

# Benefits of nucleotides in shrimp farming

The major pressure on cultured shrimps is the environment in which they are living. Adding nucleotides to the feed can help the shrimp overcome these stressful situations without losing performance.



**Recent trials confirm that shrimp feed supplemented with immuno-stimulating nucleotides increase disease resistance and growth. It also shows good results as a replacement of Artemia in larval feeding.**

**By Joachim W Hertrampf and Shravan K Mishra**

Immunity is the power of an organism to resist infections or actions of certain poisons. It can be inherited, acquired naturally or acquired artificially. Strengthening the immunity of cultured aquatic animals is an important task since bacterial and viral diseases are a major threat to aquaculture. Fish, as vertebrates, have both "non-specific" and "specific" immunity. They have an immune memory so that the animals can remember previously encountered pathogens. Shrimp, on the other hand, lack a specific immune mechanism and are apparently entirely dependent on a non-specific immune mechanism to resist infections; however, the presence of a specific immune memory in shrimp is still unclear. Immunostimulants or immunostimulatory substances influence the immune system of cultured shrimps by application via the feed. Nucleotides and glucans are probably the most promising substances for strengthening the immune system. They provide resistance to diseases and help in overcoming stress

situations in animals. While glucans are derived from the "outside" cell wall, nucleotides are obtained from the "insides" of yeast cells. Nucleotides are the basic building blocks of the nucleic acids DNA and RNA. Dietary sources of nucleotides appear to be important for supporting optimal growth and the function of metabolically active cells such as lymphocytes, macrophages and intestinal cells. The suggested modes of actions are:

- Stimulation of immune cells
- Probiotic effect in the gut
- Inhibition of pathogens, stimulation of lactobacilli
- Gut cell metabolism
- Improved protein synthesis
- Faster detoxification

## Stress reliever

A major pressure which causes stress on cultured shrimps is the environment in which the animals are living; therefore, the water and its quality. One of the parameters of water quality is the salinity. This parameter has served in two aquarium trials to study the effectiveness of nucleotides as an immunity enhancer.

In the first trial using juvenile *Penaeus monodon* with an initial live weight of 6.97g, the effect of nucleotides - by periodically changing the salinity of the water (as a stress factor) - has been tested.

Nucleotides were fed at levels of 1.0 kg/MT feed and 2.0 kg/MT feed. The daily feeding rate was 8.0% of the body weight divided into four feeding times. The

**Table 1- Response of *Penaeus monodon* on nucleotide supplemented feed under periodically changed stress (salinity) conditions.**

|  | Control           | 0.1% <sup>1)</sup>       | 0.2% <sup>1)</sup> |
|--|-------------------|--------------------------|--------------------|
| Shrimps ( <i>Penaeus monodon</i> ) (no.) | 30                | 30                       | 30                 |
| Replicates (no.)                         | 2                 | 2                        | 2                  |
| <b>Feed</b>                              |                   |                          |                    |
| Crude Protein (%)                        | 42.5              | 41.9                     | 42.8               |
| Protein digestibility (%)                | 91.2              | 90.9                     | 91.1               |
| Crude fibre (%)                          | 2.6               | 3.0                      | 3.0                |
| Ca:P-ratio (1:)                          | 1.32              | 1.32                     | 1.38               |
| Digestible energy <sup>2)</sup> (MJ/kg)  | 14.6              | 14.5                     | 14.6               |
| <b>Shrimps' performances</b>             |                   |                          |                    |
| Initial liveweight (g)                   | 6.96              | 6.96                     | 7.00               |
| Final liveweight (g)                     | 12.46             | 13.44                    | 13.90              |
| Weight gain (g)                          | 5.50 <sup>a</sup> | 6.48 <sup>b</sup>        | 6.90 <sup>b</sup>  |
| (rel.)                                   | 100.0             | 117.8                    | 124.7              |
| Feed conversion rate (1:)                | 3.24 <sup>a</sup> | 2.38 <sup>b</sup>        | 2.12 <sup>b</sup>  |
| (rel.)                                   | 100.0             | 73.5                     | 65.4               |
| Protein efficiency ratio (1:)            | 1.55 <sup>a</sup> | 1.12 <sup>b</sup>        | 1.01 <sup>b</sup>  |
| (rel.)                                   | 100.0             | 72.2                     | 65.2               |
| Molting (rel.)                           | 100.0             | 106.1                    | 127.3              |
| Mortality (%)                            | 46.7 <sup>a</sup> | 6.7 <sup>b</sup>         | 6.7 <sup>b</sup>   |
| <sup>1)</sup> Vanagen inclusion rate     |                   | <sup>2)</sup> Calculated |                    |

Different letters in the superscript denotes significance between means (P<0.05)

induced stress was applied at day 25 of the trial. Every 10 days the salinity was decreased and increased, respectively, by 10 ppt. As long as water's salinity was normal, the weight development of all groups was almost the same. However, when the salinity was reduced for the first time (at day 25), the efficacy of nucleotides in the feed was visible. For the entire trial period, the weight gain of both trial groups was significantly higher (+17.8% and +24.7%) than that of the control group. On the other hand, the differences in weight gain between both trial groups were statistically non-significant (Table 1).

In a second trial, the immune response of juvenile *Penaeus monodon* under extreme salinity conditions of 45 ppt was tested. The trial lasted for 60 days and each kilogramme of feed was supplemented with 0.2% and 0.4% nucleotides, respectively. The daily feeding rate was 8.0% of the body weight divided into four feeding times.

The extreme high salinity was applied from the first day of the trial. The results demonstrate the efficacy of nucleotides under extreme stress situations (Table 2).

**Table 2 - Response of *Penaeus monodon* on nucleotide supplemented feed under extreme salinity conditions.**

|  | Control           | 0.2% <sup>1)</sup> | 0.4% <sup>1)</sup> |
|--|-------------------|--------------------|--------------------|
| Shrimps ( <i>Penaeus monodon</i> ) (no.) | 32                | 32                 | 32                 |
| Replicates (no.)                         | 2                 | 2                  | 2                  |
| <b>Feed</b>                              |                   |                    |                    |
| Crude Protein (%)                        | 41.6              | 41.2               | 39.6               |
| Protein digestibility (%)                | 82.9              | 82.2               | 89.8               |
| Crude fat (%)                            | 6.0               | 5.6                | 5.6                |
| Ca:P ratio (1:)                          | 1.3               | 1.3                | 1.3                |
| Digestible energy (MJ/kg)                | 14.3              | 14.2               | 14.2               |
| <b>Shrimps' performances</b>             |                   |                    |                    |
| Initial liveweight (g)                   | 6.55              | 6.48               | 6.43               |
| Final liveweight (g)                     | 10.77             | 12.15              | 12.31              |
| Specific growth rate                     | 64.4 <sup>a</sup> | 87.5 <sup>b</sup>  | 91.4 <sup>b</sup>  |
| (rel.)                                   | 100.0             | 135.9              | 141.9              |
| Protein efficiency ratio                 | 1.45 <sup>a</sup> | 1.03 <sup>b</sup>  | 0.79 <sup>b</sup>  |
| (rel.)                                   | 100.0             | 71.0               | 54.5               |
| Molting (No.)                            | 107               | 110                | 114                |
| Mortality (%)                            | 25.0 <sup>a</sup> | 15.6 <sup>b</sup>  | 15.6 <sup>b</sup>  |

<sup>1)</sup> Feed fortified with "Vannagen"  
Different letters in the superscript denotes significance between means (P<0.05)

The difference in mortality between the control group and the trial group was significant. The mortality rate in the second trial was substantially lower compared with the first trial. It can, therefore, be presumed that the periodically changed salinity is a greater stress to the animals than permanent, extremely high salinity.

## Performance promoter

Aquarium trials provide basic information; however, it is not a guarantee that findings from aquaria can be repeated under pond conditions. For this reason, a pond trial under research conditions has been conducted.

The size of the control pond was 0.3ha and the size of the treatment pond was 0.4ha, respectively. The net stocking rate was 23 pieces/m<sup>2</sup>. Post larvae of *Penaeus monodon* had an initial liveweight of 0.1g. The pond trial lasted for 98 days. Nucleotides were added to the feed at a level of 0.2%. The culture conditions suffered from high salinity which ranged from 35 ppt to 41 ppt with a mean value of 38 ppt. This is remarkably high-

**Table 3 - Response of *Penaeus monodon* on nucleotide supplemented feed under pond conditions.**

|  | Control              | 0.2% <sup>1)</sup>   |
|--|----------------------|----------------------|
| Stocking density (pcs/m <sup>2</sup> ) | 23                   | 23                   |
| <b>Feed</b>                            |                      |                      |
| Crude Protein (%)                      | 44.3                 | 44.7                 |
| Protein digestibility (%)              | 91.6                 | 91.8                 |
| Calcium (%)                            | 2.11                 | 2.18                 |
| Phosphorus (%)                         | 1.35                 | 1.33                 |
| Digestible energy <sup>1</sup> (MJ/kg) | 14.2                 | 14.2                 |
| <b>Shrimps' performances</b>           |                      |                      |
| Initial liveweight (g)                 | 0.1                  | 0.1                  |
| Final liveweight (g)                   | 12.54                | 13.77                |
| Biomass produced (kg/ha)               | 1,959.0 <sup>a</sup> | 2,269.3 <sup>b</sup> |
| Feed consumed (kg/ha)                  | 3,950.0 <sup>a</sup> | 3,432.5 <sup>b</sup> |
| Feed conversion rate (1:)              | 2.02 <sup>a</sup>    | 1.51 <sup>b</sup>    |
| Protein efficiency ratio (1:)          | 1.01 <sup>a</sup>    | 0.77 <sup>b</sup>    |
| Survival rate (%)                      | 68.0                 | 71.5                 |
| Mortality (%)                          | 25.0 <sup>a</sup>    | 15.6 <sup>b</sup>    |

<sup>1</sup>Feed fortified with "Vannagen"  
Different letters in the superscript denotes significance between means (P<0.05)

er than the 18ppt to 25ppt recommended salinity.

The weight gain of the animals that were fed the nucleotide-feed was 9.8% statistically and significantly better than that of the control shrimp group (Table 3). This supports earlier findings. From the early stage of the trial, however, there was a severe bioluminescence problem in the pond of the nucleotide-fed animals. This caused difficulties in maintaining a proper and stable phytoplankton bloom. Bioluminescence problems and the frequent collapse of phytoplankton bloom causes the shrimps stress and often results in very poor growth and low survival.

Mastering such a situation indicates that the nucleotide-enriched diet has a positive impact on shrimp performance under stress conditions. This refers to the fact that nucleotide treatment increases the number of granulate hemocytes, which avoid the invasion of pathogens.

Since both ponds are not of equal size and for making the results comparable, the biomass production has been converted into yield per hectare. The nucleotide-diet group has produced 15.8% more biomass than the control group (Table 3).

The nucleotide group consumed 13.1% less feed than the control group. This is also reflected in the

**Table 4 - Performances of *Penaeus monodon* post-larvae after feeding *Artemia* and Nucleotides (glass aquaria, trial duration: 15 days per cycle).**

|                              | Artemia |       | Nucleotides |       |
|------------------------------|---------|-------|-------------|-------|
|                              | Mean    | SD+/- | Mean        | SD+/- |
| Mysis stocked (no.)          | 1,200   |       | 1,200       |       |
| Replicates (no.)             | 3       |       | 3           |       |
| <b>Post-larvae harvested</b> |         |       |             |       |
| First cycle (no.)            | 108     |       | 116         |       |
| Survival (%)                 | 27.0    | 15.0  | 29.0        | 12.0  |
| Second cycle (no.)           | 51      |       | 61          |       |
| Survival (%)                 | 12.8    | 10.0  | 15.1        | 8.0   |

feed conversion rate. The difference between both groups is 25.2% in favour of the nucleotide group. The latter has also utilised the feed's protein more efficiently. It is clear that under unfavourable pond conditions (high salinity, poor bloom maintenance) nucleotides demonstrate their strength as a stress reliever and, at the same time, its performance promoting capacity in shrimps.

## Nucleotides as the only larval shrimp feed

A major food for shrimp larvae is *Artemia*. The nutritional value of *Artemia nauplii* can be increased by adding nucleotides. In this case, *Artemia nauplii* will act as a transfer link to the early larval stage of shrimps. As a result, the immune system of shrimp larvae will be strengthened, the development will be improved and the survival rate increased.

This procedure, however, does not appear to be very efficient. Shrimp larvae were directly fed with nucleotides and were then compared with the feeding value of *Artemia nauplii*. In a two-rearing-cycle experiment in *Penaeus monodon* larvae, *Artemia* was completely replaced by nucleotides. The pre-trial period of five days started with mysis-3. In each rearing cycle there were 2,400 mysis stocked in six glass aquaria of 80 litres capacity and subdivided into two groups of three replicates. The stocking density was five larvae per litre of water. The true trial period lasted for 15 days for each cycle.

The control group (*Artemia*) was fed with 10 pieces *Artemia* per larvae at each feeding time. The other group received nucleotides at the daily rate of 20% of larvae's body weight. There were four feeding times a day with the first one at six am and the last at ten pm.

# Aquaculture

The nutritional value of the dry matter of both feed substances is markedly different and in favour of nucleotides.

In trials with post larvae parameters, such as weight gain and feed conversion, the rate cannot be recorded as the results may be very uncertain. The parameters for these kinds of trials are observations of the general health and the survival rate of post larvae as well as certain stress tests imposed. The survival rate of the first cycle was for both groups better than the survival rate for the second cycle. In comparison to the *Artemia* group, the nucleotide group has an improved survival rate of 7.4% in the first cycle and 18.4% in the second cycle, respectively. The difference, however, is statistically non-significant (*Table 4*). The general health analysis for both groups has not shown any differences. Both groups of animals exposed to various stress tests for determination of stress resistance have shown equally stress resistance capacity. For the three tests (salinity test [reducing the

salinity by 25 ppt and record survival after three hours], formalin test [animals are exposed to 200 ppm formalin, survival is recorded after two hours], and temperature test [water temperature reduced by 10°C, survival recorded after one hour]) there was a survival rate of 100%.

In addition to the immunostimulatory properties, nucleotides have a good feeding value when fed directly to early stage shrimp larvae. Microscopic observations of the animals' gut revealed that nucleotides are accepted and consumed well by the larvae. Based on the trial results, nucleotides are a suitable alternative to *Artemia* for feeding *Penaeus monodon* larvae. ●

*For all trials, the nucleotide formula Vannagen, produced by Chemoforma A.G., Augst/Switzerland has been used.*

*References are available on request from the editor  
Dick Ziggers: dick.ziggers@reedbusiness.nl*