

Evaluation of the addition of ascorbic acid to the ration of cultivated *Piaractus mesopotamicus* (Characidae) on the infestation of *Anacanthorus penilabiatus* (Monogenea)

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Abstract

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Sixty *Piaractus mesopotamicus* Holmberg, 1887 (*pacu*) fry fed a diet containing 0, 50, 100 and 200 mg ascorbic acid/kg dry feed were studied to evaluate the effect on parasitic infestation by the monogenean *Anacanthorus penilabiatus* Boeger, Husak and Martins, 1995 (Monogenea: Dactylogyridae) for a period of 24 weeks. The temperature of the aquaria was measured daily and remained between 28 and 31°C. At the beginning of the experiment, fish showed 6.15 ± 0.33 cm standard length and 8.64 ± 1.62 g average body weight. A sample of fish was examined and showed 43 ± 17 monogeneans per fish. At the end of the experiment, the gills of control and vitamin C-treated fish were collected for parasite counts. Control fish had 42.5 parasites per fish, a significantly higher number ($P < 0.05$) when compared with fish fed vitamin C, that showed 16.5 parasites per fish. Ascorbic acid fortification in the food promoted an increase in fish resistance to parasites. It is suggested that an optimum level of 139 mg/kg vitamin C supplementation either elicited better nutritional conditions by stimulating the appetite of the fish or improved the immune response.

Key words

- Ascorbic acid
- Monogenean
- *Anacanthorus penilabiatus*
- Infestation
- Fish
- *Piaractus mesopotamicus*

With the intensification of fish farming in Brazil, nutritional problems and infectious and parasitic diseases began to appear. Monogenean infestation is especially important and can be normally present asymptotically on the surface or gills of healthy fish. The confinement of fish under conditions of overcrowding, inadequate feed, high organic matter concentration and low water flow are factors that contribute to parasite reproduction. The importance of vitamin C in the immunological system of fish (1) is known. Better nutritional condition of the fish has

led to a reduction of monogenean infestation (2). Nevertheless, a large number of fish susceptible to monogenean infestation and some fish with acquired immunity show a reduction in natural immunity during epidemic outbreaks (3). Therefore, it seemed important to report the present preliminary data regarding the effect of vitamin C on monogenean infestation.

The present study was carried out for a period of 24 weeks at the Fish Nutrition Laboratory of the Aquaculture Center, UNESP, Jaboticabal, São Paulo State, Bra-

zil. Sixty *Piaractus mesopotamicus* Holmberg, 1887 (*pacu*) fry presenting 6.15 ± 0.33 cm standard length and 8.64 ± 1.62 g average body weight were analyzed. The animals were randomly assigned to twenty 100-liter aquaria, with 6 animals and 5 replicates per treatment. The temperature of the aquaria was measured daily and remained between 28.0 and 31.0°C. Water quality was analyzed weekly (4), showing the following parameters: pH 7.05-8.40; dissolved oxygen, 3.50-6.10 mg/l; electric conductivity, 180.1-195.5 μ S/cm, and alkalinity, 84.7-94.2 mg/l. The animals were fed diets containing 0, 50, 100 and 200 mg palmitate-coated ascorbic acid/kg dry feed. A sample of 20% of the fishes was examined at the beginning of the experiment for the presence of *Anacanthorus pe-*

nilabiatus Boeger, Husak and Martins, 1995 in the gills (5). At the end of the experiment, the fishes were killed and the gills were removed and placed in a glass flask with 1:4000 formalin solution, shaken in this liquid and allowed to rest for 2 h for preservation in 5% formalin solution. Data were analyzed statistically using the "Statgraphic" software package.

At the beginning of the experiment, fishes showed 43 ± 17 parasites per fish, and at the end of the experiment control fish showed elevated levels of gill monogeneans, while fish fed a diet supplemented with 50, 100 and 200 mg ascorbic acid/kg dry feed showed a significant decrease ($P < 0.05$) in monogenean infestation (Table 1). The regression study showed a relationship between vitamin C (X) and monogenean number (Y) expressed by the equation: $Y = 42.50 - 1.0746X + 0.0132X^2 - 0.000042X^3$. Our results suggest that, under laboratory conditions, 50 mg ascorbic acid/kg dry feed is sufficient to improve the growth of *P. mesopotamicus* but the optimum level is 139 mg/kg dry ration, as demonstrated by statistical analysis (Table 2). The relationship between vitamin C levels (X axis) and standard length (Y axis) can be described by the following equation: $Y = 6.15 + 0.0379X - 0.000136X^2$, $R^2 = 0.8813$.

According to Wahli et al. (6), *Salmo gairdneri* supplemented with feed containing ascorbic acid showed an improvement in resistance against the ciliate protozoan *Ichthyophthirius multifiliis*. The authors reported reduction of up to 84% of tomite infestation, indicating the presence of an immune response including specific anti-*I. multifiliis* antibodies due to the supplemented diet. The results, together with higher survival rates, suggested an influence of ascorbic acid on the immune response of the host, as also shown in our study. In Kuwait, mortality of *Epinephelus tauvina* and *Acanthopagrus cuvieri* caused by parasitic infestation of *Cryptocaryon irritans* and bacterial

Table 1 - Number of monogeneans in the gills of *P. mesopotamicus* Holmberg, 1887 fed different levels of vitamin C for 24 weeks.

Data are reported as means \pm SEM for 10 fish in each group. Averages followed by the same letter did not differ by the Tukey test ($P > 0.05$).

Ascorbic acid (mg/kg ration)	Number of monogeneans
0	42.5 ± 11.1 a
50	16.5 ± 4.7 b
100	25.0 ± 4.6 b
200	19.7 ± 2.8 b

Table 2 - Effect of ascorbic acid on body weight and length of *P. mesopotamicus* Holmberg, 1887, at the end of the experiment (24 weeks) compared to control.

Data are reported as means \pm SEM for 30 fish in each group. Averages followed by the same letter did not differ by the Tukey test ($P > 0.05$).

Ascorbic acid (mg/kg ration)	Body weight (g)	Standard length (cm)
0	91.7 ± 19.5 a	5.9 ± 0.6 b
50	102.5 ± 27.7 a	8.2 ± 0.9 a
100	103.6 ± 15.9 a	8.2 ± 0.5 a
200	106.7 ± 12.2 a	8.4 ± 0.6 a

infections was observed, but was stopped by a diet containing 500 mg ascorbic acid/kg dry feed for one week (7).

The immunological mechanisms of fish receiving dietary vitamin C supplementation may be important to avoid the occurrence of monogeneans in fish farms. The present study confirmed previous reports showing that fish submitted to better nutritional conditions can decrease monogenean infestation (2). In the present study, fish receiving vitamin C showed better appetite when compared to control, a fact that possibly improved the nutritional status of pacu, as observed by the author in a previous study (8). Martins (8) studied the effect of ascorbic acid deficiency on the growth and behavior of pacu for a similar period.

Antibodies to copepod or monogenean parasites were not detected in serum samples of *Leiostomus xanthurus*, suggesting that these parasites were not involved in the humoral immune response (9). Vladimirov (10) studied the immunological response in carp infected with *Dactylogyrus vastator* and observed that immunity depends on the intensity of infection, temperature conditions and fish species. The increase in the immunological resistance of infested fish is a consequence of the activation of their protective mechanisms and we suggest that vitamin C supplementation may improve fish health.

Important factors may be present in the

cutaneous mucus (11) and serum that play an important role against the survival of *Gyrodactylus stellatus* (12). Nevertheless, studies with *Parophrys vetulus* infested with *G. stellatus* suggested that immunity may be a factor that, in association with favorable conditions for the parasite, such as temperature changes and lack of feeding, may allow the development of epizootics (13). A relative resistance to challenge infections with ectoparasitic gill monogeneans of the genus *Pseudodactylogyrus* has been observed (14).

Fishes were measured monthly. We observed that control fish started eating 24 h after food presentation, while vitamin C-supplemented fish ate the diet during the afternoon of the same day. A humoral immune reaction was not detected in this study, but Buchmann (15) showed only a very weak humoral immune reaction to ectoparasitic monogeneans in heavily infested eels. However, further studies with *P. mesopotamicus*, an economically important freshwater fish, and their parasites such as monogeneans are needed to explain the resistance of supplemented fish. Further data related to the humoral or mucus response are also needed.

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