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Use of a unique peptide in shrimp culture for enhancing shrimp tolerance to environmental stresses

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Use of a unique peptide in shrimp culture for enhancing shrimp tolerance to environmental stresses

SUMMARY

Laboratory and field tests in which *Vital Peptide* as a nutrient was delivered to both post larvae (PLs) during their nursery phase culture via *Vital Peptide*-enriched Artemia Nauplii *(eAN)* and to young shrimp during their grow-out phase by incorporating *Vital Peptide* into the shrimp dry feed, have shown a positive effect on the shrimp's growth rate, their survival rate and, in particular, on the ability of the shrimp to withstand stress conditions.
Furthermore, the longer the duration of the feeding of PLs with *Vital Peptide*-enriched Artemia Nauplii *(eAN)* is, the better the PLs are able to withstand induced stresses.

INTRODUCTION

In recent years more and more attention has been given in shrimp culture worldwide to the aspect of shrimp tolerance in resisting the environmental stresses affecting their survival and growth parameters. The following report provides an approach to an effective delivery of the unique nutrient *Vital Peptide* to shrimp PLs during their nursery stage culture and later on throughout their grow-out stage, for enhancing their tolerance to environmental stresses, and for significantly improving their survival rates and growth parameters.

Vital Peptide

Vital Peptide, an animal protein processed from edible poultry by-products at slaughter, is characterized by a high content of low molecular weight compounds, particularly bio-active peptides.
An effective and efficient delivery of *Vital Peptide* to the shrimp at their early stages of development, as well as later on during grow-out stages, can be best achieved through Artemia Nauplii, which are a commonly used major live feed for shrimp throughout the early stages of development.

Artemia salina

Artemia, commonly known as "brine shrimp", belong to the phylum Arthropoda, class Crustacea and are a small shrimp found in the salt lakes of temperate zones.

Artemia are zooplankton such as Copepods and Daphnia, which are used both at their Nauplius and adult stages, as live food in the aquarium and in most fish and shrimp cultures worldwide.

Artemia cyst hatching

Following the incubation instructions given by the supplier of the Artemia cysts, the hatching rate should reach that given by the supplier.

A simple method for incubating Artemia cysts:

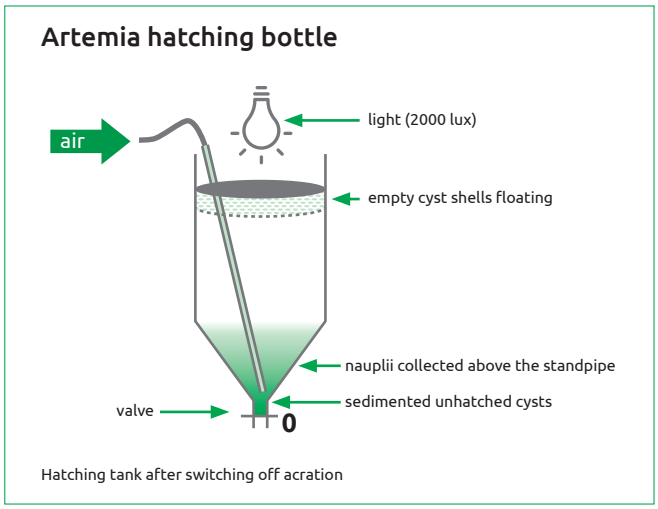

Fig. 1. *Artemia hatching container.*

Enrichment of Artemia Nauplii

Artemia are non-selective filter feeders and therefore will ingest a wide range of foods. The main criteria for food selection are particle size (25-30 µm), digestibility, and nutrient levels.

Artemia enrichment not only improves the survival, growth and success of the metamorphosis of many species of fish and crustaceans, but also increases their quality, e. g. reduced incidence of malformations, as well as improved pigmentation and stress resistance.

The same bio-encapsulation method is being developed for the oral delivery of vitamins, chemotherapeutics and vaccines to target fish and shrimp species.

MATERIALS AND METHODS

Vital Peptide, due to its high content of low molecular weight compounds and, in particular, its bio-active peptides *(Fig. 2),* appears to be a potentially a promising nutritional additive for shrimp throughout their culture.

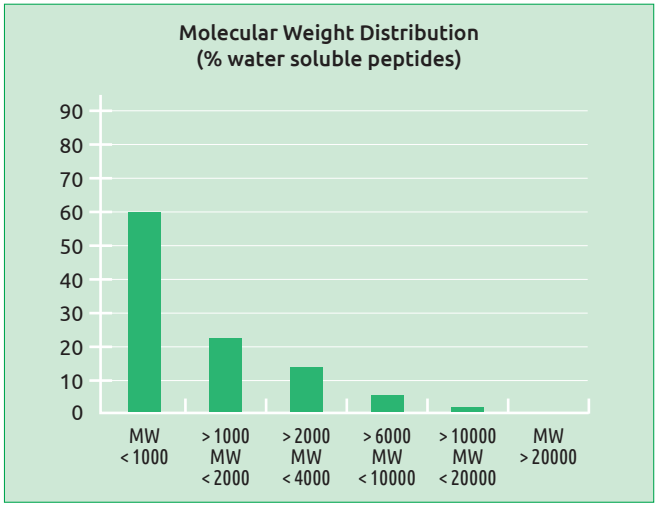

Fig. 2. *Molecular size distribution of Vital Peptide.*

The crucial question remains: what is the most efficient and effective way to deliver *Vital Peptide*, which is produced in a very fine powder form (< 200 µm), to the shrimp at their early stages of development?

Obviously, the answer is via enrichment of Artemia Nauplii with it and provision of the *Vital Peptide*-enriched Artemia Nauplii *(eAN)* to the shrimp PLs, once their active feeding has started.

The actual procedure of enrichment of Artemia Nauplii is as follows:

Newly hatched Artemia Nauplii, when their active feeding begins, about 24 h post-hatching, are kept in salt water (~30 ppt) at 27 °C under constant illumination, and provided with a gentle aeration for just a moderate water motion. The Nauplii are then supplied only with *Vital Peptide* in its very fine powder form, well-distributed over the water surface.

After 10–24 h, the Artemia Nauplii become fully enriched with *Vital Peptide.* These enriched Artemia Nauplii (*eAN*) are ready to be fed to the shrimp PLs.

The feeding of shrimp PLs with *eAN* should continue during the entire nursery phase of the culture, regardless of the supplementation of additional shrimp PLs feeds.

However, when additional supplemented feed is used, it is recommended to first feed the shrimp with the eAN and thereafter to provide the additional feed.

Recommended nursery phase culture system

Shrimp PLs are kept under intensively controlled conditions in containers (depth of around 1 m) with a water volume allowing an easy observation to ensure good control of the PLs' general behavior, feeding behavior, and their overall condition, to be observed daily.

Water and compressed air are introduced into the containers in such a way that allows only a gentle water motion in the container while the required water chemistry is guaranteed.

Bubbling air and a water current that are too strong in the rearing tank will disturb the shrimp PLs behavior and efficient feeding.

Some shading over the containers is recommended to avoid exposure to strong sunlight, but it should allow equal light over the entire container.

Grow-out phase

Vital Peptide should be added to the dry feed has to be done not simply by just mixing the dry feed with *Vital Peptide;* instead, the fry feed, whether in pelleted or granular form, must first be finely ground, into which *Vital Peptide*, also in its finely ground form, is added. They should be well mixed and then re-pelleted. Such a procedure ensures that *Vital Peptide* becomes an integrated ingredient of the dry feed to be consumed by the shrimp and not be lost or dissolved in the water before the shrimp can benefit from it.

Measurements and observations

The PLs' behavior, their feeding and mortality in the culture container are observed daily.

Once every 10 days, the weights of a representative sample of PLs (> 50) are determined.

Along with the regular observation of mortalities, a more accurate PLs survival rate is estimated at the end of the nursery phase when the shrimp harvesting takes place.

For determining the final, more accurate, survival rate, the numbers of shrimp per representative water samples are counted.

PLs feeding

Feeding of shrimp PLs is performed several times daily to ensure a continuous availability of fresh *eAN* to the PLs which are continuous feeders.

Stress tests

Once a month, representative samples (> 60) of PLs are exposed for 30 min to the following stress tests:

1. Sea water at a temperature of 13 °C.
2. Fresh water (potable water).
3. Low oxygen level.
4. Sea water at a temperature of 36 °C.

The numbers of non-survivors are counted.

RESULTS

The following results were obtained from laboratory and field trials in shrimp farms.

Stress tests results

The following are the results of four stress tests performed on PLs that were fed during their nursery phase culture for 17 days with enriched Artemia Nauplii (*eAN*) compared with PLs that were fed non-enriched Artemia Nauplii. The results show that compared with other enrichments like *AquaTrac* (a processed animal protein from edible poultry by-products at slaughter, characterized by high content of both crude protein (70 %) and in vivo shrimp digestible crude protein (95 %), pellet binding power and excellent shrimp palatability) or backing yeast, the PLs that were fed *Vital Peptide*-enriched Artemia Nauplii could, on average, best withstand the stress tests.

Furthermore, PLs under the same culture conditions that were fed dry feed, both with and without bio-flocs, withstood these stress tests, in terms of survival, even slightly better *(Tab. 1, Fig. 3)*.

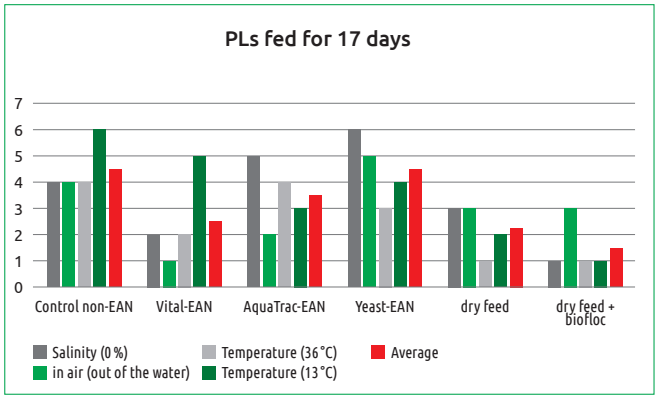

Fig. 3. *Mortality rates of stress tests performed on shrimp PLs fed, for 17 days, during their nursery phase, either enriched Artemia Nauplii (eAN) or dry feed with and without bio-flocs.*

Vital Peptide-fed PLs were, on average, more resistant to the stress tests than PLs fed enriched Artemia with other enrichments and also, performed comparably well with the dry-feed-fed PLs, both with and without bio-flocs *(Tab. 2, Fig. 4)*.

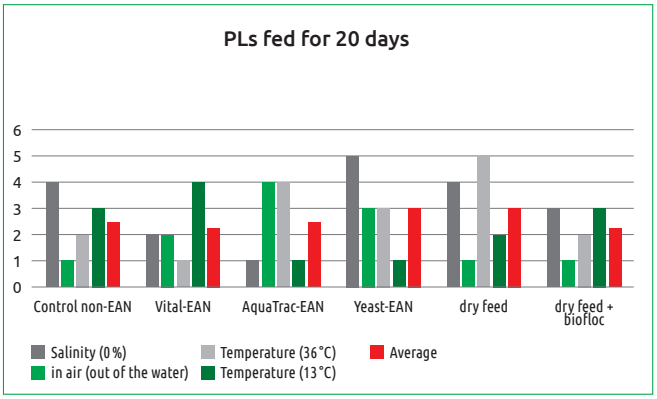

Fig. 4. *Stress test mortality performed on shrimp PLs fed for 20 days, during their nursery phase, either enriched Artemia Nauplii (eAN) or dry feed with and without bio-flocs.*

Tab. 1. Stress test mortality performed on shrimp PLs fed for 17 days, during their nursery phase, either enriched Artemia Nauplii (*eAN*) or dry feed with and without bio-flocs. (Numbers in the tables and in the graphs represent ranking positions between: 1–6, 1 for best withstanding of stress, 6 for least).

Feed	Salinity (0 %)	Low oxygen level	Temper-ature (36 °C)	Temper-ature (13 °C)	Average
Control, non-eAN	4	4	4	6	4.5
Vital-eAN	2	1	2	5	2.5
AquaTrac-eAN	5	2	4	3	3.5
Yeast-eAN	6	5	3	4	4.5
Dry feed	3	3	1	2	2.25
Dry feed + bio-floc	1	3	1	1	1.5

Tab. 2. *Stress test mortality performed on shrimp PLs fed for 20 days, during their nursery phase, either enriched Artemia Nauplii (eAN) or dry feed with and without bio-flocs. (Numbers in the tables and in the graphs represent ranking positions between: 1–6, 1 for best withstanding of stress, 6 for least).*

Feed	Salinity (0 %)	Low oxygen level	Temper-ature (36 °C)	Temper-ature (13 °C)	Average
Control, non-eAN	4	1	2	3	2.5
Vital-eAN	2	2	1	4	2.25
AquaTrac-eAN	1	4	4	1	2.5
Yeast-eAN	5	3	3	1	3
Dry feed	4	1	5	2	3
Dry feed + bio-floc	3	1	2	3	2.25

However, when the PLs under the same culture conditions were continuously fed for an additional few days with the same enriched Artemia Nauplii, the stress results again showed that

Thus, feeding shrimp PLs longer on *Vital Peptide*-enriched Artemia Nauplii (*eAN*) further enhances the shrimp's ability to withstand environmental stress conditions and in addition, enhances their growth when compared with feeding non-enriched Artemia Nauplii or enriched with other materials *(Fig. 5)*. It is therefore beneficial to feed PLs during their entire nursery phase with *Vital Peptide*-enriched Artemia Nauplii (eAN) along with dry feed plus bio-flocs for obtaining a high growth rate, a high survival rate, and the best ability to withstand environmental stresses.

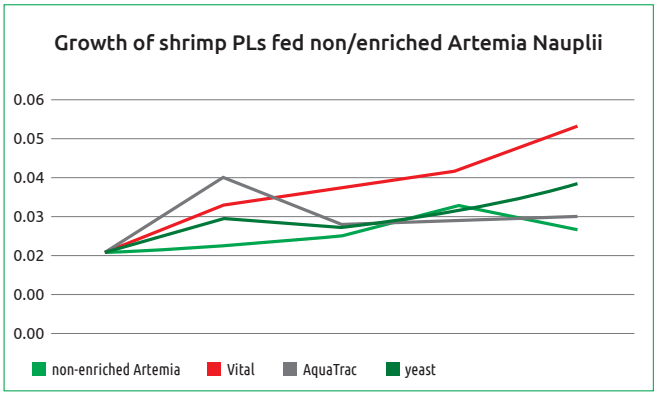

Fig. 5. Growth of shrimp PLs fed for 30 days with enriched Artemia Nauplii *(eAN)* or non-enriched Artemia Nauplii (Numbers in Gr.).

Feeding at the grow-out phase

Young shrimp that completed their nursery phase were stocked in rearing containers of 300 l at a density of 250 individuals per tank and were fed granulated dry feed either with or without *Vital Peptide* (5 %).

Within seven days of culture at the grow-out phase, shrimp groups that were fed granulated dry feed containing *Vital Peptide* showed a higher daily gain in weight compared with shrimp groups that were fed the same dry feed without *Vital Peptide*. The slight differences in the initial weight between the *Vital* and the control groups are due to the culture of the PLs at the nursery phase, during which the *Vital* groups received *Vital Peptide* enriched Artemia Nauplii *(eAN)*, while the control groups received non-enriched Artemia Nauplii *(Tab. 3)*.

Growth results at the nursery phase

Tab. 3. *Growth of young shrimp at the grow-out phase.*

Tank ID	Initial weight (DOC 1)	First Sampling (DOC 7)	Daily Gain (g/day)
Vital 1	0.62	0.94	0.046
Vital 2	0.62	1.07	0.064
Vital 3	0.68	1.01	0.047
Vital 4	0.68	1.05	0.053
Control 1	0.57	0.82	0.036
Control 2	0.57	0.90	0.047
Control 3	0.55	0.89	0.049
Control 4	0.55	0.90	0.05

DOC: day of culture

Tab. 4. *Growth of young shrimp at the grow-out phase.*

Tank ID	Water Volume (L)	Treat-ment	Initial Stock	Shrimp/ Volume (pcs/L)	Initial ABW (g)	Current ABW (g)	Daily Gain (g/day)	Est. Survival Rate (%)	Initial Biomass (g)	Est. Biomass (g)	Biomass/ Volume (kg/m3)	Cum. FCR
E1	400	Vital	80	0.20	0.62	2.82	0.063	90	49.60	203.0	0.51	2.25
E2	400	Vital	80	0.20	0.62	3.11	0.071	90	49.60	223.9	0.56	1.98
E3	400	Vital	80	0.20	0.68	2.50	0.052	90	54.40	180.0	0.45	2.74
E4	400	Vital	80	0.20	0.68	2.91	0.064	90	54.40	209.5	0.52	2.22
Average		**Vital**	**80.00**	**0.20**	**0.65**	**2.84**	**0.062**	**90.00**	**52.00**	**204.12**	**0.51**	**2.30**
C1	400	Control	80	0.20	0.57	2.38	0.052	90	45.60	171.4	0.43	2.74
C2	400	Control	80	0.20	0.57	2.41	0.053	90	45.60	173.5	0.43	2.69
C3	400	Control	80	0.20	0.55	2.16	0.046	90	44.00	155.5	0.39	3.09
C4	400	Control	80	0.20	0.55	2.71	0.062	90	44.00	195.1	0.49	2.28
Average		**Vital**	**80.00**	**0.20**	**0.56**	**2.42**	**0.053**	**90.00**	**44.80**	**173.88**	**0.43**	**2.70**

DOC: day of culture/ABW: average body weight/ADG: average daily growth

In another experiment young shrimp, again after completing their nursery phase, were stocked in rearing containers of 400 l each at a stocking density of 20 individuals per liter for grow-out. Shrimp were fed granulated dry feed containing *Vital Peptide* (5 %) in the test groups and the same feed but without *Vital Peptide* in the control groups.
After 35 days of rearing, growth parameters and survival rates were evaluated and are presented in *Tables 4 and 5*.
Shrimp that received *Vital Pepetide* in their feed reached a higher average body weight compared with the control groups, which resulted in a significant difference of the final shrimp biomass in favor of the groups receiving *Vital Peptide* in the feed. No significant difference was found in the food conversion ratio, the average daily growth, and the survival rates between the test groups and the control groups.

Tab. 5. *Growth parameters of young shrimp at the grow-out phase.*

Parameter	Vital	Control	Statistical Analysis
DOC	35	35	-
Initial ABW (g)	0.65	0.56	-
ABW (g)	2.84	2.42	s.d.
ADG (g/day)	0.062	0.053	n.s.d.
Biomass (g)	204.12	173.88	s.d.
FCR	2.3	2.7	n.s.d.

DOC: day of culture/ABW: average body weight/ADG: average daily growth
s.d. = significant different/n.s.d. = not significant different

CONCLUSIONS

Artemia Nauplii are not only a globally common live feed for fish and shrimp at their early stages of development, but they can also be used as an excellent deliverer of nutrients, medicine, etc. to baby fish and shrimp.
Artemia Nauplii are known as filter feeders, meaning that while they constantly move in the water, they filter from the water any particles small enough for them to take in.
This unique way of feeding makes it possible to deliver to the Artemia Nauplii nutrients that have been found beneficial for baby fish/shrimp but which the Artemia Nauplii in their natural form do not possess. This procedure is known as the enrichment of Artemia Nauplii *(eAN)*.
Vital Peptide, due to its high content of low molecular weight compounds and its bio-active peptides, is considered to be a potentially promising nutritional additive for shrimp, and can be most efficiently delivered to shrimp PLs, once their active feeding has started, via *Vital Peptide*-enriched Artemia Nauplii *(eAN)*.

So far, the laboratory and field tests, in which *Vital Peptide* as a nutrient has been delivered both to PLs during their nursery phase culture via *Vital Peptide* enriched Artemia Nauplii *(eAN)* and to young shrimp during their grow-out phase by incorporating *Vital Peptide* into the shrimp dry feed, have shown a positive effect on the shrimp's growth rate and their survival rate and, in particular, on the ability of the shrimp to withstand stress conditions.
Further, longer feeding of PLs with *Vital Peptide*-enriched Artemia Nauplii *(eAN)* enhances the ability of the PLs to withstand induced stresses.

In conclusion, it is advisable to feed PLs during their entire nursery phase with *Vital Peptide*-enriched Artemia Nauplii *(eAN)* along with dry feed plus bioflocs to obtain a high growth rate, a high survival rate, and a higher ability to withstand environmental stresses.

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