Service Project Report on

Investigating the Growth and Therapeutic Potential of Velox Water Additive for Sustainable Aquaculture: Boosting Shrimp (*Litopenaeus* vannamei) Growth and Immunity, Enhancing Plankton and Mitigating Vibriosis Disease

For

PUREFEED LLP

aquaculture innovation centre

Growing Together, Innovating for Tomorrow

1. Background

Greenwater aquaculture plays a vital role in sustainable shrimp farming by improving water quality, enhancing shrimp health, reducing environmental impact, and increasing productivity. This method involves cultivating beneficial microalgae, such as diatoms and phytoplankton, which help regulate water parameters, lower toxic ammonia and nitrate levels, and provide natural nutrition for shrimp larvae. Additionally, Greenwater systems foster beneficial microbial communities that outcompete harmful pathogens, reducing the risk of disease outbreaks. By promoting a balanced ecosystem, Greenwater aquaculture helps maintain stable shrimp production and ensures the long-term sustainability of farming in the region.

Purefeed LLP has developed a water additive liquid ingredient "VELOX" that enhances the phytoplankton community in the shrimp farmed water and boosts the shrimp's immune system. Aquaculture Innovation Centre (AIC) has studied its novel application in improving the growth and immune response of cultured Vannamei shrimps in both an open sheltered cement tank system and an indoor closed recirculation aquaculture system (RAS). Additionally, its potential to promote microalgae growth was evaluated in a climate-controlled algae growth chamber. AIC further conducted a disease challenge study in a biosecured Aquatic Biosafety level 2 (AqBSL2) containment facility to assess the product's effectiveness in strengthening shrimp resilience against Vibriosis disease. This research highlights its potential for innovative aquaculture solutions and the advancement of sustainable shrimp farming.

2. Study Approach

Phase I trial (Growth): Conducted at the Aquaculture Innovation Centre, Research and Development Farm (AIC-R&D farm) at 291 Neo Tiew Crescent, Lim Chu Kang, Singapore. This trial was conducted using a factorial design, with two stocking densities of specific pathogen-free (SPF) *Litopenaeus vannamei* shrimp post larvae (PL-10) viz. 50 pcs/m2 and 100 pcs/m2, each with and without the Velox additive in triplicates/experimental group. Epoxy-coated cement tanks (2.47m × 2.47m × 0.5m) with a water volume of 2.5 tons, were used for the project (Figure 1A) for an 8-week culture period, and animals were fed with 7% body weight (BW) ration per day and spaced over 5 feeding times. Feed was adjusted once every week with a representative sampling of shrimps from control and treatment tanks. Velox additive was added at a dose of 20 ppm (50 mL Velox product + 2 g yeast + 2 L culture tank water – fermented for 2 hours) daily for the first 3 weeks followed by 10x lowered dose i.e. 2 ppm (5

mL Velox product + 1 g yeast + 0.2 L culture tank water – fermented for 2 hours) for the subsequent 4 weeks of the growth period. Daily water quality parameters check included pH, Temperature, Salinity, and dissolved oxygen (DO), and weekly monitoring included nitrogenous waste levels (ammonia, nitrite, and nitrate), vibrio bacteria load and microalgae count. Survival rate (SR, %), weight gain (WG, g/fish), specific growth rate (SGR, %/day), and feed conversion ratio (FCR) were calculated.

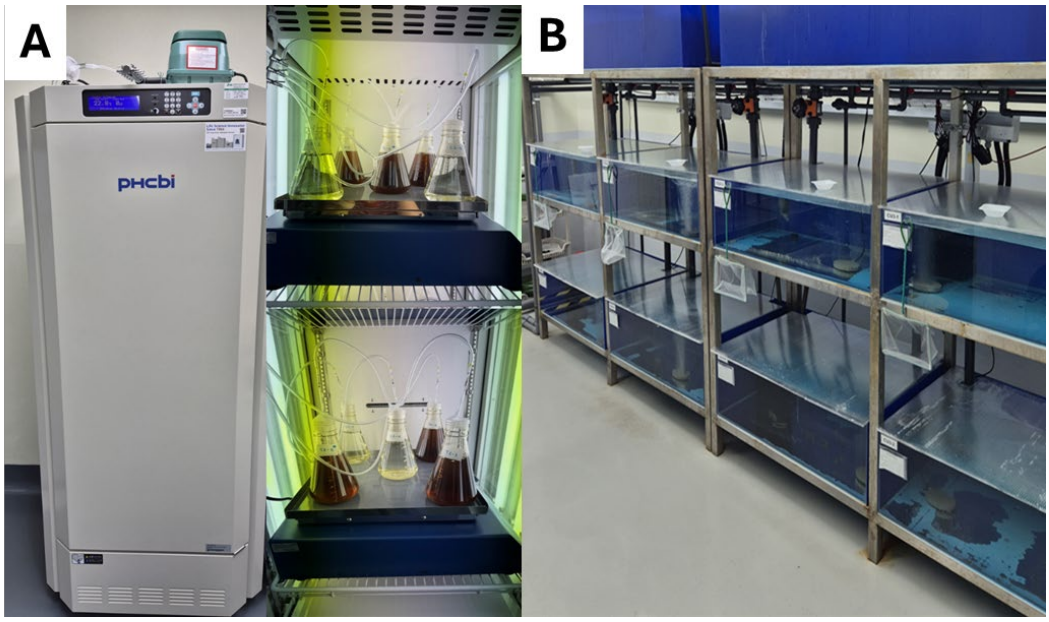
Phase II trial (Growth and Immunity): Conducted in the indoor RAS systems available at the Aquaculture Health Hub (AHH), Temasek Polytechnic, Singapore. Two stocking densities of SPF shrimp weighing ~1.3 g (50 pcs/m3 and 100 pcs/m3) each with and without the Velox additive were tested in duplicates/experimental group. A total of eight fiberglass tanks of 1-ton capacities were used for the project (Figure 1B). Velox additive was added without fermentation at a fixed dose of 2.5 ppm (2.5 mL Velox product to 1 ton of culture tank water) daily. The RAS system water parameters were maintained for dissolved oxygen (>5 mg/L), temperature (27^{o}C – 29^{o}C), pH (7.8 – 8.1), salinity (29-30 ppt), total ammonia nitrogen (<0.3 mg/L), nitrite (<0.2 mg/L), nitrate (<50 mg/L) and total alkalinity (>105 – <135 mg/L). Animals were fed with a 5% body weight (BW) ration per day and spaced over 2 feeding times over an 8-week culture period. Biweekly body weight and length measurements were made.

Immunity parameters were analysed at the end of the culture period from haemolymph collected in an anticoagulant. Total haemocyte count (THC), superoxide anion (O_2^-) radical, and prophenoloxidase (ProPO) levels were determined. Gut vibrio count was carried out in TCBS selective medium after enrichment of tissue homogenate in alkaline peptone water for 1 h at room temperature. The effect of Velox additive product on microalgae nannochloropsis was studied in a controlled algae chamber (Figure 2A) with constant temperature, HEPA-filtered air-flow, and light lux level for a period of up to 10 days or until it reached a 2-log increase in cell count.

Phase III trial (Disease tolerance/resistance): Shrimps from the *phase II* trial weighing about 7.0 – 8.0 g were moved to a 200-L tank modular RAS system located in Aquatic Biosafety Level 2 (AqBSL2) containment facility (controlled biosecured environment approved by AVS-NPARKS) (Figure 2B). All the animals were challenged with a pre-determined eighty percent mortality dose (LD_{80}) of *Vibrio parahaemolyticus* bacterial pathogen by intramuscular administration of 0.05 mL into the 4th or 5th abdominal segment. The animals were monitored for mortalities for up to seven days and relative percentage survival (RPS) was calculated.

Figure 1: (A) Epoxy-coated cement tanks for shrimp growth trial experiment. Control tank (left) and Velox additive added tank (right) (B) Indoor RAS system fiberglass tanks for shrimp growth trial.

Figure 2. (A) *Nannochloropsis* culture incubation in algae growth chamber with and without Velox additive treatment (right) (B) Modular RAS system located in a biosecured Aquatic Biosafety Level 2 containment (AqBSL2) facility



3. Results

Phase I trial (Growth): Shrimps treated with Velox showed higher final weight and weight gain compared to control groups by about 36.3% and 36.4% respectively (Table 1). The specific growth rate (SGR) was observed at 3.1 to 3.7% higher in the Velox-treated groups compared to the control groups. The feed conversion ratio (FCR) was comparatively better in tanks with lower stocking density (50 pcs/m^2) Improvement in the FCR among the Velox-treated group by 8.8%.

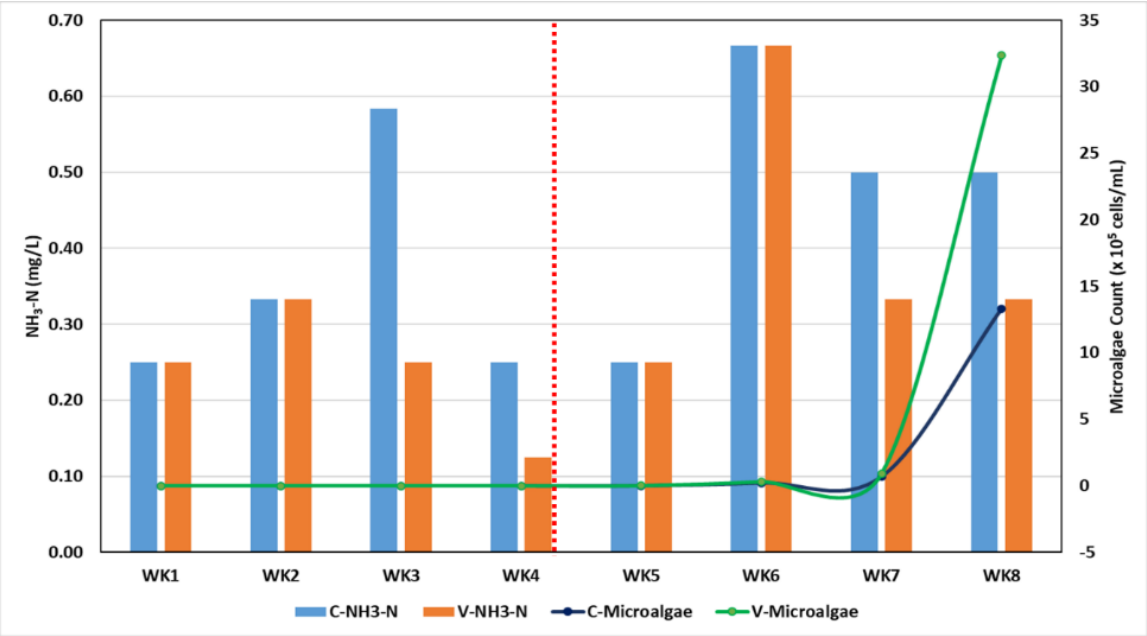
Table 1. Growth performance and feed conversion ratio (FCR) of control (C) and Velox (V) treated shrimps under two different stocking densities after 8 weeks of culture.

Treatments*	Survival rate (%)	Average final weight (g/shrimp)	Average weight gain (g/shrimp)	SGR (%/Day)	FCR
C (50)	92.62 ± 3.35	1.72 ± 0.57	1.71 ± 0.58	9.13 ± 0.56	0.74 ± 0.03
V (50)	85.49 ± 0.0	2.70 ± 1.59	2.69 ± 1.59	9.48 ± 0.99	0.68 ± 0.06
C (100)	78.35 ± 5.33	1.51 ± 0.45	1.50 ± 0.45	8.90 ± 0.51	0.81 ± 0.03
V (100)	84.90 ± 11.43	2.48 ± 1.34	2.47 ± 1.34	9.18 ± 1.15	0.82 ± 0.12

*Stocking density – The values are mean ± standard deviation, where n=3

On weeks 3 – 4 and weeks 7 – 8 a significant reduction in ammonia levels was observed in Velox treated group compared to the control group and a relativity in the increased microalgae count in treatment tanks was observed (Figure 3).

Figure 3: Total ammonia nitrogen (NH_3-N) and microalgae count in experimental groups.



Phase II trial (Growth and Immunity): A 7.36% increase in shrimp body weight (BW) was observed during week 4 among the treated shrimp group (T50) and a 5.25% increase in BW was observed during week 8 among the high stocking density treatment group (T100) (Table 2).

Table 2. Growth performance of control (C) and Velox treated (T) shrimps under two different stocking densities at 4 and 8 weeks of culture.

	WEEK-4		WEEK-8	
	BW (MEAN±SD)	**BL (MEAN±SD)**	**BW (MEAN±SD)**	**BL (MEAN±SD)**
C50	2.77 ± 0.29	7.98 ± 0.33	7.40 ± 1.19	11.11 ± 0.58
T50	2.99 ± 0.46	8.17 ± 0.34	7.10 ± 1.10	11.02 ± 0.62
C100	3.63 ± 0.79	8.49 ± 0.60	8.13 ± 1.85	11.39 ± 0.98
T100	3.65 ± 0.55	8.71 ± 0.42	8.58 ± 1.32	11.72 ± 0.68

The total haemocyte count (THC) has increased among treatment groups (T50 and T100) by 40 – 45% compared to the control groups (Figure 4A). A 16.3 – 17.5% increase in the superoxide anion (O_2^-) radical was found in haemocytes of treatment group shrimps (Figure 4B). The prophenoloxidase (ProPO) activity was higher in treatment tank shrimps with 39.1% and 48.7% among T50 and T100 groups respectively (Figure 4C).

The shrimp gut vibrio count of the control groups was 7.36 – 14.68 x 10^2 CFU/g, and Velox-treated groups presented 17.1 – 25.2 x 10^2 CFU/g. An increase of about 2.3 times higher gut colonization with healthy vibrio bacteria was observed among the T50 treatment group compared to the C50 control group and about 1.7 times higher among the T100 treatment group compared to the C100 control group

Single dose of 0.1% Velox treatment has increased the yield of microalgae Nannochloropsis in controlled environment by 35.8% used in single strength nutrient medium (1X strength) (Figure 5). Daily addition of 0.1% Velox for 5 consecutive days has enhanced the yield by 37.4%. Among the low nutrient culture medium (0.1X strength) grown cultures a single dose of 0.1% Velox has enhanced the yield of microalgae by ~ 37.2%.

Figure 4. Total haemocyte count of *L.vannamei* shrimps with and without Velox treatment (A), intracellular O_2^- radical production (B) and ProPO activity (C) in haemocytes of experimental shrimps. Values are in Mean ± SD.

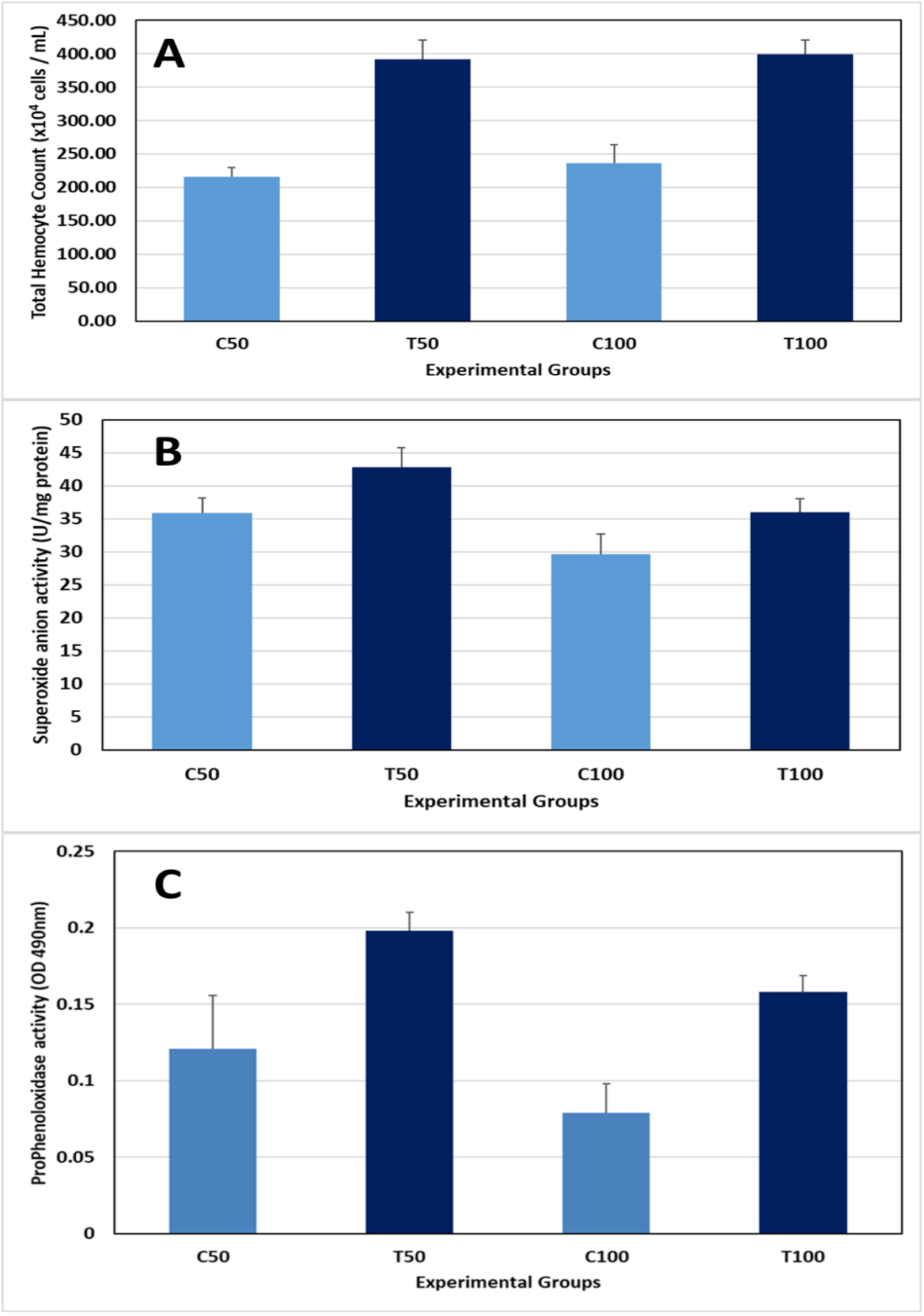
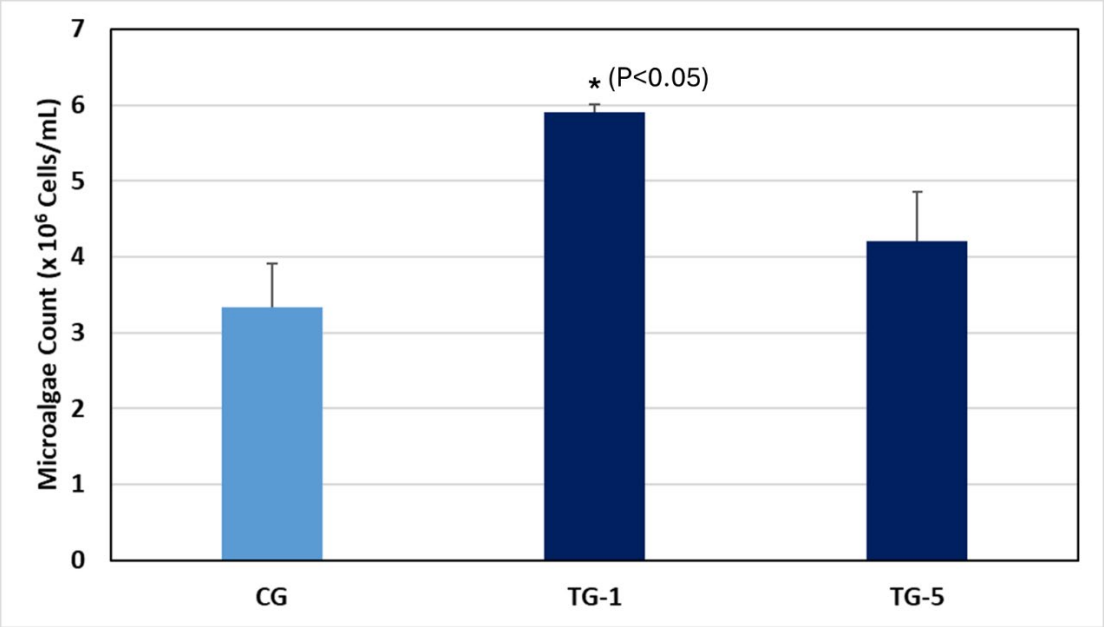
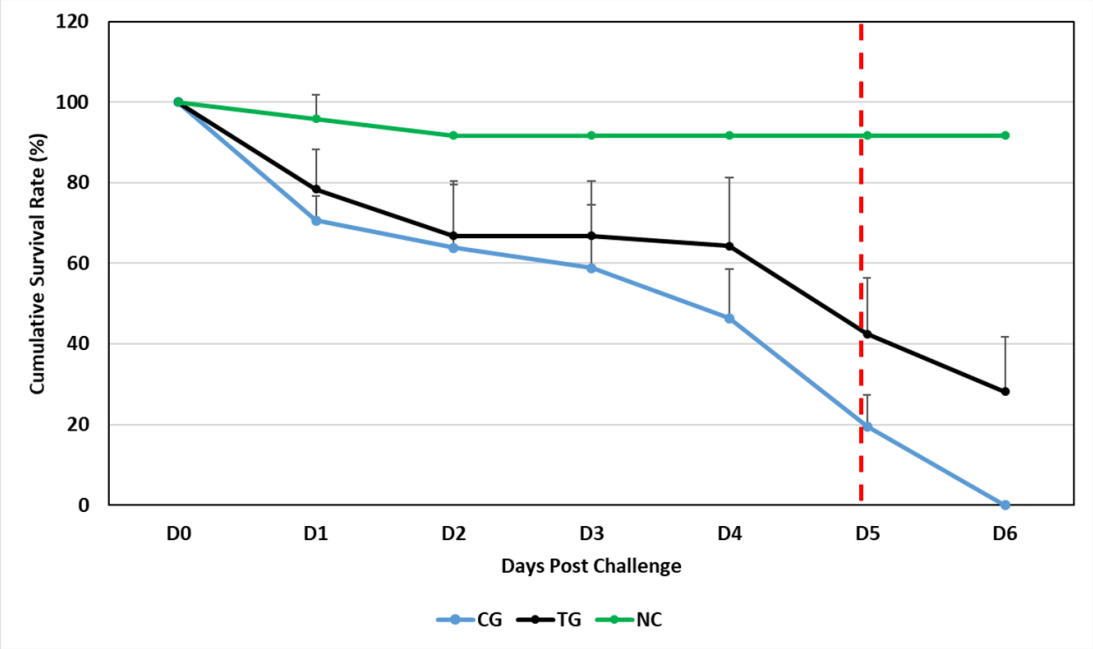


Figure 5. Microalgae Nannochloropsis growth among the control group (CG), and Velox treatment groups of single dose (TG-1) and 5-doses (TG-5) at day 7 of culture.



Phase III trial (Disease tolerance/resistance): At LD_{80} challenge dose Velox treatment group shrimps showed about 54% higher protection/survival than the control group at an 80% mortality mark in control group (Figure 5).

Figure 5. Cumulative survival rate of the control group (CG) and Velox treatment group (TG) shrimps challenged with LD_{80} dose of *Vibrio parahaemolyticus* bacteria. The red dotted line represents the 80% cumulative mortality mark of CG.



4. Conclusions

- Velox water additive has shown significant potential in enhancing green water shrimp culture, leading to improved weight gain, specific growth rate (SGR), and feed conversion ratio (FCR) in cultured shrimp. Optimizing the dosage in pond culture systems is crucial for balancing microalgae growth and managing nitrogenous waste.
- In the indoor RAS system, Velox treatment increased shrimp body weight (BW) by 5.25 – 7.4% across two different stocking densities. It has also exhibited strong immune-priming effects, with a 40-45% increase in total haemocyte count and intracellular enhancements of superoxide anion radical (~16 – 17%) and prophenoloxidase activity (39 – 48%).
- Velox significantly boosted microalgae (Nannochloropsis) yield by about 35 – 37% on day 7 with a single 0.1% dose in a controlled, nutrient-limiting environment.
- Shrimp group treated with Velox demonstrated 54% higher survival compared to the control groups at the 80% mortality threshold upon disease challenge.
- The increased disease tolerance/resistance observed in Velox-treated shrimp are strongly linked to its immune-boosting effects, which include elevated circulating haemocytes and enhanced intracellular production of prophenoloxidase and superoxide anion radicals.
- Overall, shrimp cultured with Velox water additive exhibited superior growth performance and strong protection against vibriosis. This product is expected to be even more effective in natural disease outbreaks, which progress gradually, compared to the current experimental model using an acute LD_{80} infection.