



# Assess the Impact of the Probiotics and Phytoactives Blend (PPB) on the Water Quality Parameters of the Shrimp Ponds

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## Abstract

A study was conducted to evaluate the impact of a probiotics and phytoactives blend (PPB) on water quality parameters in shrimp ponds compared to a competitor probiotics product (CPP). Four farmer-owned ponds were selected and divided into two Supplemented groups: G1-PPB (2 ponds) and G2-CPP (2 ponds). The PPB was supplemented at 600 g/ha three times a week, while the CPP was administered according to the manufacturer's recommendation. The water quality parameters analyzed included pH, salinity, alkalinity, carbonates, bicarbonates, total hardness, mineral concentrations (calcium, magnesium, potassium, and iron), and microbial count. The results showed that both PPB and CPP improved water quality parameters at similar rates 24 hours after supplementation. However, the overall performance of PPB was found to be better than CPP in maintaining optimal water quality standards for shrimp ponds. The study suggests that adding PPB to shrimp ponds can help maintain water quality standards, making it a recommended supplement for the aquaculture industry. The findings indicate that PPB is a viable option for improving and maintaining the water quality parameters necessary for healthy shrimp growth and development.

**Keywords:** Water quality; Shrimp Pond

## Abbreviations

PPB: probiotics and phytoactives blend; CPP: competitor probiotics product.

## Introduction

Around the world, shrimp culture is a highly widespread and lucrative industry. The significant market need for animal proteins can potentially be met by the shrimp business. Therefore, to improve shrimp production, intensive shrimp farming is increasing nowadays [1]. Despite the increased production, the shrimp industry is facing many challenges. Water quality determines to a great extent the success or

failure of a shrimp cultural operation. Water is an inevitable medium for shrimp farming; hence its quality has assumed a key role in shrimp culture operations owing to its various interacting effects on the productivity of cultured animals. Stressful and unfavorable environmental conditions like nutritional imbalance, mineral imbalance, and vitamin C deficiency can suppress the immune system of shrimp to a great extent, which increases disease susceptibility [2]. Due to the disease outbreaks, many shrimp farmers suffered significant economic losses, and even many of them left the activity. The disease outbreaks in shrimp farming have had a profound impact on the industry, resulting in substantial financial losses for many farmers. The decline in shrimp production and reduced market value have been further

exacerbated by the increased costs of disease treatment and management. This has led to a significant decline in the livelihoods of shrimp farmers, with some being forced to abandon their farms. The ripple effects of these losses are far-reaching, affecting not only the farmers but also their families and communities, who rely on the industry for their well-being and economic stability. Ultimately, the disease outbreaks have created an unstable environment, making it difficult for farmers to plan for the future, secure investments, and achieve a stable income. Many attempts have been implemented to increase the resistance of shrimp against various pathogens and to improve shrimp growth [3].

Citarasu reported that hormones, antibiotics, vitamins and several chemical substances were used in shrimp culture to control diseases. Even though these substances gave positive effects, they cannot be recommended because of their residual and other side effects [4]. Babu, et al. reported that antibiotics and chemicals used in shrimp culture may create various problems such as bioaccumulation, pollution, antibiotic-resistant pathogen, immunosuppression, and high expenditure [5]. Excessive use of antibiotics will be accumulated in the shrimp body, can result in residual effects, which can have long-term consequences on the environment, human health, and the sustainability of the shrimp industry [6].

The use of these substances can be costly, leading to high expenditure for shrimp farmers, which can be a significant burden, particularly for small-scale farmers. The use of hormones, antibiotics, and chemicals in shrimp farming is a complex issue that requires a holistic and sustainable solution to address its environmental, social, and economic implications. Effective management is of great significance in determining the success of shrimp culture operations. The utilization of medicinal plants in the shrimp environment is gaining momentum as it can effectively prevent disease without having adverse chemical toxicity. In the past few years, medicinal plants began to be developed as an alternative to antibiotics and chemicals used in shrimp culture [7].

The alternative herbal bio-medicinal products in shrimp culture operations, which have the characteristics of growth-promoting ability and tonic to improve the immune system, act as appetite stimulators. They increase feed consumption, induce maturation, and have antimicrobial capability and also anti-stress characteristics that will be of immense use in the culture of shrimps and other shrimps without any environmental and hazardous problems. Herbal compounds such as phenolics, polyphenols, alkaloids, quinones, terpenoids, lectins, and polypeptides have been shown to be a very effective alternative to antibiotics and other synthetic compounds e.g., Ginger (*Zingiber officinale*) and Neem

(*Azadirachta indica*).

Probiotics have many medicinal potentials to improve disease resistance against pathogens. They contain many medicinal properties for treating diseases [8-10]. Moreover, probiotics can be a safer ecological alternative tool for sustainable aquaculture.

The use of probiotics in aquaculture has many advantages in improving water quality and aquaculture health like (i) Degradation of organic matter and thus significantly reducing sludge and slime formation, thereby water quality is improved by reducing bottom sediments, (ii) Inorganic forms of nitrogen, such as ammonia, nitrite, and nitrate are also reduced, (iii) Probiotic bacteria in aquaculture ponds are antagonistic to pathogenic bacteria for food and shelter, and thereby probiotics can displace pathogenic and deleterious common bacteria like *Vibrio* sp. [11,12]. With this background, a polyherbal formulation fortified with probiotics "Probiotics and Phytoactives Blend (PPB)" was developed by Himalaya Wellness Company, Bengaluru, Karnataka, India in order to improve shrimp pond water quality. Hence, in the present study PPB was chosen for screening wherein water quality parameters viz. pH, salinity, total alkalinity, carbonates, bicarbonates, total hardness of water, conc. of calcium, magnesium, potassium & Iron, and the microbial count was assessed in various farmer-owned shrimp ponds in order to verify the claim and to determine the effect of PPB on water quality parameters of shrimp ponds and translatability of results under field conditions.

## Materials and Methods

### Probiotics and Phytoactives Blend (PPB)

PPB is a probiotic and phytoactives blend (PPB) called PondShield, developed through the M/s. Himalaya Wellness Company in Bengaluru, Karnataka, India.

PPB contains: Probiotics blend (*Bacillus subtilis*, *Bacillus licheniformis*, *Cellulomonas cartae*, *Pseudomonas denitricans*, *Rhodococcus* sp., *Saccharomyces boulardii*), Zinger (*Zingiber officinale*) and Neem (*Azadirachta indica*).

### Study Design

Four different farmers-owned shrimp ponds were selected at the Nellore district headquarters of Andhra Pradesh state, India, and treated with PPB and competitor products. The study consisted of 2 Supplementations and was designated as follows: G1-PPB (n=2) and G2-Comp-1 (n=2). The PPB was supplemented 600 g/ha 3 times a week interval. Competitor products viz. Comp-1 was administered as per the recommended dose of the manufacturer's protocol.

## Assessment of Parameters

### Water Quality Parameters

Around 50 ml of water sample was collected before (pre-application) and 24 hrs after the addition of investigational veterinary products viz. PPB and Comp-1 (post-application) in clean and sterilized plastic containers. Water quality parameters viz. pH, salinity, alkalinity, carbonates, bicarbonates, total hardness of water, the concentration of minerals (calcium, magnesium & potassium), and microbial count of pond water were accessed according to standard methods.

Water quality parameters measured were by Salinity- ERMA made in Japan ATC, Ph- PH-107 pH manufactured by MCP Medicare Products Inc, TDS and temperature- TDS-3 TDS/Temp, Ammonia- API Ammonia Kit, other parameters- spectrophotometer UV and visible spectrophotometer by following the standard procedure.

## Statistical Analysis

The data are expressed as Mean  $\pm$  SD. Data were subjected to statistical analysis using paired t-tests to draw a comparison between pre-application and post-application effects.  $p \leq 0.05$  was considered statistically significant.

## Results and Discussion

### Water Quality Parameters

There was no significant change in the water quality parameters of shrimp ponds viz. pH, salinity, total alkalinity, carbonates, bicarbonates, total hardness of water, conc. of calcium, magnesium, potassium & Iron, and microbial count were observed before and after the addition of PPB. However, the hardness of water and conc. of iron was slightly above the shrimp pond water quality standards. Upon Supplementation with, PPB there was a decrease in the hardness of water from 4575 to 4366, and the concentration of iron was reduced from 0.88 to 0.50 (Table 1).

Parameters	Standards	Before Supplementation	After Supplementation	p-value
pH	7.5 – 8.5	8.15 $\pm$ 0.05	8.25 $\pm$ 0.08	0.499
Salinity	0 – 25	16.31 $\pm$ 1.30	17.00 $\pm$ 1.44	0.185
Total Alkalinity	120 - 300	187.50 $\pm$ 12.50	200.00 $\pm$ 0.00	0.612
Carbonates	30-Oct	19.38 $\pm$ 1.48	28.33 $\pm$ 5.43	0.254
Bicarbonates	110 - 270	168.13 $\pm$ 12.32	171.67 $\pm$ 5.43	0.956
Total hardness	500 – 4000	4575.00 $\pm$ 215.27	4366.67 $\pm$ 181.96	0.484
Calcium	>100	540.00 $\pm$ 25.07	586.67 $\pm$ 33.73	0.253
Magnesium	>200	785.75 $\pm$ 61.48	722.67 $\pm$ 49.41	0.332
Iron	<0.1	0.88 $\pm$ 0.18	0.50 $\pm$ 0.00	0.141
Green Colonies	<50	20.00 $\pm$ 12.25	8.33 $\pm$ 4.77	0.705
Yellow Colonies	<300	23.75 $\pm$ 11.43	48.33 $\pm$ 11.95	0.5
Total Vibrio Count	500	43.75 $\pm$ 23.57	56.67 $\pm$ 12.29	0.652

Note: Values are expressed as Mean  $\pm$  SEM; n=8;  $p > 0.05$  as compared to before Supplementation based on paired t-test

**Table 1:** Effect of PPB on water quality parameters of shrimp ponds.

Similarly, the water quality parameters of shrimp ponds were analyzed before and after the addition of a competitor product (Comp-1), and the results depicted that conc. of calcium in shrimp pond water was significantly ( $p = 0.042$ ) increased after the addition of Comp-1 as compared to before the addition. However, conc. of calcium in the shrimp pond falls within the normal range values. Furthermore, the hardness of water and conc. of iron was slightly above the shrimp pond water quality standards; whereas, upon Supplementation with Comp-1 there was a decrease in the hardness of water (4650 Vs. 4533) and conc. iron (0.80 Vs. 0.63) was reduced (Table 2).

The analysis of water quality parameters of shrimp ponds (24 hrs. after the addition of investigational veterinary products) depicted that, there was no significant change in the water quality parameters of shrimp ponds viz. pH, salinity, total alkalinity, carbonates, bicarbonates, total hardness of water, conc. of calcium, magnesium, potassium & Iron, and the microbial count was observed following the addition of PPB and Comp-1, and values of all the water quality parameters fall within the normal reference range values. Furthermore, PPB and Comp-1 reduced the hardness of shrimp pond water more or less at the same rate (4575 Vs. 4533). The results of water quality parameters 24 hrs. after the addition of PPB and Comp-1 was represented in Table 3.

Parameters	Standards	Before Supplementation	After Supplementation	p-value
pH	7.5 – 8.5	8.08 ± 0.04	8.21 ± 0.09	0.526
Salinity	0 – 25	16.75 ± 0.88	17.50 ± 1.07	0.203
Total Alkalinity	120 - 300	200.00 ± 9.45	208.33 ± 7.22	0.537
Carbonates	30-Oct	16.25 ± 1.83	26.67 ± 6.19	0.383
Bicarbonates	110 - 270	183.75 ± 8.65	181.67 ± 11.49	0.383
Total hardness	500 – 4000	4650.00 ± 134.96	4533.33 ± 165.33	0.679
Calcium	>100	560.00 ± 26.19	*600.00 ± 29.66	0.042
Magnesium	>200	791.75 ± 40.98	738.83 ± 33.45	0.109
Iron	<0.1	0.80 ± 0.14	0.63 ± 0.09	0.561
Green Colonies	<50	14.00 ± 5.36	13.33 ± 7.64	0.547
Yellow Colonies	<300	39.00 ± 12.52	43.33 ± 15.76	0.423
Total Vibrio Count	500	56.00 ± 16.08	56.67 ± 17.56	0.93

Note: Values are expressed as Mean ± SEM; n=8, \*p<0.05 as compared to before Supplementation based on paired t-test

**Table 2:** Effect of Comp-1 on water quality parameters of shrimp ponds.

Parameters	Standards	After Supplementation with PPB	After Supplementation with Comp-1	p-value
pH	7.5 – 8.5	8.15 ± 0.05	8.21 ± 0.09	0.482
Salinity	0 – 25	16.31 ± 1.30	17.50 ± 1.07	0.296
Total Alkalinity	120 - 300	187.50 ± 12.50	208.33 ± 7.22	0.363
Carbonates	30-Oct	19.38 ± 1.48	26.67 ± 6.19	0.611
Bicarbonates	110 - 270	168.13 ± 12.32	181.67 ± 11.49	0.377
Total hardness	500 – 4000	4575.00 ± 215.27	4533.33 ± 165.33	0.092
Calcium	>100	540.00 ± 25.07	*600.00 ± 29.66	0.363
Magnesium	>200	785.75 ± 61.48	738.83 ± 33.45	0.641
Iron	<0.1	0.88 ± 0.18	0.63 ± 0.09	0.391
Green Colonies	<50	20.00 ± 12.25	13.33 ± 7.64	0.512
Yellow Colonies	<300	23.75 ± 11.43	43.33 ± 15.76	0.83
Total Vibrio Count	500	43.75 ± 23.57	56.67 ± 17.56	0.93

Note: Values are expressed as Mean ± SEM; n=8, p>0.05 as compared to after Supplementation with PondShield based on paired t-test.

**Table 3:** Comparative evaluation of post-Supplementation effects of PPB and Comp- 1 on water quality parameters of shrimp ponds.

The present trial was undertaken to evaluate the effect of PPB on water quality parameters of shrimp ponds in comparison with a competitor product. Two different farmer-owned shrimp ponds were selected and treated with PPB and Comp-1. Water quality parameters viz. pH, salinity, total alkalinity, carbonates, bicarbonates, total hardness of water, conc. of calcium, magnesium, potassium & Iron, and microbial count have been assessed in the selected farmer-owned shrimp ponds in order to verify the claim and determine the

effect of PPB on water quality parameters of shrimp ponds and translatability of results under field conditions. Study findings indicated that the performance of PPB was better than the competitor product (Comp-1) in the maintenance of water quality standards of shrimp ponds. This could be due to phytoactives present in herbal ingredients of PPB mainly through following three important actions (i) Optimizes pH fluctuations & improves dissolved oxygen (DO) content; (ii) Controls population of pathogenic bacteria and promotes

the growth of beneficial bacteria, and (iii) Supports phytoplankton growth. These three pivotal actions of PPB cause enhancement in the growth and survival of shrimp culture species.

Ammonium and nitrite, toxic metabolites originating in the feces, underused feed, and waste in shrimp systems can result in an enormous economic loss [13,14], since they can affect the physiology, immunity, survival, and growth of animals [15,16]. Traditionally, toxic metabolites have been controlled by biofilters and water exchange [17-19]. Specifically, in re-circulating shrimp culture systems (RAS), parameters of water quality need to be regularly controlled (19). The zero-water exchange (under sufficient management of carbon: nitrogen ratio) leads to an accumulation of organic matter and nutrients in shrimp culture systems. It normally develops the microbial community, and the high diversity of microorganisms promotes the stabilization of the system by taking the nitrogen compounds which generate in-situ microbial protein [20], improvement of nutrition [21], reduction of food conversion ratio (FCR), and feed costs, besides promoting the health of the organisms which are cultured [21]. In nature, the control of toxic compounds (ammonium and nitrogenous compounds) is potentially carried out by denitrifying bacteria; this is a role that probiotics might play. Recently, different kinds of probiotics have proven effective in ammonia nitrogen degradation. Accordingly, these eco-friendly additives can contribute to improving water quality [22]. For example, *Bacillus subtilis* has been widely administered as an appropriate probiotic agent to control water quality [23]. The administration of *B. subtilis* as a water additive in the rearing water of olive flounder (*Paralichthys olivaceus*) resulted in a significant reduction of ammonia levels and shrimp mortality [24]. Gram-positive bacteria, especially *Bacillus* spp., have been able to convert organic substances to carbon dioxide more efficiently when compared with Gram-negative bacteria converting a larger number of organics into bacterial biomass or slime [25]. Probiotic use in the rearing water to improve water quality, and their administration to purify wastewater from shrimp farms is helpful in areas with decreasing surface water, since the water can be reused for shrimp cultural activities after supplementation.

Neem (*Azadirachta indica*) is a medicinal plant containing diverse phytoactive substances with wide variety of biological properties. The extract of neem *A. indica* has been widely applied as an environmental insecticide, especially in shrimp ponds to control shrimp diseases and parasites in several countries around the world. According to Dunkel and Ricilards neem, products from neem have been stated to be effective in controlling shrimp predators and parasites in

shrimp culture systems [26].

Furthermore, Ginger (*Zingiber officinale*), an underground stem or rhizome belonging to the family Zingiberaceae, is widely used around the world in food as a spice. Ginger contains a wide variety of biologically active compounds like alkaloids, flavonoids, polyphenols, saponin, steroids, and tannin as well as nutritional molecules like fiber, carbohydrates, vitamins, carotenoids, and minerals [27]. The species is also rich in natural antioxidant components such as gingerols, shogaols, and Zingerone [28]. A diet enriched with ginger has been shown to promote growth, immunostimulation, digestion stimulation, and improvement of protein and lipid metabolism as well as antioxidant, antihyperglycaemic, antiviruses, antimicrobial, and parasites properties in rearing shrimp [29].

Summarily, it was evident from the findings of this study that PPB plays a pivotal role in maintaining the shrimp water quality parameters studied viz. pH, salinity, total alkalinity, carbonates, bicarbonates, total hardness of water, conc. of minerals (calcium, magnesium, potassium & Iron) and microbial count did not get affected adversely following the addition of PPB. In addition, it was observed from the microbial count that green colonies, yellow colonies, and total vibrio count were reduced following supplementation with PPB as compared to a competitor product. It was understood from the findings of Rajinikanth, et al., that the use of probiotics in shrimp ponds has a significant role in the growth, survival, and disease resistance of shrimp [30]. Concurrently, it was revealed through the results of our study that the addition of PPB in shrimp pond would also have pivotal role in the augmentation of shrimp growth and enhancement of survival and disease resistance of shrimp.

## Conclusions

Overall, the study's findings have the potential to make a significant positive impact on the aquaculture industry and the environment and can help promote a more sustainable and responsible future for the industry. However, further studies in more shrimp ponds in different geographical locations were recommended to see the translatability of results under field conditions.

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## Conflict of Interest

The authors declare that they have no conflicts of interest to report.

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