Oscars, *Astronotus ocellatus*, Have a Dietary Requirement for Vitamin C[1,2,3]

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ABSTRACT We found that vitamin C is an essential nutrient for an Amazonian ornamental fish, the oscar (*Astronotus ocellatus*). This was demonstrated by the absence of L-gulonolactone oxidase activity, the enzyme responsible for the biosynthesis of vitamin C, in liver or kidney of oscars and by a feeding trial in which oscars without vitamin C dietary supplementation developed clinical deficiency signs. Fish weighing 29.2 ± 1.9 g were divided into four groups, and each group was fed a casein-based semipurified diet containing 0, 25, 75 or 200 mg ascorbic acid equivalent (AA)/kg diet for 26 wk. Vitamin C was supplemented in the diets as L-ascorbyl-2-polyphosphate, a mixture of phosphate esters of ascorbate, which is more stable to oxidation than AA. At the end of 26 wk, fish fed no AA had significantly lower weight gain than fish fed the AA-supplemented diets ($P < 0.05$). Oscars without dietary AA supplementation gained only 37% of their initial weight, compared with 112, 102 and 91% gained by fish fed 25, 75 and 200 mg AA/kg diet, respectively. After 25 wk without dietary supplementation of AA, fish began to develop clinical deficiency signs, including deformed opercula and jaws, hemorrhage in the eyes and fins, and lordosis. Histology indicated that fish without AA supplementation had deformed gill filament support cartilage and atrophied muscle fibers. Collagen content of the vertebral column was significantly lower in fish devoid of dietary AA ($P < 0.05$). Liver AA concentration varied in proportion to dietary concentration of AA. The minimum dietary AA concentration tested in this study, 25 mg AA/kg diet, was sufficient to prevent growth reduction and AA deficiency signs in oscars. J. Nutr. 128: 1745–1751, 1998.

KEY WORDS: • *ascorbic acid deficiency* • *L-gulonolactone oxidase* • Astronotus ocellatus • *ornamental fish*

The oscar, *Astronotus ocellatus*, is a cichlid native to the Amazon basin that has worldwide commercial value as an ornamental fish. In its native range, however, the oscar or *acará-açu* is also appreciated for its meat, which has a firm consistency and lacks intramuscular bones. In the Northeast region of Brazil, several programs have been initiated to populate reservoirs with this species because of its early maturation (10 to 12 mo) and relatively high fecundity. Additionally, it reproduces in still waters and undertakes parental care, which facilitate its establishment in reservoir conditions (Fontenele 1951, Fontenele and Nepomuceno 1983). The natural diet of the oscar includes fruits, snails and small fish; it is an omnivore, with carnivorous tendencies (Ferreira 1981).

The nutritional requirements for commercially cultured food fishes such as channel catfish, *Ictalurus punctatus*, and rainbow trout, *Oncorhynchus mykiss*, are well studied (NRC 1993), whereas data on nutrient requirements for ornamental fishes are scarce. Requirements vary among the many species of fish, however, because of differences in physiologic and morphologic characteristics. Many fish species cannot synthesize ascorbic acid (AA),[5] or vitamin C, which is essential for fish growth, reproduction, and health (Dabrowski 1990, Soliman et al. 1986, Wilson 1973, Yamamoto et al. 1985). The inability to synthesize vitamin C is due to a lack of the enzyme L-gulonolactone oxidase (GLO, EC 1.1.3.8), which catalyzes the conversion of L-gulonolactone to AA (Roy and Guma 1958).

Vitamin C acts as a metabolic antioxidant, detoxifying numerous peroxide metabolites, thus protecting cell membranes and other intracellular components and processes that are sensitive to oxidation (Masumoto et al. 1991, Sandel and Daniel 1988). It is also a cofactor in the hydroxylation of proline and lysine in the synthesis of collagen (Chaterjee 1978), a component of connective tissues, blood vessels, bone matrix and scar tissue in wound repair. Clinical signs of vitamin C deficiency in channel catfish and salmonids include reduced growth, structural deformities (scoliosis, lordosis and abnormal

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[5] Abbreviations used: AA, ascorbic acid; AAPP, L-ascorbyl-2-polyphosphate; GLO, L- gulonolactone oxidase.

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support cartilage of the eye, gill and fins), abnormal pigmentation, increased capillary fragility, reduced immune responses, slow wound repair and reduced reproductive performance (Halver et al. 1969, Lim and Lovell 1978, Sandnes et al., 1984). Additionally, dietary levels of vitamin C higher than the ones required for maximum growth increase resistance against bacterial infections in channel catfish (Durve and Lovell 1982, Li and Lovell 1985, Liu et al. 1989). When commercial aquaculture feeds are supplemented with AA, the vitamin's instability is a major problem. Ascorbic acid is heat labile and prone to oxidation. Recent studies indicate that AA derivatives that include sulfate and phosphate moieties are resistant to oxidation but retain vitamin C activity for fish (Abdelghany 1996, Dabrowski et al. 1990, El Naggar and Lovell 1991a, Grant et al. 1989, Robinson 1990, Sandnes et al. 1992, Soliman et al. 1986, Wilson et al. 1989). L-Ascorbyl-2-polyphosphate (AAPP) is a mixture of phosphate esters of ascorbate (mono-, di- and triphosphate) and free AA, which is more stable to oxidation than AA (Grant et al. 1989). This form of AA was evaluated for the oscar in this study.

The vitamin C requirement of some fish species of commercial importance has been investigated. Channel catfish require as little as 11 mg AA/kg diet for maximum weight gain and for absence of deficiency signs (El Naggar and Lovell 1991a), whereas rainbow trout require up to 100 mg/kg (Halver et al. 1969). Few studies, however, address the requirement for vitamin C of tropical freshwater species. In tropical cichlids, 50 mg AA equivalent/kg diet allows maximum weight gain and absence of deficiency signs in blue tilapia (*Tilapia aurea*) (Stickney et al. 1984), whereas dietary levels >40 mg AA equivalent/kg diet are required to prevent growth reduction in juvenile hybrid tilapia (*Oreochromis niloticus*, L. × O. *aureus*), and a dietary level of 79 mg AA equivalent/kg diet is recommended for maximum growth (Shiau and Jan 1992). Chavez de Martinez (1990) reported a vitamin C requirement of 40 mg AA equivalent/kg diet for normal growth, and 110 mg/kg to ensure fish health of a native Mexican cichlid, *Cichlasoma urophtalmus*. No studies have been published to date to determine the dietary AA requirement of the oscar.

This study had the following goals: *1*) to determine if the oscar requires a dietary source of vitamin C; *2*) to establish the dietary concentration of vitamin C required for normal growth and absence of clinical deficiency signs; and *3*) to characterize the clinical signs associated with vitamin C deficiency in the oscar, if such deficiency occurs in this species.

MATERIALS AND METHODS

Experimental conditions

Water and tank system. Juvenile oscars ($n = 48$) averaging 29.2 ± 1.9 g were randomly distributed in twenty-four 54-L tanks arranged in a water-recirculating system, which included a mechanical filter, a trickling-type biological filter and a water pump. Each tank housed two fish that were separated by a perforated plastic division to prevent injuries due to aggression. Tanks were siphoned and scrubbed once a week to remove waste and to prevent algal growth; at this time, an equal mixture of charcoal-filtered city-tap water and reverse-osmosis–filtered water was used to replace 50% of the total volume of the water in the system. A 12-h light:dark cycle was used. Water temperature was kept at 28.7 ± 0.4°C, and water quality parameters such as ammonia, nitrite and pH were monitored five times a week using a commercially available water analysis kit (Hach Chemical, Ames, IA). Dissolved oxygen was monitored only once a week because levels >6 mg/L were easily maintained by spraying the water into the biofilter before it was distributed to the tanks, and by an air stone placed in each tank.

Diets and fish. Oscars were fed a casein-based semipurified basal diet (NRC 1993) without AA for 11 wk before consuming the experimental diets. The percentage ingredient composition of the basal diet is shown in **Table 1**. The basal diet was then supplemented with AAPP (STAY-C, 29.1% AA activity, Hoffmann-LaRoche, NJ) to contain 25, 75 and 200 mg equivalent AA/kg diet. When supplemented, AAPP replaced cellulose. Dry ingredients were blended using a Hobart mixer, following the addition of oil and water. This mixture was passed through a food grinder (die diameter, 2.5 mm). Resulting strings were broken, and pellets (35% moisture) were stored in hermetically sealed plastic bags at −26°C until fed. These diets, plus the basal diet without AA, were fed to oscars to satiation twice a day, 5 d/wk, and once a day on weekends, for 26 wk. Each diet was randomly assigned to six tanks containing two fish each ($n = 12$). Fish average weight at the onset of feeding the experimental diets was 51.27, 48.92, 47.61 and 49.72 g for the dietary AA concentrations of 0, 25, 75 and 200 mg/kg diet, respectively. These initial weights were not significantly different ($P > 0.05$).

Fish were anesthetized with tricaine methane sulfonate (MS-222), weighed and closely examined for any structural deformities once a month until wk 14, and twice a month from then on. Six fish per dietary treatment were sampled at wk 18 for measurement of the collagen content of the vertebrae, hematocrit, vertebral column integrity by X-ray and gill histology. At wk 26, at the end of the experimental period, the remaining six fish in each group were sampled for the determination of liver AA, vertebral column integrity by X-ray and total body length. At this time, histologic examinations were performed on the gills from four fish in each group. In addition, one fish in each group was selected for histologic evaluation of multiple tissues; tissues sampled included skin, muscle, fin, eye, brain, kidneys, liver, heart, pancreas, gonads, spleen and gastrointestinal tract. Fish were killed by an overdose of MS-222. Procedures adopted for fish handling and care complied with the Institutional Animal

TABLE 1

Composition of the basal diet

Ingredient[1]	Amount
	g/kg diet
Vitamin-free casein	36.0
Gelatin	10.0
Dextrin	33.0
Cellulose	5.6
Carboxymethylcellulose	3.0
Lipid[2]	7.0
Mineral mix[3]	4.0
Ascorbic acid-free vitamine mix[4]	1.4
Protein, %	39.4
Estimated digestible energy,[5] *kJ/kg*	12,623

[1] All ingredients except lipids and vitamin premix were obtained from United States Biochemical, (Cleveland, OH).

[2] Lipid source was 3% cod liver oil plus 4% soybean oil. The antioxidant Santoquin was added to the oils to give a final concentration of 200 mg/kg diet. The lipid sources and Santoquin were obtained from ICN Pharmaceuticals (Costa Mesa, CA).

[3] Williams and Briggs salt mixture of was used and contained (g/100 g mixture): calcium carbonate, 20.71; calcium phosphate, dibasic, anhydrous, 32.28; cupric sulfate, anhydrous, 0.1429; ferric citrate, 1.6857; magnesium sulfate, anhydrous, 6.57; manganous sulfate, monohydrate, 0.4400; potassium iodate, 0.0086; potassium chloride, 20.86; sodium phosphate, dibasic, anhydrous, 17.14, and zinc carbonate, 0.1514. This salt mixture was supplemented with (mg/kg diet) cobalt chloride, 1.0; aluminum potassium sulfide, 0.7; and sodium selenite, 0.08.

[4] The vitamin mixture was made by ICN Pharmaceuticals (Aurora, OH) and provided the following diluted in cellulose (mg/kg diet): thiamin, 10; choline chloride, 3,000; niacin, 150; riboflavin, 20; pyridoxine, 20; calcium panthotenate, 200; vitamin B-12, 0.06; *dl*-α-tocopherol, 50; menadione Na-bisulfite, 80; inositol, 400; folic acid, 5; and biotin, 2; retinyl acetate, 12 (6000 IU); and cholecalciferol, 6 (6000 IU).

[5] Digestible energy for channel catfish (NRC 1993).

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Analytical procedures

l-Gulonolactone (GLO) oxidase activity. The GLO activity was measured in liver and kidney of two oscars, average weight 15.5 ± 0.3 g, following the procedure of Ayaz et al. (1976) in which a 2,4-dinitrophenylhydrazine derivative of ascorbate is measured in a spectrophotometer at 524 nm. A sample of rat liver was also assayed as a positive control. Minor modifications were introduced including the following: 1) the incubation temperature was lowered from 37 to 30°C, and 2) an additional blank per sample was included to account for interfering substances, where the AA was destroyed (90°C for 1 h) (Dabrowski and Hinterleitner 1989).

Histology. Gill samples were taken at wk 18 and 26 for histologic preparation. Both of the second gill arches were removed, fixed and stored in 10% buffered formalin until processed routinely for histology. Other tissues were sampled from fish at wk 26 and were fixed and stored in 10% neutral buffered formalin until processed for histology. Paraffin-embedded tissues were sectioned 5 μm thick and stained with hematoxylin and eosin.

Vertebral collagen. Collagen in the vertebra was measured at wk 18, following the procedure described by Mustin and Lovell (1992). A minor modification was introduced to facilitate the removal of the vertebral column, which consisted of boiling the whole fish before removing the vertebrae. This procedure was done after all other tissue samples had been taken.

Ascorbic acid in the liver and feed. Livers were removed from three fish in each group, rinsed in distilled water, frozen in liquid nitrogen and stored at −26°C until AA analyses. Diets were sampled at the beginning of the experimental period and kept frozen (−26°C) until analysis. Ascorbic acid in the livers and diets was determined following a procedure described by Wang et al. (1988). The assay was performed by using HPLC (Knauer pump, model 64, Sonnteck, Woodcliff Lake, NJ), equipped with an electrochemical detector with a glassy carbon electrode and type TL-5A flow cell (model LC-4B, Bioanalytical Systems, West Lafayette, IN), and a reverse-phase (250 × 4.6 mm) column packed with 5-mm particles (Alltech C-18). In the diets, L-ascorbyl-2-polyphosphate esters were assayed by phosphatase digestion followed by HPLC determination of the released L-ascorbic acid. The detection limit of this method is 0.4 ng in 20 μL extract, which is equivalent to 10 mg AA/kg in the feed.

Hematocrit. Hematocrit was determined by the microhematocrit method of Snieszco (1960), in which blood from the caudal vein is withdrawn into heparinized capillary tubes and centrifuged at 17,000 × *g* for 4 min at 22°C.

Statistical analysis. Differences among dietary treatments were tested by ANOVA, and means were compared using Tukey's multiple comparison test (Steel and Torrie 1980). Data analyses were performed using the statistical program Sigma Stat, version 2.0 (Jandel Corporation, San Raphael, CA). Differences were considered significant at *P*-levels < 0.05.

RESULTS

One fish from the group that did not receive dietary AA crossed the plastic screen that was separating it from its tank mate and was killed by injuries due to aggression. No other mortality occurred during the feeding trial.

There was no detectable activity of GLO either in liver or kidney of oscars. The average values observed were lower than the assay detection limit of 0.2 μmol AA/(g tissue · h).

In the feeding trial, fish fed the diet without AA had a significantly lower weight gain than fish fed the AA supplemented diets ($P < 0.05$). During a 26-wk period, they gained only 37% of their initial weight, whereas fish fed 25, 75 and 200 mg AA/kg diet gained 112, 102 and 91% of their initial weight, respectively (**Fig. 1**). Above the dietary supplementation of 25 mg AA /kg of diet, there was no significant difference in weight gain among fish fed diets supplemented with AA

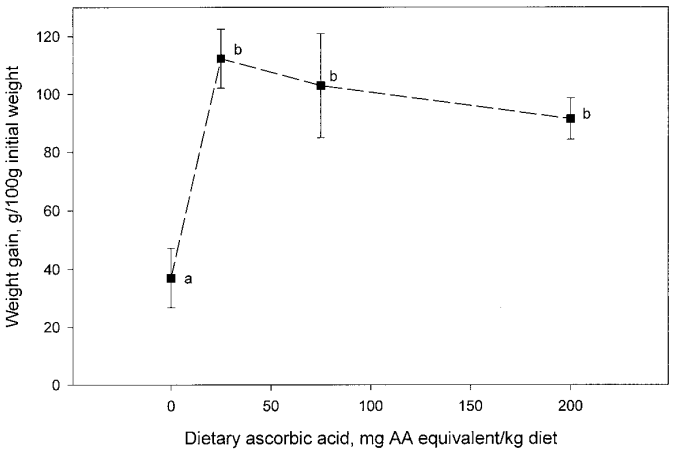


FIGURE 1 Weight gain of oscars, expressed as a percentage of initial weight, after 26 wk of consuming diets containing 0, 25, 75 and 200 mg ascorbic acid (AA)/kg diet. Values are means ± sem; $n = 12$ to wk 18, and $n = 6$ after wk 18. Squares followed by the same letter are not different ($P > 0.05$).

in the concentrations tested in this study. Fish without AA supplementation stopped growing at wk 14, and began to lose weight at wk 22 (**Fig. 2**). Fish fed the AA-supplemented diets did not stop growing during the 26-wk feeding period and reached a significantly greater weight than did fish without dietary AA.

At the end of 26 wk, no AA was detected in livers of oscars without AA supplementation, whereas an average of 41.58, 156.13 and 337.25 nmol AA/g liver was found in fish fed diets supplemented with 25, 75, and 200 mg AA/kg diet, respectively (**Fig. 3**). The measured concentrations of AA in the diets were 0, 24.2, 63.8 and 167.3 mg/kg for the formulated dietary targets of 0, 25, 75 and 200 mg/kg diet, respectively (Fig. 3).

Fish without dietary AA developed deformities in the vertebral column, as shown in **Figure 4***B*. After 26 wk, these fish were significantly shorter ($P < 0.05$) than those fed the AA-supplemented diets; this made them appear plump compared with fish receiving dietary AA supplementation. Total body length at the end of the experimental period was 13.66 ± 1.44 (mean ± SD), 17.76 ± 0.71, 16.25 ± 1.75 and 16.36 ± 0.49 cm for fish fed 0, 25, 75 and 200 mg AA/kg diet. Other clinical signs, such as hemorrhage in the eye and fins, opaque eye corneas and eroded fins, were detected as early as wk 14 in these fish (**Table 2**). At the end of 26 wk, all fish without dietary AA supplementation showed some type of bone deformity or other clinical signs (Table 2), whereas fish receiving AA supplementation did not.

Results of histologic evaluation of the gills from fish sampled at 18 wk were equivocal. In some sections of gills from the fish receiving no dietary AA, primary lamellae appeared to be shortened and there was mild bending of cartilaginous gill filament supports compared with gills from fish in the other groups. Mild swelling and disorganization of chondrocytes in the filament supports were also present. However, these findings were somewhat subjective and inconsistently present within the group receiving 0 AA and even within different sections of gill from the same fish. After 26 wk of consuming the experimental diets, there were obvious and consistent histologic differences between the fish receiving 0 AA and the other three groups; no differences were seen between fish in the three groups that received different levels of AA supplementation. Within the gills, primary lamellae of the fish con-

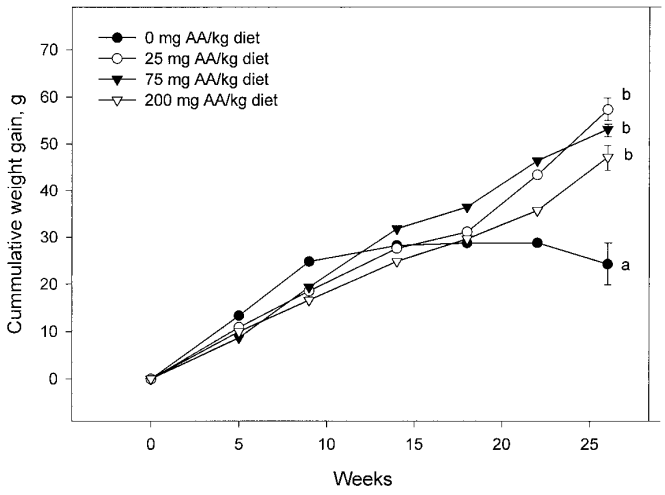


FIGURE 2 Cumulative weight gain/fish (g) at 5, 9, 14, 18, 22 and 26 wk of consuming diets containing 0, 25, 75 and 200 mg ascorbic acid (AA)/kg diet. Symbols represent the mean of 12 fish/diet up to wk 18, and 6 fish/diet from then on. Average initial weights were 51.27, 48.92, 47.61 and 49.72 g for 0, 25, 75 and 200 mg AA/kg diet, respectively. Symbols followed by the same letter are not different at wk 26 ($P > 0.05$), and SEM were 8.2, 4.7, 1.2 and 6.2 g for the dietary treatments 0, 25, 75 and 200 mg AA/kg diet, respectively.

suming 0 AA tended to be shorter and thinner than those in the other fish. The supplemented fish had bony filament supports with centers and bases composed of well-organized cartilage (**Fig. 5**A); filament supports in the unsupplemented fish were thin, contained little bone and were composed primarily of poorly organized cartilage with large, irregularly shaped chondrocytes separated by low amounts of extracellular cartilaginous matrix (Fig. 5B). Multifocally, there were bending deformities of the filament supports. Moderate amounts of loose, mucinous connective tissue were located in the centers

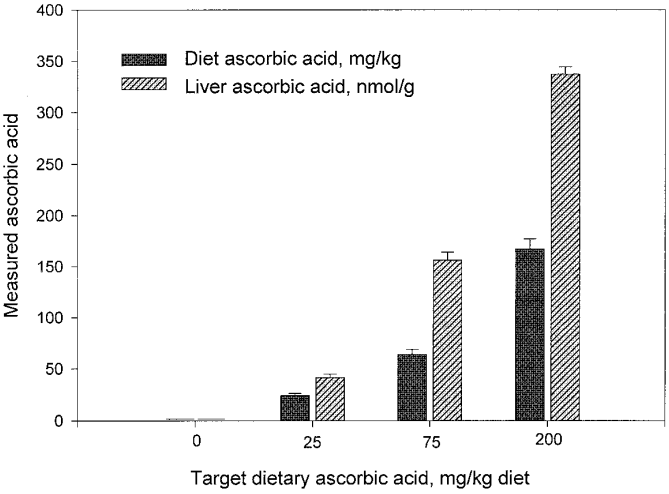


FIGURE 3 Measured ascorbic acid in livers and experimental diets of oscars after 26 wk of consuming diets containing 0, 25, 75 and 200 mg ascorbic acid (AA)/kg. Values for ascorbic acid in the liver represent the mean of three fish per dietary treatment, and SEM were 3.4, 7.8 and 7.1 nmol/g for the dietary treatments 25, 75 and 200 mg AA acid/kg diet, respectively. Values for ascorbic in the diet represent the mean of three samples per dietary treatment, and the SEM were 2.1, 5.2 and 10.3 mg/kg for the dietary treatments 25, 75 and 200 mg AA/kg diet, respectively.

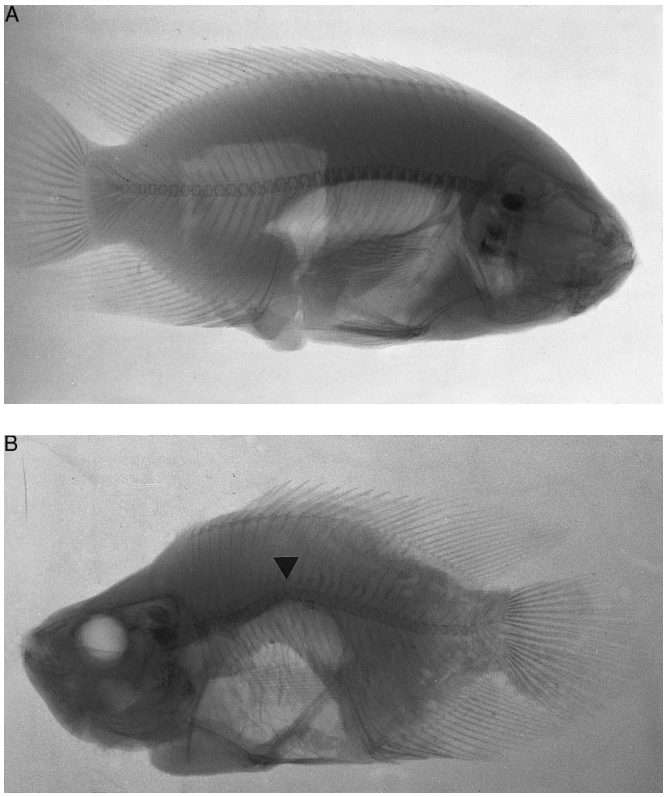


FIGURE 4 Radiographs of oscars that (*A*) received 25 mg ascorbic acid (AA)/kg diet, and (*B*) did not receive AA supplementation; the arrowhead indicates deformity in the vertebral column. Both radiographs were taken at the end of the experimental period.

of the primary lamellae, surrounding the filament supports (Fig. 5B). The dermis of the skin and fins of the unsupplemented fish was thin, and the hypodermis contained large amounts of mucinous connective tissue similar to that in the gills. Compared with supplemented fish (**Fig. 6**A), most of the skeletal

TABLE 2

Percentage of fish without dietary ascorbic acid (AA) supplementation that showed clinical signs when they first appeared, and at the end of the experimental period[1]

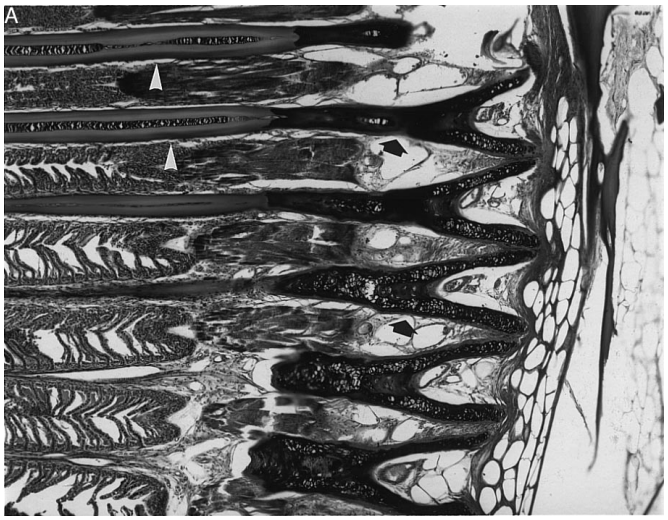
	Onset of the deficiency signs[2]	End of the experimental period[3]
Clinical signs	% affected fish	
Hemorrhage in the eyes and fins	17	100
Opaque eye lenses	17	33
Eroded fins	17	66
Deformed opercula	—	66
Deformed jaw	—	33
Lordosis[4]	17	100

1 Fish receiving the dietary AA supplementation did not show any of these clinical signs.

2 The 25 wk without AA consisted of 11 wk of pretrial depletion and 14 wk on trial.

3 The 37 without AA consisted of 11 wk of pretrial depletion and 26 wk on trial.

4 Radiographs of six fish/diet were taken.



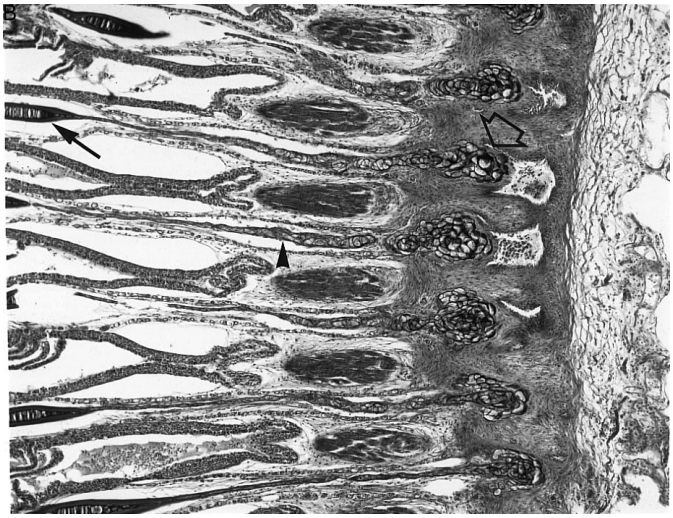


FIGURE 5 Photomicrograph of a histologic preparation from gills of an oscar that (*A*) received 75 mg ascorbic acid (AA)/kg diet for 26 wk. Note that gill filament supports (arrowheads) within the primary lamellae are composed of well-formed bone with cores of cartilage. Bases of these filament supports consist of well-organized cartilage (arrows). Hematoxylin and eosin stain (H & E), 75X magnification. (*B*) The fish did not receive dietary AA. Sample taken after other fish in the study had received AA supplementation for 26 wk. Filament supports (arrowhead) are thin and composed primarily of cartilage; only small areas of bone with cartilage cores are present (closed arrow). Cartilage within the filament supports and their bases (open arrow) contain large, poorly organized chondrocytes and scant amounts of matrix. The connective tissue around the supports is loose and mucinous; H & E 75X.

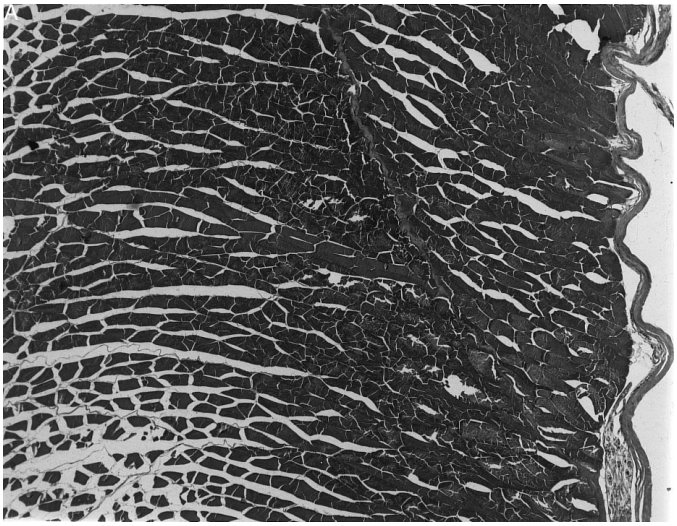
muscle fibers from the vitamin C–deficient fish were small, round, and loosely packed (**Fig. 6B**). Moderate edema of the gastric submucosa was also present in these fish; there were no other prominent histologic differences in the fish from the different groups.

After 18 wk of consuming the experimental diets, fish receiving diets without AA had significantly less collagen in their vertebrae ($P < 0.05$). Vertebral collagen concentrations were 26.23 ± 3.27, 32.38 ± 2.83, 32.69 ± 3.30 and 32.32 ± 3.31 mg/100 mg for fish receiving 0, 25, 75 and 200 mg AA/kg diet, respectively. Hematocrit values were 23.0 ± 5.33, 29.4 ± 4.16, 28.2 ± 5.63 and 28.0 ± 4.00 for fish fed 0, 25, 75 and 200 mg AA/kg diet; these values were not significantly different ($P > 0.05$).

Water quality variables remained in acceptable ranges during the 26-wk experimental period. Dissolved oxygen, ammonia, nitrite and pH values averaged 6.5, 0.05 and 0.03 mg/L, and 7.7, respectively.

DISCUSSION

This study confirmed that AA is an essential nutrient for normal growth and collagen formation for the oscar. When this nutrient was absent from the diet, oscars developed clinical signs of AA deficiency. Studies conducted with other freshwater tropical species of the cichlid family indicate a dietary requirement between 40 and 80 mg AA/kg diet to ensure normal growth and absence of deficiency signs (Chavez de Martinez 1990, Shiau and Jan 1992, Stickney et al. 1984), whereas a dietary concentration as low as 11 mg AA/kg diet is reported to be enough for channel catfish (El Naggar and Lovell 1991a). The minimum dietary level tested in this study that prevented the clinical signs of scurvy was 25 mg AA/kg



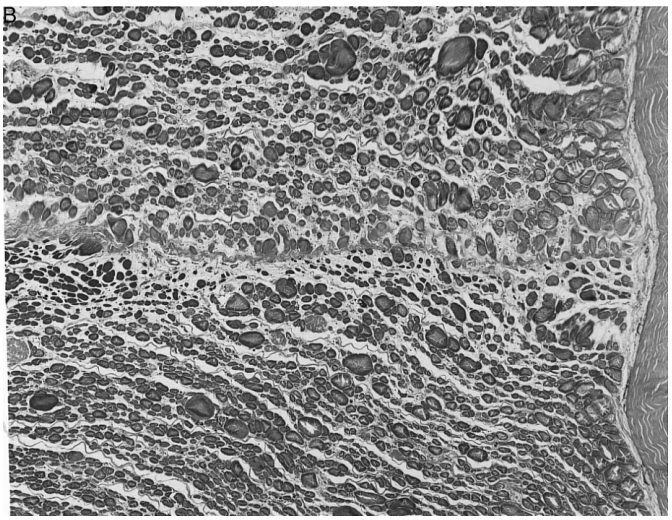


FIGURE 6 Photomicrograph of a histologic preparation from skeletal muscle of an oscar that (*A*) received 200 mg ascorbic acid (AA)/kg diet for 26 wk. Muscle fibers are closely packed and angular (spaces between fibers are artifacts of histologic processing); hematoxylin and eosin (H & E) stain, 48X. (*B*) The fish did not receive dietary AA. Sample taken after 26 wk on trial. Note that most myofibers are small, rounded, and loosely packed; H & E , 48X.

diet. This dietary level resulted in a concentration of 41.45 nmol AA/g of liver. In this study, the other dietary concentrations tested (75 and 200 mg AA/kg diet) did not promote additional weight gain or an increase in collagen formation compared with the dietary level of 25 mg AA/kg diet. Whether or not 25 mg AA/kg diet is the minimum dietary concentration of AA required by the oscar to prevent deficiency signs or reduction in growth is not known because dietary concentrations <25 mg AA/kg diet were not tested.

The average vertebral collagen concentration of 32 mg/100 mg found for oscars fed ≥25 mg AA/kg diet was somewhat higher than the average values of 27 mg/100 mg (Mustin and Lovell 1992) and 23 mg/100 mg (Wilson and Poe 1973) reported for channel catfish fed adequate levels of AA. The reason for this difference is not clear and may result from species differences.

Shiau and Jan (1992) reported that anemia is common in animals with ascorbic acid deficiency because there is a reduction in the absorption and redistribution of iron and consequently a reduction in the synthesis of hemoglobin. Hybrid tilapia without dietary AA have lower hematocrit than fish receiving dietary AA supplementation (Shiau and Jan 1992). This study found that fish without dietary AA supplementation had lower hematocrit than fish receiving dietary AA supplementation but this value was not significantly different ($P > 0.05$) from that in the fish receiving the other dietary treatments.

Oscars without AA supplementation presented typical clinical signs of scurvy as reported for other fish species, such as reduced growth, impaired collagen formation and lordosis. A severe atrophy of the muscle fibers was observed in oscars without AA supplementation as in rainbow trout (Frischknecht et al. 1994). Scoliosis, which is a lateral curvature of the spine, is a clinical sign of AA deficiency in fusiform-shaped fish such as rainbow trout (Halver et al. 1975) and channel catfish (Lim and Lovell 1978). In this study, oscar did not present scoliosis, which could be due to its laterally compressed body shape.

Histologic changes present in the gills of the unsupplemented fish were similar to those reported in other species of fish (Frischknecht et al. 1994, Halver et al. 1969, Lim and Lovell 1978, Martins 1995, Roberts 1989). The lack of bony and cartilaginous matrix in the gill filament supports resulted from interference with proper collagen formation. The loose mucinous connective tissue present in the gills and hypodermis of the skin and fins of the vitamin C–deficient fish was also caused by an inability to form normal collagen. Skeletal muscle lesions in these fish were likely produced by a combination of the loss of collagen formation and abnormalities in the skeleton that prevented normal muscular movements, resulting in myofiber atrophy and/or prevention of normal myofiber development.

Oscars without dietary AA supplementation took 25 wk (11 wk of basal diet in the pretrial depletion period plus 14 wk in the trial) to start presenting clinical AA deficiency signs. This period is long compared with the 6 and 6.4 wk taken for juvenile hybrid tilapia (Shiau and Jan 1992) and a native Mexican cichlid, *C. urophthalmus* (Chavez de Martinez 1990), respectively, to start presenting deficiency signs. Initial weights of the cichlids in the latter studies were only 1.12 and 0.17 g, respectively, vs. 29.2 g for the oscars in this study. Younger fish have a faster growth rate that could explain the difference between the time required for the onset of AA deficiency signs reported in these studies. Halver et al. (1975) states that a 10-fold increase in weight would be required to deplete tissue stores of ascorbate and for external signs of vitamin C deficiency to be evident in fish. Oscars fed a scorbutogenic diet and kept at 28.7°C average water temperature in this study presented external clinical signs of AA deficiency after a 2.8-fold increase in weight.

Dietary ascorbate supplementation equimolar to or greater than 320 mg AA/kg diet is necessary for kidney and liver saturation in juvenile rainbow trout (Matusiewicz et al. 1995). These results derive from feeding trials using graded levels of AAPP and ascorbate intraperitoneal injection experiments. In this study, increments in the dietary ascorbate supplementation up to 200 mg AA/kg diet in the form of AAPP resulted in increments of liver ascorbate concentration. This suggests that, even with the higher AAPP dietary level used in this study, AA tissue saturation may not have been reached in the oscar. However, an AA liver concentration of only 41.45 nmol/g was enough to prevent growth reduction and clinical signs of AA deficiency in the oscar after 26 wk of feeding. El Naggar and Lovell (1991b) reported that AAPP is 1.74 times more effective than AA in maintaining tissue concentrations of ascorbate in channel catfish. Further studies are required to compare the efficacy of alternative dietary sources of vitamin C for oscars.

In conclusion, this study demonstrated the necessity of dietary supplementation of vitamin C for an ornamental fish, the oscar. A dose of 25 mg AA equivalent/kg diet was sufficient to prevent reduction in growth or the development of the clinical signs of scurvy in the oscar. However, the AA dietary requirement may be even lower for this species because dietary concentrations <25 mg AA/kg diet were not tested.

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