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Effect of ginger, garlic and fenugreek powder supplemented diets on survival of pacific white leg shrimp

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Abstract
The usefulness of growth prompters and probiotics in maintaining water quality and thereby in enhancing growth rate and survival rate is described in the present investigation based on the efficacy of *L. vannamei* was fed with three different herbal powder of ginger, garlic and fenugreek of varying levels of 1%, 2.5%, 5%, 2%, 4%, 6%, 0.5%, 1% and 1.5% of concentrations respectively mainly to assess the optimal growth-promoting potential and survival rate. The feeding trial was continued for 63 days with triplicates in each treatment. The highest weight gain was observed in GP 4% supplemented diet fed vannamei compared to all other garlic supplemented diets. The survival rate of the vannamei fed diets containing garlic powder was significantly ($P < 0.01$) higher in GP 6% (91.6%) compared to control (58%). A similar trend of elevated survival rates was noticed in vannamei at ZP 2.5 (91.3%) and FP 1.5% (83.3%) compared to control. The growth-promoting ability of herbs with varying concentrations higher in GP 4% followed by ZP 2.5% and FP 1% supplemented in vannamei diets. The result shows that herbs plays a vital role in growth, survival and disease resistance of the animal by maintaining good water quality parameters throughout the study period.

Keywords: *Litopenaeus vannamei*, survival, supplemented diets, herbs, ginger, garlic and fenugreek

1. Introduction
The flavouring plants have a large sort of properties such as: inhibitor, antimicrobial, anticarcinogenic, analgesic, insecticidal, antiparasitic, anticoccidial, growth promoters, appetency improvement, stimulant of secretion of gall and biological process accelerator activity, laxatives and antidiarrhea, hepatoprotection (Coutteau *et al*., 2011) [4]. Garlic, garlic L., has been used for the treatment of the many diseases since precedent days as reported within the Codex Ebers (1550 BC) wherever associate degree Egyptian medical papyrus delineated many therapeutic formulas supported the garlic as a helpful remedy for a range of diseases like heart issues, headache, bites, worms and tumors. Garlic (Allium sativum) has many helpful effects for human and animals, exhibiting antimicrobial, inhibitor, and medicinal drug properties Sivam (2001). Garlic will facilitate within the management of pathogens, particularly microorganism and fungi, and increase the welfare of fish (Corzo 2007) [3]. Ginger (Zingiber officinalis) belongs to family Zingiberaceae family. The part of the plant used is that the rootstock, a very important spice. The utilization of spices as food and feed additives has been practiced wide since precedent days. Till date, no study has been administrated on the shrimp with Z. officinalis as associate degree flavouring course. Therefore, within the gift study, Z. officinalis was chosen and therefore the stimulatory impact verified. numerous percentages of Z. officinalis was ready and fed to postlarvae (PL-1–30) of Penaeus mammal genus through the live feed genus Chirocephalus franciscana, as a result of its versatile characteristics, like style, high nutritious worth, non-selecting filter-feeding capability and non-contamination of the culture water. Ginger will increase the exocrine gland and gut enzyme (Platel and Srinivasan, 2000) [11]. Fenugreek (Trigonella foenumgraecum) is associate degree annual herb that belongs to the rosid dicot family wide full-grown in Asian nation, India, Egypt, and Middle Eastern countries. Fenugreek has additionally been reported to exhibit medical specialty properties like anticancer, antiviral, antimicrobial, medicament, hypotensive and inhibitor activity (Cowan *et al*., 1999 and Shetty *et al*., 1997) [5, 15].

2. Materials and Methods

Litopenaeus vannamei (1000 numbers) were obtained from CP Hatchery, Nellore. Shrimp seed were packed in double plastic bags filled with oxygen and water in the ratio of 3:1 in each bag and the density of shrimp was 300/bag. Post larvae (PL10) transported by road in plastic bags containing 15 ppt saline water. PL transferred to the same salinity water in cement tanks of the wet lab. Acclimatization was carried out over 10 days. During this period the seed were fed apparent satiation with control diet. The number of shrimp seed to be packed in oxygen inflated polythene bags was calculated as per the following formula. N = (DO – 2) X V/CH Where: DO: Dissolved oxygen content of water (mg/l), V: Volume of water used for transport (Lt), C: Rate of oxygen consumption of shrimp (ml/kg of shrimp), H: Duration of transport (Hours). The aquarium tanks used for experiments were of size 60x30x30 cm. thirty aquariums including control were stalked on iron racks. Aquariums were located in a secured place where there is no direct sunlight and covered all the sides with black paper to avoid algal growth in the tank. Water in the aquariums was aerated by using air stones connected to the air compressor. Filters are used for filtering the aquarium water. The underground water was taken into a tank and allowed to aerate for 48 hours and was used for filling the aquaria. Salinity was checked before taking the water into aquarium. The water is allowed to pass through bio filter for 24 hours before introducing the shrimps into the aquaria. In each aquarium 12 numbers of shrimps with initial average weights of 3.2±0.11gm were introduced and triplicates were maintained for each treatment. Regular water exchange of 25% was done every day. Left over feed, excreta and other debris was siphoned off from the bottom of the tank without disturbing the shrimps at every 2 hours. Garlic, ginger and fenugreeks were purchased in sufficient quantities from local market. The ingredients were sun dried for 2 weeks and powdered at required quantities before feed preparation. Ten experimental diets (Plate 8) were prepared by supplementing a basal formulated diet with different levels 0% (Control), 2%, 4%, 6%, 1%, 2.5%, 5%, 0.5%, 1%, & 1.5% of garlic, ginger and fenugreek powders respectively. The growth parameters of all the shrimps of each aquarium were individually estimated by taking their total body length and weight at 7 days interval. Statistical analyses were performed using web agri stat package (WASP) version 2.0. The data obtained on Growth, Survival and Food Conversion Ratio was statistically analysed by applying Randomized Block Design (RBD) of two-way classification.

3. Results & Discussion

3. Survival Rate

Survival percentages of *L. vannamei* in various experimental treatments are presented in table 1 and figure 1. The survival percentage throughout the period of experiment was lowest for the control and by the final sampling (63th day) the survival percentage was highest 91.6 (GP 6%) and lowest 58.0 (control). Statistical analysis has shown that F- value is found to be significant among treatments. Since F- value is found to be significant, the pair- wise comparison of any two treatments could be done by computing RBD two way classifications. The treatment GP 6% had shown highest survival rate when compared to the other treatments. The subsequent positions were occupied by Treatments GP 4%, GP 2% followed by control. Treatment GP 6% has shown significant difference from all other treatments. There was significant difference in between experimental period also. Survival rate followed in the chronological order of treatments: GP 6% > GP 4% > GP 2% > control.

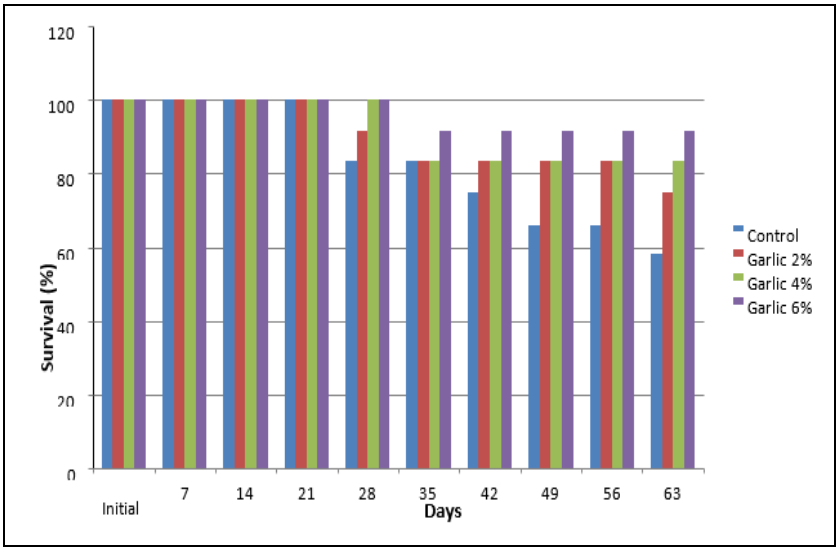

Fig 1: The percentage Survival rate in *L. vannamei* fed with different concentrations of garlic powder supplementation

Table 1: The percentage Survival rate in *L. vannamei* fed with different concentrations of garlic powder supplementation

Control (Final survival)/ %	56	58	60
GP 2% (Final survival)/%	72	75	78
GP4% (Final survival)/%	81.3	83.3	85.3
GP6% (Final survival)/%	91.4	91.6	91.8

Diet plays a vital role in the strengthening of defense system by providing essential nutrients. Recent trends in world aquaculture focused to improve food security and to use herbal supplementation in diets will drive out possibility of heavy synthetic antibiotics use and residues development in the environment. Garlic as a natural antibiotic is one of the most effective natural immuno stimulants and also contains antioxidative properties. Survival rate was improved in the *L. Vannamei* fed with garlic supplemented diet compared to control diet, progressive increasing in the survival rate was observed with the increase of in level of inclusion (Fig. 2).

The treatment GP 6% was showed highest survival rate compared to other treatment with maximum critical difference (P<0.01) (Table 1). also reported dietary garlic powder supplementation has increased the survival rate of *L. Vannamei* fry compared with the control. Similar results of highest survival rate were also observed in *M. rosenbergii* fed with herbal supplemented diets compared to control diet (Poongodi *et al*., 2012) [12]; Rebecca and Bhavan, (2014). It was also observed in *Dicentrarcus labrax* (Saleh *et al*., 2015) fed with GP supplemented diet. Disease incidence, stress factors and cannibalism may have contributed to decrease in survival rate in control group. The improved survival rate in treatment groups is attributed to the constituents of garlic like immunostimulants, antistress factors, antioxidants and antimicrobial factors. Further study is needed for identification of specific factors in the garlic which are contributing to the improvement of survival rate in *L. vannamei.*

Survival percentages of *L. vannamei* shrimp in various experimental treatments are presented in table 1 and figure 3. The survival percentage throughout the period of experiment was lowest for the control among the all treatments. By the final sampling (63th day) the survival percentage was highest 91.6 (ZP 2.5%) and lowest 58.0 (control). Statistical analysis has shown that F- value is found to be significant among treatments. Since F- value is found to be significant, the pair-wise comparison of any two treatments could be done by computing RBD two way classifications. The treatment ZP 2.5% had shown highest survival rate when compared to the other treatments. The subsequent positions were occupied by Treatments ZP 5%, ZP 1% followed by control. Treatment ZP 2.5% has shown significant difference from all other treatments. There was significant difference in between experimental period also. Survival rate followed in the chronological order of treatments as ZP 2.5% > ZP 5% > ZP 1% > control.

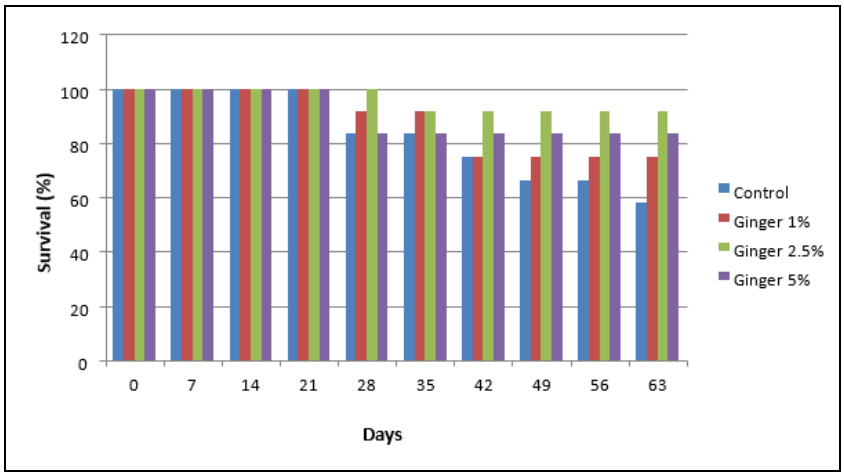

Fig 2: Survival percentage in *L. vannamei* fed with different concentrations of ginger powder supplementation

Table 2: Survival of *L. vannamei* fed with different concentration of ginger powder including control (Triplicates)

Control (Final survival)/ %	56	58	60
ZP 1% (Final survival)/%	72	75	78
ZP2.5% (Final survival)/%	91.3	91.6	91.9
ZP 5% (Final survival)/%	80.3	83.3	86.3

Present day practice of high stocking density results in infectious diseases, which in the major problem causing heavy economic lass to formers (Logambal *et al*., 2000). The use of hormones, antibiotics and several other chemicals pave the way for increase of cost of production and also residual effect on the environment. Zinger has been recommended for use as anti-inflammatory, antimicrobial nature and other beneficial effects (Kim *et al*., 2007). This study was revealed that *L. vannamei* fed with ZP 2.5% diets showed highest survival and found significant (P<0.01) compared to other treatments (Table 2 and 20; Fig.3). The constituents of Zinger, like anti-microbial factor and anti-stimulant factor may have contributed for the evaluated survival in experimental group compared to control similar trend of evaluation was found in the survival rate of *M. rosenbergii* fed with ginger diet compared to control group (Poongodi *et al*., 2012) [12]. Similar positive observation was reported by Citarasu *et al*. (1998), with increased survival in the post larvae (PL -20) of *P. indicus* fed with herbal products. Venkataramalingam *et al*., (2007) also observed increase in survival of *P. monodon* larvae with increase in percentage of Zinger enriched artemia utilization as feed. The results in the present study were also in agreement with the finding of earlier workers observation in *M. rosenbergii* (Rebecca and Bhavan, 2014, El-Desouky *et al*., 2012); in *L.vannamei* (Chang *et al*., 2012); in *Onchorhynchus mykis* (Nya and Austin, 2009).

Survival percentages of *L. vannamei* shrimp in various experimental treatments are presented in table 2 and figure 4. The survival percentage throughout the period of experiment was lowest for the control among the all treatments. By the final sampling (63th day) the survival percentage was highest 83.3 (FP 1.5%) and lowest 58.0 (control). Statistical analysis has shown that F- value is found to be significant among treatments. Since F- value is found to be significant, the pair-wise comparison of any two treatments could be done by computing RBD two way classifications. The treatment FP 1.5% had shown highest survival rate when compared to the other treatments. The subsequent positions were occupied by Treatments FP 1%, FP 0.5% followed by control. Treatment FP 1.5% has shown significant difference from all other treatments. There was significant difference in between experimental period also. Survival rate followed in the chronological order of treatments as FP 1.5% > FP 1% > FP 0.5% > control.

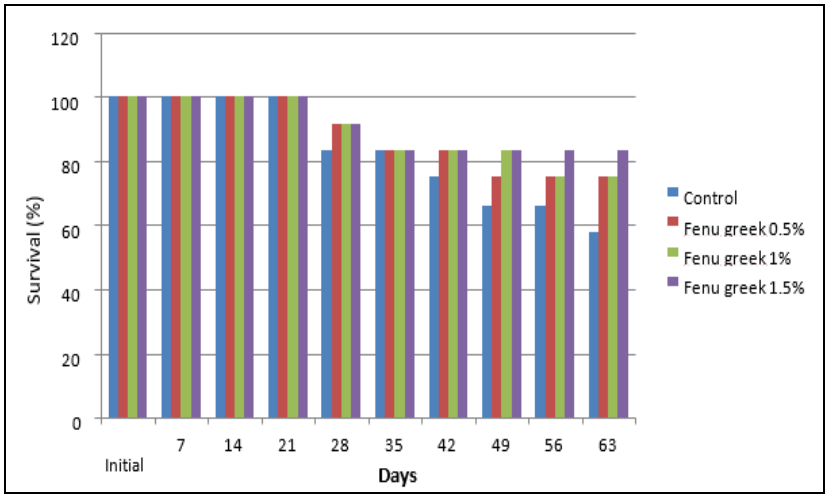

Fig 3: Specific growth rates (%) in *L. vannamei* fed with different herbal supplementation

Table 3: Survival of *L. vannamei* fed with different concentration of Fenugreek powder including control (Triplicates)

Control (Final survival)/ %	56	58	60
FP 0.5% (Final survival)/%	72	75	78
FP1% (Final survival)/%	72	75	78
FP1.5% (Final survival)/%	80.3	83.3	86.3

Present day practice of high stocking density results in infectious diseases, which in the major problem causing heavy economic lass to formers (Logambal *et al*., 2000). The use of hormones, antibiotics and several other chemicals pave the way for increase of cost of production and also residual effect on the environment. Zinger has been recommended for use as anti-inflammatory, antimicrobial nature and other beneficial effects (Kim *et al*., 2007). similar trend of evaluation was found in the survival rate of *M. rosenbergii* fed with ginger diet compared to control group (Poongodi *et al*., 2012) [12]. Similar positive observation was reported by Citarasu *et al*. (1998), with increased survival in the post larvae (PL -20) of *P. indicus* fed with herbal products. Venkataramalingam *et al*., (2007) also observed increase in survival of *P. monodon* larvae with increase in percentage of Zinger enriched artemia utilization as feed. The results in the present study were also in agreement with the finding of earlier workers observation in *M. rosenbergii* (Rebecca and Bhavan, 2014, El-Desouky *et al*., 2012); in *L.vannamei* (Chang *et al*., 2012); in *Onchorhynchus mykis* (Nya and Austin, 2009).

3.2 Specific growth rates

3.2.1 Specific growth rates of *L. vannamei* fed with different herbal supplementation

Specific growth rates for *L. vannamei* treated with different diets were calculated and presented in table 3 and figure 5. The specific growth rates by end of the experimental period (63 days) were calculated for all treatments. Control group was showed the lowest Specific Growth Rate of 0.41g and the highest value was in GP 4% with 0.63g. The treatments that stood second and third positions were ZP 2.5% (0.62g) and FP 1% (0.58g). These were followed by FP 1.5% (0.55g), GP 2% (0.52g), ZP 5% - FP 0.5% (0.46g) and GP 6% (0.42g) respectively.

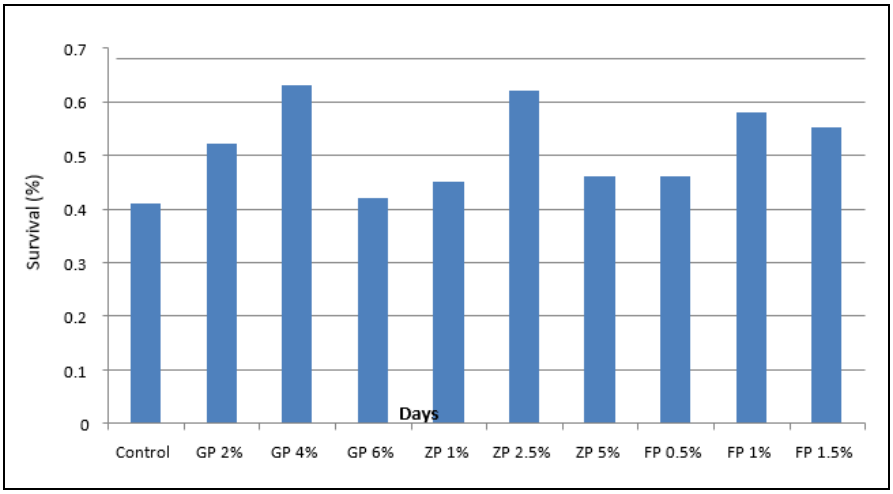

Fig 4: Specific Growth Rates of *L. vannamei* fed with different herbal supplementation

3.3 Feed conversion ratio

3.3.1 Feed conversion ratio of *L. vannamei* fed with different concentrations of garlic powder supplementation

The range for Feed Conversion Ratio observed during the period of experiment was 1.24(GP 4%) – 1.95(control). During the first sampling (7thday) Feed Conversion Ratio ranged between 1.60 and 1.95 and the highest during this period was recorded for control and the lowest was for GP 6%. Sampling on the 14thday shown the highest value 1.88 for control and the lowest 1.55 for GP 6%. The highest value of 1.90 was observed for control on 21th day while the lowest of 1.46 was recorded for GP 4%. The sampling on 28th day

recorded control with highest Feed Conversion Ratio value 1.91 and lowest value of 1.41 for treatment of GP 2%. The highest value of 1.88 was observed for control on 35th day while the lowest of 1.38 was recorded for GP 4%. Sampling on 42ndday recorded highest value of 1.83 for control and lowest value of 1.29 for GP 4%. The last sampling on 63th day recorded control with highest Feed Conversion Ratio value 1.7 and lowest value of 1.26 for treatment of GP 4%.

The Feed Conversion Ratio was subjected to analysis of variance (ANOVA) and presented in table 3. Statistical analysis has shown that F- value is found to be significant among treatments. Since F- value is found to be significant, the pair- wise comparison of any two treatments could be done by computing RBD two-way classification. The control was found to be significantly superior when compared to the other Treatments. The GP 6% and GP 2% were occupied second and third positions in feed utilization. There was a significant difference between the experimental periods also.

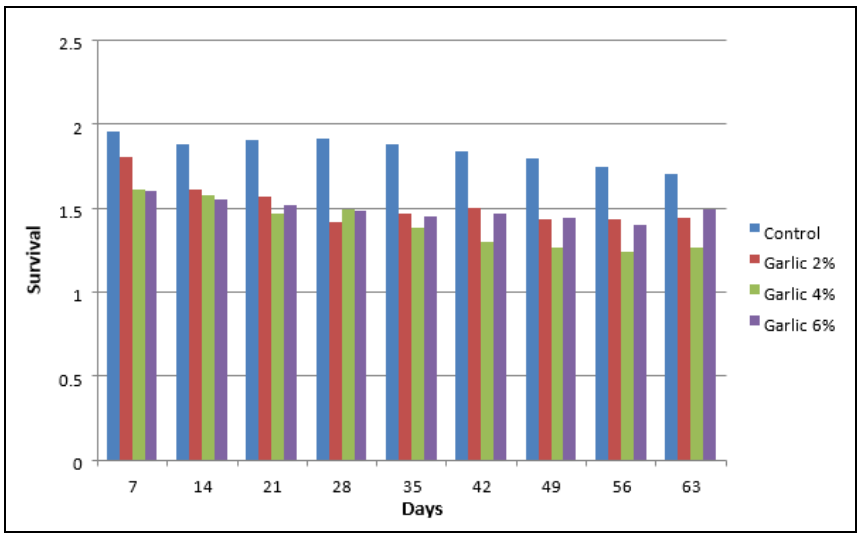

Fig 5: Feed conversion ratio of *L. vannamei* fed with different concentrations of garlic powder supplementation

The Shrimp digestive system is activated particularly in the larval and post larval stages, where the plant extract would have greatest effect (Sankar *et al*., 2011). The higher level of enzymatic activity obtained with herbal supplemented diet improve the feed utilization. In the present study FCR decreased consequentially with the increase of garlic concentration in the treatment diet. Lowest treatment average with significance during critical difference comparison noticed in the GP 4% level of inclusion (P<0.01). This indicates the superiority of GP 4% inclusion diet in the treatment diet. Labrador *et al.* (2016) were observed in L. vannamei fed with GP 6% diet showed lowest FCR value (1.27± 0.16) among all treatment diets. Poongodi *et al*., (2012) [12] also noticed similar trend of superior performance by garlic supplemented diets in *M. rosenbergii* compared to ginger, turmeric and fenugreek supplemented diets. Rebecca and Bhavan (2014) were reported similar performance of garlic supplemented diets in *M. rosenbergii*. The results were also in agreement with finding of earlier workers studies in *P. monodon* and in *P. indicus*.

4. Conclusion

The Growth promoter and survival rate of L. vannamei were done using herbs. The shrimps were fed with herbal feeds. This study was revealed that *L. vannamei* fed with ZP 2.5% diets showed highest survival and found significant (P<001) compared to other treatments. Growth promoting substance digestive enzymes activators and other bio active principles of garlic might have contributed for the increase of feed utilization in the garlic supplemented diet. The constituents of Zinger, like anti-microbial factor and anti-stimulant factor may have contributed for the evaluated survival in experimental group compared to control upon the biochemical evaluation protein shows highest value followed by other biochemical constituents like amino acids, carbohydrates and lipids. The information generated from present investigation might contribute to the incorporation of the herbs in commercial aquaculture as supplement in formulated shrimp feed to achieve good growth rate and survival.

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