Fisk.Dir. Skr., Ser. Ernæring, Vol. IV, No 1, 3-32 (1991)

VITAMIN C IN FISH NUTRITION - A REVIEW

By

KJARTAN SANDNES
Institute of Nutrition, Directorate of Fisheries,
P. O. Box 1900, N-5024 Bergen, Norway

CONTENTS

1. A BRIEF HISTORY OF VITAMIN C

A requirement of vitamin C in fish was demonstrated for the first time in 1965 (Kitamura *et al.*, 1965), but more than 200 years before, the first experimental work describing how to cure scurvy in man — the classic *A Treatise Of the Scurvy* by James Lind — was published in Edinburgh in 1753. A breakthrough in the history of vitamin C came when the Norwegian scientists Holst and Frølich (1907) discovered that guinea pigs were susceptible to scurvy, and this finding opened for attempts to isolate and identify the antiscorbutic factor. This factor was named vitamin C by Drummond (1920), and was identified by Waugh and King in 1932 (Waugh and King, 1932). The following year vitamin C was named «ascorbic acid» by Szent-Giörgy and Haworth (1933).

One year later McCay and Tunison (1934) reported that brook trout *(Salvelinus fontinalis)* fed formalin preserved meat for one year developed spinal deformities, but the symptoms were not associated with the antiscorbutic factor recently described by Waugh and King (1932). As cited by Poston (1967), Hewitt in 1937 reported that sick trout with «fatty livers» responded favorably to dietary vitamin C, while McLaren *et al.* (1947) observed hemorrhages in liver, kidney and intestine of rainbow trout *(Oncorhynchus mykiss)* fed diets low in vitamin C.

In 1961 rainbow trout with deformed vertebrae (scoliosis and lordosis) were found in many fish ponds in Japan. The fish had mainly been fed artificial dry diets, and the observations initiated experimental studies by Kitamura *et al.* (1965), showing for the first time a specific requirement for ascorbic acid (AA) in fish. Since then a substantial increase in intensive aquaculture production has taken place, and with concomitant increased efforts to investigate the various aspects of vitamin C in fish nutrition.

In recent years vitamin C deficiency has caused substantial losses in commercial fish farming. A few years ago between 40 and 80 % of rainbow trout fingerlings (5-20 g) died in many of the Yougoslavian fish farms showing signs of discoloration and deformed spine. Analyses of the feed revealed no detectable level of vitamin C, and the cause of the mortality was mainly attributed to a deficiency of this vitamin (Teskeredzic *et al.*, 1989). This incident clearly demonstrated the need for a safe supplementation and feed quality control with respect of vitamin C in fish diets.

2. CHEMICAL PROPERTIES

2.1 Chemistry

In IUPAC terms, L-*threo*-2-hexenono-1,4-lactone, is the correct name for the trivial name L-ascorbic acid (AA). Ascorbic acid is one of a pair of enantio-

mers having this structure. The other three isomers (D-ascorbic acid, L-isoascorbic acid, D-isoascorbic acid) posess 0 — 5 % vitamin C activity compared to AA (Hay *et al.*, 1967). The hydroxyl groups in positions 2 and 3 of AA are acidic and ionize with pK_1 4.17 and pK_2 11.79, respectively (Crawford and Crawford, 1980). These hydroxyl groups have to be unsubstituted for antioxidant activity in foods and feeds. However, great efforts have been made to prepare chemical derivatives involving the 2 and 3 positions which possess biological vitamin C activity and resistance to oxidation. A comprehensive list of the physical properties of AA has been given by Jaffe (1984).

Ascorbic acid has several reactive positions available for the synthesis of derivatives as reviewed by Tolbert *et al.* (1975), but in the present paper only the 2-OH and 5/6-OH derivatives which have been investigated as sources of vitamin C in fish will be discussed. These are:

Ascorbate-2-sulphate (AS)
Ascorbate-2-monophosphate (AmP)
Ascorbate-2-polyphosphate (ApP)
Ascorbyl palmitate (APt).

In the following the abbreviations given (AA and its derivatives) will be used, and in addition the term «vitamin C» is referred to where general vitamin activity is discussed.

2.2 Analysis

Numerous papers have been published on the analysis of AA, but the majority of the methods describe determination of AA in foods for human consumption, especially fruits and fruit juices which contain high levels of AA. Basically, two analytical approaches are used — direct and indirect methods - the latter involving derivatization before detection. The analytical principles of AA determination have been reviewed by Pachla *et al.* (1985), and include spectrophotometric (using a redox indicator or chromogen formation following derivatization), electrochemical, enzymatic and chromatographic methods.

No standard method has been in general use in fish nutrition studies. This makes comparisons of data on tissue levels difficult. Recently the direct chromatographic methods have gained wider use, but most of the nutritional studies published on vitamin C have been based on indirect methods involving derivatization.

Analytical methods have been described to determine the AA derivatives AS (Terada *et al.*, 1978; Tucker, 1983; Felton and Halver, 1988; Dabrowski

and Hinterleitner, 1989; Schüep *et al.*, 1989; Wang and Seib, 1990; Sandnes *et al.*, 1990), AmP (Dabrowski, 1990a) and ApP (Felton and Halver, 1988; Wang *et al.*, 1988) in feeds and tissues. Questions have been raised whether the indirect AS methods detect AS specifically in fish tissues (Dabrowski and Hinterleitner, 1989; Waagbø *et al.* 1989; Sandnes *et al.* 1990). As the assay techniques are important in relation to the somewhat controversial discussions on AA metabolism in fish, the analytical principles applied in metabolic studies will be discussed more thoroughly in Chapter 4.

3. VITAMIN C IN FISH FEEDS

3.1 Antioxidant activity

The mechanisms of AA oxidation in feeds can be explained either by a one- or a two-electron transfer. Oxidation of feed ingredients initiated by free radicals are terminated by reduction with AA, as one electron is transferred to the free radical from the ascorbate anion the result being formation of the ascorbate radical. Initiation of lipid autoxidation by superoxide radical ($O_2^{-\cdot}$), perhydroxyl radical ($HO_2^{\cdot}$), hydroxyl radical ($HO^{\cdot}$) and singlet oxygen ($O^{\cdot}$) can be retarded by AA (Fessenden and Verma, 1978; Nanni *et al.*, 1980; Cabelli and Bielski, 1983). The ascorbate radical can act as both an oxidizing agent and a reducing agent, and it can react with itself to give a 1:1 mixture of AA and dehydroascorbic acid (DHA) according to Liao and Seib (1988).

The oxidation of AA by two-electron transfer involves reaction with dioxygen in the presence of metals, espesially cupric and ferric ions. The reaction mechanisms are described by Liao and Seib (1988), showing that transition metals catalyze the autoxidation of AA by joining AA and oxygen together in a ternary complex which dissociates into DHA, hydrogen peroxide, and the metal ion. DHA is unstable and is rapidly hydrolyzed to 2,3-diketogulonic acid, thereby loosing the vitamin C activity. Autoxidation is the major cause of AA destruction in feeds, and limiting oxygen, Cu(II) and Fe(III), and the complexing between these ions and AA would reduce the losses (Bauernfeind, 1982). Metal chelating agents retard the autoxidation of AA (Liao and Seib, 1988).

The rate of AA degradation in feeds is also influenced by other reducing substances present, destruction by light and acid catalyzed dehydration of AA (Liao and Seib, 1988). Enzymes oxidizing AA are present in plants (Loewus and Loewus, 1987), and may be active in feeds containing vegetable products (Bender, 1973).

The antioxidant mechanisms of ascorbyl palmitate (APt) are basically the

same as of AA. Palmitic acid esterification of AA hydroxyl position 6 and/or position 5 leaves position 2 and position 3 unsubstituted for antioxidant activity, while the lipid solubility is increased (Cort, 1982; Takahashi *et al.*, 1986).

The antioxidant effect of AA and APt is more effective in combination with tocopherol, as tocopherol reacts with the free radicals first. AA and APt regenerate tocopherol until all ascorbate is consumed (Lambelet *et al.*, 1985).

3.2 Stability

Among the approaches to overcome the problem of AA instability in fish feeds has been microencapsulation or coating of crystalline AA. Hilton *et al.* (1977a) found that AA coated with ethyl cellulose was more stable than crystalline AA in a practical trout diet, but losses due to water addition, cold pelleting, and drying were approximately 90 %. Lovell and Lim (1978) compared AA and AA coated with ethyl cellulose, and reported losses of 23-34% (AA) and 10-24% (coated AA) after steam pelleting and 55-69% (AA) and 40-55% (coated AA) after extrusion. A total loss of 70 — 80 % of the supplemented AA coated with ethyl cellulose was reported by Sandnes and Utne (1982) after steam pelleting and storage at 4°C for 24 weeks, while virtually no AA could be detected after 16 weeks when the feed was stored at room temperature.

A product coated with triglyceride supplemented in the diet equivalent to 1000 mg AA/kg was found to contain 115 mg/kg after cold pelleting and drying at 30°C for 48 hours (Sandnes *et al.*, 1984). Soliman *et al.* (1987) found this product to have a potential as a vitamin C source, showing a two fold retention after processing compared to AA. In another study the coated AA product appeared to be equal to AA as regards steam pelleting and storage stability, while a synthetic polymer coating increased the stability of AA compared to ethyl cellulose and lipid coating, especially during storage of the feeds (Skelbaek *et al.*, 1990). The oxidation of AA is temperature dependent, and keeping the diets frozen has been shown to minimize the losses (Hilton *et al.*, 1977a; Sandnes and Utne, 1982). However, in commercial farming of fish using dry feeds this is not economically and practically feasible.

Lovell (1984) suggested application of AA on the surface of fish feeds after processing. A suspension of AA or coated AA products in the lipid source sprayed onto the pellets have been used by commercial feed producers in Norway, and unpublished results from our institute have shown that this method improves the retention of supplemented AA. However, upon handling and transport of the feed, a significant amount of the supplemented AA was recovered in the crumble loss and thus not longer available for the fish.

It should be noted that the quality of the lipid source affects AA stability in feeds, as Hung and Slinger (1980) showed that oxidized fish oil (peroxide value higher than 120 meq/kg oil) significantly reduced the stability of AA in a practical trout diet.

The AA derivates AS (Halver *et al.*, 1975; Schüep *et al.*, 1989); Sandnes *et al.*, 1989), AmP (Shigueno and Itoh, 1988) and ApP (Grant *et al.*, 1989; Sandnes *et al.*, 1989) have been found to be far more stable in fish feeds than the crystalline form. However, feed stability is not the only criterion on which to base the use of these compounds as vitamin C sources inpractical fish diets. It has to be proved that they are absorbed and possess vitamin C activity in the living tissues. These aspects are discussed in the following chapter.

4. METABOLISM

4.1 Biosynthesis

Ascorbic acid is required by eucaryotic cells, but the capacity to synthesize AA is absent in invertebrates, primates and fish (Fenster, 1989). In these animals the missing step in the biosynthesis of AA from glucose or galactose is the inability to convert L-gulonolactone to 2-keto-L-gulonolactone. (Chatterjee, 1978). The enzyme which catalyzes the conversion is L-gulonolactone oxidase (EC, 1.1.3.8). 2-keto-L-gulonolactone is transformed by spontaneous isomerization into its tautomeric form, AA (Hornig *et al.*, 1984).

Ikeda and Sato (1964; 1965) demonstrated that carp *(Cyprinus carpio)* was able to convert intraperiteonally administered ^{14}C labelled D-glucose and D-glucuronolactone (an intermediate in the biosynthesis of AA) to AA-^{14}C. Yamamoto *et al.* (1978) found L-gulonolactone oxidase acticity in carp liver of about one third of the level found in the rat, but no activity could be detected in rainbow trout. These results compare with studies by Soliman *et al.* (1985) who investigated tilapias, cyprinids and salmonids, and detected enzyme activity only in liver and kidney tissues of carp (cyprinid) and in the kidney of *Oreochromis spilurus* and *Oreochromis aureus* (tilapias). Consistent with these reports, Sato *et al.* (1978b) found that AA was not essential in the diet of carp, regardless of body weight. However, data presented by Dabrowski *et al.* (1988) indicate that an exogenous supply of AA may be necessary in the young common carp. This has also been suggested for another cyprinid, the Indian major carp *(Cirrhina mrigala)*, as reported by Mahajan and Agrawal (1980a).

4.2 Tissue storage

The most debated aspect of AA metabolism in fish is the question whether there is a storage form of vitamin C. Ascorbate-2-sulphate was suggested by

Halver *et al.* (1975) to be a storage form. Reports from Halver and his group (Tucker and Halver, 1984 a,b; Tucker and Halver, 1986) suggest that there are two or more body pools that include a store of AS which is readily interconverted in metabolizing tissues to and from AA. A sulphatase has been proposed to modulate the cellular levels of AA and AS in fish (Benitez and Halver, 1982).

The proposal that AS is a storage form of vitamin C in fish was partly based on analyses using a modification of the classical colorimetric method published by Roe and Kuether in 1942, measuring the absorbance at 515 nm produced when dinitrophenylhydrazine (DNPH) is coupled with dehydroascorbic acid. In the modified assay (Tucker, 1983) AA was determined after incubation of the sample for 3 hours at 37°C while AS was acid-hydrolyzed to AA at 100°C. Differences in incubation times and temperatures allow for analysis of total ascorbate (AA+AS) and for AA while AS is calculated by difference.

The first paper which reported on AS in fish tissues using a direct HPLC method was published by Murai *et al.* (1978). They fed channel catfish *(Ictalurus punctatus)* different levels of AA and AS in the diet, but were unable to detect AS in blood or liver from fish in any group.

In a study on vitamin C and reproduction in rainbow trout Waagbø *et al.* (1989) used the DNPH method as well as a fluorometric method (Roy *et al.*, 1976) for the determination of vitamin C in various fish tissues. The DNPH method seemed to overestimate vitamin C, and the fraction identified as AS by Tucker (1983) was termed «bound ascorbic acid» by Waagbø *et al.* (1989) after a critical evaluation of the method used. The fluorometric method used by Waagbø *et al.* (1989) involves quinoxaline formation following derivatization of dehydro AA with o-phenylenediamine (OPDA) using N-bromosuccinimide as oxidant. This method has been compared with AOAC titrimetric and fluorometric methods. The correlation coefficient was 0.979 and the average recovery was 99.3 % compared with the official methods (Pachla *et al.*, 1985).

Dabrowski and Hinterleitner (1989) using another modified DNPH assay with correction for interfering substances were not able to detect AS in fish tissues. By the same method they found AS in Artemia cysts as have been reported earlier (Mead and Finamore, 1969).

The DNPH principle of vitamin C determination has several drawbacks related to the specificity of the hydrazone formation (Zloch *et al.*, 1971; Baker *et al.*, 1973; Terada *et al.*, 1977; Dabrowski and Hinterleitner, 1989). An attempt to improve the specificity was described by Schüep *et al.* (1984) using chromatographic separation of the reaction products after extraction with an organic solvent. This method was modified by Sandnes *et al.* (1990) to detect

AS separately (standard) by means of varying incubation times and temperatures. Applied on liver tissue of Atlantic salmon the ozazone formed by the «AS» incubation procedure increased with increasing vitamin C in the diet (Sandnes *et al.*, 1990). However, using a direct HPLC assay (Schüep *et al.*, 1989) no AS could be detected in the same samples (Sandnes *et al.*, 1990), nor in liver of plaice *(Pleuronectes platessa)* as reported by Rosenlund *et al.* (1990). Thus this fraction was termed «bound AA» by these authors. In summary, the results indicate the possibility of a storage form of vitamin C in fish, but probably not in the form of AS.

4.3 Bioactivity of AA derivatives

The first AA derivative which gathered interest as a vitamin C source in fish nutrition was ascorbate-2-sulfate (AS). This compound was detected in the brine shrimp *Artemia salina* and was postulated to be a storage form of AA (Mead and Finamore, 1969; Bond *et al.*, 1972). Studies on AS in fish nutrition were undertaken by Halver and his group who in a series of reports claimed AS to be readily absorbed and equivalent to AA as a vitamin C source in fish (Tucker and Halver, 1984 a, b; Halver *et al.*, 1975; Tucker and Halver, 1986).

Other studies have not confirmed these results, and indicate that AS has a lower vitamin C bioactivity than AA. Only a few authors have presented analytical data on tissue levels of AA forms in feeding studies with fish. The experiments indicate that AS possesses some vitamin C activity in fish, but no authors have reported AS to be equivalent to AA with respect to organ retention of AA or AS (Murai *et al.*, 1978; Tsujimura *et al.*, 1978, 1981; Soliman *et al.*, 1986a).

Recent studies by Sandnes *et al.* (1990) and Dabrowski *et al.* (1990a) confirm these findings. Thus Sandnes *et al.* (1990) reported AA to have 4 - 5 times higher bioactivity in Atlantic salmon than AS. The results showed that within reasonable feed supplementation levels AS does not provide adequate supplies of vitamin C to secure optimal physiological functions as demonstrated by tissue analyses of AA and haematological parameters. In rainbow trout, Dabrowski *et al.* (1990a) showed that the total AA concentration in viscera of fish fed 500 mg AS/kg feed was one sixth of that in fish fed an equivalent AA diet. Dabrowski (1990a) demonstrated hydrolysis of AmP in intestinal extracts from rainbow trout. In an *in vitro* study carried out by Sandnes and Waagbø (1991a), an extract from the gastrointestinal tract of Atlantic salmon was incubated with AS or AmP. The concentration of AS in the medium was unaffected, while AmP was removed, thus indicating the presence of phosphatase activity and lack of sulphatase activity in the gastrointestinal extract.

Recent studies have shown that phosphate derivatives of AA posess good vitamin C activity in aquatic animals. A polyphosphorylated form of AA, L-ascorbyl-2-polyphosphate (ApP), was demonstrated to have vitamin C activity in channel catfish (*Ictalurus punctatus*) (Grant *et al.*, 1989; Robinson *et al.*, 1989; Wilson *et al.*, 1989), in fathead minnow (*Pimephales promelas*) and guppy (*Poecilia reticulata*) (Grant *et al.*, 1989) and in Atlantic salmon (*Salmo salar*) (Sandnes *et al.*, 1989). Ascorbate-2-monophosphate (AmP) has vitamin C bioactivity in shrimp *(Penaeus japonicus)* as reported by Shigueno and Itoh (1988) and in Atlantic salmon (Sandnes and Waagbø, 1991b; Sandnes *et al.*, 1991). Phosphate derivatives of AA are probably absorbed as AA after hydrolysis in the gastrointestinal tract, as demonstrated in an *in vitro* experiment by Sandnes and Waagbø (1991a).

A few reports have been published on the use of ascorbyl palmitate (APt) as a vitamin C source in fish diets. From studies with channel catfish, Brandt *et al.* (1985) concluded that APt prevented scurvy and provided sufficient vitamin C for growth and feed conversion similar to fish fed AA in the diet. Soliman *et al.* (1986a) found similar results in juvenile tilapias *(Oreochromis niloticus),* but reported that fish fed APt had higher hepatosomatic indexes and carcass lipid contents than fish fed other sources of vitamin C. In a study with rainbow trout fry, Albrektsen *et al.* (1988) reported reduced growth during the first 8 weeks of the experiment in fish fed APt in the diet compared to AA. It was shown that APt resulted in lower tissue levels of AA, but an *in vitro* assay demonstrated the presence of enzyme activity hydrolyzing APt in the gut.

As far as the combination of feed stability and vitamin C bioactivity are concerned, data hitherto reported support that the phosphate derivatives of AA are superior to AA, AS and APt as vitamin C sources in fish diets.

5. BIOLOGICAL FUNCTIONS AND DIETARY REQUIREMENTS

Unlike the B vitamins AA has no coenzyme function, but acts as a cofactor in various hydroxylation reactions in living tissues, of which the hydroxylations of collagen proline and lysine in connective tissues have been mainly studied. Ascorbic acid is further regarded as an important general modulator of redox systems in the body. A comprehensive general review on the biological functions of vitamin C is beyond the scope of this article. For further informations is referred to Levander and Cheng, 1980; Hornig *et al.*, 1984; Halliwell and Gutteridge, 1989; Padh, 1991; Slater and Block, 1991.

Exact dietary requirement studies have been difficult to carry out due to the instability of AA in fish feeds. Most studies were conducted without

control of the AA content in the diet by means of analyses of AA throughout the feeding period. In many experiments the requirement was evaluated on the basis of supplementation levels, which most probably tended to overestimate the requirement. As feed stable vitamin C sources now become available such studies will be more accurate provided that the bioactivity of the compound used is known.

5.1 Growth and development

The symptoms of AA deficiency in fish were described by Halver *et al.* (1975) including lordosis, scoliosis, distortion of support cartilage, hyperplasia of gill tissue, shortened opercles and petechial haemorrhages. External signs of AA deficiency have been described in rainbow trout (Kitamura *et al.*, 1965; Halver *et al.*, 1969; Hilton *et al.* 1977b, 1978; Meier and Wahli, 1990), brook trout (Poston, 1967), coho salmon, (*Oncorhynchus kisutch)* (Halver *et al.*, 1969), cherry salmon, (*Oncorhynchus masou*) (Halver *et al.*, 1975), Indian major carp, (*Cirrhina mrigala*) (Mahajan and Agrawal, 1980), common carp, (*Cyprinus carpio*) (Dabrowski *et al.*, 1988), guppy, (*Poecilia reticulata)* (Halver *et al.*, 1975), yellowtail, (*Seriola quinqueradiata)* (Sakaguchi *et al.*, 1969), channel catfish (Lovell, 1973; Lim and Lovell, 1978; Miyazaki *et al.*, 1985), Japanese eel, (*Anguilla japonica)* (Arai *et al.*, 1972), milkfish, (*Coregonus lavaretus)* L (Zitzov and Millard, 1988), plaice, (*Pleuronectes platessa)* (Rosenlund *et al.*, 1990), *Tilapia zilli* (Anadu *et al.*, 1990), Mexican native chichlid, (*Cichlastoma uropthalmus)* Gunther (Chavez de Martinez, 1990) and Atlantic salmon, (*Salmo salar)* (Sandnes *et al.*, 1991).

It is generally agreed that the deficiency symptoms seen in fish are caused by impaired collagen and support cartilage formation (Halver *et al.* 1975; Wilson and Poe, 1973; Lim and Lovell, 1978; Sandnes et al, 1991). Ascorbic acid functions as a cofactor for the enzyme-catalyzed hydroxylations of proline and lysine in collagen biosynthesis, where AA maintains enzyme bound iron in its divalent state (Barnes and Kodicek, 1972; Hornig *et al.*, 1984). Collagen is an abundant protein in fish, and the highest concentrations are found in skin and bones (Yoshinaka *et al.*, 1990). Collagen synthesis and the role of AA in the formation of connective tissues in rainbow trout have been extensively studied by Sato and his group (Sato *et al.*, 1978a; 1982; 1984; Yoshinaka *et al.*, 1978).

Based on studies on hydroxylation of collagen proline, Sato *et al.* (1982) estimated that the minimum dietary AA requirement to maintain normal collagen formation in rainbow trout was 50 - 100 mg per kg diet. This is in accordance with other studies in salmonids, where the minimum dietary

requirement in young fish has been suggested to be in the range of 40 — 100 mg/kg diet (Halver *et al.* 1969; Halver 1972; Hilton *et al.* 1978; Sandnes 1982). In coho salmon it was found that 50 mg/kg prevented deficiency symptoms and 100 mg/kg was needed to support optimal growth (Halver *et al.* 1969). Studies by Dabrowski *et al.* (1990a) showed that 20 mg AA/kg diet prevented skeletal deformity in rainbow trout, but Dabrowski *et al.* (1990a) suggested that a supplementation level of 500 mg AA/kg is necessary to maintain body AA concentration.

However, in the above cited reports crystalline AA or encapsulated crystalline AA were used as vitamin C sources. These are labile in fish feeds and AA is highly soluble in water. It is possible that oxidation of AA in the experimental diets and leaching may have tended to overestimate the requirement. Thus Sandnes *et al.* (1991), using a calcium salt of ascorbate-2-monophosphate (AmP) as the dietary vitamin C source in a requirement study with Atlantic salmon fry, found that the minimum dietary requirement for growth and collagen formation was in the range of 10 - 20 mg AA equivalents per kg diet. The Ca salt of AmP exhibit good vitamin C bioactivity in fish (Sandnes and Waagbø, 1991b), it is stable and less soluble in water than AA.

As no comparable studies using other sources of vitamin C have been carried out with Atlantic salmon, it may be argued that the requirement may be lower in this species than in other salmonids. Further well controlled studies with different salmonid species using bioactive and stable vitamin C forms are needed to clarify this.

In addition to the salmonids, the metabolism and dietary requirement of vitamin C have been most widely studied in channel catfish. In this species the AA requirement for growth and collagen formation has been found to be in the range of 30 — 60 mg per kg diet (Andrews and Murai, 1975; Lim and Lovell, 1978).

Unfortunately, data presented on minimum dietary AA requirements in other fish species are not so extensive as to allow for recommendations for dietary supplementations. The results may be inconclusive, and experimental design and control are not appropriate in many cases to evaluate the results. Wide variations in AA requirements have been reported within the same species, as well among species. Thus it is still discussed whether carp requires dietary vitamin C or not (Sato *et al.*, 1978b; Dabrowski *et al.*, 1988), and AA requirements for growth have been proposed to range from 40 mg/kg diet in the Mexican native chichlid (*Cichlasoma uropthalmus)* (Chavez de Martinez, 1990) to 30.000 mg/kg in *Tilapia zilli* (Anadu *et al.*, 1990).

Studies to evaluate dietary AA requirements for growth have been carried out with young fish. There is evidence that the need for dietary vitamin C decreases with increasing fish size or age (Sato *et al.*, 1978a; Hilton *et al.*, 1978;

Waagbø *et al.*, 1989). However, data are lacking on vitamin C requirements in older fish during the growth phase until maturation.

5.2 Wound healing

Physical factors and infections frequently result in wounds in fish, which further negatively affect disease resistance and the quality of fish as food. Collagen formation is necessary for optimal wound repair in animals, and the essential role of AA in the biosynthesis of collagen has promoted interest to study the effects of dietary AA related to wound healing in fish.

Halver *et al.* (1969) studied wound healing after 24 weeks of a feeding trial with rainbow trout and coho salmon which were fed 0, 50, 200, 400 or 1000 mg AA per kg diet. A 1 cm incision was made through the abdominal wall at the mid line and a similar wound was made into the musculature above the lateral line. After closing the wounds with a gut suture, the fish were fed the experimental diets for another 3 weeks before histological examination of the wounds were performed. The results showed (Halver *et al.*, 1969) that coho salmon and rainbow trout fed the AA deficient diet completely failed to repair the wounds, which was partially plugged with poorly clotted blood. Wound repair was related to the dietary content of AA, and coho salmon healed somewhat faster than rainbow trout fed the same dietary AA levels. Based on this study it was suggested that 1000 mg AA/kg diet is needed for optimal wound and tissue repair (Halver *et al.*, 1969; Halver, 1972, Ashley *et al.*, 1975; Halver *et al.*, 1975).

Lim and Lovell (1978) studied wound repair in channel catfish as a function of dietary vitamin C. An incision was made 1 cm in length and 0.2 cm in depth parallel to the lateral line and closed with surgical suture. Fish were fed 0, 30, 60, 90 or 120 mg AA/kg diet, and the wound repair study was carried out between the 15th and 17th week of the feeding experiment. The rate of wound repair was faster than in salmonids (Halver *et al.*, 1969), and showed almost complete healing of deep muscle in 10 days when fed 60 mg AA/kg. The findings were discussed in view of the report on salmonids by Halver *et al.* (1969), and it was indicated that species differences could exist related to AA and biosynthesis of collagen.

5.3 Reproduction

The build-up of the yolk material in the growing oocytes in oviparous teleost fish is called vitellogenesis. Egg yolk proteins are synthesised in the liver under hormonal control, involving estrogenic hormones. The estrogenic hormones are synthesised in the ovarian follicles and released into the bloodstream.

These hormones are taken up by the liver where they stimulate the synthesis of a specific egg yolk protein - vitellogenin — which is transported by the blood to the oocytes where it is absorbed. Vitellogenin comprises two main protein classes, the phosvitins and the lipovitellins, which constitute about 90% of the fish egg mass. This is a brief description of the processes of maturation in oviparous teleosts, for more details it is referred to comprehensive reviews edited by Richter and Goos (1982) and by Hoar *et al.* (1983).

High concentrations of AA in fish ovaries have been reported by many authors (Sandnes, 1984; 1988). Ovary levels of AA vary during the reproductive cycle in the crucian carp, *(Carassius carassius)* (Seymour, 1981 a,b), in cod, *(Gadus morhua)* (Sandnes and Braekkan, 1981) and in sea trout, *(Salmo trutta)* (Sandnes, 1984). This is accordance with findings in mammals (Lutwak-Mann, 1958). The overall high levels and seasonal variations of AA in ovaries have been suggested to reflect a requirement for AA in hydroxylation reactions in steroidogenesis in the ovarian follicle cells (Hilton *et al.* 1979; Seymour 1981a).Thus Seymour (1981b) showed that administration of pituitary extracts depleted ovarian AA in carp. Waagbø *et al.* (1989) further demonstrated significantly higher levels of circulating 17-ß-estradiol in maturing rainbow trout fed a high dietary level of AA (2000 mg/kg) than in fish fed without AA in the diet. In accordance with this finding, different levels of vitellogenin was found in the blood (Waagbø *et al.* 1989). They showed that the haemoglobin concentration was significantly negatively correlated to the gonadosomatic index in rainbow trout fed no AA in the diet, but not in fish fed dietary AA.

Komarov and Knyazeva (1984) reported an increased level of total serum protein in rainbow trout fed a vitamin C enriched (700 mg/kg) diet, but the results are difficult to interpret as a true control group apparently was lacking.

The reports cited indicate that AA participates in the metabolism of sex steroids in fish similarly as in mammals (Biswas, 1969; Biswas and Deb, 1970), although the specific mechanisms have not been studied. The results do not, however, give any information on the dietary levels needed to cover the requirement of AA for optimum gonadal function and vitellogenesis. Taking into account the profound biochemical and physiological changes occuring in maturing fish compared with the immature stages, a higher dietary level of AA than needed by juvenile fish is likely in fish with rapid gonadal growth.A further aspect of AA requirement in broodstock fish is the transfer of AA into the eggs to support the need for normal embryonic development and hatching success. Sandnes *et al.* (1984) showed that maturing rainbow trout fed a dry diet supplemented with AA which contained 115 mg AA/kg feed after processing produced eggs with a significantly improved hatching rate than did fish fed the same diet without AA supplementation. The diets were fed for

3 months prior to spawning. Egg AA levels were 31 μg/g (w.w) and 15 μg/g, respectively. Komarov and Knyazeva (1984) also presented results indicating similar effecs on egg hatching success in this species.

Soliman *et al.* (1986b) found that supplementation of 1250 mg AA/kg in the broodstock feed significantly increased egg hatchability in tilapias *(Oreochromis mossambicus)*. The study also demonstrated that AA in the broodstock diet could be transferred via the eggs to the newly hatched fry to support vitamin C activity during the early stages of life (Soliman *et al.* 1986b).

A comparison of AA in eggs from steelhead and rainbow trout fed diets containing either 800 ppm or 1400 ppm AA carried out by Ridelman (1981) showed fewer blind lots of eggs from fish fed the higher level of AA. (Cited by Iwamoto and Sower (1985)).

Little is known about the metabolism of AA in developing fish eggs after fertilization. It has been shown in sea urchin eggs that the onset of collagen synthesis takes place during gastrulation and increases to a plateau in the late pluteus stage (Golob *et al.* 1974). Mizoguchi and Yasumasu (1982) found that archenteron formation in sea urchin embryos could be induced by ascorbate and α-ketoglutarate, and suggested that this could be a result of a stimulated procollagen synthesis. Unpublished results from our institute showed an increase in the amount of egg protein hydroxyproline in rainbow trout eggs during embryonic development. The results indicate that collagenous connective tissue is formed during incubation. It should be noted that the shell of fish eggs does not contain collagen, but a keratine-like material which does not contain hydroxylated proline or lysine (Waagbø *et al.* 1989).

In summary, AA is necessary for optimal reproduction performance in fish. This is related to vitellogenesis and embryonic development. Incorporation of AA in the eggs during ovarian growth is important for a successful larval development after hatching. The data available are not sufficient to suggest a broodstock dietary requirement of AA in any species of fish, but the requirement may be higher than during the immature stages.

5.4 Interactions with other nutrients

It is well established that the requirement of vitamin C is influenced by other nutrients, either in the diet or through its biological function and metabolism. Factors affecting the stability of AA in fish feeds were discussed in Chapter 3, and the impacts of environmental factors upon vitamin C requirement and metabolism in fish will be dealt with in Chapter 5.5.

The interaction of vitamins, minerals and diet composition in fish was recently discussed in a review by Hilton (1989). This covers interactions

between AA and vitamin E, copper and iron. Only new data on these subjects will be presented in this paper. For general information on the interactions between AA and other nutrients it is referred to reviews edited by Levander and Cheng (1980) and Halliwell and Gutteridge (1989).

A recent paper by Maage *et al.* (1990) reported on interactions between dietary vitamin C forms (AA and AS) and the metabolism of trace elements in Atlantic salmon related to stimulation of the humoral defense system. This study demonstrated anaemia in vitamin C deprived fish despite significantly elevated iron concentrations in the liver. The concentrations of cadmium in the liver, gills and kidney were not affected by AA or AS in the diet, or by the supplementation levels of these forms (0, 500 and 5000 mg AA/kg; and equivalent amounts of AS). Fish fed the highest dietary level of AA showed significantly reduced selenium concentrations in the liver, but this effect was not seen in salmon fed an equivalent amount of AS in the diet.

In response to stimulation of the humoral defense system by injection of an artificial soluble antigen (NIP_{11}-LPH), fish in all groups showed increased levels of hepatic metallothionein, copper, zinc and cadmium, while hepatic selenium and iron levels were less affected. In vitamin C deprived fish the total iron content in the spleen increased nearly threefold following antigen injection, while the contents were approximately doubled in fish which had been fed any form or level of vitamin C in the diet (Maage *et al.*, 1990).

Dabrowski and Køck (1987) found that the concentrations of iron and zinc in the contents of the posterior intestine were lower in rainbow trout fed AA in the diet compared to fish fed AS supplemented and vitamin C deficient diets. It was suggested that AA enhanced the absorption of these elements in rainbow trout.

Ascorbic acid deficiency has been associated with a decreased absorption of calcium by gill, skin, bone and muscle in fish (Pang, 1971; Mahajan and Agrawal, 1980). Dabrowski *et al.* (1990a) found reduced body levels of calcium in rainbow trout fed a vitamin C free diet for 84 days, but this was not seen at day 176 of the experiment.

A better understanding of the interactions between AA, AA derivatives and trace elements and minerals in fish nutrition is needed, especially related to the content and chemical form of iron in the diets and to the absorption, redistribution and general metabolism of iron in fish.

Recent studies with Atlantic salmon have shown that excess dietary iron may excert significant negative impacts upon resistance to infectious diseases and fish health in general (Bentsen *et al.*, 1991). It is known that feed iron in terrestrial animals is absorbed by the intestinal mucosa from two separate pools of heme and nonhaeme iron. Haeme iron is well absorbed and relatively little affected by AA, while AA is a powerful enchancer of nonhaeme iron

absorption which normally constitutes the major dietary pool. Ascorbic acid plays a minor role on the absorption of insoluble inorganic iron, such as ferric oxide and ferric hydroxide, which are common feed contaminants (Lynch and Cook, 1980). Further, AA functions as a regulator in transport and redistribution of iron in the body as discussed by Maage *et al.* (1990), and iron is an important growth promotor for microorganisms. These aspects should be evaluated further in fish nutrition studies in order to secure an optimal balance between supplementation levels and chemical forms of iron as well as vitamin C in the diets.

5.5 Stress and environmental factors

Vitamin C studies in fish have also been focused on the possibilities to reduce negative impacts caused by stress and environmental factors upon health and disease resistance . These subjects have long been regarded as important topics of research in intensive production of land-living species, especially in poultry (Perek, 1984). Stress increases the susceptibility to infections in fish (Maule *et al.*, 1989), and modern fish farming implies several forms of handling such as netting, grading, vaccination and transport. Stocking density, pollution and poor water quality are also factors known to impose stress reactions in fish.

The response to a wide variety of stressors is controlled by adrenocoticotropic hormone (ACTH) from the pituitary which controls the release of catecholamines (adrenaline and noradrenaline) and corticosteroids from the chromaffin and interrenal tissues in the head kidney (primary response). Among the physiological changes caused by the release of these hormones (secondary response) are dilation of gill filament arteries, an increase in stroke volume of the heart, increased glycogen metabolism, and depression of the immune response (Mazeaud and Mazeaud, 1981; Gratzek and Reinert, 1984; Wiik *et al.*, 1989).

In the body, AA interacts in the metabolism of catecholamines and corticosteroids. It is required for the biosynthesis of noradrenaline from dopamine where AA acts as a co-substrate for the enzyme ß-monooxygenase (Levine *et al.*, 1985). It also affects the metabolism of corticosteroids as demonstrated experimentally by application of exogenous ACTH resulting in changes in AA levels of the body similar to those occuring in states of stress (Dvorak, 1984). This has also been demonstrated in fish (Wedemeyer (1969) showing a reduction in head kidney AA after different forms of stress or ACTH injection.

It is known that stocking density imposes stress in fish, and a study by Lovell and Lim (1978) indicated that the requirement of AA is increased in channel catfish stocked at high densities.

An experiment conducted by Sandnes and Waagbø (1991a) studied primary (cortisol) and secondary (serum glucose) stress responses in Atlantic salmon *(Salmo salar)* with different AA status, using ascorbate-2-monophosphate (AmP) as dietary vitamin C source. Following moderate physical stress, the circulating levels of cortisol and glucose increased, but these parameters were not significantly affected by the AA status of the fish. The concentrations of AA in head kidney and liver reflected the dietary intake of AmP, but were not significantly influenced by the stress applied to the fish.

The effects of salinity, temperature fluctuations and capture stress on the content of AA in tissues of striped mullet *(Mugil cephalus)* were investigated by Thomas (1984). All treatments led to changes in AA concentrations in the brain, gills, kidney and liver, but the pattern of fluctuations varied. Conclusions on how AA metabolism is affected by the parameters studied can hardly be drawn from this report, but the author suggested an involvement of AA in osmo- or ionregulatory functions of teleost gills, salinity and thermal adaption mechanisms in neural tissue, and the response of renal tissue to adverse environmental stimuli.

Hilton *et al.* (1979) reported that AA concentrations in rainbow trout brain were consistently higher than in other tissues. Studies on the uptake of radiolabelled AA in the brain after intraperitoneal injection indicated a long time to reach an equilibrium, and suggest the existense of a bloodbrain barrier to AA in the central nervous system (Hilton *et al.*, 1979). Brain AA depletion as a response to stress was studied by Tucker *et al.* (1987) in rainbow trout, showing that the loss of brain AA after keeping the fish for five days in metabolism chambers was significantly higher than in fish which was kept as control. A pool of AA in the brain which does not exchange with the body pool was proposed, and Tucker *et al.* (1987) suggested that the decrease of AA in the brain and in adrenals following stress is a result of excretion of AA metabolites rather than AA itself.

The relationships between AA status and the brain levels of the neurotransmitters serotonin, 5-hydroxyindoleacetic acid, noradrenaline and dopamine were studied by Johnston *et al.* (1989) in rainbow trout. The results showed a significant relationship between brain serotonin, brain AA and weight gain after 12 weeks of feeding the test diets differing in the content of AA, but not after 24 weeks. In the biosynthesis of serotonin, AA may facilitate the conversion of tryptophan to 5-hydroxytryptophan by maintaining the cofactor tetrahydrofolate of tryptophan 5-monooxygenase in its active, reduced form (Johnston *et al.*, 1989).

Fish breathe and eat in water, and this obvious fact implies that factors present in the aquatic environment have a direct impact upon the life functions. Pollutants as well as variable concentrations of naturally occuring

components in the water affects fish physiology. Such environmental factors can act either directly by interfering in metabolic processes, or indirectly by releasing a stress reaction as discussed above. In most cases probably a combination of these effects occurs.

Agrawal *et al.* (1978) demonstrated that AA protected against the organochlorine pesticide aldrin in *Channa punctatus*. Fish fed 0.25 % aldrin in a diet supplemented with 1250 mg AA/kg showed a mortality of 25 % in 30 days, while the mortality was decreased tenfold when an additional amount of 5000 mg AA was included per kg feed. Mortality was preceded by hematological changes like polycythaemia and leucocytosis, and with increased thrombocytes and decreased neutrophil populations. It has further been demonstrated that organochlorine pesticides induce the hepatic microsomal enzyme system in rats, which in turn stimulates biosynthesis of AA (Agrawal *et al.*, 1978), thus supporting a theory of a beneficial role of AA in animals exposed to organochlorine pollution. An increased AA requirement has further been demonstrated in channel catfish exposed to toxaphene (an insecticide), resulting in a depletion of hepatic reserves (Mayer *et al.*, 1978).

Nitrite is an intermediate product of nitrification, and may reach toxic levels in unfavourable intensive production of channel catfish. The toxicity of nitrite is related to its ability to oxidize haemoglobin to methaemoglobin which can not carry oxygen. Wise *et al.* (1988) showed that high dietary levels of AA (800 — 8000 mg/kg) significantly reduced nitrite - induced methaemoglobinaemia when fed 24 or 48 hours prior to nitrite exposure in channel catfish. If fish were not fed AA throughout the last 65 hours prior to nitrite exposure, no protection of AA could be detected.

Thomas (1987) reported that exposure to water-soluble fractions of oil caused a depletion of AA in brain, gill, kidney and liver tissues of striped mullet (Mugil cephalus). Liver AA concentrations were significantly depleted after one week of oil exposure, but returned to control levels after 20 days. According to Thomas (1987) care should be taken to ensure sufficient AA reserves in fish cultivated in oil contaminated environments.

The effects of acute and chronic cadmium exposures on AA status and cadmium accumulation in the tissues of juvenile striped mullet were studied by Thomas *et al.* (1982). Ascorbic acid was depleted in the liver and the gills accompanied by massive accumulation of cadmium in these organs, while the kidney and brain were less affected. In rainbow trout Yamamoto and Inoue (1985) demonstrated that the survival time of fish fed a diet containing 6000 mg AA/kg was significantly extended compared to fish fed vitamin C free diets when exposed to 0.14 ppm cadmium in the water.

The effect of AA on dietary and waterborne copper metabolism and excretion has recently been reviewed by Hilton (1989). According to the

review, AA does not appear to affect absorption, excretion and the general copper metabolism in rainbow trout. This is in direct contrast to reported effects of AA on waterborne copper in carp and rainbow trout where it has been shown that dietary AA reduces the toxicity and tissue retention of this element. It is not immediately obvious why such differences should exist, and according to Hilton (1989) further studies are warranted to clarify these questions.

5.6 Immunology and disease resistance

Considerable research has been done to find means to prevent and treat fish diseases, but little work has been carried out to determine the relationships between nutritional status, immunocompetence and disease resistance. Most of the research which has been conducted in this field has focused upon mammals and birds, where effects of vitamin C on immune functions have long been recognized and have been a topic of debate for many years. A large number of papers have been published on these aspects as reviewed by Beisel (1982) and Diplock (1991).

In a recent review on nutrition and immunity in fish, Landolt (1989) stated that studies in this field have been hampered by the fact that fish immunology is in its infancy, and that basic immune functions are still incompletely characterized. However, during the last decade some studies have been carried out, of which most interest has been focused on immunological functions and disease resistance related to dietary intake of vitamin C.

Durve and Lovell (1982) showed that a dietary supplementation of 30 mg vitamin C per kg supported normal growth and prevented deficiency symptoms in channel catfish, but increased resistance against infection by *Edwardsiella tarda* was seen at a supplementation level of 150 mg/kg at a water temperature of 23 °C. At 33 °C, the protective effect of vitamin C was significantly less. The authors suggested that the vitamin C requirement for resistance to infection in fish could be lower at higher water temperatures.

In a further study with channel catfish Li and Lovell (1985) found that mortality rates of fish experimentally infected with *Edvardsiella ictaluri* decreased with increases in dietary AA levels, ranging from 100% for fish fed an AA deficient diet to 15% for fish fed 300 mg AA/kg diet and 0 for fish fed 3000 mg AA/kg. No differences were found in antibody production against *E. ictaluri*, total complement activity, or phagocytic engulfment of *E. ictaluri* by peripheral phagocytes among fish fed diets containing 30-300 mg AA/kg, but the dose level of 3000 mg AA/kg significantly enhanced antibody production and complement activity.

A study carried out by Liu *et al.* (1989) did not confirm that increasing levels of dietary AA increased complement hemolytic activity and antibody titers in channel catfish. However, fish receiving 1000 mg AA/kg diet showed increased resistance to *Edvardsiella ictaluri*, and exhibited a 100 fold LD_{50} compared to fish receiving a diet without supplementation of AA.

In red sea bream *(Pagurus major)* fed purified diets supplemented with 0 and 1000 mg AA/kg, Yano *et al.* (1988) found no effects of diet on complement activity, while fish fed the high dietary AA supplementation showed a significant higher natural hemagglutinin titer.

Studies have also been carried out to evaluate the significance of AA on immune functions and disease resistance in the salmonid fishes. In rainbow trout Blazer (1982) showed that AA deficiency significantly depressed iron - binding capacity and phagocytosis and reduced the cellular response to *Yersinia ruckeri*. The cellular response to sheep red blood cells was not affected by dietary AA.

Wahli *et al.* (1986) studied the effect of dietary AA on the disease resistance of rainbow trout infected with the parasite *Ichtyophthirius multifiliis* which is a holotrichous ciliate. Trout fed 0 and 5000 mg AA/kg diet for five days were infected with 8.800 and 11.500 tomites per fish, respectively. Fifty days after infection no parasites could be detected on fish which had survived the infection. With the lower infectious dose the fish survival rate increased from 48% (0 mg AA/kg) to 98% (5000 mg AA/kg), while the respective values were 0% and 82% in trout infected with the highest number of zoospores. Serum from experimentally infected fish was examined for tomite-immobilizing activity, IgM and spesific antibodies against *I. multifiliis* (Wahli *et al.*, 1986). According to the authors the results definitely indicated the presence of an immune response against this parasite, and that this response may be enhanced by dietary AA.

Navarre and Halver (1989) studied the effects of high levels of dietary AA on disease resistance and humoral antibody production against *Vibrio anguillarum* in rainbow trout. Young fish were fed purified experimental diets supplemented with 0, 100, 500, 1000 and 2000 mg AA/kg for 28 weeks. Resistance to bacterial infection was related to dietary AA, and was reflected in improved survival of challenged fish fed 500, 1000 and 2000 mg AA in the diet. Higher antibody production was seen in fish fed these diets, and Navarre and Halver (1989) concluded that rainbow trout may survive bacterial challenge and improve antibody production when the diet contains 500 —1000 mg AA/kg.

Bell *et al.* (1984) did not find any positive effects on survival time of sockeye salmon *(Oncorhynchus nerka)* inoculated with *Renibacterium salmoninarum* which causes bacterial kidney disease (BKD). In these studies Bell *et al.* (1984) used

Na-ascorbate-2-sulphate (AS) as dietary vitamin C source. In view of recent findings demonstrating a reduced vitamin C bioactivity of this compound in salmonids (Dabrowski *et al.*, 1990b ; Sandnes *et al.*, 1990), the results reported by Bell *et al.* (1984) may not be significant in relation to effects of vitamin C. Survival time was in fact found to be inversely related to the dietary AS level when the diets contained low levels of Zn and Mn, and fish from the same dietary treatments which were vaccinated against furunculosis *(Aeromonas salmonicida)* did not show any differences in antibody response (Bell *et al.*, 1984).

In Atlantic salmon parr, Sandnes *et al.* (1990) studied the production of antibodies against a soluble artificial antigen (NIP_{11}-LPH) following a feeding period of six weeks when fish were fed different equivalent levels of AA and AS in the diets (0, 500 and 5000 mg AA/kg). The antibody response was somewhat reduced in fish deprived of vitamin C, but there were no differences between fish fed 500 and 5000 mg/kg, irrespective of chemical form. Further, as the vitamin C status was significantly lower in fish fed AS compared to AA as explained by a reduced bioactivity of AS, the experiment indicated that the production of antibodies is not dependent on a high physiological status of AA (Sandnes *et al.*, 1990).

Erdal *et al.* (1991) fed Atlantic salmon three diets differing in the contents of AA and AS and measured general resistance after challenge with *Vibrio salmonicida*, development of spesific immune response after vaccination against *Yersinia ruckeri* and survival after challenge. No significant differences in general or spesific resistance were found, but unfortunately the experimental design renders interpretation of the results difficult. Thus the experimental diets were supplemented either with: 90 mg AA and 218 mg AS per kg feed (Diet 1), 2980 mg AA/218 mg AS (Diet 2) or 90 mg AA/4770 mg AS (Diet 3).

Olivier *et al.* (1989) fed a practical diet supplemented with 0, 50, 100, 200, 500, 1000 and 2000 mg AA per kg to Atlantic salmon for 22 weeks. For all diets, no effects on non - spesific resistance factors were found. Groups of fish fed 0, 100, 500 and 2000 mg AA/kg were immunized against *Aeromonas salmonicida* and *Vibrio anguillarum.* One month after vaccination analyses of complement activity and humoral antibody response revealed no effects of dietary AA, and protection was observed in all vaccinated groups following a live challenge.

The papers cited on the humoral response in Atlantic salmon related to dietary vitamin C intake were confirmed by Hardie *et al.* (1991) in a feeding study with Atlantic salmon lasting for 23 weeks. However, they found that serum complement activity was significantly reduced in AA depleted fish, and on bath challenge with a virulent strain of *Aeromonas salmonicida*, a significant increase in mortality was seen in AA depleted fish compared to fish

fed 310 mg AA/kg and 2750 mg AA/kg. Factors like serum protein level, differential leucocyte count, respiratory burst (superoxide anion) activity and erythrophagocytosis, as well as lymphokine (MAF) production following immunization, were unaffected by dietary AA (Hardie *et al.*, 1991).

In summary, there is evidence that high dietary doses of vitamin C protect against infectious diseases in channel catfish, but no clear conclusions can be drawn as regards the salmonids. Further studies are needed to acquire more knowledge on how the immune system and disease resistance are affected by vitamin C in the salmonid species. Feeding experiments should include challenge tests with fish fed a range of dietary vitamin C levels in order to give recommendations on dietary supplementations when feeding fish susceptible to infectious diseases in practice.

5.7 Other physiological effects

In addition to the topics discussed, the literature on vitamin C in fish nutrition describes a wide range of different physiological effects caused by a deficiency of this vitamin. It is not possible to give a comprehensive review on all factors studied, and it is important to bear in mind that some of the effects reported may not exclusively have a metabolic origin as a result of vitamin C deficiency, but simply have been caused by a decreased feed intake in fish fed diets devoid of vitamin C.

REFERENCES

ANADU, D. I., ANOZIE, O. C. and ANTHONY, A. D., 1990. Growth responses of *Tilapia zillii* fed diets containing various levels of ascorbic acid and cobalt chloride. Aquaculture, 88:329-336.

ANDREWS, J. W. and MURAI, T., 1975. Studies on vitamin C requirements of channel catfish. J. Nutr., *105*: 557-561.

ALBREKTSEN, S., LIE, Ø. and SANDNES, K., 1988. Ascorbyl palmitate as a dietary vitamin C source for rainbow trout *(Salmo gairdneri)*. Aquaculture, *71*: 359-368.

AGRAWAL, N. K., JUNEJA, C. J. and MAHAJAN, C. L., 1978. Protective role of ascorbic acid in fishes exposed to organochlorine pollution. Toxicology, *11*: 369-375.

ARAI, S., NOSE, T. and HASHIMOTO, Y., 1972. Qualitative requirements of young eels *Anguilla japonica* for water soluble vitamins and their deficiency symptoms. Bull. Freshwater. Fish. Res. Lab., *22*: 69-83.

ASHLEY, L. M., HALVER, J. E. and SMITH, R. R., 1975. Ascorbic acid deficiency in rainbow trout and coho salmon and effects on wound healing. In: The Pathology of Fishes, Ed. W. E. Ribelin and G. Migaki, pp 769-786.

BAKER, E. M., HAMMER, D. C., KENNEDY, J. E. and TOLBERT, B. M., 1973. Interference by ascorbate-2-sulfate in the dinitrophenylhydrazine assay of ascorbic acid. Anal. Biochem. *55*: 641-642.

BARNES, M. J. and KODICEK, E., 1972. Biological hydroxylations and ascorbic acid with special regard to collagen metabolism. Vit. and Horm., *30*: 1-43.

BAUERNFEIND, J. C., 1982. Ascorbic acid technology in agricultural, pharmaceutical, food, and industrial applications. In: Ascorbic acid: Chemistry, Metabolism and Uses. Ed. P. A. Seib and B. M. Tolbert, Adv. Chem. Ser. No. 200. Am. Chem. Soc., Washington D. C., 395-497.

BEISEL, W. R., 1982. Single nutrients and immunity. Am. J. of Clin. Nutr. (supplement), *35*(2): 417-468.

BELL, G. R., HIGGS, D. A. and TRAXLER, G. S., 1984. The effect of dietary ascorbate, zinc and manganese on the development of experimentally induced bacterial kidney disease in sockeye salmon *(Oncorhynchus nerka)*. Aquaculture, *36*: 293-311.

BENDER, A. E., 1973. Loss of vitamins in industrially handled foods and its prevention. Bibl.«Nutr.Diet.» *18*: 92-113.

BENITEZ, L. V. and HALVER, J. E., 1982. Ascorbic acid sulfate sulfhydrolase (C_2 sulfatase): The modulator of cellular levels of L-ascorbic acid in rainbow trout. Proc. Natl. Sci., *79*: 5445-5449.

BENTSEN, H. B., RØRVIK, K. A. and LARSEN, H. J., 1991. Effects of varying contents of iron and n-3 fatty acids in the feed related to antibody production in farmed salmon (in norwegian). Abstract. Workshop, National program on fish health, Norway.

BISWAS, N. M., 1969. Δ^5-3ß-hydroxysteroid dehydrogenase in toad testis: synergistic action of ascorbic acid and luteinizing hormone. Endocrinology, *85*: 981-983.

BISWAS, N. M. and DEB, C., 1970. *In Vitro* studies on the effects of ascorbic acid and dehydroascorbic acid on Δ^5-3ß-hydroxysteroid dehydrogenase in toad testis. Endocrinology, *87*: 170-173.

BLAZER, V. S., 1982. The effects of marginal deficiencies of ascorbic acid and α-tocopherol on the natural resistance and immune response of rainbow trout *(Salmo gairdneri)*. Ph. D. Thesis, Univ. of Rhode Island, 121 pp.

BOND, A. D., MCCLELLAND, B. W., EINSTEIN, J. R. and FINAMORE, F. J., 1972. Ascorbic acid-2-sulfate in the brine shrimp *Artemia salina*. Arch. Biochem. Biophys., *153*: 207-214.

BRANDT, T. M., DEYOE, C. W. and SEIB, P. A., 1985. Alternate sources of vitamin C for channel catfish. Prog. Fish Cult., *47*(1): 55-59.

CABELLI, D. E. and BIELSKI, B. H. J., 1983. Kinetics and mechanism for the oxidation of ascorbic acid/ascorbate by HO_2/O_2^- radicals. A pulse radiolysis and stopped-flow photolysis study. J. Phys. Chem., *87*: 1809-1812.

CHATTERJEE, I. B., 1978. Ascorbic acid metabolism. World Rev. of Nutr. and Diet., *30*: 69-87.

CHAVEZ de MARTINEZ, M. C., 1990. Vitamin C requirement of the mexican native cichlid *Cichlasoma urophthalmus* (Gunther). Aquaculture, *86*: 409-416.

CORT, W. M., 1982. Antioxidant properties of ascorbic acid in foods. In: Ascorbic acid: Chemistry, Metabolism and Uses, Ed. P. A. Seib and B. M. Tolbert, Adv. Chem. Ser. No. 200, Am. Chem. Soc., Washington D. C., 533-550.

CRAWFORD, T. C. and CRAWFORD, S. A., 1980. Synthesis of Lascorbic acid. Adv. Carbohydr. Chem., *37*: 79-155.

DABROWSKI, K., 1990a. Assay of ascorbic phosphates and *In vitro* availability assay of ascorbic mono- and polyphosphates. J. Sci. Food Agric., *57*: 409-420.

DABROWSKI, K. and KØCK, G., 987. Absorption of ascorbic acid and ascorbic sulfate and their interaction with minerals in the digestive tract of rainbow trout *(Oncorhynchus mykiss)*. Can. J. Fish. Aquat. Sci., *46*: 1952-1957.

DABROWSKI, K. and HINTERLEITNER, S., 1989. Applications of a simultaneous assay of ascorbic

acid, dehydroascorbic acid and ascorbic sulphate in biological materials. Analyst, *114*: 83-87.

DABROWSKI, K., HINTERLEITNER, S., STURMBAUER, C., EL-FIKY, N. and WIESER W., 1988. Do carp larvae require vitamin C ? Aquaculture, *72*: 295-306.

DABROWSKI, K., EL-FIKY, N., FRIGG, M. and WIESER, W., 1990a. Requirement and utilization of ascorbic acid and ascorbic sulfate an juvenile rainbow trout. Aquaculture, *91*: 317337.

DABROWSKI, K., LACKNER, R. and DOBLANDER, C., 1990b. Effect of ascorbate on the concentration of tissue ascorbic acid, dehydroascorbic acid, ascorbic sulfate, and activity of ascorbic sulfate sulfhydrolase in rainbow trout *(Oncorhynchus mykiss)*. Can. J. Fish. Aquat. Sci., *47*: 1518-1525.

DIPLOCK, A. T., 1991. Antioxidant nutrients and disease prevention: an overview. In: T. F. Slater and G. Block (Eds.) Antioxidant vitamins and *b*-carotene in disease prevention. Am. J. Clin. Nutr. (supplement), *53*(1): 189193.

DRUMMOND, J. C., 1920. The nomenclature of the so-called accessory food factors (vitamins). Biochem. J., *14*: 660.

DVORAK, M., 1984. Ascorbic acid, stress resistance and reproduction in swine. In: I. Wegger, F. J. Tagwerker and J. Moustgaard (Editors), Ascorbic acid in domestic animals. The Royal Danish Agricultural Society, Copenhagen, pp. 80-86.

DURVE, V. S. and LOVELL, R. T., 1982. Vitamin C and disease resistance in channel catfish *(Ictalurus punctatus)*. Can. J. Fish. Aquat. Sci., *39*: 948-951.

ERDAL, J. I., EVENSEN, Ø., KAURSTAD, O. K., LILLEHAUG, A., SOLBAKKEN, R. and THORUD, K., 1991. Relationship between diet and immune response in Atlantic salmon *(Salmo salar* L.) after feeding various levels of ascorbic acid and omega-3 fatty acids. To be published.

FELTON, S. P. and HALVER, J. E., 1988. Separation of three commercial forms of vitamin C (ascorbic acid, ascorbic-2-sulfate and ascorbate-2-polyphosphate) by HPLC. Soc. Exp. Biol. Med., *190*: 1-4.

FENSTER, R., 1989. Vitamin C in fish nutrition. Animal Nutrition events, F. Hoffmann-La Roche & Co. Ltd.

FESSENDEN, R. W. and VERMA, N. C., 1978. A time-resolved electron spin resonance study of the oxidation of ascorbic acid by hydroxyl radical. Biophys. J., *24*: 93101.

GOLOB, R., CHETSANGA, C. J. and DOTY, P., 1974. The onset of collagen synthesis in sea urchin embryos. Biochem. Biophys. Acta, *349*: 135-141.

GRANT, B. F., SEIB, P. A., LIAO, M. L. and CORPRON, K. E., 1989. Polyphosphorylated L-ascorbic acid: A stable form of vitamin C for aquaculture feeds. Journ. W. Aqua. Soc., *20*(3): 143-157.

GRATZEK, J. B. and REINERT, R., 1984. Physiological responses of experimental fish to stressful conditions. Natl. Cancer Inst. Monogr., *65*: 187-193.

HALLIWELL, B. and GUTTERIDGE, J. M. C., 1989. Free radicals in biology and medicine (2nd. ed.). Clarendon Press, Oxford.

HALVER, J. E., 1972. The role of ascorbic acid in fish disease and tissue repair. Bull. Jap. Soc. Scient. Fish., *38*(1): 79-93.

HALVER, J. E., ASHLEY, L. M. and SMITH, R. R., 1969. Ascorbic acid requirements of coho salmon and rainbow trout. The Am. Fish. Soc., *98*: 762-771.

HALVER, J. E., SMITH, R. R., TOLBERT, B. M. and BAKER, E. M., 1975. Utilization of ascorbic acid in fish. Ann. N. Y. Acad. Sci., *258*: 81-102.

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, in press.

HAY, G. W., LEWIS, B. A. and SMITH, F., 1967. The vitamins. Vol. 1 (2nd edn.), ed. W. H. Sebrell Jr. and R. S. Harris. AP, Inc., N.Y., 307-346

HILTON, J. W., 1989. The interaction of vitamins, minerals and diet composition in the diet of fish. Aquaculture, *79*: 223-244.

HILTON, J. W., CHO, C. Y. and SLINGER, S. J., 1977a. Factors affecting the stability of supplemental ascorbic acid in practical trout diets. J. Fish. Res. Board Can., *34*: 683687.

HILTON, J. W., CHO, C. Y. and SLINGER, S. J., 1977b. Evaluation of the ascorbic acid status of rainbow trout *(Salmo gairdneri)*. J. Fish. Res. Board Can., *34*: 22072210.

HILTON, J. W., CHO, C. Y. and SLINGER, S. J., 1978. Effect of graded levels of supplemental ascorbic acid in practical diets fed to rainbow trout *(Salmo gairdneri)*. J. Fish. Res. Bd. Can., *35*: 431-436.

HILTON, J. W., BROWN, R. G. and SLINGER, S. J., 1979. The half-life and uptake of ^{14}C-1-ascorbic acid in selected organs of rainbow trout *(Salmo gairdneri)*. Comp. Biochem. Physiol. *62A*: 427-432.

HOAR, W. S., RANDALL, D. J., DONALDSON, E. M. (Ed), 1983. Fish physiology, Reproduction, *Vol. IX*. AP.

HOLST, T. and FRØLICH, A., 1907. J. Hyg., *7*: 634.

HORNIG, D., GLATTHAAR, B. and MOSER, U., 1984. General aspects of ascorbic acid function and metabolism. In: Ascorbic acid in domestic animals. I. Wegger, F.J. Tagwerker and J. Moustgaard (Editors). The Royal Danish Agricultural Society, Copenhagen, pp. 196-205.

HUNG, S. S. O. and SLINGER, S. J., 1980. Effect of oxidized fish oil on the ascorbic acid nutrition of rainbow trout *(Salmo gairdneri)*. Internat. J. Vit. Nutr. Res., *50*: 393400.

IKEDA, S. and SATO, M., 1964. Biochemical studies on L-ascorbic acid in aquatic animals-III. Biosynthesis of L-ascorbic acid by carp. Bull. Jap. Soc. Sci. Fish., *30*(4): 365-370.

IKEDA, S. and SATO, M., 1965. Biochemical studies on L-ascorbic acid in aquatic animals-IV. Metabolism of L-ascorbic acid-1-^{14}C in carp. Bull. Jap. Soc. Sci. Fish., *31*(10): 814-818.

IWAMOTO, R. N. and SOWER, S. (Ed), 1985. Salmonid reproduction — an international symposium. Review papers. Univ. of Washington, Seattle, USA.

JAFFE, G. M., 1984. Ascorbic acid. In: Encyclopedia of chemical technology, Vol. 24 (3rd edn.), Ed. K. Othmer, John Wiley and Sons, N. Y., 8-40.

JOHNSTON, W. L., MACDONALD, E. and HILTON, J. W., 1989. Relationships between dietary ascorbic acid status and deficiency, weight gain and brain neurotransmitter levels in juvenile rainbow trout, *Salmo gairdneri*. Fish Physiol. and Biochem., *6*(6): 353-365.

KITAMURA, S., OHARA, S., SUWA, T. and NAKAGAWA, K., 1965. Studies on vitamin requirements of rainbow trout, *Salmo gairdneri-l*. On the ascorbic acid. Bull. Jap. Soc. Sci. Fish., *31*(10): 818-825.

KOMAROV, I. P. and KNYAZEVA, L. M., 1984. Effect of vitamin C-enriched feed on the physiological state of spawning rainbow trout. Aqua. Hydr. and Ichtyol., *2*: 43-48.

LAMBELET, P., SAUCY, F. and LOLIGER, J., 1985. Chemical evidence for interactions between vitamins E and C. Experentia, *41*: 1384-1388.

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

LEVANDER, O. A. and CHENG, L., 1980. Micronutrient interactions: Vitamins, minerals, and hazardous elements. Ann. N. Y. Acad. Sci., Vol. 355.

LEVINE, M., MORITA, K., HELDMAN, E. and POLLARD, H. B., 1985. Ascorbic acid regulation of noepinephrine biosynthesis in isolated chromaffin granules from bovine adrenal medulla. J. Biol. Chem., *260*(29): 15598-15603.

LIAO, M. L. and SEIB, P. A., 1988. Chemistry of L-ascorbic acid related to foods. Food Chemistry, *30*: 289-312.

Li, Y. and Lovell, R. T., 1985. Elevated levels of dietary ascorbic acid increase immune responses in channel catfish. J. Nutr., *115*: 123-131.

Lim, C. and Lovell, R. T., 1978. Pathology of the vitamin C deficiency syndrome in channel catfish *(Ictalutus Punctatus)*. J. Nutr., *108*: 1137-1146.

Lind, J., 1753. A treatise of the scurvy. Edinburgh, Scotland.

Liu, P. R., Plumb, J. A., Guerin, M. and Lovell, R. T., 1989. Effect of megalevels of dietary vitamin C on the immune response of channel catfish *Ictalurus punctatus* in ponds. Dis. Aquat. Org., *7*: 191-194.

Loewus, F. A. and Loewus, M. W., 1987. Biosynthesis and metabolism of ascorbic acid in plants. CRC Crit. Rev. Plant Sci., *5*(1): 101-119.

Lovell, R. T., 1973. Essentiality of vitamin C in feeds for intensively fed caged channel catfish. J. Nutr., *103*: 134-138.

Lovell, R. T., 1984. Ascorbic acid metabolism in fish. In: Ascorbic acid in domestic animals. I. Wegger, F.J. Tagwerker and J. Moustgaard (Editors). The Royal Danish Agricultural Society, Copenhagen, pp. 196-205.

Lovell, R. T. and Lim, C., 1978. Vitamin C in pond diets for channel catfish. Trans. Am. Fish. Soc., *107*(2): 321-325.

Lutwak-Mann, C., 1958. The dependence of gonadal functions upon vitamins and other nutritional factors. Vit. and Hormones, *16*: 35-75.

Lynch, S. R. and Cook, J. D., 1980. Interaction of vitamin C and iron. In: Micronutrient interactions: Vitamins, minerals, and hazardous elements, Ed. O. A. Levander and L. Cheng, Ann. N. Y. Acad. Sci., *355*: 32-44.

Maage, A., Waagbø, R., Olsson, P. E., Julshamn, K. and Sandnes, K., 1990. Ascorbate-2-sulfate as a dietary vitamin C source for Atlantic salmon *(Salmo salar)*: 2. Effects of dietary levels and immunization on the metabolism of trace elements. Fish Physiol. and Biochem., *8*(6): 429-436.

Mahajan, C. L. and Agrawal, N. K., 1980a. Nutritional requirement of ascorbic acid by indian major carp, *Cirrhina Mrigala*, during early growth. Aquaculture, *19*: 37-48.

Mahajan, C. L. and Agrawal, N. K., 1980b. The role of vitamin C in calcium uptake by fish. Aquaculture, *19*: 287-294.

Maule, A. G., Tripp, R. A., Kaattari, S. L. and Schreck, C., 1989. Stress alters immune function and disease resistance in chinook salmon *(Oncorhynchus tshawytscha)*. J. of Endocrin., *120*: 135-142.

Mayer, F. L., Mehrle, P. M. and Crutcher, P. L., 1978. Interactions of toxaphene and vitamin C in channel catfish. Trans Am. Fish. Soc., *107*: 326-333.

Mazeaud, M. M. and Mazeaud, F., 1981. Adrenergic responses to stress in fish. In: A. D. Pickering (Ed.), Stress in fish, AP, London/New York, pp. 49-75.

Mccay, C. M. and Tunison, A. V., 1943. The nutritional requirements for rainbow trout. Cortland hatchery rep. 1933. State of N. Y. Conservation Dept., Albany, New York.

Mclaren, B. A., Keller, E., O'Donnel, D. J. and Elvehjem, C. A., 1947. The nutrition of rainbow trout, I. Studies of vitamin requirements. Arch. Biochem., *15*: 169-177.

Mead, C. G. and Finamore, F. J., 1969. The occurence of ascorbic acid sulfate in the brine shrimp *Artemia salina*. Biochemistry, *8*: 2652-2655.

Meier, W. and Wahli, T., 1990. Vitamin C deficiency in the rainbow trout. Animal nutrition events, F. Hoffmann-La Roche Ltd.

Miyazaki, T., Plumb, J. A., Li, Y. P. and Lovell, R. T., 1985. Histopathology of the broken-back syndrome in channel catfish. J. Fish Biol., *26*: 647-655.

Mizoguchi, H. and Yasumasu, J., 1982. Archenteron formation induced by ascorbate and

α-ketoglutarate in sea urchin embryos kept in SO_4^{-2}-2 artificial seawater. Develop. Biol., *93*: 119-125.

MURAI, T., ANDREWS, J. W. and BAUERNFEIND, J. C., 1978. Use of L-ascorbic acid, ethocel coated ascorbic acid and ascorbate 2-sulfate in diets for channel catfish, *Ictalurus punctatus*. J. Nutr. *108*: 1761-1766.

NANNI, E. J., STALLINGS, M. D. Jr. and SAWYER, D. T., 1980. Does superoxide ion oxidize catechol, α-tocopherol, and ascorbic acid by direct electron transfer? J. Am. Chem. Soc., *102*: 4481-4485.

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.

OLIVIER, G., LALL, S. P., WEERAKOON, D. E. M. and HINES, J. A., 1989. The effect of vitamin C deficiency and excess on immune responses in Atlantic salmon *(Salmo salar)*. Abstract. Int. Conf. on Phylogeny of Immunity, CNRS, France.

PACHLA, L. A., REYNOLDS, D. L. and KISSINGER, P. T., 1985. Analytical methods for determining ascorbic acid in biological samples, food products, and pharmaceuticals. J. Assoc. Off. Anal. Chem. *68*(1): 1-12.

PADH, H., 1991. Vitamin C: Newer insights into its biochemical functions. Nutr. Rev., *49*(3): 65-70.

PANG, P. K. T., 1971. The effects of complete darkness and vitamin C supplement on the killfish, *Fundulus heteroclitus*, adapted to sea water. J. exp. Zool., *178*: 15-22.

PEREK, M., 1984. Ascorbic acid and the endocrine system with emphasis on the stress problem in poultry. In: Ascorbic acid in domestic animals. I. Wegger, F.J. Tagwerker and J. Moustgaard (Editors). The Royal Danish Agricultural Society, Copenhagen, pp. 185-193.

POSTON, A. H., 1967. Effect of dietary L-ascorbic acid on immature brook trout. Fish. Res. Bull., *30*: 46-51.

RICHTER, C. J. J. and Goos, H. J. (Ed), 1982. Proceedings of the international symposium on reproductive physiology of fish. Wageningen, the Netherlands, 2-6 August, 1982. Pudoc.

ROBINSON, E. H., BRENT, J. R. and CRABTREE, J. T., 1989. AsPP, an ascorbic acid, resists oxidation in fish feed. Feedstuffs, *10*: 64-70.

ROE, J. H. and KUETHER, C. A., 1943. A color reaction for dehydroascorbic acid useful in the determination of vitamin C. Science, *95*: 77.

ROSENLUND, G., JØRGENSEN, L., WAAGBØ, R. and SANDNES, K., 1990. Effects of different dietary levels of ascorbic acid in Plaice *(Pleuronectes platessa L.)* Comp. Biochem. Physiol., *96A*(3): 395-398.

ROY, R. B., CONETTA, A. and SALPETER, J., 1976. Automated fluorometric method for the determination of total vitamin C in food products. J. Assoc. Off. Anl. Chem., *59*(6): 1244-1250.

SAKAGUCHI, H., TAKEDA, F. and TANGE, K., 1969. Studies on vitamin requirements by yellowtail. I. Vitamin B_6 and vitamin C deficiency symptoms. Bull. Jap. Soc. Sci. Fish., *35*: 1201-1206.

SANDNES, K., 1982. Vitamin C in aquaculture. Analysis. Stability in dry feed. Requirement in rainbow trout *(Salmo gairdneri)* (in Norwegian). Cand. real. thesis, University of Bergen, Norway.

SANDNES, K., 1984. Some aspects of ascorbic acid and reproduction in fish. In: Ascorbic acid in domestic animals. I. Wegger, F.J. Tagwerker and J. Moustgaard (Editors). The Royal Danish Agricultural Society, Copenhagen, pp. 213-217.

SANDNES, K., 1988. The effect of vitamin C on reproduction in fish. Animal Nutrition Events, Roche Seminar on Vitamin Nutrition in Fish, Trondheim, august 1987.

SANDNES, K. and BRAEKKAN, O. R., 1981. Ascorbic acid and the reproductive cycle of ovaries in cod (Gadus morrhua). Comp. Biochem. Physiol., *70A*: 545-546.

SANDNES, K. and UTNE, F., 1982. Processing loss and storage stability of ascorbic acid in dry fish feed. Fisk. Dir. Skr. Ser. Ernæring, *2*(2): 39-44.

SANDNES, K. and WAAGBØ, R., 1991a. Enzymatic hydrolysis of ascorbate-2-monophosphate and ascorbate-2-sulphate *in vitro* and bioactivity of ascorbate-2-monophosphate in Atlantic salmon *(Salmo salar)*. Fisk. dir. Skr. Ser. Ernæring *3*:33–39.

SANDNES, K. and WAAGBØ, R., 1991b. Effects of dietary vitamin C and physical stress on head kidney and liver ascorbic acid, serum cortisol, glucose and hematology in Atlantic salmon *(Salmo salar)*. Fisk. Dir. Skr. Ser. Ernæring *3*:41–49.

SANDNES, K., ULGENES, Y., BRAEKKAN, O. R. and UTNE, F., 1984. The effect of ascorbic acid supplementation in broodstock feed on reproduction of rainbow trout *(Salmo gairdneri)*. Aquaculture, *43*: 167-177.

SANDNES, K., ØINES, S., BARBÅRD, S. and WAAGBØ, R.,1989. Different sources of vitamin C in feed for Atlantic salmon *(Salmo salar)*. Abstract, The Third Intern. Symp. on Feeding and Nutr. in Fish, Toba, Japan.

SANDNES, K., HANSEN, T., KILLIE, J-E. A. and WAAGBØ, R., 1990. Ascorbate-2-sulfate as a dietary vitamin C source for Atlantic salmon (Salmo salar): 1. Growth, bioactivity, haematology and humoral immune response. Fish Physiol. and Biochem., *8*(6): 419-427.

SANDNES, K., TORRISSEN, O. and WAAGBØ, R., 1991. Requirement of vitamin C in Atlantic salmon *(Salmo salar)* using ascorbate-2-monophosphate as dietary source. To be published.

SATO, M., YOSHINAKA, R. and IKEDA, S., 1978a. Dietary ascorbic acid requirement of rainbow trout for growth and collagen formation. Bull. Jap. Soc. Sci. Fish., *44*(9): 1029-1035.

SATO, M., YOSHINAKA, R., YAMAMOTO, Y. and IKEDA, S., 1978b. Nonessentiality of ascorbic acid in the diet of carp. Bull. Jap. Soc. Sci. Fish., *44*(10): 1151-1156.

SATO, M., KONDO, T., YOSHINAKA, R. and IKEDA, S., 1982. Effect of dietary ascorbic acid levels on collagen formation in rainbow trout. Bull. Jap. Soc. Sci. Fish., *48*(4): 553556.

SATO, M., YOSHINAKA, R. and NAKAMOTO, K., 1984. Distribution and some properties of prolyl hydroxylase in rainbow trout. Bull. Jap. Soc. Sci. Fish., *50*(6): 1009-1013.

SCHÜEP, W., VUILLEMUIER, J. P., GYSEL, D. and HESS, D., 1984. Determination of ascorbic acid in body fluids, tissues and feedstuffs. In: Ascorbic acid in domestic animals. I. Wegger, F.J. TAGWERKER and J. MOUSTGAARD (Editors). The Royal Danish Agricultural Society, Copenhagen, pp. 50-55. Schüep, W., Marmet, J. and Studer, W., 1989. Stability of ascorbate-2-sulfate in trout feed measured by HPLC. Aquaculture, *79*: 249-258.

SEYMOUR, E. A., 1981a. Gonadal ascorbic acid and changes in level with ovarian development in the crucian carp *Carassius Carassius* (L.) Comp. Biochem. Physiol., *10A*: 451-453.

SEYMOUR, E. A., 1981b. The effects of powdered carp pituitary on ovarian development, ovarian ascorbic acid and ovulation in *Carassius carassius* L. exposed to various photoperiod and temperature regimes. J. Fish Biol., *19*: 675-682.

SHIGUENO, K. and ITOH, S., 1988. Use of Mg-ascorbyl-2-phosphate as a vitamin C source in shrimp diets. Journ. W. Aqua. Soc., *19*(4): 168-174.

SKELBAEK, T., ANDERSEN, N. G., WINNING, M. and WESTERGAARD, S., 1990. Stability in fish feed and bioavailability to rainbow trout of two ascorbic acid forms. Aquaculture, *84*: 335-343.

SLATER, T. F. and BLOCK, G., 1991. Antioxidant vitamins and ß-carotene in disease prevention. Am. J. Clin. Nutr., supplement to vol. *53*(1).

SOLIMAN, A. K., JAUNCEY, K. and ROBERTS, R. J., 1985. Qualitative and quantitative identifica-
335-343.

tion of Lgulonolactone oxidase activity in some teleosts. Aquacult. and Fish. Manag., *1*: 249-256.

SOLIMAN, A. K., JAUNCEY, K. and ROBERTS, R. J., 1986a. The effect of varying forms of dietary ascorbic acid on the nutrition of Juvenile tilapias *(Oreochromis niloticus)*. Aquaculture, *52*: 1-10.

SOLIMAN, A. K., JAUNCEY, K. and ROBERTS, R. J., 1986b. The effects of dietary ascorbic acid supplementation on hatchability, survival rate and fry performance in *Oreochromis mossambicus* (Peters). Aquaculture, *59*: 197208.

SOLIMAN, A. K., JAUNCEY, K. and ROBERTS, R. J., 1987. Stability of L-ascorbic acid (vitamin C) and its forms in fish feeds during processing, storage and leaching. Aquaculture, *60*: 73-83.

SZENT-GYÖRGY, A and HAWORTH, W. N., 1933. Nature, *131*: 23.

TAKAHASHI, M., NIKI, E., KAWAKAMI, A., KUMASAKA, A., YAMAMOTO, Y., KAMIYA, Y. and TANAKA, K., 1986. Oxidation of lipids. XIV. Inhibition of oxidation of methyl linoleate by fatty acid esters of L-ascorbic acid. Bull. Chem. Soc. Jpn., *59*: 3179-3183.

TERADA, M., WATANABE, Y., KUNITOMO, M. and HAYASHI, E., 1978. A simple method for measuring the tricarboxylic acid cycle and pentose phosphate pathway in adipose tissue. Anal. Boichem., *84*: 609-614.

TESKEREDZIC, Z., TESKEREDZIC, E. and MALNAR, L., 1989. High mortality of rainbow trout *(Salmo gairdneri)* in Yougoslavian fish farms caused by inadequate feed quality. Aquaculture, *79*: 391-395.

THOMAS, P., 1984. Influence of some environmental variables on the ascorbic acid status of mullet, *Mugil cephalus* L., tissues. I. Effect of salinity, capture stress, and temperature. J. Fish Biol., *25*: 711-720.

THOMAS, P., 1987. Influence of some environmental variables on the ascorbic acid status of striped mullet, *Mugil cephalus* Linn., tissues. III. Effects of exposure to oil. J. Fish Biol. *30*: 485-494.

THOMAS, P., BALLY, M. and NEFF, J. M., 1982. Ascorbic acid status of mullet, Mugil cephalus Linn., exposed to cadmium. J. Fish Biol., *20*: 183-196.

TOLBERT, B. M., DOWNING, M., CARLSON, W., KNIGHT, M. K. and BAKER, E. M., 1975. Chemistry and metabolism of ascorbic acid and ascorbate sulfate. In: Second conference on vitamin C, King, C. G. and Burns, J. J.(Editors). Annals of the New York Academy of Sciences, *258*: 48-70.

TSUJIMURA, M., YOSHIKAWA, H., HASEGAWA, T., SUZUKI, T., KASAI, T., SUWA, T. and KITAMURA, S., 1978. Studies on the vitamin C activity of ascorbic acid 2-sulfate on the feeding test of newborn rainbow trout. Vitamins (Japan), *52*(1): 35-43.

TSUJIMURA, M., FUKUDA, T., KASAI, T. and KITAMURA, S., 1981. Hydrolysis of L-ascorbic acid 2-sulfate to L-ascorbic acid in freshwater fishes. Bull. Jap. Soc. Sci. Fish., *47*(3): 435.

TUCKER, B. W., 1983. Studies on vitamin C metabolism in rainbow trout. Ph. D. dissertation. Univ. of Washington, Seattle, USA.

TUCKER, B. W. and HALVER, J. E., 1984a. Distribution of ascorbate-2-sulfate and distribution, half-life and turnover rates of (1-^{14}C) ascorbic acid in rainbow trout. J. Nutr., *114*: 991-1000.

TUCKER, B. W. and HALVER, J. E., 1984b. Ascorbate-2-sulfate metabolism in fish. Nutr. Rev., *42*: 173-179.

TUCKER, B. W. and HALVER, J. E., 1986. Utilization of ascorbate-2-sulfate in fish. Fish. Physiol. Biochem., *2*: 151-160.

TUCKER, B. W., TOLBERT, B. M., HALVER, J. E. and BALABAN, M., 1987. Brain ascorbate depletion as a response to stress. Int. J. Vit. Nutr. Res., *57*: 289-295.

WAAGBØ, R., THORSEN, T. and SANDNES, K., 1989. Role of dietary ascorbic acid in vitellogenesis in rainbow trout *(Salmo gairdneri)*. Aquaculture, *80*: 301-314.

WAHLI, T., MEIER, W. and PFISTER, K., 1986. Ascorbic acid induced immune-mediated decrease in mortality in *Ichtyophthirius multifiliis* infected rainbow trout *(Salmo gairdneri)*. Acta Tropica, *43*: 287-289.

WANG, X. Y. and SEIB, P.A., 1990. Liquid chromatographic determination of a combined form of L-ascorbic acid (Ld-ascorbate-2-sulfate) in fish tissue by release of L-ascorbic acid. Aquaculture, *87*: 65-84.

WANG, X. Y., LIAO, M. L., HUNG, T. H. and SEIB, P. A., 1988. Liquid chromatographic determination of L-ascorbate 2-polyphosphate in fish feeds by enzymatic release of L-ascorbate. J. Assoc. Off. Anal. Chem. *71*(6): 1158-1161.

WAUGH, W. A. and KING, C. G., 1932. Isolation and identification of vitamin C. J. Biol. Chem., *97*: 325-331

WEDEMEYER, G., 1969. Stress-induced ascorbic acid depletion and cortisol production in two salmonid fishes. Comp. Biochem. Physiol., *29*: 1247-1251.

WIIK, R., ANDERSEN, K., UGLENES, I. and EGIDIUS, E., 1989. Cortisol-induced increase in susceptibility of Atlantic salmon, *Salmo salar*, together with effects on the blood cell pattern. Aquaculture, *83*: 201-215.

WILSON, R. P. and POE, W., 1973. Impaired collagen formation in the scorbutic channel catfish. J. Nutr., *103*: 13591364.

WILSON, R. P., POE, W. E. and ROBINSON, E. H., 1989. Evaluation of L-ascorbyl-2-polyphosphate (AsPP) as a dietary ascorbic acid source for channel catfish. Aquaculture, *81*: 129-136.

WISE, D. J., TOMASSO, J. R. and BRANDT, T. M., 1988. Ascorbic acid inhibition of nitrite-induced methemoglobinaemia in channel catfish. Prog. Fish Cult., *50*: 77-80.

YAMAMOTO, Y. and INOUE, M., 1985. Effects of dietary ascorbic acid and dehydro-ascorbic acid on the acute cadmium toxicity in rainbow trout. Bull. Jap. Soc. Sci. Fish., *51*(8): 1299-1303.

YAMAMOTO, Y., SATO, M. and IKEDA, S., 1978. Existence of L-gulonolactone oxidase in some teleosts. Bull. Jap. Soc. Sci. Fish., *44*(7): 775-779.

YANO, T., NAKAO, M., FURUICHI, M. and YONE, Y., 1988. Effects of dietary choline, pantothenic acid and vitamin C on the serum complement activity of red sea bream. Bull. Jap. Soc. Sci. Fish., *54*(1): 141-144.

YOSHINAKA, R., SATO, M. and IKEDA, S., 1978. In vitro formation of collagen in skin of ascorbic acid deficient rainbow trout. Bull. Jap. Soc. of Sci. Fish., *44*(10): 1147-1150.

YOSHINAKA, R., SATO, K., SATO, M. and Anbe, H., 1990. Distribution of collagen in body of several fishes. Bull. Jap. Soc. Sci. Fish., *56*(3): 549.

ZITZOV, R. E. and MILLARD, J. L., 1988. Survival and growth of lake whitefish *(Coregonus clupeaformis)* larvae fed only formulated dry diets. Aquaculture, *69*: 105-113.

ZLOCH, Z., CERVEN, J. and GINTER, E., 1971. Radiochemical evaluation of the 2,4-dinitrophenyl-hydrazine method for determination of vitamin C. Anal. Biochem., 43: 99-106.