Ascorbic acid (vitamin c) in fish diets

Ascorbic acid (Vitamin C) is an important nutrient in fish diets and especially in freshwater fish. The biosynthesis of Vitamin C does not occur in fish, and therefore must be supplied in the food. The fish requirements are approximately 200 mg/kg food but these are related to many factors like influence of stress, the growth rate, the size of the fish, temperature of water and to the other nutrients presented in the diet. The quantity of vitamin C in the diet is also related with the presence of diseases in the fish plant. The active form is ascorbic acid and the inactive dehydroascorbic acid is white crystalline compounds. Also, a chemically synthesized form, L-ascorbate-2-phosphate is readily used by fish as a vitamin C source. Cabbage, Citrus fruits, liver, kidneyand mostly in glandular tissues there are good sources of the vitamin. Also fish food made from whole fish has good quantities of this vitamin. When this nutrient is supplied in the fish meal the food must be protected from aerobic oxidation which turns ascorbic acid in its inactive form. The most important signs of the deficiency of vitamin C are anorexia, reduction of growth, reduced bone collagen, lordosis, scoliosis, haemorrhagies in the fins and in the internal organs, problems in the gill filaments, erosion of the fins, and increased mortality. Regarding the interactions with other nutrients the vitamin C demand is increased when diets are deficient with tryptophan. Also this vitamin is very instable to oxidation in presence of elements like cooper or iron because increase oxidation and inactive ascorbic acid into dehydroascorbic acid.

Key words: Ascorbic acid, requirements, deficiency.

Introduction

(Ascorbic acid) Vitamin C can be synthesized by most animals in quantities sufficient to sustain normal functions of life, but most fishes this phenomenon is not seen because they lack the enzyme L-gloconolactone oxidase that synthesizes vitamin C from glucose (Darias et al 2011). Thus, for the above reason vitamin C is essential and should definitely take it with food. Fishes are newly added to the group of animals (after humans and birds) that require vitamin C from the diet (Dabravski et al 2001). This is because aquaculture is a relatively new science developed mostly these last two decades (De Silva, 2001) and research in fish feeding is also new. Kitamura et al. (1965) were the first to explain a critical need for ascorbic acid in trout and four years later Halver (1969) demonstrated the need for vitamin C in salmon. Later in 2001 Dabrowski prepared a full review for vitamin C in his book " Ascorbic acid in aquatic animals".

Chemical forms and its analogs

Vitamin C exists in two forms, the reduced form (ascorbic acid) and the oxidized form (dehydroascorbic acid) (Figure 1). Both have vitamin C activity but the one that predominates is the reduced form. This is called the active form of the compound. Also, both forms are often reversed. The active form (ascorbic acid) is a white, crystalline and odorless compound which is soluble in water and insoluble in fat. This reduced form is stable in acids but very unstable in alkaline solutions which make the compound to lose its activity. Also, its activity can be damaged if vitamin C is in contact with the heat and atmospheric oxidation. One derivate like L-ascorbate-2-sulfate (vitamin C2S), is heat resistant and can be stored in the tissues of salmonids if they are treated with excess dietary of vitamin C (Halver, 1985). This resistant form is also found in artemia cysts and in many other animals (when it is in the form of sulfate) (Tucker and Halver, 1984). Another vitamin C sources compound that feed manufacture industry is interested are synthetized derivatives with other electron-dense groups coupled on the 2-position of ascorbic acid (Halver 2002).

Requirements

Rainbow trout (Oncorhynchus mykiss) is one of the most studied species of fishes associated with requirements for vitamin C. It is noted that an amount of 100mg/kg dry weight of ration would be enough for trout. In trout with muscular injury should be given five times the normal dose of 500 mg/kg dry ration to cope with this situation. Also a high level of Vitamin C is added in the ration would make the rainbow trout to face with an infectious disease (Halver 2002). Navarre and Halver (1989) showed that if the trout injected with Vibrio anguillarum they were resistant to the agent with an excess dose of vitamin C in the ration. The same can be said for viral diseases to trout if in ration was added an excess of ascorbate-2-phosphate (Anggawati-Satyabudhy et al. 1989). However, Halver (2002) states that a dose of 200 mg/kg of dry ration would be sufficient in the case of rainbow trout. Regarding the common carp (Cyprinus carpio) Ikeda and Sato (1964) emphasize that an adult individual can synthesize small amounts of vitamin C, but this is related to the size of the fish. For bigger fishes was expected higher vitamin C synthetization.

For the Atlantic salmon (Salmo salar) the requirements for water soluble vitamins are lower than those referred in previous works. To get a liver storage and a good enzymatic activity should increase vitamin C dosage beyond the levels we give to simply increase the weight. There is evidence in laboratory that the increase in diet of vitamin C has a positive effect on resistance to disease and a better immunity response. Ascorbic acid protects phagocytic cells and also defends tissues from oxidation (Gatlin, 2002).

Regarding gilthead sea bream (Sparus aurata), there is no quantitative data on requirements for vitamin C. If seabream ration consists on fishmeal, there is not necessary any supplement of vitamin C that affect on growth performance (Henrique et al., 1998), but supplementary levels are recommended for having resistance to stress, renal function and wound-healing (Alexis, Karanikolas and Richards, 1997; Henrique et al., 1998). According to NRC (1993) the recommendations for salmonids may be applied to sea bream too. Woodward (1994), suggest that that, without specific data, the vitamin requirements established for salmonids can be applied to other teleost.

Also the data for vitamin requirements for European sea bass (Dicentrarchus labrax) are extremely scarce. There are reports that sea bass requirements for vitamin C are below 50mg/kg dry diet. For individuals in fattening these requirements are reduced to less than 10 mg/kg, but to have a sufficient content in the liver the diet must have higher quantities than that (Fournier et al. 2000). Kaushik et al. (1998) suggest that the vitamin C requirements for the sea bass like for sea bream would refer to the requirements of rainbow trout.

For the on growing gray mullet (Mugil cephalus) El-Dahhar (2006) applied one experiment with different levels of ascorbic acid in the diet and concluded that dietary requirements for the best growth, survival and feed utilization of this species was 40-mg/kg dry diet. While, at hybrid tilapia (Oreochromis niloticus x Oreochromis aureus) higher levels of vitamin C in the diet can spare the addition of vitamin E.

Problems of deficiency

When the fish has a diet deficient in vitamin C, generally observable signs associated with defective collagen formation. Thus, was observed hyperplasia of collagen and cartilage tissue, kyphosis, scoliosis, lordosis, resorbed opercula, deformities and hyperplasia of jaw poor regeneration of the wounds, (Halver et al., 1969). Different authors observed the same signs in different fish species like; rainbow trout (Falahatkar et al 2011), Atlantic salmon (Halver 2002), yellowtail (Kanazawa et al 1992), zebra danio (Newsome and Piron 2006), common carp (Dabrowski et al., 1989), tilapia (Stickney et al 2009), gilthead sea bream (Alexis et al 1997) Atlantic halibut (Lewis mcCrea and Lall 2010), cobia (Zhou et al 2012).

Dabrowski et al. (1995) observed ascorbic acid deficiencies related with reproductive functions in in tilapia, rainbow trout, cod and yellow perch. These deficiencies were observed during whole vitelogenesis, but embryonic viability was observed only in rainbow trout and tilapia. Also, was detected a decrease of rainbow trout fecundity and egg production. In a parallel study by the same author was observed that diets with deficient vitamin C had a reduction of yolk in the eggs of rainbow trout.

In tilapia (Tilapia aurea) feeding with a diet with lower than 25mg/kg vitamin C were observed pathological changes like scoliosis hemorragies in the mouth, fins and swim bladder (Stickney et al 2009). For discus fish (symphysodon spp.) for many years the vitamin c deficiency was related with the cause of the parasitic disease hole in head. This speculation led to the fact that adding vitamin c in the fish diet the disease desapeared. In fact, later was seen that hole in head was not related to vitamin C, but its effect healing wounds caused the healing of the lesions of the Hole in head (Lowel 1989). In gray mullet levels of vitamin C which accumulates in liver, gill and brain may be depleted significantly if the fish is exposed to cadmium (Thomas et al 2006).

Feeding the Walleyes Stizostedion vitreum with a vitamin C – free diet caused high mortality rate and retarded growth for the rest of the group. Also were presented the classical signs of vitamin C deficiency explained above for other fish species (Macconnell and Barrows 1993). While in vitamin C free-diets was observed increased mortality, retarded growth and bad feed conversion rate for juvenile hybrid striped bass (Sealey and Gatlin 2002).

In Atlantic halibut (Hippoglossus hippoglossus) the most presented signs of vitamin C deficiency were scoliosis and lordosis mostly in the hemal region of the vertebras. These experimental findings were similar with signs found in commercial halibut hatcheries (Lewis-McCrea and Lall 2010).

Interaction with other nutrients

D-ascorbic acid, 6-deoxy-L-ascorbic acid and L-glucoascorbic acid are isomers of the active form (ascorbic acid), but have very low or no activity.

Because of their close similarity these compounds compete with the active form for the sites of different enzymes and chemical reactions in organism (Halver 2002).

Lim et al (2000) investigated the interaction between vitamin C and iron if they are supplemented in the diet of catfish (Ictalurus punctatus). If the vitamin C was supplemented in high doses (3000 mg/kg) there was a significant effect on survival. Also there are reports that mention the important role of the combination of vitamins D and C ossification process in fishes and in formation of skeletal deformations (Darias et al 2011). There is also an interaction between ascorbic acid and T4 which was investigated in freshwater catfish, (Heteropneustes fossilis). If the fish is exposed to the organophosphate pesticide chlorpyrifos, the activity of different enzymes is reduced drastically. When the combination of thyroxine (T4) and vitamin C was administered to the fish, the activities of enzymes returns in the levels of control (Tripathi and Shasmal 2010).

Another interaction between vitamins C and E is reported in Atlantic salmon from Hamre et al. (1997). From the results of the study these authors says that diets with vitamins C and E have two different mechanisms of interaction: a synergistic effect against oxidation on water and lipid phases and a regeneration effect of vitamin E from its radical by ascorbic acid. For a synergistic effect in the interaction between vitamins C and E was also reported in immune system of gilthead sea bream by Ortuño et al (2001). According to the results of the study both vitamins produced a synergistic effect on the respiratory burst activity of the phagocytes of the fish. The same combination of vitamins C and E in the diet increased growth rate, and semen quality in yellow perch, (Perca flavescens) (Jun Lee and Dabrowski 2004). Meanwhile, Frischknecht et al (2006) reports that there is a correlation between vitamins C and E, but need to work more on mechanisms of these interactions.

There are reports for effects of interaction of vitamins C and E also on reproductive performance of different fishes. Thus, the addition of vitamins C and E by injection effects on reproduction of the Japanese eel (Anguilla japonica) (Furuita et al 2009). After the injection the hatchling rate and the survival rate of larvae increased. Also increased the accumulation of vitamin in broodstock and eggs. In a recent study Nguyen et al (2012) showed another effect of combination between vitamins C and E on reproductive performance, of kuruma shrimp (Marsupenaeus japonicas). These authors suggest that the diet of kuruma shrimp must contain the supplement of vitamins C and E in because their combination gives better egg hatchability and better quality of larvae.

Conclusions

The vitamin C is an essential nutrient in fishes and therefore must be supplied in the diet.