Supplementation of an immunomodulator, Ergosan in early larvae rearing of Indian white prawn, *Fenneropenaeus indicus*

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Efficacy of an algine-based immunomodulator, Ergosan was tested on growth and survival of early larval stages (nauplii – PL3) of Indian white shrimp, *Fenneropenaeus indicus* in fibreglass tanks (10,000 l) for two larval rearing cycles. Larvae fed on a diet with Ergosan were considered as treatment and those fed on a diet without Ergosan as control. Both control and treatments were replicated in five tanks and arranged in a completely randomized design. Significant difference ($P < 0.01$) in survival was observed between control and treatment. However, growth of larvae was not influenced by the supplementation of Ergosan. Total colony forming units, yellow, green *Vibrio* and luminescent bacterial colonies were found to be significantly decreased in treatment tanks. Thus Ergosan was found to be a safe immunomodulator for improving larval survival and reducing bacterial load in rearing water.

Keywords: Bacterial colonies, immunomodulator, larval rearing, supplementation, white shrimp.

IMMUNOSTIMULANTS are compounds that stimulate the nonspecific immune system when administered alone, or stimulate the specific immune mechanisms when administered along with an antigen, thereby rendering animals more resistant to microbial and parasitic infections[1,2]. Use of immunomodulators is an effective means to increase the immunocompetency and disease resistance of shrimp[3,4]. It includes structural elements of bacteria, β-1,3-glucan products from bacteria and mycelial fungi, β-1,3/1,6-glucans from the cell wall of baker's yeast, complex carbohydrate structures, seaweed, animal or plant extracts, nucleotides, nutritional factors, cytokines and other synthetic products[5,6]. They mainly facilitate the function of phagocytic cells and increase their bactericidal activities[7]. Ergosan (Schering-Plough Aquaculture, UK) is an immunomodulator derived from brown macro and micro algae (*Laminaria digitata* and *Ascophyllum nodosum*), and contains ingredients such as algines and polysaccharides which are known to strengthen the natural defence systems in juvenile shrimp[8]. Therefore a study was conducted to evaluate the efficacy of Ergosan on growth and survival of larvae (PL, post larvae) of Indian white prawn, *Fenneropenaeus indicus*.

The experiment was conducted for a period of one month in fibreglass tanks (10,000 l) in the larval rearing unit of shrimp hatchery, National Aquaculture Group, Saudi Arabia. The effect of Ergosan was tested in two larval rearing cycles. There were control and treatment for each cycle, and both were duplicated in five tanks. Larvae fed on a diet with Ergosan were considered as treatment and those fed on a diet without Ergosan were designated as control. Newly hatched nauplii were stocked at the rate of 120/l in each fibreglass tank and all tanks were assigned in a completely randomized design. Ergosan was administered from Zoea-1 to PL3 stage immediately after a micro encapsulated diet, Royal Caviar (Bernaqua, Belgium). Required quantity of Ergosan was weighed and divided into equal portions based on a feed table for the day; Table 1 provides details of the supplementation schedule. The portion to be fed for one time was mixed in 3 l of sea water and uniformly distributed to all treatment tanks after water exchange. Water exchange took place at the rate of 50% every morning. At PL3 stage, all PL in each control and treatment tank were harvested, and survival and growth assessed. Sub-sample of 150 PL was collected from each control and treatment tank to record individual length and weight. Random water and PL samples were collected daily before water exchange for microbial analysis. These samples were used to estimate total colony forming units (CFU), yellow and green *Vibrio*, and luminescent bacteria colonies[9]. One-way analysis of variance (ANOVA) was used to calculate the statistical deference in survival, growth and bacterial load between control and treatment means[10].

Table 2 provides details on PL survival and growth. In both cycles, significant ($P < 0.01$) increase in survival was observed in treatment tanks compared to control. No significant difference ($P > 0.01$) was observed on average length and weight of PL between control and treatment. Total CFU, yellow *Vibrio* colonies and green *Vibrio* colonies were found to have significantly ($P < 0.01$) decreased in water in treatment tank compared to control (Figure 1).

Table 1. Supplementation schedule of Ergosan

Day	Larvae	Feeding frequency and feed (g)/time	Total feed (g/tank/day)
1	Nauplius	No feeding	No feeding
2	Zoea 1	5 × 0.2	1
3	Zoea 2	5 × 0.2	1
4	Zoea 3	5 × 0.2	1
5	Mysis 1	5 × 0.4	2
6	Mysis 2	5 × 0.4	2
7	Mysis 3	5 × 0.4	2
8	PL 1	4 × 0.6	2.5
9	PL 2	4 × 0.6	2.5
10	PL 3	4 × 3.1	12.5

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Table 2. Survival and growth of larvae fed with Ergosan

Hatchery cycle	Experiment	Nauplii stocked/tank Mean ± SD	PL3 harvested Mean ± SD	Length (mm) Mean ± SD	Weight (mg) Mean ± SD	Survival (%) Mean ± SD
1	Control	120,000 ± 5374	98,068 ± 2324	5.0 ± 0.1	2.1 ± 0.1	82 ± 1.9
	Treatment	120,000 ± 2720	116,011 ± 2182*	6.0 ± 0.2	2.3 ± 0.2	97 ± 1.8
2	Control	120,000 ± 3814	91,870 ± 3587	4.9 ± 0.2	2.2 ± 0.1	80 ± 2.3
	Treatment	120,000 ± 4990	113,422 ± 4821*	5.6 ± 0.3	2.3 ± 0.2	94 ± 3.1

*$P < 0.01$ (one-way analysis of variance).

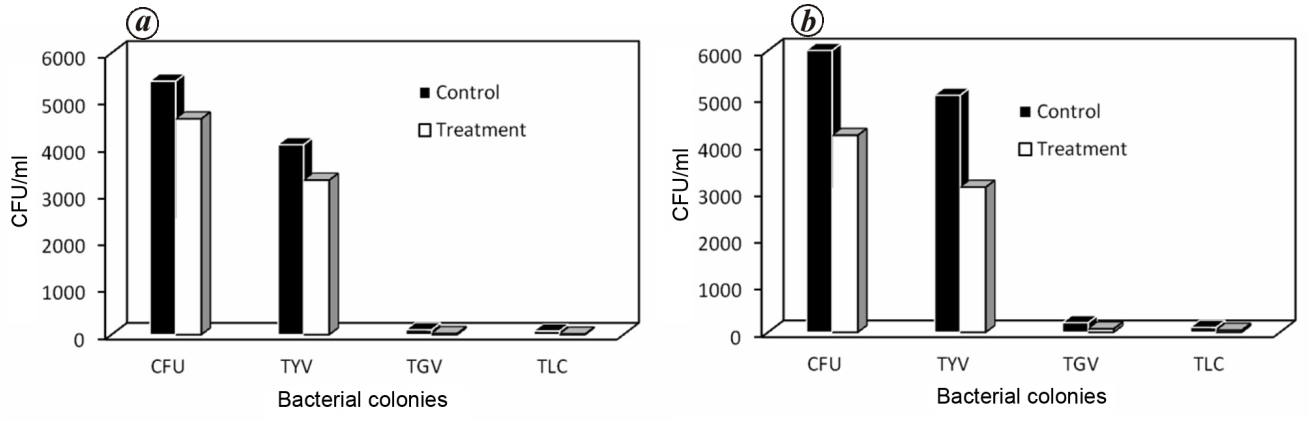

Figure 1. Microbial load in rearing water cycle 1 (***a***) and cycle 2 (***b***). CFU, Colony forming units; TYV, total yellow *Vibrio*; TGV, total green *Vibrio*; TLC, total luminescent colony.

Results of the study indicate that Ergosan could improve larval survival rate and effectively control bacterial load in rearing water. Larvae fed with Ergosan were found to be healthy and active during the rearing period compared to control. Similar observations were also reported in tiger prawn and Pacific white shrimp larvae when Ergosan was administered during larval rearing phase[11,12]. As Ergosan is an algine-based, finely powdered product, early stages of shrimp could consume the fine particles and perform better digestion[13]. It is reported that in trials with *Litopenaeus vannamei* larvae, survival from PL4 to PL7 was 71.3% compared to 61.6% in the control group[2,11]. Similarly, in *Panaeus monodon*, the survival from Zoea-1 to PL12 was 53% in treatment when compared to only 30% in control[12]. According to the manufacturers, the algine content in Ergosan enhances oxygen uptake and transfer across cell membranes, thus enabling the cells to perform more effectively[8]. In addition, it increases the proliferation and metabolic activity of lymphocytes and macrophages, and thereby promotes cellular and humoral immunity[14].

Ergosan is an algal product containing alginic acid, developed for aquaculture operations. It has immunomodulatory activity, when administered orally to intermoult stage of white shrimp for 15 days[13]. Examination of haemolymph proteins using SDS–PAGE did not reveal any obvious differences between control and Ergosan-treated shrimp[2]. Similarly, total haemocyte counts were found to be roughly equal for both control and experimental samples. However, differential analysis of haemocyte populations revealed marked changes in terms of the relative levels of hyaline, semi-granular and particularly, granular haemocytes between the two groups[15]. Enhancement of *in vitro* antimicrobial activity of haemolymph towards two shrimp pathogenic *Vibrio* isolates was recorded for shrimp fed with Ergosan. Shrimp fed with Ergosan also showed an increase in relative growth when compared to control group.

Previous studies have revealed the effects of Ergosan on fish growth, immunity and hematological parameters[2,15–17]. It is suggested that a combination of Ergosan and Hilyses yeast enhanced the serum lysozyme activity in rainbow trout[7,18]. According to these authors, lysozymes have an important role in innate immunity by lysis of bacterial cell walls, especially Gram-positive bacteria and stimulate phagocytosis of bacteria; which may be the reason for the reduction of yellow, green and luminescent *Vibrio* colonies in the treatment water in the present study.

Based on the results, it can be concluded that substantial reduction of microbial colonies in the treatment water and on larvae is due to the lysozyme activity of Ergosan. The resulting high survival and larval quality may be due to the combined effect of improved feed digestion and destruction of harmful bacteria colonies. Thus Ergosan can be safely used as a natural immunomodulator to improve water quality, larval survival and health of Indian white prawn, *F. indicus*.

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Spectral modelling of estuarine coloured dissolved organic matter

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Measuring coloured dissolved organic matter (CDOM) holds advantage over dissolved organic carbon (DOC) determination, as it can be remotely estimated unlike the latter, for which it can potentially act as a proxy. The CDOM absorbance, by definition, falls exponentially with wavelength of light (λ) in the ultraviolet-visible region. Investigating over 800 absorption spectra of water samples from the tropical monsoonal Godavari estuary and the Chilika brackish water lagoon, we found that the spectral slope (S) of the 330–440 nm region ($S_{330–440}$) is best suited to retrieve CDOM and its exponential character.

Keywords: CDOM, Chilika lagoon, Godavari estuary, spectral slope, $S_{330–440}$, UV-visible absorbance.

COLOURED (or chromophoric) dissolved organic matter (CDOM) is the optically active fraction of DOM[1]. The CDOM measurement is credited as an alternative means of quantifying dissolved organic carbon, although the conversion equations are region-specific[2–7]. The ease of CDOM measurement in terms of absorption coefficient (or absorbance) in the UV-visible region of electromagnetic radiation provides an advantage in the study of the cycling of DOM. The source, structure and interactions in

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