EFFECTS OF PETROLEUM EXPOSURE IN TAMBAQUI

FED DIETS SUPPLEMENTED WITH VITAMIN C

Matsuo, A. Y. O.
Instituto Nacional de Pesquisas da Amazônia – INPA
Alameda Cosme Ferreira, 1756 – Aleixo – Manaus (AM)-Brazil
Phone: +55 (92) 643-3187
Fax: +55 (92) 643-3186
E-mail: aline@inpa.gov.br

Chagas, E. C.
Embrapa Amazônia Ocidental
CP 319, CEP: 69011-970 – Manaus (AM)-Brazil
Phone: +55 (92) 621-0300
Fax: +55 (92) 622-1100
E-mail: edsandra@cpaa.embrapa.br

Val, A. L.
Instituto Nacional de Pesquisas da Amazônia – INPA
Alameda Cosme Ferreira, 1756 – Aleixo – Manaus (AM)-Brazil
Phone: +55 (92) 643-3188
Fax: +55 (92) 643-3186
E-mail: dalval@inpa.gov.br

Abstract

Vitamin C supplementation in the diet may be important in fish subjected to environmental pollution such as petroleum hydrocarbons. Juvenile tambaqui (*Colossoma macropomum*) were fed diets containing different vitamin C levels (0, 100, 200, and 400 mg AA (ascorbic acid)/kg) for 4 weeks. Diets were prepared using a stable source of vitamin C (L-ascorbyl-2-polyphosphate). Feeding was discontinued after four weeks and the fish (N=8 per treatment) were exposed to 12.5 ppm of crude oil for 96 h. Fish were subsequently sacrificed, and blood samples were taken for analyses of hematocrit, glucose, alkaline phosphatase, alanine amino-transferase, and aspartate amino-transferase. Hematocrit values were not significantly correlated with vitamin C levels in the diet, but they were slightly lower in the petroleum exposed group (36.5±0.9 versus 34.7±1.1 for control and petroleum groups, respectively).

Plasma glucose levels for the controls were higher for fish fed 0 and 100 mg AA/kg and lower in those fish fed 200 and 400 mg AA/kg. In the petroleum exposed fish, values dropped slightly, except for fish fed 400 mg AA/kg, which had glucose levels up to 110 mg/dl. Alanine amino-transferase levels were not affected by vitamin C (9.6±0.7 U.I./L), but increased significantly when exposed to 12.5 ppm of crude oil (15.025±1.1 U.I./L). Higher concentrations of vitamin C appeared to reduce significantly aspartate amino-transferase levels, except for those fish supplemented with 400 mg AA/kg and exposed to crude oil (in which levels of aspartate amino-transferase were significantly elevated).

Introduction

Vitamin C (ascorbic acid; AA) supplementation in the diet of tropical fish is an important factor for stress resistance, effective response of the immune system, and normal development (Waagbo, 1994). Dietary requirements vary according to species, developmental stage, environmental factors (temperature, water quality, etc.), and metabolic function (e.g., disease factors, endogenous reactions). Nutritional status in fish is also an important factor in determining their ability to withstand exposure to environmental pollutants and carcinogens such as petroleum compounds. Most petroleum compounds, like benzo(a)pyrene are not reactive, but they may be converted endogenously and bioactivated, eventually becoming carcinogenic. It appears that vitamin C stimulates enzymes (e.g., transferases) that act on detoxifying pollutants. Vitamin C appears to be required in different concentrations in different tissues for detoxification processes and other protective functions resulting from crude oil exposure (Thomas, 1987). The goal of this study was to determine the effects of petroleum exposure in the tambaqui, a tropical fish native to the Amazon Basin, under different dietary vitamin C supplementation regimes.

Materials and Methods

Juvenile tambaqui (*Colossoma macropomum*; 83.29 ± 2.93 g) were fed daily, for four weeks, diets containing different concentrations of vitamin C (0, 100, 200, and 400 mg AA/kg). Diets were prepared using a stable source of vitamin C (L-ascorbyl-2-polyphosphate (Rovimix Stay-C35; Hoffmann La Roche, Switzerland)), from which pellets were made using a meat grinder. Feeding was discontinued after four weeks and the fish (N=8 per treartment) were exposed to 12.5 ppm of crude oil from Urucu (Coari, Amazonas, Brazil) for 96 h. One of the fish fed 0 mg AA/kg did not survive petroleum exposure. The fish were sacrificed at the end of 96 h exposure using an overdose of anesthetic (MS-222;

Sigma Co.), and blood samples were taken for further analysis. Hematocrit was determined using capillary tubes centrifuged for 5 minutes at 10,000 rpm. Plasma was isolated immediately following centrifugation, and glucose analyses were performed in duplicate using Doles Reagent Kit (Glucox500®). Alkaline phosphatase, alanine amino-transferase, and aspartate amino-transferase were also analysed in plasma using Doles Reagent Kit (Fosfatases® and Transferases®).

Results and Discussion

Although there was a tendency for hematocrit values in tambaqui to be slightly lower in the petroleum-exposed group, the influence of dietary vitamin C was not significant (Table 1). Alkaline phosphatase levels in plasma varied according to different vitamin C levels in the tambaqui diet, and it was higher (though not statistically so) in fish exposed to petroleum fraction, except in the group fed 0 mg AA/kg diet.

Table 1. Hematocrit and alkaline phosphatase (plasma) levels in tambaqui fed different dietary levels of vitamin C and subsequently exposed to 12.5 ppm petroleum (crude oil) for 96 h. Values are expressed as mean ± SE.

	Treatment	*0 mg AA/kg*	*100 mg AA/kg*	*200 mg AA/kg*	*400 mg AA/kg*
Hematocrit (%RBC)	Control	35.4±0.5	36.8±1.5	36.5±1.0	37.4±0.9
	Petroleum exposed	34.9±1.4	34.3±1.4	34.4±0.6	35.4±1.0
Alkaline phosphatase (U.I./L)	Control	57.8±1.2	45.1±3.2	40.5±1.4	52.5±2.3
	Petroleum exposed	51.4±3.7	54.0±2.5	49.9±5.8	61.2±3.7

Plasma glucose levels for the control group were higher in fish fed a diet low in vitamin C (0 and 100 mg AA/kg) and lower in those fish fed 200 and 400 mg AA/kg (Figure 1a). In the petroleum exposed fish, however, values

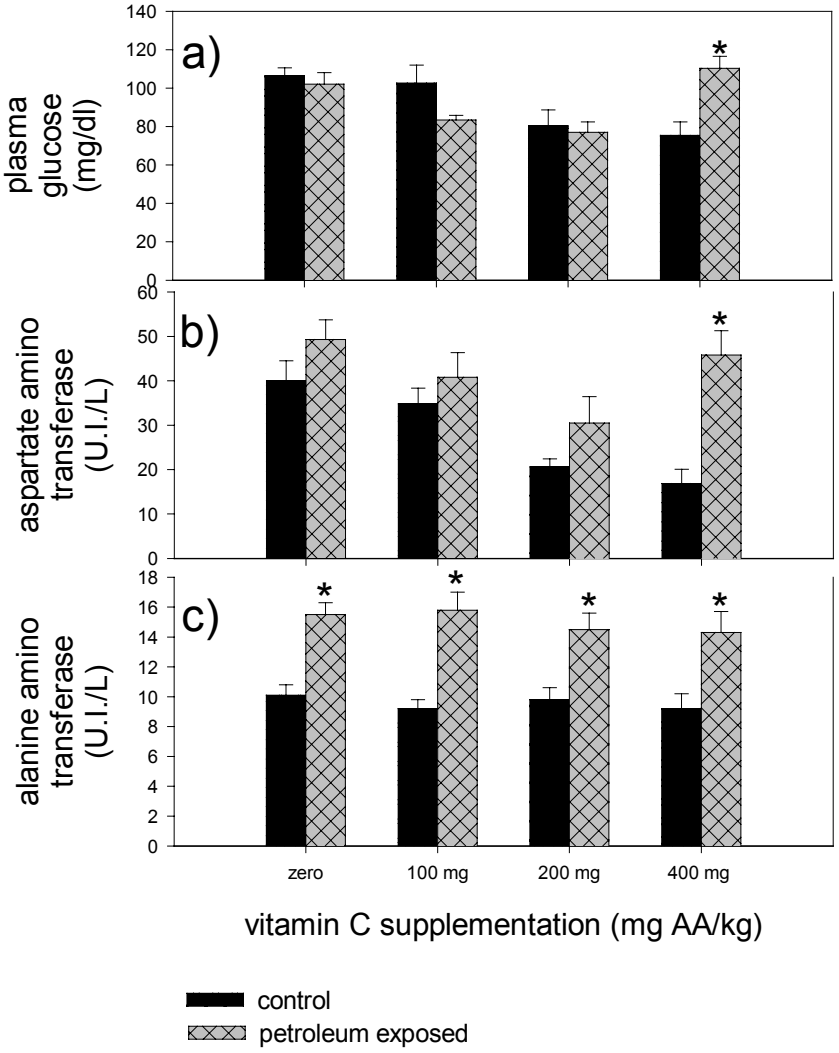

Figure 1. Effect of vitamin C supplementation in tambaqui diet following exposure to 12.5 ppm petroleum (crude oil) for 96 h. a) Plasma glucose; b) aspartate amino-transferase; c) alanine amino-transferase. Asterisks (*) indicate significant differences in relation to respective controls.

dropped slightly except for the group fed 400 mg AA/kg, which had high glucose levels (up to 110 mg/dl). Hyperglycemia is known to be a common response in fish under stress conditions and fed a low vitamin C diet (Chagas, 1998; Henrique *et al.*, 1998). It can also be a common consequence of supplementation of high doses of vitamin C, because ascorbic acid and glucose are structurally very similar (they both compete to reach tissues and organs through a common cellular transport system).

Vitamin C also appeared to affect aspartate amino-transferase levels, which were significantly lower in fish fed diets with higher concentrations of vitamin C (Figure 1b). This effect was consistent in all treatments, except for those fish receiving a diet supplemented with 400 mg AA/kg and exposed to crude oil; these fish experienced significantly elevated levels of aspartate amino-transferase in relation to the control. It is unclear, however, why this was the case.

Alanine amino-transferase levels were not affected by vitamin C supplementation, but they increased on average 57% when exposed to 12.5 ppm of crude oil (Figure 1c). The supplementation of vitamin C seems to reduce the negative physiological effects of environmental pollutants, thus helping fish cope with exposure to toxic substances.

References

Chagas, E. C. 1998. Efeito do ácido ascórbico (vitamina C) sobre a resistência ao estresse hipóxico em *Colossoma macropomum* (Cuvier, 1818). BSc. Thesis, Fundação Universidade do Amazonas, Manaus, Amazonas, 39 pp.

Henrique, M. M. F., E. F. Gomes, M. F. Gouillou-Coustans, A. Oliva-Teles, and S. J. Davies. 1998. Influence of supplementation of practical diets with vitamin C on growth and response to hypoxic stress of seabream, *Sparus aurata*. Aquaculture 161: 415-426.

Thomas, P. 1987. Influence of some environmental variables on the ascorbic acid status of striped mullet, *Mugil cephalus* Linn. Tissues. III. Effects of exposure to oil. J. Fish Biol. 30: 485-494.

Waagbo, R. 1994. The impact of nutritional factors on the immune system in Atlantic salmon, *Salmo salar*: a review. Aquacult. Fish. Manag. 25: 175-197.

Acknowledgements

We thank C. A. Marantz for reviewing the manuscript. This project was supported by CNPq and Inpa (PPI#3240).