*	
B lymphocytes	Rainbow trout	2	150-1000	Verlhac et al., 1998	
	Rainbow trout	3	20-200-2000- 4000	Verlhac et al., 1995	
	Atlantic salmon	8	60-1000	Verlhac & Gabaudan, 1994	
Antigen-specific proliferation	Rainbow trout	in vitro	increase	Hardie et al., 1993	

Figure 22:
Influence of vitamin C on lymphocyte proliferation in Atlantic salmon fed for two months.
 (AAP = ascorbate phosphate).
 Verlhac and Gabaudan, 1994.





Antibody response

Antibodies to	Sampling time after vaccination	Species	Feeding (weeks) before/after vaccination	Vitamin C doses	Reference
<i>Y. ruckeri</i>	Kinetics	Rainbow trout	2	150-1000-4000	Verlhac et al., 1996
	8 weeks	Rainbow trout	2	150- 1000	Verlhac et al., 1998
	Kinetics	Rainbow trout	2	150-1000	Verlhac et al., 1998
	Kinetics	Rainbow trout	3/end	20-200-2000-4000	Verlhac et al., 1995
	5, 7 weeks	Rainbow trout	4/end	60- 2000	Dunier et al., 1995
<i>V. salmonicida</i>	11, 17 weeks	Rainbow trout	10/end	40-400-2000- 4000	Waagbø et al., 1993*
<i>V. anguillarum</i>	4 weeks	Atlantic salmon	12/end	50 to 2000 (<i>vit E 350</i>)	Lall et al., 89
	Kinetics	Rainbow trout	28/end	100- 500-1000-2000	Navarre & Halver, 1989
<i>A. salmonicida</i>	4 weeks	Rainbow trout	12/end	50 to 2000 (<i>vit E 350</i>)	Lall et al., 1989
<i>E. ictaluri</i>	3 weeks	Channel catfish	9/end	30-60-150-300- 3000	Li & Lovell, 1985
	13 weeks	Channel catfish	9/end	100-500-1000-4000	Liu et al., 1989
IHN virus	3 weeks	Rainbow trout	6/end	20-80-320	Anggawati-S. et al., 1989

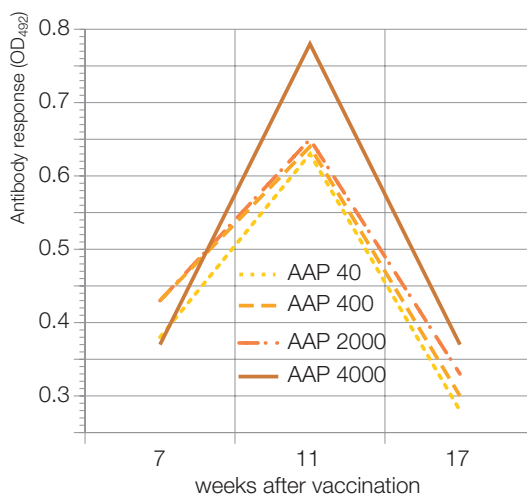


Figure 23: Influence of vitamin C on serum specific antibody levels after vaccination against cold water vibriosis in Atlantic salmon fed for ten weeks prior to injection and until the end of the experiment. (AAP = ascorbate phosphate). Adapted from Waagbø et al., 1993.



Complement (classical pathway of activation)

Species	Feeding (weeks)	Vitamin C doses	Reference
Rainbow trout (<i>vaccinated</i>)	2	150- 1000	Verlhac et al., 1998
Rainbow trout	2	150-1000-4000 (combined with glucan)	Verlhac et al., 1996
Rainbow trout	3	20-200-2000-4000	Verlhac et al., 1995
Rainbow trout	8	60-1000	Verlhac & Gabaudan, 1994
Rainbow trout	10	200- 1000	Verlhac et al., 1993
Rainbow trout	20	60-1000	Verlhac & Gabaudan, 1994
Rainbow trout	32	30-2000 (combined with vitamin E)	Wahli et al., 1998
Atlantic salmon	3	150-1000 in combination with lactoferrin	Lygren et al., 1999
Atlantic salmon	8	60-1000	Verlhac & Gabaudan, 1994
Atlantic salmon (<i>after infection</i>)	20	50 to 2000 (<i>vit E 350</i>)	Lall et al., 1989
Atlantic salmon	26	50-310- 2750	Hardie et al., 1991
Atlantic salmon (<i>before infection</i>)	27	40-400-2000-4000	Waagbø et al., 1993
Atlantic salmon (<i>surviving from infection</i>)	36	40-400-2000-4000	Waagbø et al., 1993
Channel catfish	9	100-500-1000-4000	Liu et al., 1989
Channel catfish	20	30-60-150-300- 3000	Li & Lovell, 1985

3.3 Vitamin C and resistance to disease

Non-vaccinated fish

Disease	Mode of infection	Species	Feeding (weeks) before/after infection	Vitamin C doses	Reference
<i>Vibriosis</i>	I.p.	Rainbow trout	28/end	100-500- 1000-2000	Navarre & Halver, 1989*
	Bath	Rainbow trout	28/end	100- 500-1000-2000	Navarre & Halver, 1989
<i>ERMD</i>	Bath	Rainbow trout	32	30- 2000 <i>(combined with vitamin E)</i>	Wahli et al., 1997
<i>VHS</i>	Bath	Rainbow trout	32	30-2000	Wahli et al., 1997
<i>Ich</i>	Bath	Rainbow trout	8	0-50- 2000	Wahli et al., 1995*
		Rainbow trout	32	30- 2000 <i>(combined with vitamin E)</i>	Wahli et al., 1997
<i>IHN</i>	Bath	Rainbow trout	6/end	20- 80-320	Anggawati-S. et al., 1989
<i>Vibriosis</i>	I.p. or bath	Atlantic salmon	22	50 to 2000 <i>(vit E 350)</i>	Lall et al., 1989
<i>Furunculosis</i>	I.p. or bath	Atlantic salmon	22	50 to 2000 <i>(vit E 350)</i>	Lall et al., 1989
	Bath	Atlantic salmon	26	50-310-2750	Hardie et al., 1991
	Cohabitant + i.p.	Atlantic salmon	27/end	40-400-2000- 4000	Waagbø et al., 1993
	Cohabitant	Atlantic salmon	3 + 1 without C/?	150-1000 <i>(combined with lactoferrin)</i>	Lygren et al., 1999
<i>ISA</i>	I.p.	Atlantic salmon	3 + 1	150-1000	Lygren et al., 1999
			without C/?	<i>(combined with lactoferrin)</i>	
<i>Enteric septicaemia</i>	Bath	Channel catfish	8/end	25-50-100-1000-2000	Li et al., 1993
	Bath	Channel catfish	8/end	100-250-500-1000-2000	Li et al., 1993
	Bath	Channel catfish	13/end	30-60-150-300- 3000	Li and Lovell, 1985
	Bath	Channel catfish	14/end	60- 150	Durve and Lovell, 1982
	Bath	Channel catfish	14	0-50- 3000 <i>(combined with lactoferrin)</i>	Lim et al., 2000
<i>Haemorrhagic septicaemia</i>	I.p.	Bagrid catfish	9	10- 100	Anbarasu & Chandran, 2001

Vaccinated fish

Disease	Mode of infection	Species	Feeding (weeks) before/after infection	Vitamin C doses	Reference
<i>IHN</i>	Bath	Rainbow trout	6/end	20- 80-320	Anggawati-S. et al., 1989
<i>Enteric septicaemia</i>	Bath	Channel catfish	13/end	30-60-150-300-3000	Li and Lovell, 1985
				<i>(100% vaccine efficiency)</i>	
	I.p.	Channel catfish	13/end	100-500- 1000-4000	Liu et al., 1989
<i>Haemorrhagic septicaemia</i>	I.p.	Bagrid catfish	9	10- 100	Anbarasu & Chandran, 2001

ERMD: Enteric redmouth disease
VHS: Viral haemorrhagic septicaemia
Ich: Ichthyophthiriosis
IHN: Infectious hepatic necrosis
ISA: Infectious salmon anemia



Figure 24:
Resistance of non-vaccinated rainbow trout against *ichthyophthiriosis* in relation to dietary vitamin C. Wahli et al., 1995.

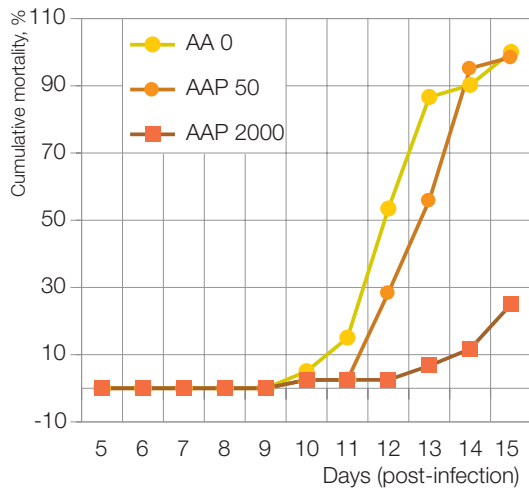
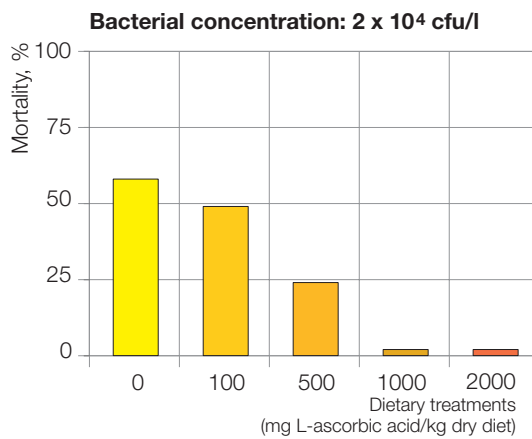
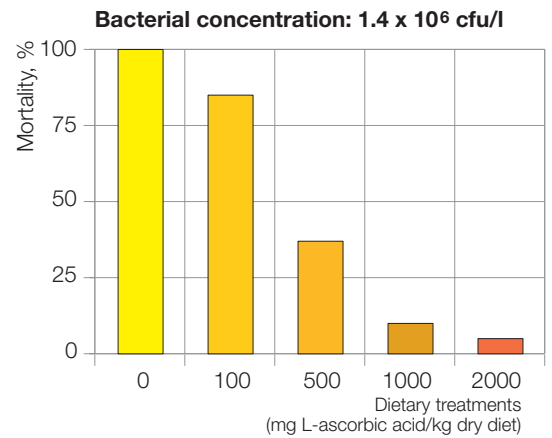
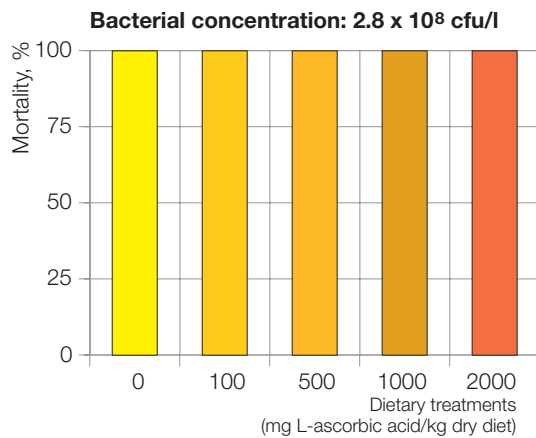


Figure 25:
Resistance of non-vaccinated rainbow trout to vibriosis in relation to dietary vitamin C at three infection doses. Navarre and Halver, 1989.



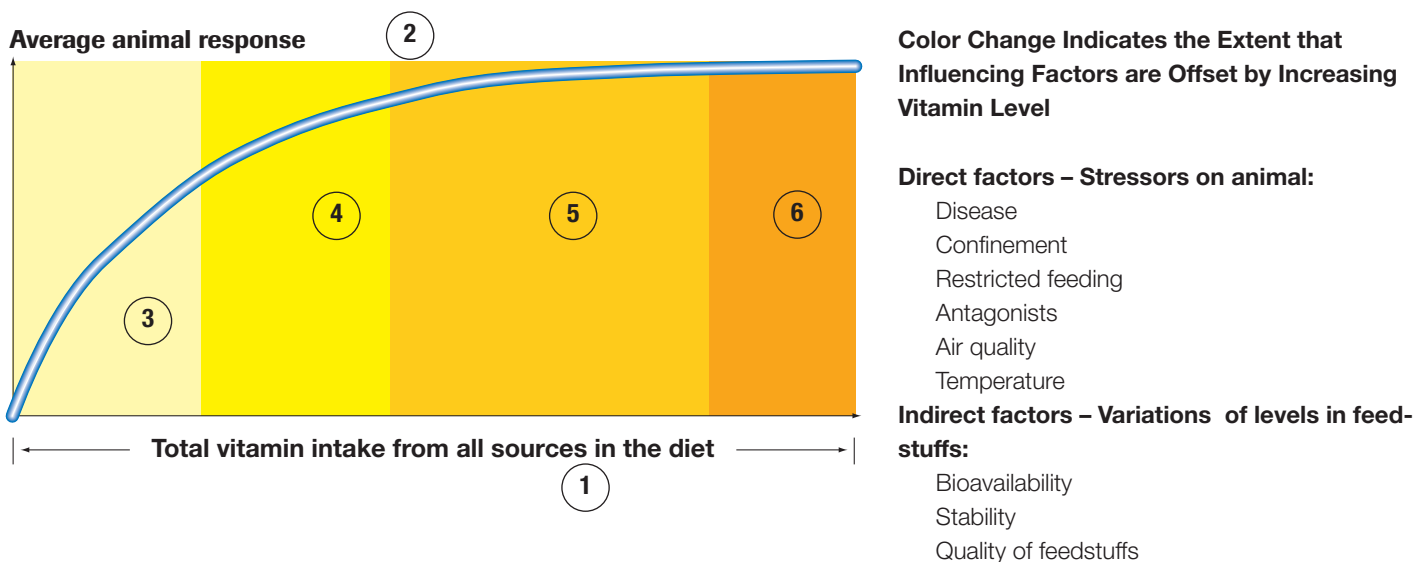
4. Practical guidelines for vitamin C administration

Optimum Vitamin Nutrition concept

Roche vitamin supplementation guidelines are designed to provide optimum vitamin nutrition under typical industry conditions. The concept of optimum vitamin nutrition presented in figure 26 indicates vitamin allowances lead-

ing to an optimisation of the overall animal response (growth, feed efficiency, reproductive performance and immunity).

Figure 26:
Optimum Vitamin
Nutrition concept.



Legends

- 1 'Vitamin Allowances'** describes the total quantity of vitamins from all dietary sources, i.e., natural content of the feedstuffs plus supplementation.
- 2 'Average animal response'** refers to any average productivity or health response of animals to vitamin intake, i.e. growth rate, feed efficiency, reproductive performance, welfare, health or immunity.
- 3 'Deficient' or 'marginal'** vitamin intake means a level of supplementation below the requirements published by NRC, ARC and other officially published vitamin recommendations. Such vitamin supply puts animals at risk of developing clinical deficiencies and disorders resulting from inadequate vitamin intake.

- 4 'Sub-optimum'** intake relates to supplementation levels, which typically meet or slightly exceed the NRC, ARC and other officially published vitamin recommendations. These levels should prevent sub-clinical deficiency signs under good conditions (however, with stress and diseases, sub-clinical deficiency might occur), but are by no means adequate to permit optimum health and productivity.
- 5 'Optimum'** intake offsets negative factors influencing animal health and performance, thus allowing to take advantage of the performance potential of modern animal breeds.
- 6 'Special applications'** levels of vitamin supplementation are safe, and focused in improving certain attributes e.g. meat quality and immunity.



General recommendations for vitamin C in fish

Since no vitamin C synthesis occurs in fish, they are dependent on a vitamin C supplementation via the feed. To optimise stability of vitamin C during feed production and storage as well as bioavailability in the fish, the use of vitamin C in phosphorylated form is recommended.

Based on the combination of experimental results obtained in several controlled studies and field experience, the following general inclusion rates of vitamin C in feeds for aquaculture species are recommended:

Species	Recommended vitamin C level (mg/kg feed)*
Salmon	150-250
Trout	150-250
Carp	150-250
Tilapia	150-250
Catfish	150-250
Seabream/seabass	150-250
Eel	150-300
Shrimp	250-500

* Vitamin C-activity in phosphorylated form

Vitamin C for optimal health status

Based on findings on the influence of vitamin C supplementation on immune response and on increasing field experience in this important area of fish nutrition, the following vitamin C levels are recommended whenever

the immune system is challenged (stressing situations, e.g. handling and grading, vaccination, winter wounds, disease outbreaks and release of smolt into the sea) and after reduced intake during winter:

Species	Dose (mg/kg)*	Duration
Salmon	1000	2–4 weeks before
Trout		and at least
Catfish		2 weeks after

* Vitamin C-activity in phosphorylated form

Frequency

This feeding regime should be repeated whenever the immune system is challenged.

Combining the general vitaminC-recommendations with those for optimal health status enables the fish to

achieve optimum health and productivity as described in the concept for Optimum Vitamin Nutrition.

References and bibliography

References and bibliography

- Amar E.C, Kiron V., Satoh S. and Watanabe T. 2001. Influence of various dietary synthetic carotenoids on bio-defence mechanisms in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research*. 32 (Suppl 1), 162-173.
- Anbarasu K. and Chandran M.R. 2001. Effect of ascorbic acid on the immune response of the catfish, *Mystus gulio* (Hamilton), to different bacterins of *Aeromonas hydrophila*. *Fish and Shellfish Immunology*. 11, 347-355.
- Anggawati-Satyabudhy A.M., Grant B..F. and Halver J.E. 1989. Effects of L-ascorbyl phosphates (AsPP) on growth and immunoresistance of rainbow trout (*Oncorhynchus mykiss*) to Infectious Hematopoietic Necrosis (IHN) virus. In: *Proceedings of the Third International Symposium on Feeding and Nutrition in Fish*, Toba, Japan, August 28 - September 1 (ed. by M. Takeda and T. Watanabe), Tokyo University of Fisheries, Tokyo. pp. 411-426.
- Blazer V.S. 1982. The effects of marginal deficiency of ascorbic acid and alpha-tocopherol on the natural resistance and immune response of rainbow trout (*Salmo gairdneri*). PhD thesis, Univ. Microfilms Intern.-USA, pp. 113.
- Deby C. 1991. La biochimie de l'oxygène. *La Recherche*. 228, 22, 56-64.
- Dunier M., Vergnet C., Siwicki A.K. and Verlhac V. 1995. Effect of lindane exposure on rainbow trout (*Oncorhynchus mykiss*) immunity. IV. Prevention of non-specific and specific immunosuppression by dietary vitamin C (ascorbate-2-polyphosphate). *Ecotoxicology and Environmental Safety*, 30, 259-268.
- Durve V.S. and Lovell R.T. 1982. Vitamin C and disease resistance in channel catfish (*Ictalurus punctatus*). *Canadian Journal of Fisheries and Aquatic Science*. 39, 948-951.
- Gabaudan J. and Verlhac V. 1992. Biological efficacy of Rovimix Stay-C as a source of vitamin C for Salmonids. Poster presentation at the International Symposium on Cultivation of Atlantic salmon, 16-20.08 1992. Bergen, Norway.
- Gabaudan J., Verlhac V., Gadiant M. and Hofmann P. 1993. Stability and utilization of vitamin C derivatives in rainbow trout (*Oncorhynchus mykiss*). Presentation at the EIFAC Workshop on Methodology for Determination of Nutrient Requirements in Fish., 29.06-01.07 1993. Eichenau, Germany.
- Halver J.E. 1972. The role of ascorbic acid in fish disease and tissue repair. *Bulletin of the Japanese Society of Scientific Fisheries*. 38 (1), 79-92.
- Hardie L.J., Fletcher T.C. and Secombes C.J. 1991. The effect of dietary vitamin C on the immune response of the Atlantic salmon (*Salmo salar L.*). *Aquaculture*, 95, 201-214.
- Hardie L.J., Mardsen M.J., Fletcher T.C. and C.J. Secombes. 1993. In vitro addition of vitamin C affects rainbow trout lymphocyte responses. *Fish and Shellfish Immunology*. 3, 207-219.
- Lall S. P., Olivier G., Weerakoon D.E.M. and Himes J.A. 1989. The effect of vitamin C deficiency and excess on immune response in Atlantic salmon (*Salmo salar L.*). In: *Proceedings of the Third International Symposium on Feeding and Nutrition in Fish*, Toba, Japan, Aug. 28-Sept. 1 (ed. by M. Takeda and T. Watanabe), Tokyo University of Fisheries, Tokyo. pp. 427-441.
- Li M.H., Johnson M.R. and Robinson E.H. 1993. Elevated dietary vitamin C concentrations did not improve resistance of channel catfish, *Ictalurus punctatus*, against *Edwardsiella ictaluri* infection. *Aquaculture* 117, 303-312.
- Li Y. and Lovell R.T. 1985. Elevated levels of dietary ascorbic acid increase immune response in channel catfish. *Journal of Nutrition* 115, 123 -131.
- Lim C. and Lovell R.T. 1978. Pathology of the vitamin C deficiency syndrome in channel catfish (*Ictalurus punctatus*). *Journal of Nutrition*. 108, 1137-1146.
- Lim C., Klesius P.H., Li M.H. and Robinson E.H. 2000. Interaction between dietary levels of iron and vitamin C on growth, hematology, immune response and resistance of channel catfish (*Ictalurus punctatus*) to *Edwardsiella ictaluri* challenge. *Aquaculture*. 185, 313-327.

- Liu P.R., Plumb J.A., Guérin M. and Lovell R.T. 1989. Effects of megalevels of dietary vitamin C on the immune response of channel catfish, *Ictalurus punctatus*, in ponds. *Diseases of Aquatic Organisms*. 7, 191-194.
- Lygren B., Sveier H., Hjeltnes B. and Waagbø R. 1999. Examination of the immunomodulatory properties and the effect on disease resistance of dietary bovine lactoferrin and vitamin C fed to Atlantic salmon (*Salmo salar*) for a short-term period. *Fish and Shellfish Immunology*. 9, 95-107.
- Navarre O. and Halver J.E. 1989. Disease resistance and humoral antibody production in rainbow trout fed high levels of vitamin C. *Aquaculture* 79, 207-221.
- Roberts M.L., Davies S.J. and Pulsford A.L. 1995. The influence of ascorbic acid (vitamin C) on non-specific immunity in the turbot (*Scophthalmus maximus L.*) *Fish and Shellfish Immunology*, 5: 27-38.
- Roitt I. 1988. *Essential Immunology*. 6th Edition. Blackwell Scientific Publications.
- Roitt I., Brostoff J. and Male D. 1989. *Immunologie Fondamentale et Appliquée*. 2nd edition. MEDSI/McGRAW-HILL Eds.
- Verlhac V., N'Doye A., Gabaudan J., Troutaud D. and Deschaux P. 1993. Vitamin nutrition and fish immunity: influence of antioxidant vitamins (C and E) on immune response of rainbow trout. In: *Fish Nutrition in Practice, Les Colloques (INRA eds)*, 61: 167-177.
- Verlhac V. and Gabaudan J. 1994. Influence of vitamin C on the immune system of salmonids. *Aquaculture and Fisheries Management* 25, 21-36.
- Verlhac V., Gabaudan J. and Schüep W. 1995. Immunomodulation in fish: II. Effect of dietary vitamin C. In: *Proceedings of the 2nd Roche Aquaculture Centre Conference on Nutrition and Disease*. June, 15th. 1995, Bangkok, Thailand. Eds: K. Kurmaly.
- Verlhac V., Gabaudan J., Obach A., Schüep W. and Hole R. 1996. Influence of dietary glucan and vitamin C on non-specific and specific immune response of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 143, 123-133.
- Verlhac V., Obach A., Gabaudan J. Schüep W. and Hole R.. 1998. Immunomodulation by dietary vitamin C and glucan in rainbow trout (*Oncorhynchus mykiss*). *Fish and Shellfish Immunology*. 8, 409-424.
- Verlhac V., Gabaudan J. and Schüep W. Influence of dietary vitamin C on the immune response of rainbow trout in relation to ascorbic acid content of the immune cells. Not published.
- Waagbø R., Glette J., Raa-Nilsen E. and Sandnes K. 1993. Dietary vitamin C, immunity and disease resistance in Atlantic salmon (*Salmo salar*). *Fish Physiology and Biochemistry*, 12(1): 61-73.
- Wahli T., Frischknecht R., Schmitt M., Gabaudan J., Verlhac V. and Meier W. 1995. A comparison of the effect of silicone coated ascorbic acid and ascorbyl phosphate on the course of ichthyophthiriosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases*. 18, 347-355.
- Wahli T., Verlhac V., Gabaudan J., Meier W. and Schüep W. 1998. Influence of combined vitamins C and E on immunity and disease resistance of rainbow trout (*Oncorhynchus mykiss*). *Journal of Fish Diseases*. 21, 127-137.
- Wahli T., Verlhac V., Girling P., Gabaudan J. and Aebischer C. 2003. Influence of dietary vitamin C on the wound healing process in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 225, 371-386.



General bibliography and non cited publications

Ascorbic acid in domestic animals. 1992. Proceedings of the 2nd symposium. 9-12 October 1990, Kartause Ittingen, Switzerland. Wenk C., Fenster R. and Völker L. (Eds).

Annual Review of Fish Diseases. 1992. Faisal M. and Hetrick F.M. Eds. Pergamon Press Ltd, USA. Volume 2.

Andersen F., Lygren B., Maage A. and Waagbø R. 1998. Interaction between two dietary levels of iron and two forms of ascorbic acid and the effect on growth, antioxidant status and some non-specific immune parameters in Atlantic salmon (*Salmo salar*) smolts. *Aquaculture*. 161, 437-451.

Areechon N., Chuajan T., Hunter B. and Jangsutthivonrawat W. 2000. Bioavailability and effects on growth, survival and stress tolerance of vitamin C monophosphate form in hybrid catfish. In the Proceedings of the 6th Roche Aquaculture conference Saia Pacific, 29.09.2000, Bangkok, Thailand.

Blazer V.S. 1991. Piscine macrophage functions and nutritional influence: a review. *Journal of Aquatic Animal Health*, 3:77-86.

Combs G.F. 1992. The Vitamins: Fundamental aspects in Nutrition and Health. Academic Press, Inc.

Dabrowski K., Lee K.J., Guz L., Verlhac V. and Gabaudan, J. 2004. Effects of dietary ascorbic acid on oxygen stress (hypoxia or hyperoxia), growth and tissue vitamin concentrations in juvenile rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*. 233, 383-392.

Dalmo R.A., Ingebrigtsen K. and Boegwald J. 1997. Non-specific defence mechanisms in fish with particular reference to the reticuloendothelial system. *Journal of Fish Diseases*. 20, 241-273.

Datta M. and Kaviraj A. 2003. Ascorbic acid supplementation of diet for reduction of deltamethrin induced stress in freshwater catfish *Clarias gariepinus*. *Chemosphere*. 53, 883-888.

Gabaudan J. and Verlhac V., 2001 Critical review of the requirement of ascorbic acid in cold and cool water fishes (salmonids, percids, plecoglossids and flatfishes). In *Ascorbic acid in aquatic organism, Status and Perspective*, Edited by Konrad Dabrowsky, CRC Press.

Lall S.P. and Olivier G., 1993. Role of micronutrients in immune response and disease resistance in fish. In: *Fish Nutrition in Practice, Les Colloques (INRA eds)*, Paris, France. 61: 101-118.

Landolt M.L. 1989. The relationship between diet and the immune response of fish. *Aquaculture*, 79: 193-206.

Le Grusse J. and Watier B. 1993. Les vitamines: données biochimiques, nutritionnelles et cliniques. CEIV Eds, Produits Roche, France.

Lygren B., Hamre K. and Waagbø R. 1999. Effects of dietary pro- and antioxidants on some protective mechanisms and health parameters in Atlantic salmon. *Journal of Aquatic Animal Health*. 11, 211-221.

Palace V.P., Brown S.B., Baron C.L., Fitzsimons J., Woodin B., Stegeman J.J. and Klaverkamp J.F. 1998. An evaluation of the relationships among oxidative stress, antioxidant vitamins and early mortality syndrome (EMS) of lake trout (*Salvelinus namaycush*) from Lake Ontario. *Aquatic Toxicology*. 43, 195-208.

Stoskopf M.K. 1993. *Fish Medicine*. W.B. Saunders Company, Harcourt Brace Jovanovich, Inc.

Waagbø R., 1994. The impact of nutritional factors on the immune system in Atlantic salmon, *Salmo salar* L.: a review. *Aquaculture and Fisheries Management*, 25: 175-197.



DSM Nutritional Products
P.O. Box 3255
CH-4002 Basel
Switzerland

www.dsmnutritionalproducts.com