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Effect of Vitamins C and E and B-1, 3 Glucan as Immunomodulators in *P. monodon* Disease Management

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Abstract: Culture of *Penaeus monodon*, tiger shrimp is one of the most profitable ventures in aquacultures sector in India and in many countries of the world. Vibriosis is one of the most serious threats in cultured marine fish and shellfish. The occurrence of infectious diseases in shrimp culture has reduced production levels during the last decade. To overcome the disease problem pertaining to *Penaeus monodon* culture, Chemotherapeutants, antibiotics and few vaccines are used. Vitamins C and E and β-1,3 glucan are considered as the potential immunomodulators in shrimp disease management, among various immunomodulators. During every water exchange six ml of pure culture of *V. parahaemolyticus* at a density of 10^5 cfu/ml was inoculated into rearing water. Health status of the experimental animals was recorded by observing the colour and attachment of the body, swimming behavior and feed intake. Vitamins C and E also showed their efficacy as immunostimulants. Vitamin C exhibited more immunostimulant effects rather than vitamin E. It is concluded that the present investigation that the combination of β-1,3 glucan and vitamin C will be effectively used as immunomodulators as they are capable of enhancing the immune response of penaeids against potential pathogens like vibrios, by decreasing the pathogenic load and increasing the growth and survival rates of the cultured species.

Key words: *Penaeus monodon* · Chemotherapeutants · Vitamins C and E and β-1,3 glucan · Immunomodulators · *V. parahaemolyticus*

INTRODUCTION

Shrimp culture industry has often been affected by infectious diseases mainly of bacterial and viral etiology causing loss of production [1]. As there are limitations in using antibiotics or vaccines for management of diseases in shrimp culture, there is growing interest in using immunostimulants. A range of preparations such as live and dead bacteria, glucans, peptidoglycans and lipopolysaccharide (LPS) have been used to stimulate the immune system of shrimp [2]. Recently, scope for vaccination of shrimp has also been demonstrated [3]. Bacterin prepared from Vibrio harveyi was found to enhance levels of hemolymph agglutinins in Penaeus monodon [4]. Use of *Vibrio alginolyticus* probiotic in *Penaeus vannamei* culture could effectively improve the survival and growth by competitively excluding potential pathogenic bacteria [5]. Health status of the cultured species plays a major role in the success of shrimp culture operation. As already discussed, several virulent pathogens like vibrios and virus cause diseases to shrimps in general and Penaeids (*Penaeus monodon*) in particular. World wide, the penaeids are being cultivated extensively, although everyone is aware of the possibility of infectious disease outbreaks in penaeids. It is obvious that economically important farm-reared shrimps are belonging to the family of Penaeidae in decapod crustaceans. *Penaeus monodon*, the black tiger shrimp exhibits the highest growth rate of all cultured species [6]. It is the most valuable indigenous aquatic species in Asia and dominates the production of all penaeid species[7]. Unfortunately, these species are highly susceptible to pathogenic attack and disease occurrence. A variety of polysaccharides from a variety

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of sources have the ability to stimulate the immune system, so behaving as immunomodulators. Interest in glucans has increased after experiments showing that zymosan stimulates macrophages via the activation of the complement system [8].

To overcome the disease problems pertaining to *Penaeus monodon* culture, Chemotherapeutants, antibiotics and few vaccines are used. However as on date, no effective vaccine is available against microbes in shrimp culture. As everyone knows, chemical and antibiotic treatments invariably lead to environmental hazards, antibiotic resistance and the deterioration of end product. This necessitates the importance of some other remedial measures like the development of vaccines. The very unfortunate thing is that the inability of the development of vaccines in invertebrates in general and shrimps in particular. However it is essential in shrimp disease management to go for such a type of treatment, which resembles vaccination. Fortunately a group of biological and synthetic compounds is available, which can enhance the non-specific defense mechanisms in animals [9].

The immune system of shrimp is rather poorly understood. While it is established that the fish have well characterized specific defense mechanism, there is no such defense mechanism in shrimp and crustaceans [10]. However shrimps have a wide array of non-specific factors, both cellular as well as humoral, which are involved in defense against pathogens [11]. It may be possible to stimulate the non-specific defense mechanism system of shrimp and enhance the disease resistance. The encouraging results obtained in finfish with immunostimulants paved the way for similar studies in shrimps.

Immunomodulator are exactly not like vaccines, which can trigger the production of specific antibodies towards a specific pathogen (specific defense mechanism). These compounds may serve as alternatives to vaccines, antibiotics and other chemicals in the protection of cultured shrimps against various diseases. Such compounds are collectively called as immunomodulators or immunostimulants. These eco-prophylactic agents can increase the barrier of infection against specific and opportunistic pathogens [12] .

Immunomodulator is a chemical drug, stressor or action that elevates the non-specific defense mechanism. Non-specific immunostimulation refers to a condition in which the immune response is stimulated to respond towards a variety of antigens. As the aquatic animals encounter a variety of pathogens in the environment, highest priority is given on non-specific stimulation of immune system using immunostimulants. They may be grouped by function or by origin. Some common immunomodulators are vitamins C and E, glucan preparation and lipopolysaccharides [13,14]. Their modes of actions are modifying the cell membranes, stimulating the macrophage lymphocytes, natural killing cell activities, activating the compliment system, acting as carriers/vehicles, reservoirs/depots, including inflammation and cytokine production. Collectively they can activate the immune system of animals and render them more resistant to infections by virus, bacteria, fungi and parasites [15].

The immunomodulating effects of *β-glucans* are well established during the development of immune reactions [16]. Original studies on the effects of β-1,3-glucans on the immune system focused on mice [17,18]. Subsequent studies demonstrated that β-1,3-glucans have strong immunostimulating activity in a wide variety of other species, including earthworms[19] shrimps [20], fish[21], rats [22,23] rabbits, [24,25] guinea pigs, sheep [26], pigs [27-29], cattle (Buddle *et al.* 1988) [30] and humans[31] .

Vitamins C and E and β-1,3 glucan are considered as the potential immunomodulators in shrimp disease management, among various immunomodulators [32,33,]. Nutritional factors definitely influence the immune system of shrimp. Vitamin C is the dietary requirement for the optimum growth and disease resistance in shrimps. From the forgoing accounts, it is understood that vitamins C and E and β-1,3 glucan have great immunomodulatory effects against microbial diseases. Therefore the present work was designed to ascertain the immunomodulatory effects of vitamins C and E and β-1,3, glucan on *Penaeus monodon*.

MATERIALS AND METHODS

Vibrio parahaemolyticus strain (which was isolated from diseased shrimp) was maintained on tryptic soy agar (TSA) slopes for 18 hrs. Then it was sub-cultured into nutrient broth for further experiment.

Experimental Design: Shrimps having the size of approximately 9 g were collected from the pond. They were acclimatized in well-aerated estuarine water for five days. Six shrimps having the same size were put in individual trough of 60 litres water capacity for rearing. All the troughs were filled with filtered estuarine water and supplied with continuous aeration. Totally four tanks were maintained. The shrimps were given the following feeds.

C-control Feed (Without Any Immunomodulator)
T1-Vitamin-C (100mg /kg of feed)
T2-Vitamin-E (100 mg/kg of feed)
T3-β 1, 3 glucan. (150mg of yeast, *Saccharomyces cerevisiae* wall fraction /kg of feed)

The feeds used for the experiments were prepared freshly. Egg white (albumin) was used as a binder. β-1, 3 glucan was extracted from the cell wall of baker's yeast *(Saccharomyces cerevisiae)*. The water exchange was made once in three days. During every water exchange six ml of pure culture of *V. parahaemolyticus* at a density of 10^5 cfu/ml was inoculated into rearing water. Feed was given daily at the rate of 10% of the body weight. Dissolved oxygen was measured as 6 ppm. Water temperature ranged from 28 to 29°C. The pH was maintained at a level of 7.8-7.9 and the salinity was in between 19 and 20 ppt. daily records were maintained on the amount of feed given, left-over feed and faecal output. After 20 days the experiments were terminated. Growth and survival rates were recorded. *V. parahaemolyticus* was quantified in water and animal samples. Health status of the experimental animals was recorded by observing the colour and attachment of the body, swimming behavior and feed intake. Immunity of the experimental animals was studied by counting the total haemocytes as described by [35] The results were made into table, figure and statistical analyses.

Table 1: Growth rate of experimental animals

Treatment	Growth rate (increment in wt) (% of initial wt)
C	2
T1	7
T2	6
T3	8

Table 2: Survival rate of experimental animals

Treatment	Survival rate (%)
C	40
T1	88
T2	79
T3	90

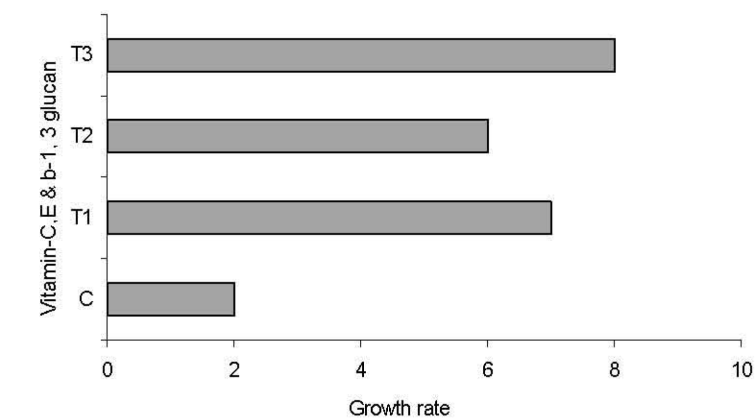

Fig. 1: Growth rate of Experimental animals

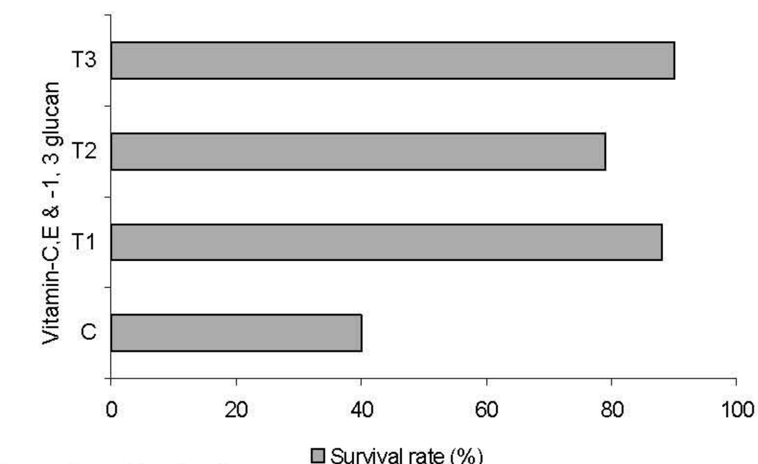

Fig. 2: Survival rate of experimental animals

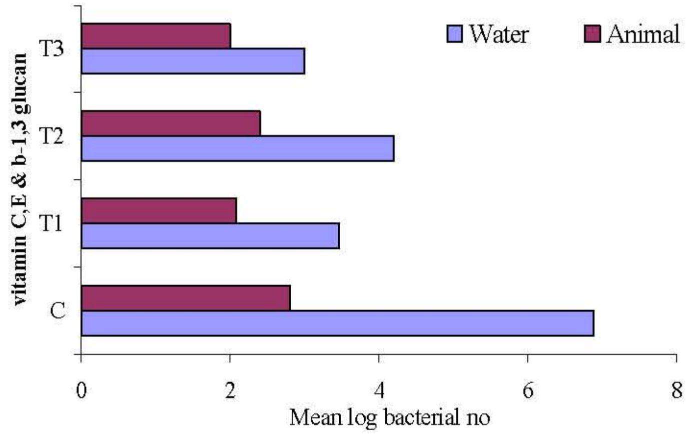

Fig. 3: *V.parahaemolyticus* in experimental tanks

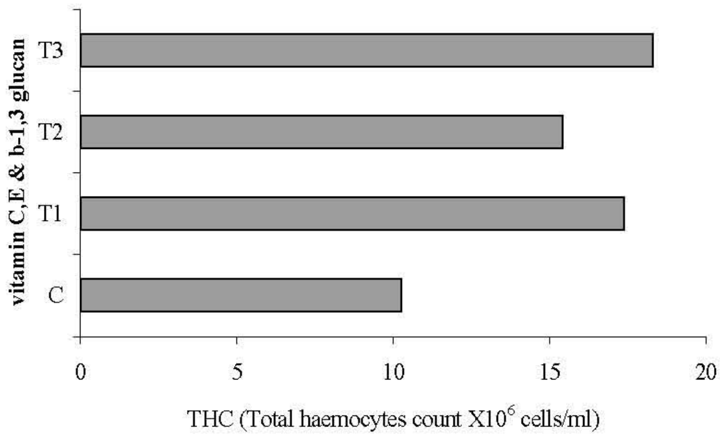

Fig. 4: Total Haemocytes count (THC) in experimental animals

Table 3: *V.parahaemolyticus* in experimental tanks

	Vibrio parahaemolyticus	
Treatment	Water (CFU/ml)	Animal (CFU/gm)
C	$7.6x10^6$	$6.4x10^2$
T1	$2.9x10^3$	$1.2x10^2$
T2	$1.7x10^4$	$2.3x10^2$
T3	$9.2x10^2$	$9.0x10^1$

Table 4: Total Haemocytes count (THC) in experimental animals

Treatment	THC ($X10^6$ cells/ml) Total haemocytes count
C	10.25
T1	17.37
T2	15.41
T3	18.29

RESULTS

Vitamins C and E and β-1,3 glucan increased the growth and survival rates than control animals. Among these, vitamin C and β-1, 3 glucan have tremendously increased the growth and survival rates (90%). Similarly vitamin C and β-1, 3, glucan reduced the population of *V.parahaemolyticus* drastically both in rearing water and in reared animals. Health status of the experimental animals was good as there were not much deformity in their body as well as they did not show any erratic movement. The control animals showed erratic movement, sluggish in nature and discolouration, attachment and deformities of their body. Total haemocytes count was more in experimental animals ($17X10^6$, $15X10^6$ and $18X10^6$ cells/ ml) in vitamins C, E and β-1, 3 glucan treated ponds respectively than in control animals ($10X10^6$ cells/ml).

DISCUSSION

The results of the present study clearly illustrate the importance of immunomodulators in shrimp culture. Among three commonly used immunomodulators, β-1,3 glucan was found to be more effective in controlling pathogenic vibrios, enhancing health status, boosting up the immunity and promoting growth and survival rates.

This coincides with the previous work [14] who concluded that β-1,3 glucan could be used as immunostimulants in penaeid rearing. The immune enhancers could increase the immune response and reduce the bacterial pathologies without affecting the environment and the cultured species, which are usually happening in antibiotic and other chemotherapeutic treatments. Also he mentioned that β-1, 3 glucan had positive effect as it would increase the resistance of the cultured animals against the dangerous white spot disease. [34] also pointed out that WSSV as secondary infection can be resisted by such shrimp, which are immunized against bacteria. From the present work and earlier reports, it is evident that β-1, 3 glucan as immunostimulant, can reduce disease outbreaks and mortality of the cultured animals and ultimately cause better growth and survival rates. In the present finding maximum of 90% survival was obtained whereas in the control animals the survival rate was very poor as only 40% of the survival was observed. Likewise maximum growth increment was attained in β-1,3 glucan treated tank. This is supported by previous findings [35] who also observed the same trend in crabs and penaeids. β-1, 3 glucan showed vibriocidial activity as it reduced the population of *V. parahaemolyticus* drastically.

This is in concordance with the findings of previous researchers. [36] reported the induction of vibriocidal activity in the haemolymph of *P. monodon* fed with a diet containing yeast β-glucan. Enhanced phenoloxidase activity has been demonstrated in *P.monodon* treated with immunostimulants like β-1,3 glucan by immersion[37]. It is apparent that the animals treated with β-1,3 glucan exhibited higher counts of total haemocytes. Generally the haemocytes constitute the principal effectors of the immune response of crustaceans. Their function involves cellular mediation mechanisms, consisting of reactions such as phagocytosis, encapsulation, cytotoxicity, etc and humoral factors such as coagulation's proteins, hydrolytic agglutinins (lectins) and antimicrobial peptides, which are produced by cellular factors. Increased haemocytes counts with β-1,3 glucan were also recorded by Rodriguez *et al.* [14] (2007) in penaeid, *P.vannamei.*

Vitamins C and E also showed their efficacy as immunostimulants. Vitamin C exhibited more immunostimulant effects rather than vitamin E. It agrees with the several earlier findings. Proved the efficacy of vitamin C as immunostimulant in channel catfish (*Ictalarus punctatus*) [38]. Vitamin C supplement feed could enhance the growth rate in channel cat fish[39]. Concluded that vitamin C will decrease the mortality and increase the growth in young rainbow trout, *Salmo gairdneri* challenged by vibrios[40]. Several researchers [32,33] (Shiau and Jan, 1992; Shiau and Hsu, suggested that vitamin C would increase immunity and prevent disease syndromes in fish and shrimp.

Few works are also available to support the ability of vitamin E in enhancing immunity. According to [41,42] vitamin-E could also be used as immunomodulator in fish and shrimp. However in the present study unlike β-1, 3 glucan and vitamin C, much efficacy was not observed in the case of vitamin E treatment trials. Hence based on the present investigation and the earlier works it can be concluded that the combination of β-1, 3 glucan and vitamin C will be effectively used as immunomodulators as they are capable of enhancing the immune response of penaeids against potential pathogens like vibrios and WSSV by decreasing the pathogenic load and increasing the growth and survival rates of the cultured species.

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