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Effect of garlic supplemented diets on growth and survival of Pacific white leg shrimp, *Litopenaeus vannamei* juveniles

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Abstract
A study was carried out to evaluate the effect of adding three levels of garlic powder at 0.1, 0.5 and 1.0% in a basal diet (commercial pellet feed) of Pacific white leg shrimp, *Litopenaeus vannamei* juveniles (5.51±0.34 g) on growth performance, survival rate. The experiment was carried out for 49 days and *L. vannamei* were fed on four experimental diets with 0% (G_0), 0.1% (G_1), 0.5% (G_2) and 1.0% (G_3) of garlic powder in basal diet. The dietary garlic inclusion at three different levels had significantly ($p< 0.05$) higher. Average weight gain (AWG), SGR, better FCR and survival rate compared to control diet. However, survival rate was not significant between G_1 and G_0. Among the garlic inclusion diets a significantly ($p< 0.05$) higher AWG (10.39 ± 0.02) and survival rate (92.50 ± 0.50), better FCR (1.13 ± 0.03) and SGR (2.74 ± 0.03) observed in G_2 followed by G_3 and lowest in G_1. No significant ($p< 0.05$) difference in FCR and survival observed between G_2 and G_3.

Keywords: AWG, FCR, garlic diets, *L. vannamei*, SGR, survival

Introduction
Pacific white shrimp, *Litopenaeus vannamei*, is one of the most intensively cultivated shrimp in the world [1]. In Asia it has been first introduced in China, later it has spread to East, Southeast and South Asian countries. After the devastation of *P. monodon* culture with white spot syndrome virus, *L. vannamei* came as lucrative species in Indian aquaculture industry [2]. At present, *L. vannamei* is a popular cultured species in India and its culture was initially taken up by Andhra Pradesh state during 2009 [3]. *L. vannamei* culture is growing at the exponential phase in the country, with remarkable growth both in terms of culture area and export value until the year 2014 [4]. However, in recent years *L. vannamei* faming is suffering with diseases of various aetiological agents as well as morbidity associated with poor management practices causing catastrophic influence on the farmer's economy [5].

Intensive aquaculture of shrimp led to economic growth but, unfortunately, the outbreak of viral and bacterial diseases resulted in drastic reduction in vannamei production in many countries [6], including India [4]. Among the various bacterial diseases of *L. vannamei*, infections caused by *Vibrio* spp. were considered as an important problem causing early mortality syndrome (EMS) by *V. parahaemolyticus* [7], white gut and white fecal syndrome by *V. parahaemolyticus*, *V. fluvialis*, *V. alginolyticus* and *V. mimicus* [8,9].

As treatment against vibriosis, wide ranges of antibiotics are being orally administered to shrimp. However, inappropriate or excessive use of antibiotics in aquaculture resulted in development of drug resistant bacterial strains in host animals [10], negative effects on growth performance of cultured species and potential harm to human health and environment [11].

Hence, the major shrimp importing countries such as European Union (EU), USA refused to import aquaculture products having antibiotic residues. So the use of antibiotics have been limited or banned in many countries including India. Therefore, researchers have intensified effort to exploit the alternative strategies to treat bacterial diseases in shrimp farming such as plant based products termed 'phytobiotics' as dietary supplements that enhance growth performance, immune response and consequently their resistance to pathogens [12, 13].

The administration of phytobiotics in aquaculture is a relatively new area of research showing promising results. Phytobiotics are plant derived, natural compounds embedded into diets which enhance animal productivity [14]. Garlic (*Allium sativum*) is one of the known medicinal plants used as an immunostimulant with a reputation as natural antibiotic [15].

Garlic has long been used as a therapeutic measure for the treatment of many diseases in humans and livestock, since ancient times as reported in the Codex Ebers (1550 BC). The bioactive components of garlic include sulphur containing compounds such as allin, diallyl sulphides and allicin [16]. Allicin (dially thiosulfinate) is the most abundant compound representing about 70% of all thiosulfinates present, or formed in crushed garlic [17]. The garlic has antibacterial, antiviral, antifungal and antiprotozoal and also has beneficial effects on the cardiovascular and immune system [18].

In aquaculture operations, garlic promotes growth, enhances the immune system, stimulates appetite, and strengthens the control of pathogens, especially bacteria and fungi [19]. However, studies on the application of garlic as phytobiotic in shrimp are very limited. The potential use of garlic powder as additive on the growth and survival of *L. vannamei* was studied by [13]. The present study aims to evaluate the phytobiotic effect of garlic powders on the growth and survival in *Litopenaeus vannamei* juveniles.

Materials and methods:

Conditioning of Experimental Shrimp

Healthy juveniles of *L. vannamei* (average length 8.4±0.3 cm and average weight 5.51±0.34 g) were procured from a private shrimp farm located at Korutur village of Indukurpeta mandal, SPSR Nellore district, Andhra Pradesh under minimum stress in oxygen filled polyethylene bags containing 3 ppt salinity water to the college wet laboratory and acclimatized to lab conditions. The shrimp were then stocked in rectangular flat bottom fibreglass reinforced plastic (FRP) reservoir tank of one ton capacity with sufficient aeration and maintained for a period of two weeks. The water salinity in reservoir tank was gradually raised from 3 ppt to 12 ppt during this period. The shrimp were fed on commercial pellet diet (34.57% crude protein) twice daily at 08:00 Am and 08:00 Pm to their satiation. The left over feed and faeces were siphoned out daily prior to each feeding with a water exchange rate of 5-10%.

Experimental Rearing Facility:

The experiment was carried out in the wet lab of the College of Fisheries Science with adequate aeration and moderate light condition during the day. Rectangular flat bottom HDPE tanks with the size of 30x60x40 cm were used for the experiment. During the experimental period the treated sea water was diluted to 12 ppt salinity using fresh water drawn from bore well and the experimental tanks were filled up to 23 cm height after fine filtration using nylon bolting cloth. For maintaining optimal DO content (6-8 ppm) in the experimental tanks a roots air blower of 0.1 hp capacity was used. Two aeration pipes with diffuser stones were provided per replicate tank.

Preparation of Experimental Diets

In the present experiment, Spray dried powder of garlic cloves was procured from M/s Natural Dehydrate Pvt Ltd, Chennai were used as phytobiotic. A commercial vannamei pellet feed (CP Aquaculture pvt Ltd, Chennai, India) was used as the basal diet. For the experiment, three experimental diets with three different concentrations each of garlic (G_1, G_2, and G_3) powders were prepared. For each experiment a control with basal diet was maintained separately as control. The levels of inclusion of each phytobiotic are shown in Table 1. Garlic powder at concentrations of 1, 5 and 10 g per kg of basal diet were initially dissolved in egg white and then mixed thoroughly with basal diet to form a uniform coating of phytobiotic on pellets. The garlic coated basal diets each at 3 different concentrations were then dried overnight at 50 ºC in hot air oven, packed in air tight plastic bottles and stored at 4 ºC until use.

Experimental Design and Procedure

The experimental setup consisted of four treatments with garlic supplemented diets *viz*., G_0, G_1, G_2 and G_3 with four replicates for every treatment. For experimental setup uniform sized juveniles of *L. vannamei* (average weight 5.60±0.07 g) were selected from the stock population and each of 16 replicate tanks were stocked with 15 shrimp. Throughout the experimental period continuous aeration was provided to all the experimental tanks. The water was exchanged in the experimental tanks at about 10-15% at every three days interval. The shrimp in the experimental tanks of set up-1 and 2 were fed three times daily (7:00 Am, 2:00 Pm and 9:00 Pm) at 4% body weight per day with pellet feed containing graded levels of garlic (G_0, G_1, G_2 and G_3) respectively. During the experimental period, feeding rate was adjusted once in a week according to shrimp weight gain. Proximate composition of the pellet feed used during the experimental period is given in Table 2.

Table 1: Inclusion level of phytobiotic in experimental diets

Phytobiotic	Level of inclusion/kg feed	Diet Code
Garlic	0 g	G_0
	1 g	G_1
	5 g	G_2
	10 g	G_3

Table 2: Proximate composition of commercial vannamei pellet feed used for feeding of *L. vannamei* juveniles.

Feed type	Protein (%)	Fat (%)	Ash (%)	Fiber (%)	Moisture (%)	NFE (%)
Sinking pellet	34.57	5.23	5.31	5.54	9.74	49.35

*Net faecal energy

Water Quality Parameters

The water pH, dissolved oxygen (DO), temperature were measured on daily basis and total ammonia nitrogen (TAN) was measured at weekly intervals. Salinity was measured at three days interval during water exchange in the experimental tanks in order to maintain salinity of 12 ppt. All the water quality parameters were measured at 10:00 Am by the following methods (Table 3)

Table 3: Water quality parameters

Parameter	Instrument
Temperature (ºC)	Mercury bulb thermometer with an accuracy of 0.1˚C
pH	Universal pH indicator method
DO (ppm)	Aqua check DO kit - (WT028A, Himedia Laboratories Pvt. Ltd)
Salinity (ppt)	Portable salinity refractometer (ERMA, RHS- 28 with built in automatic temperature compensation system)
Total Ammonia Nitrogen(NH3-N) (mg/l)	Spectrophotometric method at wave length 640 nm [20]

Growth Parameters

During the experimental period shrimps were subjected to growth assessment at weekly interval. The growth parameters such as Average body weight gain (AWG), specific growth rate (SGR) and food conversion ratio (FCR) were observed during the experimental period.

Average Weight Gain (g) = Final body weight – Initial body

$$\text{Specific Growth Rate (\%)} = \frac{\ln(\text{Final Weight}) - \ln(\text{Initial Weight}) \times 100}{\text{Experimental period in days}}$$

$$\text{Food Conversion Ratio} = \frac{\text{Feed intake on a dry matter basis}}{\text{Weight gain on wet weight basis}}$$

$$\text{Survival Rate (\%)} = \frac{\text{Total number of shrimps survived}}{\text{Total number of shrimps stocked}} \times 100$$

Statistical Analysis

Statistical analysis of the experimental data was carried out using statistical software SPSS version 20 (IBM 2012). One way analysis of variance (ANOVA) was used to test the significance of differences. Statistical significance level was set at ($p< 0.05$) and the difference between treatments means were tested using Duncan's multiple range tests.

Results and Discussion

Water quality parameters

The physico-chemical parameters of water were in optimal range in all the treatment tanks during the dietary garlic experiment. The minimum and maximum temperature, DO, pH and total ammonia nitrogen (TAN) values were presented in table 4. There was no significant difference ($p>0.05$) in the water temperature, DO, pH and TAN among the four experimental groups.

Table 4: The water quality parameters value ranges during experiment period.

Parameter	Range
Temperature ^{0}C	28.24±0.01 – 30.36±0.01
DO (ppm)	6.82±0.06 - 8.24±0.04
pH	7.14±0.06-7.81±0.06
Total Ammonia nitrogen (ppm)	0.012±0.03 - 0.088±0.0020

The mean water quality parameters recorded in all the treatment tanks during the two experiments did not vary significantly ($p > 0.05$) with the inclusion of garlic powders in the diets at increasing levels as well in the controls. The mean values of water temperature, DO, pH, salinity, TAN were well within acceptable ranges for the culture of *L. vannamei juveniles* [21.22].

Growth Performance

ABW, SGR and FCR

Data on growth performance of *L. vannamei* juveniles in all the experimental tanks were observed at weekly intervals. The level of inclusion of garlic in the diet showed significant ($p< 0.05$) difference among the four treatment groups in average body weight gain (g), SGR, FCR and survival of shrimp (table 5 and fig. 1). Overall after 49 days, among all treatments, the highest average body weight gain of 2.74±0.03 g in treatment G_2 and the lowest average body weight gain of 6.37±0.05 g in control (G_0), the highest Specific Growth Rate was recorded 2.74±0.03 in G_2 and lowest was recorded 2.45±0.05 in control group (G_0) and the highest feed conversion ratio value of 1.65±0.05 in control group (G_0) and lowest value of 1.13±0.03 in treatment G_2.

In aquaculture operations, garlic promotes growth, enhances the immune system, stimulates appetite, and strengthens the control of pathogens, especially bacteria and fungi [19]. Most aquatic garlic research has involved fresh garlic extracts, with experimental subjects either fed a garlic-added feed or treated with a garlic juice immersion [23].

The results of the present study showed that dietary supplementation of garlic powder at 0.1 (G_1), 0.5 (G_2) and 1% (G_3) had a significant effect on the average body weight (ABW), SGR and FCR of *L. vannamei* juveniles than that in control (G_0). These results are in accordance with those of previous studies in European sea bass, *Dicentrarcus labrax* [24], Nile tilapia [25], *C. carpio* [26], *Acipenser ruthenus* [27], *Lates calcarifer* [28], *Epinephelus coioides* [29] and *Onchorhynchus mykiss* [30].

The effect of garlic powder supplemented diets at 2, 4 and 6% on the growth of *L. vannamei* revealed significantly better in treatments than control and the shrimp fed on 6% garlic powder diet obtained non-significant highest final weight than other groups [13]. However, [31] reported no significant difference in growth performance of *L. vannamei* fed on basal diet and six herbal formulated diets with different combinations of Astragalus polysaccharides (APS), chlorogenic acid (CGA) and allicin, for 21days. Similarly, [32] reported that SGR and FCR in *Labeo rohita* fed with 0.5 and 1% garlic diet was not significantly different from that of the control.

The differences observed in the results of the above studies might be due to the difference in mode of garlic administration, dosage levels, life stages and variation in intestinal morphology and gut micro flora [33].

After 49 days of feeding trial in the present study, shrimp fed 0.5% (G_2) garlic supplemented diet exhibited significantly higher average body weight (ABW) and SGR than that of 1% (G_3) and 0.1% (G_1). [34] Found that the growth rate of *Colossoma barchypomum* fish increased and then decreased with increasing amounts of allicin.

Survival Rate

The effect of garlic inclusion level on the survival rate (%) of *L. vannamei* juveniles is shown in table 5. Significantly ($p< 0.05$) higher survival rate of shrimp was observed in G_2 (92.50±0.50) followed by G_3 (90.00±0.81) than G_1 (77.50±0.50) and G_0 (72.51±0.95). Survival rate was not significantly different between G_1 and G_0.

[13] Observed that the effect of garlic powder supplemented diets at 2, 4 and 6% on the survival of *L. vannamei* revealed significantly better in treatments than control. Lower survival rate in the Control group may be due to cannibalism. This usually occurs in some stages of shrimp culture where stronger individuals attacks and devour the weak ones and usually targeting the appendages [35] and thus contributes to the survival reduction. In the present study the survival rate of shrimp were significantly better in G_2 followed by G_3 than G_1. This indicates that incorporation of garlic in shrimp diet had increased the survival of the cultured species. The improved survival rate of the treatment groups is attributed from the

bioactive elements of garlic including sulfur containing compounds such as allin, diallysulphides and allicin [16], but further investigations are needed to clarify the underlying mechanisms.

Table 5: Effect of garlic supplementation on the growth performance and survival (%) of *L. vannamei* (mean±SD) juveniles observed at the end of experiment.

Treatments	G_0	G_1	G_2	G_3
Initial	5.60^{a}±0.03	5.64^{a}±0.04	5.62^{a}±0.03	5.60^{a}±0.06
Final	11.97^{a}±0.09	12.68^{b}±0.05	16.01^{d}±0.07	14.96^{c}±0.03
ABW (g)	6.37^{a}±0.05	7.04^{b}±0.06	10.39^{d}±0.02	9.36^{c}±0.06
SGR	2.45^{a}±0.05	2.51^{b}±0.02	2.74^{d}±0.03	2.67^{c}±0.03
FCR	1.65^{c}±0.05	1.48^{b}±0.04	1.13^{a}±0.03	1.18^{a}±0.02
Survival (%)	72.51^{a}±0.95	77.50^{a}±0.50	92.50^{b}±0.50	90.00^{b}±0.81

The mean with different superscript letter in each row represents significant difference. The ANOVA was done at $p< 0.05$ for separation of means. The significant difference between treatments means was tested using Duncan's multiple range test (Duncan, 1995).

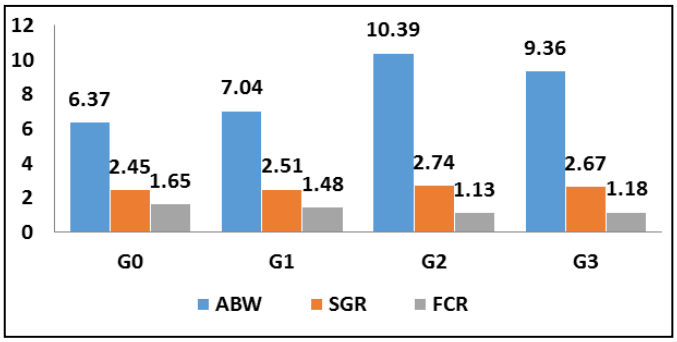

Fig 1: The Average Weight Gain (g) (AWG), SGR and FCR of *L. vannamei* in garlic supplemented diets.

Conclusion

The results of the present study indicated 0.5% of garlic powder supplementation is optimal for the growth and survival of *L. vannamei* juveniles. Higher concentration of garlic powder (1%) did not show significant improvement in growth and survival in *L. vannamei*. Further, it is evident from different studies that, the optimum feeding amount of garlic is species-specific.

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