Effects of Dietary Vitamin C on Growth, Tolerance to Aquaculture-Related Stressors, and Antibody Production in Channel Catfish

THESIS

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By

Patricia M. Mazik
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ABSTRACT

The effects of dietary vitamin C on growth, tolerance to aquaculture-related stressors, and antibody production in channel catfish (Ictalurus punctatus) were investigate by feeding selected amounts of vitamin C. Three experimental diets were prepared with either 0 (vitamin C-free), 78 (normal), or 390 (high level) mg of dietary vitamin C per kg of feed. After 120 days, we subjected fingerlings to several stressors including ammonia, net confinement, and low dissolved oxygen. The effects of dietary vitamin C on growth, caudal fin malformation (tail erosion), and the development of antibody titers after immunization were also studied. Channel catfish fed a vitamin C-free diet grew less, had a higher incidence of fin malformation, had a lower tolerance to ammonia, and died of hypoxia at higher dissolved oxygen levels than the fish fed the normal or high level diets. Fish fed the normal and high level vitamin C diets exhibited similar growth, antibody production, and tolerance to stressors. Dietary vitamin C had no observable effect on the tolerance of the fish to stress induced by confinement in a net.

INTRODUCTION

Vitamin C has been established as a dietary requirement for optimum growth, disease resistance, and collagen formation in fishes (Dupree 1966; Lovell 1973; Wilson and Poe 1973; Andrews and Murai 1975). Reports indicate fish suffering from a vitamin C deficiency gain less weight, do not feed as efficiently, and have lower survival than fish receiving adequate concentrations of vitamin C. A high incidence of scoliosis, lordosis, fin malformation, and depigmentation has also been reported (Lovell 1973).

Thirty mg of vitamin C per kg of feed was found sufficient to meet the dietary requirement of channel catfish (Ictalurus punctatus) for normal growth and prevention of deficiency symptoms, but 60 mg/kg was necessary for maximum growth and wound repair (Lim and Lovell 1978). Elevated levels of vitamin C (> 150 mg/kg) may also contribute to the resistance of channel catfish to disease (Durve and Lovell 1982).

During their production and stocking, channel catfish fingerlings are often netted and crowded into holding tanks. They may be exposed to high levels of metabolic wastes such as ammonia, to drastic temperature changes, and to low dissolved oxygen concentrations. If a high level of vitamin C can reduce the effects of aquaculture-related stressors on channel catfish fingerlings, then it may increase

productivity. The object of this study was to determine the effects of dietary vitamin C on the responses of channel catfish to certain aquaculture-related stressors. Specifically, we examined the effects of vitamin C on: 1) tolerance to ammonia toxicity; 2) tolerance to net confinement; and 3) tolerance to low dissolved oxygen. We also studied the effects of dietary vitamin C on growth, caudal fin malformation (tail erosion), and the development of antibody titers.

MATERIALS AND METHODS

Feed used in this study was an experimental catfish diet containing 40% protein. The main ingredients included: menhaden fish meal, 12.5%; soybean meal(49.5% protein), 35%; blood meal, 5%; wheat midds, 24.5%; wheat, 18%; and fish oil, 2.0% (0.5% of the fish oil was withheld to use in application of vitamin C). The remaining ingredients were added as g per kg of feed: U.S. Fish and Wildlife Service Trace Mineral Mix No. 2, 1.0 g; U.S. Fish and Wildlife Service Vitamin Mix No. 30 (U.S. Fish and Wildlife Service Trout Feed Formulation Specification, January 1983, Bulah Fish Technology Center, P.O. Box 245, Bulah, WY 82712); choline chloride (50%), 1.75 g; and calcium carbonate, 23.26 g. We manufactured the feed as a vitamin C-free, dry, sinking pellet; and screened it into five sizes according to projected fish growth. Three experimental diets were prepared and supplemented with 0, 78, or 390 mg/kg vitamin C corresponding to vitamin C-free, normal, or high level diets respectively. The vitamin C was suspended in fish oil and sprayed uniformly on the feed. The vitamin C-free diet was prepared by spraying the feed with only fish oil. The diets were stored at -70 C.

Channel catfish fry of approximately 0.03 g each were obtained from the San Marcos State Fish Hatchery, San Marcos, Texas, transported to the National Fish Hatchery and

Technology Center, San Marcos, Texas, and stocked into nine 52-L fiberglass tanks. The tanks were supplied with well water (temperature, 23 C; pH, 7.9; hardness, 288 mg/L; alkalinity, 252 mg/L; dissolved oxygen > 6.5 mg/L) at a flow rate of 1.0 L per minute (>1.0 turnovers/hour). The fry were randomized into three replicates of each feed level and placed on either the vitamin C-free, normal or high level diet. After 28 days, 400 fish from each tank were randomized and stocked into each of nine 1200-L circular fiberglass tanks supplied with the same well water at a flow rate of 15.1 L per minute (1.5 turnovers/hour). Dissolved oxygen and temperature were measured daily, with pH, hardness, and alkalinity measured weekly. Fish were fed to satiation four times per day in the 52-L tanks, then twice per day after being stocked into the 1200-L tanks. A bacterial disease which occurred eight weeks into the study was treated with a ten day terramyacin treatment (2.4 g terramyacin/ten day ration) mixed in the feed. Previous studies (Durve and Lovell 1982) indicated that 90 days was sufficient for the development of vitamin C deficiency symptoms. Since our water temperature (23 C) was cooler than the optimum temperature for channel catfish (28 - 30 C), the present study was extended to 120 days.

After 120 days, 100 fish from each of the nine tanks were transported to the facilities at Southwest Texas State University, San Marcos, Texas for the stress and antigen

challenges. Fish were transported in 60-L aerated containers, 100 fish per container (< 1.0 g fish/L water). Transport time was approximately 20 minutes. Test water quality used in all experiments except the ammonia challenges was similar to culture water quality.

Ammonia toxicity tests were conducted in glass aquaria with a water volume of 15-L. The aquaria were placed in a 23 C water bath to maintain constant temperature. Test water was aerated to maintain dissolved oxygen levels near saturation. Ten fish were placed into each aquarium 24 hours prior to ammonia exposure. Water quality was measured in selected aquaria every 24 hours (Table 1). Twenty-four, 48, 72, and 96-hour median lethal concentrations (LC50) were determined according to Thompson (1947). The ammonia concentration, as ammonium chloride, was increased from aquarium to aquarium using a geometric progression factor of two in a series of four aquaria per replicate (from 8 - 64 mg/L NH_3-N). One aquarium for each feed level served as a control. Dead fish were removed every 24 hours and weighed to the nearest 0.01 g. An LC50 was determined for each of the nine experimental tanks.

Tolerance to net confinement was determined by placing 60 fish from each of the nine tanks in a shallow net arranged in a manner which caused the fish to be in continuous contact with the net and each other. Test water was constantly aerated to keep dissolved oxygen levels near

Table 1. Water quality characteristics in test aquaria during ammonia toxicity tests (range, mean, or mean ± SE). Numbers of tanks sampled are given in parentheses.

Character	24 h	48 h	72 h	96 h
pH[a]	7.9 - 8.2 (24)	8.0 - 8.1 (18)	8.0 - 8.2 (19)	8.0 - 8.1 (18)
D.O. (mg/L)[b]	7.6 ± 0.0 (28)	7.8 ± 0.1 (28)	7.7 ± 0.0 (22)	7.7 ± 0.0 (25)
Temperature (C)[b]	23 C (28)	23 C (28)	23 C (22)	23 C (25)
Alkalinity (mg/L)[c]	157.4 ± 6.1 (10)	153.3 ± 5.5 (10)	149.6 ± 6.5 (10)	139.2 ± 5.4 (10)
Hardness (mg/L)[c]	206.3 ± 5.5 (10)	188.6 ± 4.6 (10)	182.4 ± 7.2 (10)	175.8 ± 5.2 (10)
Ammonia (% Nominal)[d]	104.4 ± 1.9 (24)	105.6 ± 1.8 (17)	103.5 ± 2.2 (20)	106.2 ± 1.7 (18)

a. pH meter
b. dissolved oxygen/temperature meter
c. Hach Chemical Company (1973)
d. direct nesslerization (Standard Methods 1980)

saturation. The fish were confined in the net for ten days.

After the ten days of net confinement, five fish from each net were subjected to low dissolved oxygen tests. Fish were placed in sealed jars (one fish/jar) containing 100 mL of saturated well water (>8.0 mg/L dissolved oxygen). The initial temperature, dissolved oxygen, and time were recorded. Jars were placed in a 23 C water bath to maintain a constant temperature. Upon death of the fish, elapsed time, residual oxygen, and individual fish weight were recorded.

The percentage of fish with fin malformations was determined by observing the caudal fins of 55 fish from each of the nine tanks at day 120. Fin malformation was assessed based on descriptions by Lim and Lovell (1978).

After 270 days, disease resistance was determined by injecting the normal and high level diet fish with a 0.5 mL mixture of mineral oil and the antigen of Brucella abortus. Fish used in this study were raised to approximately 70 g on the experimental diets, then converting to a Glencoe pellet diet (Glencoe Mills, Glencoe, MN) after 210 days. Because the fish fed the vitamin C-free diet did not achieve sufficient size, they were excluded from this study. The fish raised on the normal diet were fed only the Glencoe pellet (reported by the manufacturer to contain 70 mg/kg vitamin C), while the fish raised on the high level diet were fed the Glencoe diet with additional vitamin C sprayed

on with fish oil. The additional vitamin C was calculated to increase the vitamin C level in the feed to 390 mg/kg. The normal diet received only fish oil. The fish were fed their respective diets throughout the 28 day study. Ten fish from both treatment groups were each immunized twice, seven days apart. Fourteen and 28 days after the initial injection, the fish were bled with a syringe from the hemal arch, the blood allowed to clot, and the serum removed for analysis. Each fish was bled only once. Antibody titers were determined according to the instructions of the manufacturer of the antigen and positive control serum (Cooper Diagnostics, Round Lake, Illinois).

One- and two-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls multiple-range test, and linear regression (Woolf 1968) were used to test treatment effects for significance of difference where appropriate. An $\alpha = 0.05$ was established as the level for significance in all tests.

RESULTS AND DISCUSSION

The fish treated with the normal and high level diets grew significantly better (one-way ANOVA) than the vitamin C-free treatment group during the 120 day study (Figure 1). Andrews and Murai (1975) and Lovell (1973) also have shown fish fed vitamin C-free diets fed less and gained less weight.

The normal and high level treatment group fish subjected to ammonia had similar LC50 values (two-way ANOVA) (Figure 2). Fish which received the vitamin C-free treatment had significantly lower LC50 values than the other two treatments. The 24-hour LC50 values were similar to those reported by Tomasso et al. (1980a).

No mortalities occurred during the ten day net confinement experiment. Experiments done on largemouth bass (Micropterus salmoides) have reported significantly elevated corticosteroid levels and mortalilty after 48 hours of confinement (Carmichael 1984a and Carmichael et al. 1984b). Experiments on hybrid striped bass (Morone chrysops x Morone saxatilis) reported significantly elevated cortcosteroid levels after just ten minutes and mortality after 48 hours of net confinement (Tomasso et al. 1980b). The results indicate channel catfish are able to tolerate net confinement for longer time periods in comparison to largemouth or hybrid striped bass.

Figure 1. Weights (mean ± SE) of channel catfish raised for 120 days on either 0, 78, or 390 mg/kg dietary vitamin C. Treatment groups with statistically similar means share a common line at the top of the figure. Numbers of fish weighed are given in or above columns (one-way ANOVA).

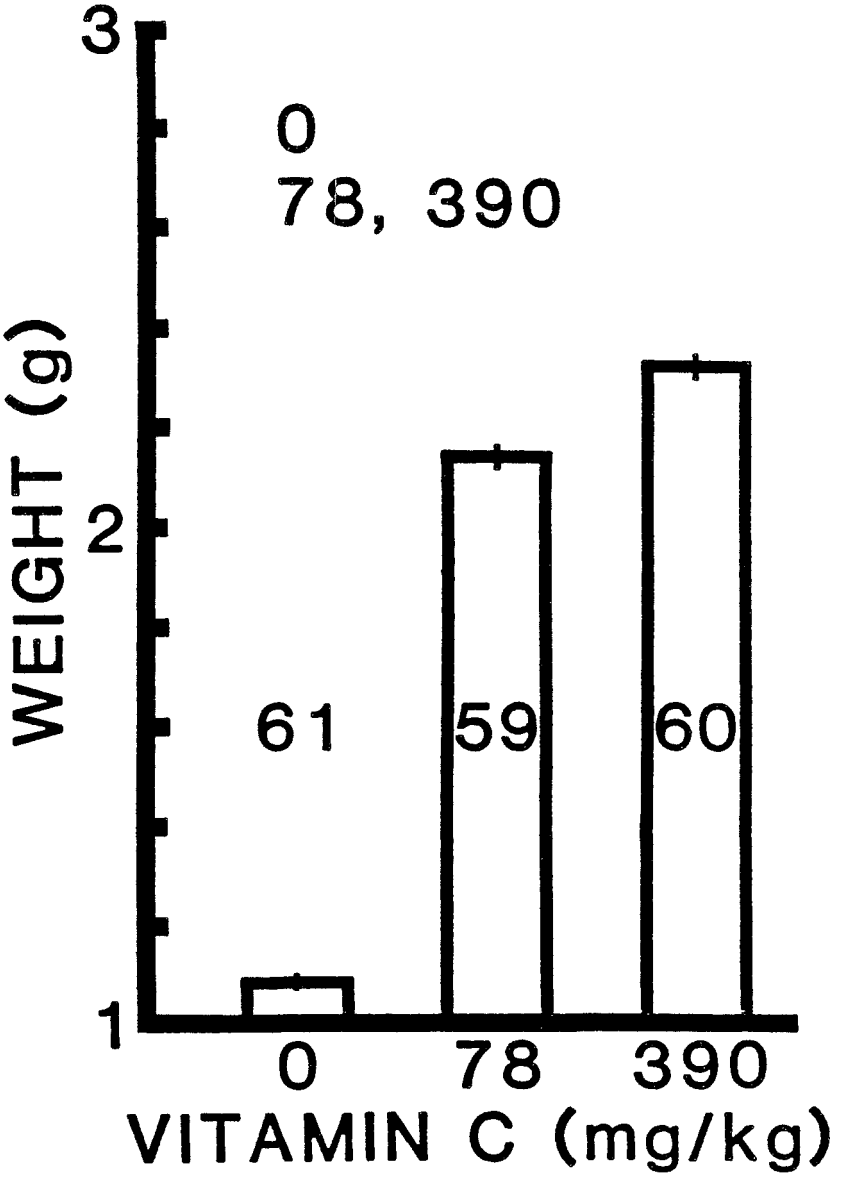
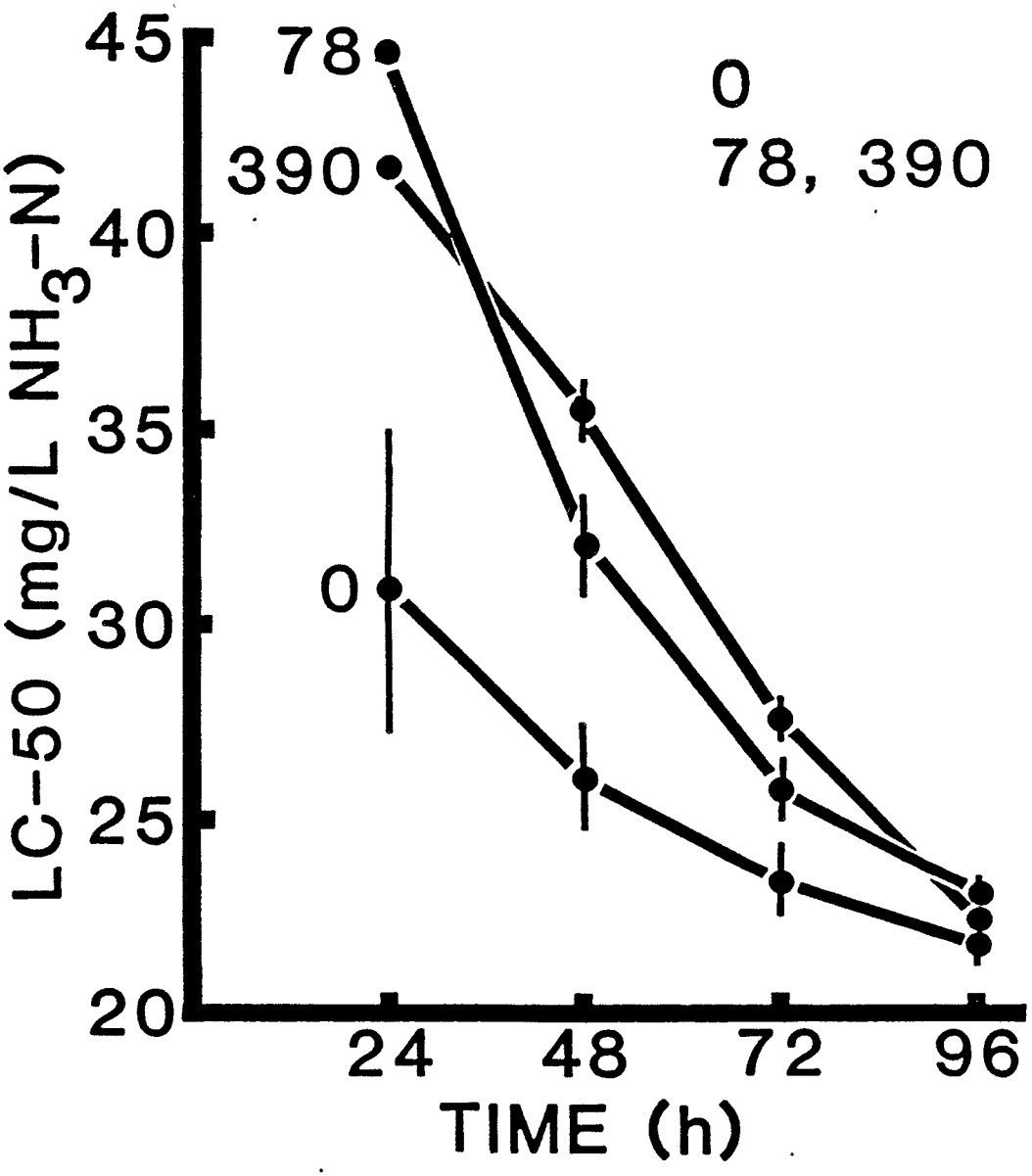

3
2
1
WEIGHT (g)
0
78, 390
61
59
60
0
78
390
VITAMIN C (mg/kg)

Figure 2. Median lethal concentrations (LC50) of ammonia to channel catfish fed 0, 78, or 390 mg/kg dietary vitamin C (mean ± SE). Treatment groups with statistically similar means share a common line at the top of the figure (two-way ANOVA).


45
40
35
30
25
20
LC-50 (mg/L NH_3-N)
78
390
0
0
78, 390
24
48
72
96
TIME (h)

Low dissolved oxygen tests showed the fish fed the vitamin C-free diet died at significantly higher (one-way ANOVA) residual oxygen levels than the fish fed the normal and high level diets (Figure 3). There were no significant differences between the normal and high level vitamin C groups. Moss and Scott (1961) suggested fish size (weight) in channel catfish did not affect the critical dissolved oxygen level (residual oxygen level). Regression analyses of fish weight and residual oxygen conducted within feed level groups indicated differences among the residual oxygen levels of the three treatment groups were the result of the vitamin C concentrations and not weight.

Tail malformations were more common in the vitamin C-free treatment group than in the normal or high level groups (Figure 4). Similar results have been reported by Lim and Lovell (1978). Vitamin C is a coenzyme required for the conversion of proline to hydroxyproline, a constituent of collagen. As collagen is necessary in fin formation, the absence of vitamin C will inhibit the above reaction, perhaps causing the fin malformation (Mehrle and Mayer 1975a; Mehrle and Mayer 1975b; Lim and Lovell 1978; Mauck et al. 1978).

All fish tested had undectable antibody titers to Brucella antigen prior to immunization. Antibody titers developed in response to immunization, but there were no significant differences (two-way ANOVA) between the normal

Figure 3. Residual oxygen levels for channel catfish fed 0, 78, or 390 mg/kg dietary vitamin C (mean $\pm$ SE). Treatment groups with statistically similar means share a common line at the top of the figure. Number of fish observed are given in columns (one-way ANOVA).

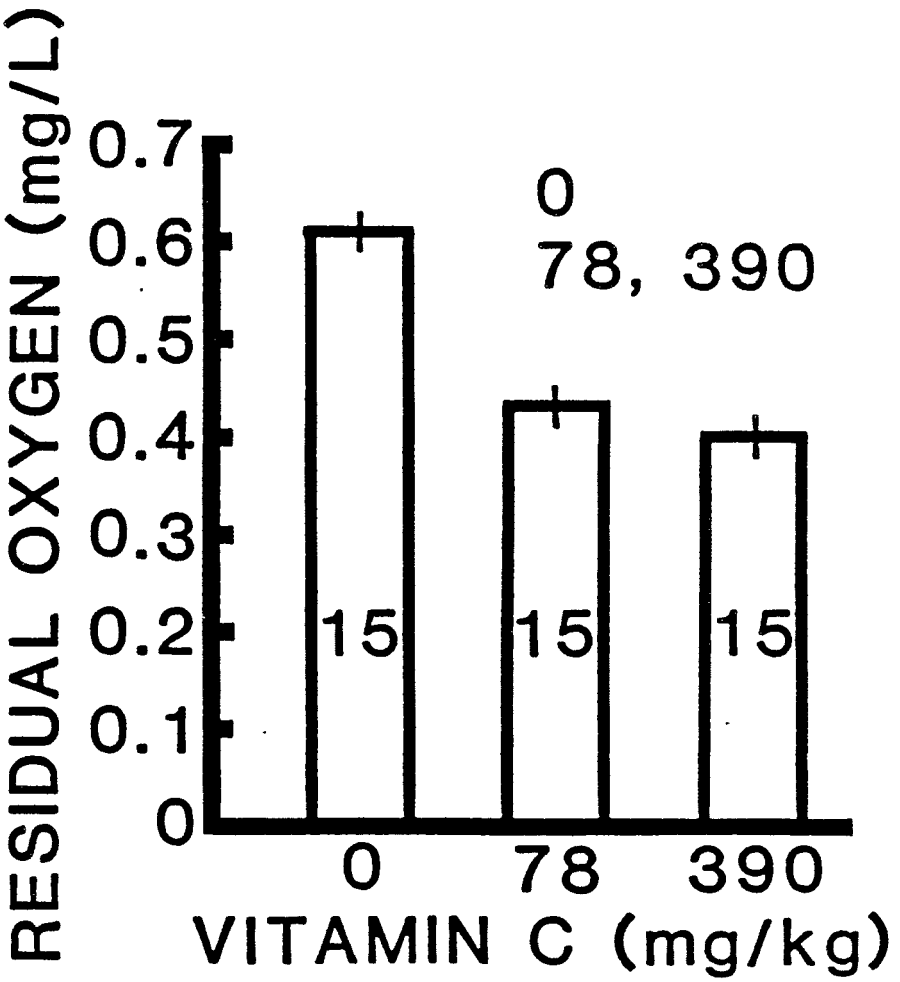
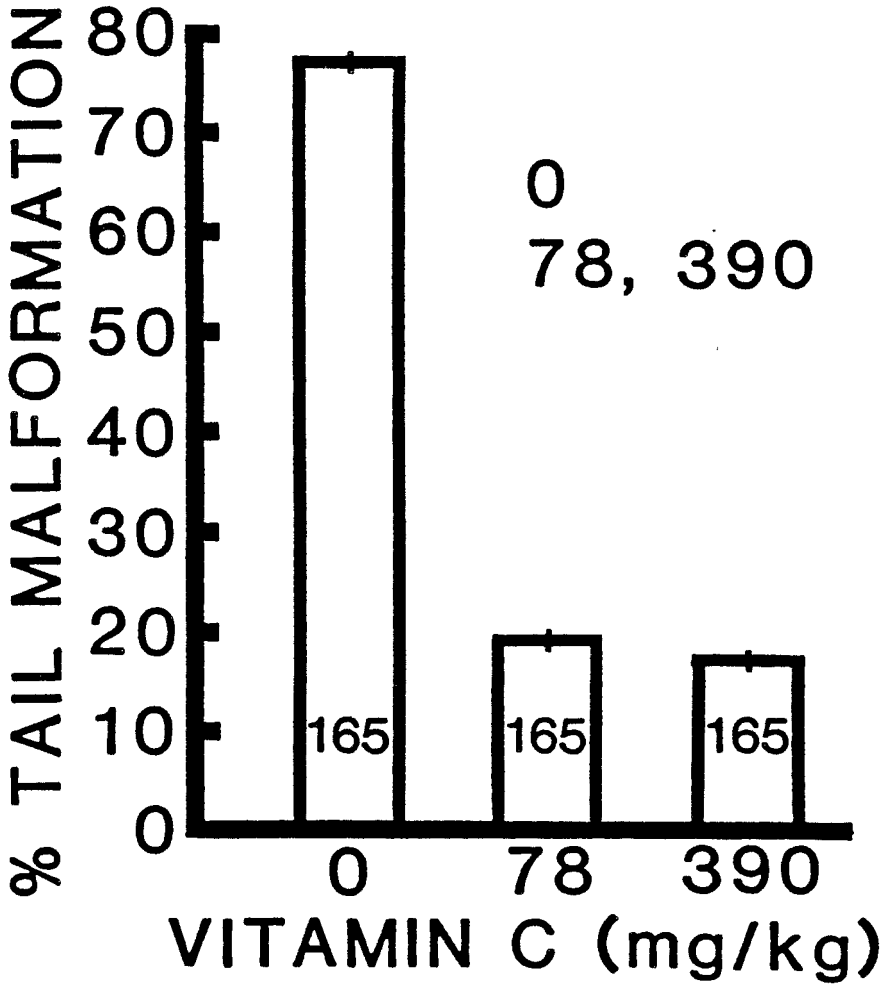

RESIDUAL OXYGEN (mg/L)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
78, 390
15
15
15
0
78
390
VITAMIN C (mg/kg)

Figure 4. Percent tail malformation of channel catfish fed 0, 78, or 390 mg/kg dietary vitamin C (mean ± SE). Treatment groups with statistically similar means share a common line at the top of the figure (one-way ANOVA).


% TAIL MALFORMATION
80
70
60
50
40
30
20
10
0
165
165
165
0
78, 390
0
78
390
VITAMIN C (mg/kg)

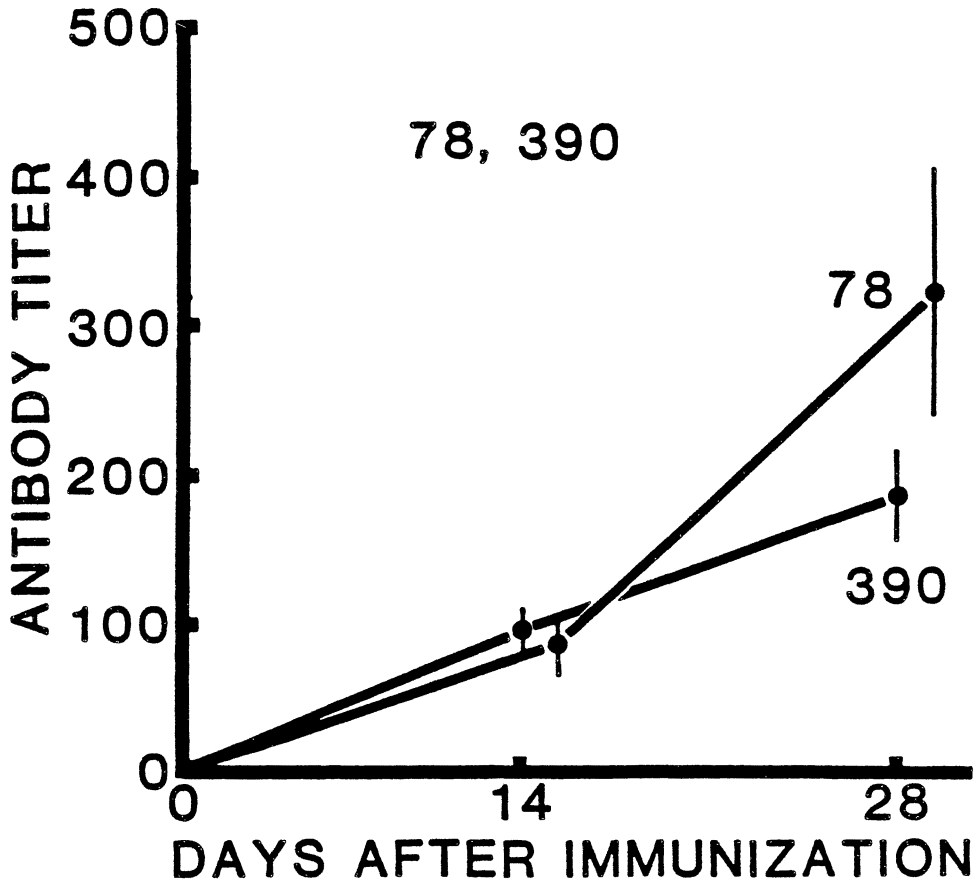
and high level vitamin C treatments (Figure 5).

Percent survival was affected by a bacterial disease eight weeks into the study. Survival was 68%, 82%, and 87% before the disease and 57%, 57%, and 48% at the end of the study for the vitamin C-free, normal, and high level diets respectively. Accurate conclusions regarding treatment effects on disease resistance cannot be determined from this type of data without a substantially larger sample size.

Experiments were also conducted to determine the effects of feeding high dietary levels of vitamin C prior to the induction of stress. After 120 days, the fish remaining in the three feed level groups were all placed on the high level diet. At 130 and 140 days, 100 fish from each replicate were transported to the lab and subjected to the above stressors. General trends indicated feeding the high level diets for short periods of time did not increase the tolerance of the vitamin C-free or normal treatment groups to stressors. However, the inconsistant results of these experiments preclude reaching any conclusions about the value of the short-term feeding of high dietary levels of vitamin C.

The results of these experiments indicate the fish fed normal and high level vitamin C diets grew better and were able to tolerate ammonia and low dissolved oxygen significantly better than the fish fed the vitamin C-free diet. Fish fed the normal and high level vitamin C diets

Figure 5. Antibody levels in response to the antigen of Brucella abortus in channel catfish fed 0, 78, or 390 mg/kg dietary vitamin C (mean ± SE). Treatment groups with statistically similar means share a common line at the top of the figure (two-way ANOVA).


500
400
300
200
100
0
ANTIBODY TITER
78, 390
78
390
0
14
28
DAYS AFTER IMMUNIZATION

also had a lower percent of tail malformation. Concentrations of dietary vitamin C of up to five times the normal level, however, did not increase growth rates, tolerance to ammonia and dissolved oxygen, or antibody production. Low dietary levels of vitamin C and feeding high dietary levels prior to stress were not found to be advantageous in the tolerance of aquaculture-related stressors in this study.

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