

# Improved Nutrition Enhances Immune Competence, Disease Resistance In Penaeid Shrimp

Peter Coutteau  
petercouteau@compuserve.com  
S. Ceulemans

INVE Technologies nv  
Aquaculture Division – Farm Nutrition  
Oeverstraat 7  
B-9200 Baasrode, Belgium

L. Chim  
D. Saulnier  
P. Lemaire

IFREMER  
Centre Océanologique du Pacifique  
Tahiti, French Polynesia

Most disease problems in shrimp production are complex and still poorly understood. Sustainable management strategies and regulations restrict the number of drugs available to treat pathogens. Contrary to agriculture, the economics of drugs in aquaculture are not appealing for the pharmaceutical industry to invest in the development of novel, acceptable therapeutics. Vaccines are likely to be ineffective in crustaceans, which lack a specific immune system similar to that of vertebrates. Therefore, shrimp aquaculturists must consider seed-stock quality, husbandry procedures, and healthy nutrition as the major tools to control disease.

## Improving Health Via Feed

Current nutritional research efforts are directed towards the identification of requirements for key nutrients affecting the health and immunology of



The goal: a healthy harvest of *Penaeus monodon*.

shrimp, such as vitamin C and E, phospholipids, essential fatty acids, trace minerals and carotenoids.

One of the most promising areas of development for strengthening the non-specific defense mechanisms and protect shrimp against disease is the administration of immunostimulants. Various compounds have been identified, mostly derived from the cell envelope of microorganisms, such as polysaccharides, lipoproteins, and lipopolysaccharides. Preliminary results indicated that the efficacy of various commercially available immunostimulants to improve stress and/or disease resistance of fish and shrimp strongly depends on the type of the product, and on the supply of adjuvant nutri-

The design of specialty premixes to improve disease resistance is based on the selection of the appropriate immunostimulants in combination with a balanced supply of key nutrients.

ents that are essential to support the buildup of the immune system. Therefore, the design of specialty premixes to improve disease resistance is based on the selection of the appropriate immunostimulants in combination with a balanced supply of key nutrients to support the enhancement of the immune system.

## Experimental Conditions, Design

Progress in research on disease control of shrimp is hampered by methodological difficulties to assess health status, and limited knowledge of crustacean immunology. In our study, the effect of an immunostimulating specialty premix on disease resistance of *Penaeus stylirostris* was evaluated using haemolymph parameters (ex vivo) as well as a challenge test (in vivo).

Experiments were conducted at the IFREMER Centre Océanologique du Pacifique in Tahiti. Juvenile shrimp (*Penaeus stylirostris*,  $5.1 \pm 0.5$  g) were collected from a commercial pond and transferred to the laboratory, where

they were maintained in 500-l tanks (70 shrimp/tank) and fed a local commercial feed until the start of the trial.

For each treatment, two groups of animals were stocked:

1. Animals used for the analysis of haemolymph parameters: 12 shrimp/200-l tank (5 tanks = 60 shrimp/treatment).



Extracting haemolymph sample to determine total count of circulating haemocytes and their phagocytosis activity. Photo by K. Vande Braak.

2. Animals used for the challenge test: 50 shrimp/500-l tank (3 tanks = 150 shrimp/treatment).

The experiment was performed at 26-28° C and 35 ppt salinity. Individual growth was followed during the trial by tagging 45 shrimp per treatment in the 500-l tanks. Shrimp were fed on the experimental feeds during 21 days prior to the extraction of haemolymph or exposure to the challenge test.

### Feed Preparation, Performance

Experimental feeds were produced by cooking-extrusion. A high performance formulation served as control feed (40% crude protein/8% crude fat), in which 1% of filler (cellulose) was replaced with an immunostimu-

lating premix (INVE Aquastim-S, Table 1).

Shrimp activity was excellent throughout the trial and no mortality was observed during the three-week feeding period. Individual weight gain, measured

on tagged shrimp, averaged 3.6-3.9 g over the three-week period (~1.2-1.3 g/week). Average weight gain was 9% higher in the shrimp fed the Aquastim-S supplemented feed, compared to the control group. Similarly, Sung et al. (1994) showed growth enhancement due to immunostimulation in postlarval tiger shrimp (*P. monodon*).

### Haemolymph Parameters

The molting stage was determined under the binocular microscope using the method of Drach and Tchernigovtzeff (1967). Haemolymph was extracted between the last cephalothorax segment and the first abdominal segment using a 1-ml sterile syringe, and only from animals in intermolt stage (stage C or D<sub>0</sub>) to avoid interference of molting stage.

### Total Haemocyte Count

Total numbers were counted in microtiter plates under an optical micro-

scope without discrimination of the three types of haemocytes using the method of Le Moullac et al. (1997).

### Haemocyte Phagocytosis Activity

Phagocytosis is an important component of the immune system of shrimp, responsible for the elimination of foreign bodies in the shrimp haemolymph. It generates a liberation of free radicals during the so-called “respiratory burst,” which results in a strong microbicidal action. The color change of Nitro Blue Tetrazolium (NBT) is used to evaluate phagocytosis activity through the quantification of the anion superoxide O<sub>2</sub><sup>-</sup>, which is the first product released during the respiratory burst. The oxidation of NBT (yellow) to formazan (blue) is a measure of the oxidative activity of the haemocytes. The NBT activity of the haemocytes is measured as such (basal NBT activity), or after immunostimulation of the haemocytes in vitro by exposure to an immunostimulant (stimulated NBT activity) following the method of Secombes (1990).

### Effect of Feed On Haemolymph Parameters

The total count of haemocytes circulating in the haemolymph showed a high variability between individual shrimp (Table 2). Nevertheless, the total haemocyte count was significantly lower in the Aquastim-S treatment compared to the control group. Immunostimulation may decrease the concentration of circulating haemocytes in the haemolymph, due to infiltration of haemocytes in immunostimulated tissues and organs.

The microbicidal activity of the haemocytes, measured as basal as well as stimulated NBT activity, increased for both treatments during the trial compared to the initial value for shrimp fed the commercial feed. The boost of phagocytosis activity was most pronounced in the Aquastim-S group, showing a 2-fold increase of basal NBT and a 2.4-fold increase of stimulated NBT compared to the initial values (an increase of 33% and 84% compared to the control feed). The ratio of stimulated NBT/basal NBT, which is a measure of the microbicidal capacity of the haemocytes, increased from

Table 1. Formulation of experimental diets (% of diet).

Raw Material	Control	Aquastim-S
Standard fishmeal (72% CP basis)	33.9	33.9
Shrimp head meal	5.0	5.0
Defatted soybean meal	11.0	11.0
Wheat gluten	2.0	2.0
Wheat flour	16.2	16.2
Wheat middlings	22.6	22.6
Fish oil	1.3	1.3
Shrimp premix*	5.0	5.0
Cellulose (filler)	3.0	2.0
INVE Aquastim-S 10-00**		1.0
Total	100.0	100.0

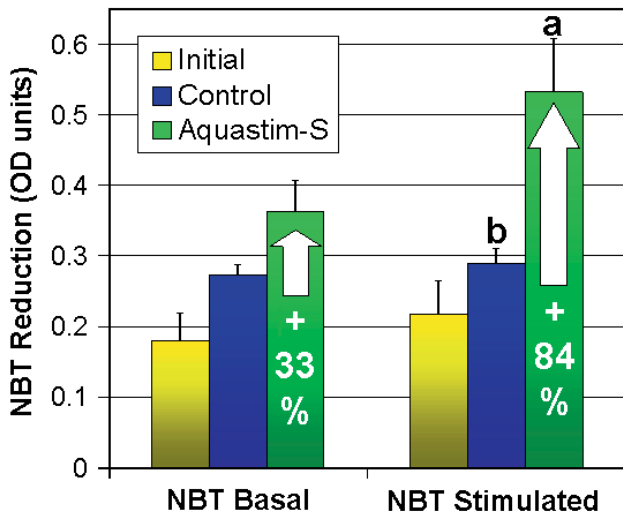
\* Nutritional concentrate providing vitamins, minerals, trace elements, phospholipids, essential amino acids, cholesterol, attractants.

\*\* Specialty concentrate providing blend of selected immunostimulants and nutrients.

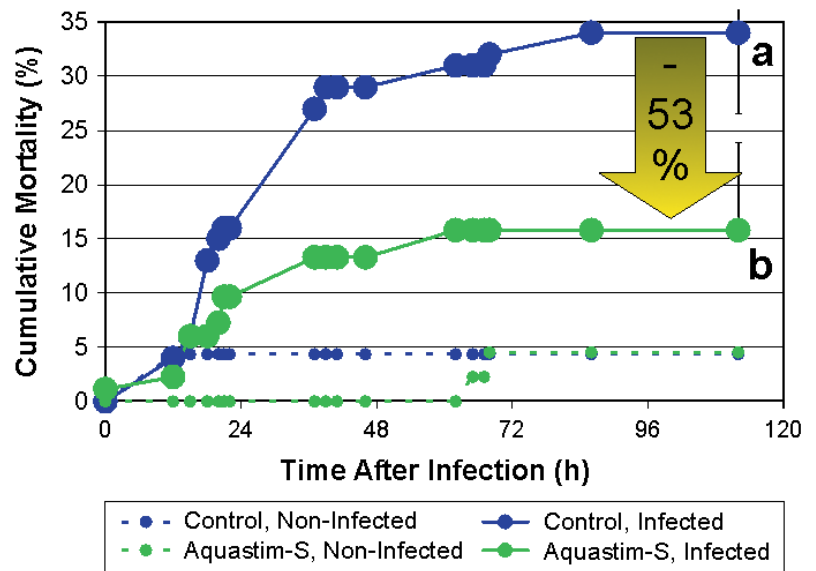
Table 2. Evolution of total haemocyte count (per mm<sup>3</sup> of haemolymph) between shrimp acclimated to commercial feed and shrimp fed experimental feeds during 21 days.

	Feed	Number Shrimp	Average	Std. Error
Day 0 (initial)	Commercial feed	24	36,250	2352
Day 21	Control	32	44,506 a	2170
Day 21	Aquastim-S	26	32,969 b	2605





**Figure 1.** Evolution of basal (NBTb) and stimulated (NBTs) phagocytosis activity between shrimp acclimated to commercial feed and shrimp fed experimental feeds during 21 days. Data represent average and standard error of 11 and 24 individuals, respectively, for initial and final shrimp. Different superscripts indicate significant treatment differences (Student t-test,  $P < 0.05$ ).



**Figure 2.** Cumulative mortality (%) during experimental infection for shrimp fed two experimental feeds. Data represent average and standard deviation of two (non-infected controls) or four (challenged shrimp) tanks each initially stocked with 25 shrimp. Different superscripts indicate significant differences (Student t-test,  $P < 0.05$ ).

1.1 to 1.5 due to the supplementation of Aquastim-S to the feed.

### Challenge Test

An experimental infection was performed with a highly virulent bacterial strain of *Vibrio penaeicida* (AM101). Shrimp were immersed during 2 h in 10 l of seawater containing 105 CFU/ml of AM101, a dosage pre-tested to target appropriate mortalities in the challenge. Control groups received the same treatment as the challenged shrimp but were not exposed to bacteria during the immersion. After the experimental infection, the shrimp were rinsed with seawater and transferred to tanks containing 25 shrimp per 100 l of prefiltered seawater (1  $\mu$ m). The water was continuously aerated and temperature kept at 24-26° C. Mortality was determined three times/day during five consecutive days. Feeding was continued during the follow up period. Infected groups were run with four tanks (~100 shrimp) per treatment, whereas non-infected control groups were run with two tanks (~50 shrimp) per treatment.

### Sensitivity

The sensitivity to the challenge test was expressed by the total mortality observed during five days post-infection and the cumulative mortality index, which was calculated as the

sum of cumulative mortality observed over the duration of the follow up period. The cumulative mortality index takes into account the total mortality as well as the spread of mortality over time.

### Effect of Feed On Challenge Mortality

A limited mortality was observed in the non-infected groups during the follow up period of five days (5% in both groups, Figure 1). After five days, the bacterial infection resulted in a total mortality of 16% and 34% in the Aquastim-S and control group, respectively (a reduction of mortality with 53% due to the feed supplementation, Figure 2). Similarly, the cumulative mortality index was significantly higher in the control group compared to that of the Aquastim-S group (377 $\pm$ 77 and 190 $\pm$ 78, respectively).

### Conclusion

Our results demonstrated that nutrition is an effective tool to enhance health status of penaeid shrimp. The supplementation of a blend of selected immunostimulants and nutrients to

a high quality feed for *Penaeus stylirostris* resulted in an enhancement of immune competence and resistance to disease after three weeks of feeding, as indicated by:

- A reduction of the total number of haemocytes in circulation in the haemolymph (with 26% compared to the control group)
- A boost of the phagocytosis activity of the haemocytes (33% and 84% increase of basal NBT and stimulated NBT activity, respectively, compared to the control group)
- Improved survival in a challenge test (53% less mortality compared to the control group following an experimental infection with *Vibrio penaeicida*).

Note: Cited references are available from the first author.

Supplementation of selected immunostimulants and nutrients to a high-quality feed for *Penaeus stylirostris* resulted in an enhancement of immune competence and resistance to disease after three weeks.