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The minimum dietary requirement of vitamin C in Atlantic salmon (*Salmo salar*) fry using Ca ascorbate-2-monophosphate as dietary source

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Abstract

The minimum dietary vitamin C requirement for optimal growth and normal development in Atlantic salmon (*Salmo salar*) fry at the onset of feeding was studied, using Ca ascorbate-2-monophosphate (AP) as dietary source. The requirement was established by means of a feeding study lasting for 23 weeks from the beginning of feeding. The practical diets used were supplemented with AP at levels of 0, 10, 20, 40, 80 and 160 mg ascorbic acid (AA) equivalents/kg. Growth, mortality, hydroxyproline content in skin and backbone, and AA in liver were recorded to evaluate the results. The results suggest that the minimum dietary requirement for optimal growth and normal development is in the range of 10-20 mg AA equivalents/kg dry diet during the period studied.

Introduction

Fish require a dietary source of vitamin C for growth and development. The minimum dietary requirement has been investigated in several species (Mahajan and Agrawal 1980, Cowey 1988; Chavez de Martinez 1990), but no such studies have been carried out on Atlantic salmon (*Salmo salar*). In the salmonid fishes, the dietary requirements for optimum growth in rainbow trout (*Salmo gairdneri*) have been found to be in the range of 40-100 mg ascorbic acid (AA)/kg diet (Halver *et al.* 1969; Hilton *et al.* 1978; Halver 1982; Sandnes 1982; Sato *et al.* 1982). In coho salmon (*Oncorhynchus kisutch*) a dietary content of 50 mg/kg prevented deficiency symptoms, 100 mg/kg supported optimal growth, while higher levels were needed to maintain high tissue reserves or to promote rapid wound repair (Halver *et al.* 1969).

Fish seem to be most vulnerable to vitamin C deficiency during the early stages of life when the specific growth rate is high and leaching of AA is severe due to the small size of the feed particles. In rainbow trout a reduced dietary AA requirement with increasing age has been demonstrated (Hilton *et al.* 1978; Sato *et al.* 1978; Waagbø *et al.* 1989), but the reason for this is unknown.

Unlike other water soluble vitamins, AA has no coenzyme function, but is known to affect several physiological functions in the body. A prime function of AA appears to be that of a reducing agent maintaining metaloenzymes in a reduced and active state (Padh 1991). Examples of physiological effects of AA demonstrated in fish are related to reproduction (Sandnes *et al.* 1984; Soliman *et al.* 1986; Waagbø *et al.* 1989), immune functions and disease resistance (Durve and Lovell 1982; Navarre and Halver 1989; Hardie *et al.* 1991), iron metabolism and hematology (Hilton *et al.* 1978; Maage *et al.* 1990; Sandnes *et al.* 1990), lipid metabolism (John *et al.* 1979; Waagbø *et al.* 1989), interactions with other micronutrients (Hilton 1989, Maage *et al.* 1990), stress (Wedemeyer 1969), brain neurotransmitters (Johnston *et al.* 1989) and en-

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vironmental factors (Pang 1971; Thomas and Neff 1984). The AA requirement is thus influenced by many factors, and it is therefore necessary to establish the minimum required dietary level to avoid deficiency symptoms and to support growth under a variety of normal conditions.

The biochemical reaction in which AA has been most studied and has been shown to have a specific function in mammals, is as a cofactor in the hydroxylation of proline to hydroxyproline (OH-proline) in collagen, which is important for the strength of the connective tissues (Barnes and Kodicek 1972; Padh 1991). This function of AA has also been demonstrated in aquatic animals, and is related to the skeletal deformations seen in scorbutic fish (Wilson and Poe 1973; Yoshinaka *et al.* 1978; Sato *et al.* 1982; Albrektsen *et al.* 1988).

The instability of supplemented AA in diets complicates accurate studies on dietary requirements in fish. The substantial losses of AA during feed production, storage and leaching to the water (Hilton *et al.* 1977a; Slinger *et al.* 1979; Sandnes and Utne 1982) make it difficult to control the exact amount of AA ingested by the fish.

Vitamin C active compounds with greater stability and reduced solubility in water are now available and more accurate determinations of vitamin C requirements in fish are possible. Ca ascorbate-2-monophosphate (AP) has shown good vitamin C activity in Atlantic salmon (Sandnes and Waagbø 1991). The present study was conducted to evaluate the vitamin C requirement in young Atlantic salmon with AP as vitamin C source. Growth studies, incidence of deficiency symptoms and mortality, analyses of tissue levels of AA, and analyses of OH-proline in skin and backbone were used as criteria.

Materials and methods

Fish and diets

Two thousand Atlantic salmon fry at a stage just prior to the first exogenous feed (mean weight 0.16 g) were distributed into each of 18 tanks (1.5 × 1.5 m). Each of six experimental diets was assigned to three tanks and fed for 23 weeks. By week 11 the number of fish was adjusted to 870 in each tank. The tanks were equipped with a flow-through water system, and were supplied with adequate amounts of running fresh water supplemented with filtered salt water to give a salinity of 1-2 ppt in order to stabilize the water quality.

The experimental diets were prepared from a basal practical diet which was supplemented with Ca ascorbate-2-monophosphate (F. Hoffmann-La Roche) at levels equivalent to 0, 10, 20, 40, 80 or 160 mg ascorbic acid (AA)/kg dry diet. The basal diet contained (g/kg): fish meal (Norse LT) 700, modified maize starch (Suprex) 160, capelin oil (Norsalmoil) 130, and vitamins (-AA) and minerals according to NRC (1981). The feeds were cold pelleted (2.5 mm diam.) and dried in the laboratory. The pellets were crushed and sieved to appropriate size according to fish size.

Samples and analyses

Weight was recorded in 100 fish which were collected from each tank after 5, 9, 11, 14, 18 and 23 weeks. Analyses of AA in liver (Roy *et al.* 1976) were performed on samples collected at week 11, 14, 18 and 23. The contents of hydroxyproline (OH-proline) in skin and backbone sampled at the end of the experiment were determined according to Albrektsen *et al.* (1988). Compiled data are presented as there were no significant differences between replicates in the parameters studied.

Results and discussion

The growth, mortality, OH-proline content in skin and backbone and liver AA content are shown in Tables 1-4, respectively. Most data indicate that the minimum dietary requirement is satisfied by an AP supplementation of 10 mg AA equivalents/kg. The only possible exception is the higher OH-proline content in skin of fish fed 20 mg/kg compared to the 10 mg/kg group. A comparable effect was not seen in the content of OH-proline in the backbone. The skin seems to be a more sensitive indicator of AA status as it shows a wider range of OH-proline levels relative to dietary levels of AP. Thus, it seems appropriate to conclude that the minimum dietary requirement of AP is in the range of 10-20 mg AA equivalents/kg of dry diet. In rainbow trout, Sato *et*

Table 1. Growth (g, mean ± SD, n=3)[1] of Atlantic salmon fed different dietary levels of ascorbate-2-monophosphate in the diet

	Ascorbate-2-phosphate supplemented in the diets (L-ascorbic acid equivalents, mg/kg)					
Weeks	0	10	20	40	80	160
5	0.4±0.1	0.3±0.1	0.4±0.1	0.4±0.1	0.4±0.1	0.4±0.1
9	0.9±0.1	0.9±0.1	0.9±0.1	0.9±0.1	0.8±0.1	0.9±0.2
11	2.0±0.5	1.9±0.4	2.0±0.4	1.9±0.4	1.9±0.5	1.8±0.4
14	2.3±0.9	3.3±0.8	3.2±0.8	3.4±0.9	3.4±1.9	2.9±0.8
18	5.6±1.3	6.4±2.0	7.0±2.6	7.1±2.3	6.8±2.3	6.8±2.5
23	5.9±1.6	19.9±7.1	22.9±7.6	19.5±7.2	21.7±7.2	21.0±7.4

[1]100 fish were weighed in each of 3 replicate tanks, values represent mean of 3 tanks.

Table 2. Mortality (%) of Atlantic salmon fed different dietary levels of ascorbate-2-monophosphate in the diet for 23 weeks

	Ascorbate-2-phosphate supplemented in the diets (L-ascorbic acid equivalents, mg/kg)					
Weeks	0	10	20	40	80	160
0-11	18	13	9	15	14	17
11-23	28	2	2	1	4	1

Table 3. Hydroxyproline (% of protein) in skin and backbone of Atlantic salmon fed different dietary levels of ascorbate-2-monophosphate in the diet for 23 weeks

	Ascorbate-2-phosphate supplemented in the diets (L-ascorbic acid equivalents, mg/kg)					
Weeks	0	10	20	40	80	160
Skin	1.9±0.2	3.8±0.4	4.4±0.3	4.5±0.2	4.4±0.3	4.5±0.2
Backbone	2.3±0.2	4.0±0.2	4.0±0.2	4.2±0.2	4.2±0.1	4.3±0.2

Each value represents mean ± SD of 3 pooled samples, each prepared of 15 fish from each tank.

Table 4. Concentration of ascorbic acid in the liver (µg/g w.w.) of Atlantic salmon fed different dietary levels of ascorbate-2-monophosphate in the diet for 23 weeks

	Ascorbate-2-phosphate supplemented in the diets (L-ascorbic acid equivalents, mg/kg)					
Weeks	0	10	20	40	80	160
11	<5	6±4	9±6	12±9	21±9	47±5
14	<5	6±1	10±1	16±3	27±2	58±3
18	<5	6±1	11±1	20±3	37±3	71±9
23	<5	8±1	12±1	21±2	49±5	85±8

Each value represents means of 3 pooled samples, each prepared of 15 fish from each tank.

al. (1982) reported a minimum dietary requirement of 50-100 mg AA/kg to maintain normal collagen formation.

Growth was not severely affected until week 18, after which fish fed AP free diet ceased to grow and showed severe signs of deficiency as described by Halver *et al.* (1975). All other groups showed excellent growth during the remaining experimental period, and there were no differences according to dietary treatment.

Mortality varied somewhat during the first half of the experiment, but there were no differences that could be attributed to the dietary level of AP, and mortality levels were within the range expected during the initial phase of swim-up feeding of this species. During the remaining period of feeding, dietary AP deficiency resulted in high mortality rates (28%), relative to those of the AP-supplemented groups (1-4%).

The concentration of AA in the liver was re-

corded from week 11 onwards, and clearly demonstrated a relationship between dietary AP content and liver AA content, in accordance with previous studies (Sandnes and Waagbø 1991). The liver AA concentration was steady throughout weeks 11-23 in fish fed 10 and 20 mg AA equivalents/kg, while it increased towards the end of the study in fish fed higher dietary levels. This observation supports that the minimum dietary requirement is within the range of 10-20 mg/kg, as these levels support a steady AA concentration in liver, while it increases with feeding period in fish fed higher dietary levels.

Since a feed supplementation of AP equivalent to 10-20 mg AA/kg approximates the minimum dietary requirement in Atlantic salmon, analyses of AA in the liver indicate that a level of 10 µg AA/g wet tissue approximates the lower safe limit to avoid clinical AA deficiency. This is half the level of 20 µg/g as reported in rainbow trout by Hilton *et al.* (1977b) and Sandnes (1982). Care should be taken to compare studies using different dietary sources of vitamin C and different experimental methods, especially when comparing studies with poikilotherm animals such as fish. However, the data may indicate that there is a difference between rainbow trout and Atlantic salmon as regards the minimum dietary requirement of this vitamin.

Previous studies to establish dietary requirements for vitamin C in fish have used AA as the dietary source. The lack of stability of this compound in fish foods, and the high water solubility resulting in leaching losses (Hilton *et al.* 1977a; Slinger *et al.* 1979; Sandnes and Utne 1982), have complicated accurate studies to evaluate the requirement. The vitamin C source used in the present experiment has been shown to exhibit good bioavailability in Atlantic salmon (Sandnes and Waagbø 1991), and the physical properties of phosphate derivates of AA in feeds and water (Shigueno and Itoh 1988) minimize the problems of stability and leaching mentioned above. This implies that what is really ingested by the fish is more closely related to the feed supplementation level than when using AA as dietary source. Thus, we feel confident that the present study is the first to establish the minimum dietary requirement of vitamin C in Atlantic salmon, and reports the correct quantitative level in view of the criteria used in the experiment.

The latter statement is important, since the dietary vitamin C requirement in fish varies according to several factors. The present investigation reports on the minimum dietary requirement to support growth and normal development in Atlantic salmon fry at start feeding. Further studies using the same dietary source of the vitamin should be carried out to establish requirements for optimal smoltification performance, disease resistance and growth during the fresh water and the seawater stages, and reproduction. Further, the interaction of AP with other micronutrients in the diet should be evaluated, for instance, the effect on iron absorption which is known to be affected by AA.

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