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Research Article

Effect of Probiotics on Immunological indices of *Penaeus vannamei* (Boone, 1931) from the Culture Ponds of Ampalam and Gollalavalasa, Andhra Pradesh, India

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Abstract: The present work is carried out in commercial shrimp farms located at Ampalam and Gollalavalasa of Srikakulam District, Andhra Pradesh, India, over a period of two consecutive years i.e 2018-2019. Modified extensive shrimp farms were selected for this research work. The data was recorded from both control and experimental ponds in summer and winter crops. The PCR screened seeds of *P. vannamei* procured from the commercial hatchery located in Visakhapatnam were used. With regard to immunological parameters the experiments were categorized into two groups such as group-I and group-II. In group-I experiments the feed probiotic Pro-2 applied at the rate of 5g/kg and 10g/kg and no immunostimulant was combined. Whereas in the group-II experiments the combination of the feed probiotic Pro-2 and immunostimulant 1,3 β-Glucan was applied in the experimental ponds at the rate of 5g/kg feed probiotic +5 g/kg immunostimulant. Similarly 10g/kg feed probotic + 5 g/kg immunostimulant was administered. Apart from sound management of pond environment to maintain good conditions for commercial shrimp culture, both probiotics and immunostimulants are good candidates to replace the use of the chemicals and antibiotics in the development of sustainable shrimp farming. *Bacillus* spp., appear to behave as good probiont as well as showing potential as immunostimulant. The addition of the immunostimulant 1,3-β Glucan along with the feed probiotics resulted in increasing total haemocytic count with greater phatocytic activity.
Keywords: Phenol oxidase, Phagocytic activity, *P. vannamei.*

Introduction

Johansson *et al.,* (2000) reported that haemocytes play an important role in shrimp immune system including recognition, phagocytosis, melanization, cytotoxicity and cell-cell communication. Recently studies are mainly focused on the effects of probiotics in shrimp immunity and on the responses of haemocytes.

Many scientific workers have provided information about increase of total haemocytic count in different species of shrimps and prawns with the application of probiotic in *Macrobrachium rosenbergii* (Mujeeb Rahiman *et al.,* 2010), *P. monodon* (Rengpipat *et al.,* 2000), *P. japonicus* (Zhang *et al.,* 2011) and *P. vannamei* (Li *et al.,* 2007) respectively. Although it has been observed that in *P. vannamei* the total haemocytic count was decreased significantly when shrimp fed with *L. plantarum* as reported by Chiu *et al.,* (2007). While according to Tseng *et al.,* (2009) no such significant reaction of total haemocytic count was observed after feeding with *Bacillus subtilis.* Similar effect was also noticed in *Pseudomonas* fed *P. monodon* as reported by Alavandi *et al.,* (2004).

Fu *et al.,* (2011) studied about haemocytic phagocytosis in shrimp that was enhanced by *Bacillus subtilis* harboring a viral protein (VP28) and this was speculated by them as a significant factor in the

protection against white spot syndrome virus disease. Furthermore respiratory burst of haemocytes could also be enhanced by probiotic application as reported by Mujeeb Rahiman *et al.,* (2010).

According to Rengpipat *et al.,* (2000) and Tseng *et al.,* (2009) haemocytic phagocytosis was also increased in *P. monodon* and *P. vannamei* when fed with Bacillus S1 and *Bacillus subtilis* E 20 respectively. The purpose of the present study is to estimate the Haemocytic count, phagocytic activity and Phenol oxidase activity in response to Pro-2 and immunostimulant 1, 3 β-Glucan, a commercial brand β-ADVANTAGE.

Material and Methods

The present work is carried out in commercial shrimp farms located at Ampalam and Gollalavalasa of Srikakulam District, Andhra Pradesh, India, over a period of two consecutive years i.e 2018-2019. Modified extensive shrimp farms were selected for this research work. The data was recorded from both control and experimental ponds in summer and winter crops.

For studies on immunological indices the commercial feed probiotic used in the present study is Pro-2. It is composed of *Lactobacillus sporogenisis* with the strength of one million i.e. $1.0x10^6$ cfu/g. The immunostimulant used in the present studies is 1,3 β-Glucan, a commercial brand β-ADVANTAGE.

The experiments were categorized into two groups such as group-I and group-II. In group-I experiments the feed probiotic Pro-2 applied at the rate of 5g/kg and 10g/kg and no immunostimulant was combined.

Whereas in the group-II experiments the combination of the feed probiotic Pro-2 and immunostimulant 1,3 β-Glucan was applied in the experimental ponds at the rate of 5g/kg feed probiotic +5 g/kg immunostimulant. Similarly 10g/kg feed probotic + 5 g/kg immunostimulant was administered.

The immunological studies such as phagocytic counts, total haeamocytic count and Phenol oxidase activity were observed in the laboratories by collecting the samples randomly from the control and experimental ponds during the study period. Preparation of the haemolymph for microscopic observation, blood sample of 0.5 ml from each shrimp were withdrawn from the base of the third walking leg by a syringe containing 1.5 ml anticoagulant (K-199 + 5% L-cysteine). After collecting the haemolymph, haemocytes were counted using a haemocytometer and calculated the number of blood cells i.e. total haemocytes per cubic millimeter.

For the determination of Phenoloxidase activity spectrophotometric method was followed at 490 mm wave length, for this amount of Dopachrome formed from the L-dihydrophenylalanine (L-DOPA) was recorded as described by (Barraco *et al.,* 1995).

For the determination of phagocytic activity haemolymph was collected and used modified KC-199 medium and method followed as described by Itami *et al.,* (1994), and Weeks-Perkins *et al.,* (1995).

Percentage phagocytosis = Phagocytic haemocytes/ Total haemocytes x 100

Statistical analysis

One-way ANOVA was carried out to check the effect of days on the immune parameters, in control and experimental farms of the winter and summer crops at Ampalam, and Gollalavalasa during 2018 and 2019. These analyses were done by using IBM SPSS Version 22.0. Bar graphs were drawn by using mean values and SD of immune parameters in MS Excel 2016. All values were represented as Mean ± SD.

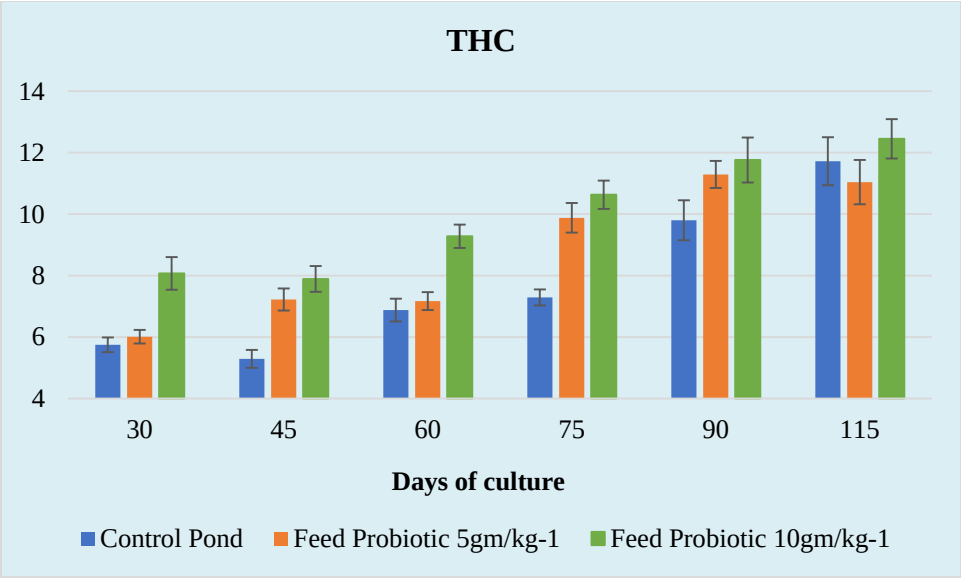

Figure 1. Total haemocytic count-Group 1

Table 1. Total haemocytic count-Group 1					
ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	44.766	2	22.383	4.918	.011
Within Groups	232.119	51	4.551		
Total	276.886	53			

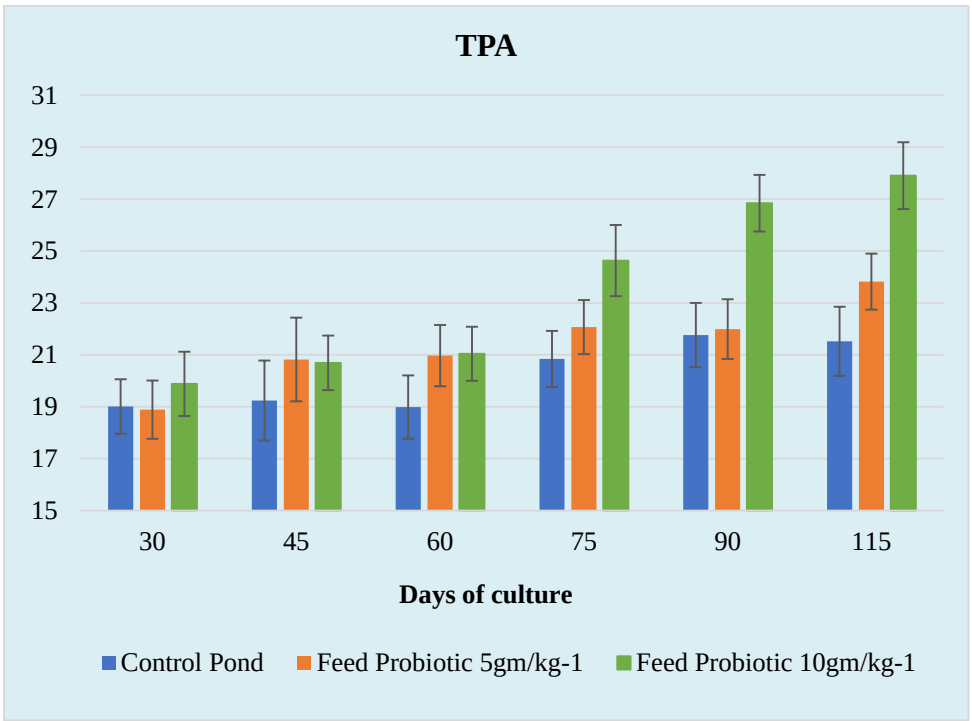

Figure 2. Total phagocytic activity-Group 1

Table 2. Total phagocytic activity-Group 1					
ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	98.507	2	49.253	8.501	.001
Within Groups	295.486	51	5.794		
Total	393.993	53			

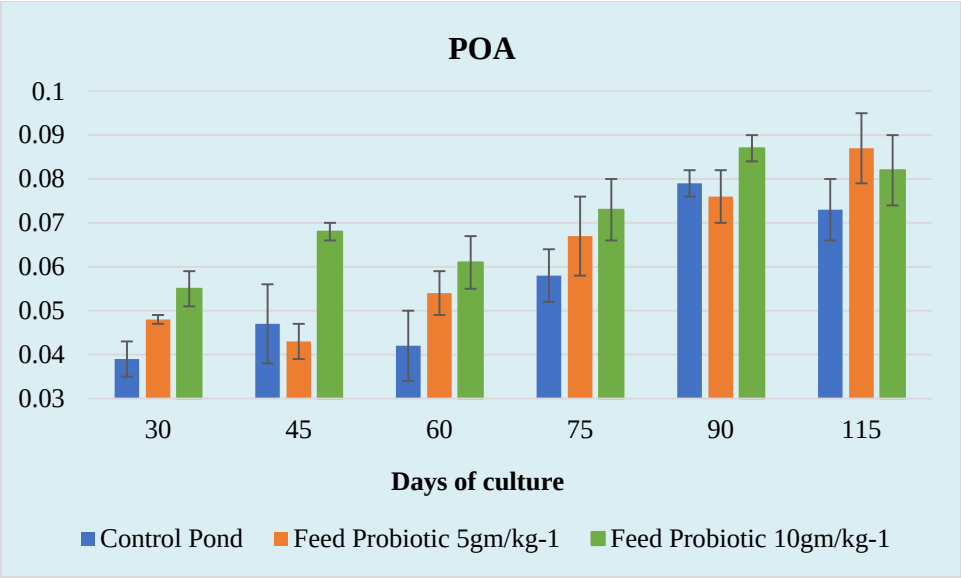

Figure 3. Phenol oxidase activity-Group-1

Table 3. Phenol oxidase activity-Group-1					
ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.002	2	.001	4.112	.022
Within Groups	.012	51	.000		
Total	.014	53			

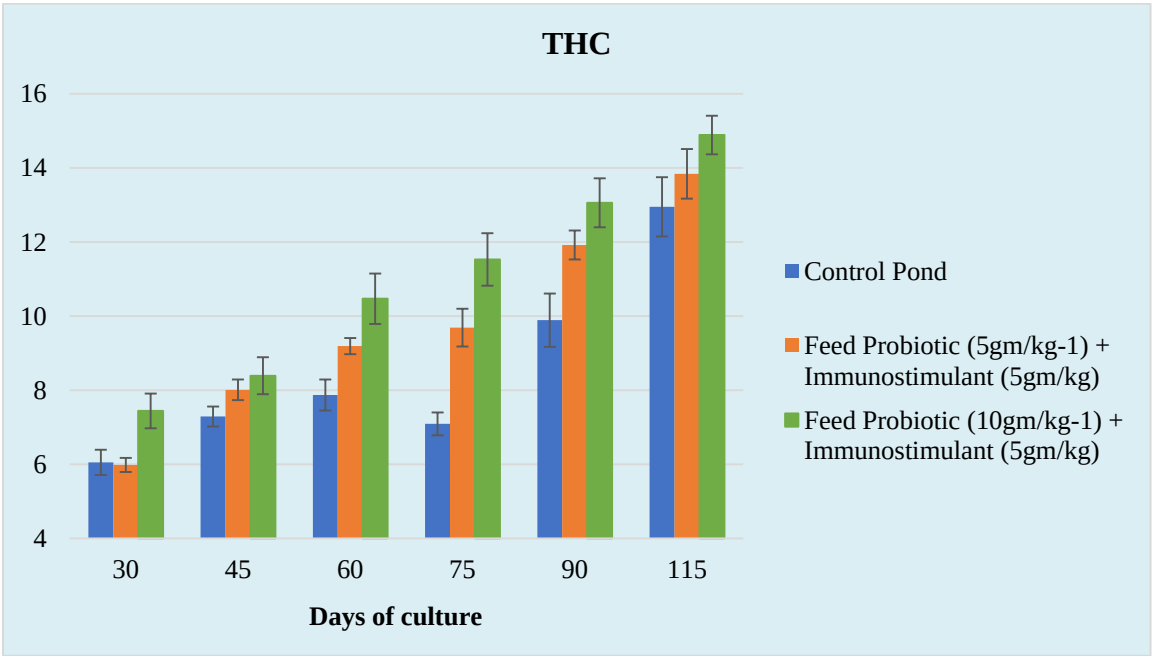

Figure 4. Total haemocytic count-Group 2

Table 4. Total haemocytic count-Group 2					
ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	53.592	2	26.796	4.025	.024
Within Groups	339.543	51	6.658		
Total	393.135	53			

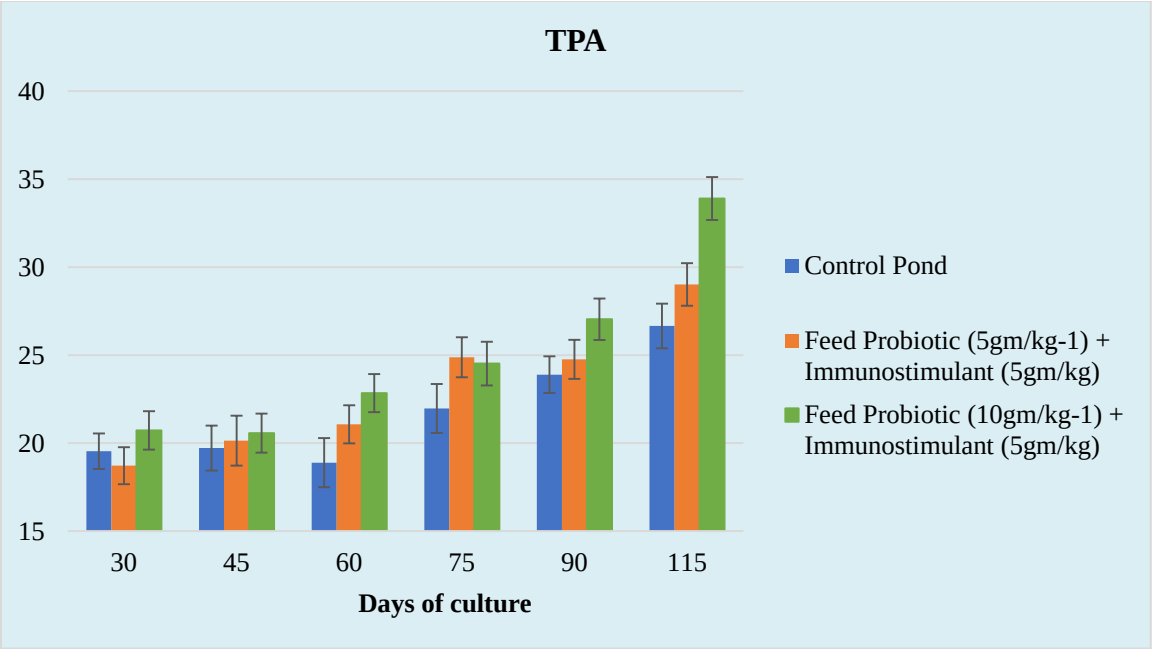

Figure 5. Total phagocytic activity-Group 2

Table 5. Total phagocytic activity-Group 2					
ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	90.282	2	45.141	2.927	.063
Within Groups	786.471	51	15.421		
Total	876.753	53			

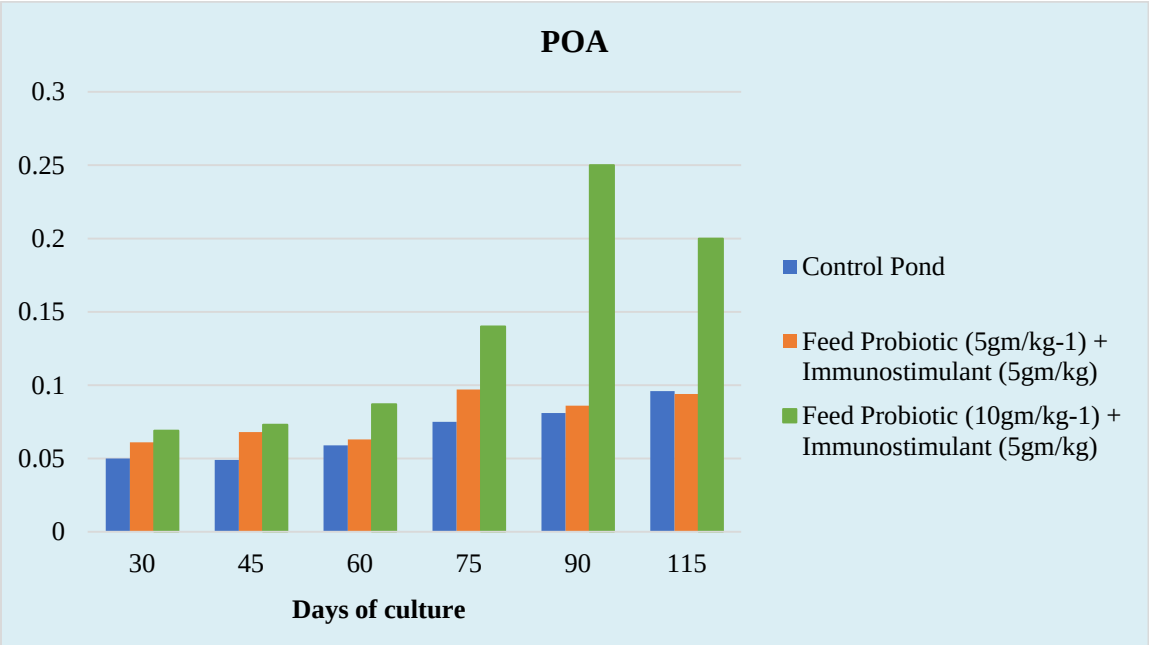

Figure 6. Phenol oxidase activity-Group 2

Table 6. Phenol oxidase activity-Group 2					
ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.049	2	.024	13.199	.000
Within Groups	.094	51	.002		
Total	.143	53			

The present findings of the mean and standard deviation values (mean±SD) for total haemocytic, phagocytic and phenol oxidase activity were categorized into group 1, and group 2.

Group-I

Total haemocyte count with feed probiotic

It is evident from the present results that the haemocytic count of group 1 was recorded at different time intervals of culture in control pond and experimental ponds (treated with feed probiotic). Mean values of control pond were ranged from 5.29±0.29 to 11.72±0.78. Similarly mean values of probiotics treated pond with 5g/kg ranged from 6.01±0.22 to 11.29±0.44. Whereas mean values of probiotics treated pond with 10 g/kg were ranged from 7.89±0.42 to 12.45±0.64 respectively **(Tables 1-3, Figures 1-3).**

Phagocytic activity with feed probiotic

The phagocytic activity of group 1 was recorded at different time intervals of culture in control pond and experimental ponds (treated with feed probiotic). Mean values of control pond were ranged from 18.99±1.22 to 21.76±1.24. Similarly mean values of probiotics treated pond with 5g/kg ranged from 18.89±1.12 to 23.82±1.08. Whereas mean values of probiotics treated pond with 10 g/kg were ranged from 19.88±1.24 to 27.90±1.29 respectively **(Tables 1-3, Figures 1-3).**

Phenol oxidase activity with feed probiotic

Phenol oxidase activity of group 1 was recorded at different time intervals of culture in control pond and experimental ponds (treated with feed probiotic). Mean values of control pond were ranged from 0.039±0.004 to 0.079±0.003. Similarly mean values of probiotics treated pond with 5g/kg ranged from 0.043±0.004 to 0.087±0.008. Whereas mean values of probiotics treated pond with 10 g/kg were ranged from 0.055±0.004 to 0.087±0.003 respectively **(Tables 1-3, Figures 1-3).**

Group II

Total haemocyte count with feed probiotic and immunostimulant

It is evident from the present results that the haemocytic count of group 2 was recorded at different time intervals of culture in control pond and experimental ponds (treated with feed probiotic and immunostimulant). Mean values of control pond were ranged from 6.05±0.34 to 12.95±0.80. Similarly mean values of probiotics (5g/kg) and immunostimulant (5g/kg) treated pond ranged from 5.98±0.19 to 13.84±0.67. Whereas mean values of probiotics (10g/kg) and immunostimulant (5g/kg) treated pond were ranged from 7.44±0.47 to 14.89±0.52 respectively **(Tables 4-6, Figures 4-6).**

Phagocytic activity with feed probiotic and immunostimulant

The phagocytic activity of group 2 was recorded at different time intervals of culture in control pond and experimental ponds (treated with feed probiotic and immunostimulant). Mean values of control pond were ranged from 18.89±1.40 to 26.66±1.27. Similarly mean values of probiotics (5g/kg) and immunostimulant (5g/kg) treated pond ranged from 18.72±1.05 to 29.02±1.21. Whereas mean values of probiotics (10g/kg) and immunostimulant (5g/kg) treated pond were ranged from 20.57±1.11 to 33.90±1.22 respectively **(Tables 4-6, Figures 4-6).**

Phenol oxidase activity with feed probiotic and immunostimulant

The phenol-oxidase activity of group 2 was recorded at different time intervals of culture in control pond and experimental ponds (treated with feed probiotic and immunostimulant). Mean values of control pond were ranged from 0.049±0.009 to 0.096±0.006. Similarly mean values of probiotics (5g/kg) and immunostimulant (5g/kg) treated pond ranged from 0.061±0.002 to 0.097±0.008. Whereas mean values of probiotics (10g/kg) and immunostimulant (5g/kg) treated pond were ranged from 0.069±0.009 to 0.25±0.002 respectively **(Tables 4-6, Figures 4-6).**

In spite of the lack of immunoglobulins shrimp have developed immune defense mechanism that could hamper the invading pathogens and one of them is through prophenoloxidase (proPO) cascade

system as reported by Fagutao *et al.,* (2009). Phenoloxidase enzyme play an important role in controlling bacterial load in haemolymph, in self-non-recognition and protect against pathogenic bacteria as reported by Hernandez-Lopez *et al.,* (1996) and Amparyup *et al.,* (2009). The application of probiotics causes to the stimulation of phenoloxidase activity in *P. vannamei* (Li *et al.,* 2007; Chiu *et al.,* 2007; Tseng *et al.,* 2009; Wang and Gu, 2010; Nimrat *et al.,* 2013), *P. monodon* (Rengpipat *et al.,* 2000), *P. japonicus* (Zhang *et al.,* 2011) and in *Macrobrachium rosenbergii* (Mujeeb Rahiman *et al.,* 2010). The stimulation of β-1-3-glucans is so specific in its active form, the phenoloxidase catalyses, the oxidation of phenols to semiquinons and quinines this is because of their high reactivity will vanish the microorganisms.

The immune response was measured in the shrimp *P. vannamei* by the total haemocytic count (THC) and percentage of phagocytosis by measuring the phagocytic count. In the present findings higher phenoloxidase activity was observed when shrimps were fed with feed probiotics along with 1-3- β-glucans in different experimental studies. Similar results were reported by Sung *et al.,* (1996) who obtained phenoloxidase activity values of 5.04 and 2.30 units in *P. monodon* in which plasma stimulated with vibrio bacterial antigen and zymogen. Similar studies conducted and observed by Alabi *et al.,* (1999). Since phenoloxidase is activated by probiotics and 1-3-β-glucan components, hence to evaluate the response of the immune system in *P. vannamei* total phenol oxidase activity was used as an indicator in the present investigation. Further by the administration of immunostimulant along with feed probiotic could induces the significant production of haemocytes and enhanced phagocytic activity was noticed in the present study.

Conflicts of interest: There is no conflict of interest of any kind.

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