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RICHARD T. LOVELL

Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, Alabama 36830

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ABSTRACT Sixteen hundred channel catfish fingerlings were stocked in 1-m³ floating cages, 400 per cage, and fed production-type diets intensively for 180 days. Half of the fish were fed a diet that was nutritionally sufficient except for being low in vitamin C and the remaining fish were fed the same formulation with supplemental vitamin C. At the end of the feeding period each fish was examined for gross signs of vitamin C deficiency and fish were sampled for examination for internal symptoms by X-ray, tissue analysis, and histological techniques. Weight gains (g), feed conversions (g gain/g feed), survival (%) and incidence of physical deformation (%) for fish fed diets with and without supplemental ascorbic acid, respectively, were 531 vs. 331; 1.29 vs. 1.88; 98.0 vs. 77.8; and 3.9 vs. 45.0. Deformities were primarily scoliosis and lordosis with depigmented areas on backs. Vitamin C deficiency resulted in higher mortality from pathogenic bacteria infestation. Differences in response to the two diets did not become apparent until after the fish had grown satisfactorily for at least 12 weeks or weighed 100 to 180 g. Dietary supplementation of vitamin C was reflected in blood, liver and anterior kidney contents of ascorbic acid but not in condition of gill filaments or opercula. J. Nutr. *103:* 134–138, 1973.

INDEXING KEY WORDS channel catfish · vitamin C deficiency · scoliosis · lordosis

Channel catfish from intensive cultures which show disease symptoms without signs of pathogen infestation are brought to the Cooperative Fish Parasite and Disease Laboratory at Auburn University with increasing frequency. Most characteristically these fish have one or more of such anomalies as scoliosis, lordosis, vertical bands of depigmentation, irregular swimming, tetany, poor growth, and mortality. The fish usually weigh 0.1 to 0.25 kg, come from a type of modified culture environment where natural aquatic food is limited, and are intensively fed commercial-type rations. The diseased fish have been fed rations not supplemented with vitamin C. Vitamin C deficiency is suspected as being responsible for the deformities because none of the above symptoms have been observed in catfish grown in artificial cultures at the Auburn University Fisheries Research Unit when diets contained supplemental vitamin C. Kitamura et al. (1) showed that rainbow trout (*Salmo gairdnerii*) and carp (*Cyprinus carpio*) developed spinal curvatures when fed diets low in vitamin C. Poston (2) made similar observations with brook trout (*Salvelinus fontinalis*) after a 34-week feeding period. Halver (3) and Coats and Halver (4) found no abnormal responses by chinook salmon (*Oncorhynchus tshawytscha*) or coho salmon (*O. kisuthch*) fingerlings in short-term (16-week) feeding trials on vitamin C-deficient diets. In 24-week feeding trials, Halver et al. (5) demonstrated acute lordosis and scoliosis in coho salmon and rainbow trout on vitamin C-deficient diets and established tentative dietary requirements for vitamin C for these two species. Dupree (6) was unable to demonstrate a need for dietary vitamin C by channel catfish fingerlings fed purified diets in metal aquariums for 36 weeks. The purpose of the present study was to obtain information

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on the response of channel catfish grown from stocking to harvest size in a culture system with limited natural aquatic food and intensively fed a high performance diet sufficient in all nutrients except vitamin C.

MATERIALS AND METHODS

Sixteen hundred channel catfish fingerlings were randomly assigned in equal numbers to four 1-m³ cages which were suspended in a 2-hectare pond at the Auburn University Fisheries Research Unit. Fish were stocked on March 15 and fed a nutritionally adequate adjustment diet for 31 days. On April 15 the fish in each cage were weighed collectively. The overall average weight per fish was 13.6 g. The fish were fed one of two experimental diets for 180 days. Feed was allotted once daily, 6 days per week, at a rate which began at 4% of biomass and decreased to 1.5% of biomass near the end of the trial. Feed allotments were adjusted biweekly based upon monthly samplings.

One-half of the fish were fed a diet that was formulated to be nutritionally adequate for satisfactory growth of channel catfish in an artificial environment. The remaining fish were fed a diet of similar composition except that it contained no supplemental vitamin C. The diets were designed by the investigator and processed by a feed manufacturing firm as extruded or "nonsinking" pellets. The composition of the experimental diets is shown in table 1. The vitamin supplement was based upon estimated quantitative needs of channel catfish, and has provided satisfactory results in artificial-type catfish cultures at the Auburn University Fisheries Research Unit. Deliberate excesses of vitamins C, thiamin, folic acid, and menadione were used to allow for possible damage by the high processing temperatures. The feed was packaged in plastic-lined bags and stored under refrigeration for the length of the feeding trials.

Epizootics of *Aeromonas liquefaciens* (bacteria) and *Trichophyra* (protozoa) in the early stage of the feeding trial caused 12.2% mortality which was relatively evenly distributed among the four cages. Effective treatments were obtained with applications of formalin and dietary antibiotic. Afterwards the fish were counted and weighed again. Approximately 25% of the fish from each cage were sampled monthly for weight measurement and inspection for anticipated signs of vitamin C deficiency.

TABLE 1

Composition of experimental diet fed with or without supplemental vitamin C to channel catfish in cages for 180 days

Ingredient	Percent
Corn	32.7
Herring meal	21.7
Peanut meal	20.3
Dehulled soybean meal	20.3
Dried corn fermentation solubles	6.7
Dicalcium phosphate	1.0
Vitamin mixture[1]	0.5
Vitamin C	0.088 or 0

[1] Vitamin mixture (mg kg diet): retinyl acetate (325 USP units, mg), 17.0; *dl*-α-tocopheryl acetate, 33.0; cholecalciferol (200 ICU/mg), 22.0; vitamin B_{12}, 0.028; riboflavin, 16.5; choline chloride, 1542.0; niacin, 99.1; pantothenic acid, 60.6; thiamin, 220.0; menadione, 4.4; pyridoxine, 5.5; folic acid, 1.1; biotin, 0.0111; ethoxyquin, 198.3.

All fish were counted, weighed, and examined for gross external signs of vitamin C deficiency at the end of the feeding period. Fish from each treatment were sampled for radiographs [2] and for examination for internal anomalies. Approximately 2 to 5 ml of blood was drawn from the caudal artery of each of six deformed fish and six fish from the control diet for ascorbic acid determination and hematocrit readings. Liver and anterior kidney tissues of each of the fish were analyzed for ascorbic acid. Methods described by Polk (7) and Wedemeyer (8) were used in the tissue analyses. Gill arches from the fish were removed, preserved in 10% formalin and subsequently sectioned and stained with Azan stain for histological examination for possible gill filament distortion.

RESULTS AND DISCUSSION

The feeding trial demonstrated that the cage-grown channel catfish needed a supplemental source of vitamin C in the diet to prevent extensive deformation of spinal columns, poor growth and feed conversion, and increased sensitivity to pathogenic bacterial infestation. Table 2 summarizes the growth response, survival and incidence of deformed fish from diets with and without supplemental vitamin C. Evidently the diet

[2] Screen-film radiographs were made, using standard diagnostic equipment and techniques, in the Radiology Department, School of Veterinary Medicine, Auburn University.

TABLE 2

Weight gain, feed conversion ratio (g feed/g gain), survival and body deformation in caged channel catfish fed diets with or without supplemental vitamin C for 180 days

Supplemental vitamin C	Cage	Avg wt gain	Feed conversion ratio	Survival[1]	Deformed fish[2]
		g		%	%
Yes	1	537.7	1.27	100.0	3.2
	2	524.7	1.31	96.0	4.6
	Avg	531.2	1.29	98.0	3.9
No	3	336.0	1.98	78.2	51.0
	4	326.8	1.80	77.4	39.0
	Avg	331.4	1.88	77.8	45.0

[1] Survival since arrest of parasite infestation in early phase of the experiment. [2] Fish showing one or more of the symptoms of scoliosis, lordosis, or depigmented area on back.

was nutritionally adequate when supplemented with vitamin C, based upon the gain by 13-g fingerlings of 513 g in 180 days with a favorable feed conversion ratio of 1.29. The average gain was 331 g with a conversion ratio of 1.88 without supplemental vitamin C. After the general epizootic in the early phase of the experiment, mortality was only 2% among the fish fed the control diet, but 22% among the fish on the vitamin C-deficient treatment. During the last 73 days of the feeding period eight to 12 dead fish per week were removed from the cages of vitamin C-deficient fish. Cause of the deaths was determined by the Cooperative Fish Parasite and Disease Laboratory to be *Aeromonas liquefaciens*.

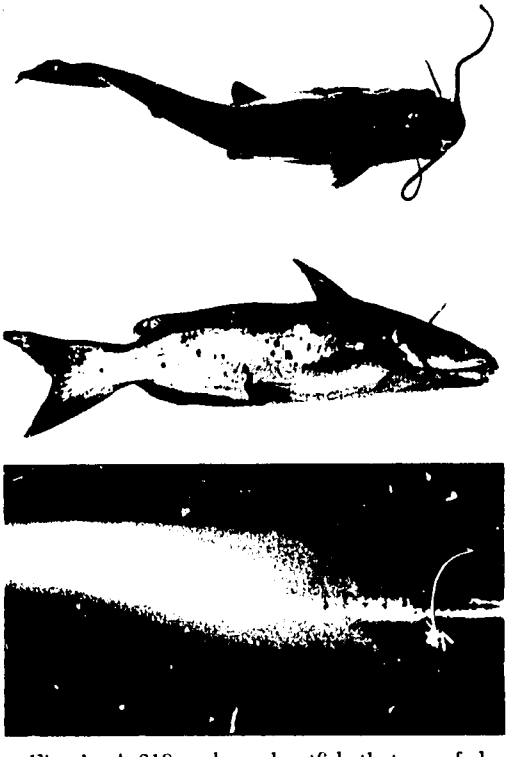

Fig. 1 A 318-g channel catfish that was fed a vitamin C-deficient diet for 180 days: top—fish in relaxed position in water showing moderate scoliosis; middle—note humped back and depigmented, vertical band posterior to the dorsal fin; bottom—radiograph showing hemivertebra.

Monthly sampling indicated that differences in weight or physical conformation between fish fed the two diets were not manifest until after the first 12 weeks of the feeding period, or until the fish weighed 100 to 180 g. All fish fed actively until approximately the last 30 days of the test when the vitamin C-deficient fish accepted feed more slowly.

Deformities were identified visually at the end of the experiment in 45% of the fish fed the vitamin C-deficient diet. Most common was scoliosis, a lateral curvature of the spinal column, usually at approximately the mid-length of the fish. In extreme cases the angle of spinal displacement was as much as 60°. There was usually external swelling near the damaged area, often with a vertical, depigmented band 0.5 to 2.0 cm wide on the back. Many fish showed lordosis, with or without scoliosis which was most commonly characterized by a large hump near and usually immediately posterior to the dorsal fin. Exposure of the vertebral column by excising

the flesh revealed hemorrhagic areas along the spinal column in many vitamin C-deficient fish which appeared normal externally. In severely deformed fish the vertebral column was separated or severely eroded with pronounced atrophy of the spinal cord. Enlarged, spongy vertebrae and hemivertebrae were often found. Figure 1 shows a fish with moderate scoliosis and lordosis, with depigmentation. The radiography shows a half-developed vertebra in the fish. Figure 2 shows radiographs of fish with more pronounced spinal deformations. Size of fish did not seem to be a relevant factor in relation to incidence or severity of deformity. Surface swimming or tetany, which have been observed in practical catfish cultures where vitamin C deficiency was suspect, were not found. Gross examination of internal organs revealed no hemorrhagic areas that could be attributed to diet. Histological examination showed no distortion of gill filaments such as was found in salmon (5). Operecula appeared normally developed.

Table 3 shows mean ascorbic acid levels in blood, liver and anterior kidney of six deformed and of six fish from the control treatment. All values were significantly ($P < 0.05$) higher for the fish fed supplemental vitamin C in the diet, although the difference in liver stores of ascorbic acid between healthy and unhealthy fish was small. Blood concentrations of ascorbic acid in channel catfish from open-pond cultures at the Fisheries Research Unit have ranged from 3 to 10 mg 100 g (unpublished data[3] indicating that ascorbic acid levels in tissues of "normal" catfish probably vary with dietary levels as has been found to occur with salmonids (5). Although the data in table 3 reflect differences in dietary levels of vitamin C, they are not projected to represent tissue levels of ascorbic acid definitive of normal or scorbutic channel catfish.

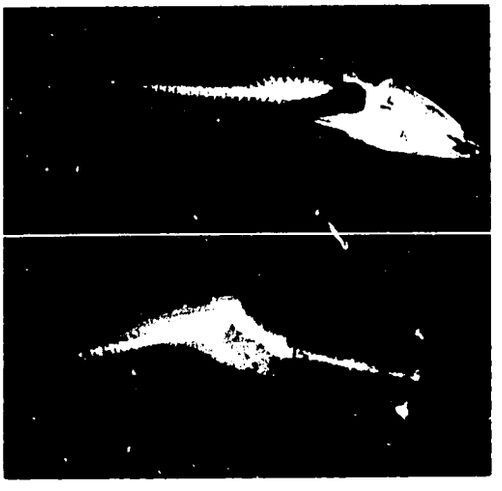

Fig. 2 Radiographs of two channel catfish, weighing approximately 350 g each and fed a vitamin C-deficient diet for 180 days, showing pronounced dorsal–ventral spinal curvature at base of dorsal fin (top) and severe lateral dislocation of vertebrae (bottom)

TABLE 3

Average ascorbic acid concentrations in blood, liver and anterior kidney of channel catfish fed diets with or without supplemental vitamin C in cages for 180 days

Supplemental vitamin C	Tissue concentrations[a]		
	Blood	Liver	Kidney
	mg/100 g		
Yes	4.4 ± 1.9[b]	7.1 ± 2.2[b]	11.0 ± 5.2[b]
No	2.1 ± 1.0[b]	5.0 ± 1.5[b]	7.2 ± 1.1[b]

[a] Averages of six fish from each diet with standard deviations. Values in each column which have the same superscript are not significantly different ($P > 0.05$).

Ikeda and Sato (9) found that carp were able to synthesize vitamin C but not in quantities sufficient to provide for rapid growth. Channel catfish probably synthesize limited quantities of the vitamin which will prevent manifestation of clinical deficiency symptoms when they are not subjected to the stress of fast growth. Dupree's (6) observation that channel catfish fed on a vitamin C-deficient diet grew as well as fish fed a complete diet may be explained on this basis since the fish grew slower on the purified diet in the metal aquariums than fish under more practical conditions.

It was apparent that the absence of supplemental vitamin C from the practical-type diet used in this study was responsible for the poor growth and spinal deformities in the fish. Other factors, independently or through interaction with vitamin C deficiency, may cause similar responses. Halver and Shanks (10) reported scoliosis and

[3] Fisheries Research Annual Report, 1970, vol. 1, part 1, Agricultural Experiment Station, Auburn University, Auburn, Ala.

lordosis in salmon fed tryptophan-free diets. Watanabe et al. (11) found that a dietary vitamin E deficiency caused lordosis in carp. Exposure to high levels of various pesticides has caused development of hemorrhages and vertebral injuries in golden shiners (12), bullheads (13), trout (14), and bluegill (15).

Vitamin C is seldom added to commercial catfish feeds although most of the major feed ingredients, such as oilseed meals, fish and animal byproducts and cereal grains and byproducts, contain insignificant quantities of this vitamin. One reason is that adverse effects attributable to dietary deficiency of vitamin C in catfish have not been demonstrated experimentally. Another is that most commercial catfish culture is in open ponds at low to moderate stocking densities where the presence of natural aquatic food usually prevents serious vitamin deficiencies. Supplementation of vitamin C in catfish diets will be necessary as the industry shifts toward more intensified culture and more artificial environments for growing the fish.

Additional research is needed to provide a more definitive demonstration of the vitamin C deficiency syndrome in channel catfish, subclinical deficiency symptoms, and minimum quantitative dietary requirements of this vitamin. The feeding trials must be conducted under conditions where vitamin C from dietary and aquatic sources is precisely controlled and graded and the fish are growing rapidly.

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