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Efficacy of Evaluation of Gut Probiotics against White Gut and White Feces Disease in *Litopenaeus vannamei* Shrimp Aquaculture Systems in Two Different Geographical Regions of Andhra Pradesh

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Abstract The aim of the study is to investigate the efficacy of gut probiotics in shrimp aquaculture system for the prevention of white gut (WG) and white feces (WF) disease in *Litopenaeus vannamei*. The study was executed in infected *L. vannamei* culture ponds in Varadarajapuram and Cheyyeru Gunnepalli in West and East Godavari districts of Andhra Pradesh. The rate of incidence of white gut and white feces infections in affected shrimp ponds were found to be at 21% in 57~67 days (11.11 gm) and 17% in 108~117 days (28.57 gm) of culture period, respectively. Further, the water quality parameters like temperature ((28.3±0.8)ºC to (30.4±1.2)ºC), salinity ((7.4±1.0) psu to (9.2±1.1) psu), dissolved oxygen ((3.3±0.5) mg/L to (5.6±0.6) mg/L), pH ((7.4±0.2) to (8.6±0.2)), and total alkalinity ((150.2±1.5) ppm to (196±1.5) ppm) were analyzed. In addition, the concentration of ammonia and nitrite in the infected shrimp pond was monitored. The simple linear regression pattern was constructed as the dependent variables between ammonia and nitrite, expressed an R^2 value of 0.952 1 (before probiotics treatment) and 0.939 4 (after probiotics treatment). Extended use of probiotics, the level of ammonia and nitrite concentration was extremely reduced in the culture pond from 0.73±0.01 to 0.038 and 0.51±0.01 to 0.001 respectively. From this study, it was concluded that the use of gut probiotic has significant impact on the retrieval of white gut and white feces disease in the *L. vannamei* aquaculture practice.

Keywords Probiotics; White gut; White feces; *L. vannamei*; Gut health; *Vibrio* spp.

Background

Shrimp aquaculture has been remained as a significant economic resource for many developing countries and served as an important source of food and livelihoods for people around the world. It contributes to over 50% of worldwide shrimp production and is regarded as the most valued aquaculture business (Mamdoh et al., 2019). Marine shrimp aquaculture has been the mainstay of aquaculture industries due to shrimp’s richness in protein supply (FAO, 2016; Mamdoh et al., 2019). Shrimp aquaculture is a common practice in developing nations in Asia and throughout the tropical world supporting non-urban communities with means of survival, and consequently reduced poverty (FAO, 2016).

The white feces syndrome has caused significant economic damage in the cultured shrimp industry in China, Indonesia, Malaysia, Thailand, Vietnam and other countries in Southeast Asia. Thus, more attention should be paid to the pathogenesis of this disease. Due to the impaired immune response and microbiota change in the intestinal tract, shrimp aquaculture industry in India faces the threat of white gut and white feces diseases. Moreover, probiotic practices in aquaculture started to be of importance for increased disease resistance, growth, and feed efficiency (Ramanathan et al., 2005). The first important bottleneck is viral disease outbreak (Karuna et al., 1994), which withered the confidence of entrepreneurs and financial institutions. Another very important problem in shrimp culture is pollution. The organic load in term of unutilized feed due to excessive feeding, fecal matter released by shrimps and dead algae, settlement at the bottom of the pond are contributing to pollution of the pond bottom (Yew-Hu, 1992).

Despite the accelerated development in Indian aquaculture of shrimp, the farming industry has been challenged by different pathogenic diseases over the past decades, which reported to cause substantial economic losses (Seibert and Pinto, 2012). An outbreak of disease has led to a significant reduction in shrimp production and the conversion of shrimp ponds into catfish ponds has forced many farmers out of business, especially in India (Karunasagar and Ababouch, 2012). Shrimp’s white gut and white feces infections are caused by opportunistic bacterial pathogens such as *Vibrio vulnificus, Vibrio fluvialis, Vibrio parahaemolyticus and Vibrio aliginolyticus*, that leads to huge losses in the *L. vannamei* shrimp farmed industry (Moriarty, 1999; Somboon et al., 2012; Mastan et al., 2015). White gut and white feces diseases severely infectshrimp’s hepatopancreas and gut, and turn it to white and pale in colour (Limsuwan et al., 2010; Durai et al., 2015; Mastan et al., 2015).

Probiotics improve the health status of the shrimp by resisting the colonization of pathogens through competition, releasing metabolites that prevent the growth of pathogens and thus increasing the shrimp resistance to diseases (Moriarty, 1998; Mamdoh et al., 2019). In severe cases, white feaces syndrome (WFS) could lead to farm losses due to decreased survival, retarded growth increased feed conversion ratios referred to as WFS (Limsuwan, 2010; Durai, 2015). Incidences of WFS are also associated with high stocking densities, poor pond bottom, high plankton blooms and bad feed management and high pollution in pond water were some of the other factors which are responsible for white fecal syndrome in *L. vannamei*. The affected shrimp show a loose exoskeleton and protozoan fouling infestation that causes a dark coloration of gills (Mastan et al., 2015).

The use of gut probiotics to control the white gut and white feces diseases in shrimp farming is being accepted by researchers due to their eco-friendly practice in aquaculture, but it still requires large scale trials. The role of gut probiotics bacteria in small scale has been studied. But the commercial level is not well-known, especially in white leg shrimp *L. vannamei* (Elumalai et al., 2013). Hence, the present study is focused to investigate the impacts of gut probiotic on the commercial culture of *L.vannamei* in infected shrimp ponds.

1 Materials and Method

1.1 Study site

For study purpose, two shrimp ponds were chosen which located in Varadarajapuram (16°42’48.5”N; 81°25’54.7”E) and Cheyyeru Gunnepalli (16º42’48.5”N; 81º25’54.7”E) villages and the farm is situated 22 and 7 km away from the Bhimavaram and Amalapuram town, respectively in Andhra Pradesh (Figure 1). The experiments were performed during the period of June to August, 2019.

Figure 1 Representative images of the map showing the study area at Varadarajapuram and Cheyyeru Gunnepalli West and East Godavari districts, Andhra Pradesh

1.2 Product composition
The commercial feed supplements were obtained from the R&D facility of Tablets (India) Limited, Chennai, (CAA Reg. No. CAA/F16/PRO/00005). It is a fine blend of ascorbic acid, essential minerals and vegetable derivatives that produce organic acids. The blend is thoroughly mixed with an inert excipient. Complex material of various organic compounds like ascorbic acid (vitamin C), bioflavonoids (vitamin P), amino acids, citric peptides, fatty acid, pectin polypeptides and tocopherols are available in the gut probiotics.

1.3 Experimental design
Initially, the two selected ponds were allowed to dry and then the bottom of the pond was scrapped up to 3 to 5 cm depth using a tractor blade to avoid top soil. The pond bottom was ploughed horizontally and vertically to a depth of 30 cm to remove the obnoxious gases, oxygenate the bottom soil and remove the hydrogen sulphide odor and to increase the fertility. The soil pH was recorded in the ponds with the help of cone type pH meter. For the availability of nutrients, required amount of the lime was applied to neutralize the acid soil based on the average pH level of the pond. Bore water with salinity ranging from 5~10 psu (practical salinity units) was pumped onto the prepared shrimp ponds. Post larvae shrimp (PLs) were subjected to laboratory analysis for monitoring their health status including the infections like White Spot Syndrome Virus (WSSV), *Enterocytozoon hepatopenaei* (EHP) and *Vibrio* spp. associated infection. With results being negative for all the aforementioned tests, PLs were stocked at a density of 45/m^2 with the water depth of 1.7 m and pond area 2.2 and 1.8 ha each ponds. Water exchange was not entertained in the ponds. The gut probiotic (feed supplement) was mixed with feed (5 g/kg feed). Then the animals fed with the probiotic mixed feeds, were examined to observe the percentage of white gut and white feces syndrome in the culture pond.

1.4 Physico-Chemical analysis
The environmental entities were analyzed in the culture pond water during the study period in four different spots with replicate sampling. Water samples were collected between 07.00 and 08.00 hrs for *in situ* examination and laboratory analysis. Collected samples were examined for pH, temperature, and salinity on the spot. The salinity in the ponds was recorded *in situ* by means of a portable hand-held optical refractometer (Atago, Japan) and was cross checked in the laboratory using Mohr-Knudsen method. pH was measured using electronic pH pen (Erma, Japan), temperature was measured using standard Celsius thermometer. The dissolved oxygen concentration was estimated by modified Winkler's method as described by Strickland and Parsons (1972). The total alkalinity of the water was measured as per APHA (1998).

1.5 Physical analysis
During the culture period, shrimps were physically investigated in the check trays at regular basis in every morning and also the weight of the samples was measured once in a week. Shrimps were examined for observe the physical signs of infection like reduction in feeding, prevalence of lethargy, muscle opacity, melanised foci, black soil in the gut, gaps in the gut and antenna cut etc., during the culture period.

1.6 Statistical analysis
Data were expressed as mean± SE. All statistical calculations were performed using SPSS 11.5 (SPSS Inc, Chicago, IL, USA). Linear regression was plotted between ammonia and nitrite using Origin 6.1 statistical package (Origin Lab Corporation, Massachussets (USA).

2 Results
In the present study, the physicochemical parameters such as temperature, dissolved oxygen, salinity, ammonia, nitrite and total alkalinity of the shrimp ponds were closely monitored in pre and post-application of top coating feed supplement (Table 1; Table 2; Table 3; Table 4). The obtained results show that the temperature of infected ponds was ranged between (28.333±0.8)ºC and (30.448±1.2)ºC and the dissolved oxygen varied from (3.356±0.1) mg/L to (6.074±0.1) mg/L (Figure 2) and pH reading ranged between 7.407±0.2 and 8.638±0.2 (Figure 3). In addition, the salinity of the infected ponds extended from (7.456±1.0) psu to (9.298±1.1) psu (Figure 3).

Table 1 Effect of Ecoclean on pH, salinity, temperature and dissolved oxygen in infected shrimp ponds at Varadarajapuram

S.N	DOC	pH	Salinity (PSU)	Temperature (°C)	DO	Remarks
1	56	8.590±0.2	6.684±1.1	29.572±0.5	4.628±0.5	Before WG and WF infection 80 kg/day feed was given
2	57	8.521±0.2	6.732±1.0	29.372±0.5	4.273±0.3	Before WG and WF infection 80 kg/day feed was given
3	58	8.099±0.2	7.817±1.1	29.114±0.7	3.629±0.8	After WG and WF infected 60 kg/day feed was given
4	59	7.796±0.2	8.885±1.0	29.909±1.1	3.641±0.7	5 g of Ecoclean applied per kg feed 2 meals/day 60 kg feed was given
5	60	7.515±0.2	7.456±1.0	30.448±1.2	3.933±0.5	2 meals/day 60 kg/day feed was given
6	61	7.655±0.2	7.504±1.1	30.191±1.1	4.021±0.5	2 meals/day 60 kg/day feed was given
7	62	7.910±0.2	8.477±1.0	30.433±1.1	4.681±0.3	2 meals/day 66 kg/day feed was given
8	63	8.396±0.2	7.880±1.0	29.845±0.9	5.153±0.6	2 meals/day 70 kg/day feed was given
9	64	8.507±0.2	8.761±1.0	29.159±1.1	5.227±0.4	1 meal/day 74 kg/day feed was given
10	65	8.638±0.2	8.682±1.1	29.247±1.0	5.288±0.5	After recovered of WG and WF 80kg/day feed was given

Note: Data were expressed as mean values of duplicate independent experiments with standard errors

Table 2 Effect of Ecoclean on Ammonia, nitrite and total alkalinity in infected shrimp ponds at Varadarajapuram

S.N	DOC	Ammonia (TAN) (ppm)	Nitrite (NO_2) (ppm)	Total Alkalinity (ppm)	Remarks
1	56	0.416±0.01	0.283±0.01	177.751±1.5	Before WG and WF infection 80 kg/day feed was given
2	57	0.385±0.01	0.277±0.01	170.905±1.5	Before WG and WF infection 80 kg/day feed was given
3	58	0.256±0.01	0.183±0.01	159.664±1.5	After WG and WF infected 60 kg/day feed was given
4	59	0.224±0.01	0.169±0.01	160.256±1.5	5 g of Ecoclean applied per kg feed 2 meals/day 60 kg feed was given
5	60	0.180±0.01	0.073±0.01	164.468±1.5	2 meals/day 60 kg/day feed was given
6	61	0.146±0.01	0.063±0.01	165.354±1.5	2 meals/day 60 kg/day feed was given
7	62	0.126±0.01	0.054±0.01	170.010±1.5	2 meals/day 66 kg/day feed was given
8	63	0.132±0.01	0.044±0.01	187.381±1.5	2 meals/day 70 kg/day feed was given
9	64	0.091±0.01	0.031±0.01	190.901±1.5	1 meal/day 74 kg/day feed was given
10	65	0.038±0.00	0.001±0.00	191.648±1.5	After recovered of WG and WF 80kg/day feed was given

Note: Data were expressed as mean values of duplicate independent experiments with standard errors

Table 3 Effect of Ecoclean on pH, salinity, temperature and dissolved oxygen in infected (21%) shrimp ponds at Cheyyeru Gunnepalli

S.N	DOC	pH	Salinity (PSU)	Temperature (°C)	DO	Remarks
1	108	8.366±0.2	8.567±1.1	29.466±0.6	5.366±0.5	Before WG and WF infection 75 kg/day feed was given
2	109	8.933±0.2	8.478±1.4	29.366±0.5	4.833±0.2	Before WG and WF infection 75 kg/day feed was given
3	110	7.933±0.2	8.492±1.1	28.333±0.8	4.133±0.2	After WG and WF infected 57 kg/day feed was given
4	111	7.666±0.2	8.409±1.0	28.733±0.9	3.566±0.5	5g of Ecoclean applied per kg feed 2 meals/day 57kg feed was given
5	112	7.407±0.2	8.208±1.0	29.266±1.2	3.333±0.5	2 meals/day 57kg/day feed was given
6	113	7.966±0.2	8.722±1.0	29.433±0.6	3.633±0.5	2 meals/day 57kg/day feed was given
7	114	8.133±0.2	8.598±1.0	29.466±0.4	4.833±0.1	2 meals/day 60kg/day feed was given
8	115	8.333±0.2	8.897±1.0	29.166±0.4	4.966±0.7	2 meals/day 65kg/day feed was given
9	116	8.466±0.2	8.802±1.1	29.966±0.7	5.133±0.4	1 meal/day 69kg/day feed was given
10	117	8.533±0.2	9.298±1.1	29.366±0.5	5.633±0.6	After recovered of WG and WF 75kg/day feed was given

Note: Data were expressed as mean values of duplicate independent experiments with standard errors

Table 4 Effect of Ecoclean on ammonia, nitrite and total alkalinity in infected shrimp ponds at Cheyyeru Gunnepalli

S.N	DOC	Ammonia (TAN) (ppm)	Nitrite (NO_2) (ppm)	Total Alkalinity (ppm)	Remarks
1	108	0.733±0.01	0.514±0.01	174.169±1.5	Before WG and WF infection 75 kg/day feed was given
2	109	0.633±0.01	0.480±0.01	171.448±1.5	Before WG and WF infection 75 kg/day feed was given
3	110	0.645±0.01	0.441±0.01	169.596±1.5	After WG and WF infected 57 kg/day feed was given
4	111	0.612±0.01	0.366±0.01	155.335±1.5	5g of Ecoclean applied per kg feed 2 meals/day 57kg feed was given
5	112	0.477±0.01	0.276±0.01	150.226±1.5	2 meals/day 57kg/day feed was given
6	113	0.339±0.01	0.258±0.01	154.657±1.5	2 meals/day 57kg/day feed was given
7	114	0.350±0.01	0.231±0.01	170.055±1.5	2 meals/day 60kg/day feed was given
8	115	0.245±0.01	0.181±0.01	183.840±1.5	2 meals/day 65kg/day feed was given
9	116	0.116±0.01	0.115±0.01	196.995±1.5	1 meal/day 69kg/day feed was given
10	117	0.094±0.00	0.022±0.00	195.636±1.5	After recovered of WG and WF 75kg/day feed was given

Note: Data were expressed as mean values of duplicate independent experiments with standard errors

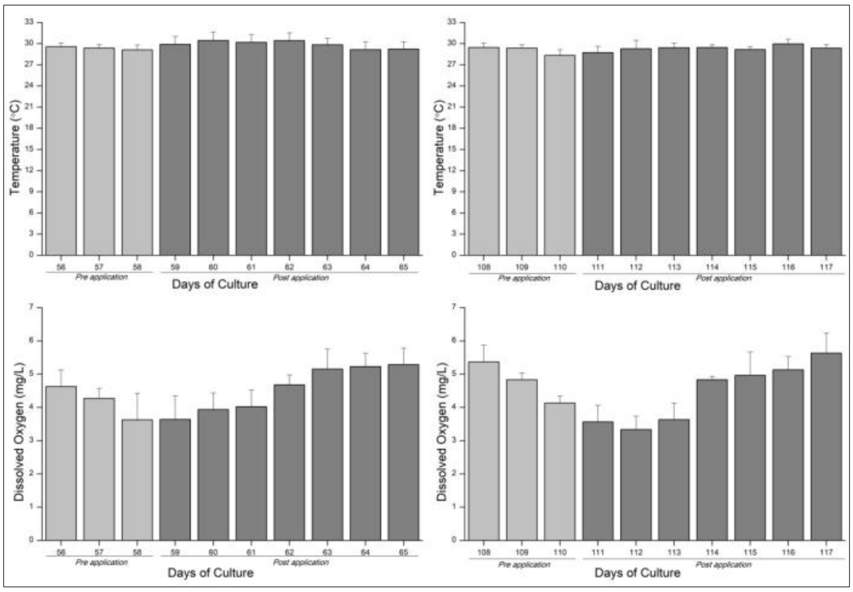

Figure 2 Impact of Gut probiotic on temperature and dissolved oxygen in white gut and white feces affected shrimp culture ponds

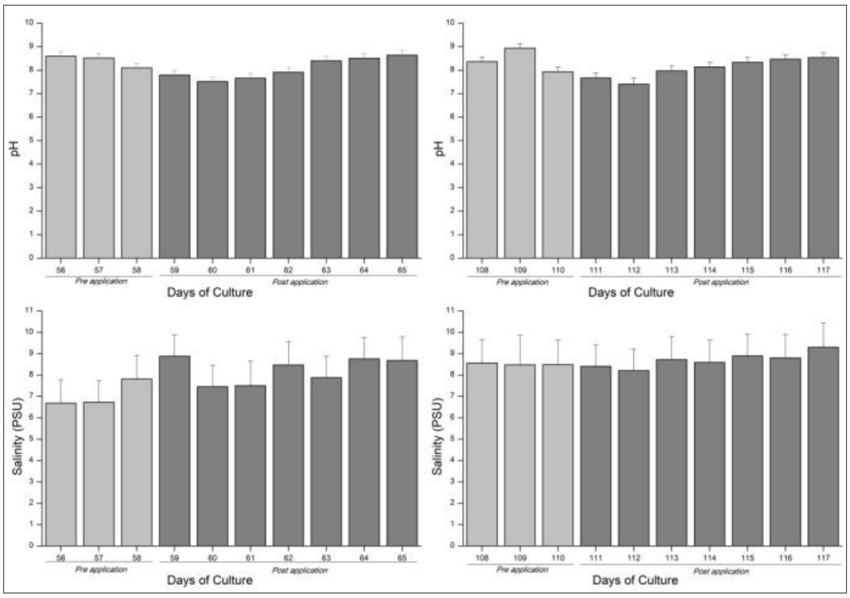

Figure 3 Impact of Gut Probiotic on pH and salinity in white gut and white feces affected shrimp culture ponds

Meantime, the concentration of unionized ammonia was higher in pre-application of gut probiotics (top coating feed supplement) in infected shrimp ponds (0.733±0.01) ppm and it was reduced to 0.038±0.00 ppm by the post-application of top coating feed supplement in white gut and white feces diseased ponds (Figure 4). The highest concentration of nitrite was marked as 0.514±0.01 in pre-application stage and reduced nitrite value was recorded as (0.001±0.00) ppm (Figure 4) in post-application of top coating feed supplement. Similarly, the total alkalinity of the infected ponds was observed and the result reveals that the gut probiotics was significantly reduced the alkalinity from (150.226±1.5) ppm to (196±1.5) ppm (Figure 5). Simple linear regression pattern constructed pre and post application of top coating feed supplement between ammonia and nitrite as dependent variable expressed R^2 values of 0.952 and 0.939 respectively (Figure 6).

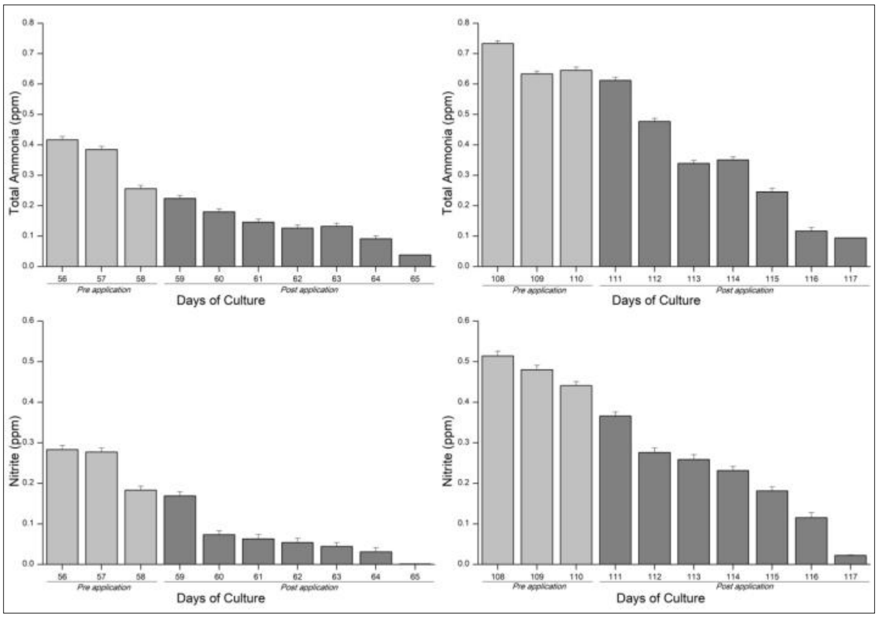

Figure 4 Impact of gut probiotic on ammonia and nitrite in white gut and white feces Affected shrimp culture ponds

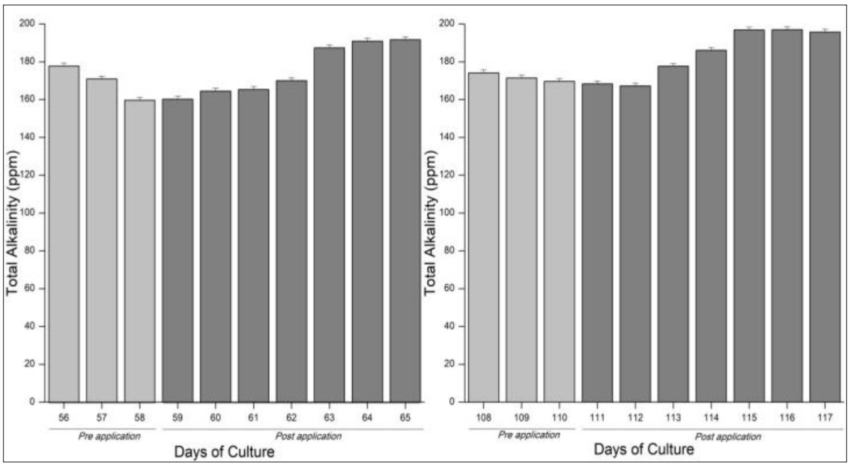

Figure 5 Impact of gut probiotic on total alkalinity in white gut and white feces affected shrimp culture ponds

During the culture period, the strings of white feces floating on the water surface was observed in 57~67 days of juvenile shrimps culture (11.11 gm) and 108~117 days of culture (28.57 gm) in Varadarajapuram and Cheyyeru Gunnepalli ponds (Figure 7a; Figure 7b). Moreover, Figure 8a and Figure 8b show a white coloured gut in infected shrimps while, the normal healthier gut region was in the shrimps which consumed top coating feed supplement. Severely affected individuals were died while others exhibited growth retardation. The prevalence of white gut and white feces disease have been noticed, which were highest (21%) in Cheyyeru Gunnepalli (Amalapuram, EG) pond and lowest (17%) in Varadarajapuram (Bhimavaram, WG) pond. Coming to feed administration, the feed consumption of infected shrimp was come down from 80 kg to 60 kg and 75 kg to 57 kg in both culture ponds. Overall, the present study reveals that the infected shrimps in the culture ponds were completely recovered from the white gut and white feces diseases by feeding the gut probiotics with commercial shrimp feed 5 g/kg (Figure 9a; Figure 9b).

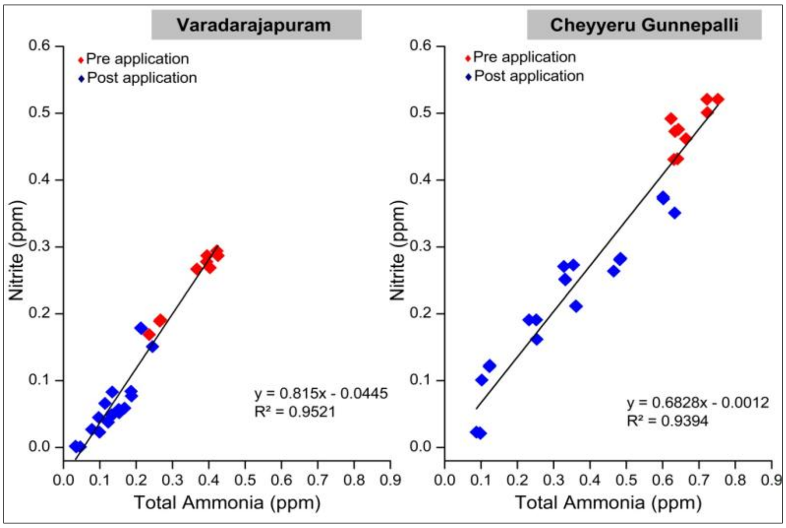

Figure 6 Impact of gut probiotic on the control of ammonia and nitrite in the white gut and white feces diseased shrimp culture ponds

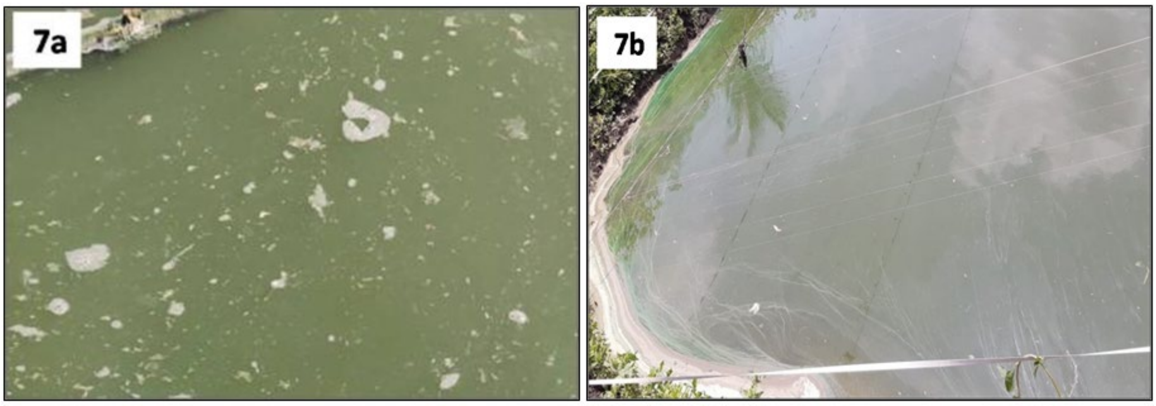

Figure 7(a; b) Representative image showing the strings of white feces on water surface of shrimp culture pond in Varadarajapuram and Cheyyeru Gunnepalli

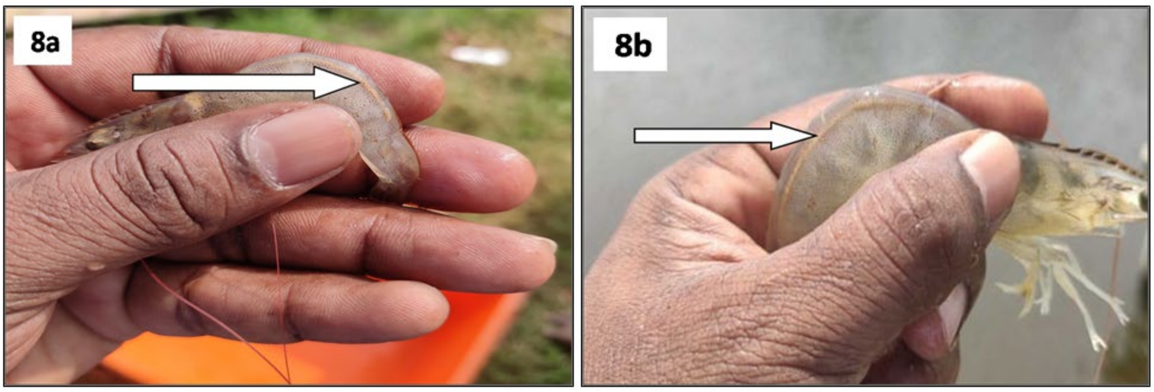

Figure 8(a; b) Representative image showing the infected shrimp with white gut and white feces disease in Varadarajapuram and Cheyyeru Gunnepalli

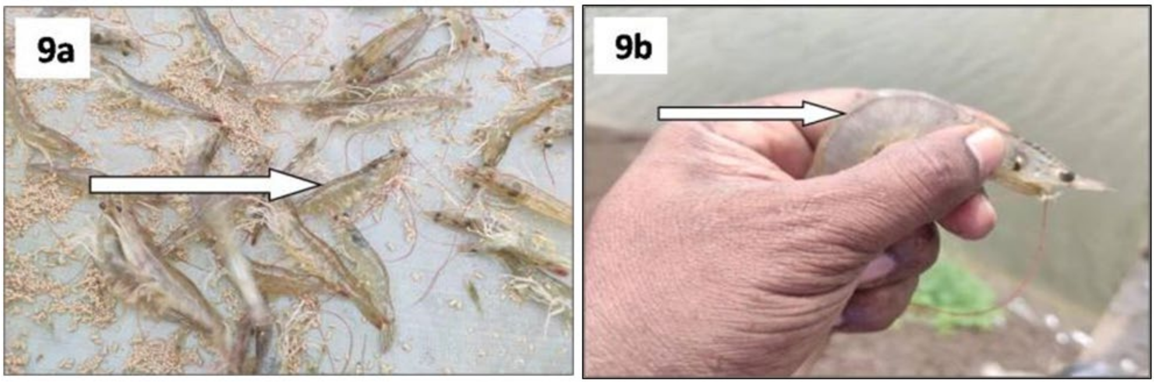

Figure 9(a; b): Representative image showing the recovered shrimps from the white gut and white feces disease by post-application of gut probiotics in infected shrimp culture pond in Varadarajapuram and Gunnepalli

3 Discussion

Shrimp farming is an aquaculture business for the cultivation of marine shrimp or prawns for human consumption. Although traditional shrimp farming has been carried out in Asia for centuries, which accounts for 55 percent of the shrimp produced globally. After *L. vannamei* was introduced to Asia, shrimp production increased significantly. *L. vannamei* became the most important shrimp species in terms of aquaculture production, it is more than 70%. The increase in shrimp production has resulted by intensification of production, which is directly linked with an increased incidence of diseases. Nowadays, there are several major diseases in shrimp farming such as white spot disease, acute hepatopancreatic necrosis disease and white feces disease. Diseases in particular are a major constraint for the sustainability of shrimp production in many countries (Flegel, 1997; Alabi et al., 2000). The environmental and physiological stress factors are often related to aquatic disease and infections; these elements have been related to an increased susceptibility to infectious diseases (Lightner and Redman, 1998). Shrimp farming has seen a significant increase in national and international markets due to its taste and market demand. Several previous studies have reported that the *L. vannamei* majorly affected by white gut and white feces diseases which reduced feed intake, growth and survival of shrimp culture (Mastan et al., 2015; Durai et al., 2015; Biju and Gunalan, 2016; Kumara and Hettiarachchi, 2017). Recently, increasing attention has been paid to the relationship between optimum growth and survival of shrimp and the superior water quality is characterized by adequate pH, salinity, temperature, dissolved oxygen, ammonia, nitrite and total alkalinity.

Temperature is one of the significant environmental parameters in shrimp aquaculture as it directly influences the metabolism, oxygen consumption, growth, moulting and survival. Sudden change in temperature may affect the shrimp immune system. The pond optimum temperature range is 25ºC~30 ºC beyond which lethal effects on shrimp has been recorded (Boyd and Fast, 1992). In the present study, the maximum temperature ranged between (28.333±0.8)°C and (30.448±1.2)°C. Temperature almost remained stable throughout the study period favoring the growth of the animal. The results of present study are well accordance with Soundarapandian (2008) who noticed that the optimum range of temperature for *P. monodon* shrimp is 26 ºC~33ºC. Temperature almost remains stable throughout the study period for favoring the growth of animal.

Likewise, dissolved oxygen plays an important role on growth and production through its direct effect on feed consumption and maturation. Lack of dissolved oxygen can be directly harmful to shrimps and cause a substantial increase in the level of metabolites and also tension hampers metabolic performances in shrimp and can reduce the growth and moulting will cause mortality (Molluae, 2001). Thus, it is important to maintain aeration in the culture ponds by setting up required aerators. With respect to dissolved oxygen level was observed, there was much variation in white gut and white feces diseased culture ponds the values ranged from (3.333±0.5) mg/L to (5.633±0.6) mg/L pre and post application of gut probiotics. Similarly, Gunalan et al. (2010) stated that the optimal range of dissolved oxygen for *L. vannamei* shrimp culture ponds is 3.5 mg/l and the low level of dissolved oxygen can cause mortality. According to Ranjan and Gopalakrishnan (2016) who noticed the dissolved oxygen in white feces infected shrimp culture ponds was recorded at maximum of 5.8 mg/L and minimum of 4.5 mg/L in the white feces diseased ponds. The obtained results of the present study indicate that the dissolved oxygen exposes acceptable range for the survival and growth of *L. vannamei* in white gut and white feces affected both shrimp culture ponds in pre and post application of gut probiotics.

Also, salinity is considered to be the most vital factor for propelling the functional responses of the shrimp biological system such as metabolism, growth, migration, osmotic behavior, and reproduction (Edward et al., 2016). The normal growth of shrimp can be achieved between 15 and 20 ppt (Chen, 1976; Cheng and Liao, 1986). In previous, Boyd (1989) also stated the range of ideal salinity for shrimp cultivation is 15~25 ppt and high or low salinity affects the moulting frequency. Even though, *L. vannamei* is euryhaline animal it is comfortable when it exposed to optimum salinity and the high salinity would weaken the shell and prone to diseases. The obtained results of the present study indicate that the salinity values varied from (6.684±1.0) psu and (9.298±1.1) psu in pre and post application of gut probiotics in white gut and white feces infected shrimp culture ponds. Though salinity is one among the key factor which could influence the growth of the shrimp, it is well balanced by the topping up water to manage the loss of water through seepage and consideration, thereby balancing the salinity of the water irrespective of the temperature. However, the white leg shrimp, *L.vannamei* is widely cultured in Central and South America and cultured animals which tolerate the utmost salinity from 2 to 45 psu (Parker et al., 1974).

The pH of the culture medium is directly related with metabolism and other physiological process of shrimps. Low pH increases the toxicity of nitrite to cultured organism (Wedemeyer and Yasulake, 1978), the toxic form of sulphide (Chein, 1992) and high pH increases the unionized ammonia (Clot and Armstrong, 1979). pH is one of the imperative environmental characteristic, which decides the survival and growth of shrimp under culture, its metabolism and other physiological process of shrimps. In the present study, obtained pH values were ranging between 7.407±0.2 and 8.638±0.2 for the pre and post application of gut probiotics in the two culture ponds. Similarly, reported by Harpaz and Karplus (1991) who recommend the favorable pH range from 7.6 to 8.6 for *L. vannamei* shrimp culture. In the light of the above statement, in a study conducted elsewhere attributed the optimum range of pH 6.8 to 8.7 should be maintained for maximum growth and production, which is very much evident of the present study as well Ramanathan et al. (2005). The most common cause of low pH in water is acidic bottom soil, liming can be used to reduce soil acidity as well as in most common cause of high pH is high rate of photosynthesis by dense phytoplankton blooms. Stated alkalinity is the amount of carbonate, bicarbonate and hydroxide contained in the water. Alkalinity becomes an important key in the water because of its ability to sustain the pH level and low alkalinity in water is poorly buffered against pH change (Boyd, 1984). The total concentration of all divalent cations in water expressed in terms of milligrams per liter of calcium carbonate is the total hardness. In most waters, total hardness and total alkalinity concentrations are approximately equal (Boyd, 1998). The total alkalinity in the ponds was well maintained with application of lime. In this study, total alkalinity in the white gut and white feces infected ponds was observed to be diversified from (150.226±1.5) ppm to (196±1.5) ppm. This result is similar to the previous report of Ranjan and Gopalakrishnan (2016), who stated that the maximum 120 ppm alkalinity was recorded in the normal pond and minimum 100 ppm in the white feces infected shrimp culture pond. Boyd (1998) studied in most waters, total hardness and total alkalinity concentrations are approximately equal. In recent days, the physico-chemical parameters of the culture ponds are adjusted manually by the application of certain components of the aquaculturists.

Shrimp excretes faecal waste in the pond. In addition, unused feed also settles in the bottom of the pond. Fecal waste and unused feed remains as the source of organic matter in the shrimp pond. The accumulation of these organic matters increases the level of toxicity, and subsequently deteriorates the water quality. With increasing in the amount of sludge, the pH of pond will decrease due to CO_2 concentration as a result of respiration process which occurs in various microorganisms as well as shrimps (Delgado et al., 2003). However, ammonia is more toxic than nitrite, generally ammonia exists in water both in ionized and unionized forms. Among them, ionized ammonia is more toxic than unionized form. Ammonia concentration depends on pH, temperature and to lesser extent of salinity. Effect of probiotics on managing the toxic gases were well noticed from the obtained results of the present study. Efficiency of gut probiotics on white gut and white feces infected shrimps in the culture ponds were well noticed. In the present results revealed that, the level of ammonia was well below this mark in both shrimp culture ponds it ranged from 0.038±0.00 to 0.733±0.01 in pre and post application of gut probiotics. Meantime, the maximum concentration of nitrite in infected shrimp culture ponds was recorded as 0.514±0.01 and post-application of gut probiotics reduce the concentration of nitrite which was recorded as (0.001±0.00) ppm. The results observed in the present study is comparable with the study made by Rutledge and Guest (1997) who noticed better water quality is essential for good shrimp production, several parameters were monitored daily and many were present within optimal ranges for the shrimp production.

In the present study, two ponds were used for *L.vannamei* culture and each pond size are 2.2 ha and 1.8 ha. Even if shrimps are bottom dwelling organisms, the depth and volume of water in a pond has a certain physical and biological consequences. Previous study was made by Ramanathan et al. (2005) who stated that the ideal pond size for shrimp culture was 1 or less than 1 ha. The pilot study was observed to find out the efficiency of gut probiotics on the white gut and white feces disease infected in shrimp culture ponds including on feed intake, growth and survival is the most important for cultivable species *L. vannamei* (Ramanathan et al., 2005; Soundarapandian et al., 2010). In the present study, the stocking density were 33 PLs/m^2 and 37 PLs/m^2 in two ponds of Varadarajapuram and Cheyyeru Gunnepalli and observed based on the water quality parameters in the *L. vannamei* culture ponds including feed intake, survival and optimum growth.

The obtained result indicated that the rate of incidence of white gut and white feces infections in affected shrimp ponds were found to be at 21% in 57~67 days (11.11 gm) and 17% in 108~117 days (28.57 gm) of culture period, respectively. The white gut and white feces infected shrimp cultures feed consumption was reduced in both Varadarajapuram and Cheyyeru Gunnepalli ponds in gradually from 80 kg to 60 kg and from 75 kg to 57 kg, respectively. In the obtained results from 56th to 65th days of culture period in the infected ponds revealed that the symptoms of white gut and white feces disease was majorly recorded in Varadarajapuram pond and 108th days of culture to 117th days of culture pond was found white gut and white feces in Cheyyeru Gunnepalli ponds shrimp culture. According to Sriurairatana et al. (2014) studied incidences of white feces syndrome in *L. vannamei* were observed after 50~60 days of stocking of the PLs in the culture ponds. Moreover, the excess feed, fecal matter and metabolites will exert tremendous influence of the water quality of the shrimp culture ponds. Still, causative agent for white feces and white gut has not notorious so far. In the present study, high density dwellers of *L. vannamei* shrimp culture ponds were found to be more prone to white gut and white feces infestation and were also associated with poor water quality, poor pond bottom, high plankton blooms and poor feed management and high pollution.

4 Conclusion

Based on the present study, it could be concluded that the gut probiotics efficiently control/treat the infections in shrimp aquatic farms. The gut probiotic mixture contains beneficial acids which greatly influenced by dietary supplementation. These beneficial acids created healthy shrimp gut and suitable environment for the shrimp culture. Further, it was revealed that the application of gut probiotics plays an important role in maintaining or promoting the gut health of the infected shrimps. The beneficial flora in the intestine of shrimps helps to reducing the white gut and white feces disease in the infected animals by maintaining stable water quality parameters by routine analysis throughout the culture period. The present study provides evidence that white gut and white feces disease are widespread in the East and West Godavari districts of Andhra Pradesh. Therefore, shrimp farmers are advised to utilize locally isolated intestinal probiotics to treat affected shrimps, as well as measures for sustainable and successful disease-free shrimp culture.

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