

ROLE OF VITAMIN C IN FISH NUTRITION

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Abstract

Biosynthesis of vitamin C in liver is known to occur in some vertebrates but the majority of fishes lacks the enzyme L-gulonolactone oxidase which plays major role in synthesis of ascorbic acid in fishes. In intensive or commercial culture the fishes fulfill the requirement of vitamin C from the feed supplied by the fish farmer. But if the feed is not fortified with adequate vitamin C it leads to deformed vertebrates and unable to cope up environmental stressors which leads to great commercial loss. In this context this review is an attempt to throw light on the role of vitamin C in fish nutritional aspect.

Introduction

Vitamin C requirement of fish was first time demonstrated in 1965 (Kitamura et al; 1965). He showed for the first time a specific requirement for ascorbic acid in fish. Teskeredzic et al., 1989 revealed that discolouration and deformed spine of Rainbow trout fingerling due to lack of Vitamin C in the artificial feed. These finding clearly demonstrated that vitamin C requirement for healthy fish reared in intensive system where they are solely depends on artificial feed.

Fenster, 1989 revealed that Vitamin C requirement by eukaryotic cell, but the capacity to synthesize vitamin C is absent in invertebrates, primates and fish.

As per Chatterjee, 1978 fish lack the capacity to biosynthesis of ascorbic acid AA from glucose or galactose is the inability to convert L-gulonolactone to 2-Keto-L-gulonolactone. They lack the enzyme L- gulonolactone oxidase (EC,1.1.3.8). Horning et al., 1984 demonstrated that 2Keto L gulonolactone is transformed by spontaneous isomerization into its tautomeric form Ascorbic acid. Chatterjee 1973 and Levine, 1986 revealed that no fish species could synthesize ascorbate and amphibians are first appeared to synthesize ascorbate.

Touhata et al., 1995 revealed that all cartilaginous and non-teleost bony fishes can synthesize ascorbate, predominantly in the kidney, but teleost fishes are unable to do so.

Moreau and Dabrowski, 1998; 2000 observed that hagfish , lampreys, shark, rays, lungfishes, sturgeons and bowfins fishes can synthesize ascorbate but never in any of the

numerous teleost fish species studied , it can be detected that the ability of ascorbate synthesis was lost in the common ancestor of teleost fish 200-210 million year ago.

Chemical properties of vitamin C

As per IUPAC term correct name of vitamin C (L-ascorbic acid) is L – threo -2-hexenono -1,4-lactone. Ascorbic acid is one of a pair of enantiomers having this structure. The hydroxyl groups in position 2 and 3 of Ascorbic acid are acid and ionic 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 food and feeds.

Analysis of vitamin C

Pachla et al 1985 revived analytical principles of Ascorbic acid determination and include spectrophotometric (using redox indicator or chromogen formation following derivatization), electrochemical, enzymatic and chromatographic methods. Direct chromatographic methods have gained wider use, but most of the nutritional studies published on vitamin C have been based on in direct methods involving derivatization .

Vitamin C in fish feed

Antioxidant feeds activity

Ascorbic oxidation in feed 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 Ascorbic acid , as one electron is transferred to the free radical of lipid form the ascorbate anion the result being formation of ascorbate radical.

Some workers like Fessenden and Verma , 1978; Nanni et al. ; 1980; Cabelli and Bielski , 1983 reported that initiation of lipid autoxidation by super oxide radical (O_2^-) , perhydroxyl radical (HO_2^-), hydroxyl radical (HO^-) and singlet oxygen (O^*) can be retarded by ascorbic acid (AA).

Stability of vitamin C

Ascorbic acid instability in fish feed has been overcome by microencapsulation or coating of crystalline ascorbic acid. Workers like Hilton et al., 1997a revealed that Ascorbic acid coated with ethyl cellulose was more stable than crystalline Ascorbic acid in practical trout diet. Lovell and Lim , 1978 compared Ascorbic acid (AA) and a coated Ascorbic acid with ethyl cellulose and reported loss of 23 -34 % (AA) and 10 -24 % (coated AA) after steam pelleting and 55 -69 % (AA) and 40 -55% (coated AA) after extrusion.

Quality of lipid source affect AA stability in feed , as Hung and Slinger (1980) showed that oxidized fish oil (peroxide value higher than 120 mg/kg oil) significantly reduced the stability of AA in a practical trout diet.

Biological function and dietary requirement of vitamin C by fishes

Vitamin C act as a cofactor in various hydroxylation reactions in living tissues , of which the hydroxylations of collagen proline and lysine in connective tissues . It is a important general modulator of redox system in the body.

Growth and development of fishes

Halver et al., 1975 described the symptom of Ascorbic acid deficiencies like lordosis , scoliosis, distortion of support cartilage , hyperplasia of gill tissue, shorted and petechial hemorrhages opercles. Ascorbic acid deficiency symptom seen in fish are caused by impaired collagen formation and support cartilage formation (Halver et al; 1975; Wilson and Poe, 1973; Lim and Lovell; 1978 ; Sandnes et al., 1991)

Ascorbic acid function as a cofactor for enzyme –catalyzed hydroxylation 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 its highest concentration is found in skin and bones (Yoshinaka et al.,1990)

Sato et al.,1982 estimated that the minimum dietary AA requirement to maintain normal collagen formation in rainbow trout was 50-100 mg/kg diet.

Fish	Minimum Requirement of Vitamin C	Reference
Salmonoid young fish	40-100mg/kg diet	Halver et al., 1969; Halver,1972; Hilton et al., 1978; Sandnes,1982.
Coho salmon	50mg/kg prevented deficiency symptom and 100mg/kg. optimal growth	Halver et al.,1969.
Rainbow Trout	20mgAA/kg diet prevent skeletal deformities/500mgAA/kg maintain body AA concentration	Dabrowski et al., 1990a.
Atlantic salmon fry	10-20mgAA equivalent per kg diet requirement for growth and collagen formation (calcium salt of ascorbate 2mono phosphate.)	Sandnes et al., 1991.
Channel catfish.	30-60 mg/kg diet.	Adrews and Murai, 1975 Li and Lovel, 1978.
<i>Cichlasoma urophthalmus</i>	40mg/kg	Chavez Martinez 1990
<i>Tilapia zilli</i>	3000mg/kg	Anadu et al., 1990.

Wound healing

Collagen formation is necessary for optimal wound repair in animals and the essential role of AA in the biosynthesis of collagen. Halver et al., 1969 observed that salmon and rainbow trout fed the AA deficient diet completely failed to repair the wounds, which was partially pluffed with poorly clotted blood. It was suggested that 1000mg AA/kg diet is needed for optimal wound and tissue repair (Halver, 1972, Ashiley et al., 1975; Halver et al., 1975). Lim and Lovell 1978 studied wound repair in channel catfish as a function of dietary vitamin C (@60mgAA/kg)

Reproduction

High concentration of Ascorbic acid in fish ovaries have been reported by many author (Sandnes,1984; 1988). Sandnes et al., 1984 showed that maturation rainbow trout fed a day diet supplemented with AA which contained 115mgAA/kg feed after processing produced eggs with a significantly improved hatching rate that did fish fed the same diet without AA supplementation . Soliman et al., 1986b found that supplementation of 1250 mgAA/kg in the broodstock feed significantly increased egg hatchability in Tilapia (*Oreochromis mossambicus*). Ascorbic acid is necessary for optimal reproduction performance in fish . This is related to vitellogenesis and embryonic development. Incorporation of AA in the egg during ovarian growth is important for successful larval development after hatching.

Interaction with other nutrients

Maage et al., 1990 reported on interaction between dietary vitamin C form (AA and AS) and metabolism of trace elements in Atlantic salmon related to stimulation of humoral defence system. This study demonstrates that anaemia in vitamin C deprived fish despite significantly elevated iron concentration in the liver. Dabrowski and Kock (1987) suggest that AA enhanced the absorption of iron and zinc in rainbow trout. There is decrease in absorption of calcium by gill, skin, bone and muscle in fish when there is ascorbic acid deficiency in the diet.(Pang,1971; Mahajan and Agrawal, 1980a and b.)

Stress and Environmental Factors

Dietary Vitamin C in fish feed found to reduce negative impact caused by stress and Environmental factor upon health and disease resistance. Lovell and Lim 1978 revealed that requirement of Ascorbic acid is increased in channel catfish stocked at high densities so as to reduce the stress. Agrawal et al., 1978 demonstrated that Ascorbic acid protected against the Organochlorine pesticide aldrin in *Channa punctatus*. Wise et al., 1988 showed that high dietary level of Ascorbic acid (800-8000mg/kg) significantly reduced nitrite induced methamoglobin aemia when fed 24 or 48 hrs prior to nitrite exposure in Channel catfish.

Immunological and disease resistance

Several studies revealed that vitamin C plays vital role in immune function of fishes. Some of the workers studies on immune function of fishes with respect to vitamin C are cited below.

Name of Fish	Minimum Requirement of Vitamin C	Physiological Activities	References
Channel Catfish	30mg/kg	Support normal growth and prevent deficiency disease.	Durve and Lovell, 1982
	150mg/kg	Increased resistance against infection by <i>Edwardsiella tadar</i> 15% mortality of fishes infected with <i>Edwardsiella ictaluri</i>	
Channel catfish	300mg/kg	Enhances antibody production and complementary activity	Li and Lovell, 1985.
Red Sea Bream (<i>Pagrus major</i>)	1000mg/kg	Higher natural hemagglutinin titre No parasitic infection	Yano,etal., 1988.
Rainbow trout	5000mg/kg	Holotrichous ciliate (<i>Ichthyophthirius multifiliis</i>) Survival increases upto 98%.	Wahli etal.,1986
Rainbow trout	500-1000mg	Survive increases and improved humoral antibody production against <i>Vibrio anguillarum</i>	Navarre and Halver, 1989.
Atlantic Salmon	310mg/kg And 2750mg/kg	Significant decrease in mortality when challenge with a virulent strain of <i>Aeromonas salmonicida</i> .	Hardie etal., 1991.

Conclusion

Intensification of aquaculture practices leads to increase in incidence of disease outbreaks due to environmental stressors and imbalance in host- pathogen interaction. This ultimately ends up great economic losses. This outbreak can be reduced by using antibiotics. But due to antibiotic problems like immunity developed by the pathogenic bacteria there is ban on antibiotics in aquaculture practices to overcome the problem of diseases one of the most successful alternative is to boost up the immunity of the aquaculture animals by using probiotics, prebiotics, vitamins and other immunostimulants. As vitamin C plays versatile role

in growth and development, wound healing, enhancing reproductive performance, coping with environmental stressors and boosting immunological parameters. There is need for standardization of dose of vitamin C for each fish species not only species but according to age groups. One other problems on which studies are to be undertaken about the stability of vitamin-C during feed manufacturing process, storing and actual use in aquaculture aquatic bodies.

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