Dietary Ascorbic Acid Requirement of Juvenile Tilapia *Oreochromis niloticus* × *O. aureus*.

Shi-Yen Shiau^{*1,2} and Fun-Lin Jan^{*1}

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A 14-week growth study was conducted to estimate the optimal ascorbic acid requirement in diets for hybrid juvenile tilapia *Oreochromis niloticus* × *O. aureus*. Purified diets with seven levels (0, 40, 60, 80, 100, 125, 150, and 200 mg/kg diet) of supplemental L-ascorbic acid were pelleted and fed to 1.12 ± 0.11 g tilapia. Results indicated that fish fed diets lacking or low (40 mg/kg) supplementary ascorbic acid had poor growth, feed conversion ratio (FCR), and protein efficiency ratio (PER); low ascorbic acid contents in the liver and body, also low haematocrit values in the blood. Deficiency of ascorbic acid resulted in a mortality of 37.78%. Weight gain analyzed by broken line regression indicated that the dietary ascorbic acid requirement for maximal growth in tilapia is 79 mg/kg.

Ascorbic acid (vitamin C) has important physiological functions in both plants and animals.¹⁾ An ascorbic acid requirement by fish was first demonstrated by Kitamura *et al.*,²⁾ which showed that vitamin C was required by both carp *Cyprinus carpio* and rainbow trout *Oncorhynchus mykiss*. Other researchers have demonstrated that ascorbic acid is needed by brook trout *Salvelinus fontinalis*,³⁾ coho salmon *Oncorhynchus kisutch*,⁴⁾ channel catfish *Ictalurus punctatus*,⁵⁻⁷⁾ and Indian major carp *Cirrhina mrigala*.⁸⁾

While it has been shown that tilapia *O. niloticus* required dietary ascorbic acid for growth,^{9,10)} and about 50 mg/kg of ascorbic acid was reported to be required by young tilapia *O. aureus*,¹¹⁾ the quantitative ascorbic acid requirements for tilapia species have not received much attention from researchers. The purpose of this study was to estimate the optimum vitamin C requirements of juvenile hybrid tilapia *Oreochromis niloticus* × *O. aureus*.

Materials and Methods

Experimental Diets and Feeding Regime

A basal diet, ascorbic acid free, was formulated (Table 1) to contain 35% protein and 11% lipid. L-Ascorbic acid (Sigma Co., St. Louis, MO, U.S.A.) was incorporated into the basal diet at level of 0, 40, 60, 80, 100, 125, 150, and 200 mg per kg of dry diet. Diet preparation has been described

previously.¹²⁾ Each diet was sealed in plastic bags and stored at -20°C until fed.

The fish were fed 5% body weight per day (dry food/whole fish), divided into two equal feedings (08.00h and 17.00h). The amount was close to the maximum daily ration for tilapia estimated from feed consumption during the adjustment period of the study. Each group of fish was weighed every other week and the amount of diet fed was adjusted accordingly. The fish were fed the test diets for a 14-week period.

Animals and Experimental Rearing System

Male tilapia were supplied by the Far East Propagation Co., Cha-Yi, Taiwan. After arrival, they were acclimated to laboratory condition for 4 weeks in a plastic tank (74W × 95L × 45H cm) supplied with aerated recirculated water. At the beginning of the experiment, 24 aquaria (30.5 × 61.0 × 33.5 cm, 60 l) were each stocked with 14 fish with an average weight of 1.12 ± 0.11 g. Each experimental diet was fed to fish in each of three aquaria. Each aquarium was part of a closed recirculating water system which had a common reservoir of water maintained at 26 ± 1°C. Water was circulated at 2 l/min through two biofilters to remove impurities and reduce ammonia levels. Dissolved oxygen levels were at least 6 ppm and pH was 6.6-6.8 throughout experimental period. Light was kept on a 12-h light/dark cycle (07.00 to 19.00 h).

*1 Department of Marine Food Science, National Taiwan Ocean University, Keelung, Taiwan 202, ROC.
(蕭 錫延, 詹 芳霖).

*2 To whom reprint requests should be addressed.

Table 1. Composition of experimental diets

(%)

Ingredients	Vitamin C content (mg/kg)							
	0	40	60	80	100	125	150	200
Vitamin-free casein* ¹	41.02	41.02	41.02	41.02	41.02	41.02	41.02	41.02
Corn starch	35	35	35	35	35	35	35	35
Cod liver oil	4	4	4	4	4	4	4	4
Corn oil	7	7	7	7	7	7	7	7
CMC* ²	2	2	2	2	2	2	2	2
Mineral mix.* ³	4	4	4	4	4	4	4	4
Ascorbic acid-free* ⁴ vitamin mix.	2	2	2	2	2	2	2	2
Ascorbic acid (mg/kg)	0	40	60	80	100	125	150	200
Alpha-cellulose	4.98	4.98	4.98	4.98	4.98	4.98	4.98	4.98
ME (kcal/100 g)* ⁵	373	373	373	373	373	373	373	373
Analyzed ascorbic acid (mg)	—	37.2	58.1	76.8	97.8	120.3	141.5	183.6

*¹ Protein content: 85.32%.*² CMC: carboxymethyl cellulose.*³ Salt No. 2 + mineral premix No. 5.³⁰⁾

Salt No. 2: Calcium biphosphate 13.58 g; calcium lactate 32.70 g; ferric citrate 2.97 g; magnesium sulfate 13.70 g; potassium phosphate (dibasic) 23.98 g; sodium biphosphate 8.72 g; sodium chloride 4.35 g.

Mineral premix No. 5: Salt mixture No. 2 100 g; AlCl₃·6H₂O 0.015 g;KI 0.015 g; CuCl 0.01 g; MnSO₄·H₂O 0.08 g;CoCl₂·6H₂O 0.1 g; ZnSO₄·7H₂O 0.3 g.*⁴ Vitamin mixture: *p*-Aminobenzoic acid 5 mg; biotin 0.6 mg; inositol 200 mg; Ca-pantothenate 10 mg; pyridoxine-HCl 4 mg; riboflavin 5 mg; nicotinic acid 20 mg; thiamine-HCl 5 mg; menadione 4 mg; vitamin A acetate 200 IU; Alpha-tocopherol 40 mg; cyanocobalamin 0.01 mg; folic acid 1.5 mg; choline chloride 400 mg.*⁵ ME: Metabolizable energy.Protein (4.5 kcal/g) as reported for trout.³¹⁾Carbohydrate (3.49 kcal/g) as reported for carp.³²⁾Lipid (8.51 kcal/g).³³⁾

Experimental Procedure

Each tank of fish was collectively weighed in water without anaesthesia. During weighing, mortality and the condition of the fish were recorded. Treatment effect was evaluated by determining percent weight gain, feed conversion ratio (FCR), and protein efficiency ratio (PER). These parameters were calculated as follows:

$$\text{weight gain (\%)} = \frac{\text{Wt (final)} - \text{Wt (initial)}}{\text{Wt (initial)}} \times 100$$

$$\text{FCR} = \frac{\text{Feed intake (g)}}{\text{Wt (final)} - \text{Wt (initial)} (\text{g})}$$

$$\text{PER} = \frac{\text{Wt (final)} - \text{Wt (initial)} (\text{g})}{\text{Protein intake (g)}}$$

Blood was collected using a heparinized syringe from the caudal vein of experimental fish for haematocrit determination.¹³⁾ The ascorbic acid content of the experimental diets were measured and the total ascorbate concentrations in liver and the whole body without liver were measured for six fish per treatment at the termination of the experiment.¹⁴⁾ The ascorbic acid requirement was determined by using broken line analysis

method.¹⁵⁾

Statistical Analysis

All data were analyzed for statistical significance using analysis of variance (SPSS/PC program). Multiple comparisons among means were made with the Duncan New Multiple Range Test.¹⁶⁾ Statistical significance was determined by setting the aggregate type I error at 5% ($P < 0.05$) for each set of comparisons.

Results

The percent weight gain, feed conversion ratio (FCR), protein efficiency ratio (PER), and mortality rate of tilapia fed diets with various levels of ascorbic acid are shown in Table 2. The weight gains were lowest for fish fed no ascorbic acid, intermediate when the supplementation level was 40 mg/kg and highest when the supplementation were 60 or above mg/kg.

PER and FCR values generally followed the weight gain data. The mortality rate of fish fed diets supplemented with any of the ascorbic

Table 2. Weight gain, feed conversion ratio (FCR), protein efficiency ratio (PER), and mortality of fingerling tilapia fed diets containing various levels of ascorbic acid for 14 weeks*

Vitamin C level (mg/kg diet)	Weight gain (% of initial)	FCR (g feed/g gain)	PER	Mortality (%)
0	1239 ± 35 ^c	1.44 ± 0.18 ^c	2.00 ± 0.10 ^c	37.78 ± 12.57 ^a
40	1362 ± 56 ^b	1.22 ± 0.06 ^b	2.34 ± 0.08 ^b	20.00 ± 5.45 ^b
60	1484 ± 21 ^a	1.05 ± 0.02 ^a	2.72 ± 0.07 ^a	11.34 ± 3.33 ^{b,c}
80	1541 ± 39 ^a	1.01 ± 0.02 ^a	2.83 ± 0.07 ^a	6.67 ± 0.00 ^c
100	1553 ± 64 ^a	1.03 ± 0.03 ^a	2.81 ± 0.09 ^a	20.22 ± 3.14 ^b
125	1551 ± 54 ^a	1.00 ± 0.04 ^a	2.83 ± 0.10 ^a	20.00 ± 5.45 ^b
150	1573 ± 60 ^a	0.95 ± 0.02 ^a	2.86 ± 0.05 ^a	11.11 ± 6.28 ^{b,c}
200	1557 ± 58 ^a	0.99 ± 0.01 ^a	2.81 ± 0.08 ^a	13.33 ± 5.44 ^{b,c}

* Figures in each column having the same superscript are not significant different ($p < 0.05$).

Table 3. Haematocrit value, liver and carcass ascorbic acid content of fingerling tilapia fed various levels of ascorbic acid for 14 weeks*

Vitamin C level (mg/kg diet)	Haematocrit (%)	liver ascorbic acid (ng/g)	carcass ascorbic acid (ng/g)
0	24.88 ± 0.71 ^b	14.21 ± 2.86 ^c	10.83 ± 1.41 ^c
40	25.44 ± 0.32 ^b	21.06 ± 0.81 ^b	16.38 ± 1.73 ^b
60	28.24 ± 1.35 ^a	31.13 ± 0.96 ^a	22.79 ± 1.22 ^a
80	28.43 ± 0.91 ^a	34.11 ± 2.61 ^a	25.09 ± 0.90 ^a
100	29.17 ± 0.47 ^a	33.11 ± 3.43 ^a	24.83 ± 1.57 ^a
125	28.24 ± 0.67 ^a	34.36 ± 1.62 ^a	24.38 ± 1.82 ^a
150	28.52 ± 0.40 ^a	39.14 ± 9.06 ^a	26.84 ± 4.30 ^a
200	29.12 ± 0.88 ^a	40.03 ± 8.12 ^a	30.40 ± 4.13 ^a

* Same as Table 2.

acid level were lower than those fed the control unsupplemented with ascorbic acid diet ($P < 0.05$).

The haematocrit value, liver ascorbic acid content, and body ascorbic acid content in fish fed diets with various levels of ascorbic acid supplementation are given in Table 3. The haematocrit values were low in fish fed the ascorbic acid-free diet and the diet supplemented with 40 mg/kg ascorbic acid, and increased in fish fed diets supplemented with 60 and above mg/kg ascorbic acid.

Both the liver and the body ascorbic acid content in fish were lowest in fish fed the ascorbic acid-free diet, intermediate in fish fed the diet supplemented with 40 mg/kg ascorbic acid and highest in fish fed diets supplemented with 60 or more mg/kg ascorbic acid.

Fish fed the ascorbic acid-free diet exhibited a range of external signs of ascorbic acid deficiency first appearing after 6 weeks. The changes include loss of pigmentation followed by lesions of the skin with loss of scales and necrosis of the fins haemorrhages all over the body. At the termination of the experiment the mortality rate of fish fed the ascorbic acid-free diet was 37.78%.

Broken line analysis was used for estimating the dietary ascorbic acid requirement for maximal growth.¹³⁾ The regression equations used in the estimating requirements were $Y = 12.90 + 0.0993 X$ and $Y = 15.40$. Since the breakpoint at 79 mg/kg gave the least mean square error, this value is estimated to be the tilapia fingerling's requirement for maximal growth.

Discussion

Ascorbic acid plays an important role in certain aspects of protein metabolism¹⁷⁾ and Ram¹⁸⁾ suggested that ascorbic acid has a specific effect on growth. This may explain the reduction in the growth performance and the protein efficiency ratio for tilapia fed a diet devoid of ascorbic acid in the present study. Similar results have been reported for channel catfish,^{19,20)} rainbow trout,²¹⁾ and coho salmon.⁴⁾

Mortality rate was reported around 98% by Soliman *et al.*¹⁰⁾ in tilapia *O. mossambicus* fed a diet without supplemental ascorbic acid in 8 weeks. It has been suggested that the dietary requirement of ascorbic acid by channel catfish,²²⁾

rainbow trout,^{21,23)} and common carp²⁴⁾ decreases with an increase in fish size. The initial body weight of fish of the present study was around 1 g compared to 6.3 mg of the Soliman's study. Different fish size may explain the differences of the mortality rate in fish fed vitamin C-free diet of two studies. Different size may also explain the differences of ascorbic acid requirement. Kesamaru *et al.*^{*} suggested that tilapia *O. niloticus* require a 200–300 mg/kg diet of ascorbic acid. The initial weight of the fish in their study was 0.05 g.

Anemia is common in animals with ascorbic acid deficiency. It is caused by reduced absorption and redistribution of iron and synthesis of haem,²⁵⁾ iron uptake in the intestine and redistribution of iron between the different body pools in fish are facilitated by ascorbic acid. The gradual lowering of haematocrit values found in the present study (Table 3) indicates that scorbutic tilapia progressively develop an anemic condition. A deficiency of ascorbic acid has also been observed to effect a reduction in haematocrit values in catfish,^{7,10)} trout,²³⁾ and snakehead fish.²⁶⁾

In the present study, after the feeding trial, 14.21 ng/g of vitamin C was obtained in the liver in tilapia fed the vitamin C-free diet (Table 3). This figure was higher than that reported by Soliman *et al.*⁹⁾ with tilapia *O. niloticus* in which 6.12 ng/g in liver was obtained. Hilton *et al.*²⁷⁾ and Albrektsen *et al.*²⁸⁾ suggested that the liver concentration of the vitamin is a good index of the ascorbic acid status of rainbow trout. In other words, the liver vitamin concentration provide good index of the status of the need of the fish. The reason for the difference of the liver vitamin C content of the two study is not clear. The extend to which of this difference of vitamin C content in liver would reflect the vitamin C requirement of tilapia is not known since the quantitative requirement was not measured in the Soliman's study.

In the present study, L-ascorbic acid was used as the source of vitamin. Under our experimental conditions with juvenile tilapia weighing around 1 g, the vitamin C requirement was around 79 mg. Soliman *et al.*⁹⁾ suggested that glyceride-coated ascorbic acid may be preferred for use in fish feed. Further study is needed to quantify the ascorbic acid requirements for tilapia, using other forms of ascorbic acid.

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