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Effect of Feeding *Artemia* Enriched with Stresstol and Cod Liver Oil on Growth and Stress Resistance in the Indian White Shrimp *Penaeus indicus* Postlarvae

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Abstract

Artemia franciscana preadults were enriched with emulsified codliver oil and stresstol, a medicinal plant product having the properties of giving tonic to resist stress and improve growth and vigor. Both enrichment and evacuation time in the *Artemia* were studied. A maximum of 127 and 72 min were required to enrich and evacuate 100% of gut of *Artemia*, respectively. *Penaeus indicus* postlarvae (PL-20) fed with preadult *Artemia* and *Artemia* enriched with emulsified fish oil, stresstol as well as oil mixed with stresstol consumed *Artemia* more or less at the same rate. However, the absorption efficiency of *P.indicus* postlarvae fed with enriched *Artemia* differed from those fed with the non-enriched *Artemia*. The non-enriched *Artemia* fed postlarvae grew at an average rate of 19.3 mg/g/day, whereas the postlarvae fed with the stresstol-enriched *Artemia* had an average growth rate of 31.3 mg/g/day. Postlarvae fed with the fish oil-enriched *Artemia* exhibited the highest growth rate at 42.2 mg/g/day. In the stress test, where the postlarvae (fed with enriched and non-enriched *Artemia* for 8 days), were exposed to 0 and 50 ppt salinities, it was revealed that the stresstol-fed postlarvae had increased resistance to stress. Hence, the use of stresstol in the larviculture of *P.indicus* shrimp is recommended.

Introduction

One of the problems in culturing shrimp larvae is food, which accounts for more than 50% of the running costs of shrimp larviculture (Pandian and Marian

1991; Marian 1995). The larval diet of shrimp consists of a wide variety and abundance of both phytoplanktonic and zooplanktonic organisms. This abundance and diversity of food organisms of different sizes and biochemical composition maximize the chances for meeting the nutritional requirements of shrimp larvae. Selection of a suitable diet is normally determined by a number of criteria, based on both the cultured animal and the culturist. For the cultured animal, it must be viewed from both the physical and nutritional points of view. From the physical point of view, availability and acceptability must be considered. From the nutritional point of view, digestibility as well as energetic and nutrient requirements are of utmost importance. For the culturists, the best diet(s) would satisfy the following: the food (s) must be readily available, cost effective, simple and versatile (Léger and Sorgeloos 1994).

One of the aspects of versatility of the larval food, *Artemia*, is its use as a carrier for various dietary components such as essential nutrients, pigments, hormones, prophylactics, therapeutics, vaccines, etc. Some components such as specific fatty acids are not only carried in the gut of the prey but also accumulated in its tissue, leading to high doses of these components in the prey organism.

It has been demonstrated that the most important essential fatty acids for brackishwater and marine crustaceans, fish and molluscs are the (n-3) highly unsaturated fatty acids (HUFA), especially eicosapentaenoic acid 20:5 (n-3) and docosahexaenoic acid 22: 6(n-3). These are essential compounds required for membrane formation, osmoregulation and synthesis of prostaglandins. They also appear to have an activating role in the immune system. Variability in 20:5 (n-3) contents as well as the absence of significant levels 22:6(n-3) in the traditional live feeds used in shrimp larviculture has stimulated research on the commercial development of (n-3) HUFA enrichment products for zooplankton organisms (eg. microcapsules, emulsions, microparticulate diets) as well as formulated feed as supplement/substitute for the zooplankton. The use of (n-3) yeast (TOPAL, INVE Aquaculture M.V.Belgium) as an algal substitute/supplement and the enrichment of *Artemia* in (n-3) HUFA clearly improved culture results (Léger *et al.* 1987). Survival and growth increased significantly when (n-3) HUFA supplementation was applied in the zoea stages (Léger and Sorgeloos 1994). Further improvements are noticed when extra (n-3) HUFA enrichment was provided in the mysis and postlarvae stages. Hatchery operators feeding (n-3) HUFA fortified diets observed more consistent results and production of more robust PL, i.e., PL-5 as big as PL–10 fed non-enriched *Artemia* (Sorgeloos 1988; Chamberlain 1988). Improved nutrition in early larval stages affects not only hatchery outputs but also survival and growth in late postlarval stages.

Several indigenous medicinal plant products have been identified and used in human and higher vertebrates as medicine/tonic to resist stress and to improve growth and vigor (Tamilvanan 1987). Plant products having these properties have been selected, enriched in preadult *Artemia* and fed to the shrimp larvae. This is the first report on the effect of feeding *Artemia* enriched with an indigenous medicinal product on growth and stress tolerance in shrimp larvae.

Materials

Experimental animals

Postlarvae of *Penaeus indicus* (PL–15) were purchased from M.M. Aquapark Hatchery, acclimated and reared up to PL-19. Subsequently, they were starved for 12 hours before the start of the experiment.

Cod liver oil

The cod liver oil, containing both saturated and unsaturated fatty acids (UNIVERSAL GENERICS PVT. LTD., Thane, India) was enriched with vitamin A and Vitamin D_3.

Stresstol

Stresstol is a medicinal product prepared from five plant materials having the properties to resist stress and improve growth, vigor and nervous stimulus. Each plant material was ground into micronized powder (<50μm). The stresstol was prepared according to Table 1.

Table 1. Properties, combination and composition of stresstol.

Sl. No	Ingredients	Actions	Active compounds	Percentage
1	*Withania somniferra*	Nervine tonic	Withanine, withananine, somniferine, somniferinine, tropine, choline, cuscohygrine, isopelletierine, etc	25
2	*Ocimum sanctum*	Antiperiodic febrifuge, Anti-bacterial vermifuge, nervinetonic, dyspepsia, expectorant, etc.	Ocimin	60
3	*Tonospora cordifolia*	Liver corrective, tonic, blood purifier digestive, etc.	Berberine	5
4	*Picrorhiza kurroa*	Antiperiodic livertonic, cathartic anthelmintic	Cathartic acid, piccrorhizin, Picrosides – I, II, and III, Apocyanacin	5
5	*Eclipta erecta*	Alterative, antifungal Hepictonic Chologogue	Ecliptine	5

Methods

Preparation of enrichment diets (Stock solution)

Emulsified fish oil

4 g of cod liver oil and 1 g of poultry egg yolk were mixed and emulsified in 100 ml water.

Stresstol

5 g of stresstol powder was mixed in 100 ml water.

Oil mixed stresstol

5 g of stresstol powder was mixed in 5 g cod liver oil; 5 g of this product was then mixed in 100 ml water.

Bioencapsulation study

The enrichment media for each nutrient i.e., emulsified fish oil, stresstol and oil mixed with stresstol were prepared by diluting 10 ml of stock solutions to 100 ml in a 500 ml beaker. To enrich the *Artemia*, 100 preadults were introduced in each enriched medium with continuous mild aeration. The *Artemia* gut was observed under the microscope at regular intervals of time (10 min). The gut saturation time as well as gut retension/evacuation time were calculated. The enriched preadults were made ready for feeding the postlarvae.

Feeding experiment

Two types of feeding experiments were performed. The first type of experiment was conducted in 8 l of water at a density of 10/l for all types of feeds with 3 replicates. Mild aeration was given continuously in order to keep the O_2 level optimal. *Artemia* were fed to the postlarvae of *P. indicus ad libitum* in order to feed the enriched *Artemia* in a shorter period without losing the enriched product. This experiment was performed for assessing mainly the resistance of the PL to osmotic shock after the termination of the feeding experiment.

In the second set of experiment, individuals of *P. indicus* postlarvae (2 no's/l) were reared in a glass aquaria (3 replicates of each) containing 1 liter of sea water. An *ad libitum* feeding regime was applied to all aquaria throughout the experiment and the given food density was adjusted 4 times a day (6.00, 10.00, 14.00 and 18.00h) at the rate of 10, 10, 20 and 60%, respectively. The unfed *Artemia* were collected and food consumption was estimated in terms of mean dry weight of enriched *Artemia*. Feces were also collected 4 times a day (before feeding) and dried in a hot air oven (80^0C). In both studies, before introducing the postlarvae, the length as well as the weight were mea-

sured. The second experiment was mainly performed for bioenergetic studies. After eight days, the postlarvae were sacrificed and the length and weight of the animals were measured. The bioenergetic parameters were calculated following the modified IBP (International Biological Programme) formula of Petrusewicz and Mac Fadyen (1970) and Marian and Murugadass (1991).

The specific growth rate was calculated by using the formula given below.

$$SGR = \frac{(\ln W_2 - \ln W_1)}{(t_2 - t_1)} \times 100$$

where,

W_2 = Final weight at time t_2

W_1 = Initial weight at time t_1

Stress study

In an attempt to evaluate the physiological condition of the postlarvae, the resistance of the postlarvae to osmotic shock was studied, following the method of Tackaert *et al.* (1989).

The postlarvae from the first experiment were fed with enriched *Artemia* for eight days and were put in 5 l glass tanks with a salinity of 0 and 50 ppt. Twenty nine animals were used for each of the 3 replicates. Survival was monitored at regular intervals of 5 minutes for 0 ppt and at 60 minute intervals for 50 ppt, until all animals had died. Postlarvae which did not react to mechanical stimulation with a soft paint brush were considered as dead. The cumulative mortality index (CMI) was calculated by summing up the mortality counts noted at each time interval

$CMI = DX_1 + DX_2 + DX_3 + \ldots\ldots\ldots DX_n$

where D is the number of dead individuals at time X_1, X_2, $X_3 \ldots X_n$.

The higher the CMI value, the lower the salinity resistance. The data were analyzed statistically following Zar (1974).

Results

Bioencapsulation

A. franciscana preadult measuring about 7±0.5 mm, enriched with emulsified fish oil, stresstol and oil mixed stresstol differed in their gut loading time (Table 2).

The enriched *Artemia*, when kept in water and free from the enrichment particles, started digestion. After 54 to 72 min, 90% of the gut was unloaded (Table 3).

Growth study

The PL-20 of *P. indicus* fed with non-enriched *Artemia* alone consumed 164 mg/g/d and the PL fed bioencapsulated *Artemia* consumed 166,172 and

Table 2. Time taken for gut loading of *A. franciscana*.

Enrichment products	Time (min) taken for gut loading (%)		
	50	90	100
Emulsified fish oil	72	109	127
Stresstol	65	89	95
Oil mixed stresstol	54	98	110

Table 3. Time taken for unloading the gut of *A. franciscana*.

Enrichment products	Time (min) taken for unloading (%)		
	10	50	90
Emulsified fish oil	25	66	68
Stresstol	12	40	72
Oil mixed stresstol	10	35	54

165 mg/g/d when fed with enriched emulsified fish oil, stresstol and oil mixed with stresstol, respectively (Table 4).

One-way ANOVA performed on the consumption rate revealed that *P. indicus* postlarvae consumed more or less the same quantity of the enriched/ non-enriched *Artemia* and the consumption did not vary significantly ($F=3, 9=1.64$; $P>0.05$). Absorption in postlarvae fed non-enriched and enriched *Artemia* varied slightly. For instance, the control group fed with non-enriched *Artemia* absorbed 87%. The stresstol enriched *Artemia* was absorbed at the lowest level (81%). When the oil mixed stresstol-enriched *Artemia* was fed to the postlarvae, absorption did not increase significantly. The overall values obtained for the absorption efficiency were analyzed statistically and they were not significantly different ($P>0.05$). The growth rate attained by postlarvae fed with *Artemia* without enrichment was 19.3 mg\g\day. The fish oil enriched *Artemia* fed postlarvae had a growth rate of 42.4 mg/g/day. When stresstol-enriched *Artemia* were fed, the growth rate decreased to 31.5 mg/g/day and to 38.7 mg/g/day, when oil mixed stresstol *Artemia* was given. *Artemia* enrich with emulsified fish oil increased the conversion efficiency from 11.9 to 25.67, i.e, 115% increased due to the effect of fish oil. When stresstol alone was fed to the postlarvae, the increase in conversion efficiency was only 53.8%, whereas the oil mixed stresstol enriched *Artemia* increased the conversion by 96.6% from the control group.

The specific growth rate of the *P. indicus* postlarvae fed with non-enriched *Artemia* was the lowest, while feeding *Artemia* enriched with fish oil resulted in the significantly ($P<0.05$) highest growth (Table 5).

Stress resistance

After eight days of culture, marked differences in the ability of postlarvae to survive the osmotic stress were detected between postlarvae fed with fish oil and stresstol-enriched *Artemia* (Table 6). The percentage of

Table 4. Bioenergetic parameters of *P. indicus* postlarvae fed with non-enriched and enriched *A. franciscana* preadults. Rates are expressed in mg/g/day and efficiencies in percentage.

Treatment products	Production rate	Consumption rate	Feces collection	Absorption rate	Absorption efficiency	Metabolic rate	Conversion efficiency
Control	19.3±2.32	164.4±4.42	3.07±0.15	142.9±3.82	86.9±2.33	123.65±3.30	11.9±1.43
Emulsified fish oil	42.4±3.36	165.7±0.97	3.08±0.27	144.3±6.85	87.0±4.13	101.9±4.85	25.6±2.03
Stresstol	31.50±1.89	171.9±6.35	4.77±0.10	138.8±5.13	80.7±2.98	107.3±3.98	18.3±1.10
Oil mixed stresstol	38.7±3.07	165.4±3.52	4.3±0.23	136.0±2.90	82.0±1.75	97.3±2.07	23.4±1.86

Table 5. Growth characteristics of *P. indicus* postlarvae fed with non-enriched and enriched *A. franciscana*.

Treatment products	Initial length	Final length	Specific growth rate
Control (C)	17.5±2.12	20.5±2.59	4.95±0.594
Emulsified fish oil (a)	17.5±2.12	21.7±3.18	9.07±1.36
Stresstol (b)	17.8±0.35	20.13±1.15	7.32±0.44
Oil mixed stresstol (c)	17.0±0.85	20.8±1.58	8.53±0.68

C Vs a: t = 3.915; P=<0.05
C Vs b: t = 4.432; P=<0.05
C Vs c: t = 5.598; P=<0.05

Table 6. Cumulative mortality index (CMI) values of *P. indicus* postlarvae fed with different diets and exposed to low and high salinity stress (0 and 50 ppt).

Diets	0 ppt		50 ppt	
	CMI	Reduction of stress	CMI	Reduction of stress
Control	937.5	0	1250	0
Emulsified Fish oil	833.3	11.1	1000	20
Stresstol	750	20	850	32
Oil mixed Stresstol	833.3	11.1	950	27

mortality for 0 and 50 ppt are illustrated in Figs. 1 and 2. In 0 ppt, stresstol fed groups had a lower cumulative mortality index than the other groups (Fig 1). After 120 minutes, 12% of postlarvae succumbed to death when no enrichment was given. This mortality increased to 56% at 240 minutes. In enriched groups, all animals succumbed to death at 420 minutes (Fig 2).

Discussion

The essential fatty acid enrichment of live prey organisms is a common practice in marine fish hatcheries and is becoming very popular in shrimp hatcheries (Sorgeloos *et al.* 1987). Fish oil contains the most important essential fatty acids for brackish water and marine shrimps (eicosapentaenoic acid and docosahexaenoic acid). These fatty acids are required not only for membrane formation and osmoregulation but also play an active role in immune systems. Marine shrimp cannot synthesize these fatty acids and they need to acquire them through their diets. Variability in essential fatty acid content as well as low fatty acid levels in traditional live feed used in shrimp larviculture has stimulated research on the commercial development of n–3 HUFA-rich algal substitute supplements (e.g. microcapsule, W-3 enriched yeast products, microparticulate diets, etc.).

It has been shown that feeding *Artemia* nauplii with high HUFA content to *P. monodon* postlarvae increased survival rates (Millamena *et al* 1988; Abelin 1991). Rees *et al* (1994) noticed the beneficial action of HUFA on the

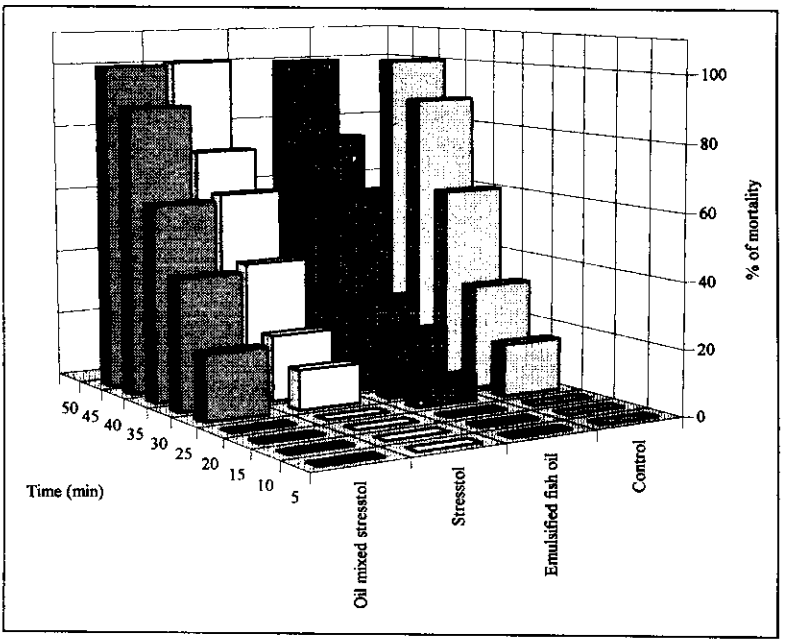

Fig. 1. Mortality (%) of *P. indicus* postlarvae exposed to salinity stress (0 ppt) after being fed non-enriched (control) and enriched *Artemia* for eight days.

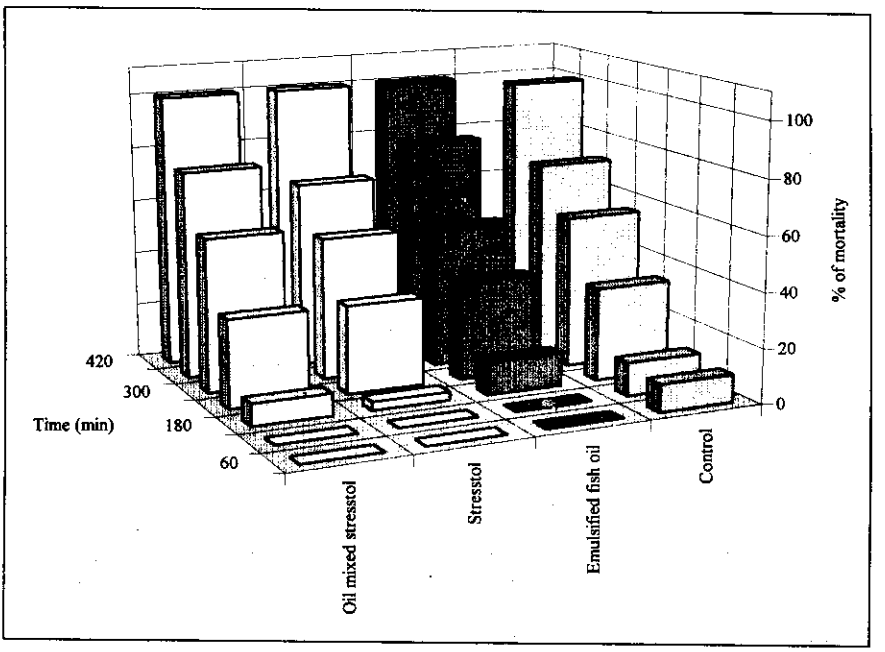

Fig. 2. Mortality of *P. indicus* postlarvae exposed to salinity stress (50 ppt) after being fed non-enriched (control) and enriched *Artemia* for eight days.

survival of *P. monodon*, but failed to see the growth-promoting effect of HUFA. But in *P. indicus*, the growth promoting effect of both emulsified fish oil and stresstol was noticed when they were delivered through the bioencapsulated *Artemia*. The stresstol further enhanced the ability of *P. indicus* to withstand stressful saline conditions. The stress tolerance power of stresstol was higher than the emulsified fish oil. In *P. stylirostris*, Léger *et al.* (1985) found the growth-promoting and stress tolerance effect of HUFA-enriched *Artemia*. Similar situations were observed by Tackaert *et al.* (1989) in shrimp larvae fed HUFA-enriched *Artemia*. Working on the hypoosmotic shock resistance power of erythrocytes of rat fed HUFA-enriched cod liver oil, Horstmark *et al.* (1987) concluded that higher resistance to hypoosmotic shock was due to the higher incorporation of n-3 HUFA in cell membranes. In *P. stylirostris* and *P. Vannamei*, the development of a more ramified structure in the gills resulted when these fish were fed with HUFA-enriched *Artemia*. The correlation of the gill structure with an increase in the exchange surface area also resulted in better resistance to osmotic shocks (See Rees *et al.* 1994).

Merchie *et al.* (1995) have shown that, like fatty acids, Vitamin C is a substance which accelerates metamorphosis while increasing stress resistance. However, stresstol has been shown as a better substance in supporting stressful conditions than HUFA, which is also considered as good for supporting growth. Further study in shrimp larval culture incorporating one or other growth promoting medicinal plant products for a longer duration (about 2 to 4 weeks) may help produce healthy and fast-growing shrimp larvae.

Conclusion

In the larviculture of *Penaeus indicus*, it is advantageous to feed *Artemia* enriched with oil-mixed stresstol to produce shrimp larvae with two times higher growth and conversion as well as better stress tolerance.

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