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# Could a diet enriched with n-3 highly unsaturated fatty acids be considered a promising way to enhance the immune defences and the resistance of *Penaeid* prawns to environmental stress?

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**Abstract:** The prawn *Penaeus stylirostris* (Stimpson), when fed for 28 days with n-3 highly unsaturated fatty acid (HUFA)-enriched feed pellets, demonstrated an enhanced resistance to variations in environmental parameters (a decrease in temperature and salinity over a 4-day period from 28 °C to 17 °C and from 35‰ to 10‰ respectively) and an improvement in their immune defence potential, i.e. increased agglutination titre of plasma and increased respiratory burst of haemocytes.

**Keywords:** crustaceans, essential fatty acids, stress, environment, osmoregulation, immunostimulation

## Introduction

During culture, Penaeid prawns may be subject to drastic variations in their environment that may lead to a decrease in their resistance to bacterial attack. Thus, in New Caledonia, a seasonal decrease in temperature to 10 °C (from 29 down to 19 °C) can occur during a rearing cycle. This may be accompanied by an increasing sensibility to a bacteria, Vibrio penaeicida (Costa, Mermoud, Koblavi, Morlet, Haffner, Berthe, Legroumellec & Grimont, 1998). This pathology shows chronic phases and acute episodes of mortality, often preceded by stressful conditions such as molting peak and temperature decrease. Deseased prawns are weak. They are soft with dark cuticle, opaque muscles, and empty gut (Costa, Mermoud, Mari, Bonami, Hasson & Lightner, 1998). It has been shown for fish that a HUFA-enriched diet increased the resistance of larvae to different stresses such as handling and variations in T°C and S‰ (Kanazawa, 1997; Furuita, Konishi & Takeuchi, 1999). HUFAs also enhance the survival of fish larvae during metamorphosis (Dhert, Lavens, Duray & Sorgeloos, 1990) and act as a growth factor in crustaceans (Kanazawa, Teshima & Sasaki 1979). The resistance of postlarvae Penaeid prawns is enhanced with a HUFA-enriched diet (Rees, Curé, Piyatiratitivorakul, Sorgeloos & Menasveta 1994). Nevertheless, no data exist on the influence HUFAs may have on the capacity of cultured prawns to regulate their osmotic pressure or on the changes effected on their immune defences. The aim of this work was to show the influence of HUFAs on the response of Penaeus stylirostris to T° and salinity stress, and to study their impact on its immune defence potential. The overall objective is to determine if the enrichment of feed pellets with HUFAs is a promising way to prevent the problems related to quick and extensive physical environmental changes (T° and S‰), whether or not these are accompanied by bacterial attack.

## Materials and Methods

The effects of HUFAs were determined on prawns fed two different diets containing high or low levels of HUFAs. Two main basic constituents of the experimental feed pellets (fish and squid meal) were lipid-free. Lipids were removed from the meal with four successive 2 hour baths in absolute ethanol at 60 °C. Cod liver oil, which is rich in HUFAs, was added to the HUFA+ feed pellets (7% w/w), whereas coconut oil, which contains a very low concentration of HUFAs, was added to the HUFA- feed pellets (7% w/w). Analyses of HUFAs (EPA=20:5 n-3 + DHA=22:6 n-3) in 5 aliquots showed concentrations of 2.06±0.12 and 14.51±0.26 $g.kg^{-1}$ for HUFA- and HUFA+ feed pellets respectively. In standard feed pellets, the concentration of HUFAs ranges usually from 7 to 9 $g.kg^{-1}$ feed pellets.

The experiments were carried out in 8 thermoregulated 150 l tanks, each containing 30 prawns (6.56 g ± 1.79 g mean weight). The prawns were subjected to a 7 day fasting period (D0 to D7) in order to normalise their physiological condition. From D7 to D28, 4 tanks were fed the HUFA+ diet and 4 tanks the HUFA- diet. From D0 to D28, the temperature and salinity were 28 °C and 35 ‰ respectively. At D28, samples were taken to test the immune system related parameters. The following measurements were carried out : plasmatic protein levels (Lowry, Rosebrough, Farr & Randall 1951), the agglutination titre of plasma (Vargas-Albores, Guzman, & Ochoa, 1993), the number of haemocytes (Le Moullac, Le Groumellec, Ansquer, Froissard, Levy & Aquacop, 1997), the respiratory burst activity of haemocytes. This last parameter was quantified *in vitro* by measuring the reduction of Nitro Blue Tetrazolium (NBT) to formazan as a measure of superoxyde anion production (Song and Hsieh, 1994) in unstimulated haemocytes

and in haemocytes stimulated with zymosan particles, to simulate a bacterial attack. From D28 to D32, the temperature and salinity were progressively lowered to 17°C and 10 ‰ respectively and the mortality rate recorded. The osmolality of the prawn haemolymph was measured at D32 (Lignot, Cochard, Soyez, Lemaire & Charmantier, 1999). A Student's *t* test was used to compare the difference of the means for each parametre.

**Results and discussion**

Survival rates at D28, expressed as % of initial number of prawn in the tanks, were 82.7 ± 6.9 in the HUFA+ tanks and 81.2 ± 1.0 in the HUFA- tanks. These values were not significantly ($p>0.1$) different from each other. At D32, after the 4 day stress period, a significant ($p<0.01$) difference in mortality between the 2 groups of tanks was observed. The average survival rates were 58.3 ± 4.8 in the HUFA+ tanks and 32.6 ± 1.3 in the HUFA- tanks. Fig. 1 shows the osmolality values at D32 in the haemolymph at the different intermoult stages (Drach & Tchernigovtzeff, 1967). The mean osmolality was always higher in HUFA+ prawns than in HUFA- prawns. A *t* test showed that this difference was significant ($P<0.5$) for stage B, and highly significant ($P<0.005$) for stages C-D0 and D1. The concentration of plasmatic proteins (table 1) was not significantly ($p>0.1$) different in HUFA+ and HUFA- prawns. The agglutination titre of plasma was significantly ($p<0.01$) higher in HUFA+ prawns: 6.00 ± 1.95 for HUFA+ prawns and 4.22 ± 1.16 for HUFA- prawns. The number of circulating haemocytes (table 1) was lower in HUFA+ prawns but this value was not significantly ($p>0.1$) different from that of the HUFA- prawns. The basic respiratory burst activity (NBTb) of haemocytes was not significantly modified by the diet (table 1). When this activity was stimulated with zymosan (NBTs), it was significantly

($p<0.05$) higher for HUFA+ prawns (O.D. = 0.289 ± 0.196) compared to HUFA- prawns (O.D. = 0.172 ± 0.195).

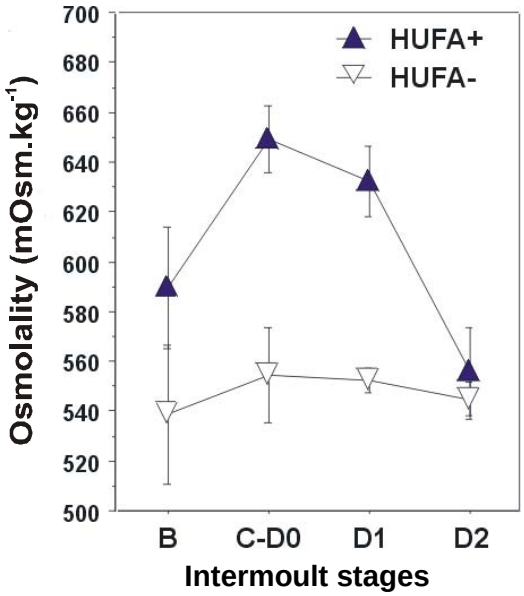
Table 1: Variation in immune defence-related parameters in relation to the level of HUFAs in the diet. Values are expressed as mean ± S.E. (Number of analyses between brackets)

| | HUFA+ Prawns | HUFA- Prawns |
|---|---|---|
| Plasmatic proteins ($mg.ml^{-1}$) | 85.0±50.7 (19) | 77.5±30.2 (20) |
| $Log_2$ Agglutination titre [a] | 6,00±1,95 (12) | 4,22±1,16 (18) |
| Haemocyte ($cells.mm^{-3}$) | 10855±5108 (19) | 15437±5736 (20) |
| NBTb.$10^5$ haemocyte[b] | 0,138±0,070 (12) | 0,129±0,145 (16) |
| NBTs. $10^5$ haemocyte[ab] | 0,289±0,196 (12) | 0,172±0,195 (16) |

[a] The difference between HUFA+ and HUFA- is significant at the $p<0.05$ level

[b] The results correspond to the quantity of NBT transformed into Formazan. They are expressed as the optical density at 630 nm (O.D.630) per $10^5$ haemocytes, calculated from the O.D.630 measured in 25 µl of haemolymph, and from the number of haemocytes in the 25 µl sample.

Figure 1: Osmolality of haemolymph at different intermoult stages for HUFA+ and HUFA- conditioned prawns, at 10 ‰ salinity.




The increase in the agglutination activity of plasma was not due to a variation in concentration of the plasmatic proteins. This increase could be due to a change in the lipid fraction of the circulating lipoproteins, which are involved in the recognition of extraneous bodies and which increase the efficiency of immune defence mechanisms such as phagocytosis (Vargas-Albores et al., 1993; Vargas-Albores, 1995). HUFAs

contribute to the maintenance of cell membrane function in fish (Hazel, 1984) and increase the phagocytic capacity of leukocytes (Kiron, Gunji, Okamoto, Satoh, Ikeda & Watanabe, 1993; Ashton, Clements, Barrow, Secombes & Rowley, 1994). In crustaceans, it has been shown that a low temperature has a deleterious effect on the structure and function of cell membranes, and that HUFAs allow an adaptation to slow temperature decreases by modifying the composition of the cell membrane, leading to an increase in its fluidity (Pruitt, 1990). Thus, it is likely that a preventive HUFA+ diet could lead to a better " homeoviscous adaptation " (Sinensky, 1974) of the cell membranes with, as a consequence, a better adaptation and resistance of prawns to environmental stress such as a important and sudden drop in temperature and salinity. This could allow not just a better regulation of the osmolality, but might also strengthen prawns' resistance to infectious diseases by improving the agglutination activity of the plasma and the phagocytic ability of the haemocytes. Although the concentration of HUFAs in the feed pellets corresponded either to a huge deficiency or to a large excess of HUFAs in the diets, compared to "normal" feed pellets, this preliminary study tends to confirm that, as for fish, a HUFA-enriched diet appears to be a promising avenue of research into preventing the observed mass mortality crises, particularly in areas where large variations in environmental conditions may occur and/or where endemic bacterial epidemics exist.

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