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Vitamin C: An Important Nutritional Factor in Fish Diets

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Authors' contributions

This work was carried out in collaboration between all authors. Author JOH designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author SHP managed the literature searches and analyses of the study performed the structural equation modeling and discuss the conclusion. All authors read and approved the final manuscript.

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ABSTRACT

Vitamin C is a micronutrient necessary for the proper functioning of fish body. It is needed for absorption of ions and nutrients and its deficiency in fish is evident mostly by structural deformities such as curvature of the spine, broken skull and hemorrhage. It is therefore important to incorporate Vitamin C into fish diets at recommended dosages for active uptake. It is a major component in the synthesis of collagen which enables fish to maintain its structural and skeletal integrity. During feed formulation, the use of phosphorylated form should be used for optimal stability in feeds because a great portion of the micronutrients can be lost during feed processing and storage. Based on all these facts, research is continuously being carried out to study the beneficial effects and how it can be adequately composed in fish feeds to meet the particular requirements of fish.

Keywords: Vitamin C; fish health; deficiency.

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1. INTRODUCTION

Vitamin C is an essential micronutrient that cannot be synthesized by fish and must be present in fish diets for proper functioning of the body. It is required for the biosynthesis of the collagen which is a pre-requisite for the formation of connective tissue and increases the absorption of iron in fish. It prevents various diseases; it is soluble in water and is easy oxidated by heat, light and metal [1].

Most animals can manufacture vitamin C in sufficient quantities for normal growth and function, but many fish cannot because they lack the enzyme L-gulonolactone oxidase for its manufacture [2]. Vitamin C facilitates the absorption of iron and is necessary for a maximum rate of immune responses and enables a good response to stressors. Fish show deficiency signs if they are fed diets deficient in vitamin C. Most commercial feed ingredients are almost completely devoid of vitamin C and it has to be supplemented in the diets. However, vitamin C is very sensitive to oxidative destruction during processing and storage and thus a significant amount is lost at that time [3].

Vitamin C acts as a reducing agent by being an electron donor to enzymes that assist in synthesizing collagen, carnitine, norepinephrine, peptide hormone, and tyrosine metabolism. It is also a chemical reducing agent [antioxidant] thereby decreasing oxidative DNA and/or protein damage lipid peroxidation, oxidants and extracellular oxidants from neutrophils. As the skeleton forms the structure of the body, collagen helps to maintain the structure of the skeleton, maintains its strength, even repair them after breaks and injuries [2]. Fig. 1 shows the structure of vitamin C.

2. VITAMIN C LEVELS AND FISH HEALTH

The importance of Vitamin C in farmed fish is well known in relation to good health and performance but it presents a challenge in the health of fish; researchers have yet to determine the dietary supplemental levels that permit optimum health and performance in various species. Further complicating the issue is the poor stability of most vitamin C products and the fact that supplemental levels which are sufficient under laboratory conditions may prove inadequate for intensive commercial operations.

The effects of ascorbic acid deficiencies or inadequacies vary with the age of the fish. For example, Gabaudan *et al.* [4] reported significant differences in weight gains between rainbow trout fed a vitamin C deficient diet and those receiving 181 g/ton (200 ppm) Vitamin C, beginning at 10 months of age. After 15 months, the vitamin C deficient fish had only half the weight gain of those receiving normal supplementation.

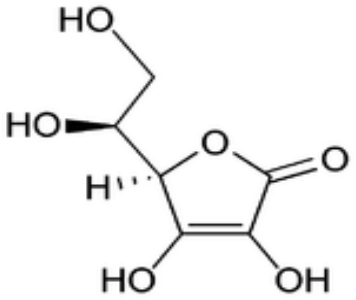

Fig. 1. Structure of vitamin C

A low dietary intake can support normal growth rates in fish despite the occurrence of both long-term clinical and subclinical deficiencies. Fish lying on their side, may exhibited abnormal swimming behavior and (or) have shortened gill covers and abnormal curvature of the spine (scoliosis) are the first clinical and macroscopic signs of the deficiency in fish [4]. The most notable pathological changes with vitamin C deficiency occur in supportive tissue (collagen, bone and cartilage), muscle development and blood-forming organs. Vitamin C deficient catfish show deformities especially scoliosis [5].

To determine optimal levels of dietary supplementation, Ikeda [6] studied young rainbow trout in a normal state of health, beginning four weeks after hatching and continuing for 20 weeks. Although there were no visible signs of deficiency with vitamin C supplementation at even 18 g/ton (20 ppm) of dry diet and reported that collagen synthesis was depressed when the fish received 45 g/ton (50 ppm) of vitamin C or less. At 90 g/ton (100 ppm), collagen synthesis stabilized. Liver tissue analysis showed that body stores of vitamin C did not peak until dietary intake reached 454 mg of vitamin C per ton (500 ppm) of dry diet. As a result, it was recommended that this level helps to maintain good health for young rainbow trout. Further in 10-month-old fish, he reported different effects with vitamin C deficiencies or inadequacies. There were no deformities in skin collagen, suggesting that the fish could biosynthesize vitamin C up to the minimum

amount necessary to maintain life. However, when fish suffered from skin wounds, there was a significant difference in regenerative capacity between fish receiving no dietary vitamin C and those receiving 1,814 g/ton (2,000 ppm). These results suggest an increased demand for vitamin C when collagen synthesis is urgently needed to promote healing.

Ikeda [6] also reported that rainbow trout fed increased dietary vitamin C showed enhanced tolerance of environmental stresses, such as reduced ambient oxygen. When water oxygen level was maintained below 0.9 ml/l for four hours, fish survivability increased in proportion to dietary vitamin C levels. Survival rate declined to 50% in the fish receiving no dietary vitamin C, compared with 80 percent for fish receiving either 272 g/ton (300 ppm) or 907 g/ton (1,000 ppm) and 90 percent of rainbow trout that received 2,722 g/ton (3,000 ppm).

Studies have shown that vitamin C-deprived fish are more susceptible to stress and seem to develop impaired immune functions. Conversely, in a summary of studies with both rainbow trout and channel catfish, Gabaudan et al. [4] noted increased survivability to bacterial, parasitic and viral infections with increased dietary levels of vitamin C.

Hardie et al. [7] studied immunological responses in Atlantic salmon wherein they found the deficient fish had significantly lower concentrations of serum complement. In addition, fish receiving 2,495 g/ton (2,750 ppm) of vitamin C had significantly greater amounts of complement than did those receiving 281 g/ton (310 ppm). One problem being faced by researchers studying vitamin c is its poor stability in many forms. By the time feed is fed, it may have only half or less of what was put in. Studies have also shown that without stabilization, 50% or more of it may be lost in feed processing, and even more may be broken down in the gut of the fish before it can be absorbed.

3. BIOLOGICAL SIGNIFICANCE OF VITAMIN C

Biologically, ascorbate acts as a reducing agent donating electrons to various enzymatic and few non-enzymatic reactions. Oxidized forms of vitamin C i.e. one- electron (semi dehydro-ascorbic acid) and two-electron (dehydroascorbic acid) can be reduced with the help of the body by glutathione and enzymes dependent on NADPH [8,9]. Ascorbate is maintained in reduced state as a result of the presence of glutathione in cells and extracellular fluids [10]. The Table 1 shows the properties of vitamin C.

4. AVAILABILITY OF VITAMIN C

4.1 Tissue Distribution of Vitamin C

Vitamin C is concentrated in many vital organs with active metabolism and is related to the dietary intake of the vitamin. Moreover, some tissues such as brain, thymus and leukocytes accumulate high concentrations of ascorbic acids. In these tissues, ascorbic acid levels seem to be retained longer in case of dietary vitamin C depletion compared to storage organs such as liver. An example of tissue distribution of vitamin C can be observed when Rainbow trout were fed vitamin C as ascorbate phosphate at the dose of 200 mg ascorbic acid equivalents per kg of feed. The very high levels found in thymus, brain and leukocytes confirm the hypothesis that ascorbic acid is important in preserving vital tissues from oxidation processes [11].

Liver, head and kidney are important storage organs for vitamin C in fish. The high level found in the head and kidney is likely to be related to the presence of lymphopoietic tissues. Trunk kidney and spleen are also able to store a large amount of vitamin C. Trunk kidney is the site of chromaffin cells which are responsible for catecholamine biosynthesis. Ascorbic acid is concentrated at the site of catecholamine formation and it is released with newly synthesized corticosteroids in response to stressors [12].

4.2 Benefits of Vitamin C

Vitamin C has many benefits especially to the health; the most clearly established functions of vitamin C involve synthesis of collagen, the fibrous protein constituent of bone, cartilage, tendon and other connective tissue. Other studies have shown a role for vitamin C in immune response, in lessening the effects of chemicals that are toxic in water and in helping prevent negative effects of water temperature fluctuations [13]. Other benefits include:

- It can act as an antioxidant that neutralizes free radicals that cause dangerous diseases.
- It can help the body to absorb iron.
- It can help maintain the structure of collagen in the body.

Table 1. Properties of vitamin C

Characteristics	
Molecular formula	$C_6H_8O_6$
Molar mass	176.12 g mol^{-1}
Appearance	White or light yellow solid
Density	1.65 g/cm^3
Melting point	190-192°C, 463-465 K, 374-378°F (decomp.)
Solubility in water	330 g/L
Solubility in ethanol	20 g/L
Solubility in glycerol	10 g/L
Solubility in propylene glycol	50 g/L
Solubility in other solvents	insoluble in diethyl ether, chloroform, benzene, petroleum ether, oils, fats
Acidity (pK_a)	4.10 (first), 11.6 (second)

- It helps increase useful cholesterol and minimizes cholesterol that is harmful to the body.
- It can help to repair damaged cells and regenerate cells in the body.
- It can strengthen and maintain the performance of the muscles of the heart.
- Minute supplementation of vitamin C at least in diets can help to reduce the risk of severe cataracts [12].

Vitamin C can enhance the growth of fish exposed to low and high dose of mercury, enhanced weight gain and survival rate [14].

Fish do not biosynthesize Vitamin C in their body because they lack the enzyme L-gulonolactone oxidase, which is the last enzyme of the biosynthetic pathway: And therefore it must therefore be supplied to the fish through the feed.

4.3 Sources of Vitamin C

The best vitamin for health is a *natural vitamin*. Some sources of natural vitamins that contain vitamin C are: Broccoli; Guava, Red Paprika, Orange, Spinach, Nuts, Green Chili and Tomatoes`.

Apart from the mentioned sources, it can also be gotten from other fruits and vegetables such as asparagus, cabbage, potatoes, kiwi, watermelon, papaya, pineapple [2].

5. SIGNS OF VITAMIN C DEFICIENCY IN FISH

Spinal lordosis and scoliosis are the most common observed signs of skeletal deformities in vitamin C deficient fish. It may appear in fish as a slight curvature of the spine (Plates 1-3). This is more visible in larvae and several fry than adult fish, opercular and gill lamellar can also be deformed as a result of the deficiency. Major signs of ascorbate deficiency include reduced growth, scoliosis, lordosis, internal and fin haemorrhage, distorted gill filaments, fin erosion, anorexia and increased mortality Anorexia and lethargy also occurs and may result to fish showing internal or external hemorrhaging. Healing of wounds will also occur at a slow rate [15].

5.1 Diagnosis of Vitamin C Deficiency

Spinal deformities in fish can result from inheritance or trauma and not necessarily deficiency of the vitamin; although **t**he use of Radiography can show and identify spinal deformities in fish.

The reliable ways of detecting vitamin C deficiency in fish include:

- When there is Low liver and kidney concentrations
- Identification of Vertebral collagen level, especially in channel catfish and rainbow trout [3].

5.2 Treatment of Vitamin C Deficiency

The basic treatment involves vitamin C supplementation and attention should be taken on the account oxidation of vitamin C that can occur during feed processing and storage. More stable derivatives of vitamin C have been developed to minimize these problems by combining ascorbic acid with phosphate or sulfate [5]. Fish are more prone to developing bacterial infections, and any of such problems that arise should be treated accordingly.

Plate 1. Vitamin c-deficient rainbow trout showing broken back syndrome
Source: Lim et al. [12]

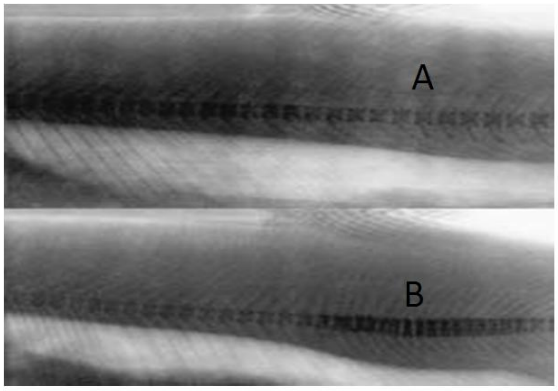

Plate 2. X-ray of a healthy fish (A) and a vitamin C deficient fish showing deformed vertebrae (B)
Source: Lim et al. [12]

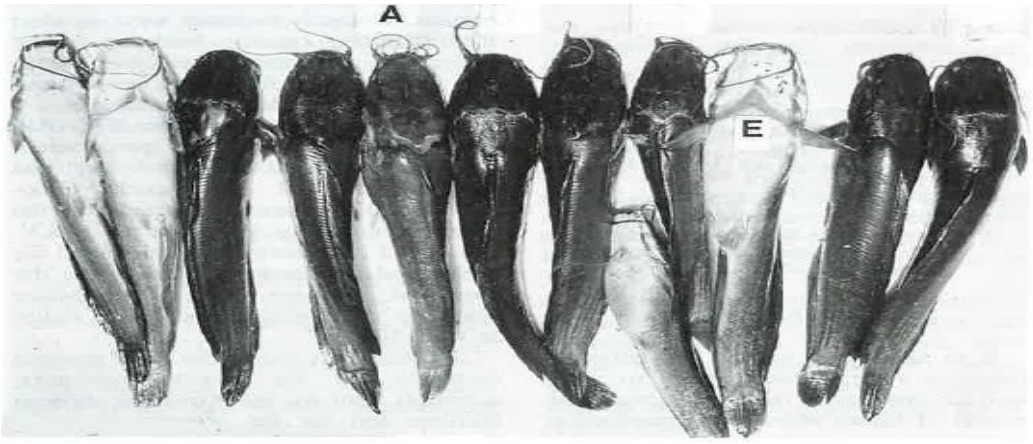

Plate 3. Broken skull disease of *Clarias gariepinus* in Nigeria. A,B,C- dorsal view of fully developed broken skull disease; D- early stage of disease condition; E- ventral view of developed disease condition

Merchie et al. [16] incorporated levels of vitamin C into feed of *Penaeus monodon* to determine the effects on stress and disease resistance and the effects on physiological condition were observed and evaluated after 4 weeks of feeding. Acsorbic acid (AA) kg^{-1} at 100, 1700 and 3400mg was combined to a diet of 230 mg AX kg^{-1} and grouped into three; 810 mg AX kg^{-1} of diet contained 200 and 1700 mg AA kg^{-1} and grouped into two diets. The biomass of the group receiving the lower dietary ApP–AX combination was significantly lower than for all other treatments, significant differences was observed in stress resistance in the groups fed 230 mg Ascorbic acid/kg diet; and a drop in mortality after an osmotic shock was observed when the vitamin C concentration in the feed was increased from 100 to 3400 mg Ascorbic Acid/ $kg^{-1.}$

5.3 General Recommendations for Vitamin C in Fish

Fish are dependent on a vitamin C supplementation via the feed since no synthesis can occur in fish. To optimise stability of vitamin C during feed production and storage as well as bioavailability in the fish, phosphorylated form of vitamin C is recommended. Based on the combination of experimental results obtained in several controlled studies and field experience, the following general inclusion rates of vitamin C in feeds for aquaculture species are recommended and shown in Table 2 below:

Table 2. Recommended vitamin levels for aquaculture fish spsecies

Species	Recommended vitamin C level (mg/kg of feed)
Tilapia	150-250
Catfish	150-250
Shrimp	250-500
Salmon	150-250
Trout	150-250

Source: adapted from Lim et al. [12]

6. CONCLUSION AND RECOMMENDATION

From this review, it is evident that the benefits of vitamin C cannot be over emphasized and must be present for normal well being and functioning in fish.

It is therefore essential that fish feeds should be incorporated with the required dosages of Vitamin C supplement for proper functioning of the body and it should be noted that vitamin C can be oxidated during processing and storage of feed, therefore vitamin C should be used in phosphorylated form is recommended for optimal stability in fish feeds.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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