JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 33, No. 1
March, 2002

Enhancement of Stress Resistance of the Guppy *Poecilia reticulata* through Feeding with Vitamin C Supplement

LIAN CHUAN LIM

Freshwater Fisheries Centre, Agri-food & Veterinary Authority of Singapore, Sembawang Research Station, Lorong Chencharu, Singapore 769194 Republic of Singapore

PHILIPPE DHERT[1]

Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Rozier 44, Gent, Belgium

WEI YEN CHEW

Freshwater Fisheries Centre, Agri-food & Veterinary Authority of Singapore, Sembawang Research Station, Lorong Chencharu, Singapore 769194 Republic of Singapore

VEERLE DERMAUX

Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Rozier 44, Gent, Belgium

HANS NELIS

Laboratory of Pharmaceutical Microbiology, Harelbekestraat 72, B-9000 Ghent, Belgium

PATRICK SORGELOOS

Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Rozier 44, Gent, Belgium

Abstract

This study investigated the use of vitamin C supplement in formulated diets and live *Artemia* juveniles to enhance the stress resistance of the guppy *Poecilia reticulata*. To evaluate the stress resistance, fish were subjected to osmotic shock in pre-aerated water containing 35 ppt sodium chloride. Ascorbyl acid-polyphosphate and ascorbyl palmitate were used as vitamin C sources for formulated diets and live *Artemia* juveniles, respectively. Results showed that guppies fed moist formulated diets supplemented with ascorbic acid at 1,000 mg/kg or 2,000 mg/kg diet displayed significantly higher levels of stress resistance than fish fed control formulated diet for 13 d. The stress resistance of fish fed a lower dose (200 mg/kg diet), however, did not differ significantly from that of the control. On the other hand, the stress resistance of guppies fed *Artemia* juveniles bio-encapsulated with 10% or 20% ascorbyl palmitate did not show significant difference from that of fish fed control *Artemia* after 20 d. The stress resistance of these three groups of *Artemia*-fed fish, however, was significantly higher than that of fish fed the control formulated diet. Biochemical analyses showed that raising the ascorbic acid level in feeds resulted in a concomitant increase in the incorporated ascorbic acid level in the whole-body tissue of the guppy. The increased stress resistance of the guppy fed a vitamin C supplemented diet has also been demonstrated in four commercial farms. The potential application of the improved stress resistance in the ornamental fish industry was discussed.

Singapore exports 30% of the world's ornamental fish, making it the global leader in the ornamental fish trade. Fish are transported live by air to some 70 countries throughout the world. In the ornamental fish export business, freight costs range from 30% to more than 100% of the fish cost (Lim and Chua 1993). In order to cut down the costs, ornamental fish have to be packed at very high densities. They undergo stress due to handling procedures (e.g., selection, sorting, etc.) prior to packing, loading and unloading processes, crowding, and adverse water quality conditions during transportation. The transport time also adds a degree of stress to the live ornamental fish, and it can take up to 48 h from time of packing to unpacking of the fish. Fish that have low stress resistance cannot withstand the stresses encountered during the transportation process, leading to relatively high post-shipment mortality within a week of arrival. This low resistance to stress is due to sub-optimal physiological conditions arising from infectious diseases, nutritional deficiencies, poor water quality, etc.

Vitamin C (or ascorbic acid, AA) is well known as a major water-soluble antioxidant, and it has been identified as a potent immuno-modulator suitable for use in aquaculture (Uma et al. 1996). It has also been found to be an effective antidote of copper intoxication in *Tilapia zillii* (Ghazaly 1994). While the AA requirement for normal growth and survival might be quite low (10–20 ppm) (Sandnes et al. 1992), a higher level is required to improve the stress resistance and the immunological response of fry and juvenile stages (Merchie et al. 1993). This was illustrated by Merchie et al. (1996) who demonstrated that, in a challenge test with *Vibrio anguillarum,* turbot larvae fed vitamin C-enriched *Artemia* nauplii at 1,500 ppm or 2,500 ppm displayed improved stress resistance and survival. Young fish fed diets supplemented with AA displayed improved biological conditions such as increases in growth rate and survival rate and decrease in deformities in the skeletal structure. Physiological conditions such as resistance to toxicants, stress resistance, and immune system functions were also enhanced with the improved nutrition (Merchie et al. 1993).

In this study, the effect of vitamin C supplementation in formulated moist diets and in *Artemia* juveniles on the stress resistance of the guppy *Poecilia reticulata* was investigated, with the objective of enhancing the condition of the fish prior to transport.

[1] Present address: INVE Technologies NV, Oeverstraat 7, B-9200, Baasrode, Belgium.

Materials and Methods

Experimental Fish

Market-sized, assorted, male guppies of about 3.5 mo old, with a mean body weight of 0.34 g, were obtained from a commercial farm in Singapore. Upon arrival, the fish were first subjected to a prophylactic treatment in four 150-L tanks (0.9 m × 0.45 m × 0.45 m depth) to get rid of any parasites and bacteria carried by the fish (Loo et al. 1998). The treatment consisted of a 24-h exposure to 10-ppm chloramphenicol and 2-ppt coarse salt, followed by a 1-h bath in 0.1-ppm malachite green, 50-ppm formalin (37% industrial grade) and 2-ppt coarse salt. All the fish were starved during the prophylactic period. After treatment, the fish were allowed to acclimatize in the tanks for another day before they were distributed randomly into a series of 60-L tanks (0.60 m × 0.30 m × 0.35 m depth), each consisting of 45 L of water, at 50 fish/tank for the experiments.

Feed Preparation

Ascorbyl polyphosphate (ApP, ascorbic-acid-2-phosphate containing 35% active ingredient of AA) was used as the vitamin C source of formulated diets. Vitamin C-boosted formulated diets were prepared daily, based on the diet used commonly in local guppy farms in Singapore (Table 1). The AA levels in the formulated diets were 200 mg/kg, 1,000 mg/kg, and 2,000 mg/kg, respectively, in the first experiment and 2,000 mg/kg in the second experiment.

For enrichment of *Artemia* juveniles, ascorbyl palmitate (AP) was used as the vitamin C source because of its stable and lipophilic characteristics, which allowed its incorporation into the booster emulsion. AP administered through live feeds has been tested successfully as a dietary vitamin C source for the European seabass *Dicentrarchus labrax* and African catfish *Clarias gariepinus* (Merchie et al. 1995b). The *Ar-*

TABLE 1. *Ingredient composition (%) of formulated diet.*

Ingredient	Control FD	FD 200	FD 1000	FD 2000
Spirulina algae	0.21	0.21	0.21	0.21
Fish meal	31.7	31.7	31.6	31.5
Wheat flour	21.1	21.1	21.1	21.0
Wheat bran	42.2	42.2	42.1	42.0
Starch	4.76	4.76	4.76	4.76
Ascorbic-acid-2-phosphate	0	0.057	0.286	0.571

temia juveniles (10-d old, 5 mm in length) used in this study were obtained from an intensive tank culture using the technique described by Lim et al. (2001). Bio-encapsulation of vitamin C in *Artemia* juveniles was performed following the technique described by Merchie et al. (1995a) for *Artemia* nauplii and rotifers. *Artemia* juveniles were bio-encapsulated with two dosages of AP at 10% and 20% respectively for 14 h before feeding to the guppy.

Feeding Experiments

Two experiments, both run in four replicates for each treatment, were conducted. The first experiment was designed to study the effect of dietary AA in the formulated diets on the stress resistance of guppy. Three groups of fish were fed formulated moist diets supplemented with AA at 200 mg/kg, 1,000 mg/kg, and 2,000 mg/kg, respectively; one group was fed the control diet (without AA supplementation). A second experiment was designed to study whether feeding the fish with *Artemia* juveniles would help to improve the stress resistance of the guppy. Five groups of fish were tested; three groups were fed *Artemia* juveniles (without AP supplement and with AP supplements at 10% and 20%, respectively) and two groups were fed formulated moist diets (with no AA supplement and with 2,000 mg/kg AA supplement, respectively). Aeration was provided through two air tubes per tank, one joined to a box filter and another to an airstone. The experimental periods were 13 d for the first experiment and 20 d for the second experiment. During these periods, the fish were fed three times per day, at 0930, 1200 and 1500 h. The daily food ratio corresponded to 6.3 g of formulated diet or an equivalent of 1,500 *Artemia* juveniles per tank. The tanks were siphoned once a day after the 1500 h feeding, and about 20% of the water in each tank was renewed daily. The water quality parameters, including temperature, pH, dissolved oxygen, and free ammonia, were monitored on day 7, 13 and 20.

Field Application

Field observations were conducted in four ornamental fish farms to study the possible application of vitamin C supplement to enhance the stress resistance of guppy in the commercial production of the fish. All the fish used in the same observation were originated from the same mass spawning batch and raised in net cages located in the same pond. There were two treatment groups in each farm, with three replicate net cages (5 m × 5 m × 2 m depth) in each group, and 1,000 market-sized male guppy (mean body weight 0.32–0.35 g) in each net cage. The fish in one set were fed formulated diet boosted with coated AA (Takeda Coated Ascorbic Acid F-90, Takeda Chemical Industries, Osaka/Tokyo, Japan) at 2,000 mg/kg diet, while the control group was fed control diet. The fish were sampled from each net cage for stress test after 7 (or 10) and 14 d to compare the stress resistance of the two groups of fish.

Stress Test

A stress test was used to evaluate the stress resistance of the guppy, following the method described by Lim et al. (2000). The

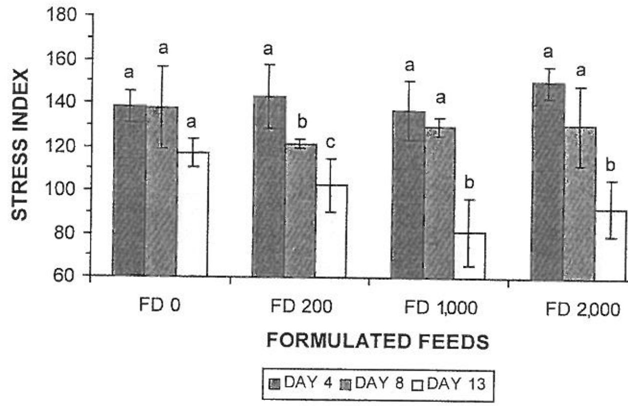

FIGURE 1. *Variation of stress indices of guppy fed formulated diets (FD) boosted with different AA levels (Control FD: 0 mg/kg; FD 200: 200 mg/kg; FD 1,000: 1,000 mg/kg and FD 2,000: 2,000 mg/kg) during the 13-d feeding period. Value represents mean of four replicates and its standard error. Any bars marked with different alphabet letters within the same diet indicate significant difference between means* ($P \leq 0.05$).

dietary treatments were evaluated using the stress tests on days 4, 8 and 13 in the first experiment, days 4, 15 and 20 in the second experiment, and days 7 (or 10) and 14 in the field observation. During the test, ten fish were randomly sampled from each tank and were subjected to osmotic shock in a 500-mL beaker containing 500 mL of 35-ppt saline solution, made up of pre-aerated culture water and coarse salt. The cumulative mortality was recorded at 3-min intervals over a 2-h period. The stress resistance of the fish was expressed as stress index, which was calculated as the average value of four replicates, obtained after the additions of all the 40 cumulative mortality readings during the observation period. A high stress index would indicate low stress resistance of the fish and *vice versa.*

Vitamin C Analyses

To verify whether AA could be incorporated into the guppy after feeding with vitamin C supplement, biochemical analyses for AA were carried out on *Artemia* juvenile samples and fish samples collected at the end of the second feeding experiment. The analyses were determined by the procedure described by Nelis et al. (1997), and the results are expressed in μg AA/g wet weight.

Statistical Analysis

Statistical evaluation was performed using software package Statistica, version 5.0 (StatSoft, Inc., Tulsa, Oklahoma, USA). Experimental data, expressed as means of stress indices and AA contents, were subjected to one-way analysis of variance (ANOVA). Differences among means were determined by Duncan's multiple range test (Hays 1988). Significance was accepted for $P \leq 0.05$.

Results

In both the first and second experiments, the water quality parameters, including temperature (range 26.0–27.5 C), pH (6.5–6.8), dissolved oxygen (7.5–7.9 mg/L) and free ammonia (< 0.02 mg/L), were all found within the optimal levels for the guppy, with no significant differences among the aquariums.

First Feeding Experiment

At the early stage of the experiment, the stress indices were more than 137 in all cases (Fig. 1). There were general improve-

TABLE 2. *Stress indices of guppy fed formulated diets (FD) with different amounts of vitamin C. Figures in parentheses after the mean values indicate the SD. Means in each row followed by different letters are significantly different (P ≤ 0.05).*

	Type of diets and concentration of vitamin C			
Days	Control	FD 200 (200 mg/kg)	FD 1,000 (1,000 mg/kg)	FD 2,000 (2,000 mg/kg)
4	138.0 a (7.2)	143.3 a (14.7)	137.3 a (13.3)	150.5 a (7.3)
8	137.8 a (18.7)	121.8 a (2.2)	129.8 a (4.3)	130.8 a (18.3)
13	117.3 a (6.4)	102.8 ab (12.4)	81.5 b (15.7)	92.8 b (13.0)

ments in the stress resistance of all the three groups of guppy fed vitamin C-boosted diets from day 4 to day 8, although only the difference in the group fed formulated diet with 200 mg/kg AA supplement was statistically significant (Fig. 1). With further feeding of the vitamin C supplements, the stress indices of these three groups of fish were reduced to less than 103 on day 13, which were significantly lower than the values recorded on day 8. In the control group, there was no significant improvement in the stress resistance throughout the experimental period, even though the mean stress index recorded on day 13 was lower than that on day 8.

On both days 4 and 8, there were no significant differences in the stress resistance among the four groups of fish fed formulated diets supplemented with different levels of AA (Table 2). By day 13, the positive effects of vitamin C supplementation on stress resistance became prominent. The mean stress indices of the two groups of guppy fed AA-boosted diets at 1,000 mg/kg and 2,000 mg/kg respectively were significantly lower than that of the control group. Guppies fed the lower dose of 200

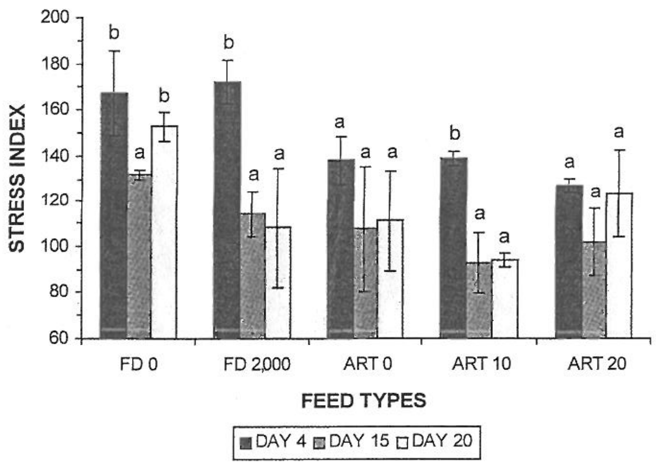

FIGURE 2. *Variation of stress indices of five groups of guppy fed formulated diets (FD) or* Artemia *juveniles (ART) boosted with different AA levels (Control FD: 0 mg/kg; FD 2,000: 2,000 mg/kg; ART 0: Control; ART 10: 10% AP; ART 20: 20% AP) during the 20-d feeding period. Value represents mean of four replicates and its standard error. Any bars marked with different alphabet letters within the same diet indicate significant difference between means (P ≤ 0.05).*

TABLE 3. *Stress indices of guppy fed formulated diets and* Artemia *juveniles, supplemented with different amounts of vitamin C. Figures in parentheses after mean values indicate the SD. Means in each row followed by different letters are significantly different (P ≤ 0.05).*

	Type of diets[1] and vitamin C concentration				
Days	FD Control	FD 2,000 (2,000 mg/kg)	ART Control	ART 10 (10% AP)	ART 20 (20% AP)
4	167.3 a (18.5)	172.3 a (9.5)	137.8 b (10.6)	138.5 b (3.1)	126.7 b (2.9)
15	132.0 a (2.0)	114.0 ab (10.1)	107.7 ab (27.5)	92.8 b (13.1)	102.0 b (14.7)
20	152.7 a (6.0)	108.3 b (26.1)	111.3 b (22.1)	94.0 b (2.8)	123.3 b (19.3)

[1] FD: Formulated diet; ART: *Artemia* juveniles; AP: Ascorbyl palmitate.

mg/kg, however, did not differ significantly from the other treatment groups, including the control group.

Second Feeding Experiment

As observed in the first experiment, the stress resistance of all the five groups of fish was quite low on day 4, as revealed by the relatively high stress indices ranging from 127–172 (Fig. 2). On day 15, there was a general improvement in the stress resistance of all the five groups of fish, and the differences were statistically significant in the two groups fed formulated diets and the group fed *Artemia* juveniles with 10% AP supplement. From day 15 to day 20, there was no significant change in the stress resistance of all the fish groups, except for the control formulated diet group where the stress resistance was significantly lower than at day 15.

On day 4 and day 15, there was no significant difference between the stress resistance of the two groups of guppy fed formulated diets, with or without AA supplement (Table 3). By day 20, however, fish fed vitamin C-boosted diet at 2,000 mg/kg displayed a significantly higher level of stress resistance than the control group. On the other hand, there was no significant difference in the stress resistance among the three groups of *Artemia*-fed guppy throughout the experiment (Table 3). All the three groups of *Artemia*-fed fish, with or without vitamin C supplement, showed significantly higher stress resistance than the group fed control formulated diet.

Whole-body Vitamin C Content in Guppy

Results of biochemical analyses showed that the whole-body AA content of the guppy sampled at the end of the second feeding experiment was linked to dietary ApP and AP supplies in formulated diets and live *Artemia* juveniles, respectively. Raising the vitamin C content in the feeds resulted in a concomitant increase in the incorporated AA level in the guppy (Table 4). A significant 3-fold increase from 15 μg/g to 46 μg/g AA was recorded in the fish receiving 2,000 mg/kg supplement. For the three groups of fish fed *Artemia* juveniles, the

TABLE 4. *Vitamin C contents (μg/g wet weight) in diets and in whole-body tissue of the guppy fed the respective diets after 20 d. Figures in parentheses after mean values indicate the SD. Means in each row followed by different letters are significantly different (P ≤ 0.05).*

	Type of diets[1] and vitamin C concentration				
Type of samples	Control FD	FD 2,000 (2,000 mg/kg)	ART Control	ART 10 (10% AP)	ART 20 (20% AP)
Diet	0[2]	2,000[2]	0a (0)	10.9 b (5.5)	19.9 b (7.5)
Fish	15.0 a (10.8)	45.9 b,c (3.6)	39.5 b (6.8)	65.9 c,d (5.1)	77.8 d (14.0)

[1] FD: Formulated diet; ART: *Artemia* juveniles; AP: Ascorbyl palmitate.
[2] AA content based on amount of ascorbyl polyphosphate incorporated in the formulated diets.

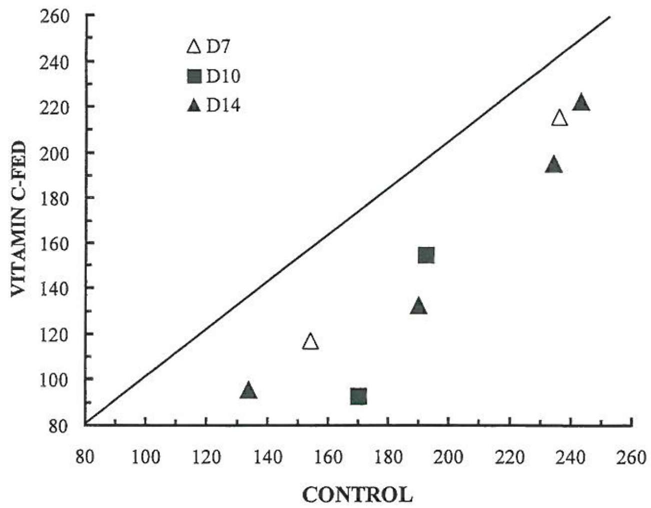

FIGURE 3. *Stress index of guppy fed with vitamin C supplement (at 2,000 mg/kg, for 7–14 d) as a function of that of control fish. Median line is plotted to show the relationship of the stress indices of the two groups of fish.*

mean AA content varied between 40 μg/g and 78 μg/g, according to the dietary AP supply. As the *Artemia* juveniles used in the experiment were fed rice bran and de-fat soybeans during culture (Lim et al. 2001), the AA content in the control *Artemia* juveniles was undetectable. As a result, the mean AA content in the group fed control *Artemia* juveniles was significantly lower than in fish fed *Artemia* juveniles boosted with vitamin C at 10% or 20% AP.

Field Application

The stress resistance data obtained from field observations in four commercial farms were plotted in Fig. 3, to show the relationship between the stress index of vitamin C-fed fish and the control fish. The results showed that all the coordinates fell below the median line, indicating that in all cases, guppy fed vitamin C supplement at 2,000 mg/kg displayed a higher level of stress resistance than the corresponding control fish.

Discussion

Our study has shown that the salinity stress test could be an effective tool for evaluation of the stress resistance of the market-sized guppy. The test was effective in distinguishing the stress resistance levels of fish fed different quality diets. In fact, fish fed AA-boosted formulated diet at 1,000 mg/kg or 2,000 mg/kg were found significantly more resilient to osmotic shock than the control fish after 13 d (Table 2). These results demonstrated that the stress resistance of the guppy could be enhanced through nutritional prophylaxis, by supplementation of vitamin C in the formulated diet. In the same experiment, guppies fed a lower dose of 200 mg/kg showed no significant difference in the stress resistance from the control group. These results suggested that under the present experimental conditions, and with the moist formulated diets used, the concentration of 200 mg/kg AA was insufficient to significantly improve the stress resistance of the fish. In all the treatment groups, the lowest stress resistance was consistently recorded on day 4 (Fig. 1). The lower stress resistance could be attributed to the handling stress arising from distribution of fish into individual

tanks at the beginning of the experiment. On both days 4 and 8, there were no significant differences in the AA levels among the four groups of fish fed formulated diets, with or without vitamin C supplement. The stress induced by prophylactic treatment could have masked the effects of vitamin C at this early stage.

The AA level in the whole-body tissue of guppy reflected the level of ApP in the formulated diet, as shown by the significantly higher incorporated AA level recorded in the fish group fed vitamin C-boosted diet than in the group fed control formulated diet (Table 4). Similarly, the incorporated AA levels in the two fish groups fed *Artemia* juveniles bio-encapsulated with vitamin C at 10% or 20% AP were also found higher than that fed control *Artemia* juveniles. These results indicate that vitamin C could be effectively incorporated into the tissues of the guppy, through feeding with either boosted formulated diet or bio-encapsulated live *Artemia* juveniles.

In contrast to the results obtained in the fish groups fed formulated diets, guppies fed *Artemia* juveniles bio-encapsulated with 10% or 20% AP for 20 d did not show any significant difference in stress resistance from those fed control *Artemia* juveniles (Table 3), despite the fact that their incorporated AA level was higher than the control *Artemia*-fed fish (Table 4). The stress resistance of all the three groups of *Artemia*-fed fish, with or without vitamin C supplement, was however significantly higher than that of the group fed control formulated diet. These results were in agreement to the results of AA analyses, which showed a significantly lower AA level in the control formulated diet group. It is noteworthy that the fish group fed control *Artemia* juveniles (40 μg/g) displayed a 2.5 times higher AA content than that fed control formulated diet (15 μg/g), and at about the same level as that fed formulated diet with 2,000 mg/kg AA supplement (46 μg/g) (Table 4). These results suggest that the *Artemia* juvenile without vitamin C enrichment would provide sufficient AA to maintain a high level of stress resistance in the guppy. The adequate level of the whole-body tissue AA required to enhance the stress resistance of the guppy was likely to be that detected in fish fed formulated diet boosted with 2,000 mg/kg AA or control *Artemia* juveniles, that is, in the range of 40–46 μg/g (Table 4). There was no significant difference in the whole-body tissue AA between the two groups of guppies fed *Artemia* juveniles boosted with 10% AA and 20% AA respectively.

Our field observations conducted in commercial farms demonstrated that in the commercial production of guppy, feeding the fish with vitamin C supplement would effectively enhance their stress resistance. Guppy cultured in net cages, after being fed formulated diet boosted with 2,000 mg/kg AA for 7–14 d, consistently displayed a higher level of stress resistance than the corresponding control fish (Fig. 3). The improved stress resistance through feeding with vitamin C supplement is likely to have potential application to the ornamental fish industry. The industry is export-oriented and almost all the fish are transported by air. Fish are constantly subjected to stress prior to and during transportation. Fish that have low stress resistance would not be able to withstand the stresses arising from the transport operation. The application of nutritional prophylaxis using vitamin C to enhance the stress resistance of the fish could be a solution to improve the situation. Vitamin C is widely considered to have beneficial effects on disease and stress resistance in both salmonids and channel catfish (Wedemeyer 1997). Liu et al. (1989) reported that channel catfish fed diets containing vitamin C at 1,000 mg/kg for 9 wk showed increased resistance to *Edwardsiella ictaluri*. Mazik et al. (1987) found that the same fish species fed diets containing up to 390 mg vitamin C/kg for 120 d exhibited a higher tolerance to ammonia than fish fed a vitamin C-free diet, but the vitamin C feeding has no observable effect on the tolerance to stress induced by confinement in net. On the other hand, Thompson et al. (1993) studied the effect of stress on the immune response of Atlantic salmon and concluded that vitamin C did not appear to ameliorate stress-induced immunosuppression in the fish. Our subsequent study using the guppy demonstrated that feeding a vitamin C supplement at 2,000 mg/kg diet for 10 d prior to shipment significantly reduced the cumulative mortality of the fish from 23% to 8% at 7-d post-shipment (Lim et al. 2000).

In conclusion, the stress resistance of the guppy could be enhanced through feeding the fish either with formulated diets fortified with AA at 1,000 mg/kg or 2,000 mg/kg or with *Artemia* juveniles, even without vitamin C enrichment. Further experiments should be conducted to apply these findings to enhance the disease and stress resistance so as to improve the post-shipment survival of the fish.

Acknowledgments

This study was funded by the Agri-food & Veterinary Authority of Singapore (Project No. FFC/E7/99).

Literature Cited

Ghazaly, K. S. 1994. Efficacy of ascorbic acid (vitamin C) on experimental copper intoxication in Tilapia zillii. Bulletin of the National Institute of Oceanography and Fisheries (Egypt) 20(2):249–257.

Hays, W. L. 1988. Statistics, fourth edition. CBS College Publishing, New York, USA.

Lim, L. C. and L. H. Chua. 1993. Transportation of ornamental fish for export—the Singapore experience. Page 1–24 *in* AQUARAMA Conference Proceedings 1993. Singapore, 24–26 June 1993. Expoconsults, Singapore.

Lim, L. C., A. Soh, P. Dhert, and P. Sorgeloos. 2001. Production and application of on-grown *Artemia* in freshwater ornamental fish farm. Aquaculture Economics and Management 5(3/4):211–228.

Lim, L. C., C. C. Wong, C. H. Koh, P. Dhert, and P. Sorgeloos. 2000. A stress resistance test for quality evaluation of Guppy (*Poecilia reticulata*) (abstract). Page 4–5 *in* Abstract book of AVA Technical Seminar. Singapore, 1 September 2000. Agri-food and Veterinary Authority of Singapore, Singapore.

Liu, P. R., J. A. Plumb, M. Guerin, and R. T. Lovell. 1989. Effect of megalevels of dietary vitamin C on the immune response of channel catfish *Ictalurus punctatus* in ponds. Diseases of Aquatic Organisms 7(3):191–194.

Loo, J. J., K. H. Ling, and L. C. Lim. 1998. Development of an improved treatment protocol for quality enhancement of Guppy prior to export. Singapore Journal of Primary Industry 26:15–20.

Mazik, P. M., T. M. Brandt, and J. R. Tomasso. 1987. Effects of dietary vitamin C on growth, caudal fin development, and tolerance of aquaculture-related stressors in channel catfish. Progressive Fish Culturists 49(1):13–16.

Merchie, G., P. Lavens, P. Dhert, M. Dehasque, H. Nelis, A. De Leenheer, and P. Sorgeloos. 1995a. Variation of ascorbic acid content in different live food organisms. Aquaculture 134:325–337.

Merchie, G., P. Lavens, P. Dhert, M. G. U. Gomez, H. Nelis, A. De Leenheer, and P. Sorgeloos. 1996. Dietary ascorbic acid requirements during the hatchery production of turbot larvae. Journal of Fish Biology 49(4):573–583.

Merchie, G., P. Lavens, P. Dhert, R. Pector, A. F. Mai Soni, H. Nelis, F. Ollevier, A. De Leenheer, and P. Sorgeloos. 1995b. Live food mediated vitamin C transfer to *Dicentrarchus labrax* and *Clarias gariepinus*. Journal of Applied Ichthyology 11:336–341.

Merchie, G., P. Lavens, H. Nelis, A. De Leenheer, and P. Sorgeloos. 1993. Effect of vitamin C incorporation in live food on the larviculture success of aquaculture species. Mededelingen van de Faculteit Landbouwwetenschappen van de Rijksuniversiteit Gent 58(4b):1999–2008.

Nelis, H., G. Merchie, P. Lavens, P. Sorgeloos, and A. De Leenheer. 1997. Liquid chromatographic determination of vitamin C in aquatic organisms. Journal of Chromatographic Science 35:337–341.

Sandnes, K., O. Torrissen, and R. Waagbø. 1992. The minimum dietary requirement of vitamin C in Atlantic salmon (*Salmo salar*) fry using Ca ascorbate-2-monophosphate as dietary source. Fish Physiology and Biochemistry 10:315–319.

Thompson, I., A. White, T. C. Fletcher, D. F. Houlihan, and C. J. Secombes. 1993. The effect of stress on the immune response of Atlantic salmon (*Salmo salar* L.). Aquaculture 114(1–2):1–18.

Uma, A., M. J. P. Jeyaseelan, and V. Sundararaj. 1996. Immunomodulators in aquaculture. Seafood Export Journal 27(6):23–26.

Wedemeyer, G. 1997. Effects of rearing conditions on the health and physiological quality of fish in intensive culture. Pages 35–71 *in* G. K. Iwama, A. D. Pickering, J. P. Sumpter, and C. B. Schreck, editors. Fish stress and health in aquaculture. Cambridge University Press, Cambridge, UK.

JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 33, No. 1
March, 2002

Effect of Dietary Inclusion of Blue Mussel Extract on Growth and Body Composition of Japanese Flounder *Paralichthys olivaceus*

KOTARO KIKUCHI

Abiko Research Laboratory, Central Research Institute of Electric Power Industry, Abiko, Chiba 270-1194 Japan

AIKO UEDA AND HARUO SUGITA

Department of Marine Science and Resources, Nihon University, Kameino, Fujisawa, Kanagawa 252-8510 Japan

SHIGENOBU TAKEDA

Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo, Tokyo 113-8657 Japan

Abstract

Dietary inclusion of a water-soluble fraction of blue mussel *Mytilus galloprovincialis* was examined as a feeding stimulant for juvenile Japanese flounder *Paralichthys olivaceus*. The control diet mainly consisted of fish meal, potato starch, and pollack liver oil. Five, 10, and 20% (weight/weight) of the control diet was exchanged with aqueous extracts of blue mussel meat in experimental groups. Fish of about 10 g in initial body weight were fed each diet to satiation, twice daily, 6 d per wk for 6 wk at 20 C. The final body weight, weight gain, and feed efficiency of fish fed the diets containing blue mussel extracts were significantly higher than those of fish fed the control diet. However, these parameters were not different among experimental groups containing blue mussel extract independent of the inclusion level of extract. A similar trend was shown in protein efficiency ratio as fish fed the control diet had a significantly lower protein efficiency ratio than the other dietary groups. Compared to the control diet, higher plasma protein and lower triglyceride were found in fish fed the diets with the extract, while other blood constituents were relatively similar for the dietary groups tested. On the other hand, whole-body crude lipid content and lipid retention of fish fed the diets with the extract were generally significantly higher than those of fish fed the control diet. Whole body crude protein was identical regardless of the dietary composition; however, protein retention of fish showed a similar trend to lipid retention.

Blue mussels are a nuisance organism for electric power plants located along the coast of Japan. Excessive mussel growth along water intake pipes constricts and impedes the inflow of cooling water. Generally, mussels are collected once or twice a year and buried in the landfill of the plant after incineration of organic matter. The removal and disposal requires considerable cost; moreover, landfill space is scarce in Japan.

Several studies on the utilization of collected mussels have been conducted to address the disposal problem; however, no effective methods other than their use as a source of fertilizer have been developed. Blue mussels *Mytilus galloprovincialis* have been used as a supplemental aquaculture feed for crustaceans such as Kuruma prawn *Penaeus japonicus* and lobster *Panulirus japonicus* in Japan. Availability of the mussels as finfish feed was demonstrated with rainbow trout *Oncorhynchus mykiss* (Grave et al. 1979; Berge and Austreng 1989) and red sea bream *Pagrus major* (Kitamura et al. 1981). We also reported that the freeze-dried meat of blue mussels can