

## Nucleotides: A stress reliever and growth enhancer in shrimp farming

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### Summary

The stress elevating properties of nucleotides were investigated in aquarium trials by changing periodically the salinity from 20 ppt to 30 ppt or maintaining a permanent high salinity (45 ppt) situation as an induced stress. Feed containing nucleotides at levels of 0.1% and 0.2%, respectively fed to shrimp (*Penaeus monodon*) developed much better than the control group without nucleotides. The weight gain improved by 17.8% and 24.7% depending on the inclusion rate of nucleotides. The feed conversion rate was improved by 27.9% and 34.8%, respectively. In a follow-up trial with a maintained salinity of 45 ppt nucleotide levels of 0.2% and 0.4% were tested. The performance of the 0.2% nucleotide group was similar to the results of the previous trial with periodically changing salinities while the 0.4% nucleotide group showed a specific growth rate which was 41.9% significantly better than that of the control group. The feed conversion rate was significantly improved by 42.9%.

A pond trial under research conditions has had the objective to confirm the nucleotide effect of the aquarium trial. The pond trial on *Penaeus monodon* lasted for 98 days with a net stocking rate of 23 pieces/m<sup>2</sup> and an initial live weight of 0.1 g. The salinity varied between 35 ppt and 41 ppt with an average of 38 ppt. Feed provided with 0.2% nucleotides produced 15.8% more biomass per ha than the control group, although the pond water of the trial group suffered from an unstable phytoplankton bloom. The feed conversion rate was 23.8% significantly different from the control group.

In addition, nucleotides have also been successfully used as the only food for post larval rearing in replacement for *Artemia*. In two-rearing-cycle experiment on black tiger shrimp (*Penaeus monodon*) larvae, *Artemia* was completely replaced by nucleotides. The pre-trial period of five days started with mysis-3 stage animals. The true trial period with three replicates per group lasted for 15 days for each cycle. The nutritional value of *Artemia* and nucleotides (based on dry matter) was substantially higher for *Artemia*, but the HCl-pepsin digestibility for nucleotides was almost double that for *Artemia*. Due to the higher fat content, the calculated digestibility was in favour of nucleotides. In comparison to the *Artemia* group, the nucleotide group had better survival rates of 7.5% (first cycle) and 18.4% (second cycle), but differences were statistically not significant. The general health analyses for both groups did not show any differences when exposed to various stress tests (salinity test, formalin test, temperature test). This means that both groups have equal stress resistance capacity. In a follow-up trial with animals from the second cycle, the long term effect of nucleotides was tested. The results confirmed the previous findings.

## **Introduction**

Immunity is the power of the 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 (Raa 1996). 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 (Soderhall and Cerenius 1992) although the presence of a specific immune memory in shrimp is still disputed.

## **Immunostimulants**

Immunostimulants or immunostimulatory substances influence the immune system of cultured shrimps by application via the feed. Raa (1996) has classified immunostimulants as follows:

- Nucleotides
- Glucans
- Bacterial products
- Products from mycelial fungi
- Peptides from animal products
- Cytokines

Among these, nucleotides and glucans are probably the most promising substances for strengthening the immune systems. They provide resistance to diseases and help overcome stress situations in animals.

However, there are specific differences between both substances. Both substances are extracted from yeast cell walls. While nucleotides are derived from the "insides" of yeast cells, glucans are derived from the "outside" cell wall (Ancieta-Probstetl et al. 2005). There are also differences in the immunostimulatory effect. According to Smith et al. (2003), the effect of glucans in shrimps may be limited and under certain situations also detrimental. Findings by Scholz et al. (1999) suggest that the application for more than 43 days results in negative response and Chang et al. (2000) proposes that the administration of glucan diet must be limited to three weeks.

Nucleotides, on the other hand, are the basic building blocks of the nucleic acids DNA and RNA. They are merely a biological active multi-compound-complex with the following main active substances:

- Purified nucleotides
- Purified RNA (Ribonucleic acid)
- Precursors of nucleotides
- Organic acids

Research suggests that nucleotides are "semi-essential" nutrients (Carver and Walker 1995; Devresse 1998). 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 (Rudolph et al. 1990). The precise mode of action has not yet been fully established. But without any doubts, it helps the shrimps to overcome immuno-suppressive conditions and in this way it stimulates the immune 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

These are reasons that nucleotides are used commercially as feed additive for the improvement of animals' growth or disease resistance (Portsmouth 1993). Several trials have demonstrated that nucleotides in shrimp feed has a positive effect on growth, feed conversion and survival of animals (Promchaiwong 1995; Applebaum S., pers. comm. 1999; Achupafas 2000; Ancieta-Probstl et al. 2005).

In the following experiments, the efficacy of nucleotides will be discussed which have been conducted by the R&D-Unit of The Waterbase Ltd., Nellore/India<sup>1</sup>.

#### *Nucleotides as stress reliever*

Stress is considered as a mental or physical tension. Cultured shrimps are always under a certain tension, although the stress as such cannot be accurately measured. The major pressure on cultured shrimp causing stress is the environment where the animals are living in this means 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 because the stronger the immunity system of an animal, the better it can manage stress situations.

In the first trial in juvenile *Penaeus monodon* at an initial liveweight of 6.97 g the effect of nucleotides by periodically changing the salinity of the water-as a stress factor-was tested. The organisation of the trial is shown in *Table 1*. 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.

Table 1. Salinity as stress relieving factor (trial set-up)

	Trial	
	1	2
Aquaria (no.)	6	6
Water capacity (l/each)	450	450
Trial duration (days)	60	60
Groups (no.)	3	3
Replicates (no.)	2	2
Stocking density (pieces/m <sup>2</sup> )	16	18
Salinity (ppt)	20+30	45

<sup>1</sup>For all trials the nucleotide formula Vamagen, produced by Chemofarma A.G., Augst/Switzerland has been used

Table 2. Response of *Penaeus monodon* fed on nucleotides supplemented feed under periodically changed stress (salinity) condition

	Control	0.1% <sup>1</sup>	0.2% <sup>2</sup>
Shrimps ( <i>Penaeus monodon</i> ) (no.)	30	30	30
Replicates (no.)	2	2	2
<i>Feed</i>			
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
<i>Shrimps' performances</i>			
Initial liveweight (g)	6.96	6.96	7.00
Final liveweight (g)	12.46	13.44	13.90
Weight gain (g)	5.50a	6.48b	6.90b
(rel.)	100.0	117.8	124.7
Feed conversion rate (1:)	3.24a	2.38b	2.12b
(rel.)	100.0	73.5	65.4
Protein efficiency ratio (1:)	1.55a	1.12b	1.01b
(rel.)	100.0	72.2	65.2
Molting (rel.)	100.0	106.1	127.3
Mortality (%)	46.7a	6.7b	6.7b

<sup>1</sup>Vanagen inclusion rate

<sup>2</sup>Calculated

Values in the same row with different letters denote significant differences between means ( $p < 0.05$ ).

The induced stress was applied at day 25 of the trial. Every 10 days the salinity was decreased and increased respectively, by 10 ppt. The control group (without nucleotides) suffered particularly from the frequently changed salinity, as shown by the losses. After each change of the salinity the cumulative mortality increased drastically. The differences in the mortality rate were statistically significant (Table 2).

As long as the water's salinity was normal, the weight development of all groups was almost the same. However, when the salinity was changed (reduced) for the first time (at day 25), the efficacy of nucleotides in the feed was visible. Both treated groups outperformed the control group. This was the case until the completion of the trial. For the whole trial period the weight gain of both treated groups was statistically significantly higher (+17.8% and +24.7%) than that of the control group. On the other hand, the differences in weight gain between both treated groups were statistically non-significant (Table 2).

Statistically significant difference was observed in feed conversion between the treated groups and the control group. This indicates that the feed was substantially better utilised by the nucleotides groups. The feed conversion between the treated groups was statistically not significant. Utilisation of feed protein by the nucleotides groups was superior to the control as shown by the protein efficiency rate.

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 kg of feed was supplemented with 0.2% and 0.4% nucleotides respectively (Table 3). The daily feeding rate was 8.0% of animal's body weight and divided into four feeding times.

Extremely high salinity was applied from the first day of the trial. A salinity of more than 35 ppt was reported to significantly affect the growth of shrimp (Boyd 1998)

Table 3. Response of *Penaeus monodon* on nucleotides supplemented feed under extreme salinity conditions

	Control	0.2% <sup>1</sup>	0.4% <sup>1</sup>
Shrimps (no.)	32	32	32
Replicates (no.)	2	2	2
<i>Feed</i>			
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
<i>Shrimps' performances</i>			
Initial liveweight (g)	6.55	6.48	6.43
Final liveweight (g)	10.77	12.15	12.31
Specific growth rate (%)	64.4a	87.5b	91.4b
(rel.)	100.0	135.9	141.9
Protein efficiency ratio (%)	1.45a	1.03b	0.79b
(rel.)	100.0	71.0	54.5
Molting (no.)	107	110	114
Mortality (%)	25.0a	15.6b	15.6b

<sup>1</sup>Feed fortified with "Vanmagen"

Values in the same row with different letters denote significant differences between means ( $p < 0.05$ )

The protein efficiency ratio of the treated groups was 29.0% and 45.0%, respectively, significantly superior to the control which was extremely poor in the high salinity situation. Under such conditions, the food intake will remain normal, but growth will be retarded because the ingested nutrients are required for reducing the osmotic pressure from which the animals are suffering from. The nucleotides, on the other hand, helped the treated animals to manage this stress situation resulting in better development.

The differences in mortality between the control and treated trial groups were significant. However, compared with the first trial the mortality rate in the control group was substantially lower in the second trial. This study showed that periodically changed salinity was a greater stressor to the animals than permanent extremely high salinity.

#### *Nucleotides as performance promoter*

As aquaria trials only provide basic information and is not a guarantee that findings from the aquaria can be repeated under pond conditions, a pond trial under research conditions was therefore conducted.

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The size of the trial ponds is 0.3 ha (control) and 0.4 ha (treatment) respectively; the net stocking rate was 23 pieces/m<sup>2</sup>. Post larvae of *Penaeus monodon* had an initial liveweight of 0.1 g. 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 was higher than the 18 ppt to 25 ppt recommended salinity by Boyd (1998).

Table 4. Response of *Penaeus monodon* fed nucleotides supplemented feed under pond conditions

	Control	0.25% <sup>1</sup>	Control=100%
Stocking density (pcs/m <sup>2</sup> )	23	23	
<i>Feed</i>			
Crude protein (%)	44.3	44.7	
Protein digestibility (%)	91.6	91.8	
Calcium (%)	2.11	2.18	
Phosphorous (%)	1.35	1.35	
Digestible energy <sup>1</sup> (MJ/kg)	14.2	14.2	
<i>Shrimps' performances</i>			
Initial liveweight (g)	0.1	0.1	
Final liveweight (g)	12.54	13.77	109.8
Biomass produced (kg/ha)	1,959.0a	2,269.3b	115.8
Feed consumed (kg/ha)	3,950.0a	3,432.5b	89.9
Feed conversion rate (1:)	2.02a	1.51b	74.8
Protein efficiency ratio (1:)	1.01 <sup>a</sup>	0.77 <sup>b</sup>	76.2
Survival rate (%)	68.0	71.5	105.4
Mortality (%)	25.0a	15.6b	62.4

<sup>1</sup> Feed fortified with "Vanmagen"

Values in the same row with different letters denote significant differences between means ( $p < 0.05$ )

The weight gain of the nucleotide-feed fed animals was 9.8% and significantly better than that of the control shrimps (Table 4). This corroborates with the findings by Promchaiwong (1995) and Achupalax (2000) in pond trials. However, from the early stage of the trial there was a severe bioluminescence problem in the pond of the nucleotide fed animals. Due to that it was difficult to maintain a proper and stable phytoplankton bloom. Bioluminescence problems and the frequent collapse of phytoplankton bloom caused stress to shrimps and often resulted in very poor growth and low survival (Armanath, pers. comm 2004).

This indicates that the nucleotide-enriched diet has a positive impact on shrimp performances under stress conditions. This is due to the fact that nucleotide treatment increases the number of granulate hemocytes as stated by Fegan (2002) and Ancieta-Pröbstl et al. (2005) and hemocytes can help to 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 ha. The nucleotide-diet group produced significantly 15.8% more biomass than the control group (Table 4).

The nucleotide group consumed 13.1% less feed than the control. This is reflected in the feed conversion rate, too. The difference between both groups is 25.2% in favour of the nucleotide group. The latter has also utilised the feed protein more efficiently. Expressed as the protein efficiency ratio the difference is 23.8%. The differences of all parameters are statistically significant.

Under unfavourable pond condition (high salinity, poor bloom maintenance) nucleotides have demonstrated its strength as a stress reliever in shrimps and at the same time its performance promoting capacity.

Table 5. Performances of black tiger shrimp (*Penaeus monodon*) post-larvae after feeding *Artemia* and nucleotides (glass aquaria /trial duration: 15 days per cycle)

	<i>Artemia</i>		Nucleotides		Difference ( <i>Artemia</i> ) = 100%)
	Mean	SD±	Mean	SD±	
Mysis stocked (no.)	1,200		1,200		
Replicates (no.)	3		3		
Post-larvae harvested					
First cycle (no.)	108	116			
Survival (%)	27.0	15.0	29.0	12.0	107.4
Second cycle (no.)	no.	51		61	
Survival (%)	12.8	10.0	15.1	8.0	118.4

### *Nucleotides as the only larval shrimp feed*

The major food for shrimp larva is *Artemia*. The nutritional value of *Artemia nauplii* can be increased by feeding them with 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 improve and the survival rate will increase.

This procedure, however, appears to be not very efficient. Shrimp larvae were therefore, directly fed with nucleotides and 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, 2,400 mysis stocked in six glass aquaria of 80 litre capacity were 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 per 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 600 hours and the last at 2200 hours. The nutritional value of the dry matter of both feed substances is quite different and in favour for the nucleotides.

In trials with post larvae, parameters such as weight gain and feed conversion rate cannot be recorded or the results may be very uncertain. The parameters for this kind of trials were observations of the *Penaeus* larvae (PL) general health, the survival rate and certain stress tests. The survival rate of the first cycle was for both groups better than the one for the second cycle. In comparison to the *Artemia* group the nucleotide group has a 7.4% (first cycle) and 18.4% (second cycle) respectively, better survival rate. However, the difference was statistically non-significant (Table 5).

The general health analysis for both groups had not shown any differences (Table 6). Animals exposed to various stress tests for determination of stress resistance had shown that both groups have equal stress resistance capacity (Table 6). For the three tests (salinity test [reducing the salinity by 25 ppt and record survival after three hours], formalin test [animals were exposed to 200 ppm formalin, survival was 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.

Table 6. Health analysis of black tiger shrimp (*Penaeus monodon*) post larvae at the end of each rearing cycle

	Artemia		Nucleotides	
	1 <sup>st</sup> cycle	2 <sup>nd</sup> cycle	1 <sup>st</sup> cycle	2 <sup>nd</sup> cycle
Activity	Good	Good	Good	Good
Size	Normal	Slender	Normal	Slender
Gut:muscle ratio	1:4	1:4	1:4	1:4
Hepatopancreas	Normal	Normal	Normal	Normal
Gut	Full	Full	Full	Full
Deformities	Nil	Nil	Nil	Nil
Size variation	Higher	Higher	Lower	Lower

## Conclusion

Supplementation of nucleotides in shrimp feed could enhance the immune system, relieve stress situations, promote animals' performances, and can replace *Artemia* in larval feeding.

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