Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26 (4): 1289 – 1304 (2022)
www.ejabf.journals.ekb.eg

Effects of dietary supplementation of Bacti-nil®Aqua on growth performance, feed utilization, immune responses, and body composition of the Pacific white shrimp, *Litopenaeus vannamei*

El-Sayed Hemdan Eissa[1], Ahmed A. El-Kady[2], Mohamed Ismail[3], Ashraf A. El-Badawi[4,5], Norhan H. Ahmed[6], Manal I El Barbary[7]*

1. Fish Research Centre, Arish University, Egypt.
2. Fish Farm and Hatchery in Kafr El-Sheikh,Egypt.
3. Marine Science Department, Faculty of Science, Port Said University, Port Said, Egypt.
4. Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharkia, Egypt.
5. Biology Department, University College, Umm-Al Qura University, Makkah, Saudi Arabia.
6. Department of Animal production, Faculty of Agriculture, Zagazig University, Egypt.
7. National Institute of Oceanography and Fisheries, NIOF, Cairo, Egypt.

*Corresponding Author: manal278@yahoo.com

ARTICLE INFO

Article History:
Received: Aug. 1, 2022
Accepted: Aug.11, 2022
Online: Aug. 25, 2022

Keywords:
Organic acids,
Immunity,
Antioxidants,
Mortality rate,
Body composition,
Litopenaeus vannamei

ABSTRACT

This study aimed to evaluate the dietary supplementation of various levels of Bacti-nil®Aqua on growth performance, nutrient utilization, immune response, antioxidant activities, mortality rate, and chemical composition of the Pacific white shrimp *Litopenaeus vannamei*. Four hundred and eighty *L. vannamei* fries (3.0 ± 0.002 g) were randomly divided into 4 treatments (T1-T4). T1 represented the negative control fed on a basal diet, the fries in treatments T2, T3 and T4 were fed on Bacti-nil®Aqua supplemented diet at three concentrations of 2, 3, and 4g/kg diet, respectively, for 60 days. The results showed that the morphometrics measurements (body and antenna length), the growth parameters (BW, BWG, ADG, and RGR) and the condition factor values were significantly ($P < 0.05$) higher in the Bacti-nil®Aqua supplemented treatments than those of the control group. Moreover, the different Bacti-nil®Aqua supplemented diets had no significant effect on the survival rate (SR) of *L. vannamei*. The chemical composition of the *L. vannamei* and feed utilization parameters exhibited no significant differences in DM and ash. The protein content of *L. vannamei* increased significantly when fed on Bacti-nil®Aqua, compared to T1. In contrast, the highest values of lipids were recorded in the control treatment (T1). Different concentrations of Bacti-nil®Aqua (T2-T4) resulted in a significant improvement in PER and PPV%, the best FCR, and the highest nonspecific immune responses (THC, phagocytosis, lysozyme activity, and phenoloxidase activity), in addition, superoxide dismutase activity compared to the control diet (T1). Overall, the current study concluded that the highest levels of Bacti-nil®Aqua (3 and 4g/kg) resulted in improved growth parameters, survival rate, feed utilization, chemical composition, and nonspecific immune responses of *L. vannamei*.

INTRODUCTION

Shrimp farming has developed rapidly and became one of the major sources of the aquaculture industry and food production in most countries **(Lukwambe *et al.*, 2019)**. It

is one of the most profitable projects of the mariculture industry **(Eissa *et al.*, 2021a)**. Shrimps are among the most remarkable species in production and growth performance all over the world **(Eissa *et al.*, 2021b)**. One of the world's largest farmed tropical prawns is the Whiteleg shrimp *L. vannamei*, a native species of the Pacific Ocean **(FAO, 2018)**. This species is considered as the main target species for the shrimp industry. *L. vannamei* is characterized by speedy growth and low protein requirements, it also has high tolerance to water salinity variability **(Wang *et al.*, 2019)**, environmental factors, such as water temperature **(Madeira *et al.*, 2015)**, pH variability **(Wang *et al.*, 2016; Han *et al.*, 2018),** and bad water quality including the depression of dissolved oxygen **(Han *et al.*, 2018)**, the presence of chemical pollutants such as ammonia, nitrite, hydrogen sulphide, and heavy metals **(Duan *et al.*, 2018; Harun *et al.*, 2019)**. Its high tolerance capacity is shown especially with the intensification culture affecting shrimp growth and survival rates and can lead to economic losses resulting from outbreaks of bacterial and viral diseases. Diseases cause a critical threat to the sustainable growth of the shrimp industry and production. Shrimp like all invertebrates has a simple body structure, and primitive immune system; they do not produce antibodies and do not have an adaptive immune system **(Loker *et al.*, 2004; Rowley & Powell, 2007; Canesi & Procházková, 2014)**. Shrimps avoid the invasion of their bodies by biological or innate immune responses, such as their epithelium, shells, and mucous layers, which act as physical and chemical barriers for pathogens. Moreover, there are variety of routes to eliminate the invasive pathogens, viz. phagocytosis by phagocytes, secretion of antibacterial agent as lectin, enzymes, lysozyme and production of cytokines that stimulate the inflammatory reactions **(Beschin, 2001; Loker *et al.*, 2004; Gowda *et al.*, 2008; Dong & Sun, 2021)**. Shrimp culturists are using chemotherapeutics such as antibiotics to manage many different diseases as an immediate resort to control them; however, antibiotics have risks due to the emergence of antibiotic resistant bacteria and their residues in the bodies of aquatic animals **(Thornber *et al.*, 2019)**. Therefore, several studies have been carried out to evaluate the effects of different alternative antimicrobial materials, such as herbs, probiotics and organic acids on growth rate, immune responses, antioxidant activity and disease control of shrimp **(Sharawy *et al.*, 2016; Sharawy *et al.*, 2017; Goda *et al.*, 2018; Huang *et al.*, 2018; Davies *et al.*, 2019; Goda *et al.*, 2019; Nasmia *et al.*, 2022)**. Organic acids are distinguished by their antimicrobial properties which destroy the bacterial cell wall and change the cytoplasmic pH. Furthermore, organic acids are distinguished by their growth-promoting action, and cost-effective application **(Nuez Ortín *et al.*, 2020)**. One of the alternative antimicrobial compounds is the Bacti-nil®Aqua; it is a mixture of organic acids specifically formulated for aquatic organisms such as shrimp. The current study aimed to investigate the efficiency of Bacti-nil®Aqua supplemented diets to promote the growth performance, feed utilization and immune response of *L. vannamei.*

MATERIALS AND METHODS

1. Experimental design

A field study was carried out in a private earthen pond fish farm in Damietta Governorate, Egypt. 480 healthy whiteleg shrimps *L. vannamei* with an average initial body weight (BW) of 3.0 ±0.03 g and an average initial total length (TL) of 7.95 ±0.05 cm were purchased from a private shrimp farm and acclimatized to the experimental conditions for two weeks. Twelve hapas (each 1.0 m width × 1.0 m length ×1.0 m water depth and was stocked with forty whiteleg shrimp) were used representing the four experimental treatments (T1-T4) in triplicate. Shrimps were incorporated in a feeding trial to test the effects of commercial dietary supplementation of Bacti-nil®Aqua on shrimp propagation. The experimental feeding period was about 60 days. It began on the 24thof October until the 23rd of December 2020, where the feed was offered to the shrimp three times per day. The daily feeding rate changed every two weeks to follow the following values of 6, 5, 4, and 3% of the fish biomass in each hapa. Shrimps in the control group (T1) received only a basal diet. In T2, shrimps were fed on a basal diet with 2g/kg Bacti-nil® Aqua, whereas those in T3 were fed a basal diet with 3 g/kg powdered Bacti-nil®Aqua, and T4 were fed on a basal diet with 4g/kg Bacti-nil® Aqua.

Hapas were fixed and arranged in the earthen pond. Water was exchanged daily by 5%, 10%, and 15% during the first, second and third 20 days of the experimental period, respectively, and the water quality was monitored daily.

2. Experimental diets

The diet and the feed additive Bacti-nil®Aqua (with 1.0 kg and 25 kg presentation) were purchased from the local market. The manufacturer of the organic acids Bacti-nil®Aqua is Nutriad International NV. Schietstandlaan 2, 2300 Turnhout, Belgium. The product was imported by EGAVET (Arab Egyptian Group for Trading and Veterinary Services, Importer: 166 King Faisal Street, Giza, Arab Republic of Egypt (ARE)). Bacti-nil®Aqua is a synergic mix of organic acids with powerful antibacterial action that reduces colonization risks of pathogenic microorganisms by promoting healthy gut microbiota in fish and shrimp, which leads to improved growth performance. Bacti-nil®Aqua was added to the artificial diets and mixed with corn oil as a binding substance, while the equal amount of oil was added in Bacti-nil®Aqua free diet (control basal diet). The Bacti-nil®Aqua was added to a powder and mash diets during the different period of the experiment by the sparing method. Experimental diets were formulated as a basal diet with the addition of different concentrations of Bacti-nil®Aqua (i.e., 0, 2, 3, and 4g/kg diet) (Table 1). During the current study, shrimps were fed on the artificial powder and mash formulated feeds at the first and last month, respectively. Feed was handfed three times per day, and the amounts used in each hapa were recorded every 2 weeks starting from the midst of December to February 2019-2020. Artificial feed

proximate composition was 38% of crude protein as shown in Table (1); the proximate analysis was done according to the method of **AOAC (2000)**.

Table 1. Formulation (g/kg) and proximate composition (%) of the experimental diets and Bacti-nil®Aqua

	Diets			
	Control	Bacti-nil®Aqua		
Ingredient	**T1**	**T2**	**T3**	**T4**
Wheat flour	120.0	118.0	117.0	116.0
Shrimp meal	250.0	250.0	250.0	250.0
Rice bran	70.0	70.0	70.0	70.0
Soybean meal	150.0	150.0	150.0	150.0
Fish meal	300.0	300.0	300.0	300.0
Bacti-nil	0.0	2.0	3.0	4.0
Fish oil	60.0	60.0	60.0	60.0
CMC	10.0	10.0	10.0	10.0
Vit.Mix	20.0	20.0	20.0	20.0
Min. mix	20.0	20.0	20.0	20.0
TOTAL	1000.0	1000.0	1000.0	1000.0
Proximate composition of diets (%)				
Dry matter	90.64	90.83	91.31	90.60
Moisture	9.35	9.02	8.76	9.30
Crude protein (N × 6.25)	38.80	38.76	38.76	38.80
Crude fat	10.96	10.80	10.85	10.87
Crude fiber	1.74	1.57	1.32	1.60
Ash	6.15	6.62	7.11	6.42
Carbohydrate (NFE)	32.975	33.087	32.291	33.017
Gross energy kcal/100g	459.467	458.56	459.22	457.61
Composition of BACTI-NIL®AQUA:				
Formic acid (85%)			12.00%	
Ammonium formate (72%)			23.00%	
Propionic acid (99%)			10.0%	
Sorbic acid (99%)			1.00%	
Caprylic Acid (100%) (Paim)			6.10%	
Capric Acid (100%) (Paim)			3.90%	
Talc (Natural mixture of Steatites &Chlorite)			2.50%	
Silicic acid, precipitated and dried1.50%			41.50%	
Total			100%	

* NFE: nitrogen free extract; Vit: vitamin; Min.: mineral CMC: carboxymethylcellulose; T2, T3 and T4 are treatments supplemented with Bacti-nil® Aquaat 2, 3, and 4 g/kg diet.

3. Water quality measurements

The examined water quality parameters were temperature (˚C), pH, dissolved oxygen (DO), salinity, ammonia (NH_4), nitrite (NO_2), and nitrate (NO_3), which were measured daily by Hanna aquaculture multi-parameter photometer (HI83303).

4. Performance evaluation and survival rate

The morphometric measurements such as body length from the tip of rostrum to tip of telson (BL), final length gain (FLG), final antenna length (FAL) and antenna length gain (ALG) were measured at the end of the experiment using a caliper.

The growth performance parameters were calculated by the following formulae:

Average body weight: BW (g) =Total weight sampled/ Total number sampled

Body weight gain (BWGg/shrimp): $BWG = W_t - W_0$; Where, W_0: the initial average weight per gram, and W_t: the final average weight at time t.

Average daily gain (g/shrimp/day): $ADG = W_t - W_0/d$; Where, W_t is the weight of the shrimp at time t; W_0 is the weight at the beginning of the study, and d is the day in the detected growth period.

Specific growth rate (SGR) = $(\ln W_t - \ln W_0) \times 100$/ number of days.

Condition factor: $CF = 100 \times [W_t (g)/L_t^3]$; where L_t: Final length of shrimp (cm).

Feed conversion ratio (FCR) = feed intake (dry matter) (g)/weight gain (g).

Survival rate (%) of the shrimp in each treatment = 100 × (initial number /final number)

Protein efficiency ratio (PER) = Body weight gain (g)/Protein intake (g).

Protein productive value (PPV %) = Retained protein (g) x 100/ protein intake (g). Where PER and PPV% were calculated according to **Jobling (1983).**

5. Evaluation of Bacti-nil®Aqua on the immunity parameters

Ten white shrimp *L. vannamei* samples from each group were used for haemolymph collection. A haemolymph sample (250μL) of an individual shrimp was collected from the base of the 3rd walking leg using a sterile 1-mL syringe containing 750 \μL of precooled (4°C) anticoagulant (0.114 M trisodium citrate, 450 MNaCl,10 mMKCl, 10 mM HEPES at pH 7.4) **(Nonwachai *et al.*, 2010)**. The haemolymph-anticoagulant mixture was used to measure the nonspecific immune responses.

Total hemocyte count (THC) was estimated according to the method of **Blaxhall (1973)**, based on the following formulation: Total hemocytes (cells/mL) = average cells number $\times 10^4 \times$ dilution factor. Phagocytotic assay was estimated according to the study of **Itami *et al.* (1994)** with some modifications. Thelysozyme activity was estimated with the turbidimetric technique described in the study of **Engstad *et al.* (1992)**. The antioxidant parameter as phenoloxidase activity was measured according to the method of **Supamattaya *et al.* (2000)**. While, superoxide dismutase activity (SOD) was estimated based on the technique of **Nishikimi *et al.* (1972)**. Their assay kits were purchased from Biodiagnostic and/or Biotechnology Co., ARE.

6. Proximate composition of *L. vannamei*

The chemical composition of *L. vannamei* samples as the percentage of dry matter (DM), crude protein (CP), crude lipid (CL), and ash were measured according to **AOAC (1995)** at the beginning and end of the experiment using four shrimps per group.

7. Statistical analyses

The results of growth parameters, survival rate, feed utilization and immunological parameters were analyzed by Tukey's multiple comparison test and one-way ANOVA using the SPSS Statistics program. Significant differences ($P < 0.05$) were noted by different letters a, b, c, and d, and no significant differences were indicated by the same letters among treatments.

RESULTS AND DISCUSSION

1- Water quality

Water quality parameters of the experimental hapas stocked with *L. Vannamei* fed Bacti-nil®Aqua-supplemented diets during the study period (24 October to 23 December 2020) (Table 2) showed no differences among water hapas during the experiment period because all hapas were in the same pond; however, differences were detected among different intervals.

Table 2. Water-quality parameters measured during the experiment with *L. vannamei*

Parameter/ day	Temperature (^oC)	Salinity (ppt)	pH	DO (mg/L)	NH_4	NO_2	NO_3
zero time	30	25	8.1	5.43	0.6	0.042	0.022
after 2 weeks	26	23	7.9	5.99	0.5	0.032	0.026
after 4weeks	23	22	8.3	6.23	0.4	0.041	0.024
after 6 weeks	20	18	8.0	7.15	0.3	0.025	0.036
after 8 weeks	18	15	7.6	7.22	0.4	0.029	0.032

*DO: Dissolved oxygen; NH_4: Ammonia; NO_2: Nitrite; NO_3: Nitrate

Increasing temperature adversely affect the growth of animals **(Brett, 1979)**. Shrimp was negatively affected when the temperature dropped to 23°C, while the growth and feed consumption of shrimp decreased when the temperature increased to 30°C **(Wyban *et al.*, 1995)**.

Nevertheless, the values of water parameters were within the optimal range for the growth of *L. vannamei*. **Liu and Manacebo (1983)** reported that the greatest growth of *L. vannamei* can result from salinity of 22 g/L. In addition, **Chiu (1988)** mentioned that the optimal level of salinity ranged from 20 to 25g/L. The optimal level of temperature ranged from 25 to 30°C **(Boyd 1998)**. While, the best growth was achieved from temperature, DO and pH ranging between 20–30°C, 4.5–6 ppm and 7.5 to 8.5,

respectively. Moreover, the total ammonia (NH_4) level should not exceed 1ppm, and nitrite must be lower than 0.1ppm. Overall, water parameters during most of the culture period were suitable for shrimp growth, except for the last 2 weeks; salinity, pH, and NH4 were within the optimum range, while DO was higher than the recommended levels for shrimp. Results of **Labrador *et al.* (2016)** showed that, when water temperature drops outside the optimal level for large periods, the production potential will be impaired. This can be verified since low temperature (≤14°C) may be sufficient to kill *L. vannamei* **(Green, 2008).**

2- Morphometric measurements and survival rate

The morphometric measurements, such as body length, final length gain, final antenna length and antenna length gain at the end of the experiment showed highly significant values ($P < 0.05$) with the Bacti-nil®Aqua dietary supplementation than the control group. Both high levels of Bacti-nil®Aqua (3 and 4%) reflected higher antenna length gain than the low level (2g/kg) (Table 3).

Table 3. Some morphometric parameters and survival rate

	T1	**T2**	**T3**	**T4**
Initial length	7.96 ±0.08	7.930 ±0.08	8.03 ±0.08	8.00 ±0.08
Final length	12.5^{b} ±0.11	13.53^{a} ± 0.11	14.00^{a}±0.11	14.03^{a} ±0.11
Length gain	4.53^{b} ±0.15	5.60^{a} ±0.15	5.96^{a} ±0.15	6.03^{a} ±0.15
Initial antennal length	7.90 ± 0.08	7.930 ±0.08	8.03 ±0.08	7.86 ±0.08
Final antenna	10.6^{c} ±0.20	13.3^{b} ±0.2	14.8^{a} ± 0.2	14.9^{a} ±0.2
Antenna length gain	2.70^{c} ±0.23	5.36^{b} ±0.23	6.76^{a} ±0.23	7.03^{a} ±0.23
Initial number of shrimp	40 ± 0.0	40 ± 0.0	40 ± 0.0	40 ± 0.0
Final number	20.66^{b} ±2.21	26.33^{a} ±2.21	29.00^{a} ±2.21	30.33^{a} ±2.21
SR%	51.66^{b} ±5.54	65.83^{a} ±5.54	72.5^{a} ±5.54	75.83^{a} ±5.54

*T1 = Control; T2, T3 and T4 are treatments supplemented with Bacti-nil®Aquaat 2, 3, and 4 g/kg diet; SR = survival rate. Means within the same row with different superscript letters (a–c) are significantly different ($P < 0.05$).

Survival rates of the *L. vannamei* fed Bacti-nil®Aqua diets were significantly higher, compared to *L. vannamei* in the control treatment. However, different Bacti-nil®Aqua-supplemented diets had no significant effect on SR (%) of *L. vannamei*. On the other hand, the 4% Bacti-nil®Aqua had the highest SR (%) among all treatments. These results indicated that the major advantages of feed supplementation with Bacti-nil®Aqua might be exclusive to health benefits, such as the promotion of gut microbiota, immune response, and resistance to diseases **(Kesselring *et al.*, 2021)**. The infections by bacterial diseases are mostly caused by exposure to adverse environmental conditions (such as poor water quality, low oxygen, high temperatures, etc.) and can lead to mass mortality of shrimp larvae **(Dash *et al.*, 2016)**.

The intensification in culture practices is increasing diseases levels, so pathogen containment is becoming increasingly important especially in shrimp farming, which faces some unique challenges compared to fish production. For example, shrimps do not have an acquired/adaptive immune system, but they depend only on the innate immunity, which is usually fast acting and nonspecific. Therefore, it is more susceptible to disease causing agents than fish and in dire need of antimicrobial usage, biosecurity measures, and good hygiene. **Nuez Ortin *et al.* (2020)** conducted the first investigation of the antibacterial efficacy of Bacti-nil® Aqua against bacterial pathogens. While, the current study is the first study focusing on the potential of Bacti-nil® Aqua as a growth promoter of *L. vannamei*

3- Growth performance indices of *L. vannamei*

Growth performance indices (BW, BWG, ADG, CF, SGR and RGR) of whiteleg shrimps fed Bacti-nil®Aqua-supplemented diets are presented in Table (4). The BW, BWG and ADG of *L.vannamei* fed diets supplemented with different concentrations of Bacti-nil®Aqua (T2-T4) at different intervals were significantly higher ($P < .05$) than the control diet (T1). Therefore, the different studied concentrations of Bacti-nil®Aqua enhanced the growth performance and the nutrient utilization of *L.vannamei*. In addition, the T3 andT4 reflected higher values from those recorded in T2, with no significant differences between T3 and T4 in all intervals. The changes in the feeding rate % every two weeks (from 6, 5, 4, and 3% of shrimp biomass through the 4different feeding intervals) had no significant effect on weight (%), BWG and ADG of *L. vannamei* in all feeding intervals in both T3 and T4. Nevertheless, significant changes were recorded between some feeding intervals in T1 and T2, especially with the low feed rates 4 and 3%. On the other hand, the CF values decreased with the use of the Bacti-nil®Aqua.

In the same trend, the results showed that *L. vannamei* fed on the highest level of Bacti-nil®Aqua (T4) reflected the highest significant improvement in RGR and SGR, followed by T3 and T2; while, the control group (T1) exhibited the worst values. On the other hand, *L. vannamei* fed diets supplemented with 3 and 4g/kg Bacti-nil®Aqua (T3 and T4) recorded the highest FBW and BWG, ADG when compared to T2.

No previous studies have demonstrated that supplementing Bacti-nil®Aqua to the diets of *L. vannamei* significantly enhanced the growth performance, while the current study showed that the optimal concentrations of 3 and 4% Bacti-nil®Aqua gave both the overall best results. These findings are supported by many studies reporting that feed additives lead to positive impacts on growth rates, immunological responses and resistance to diseases **(Sirirustananun *et al.*, 2011; Schleder *et al.*, 2017)**.

4- Body proximate composition and feed utilization indices

The effects of Bacti-nil®Aqua-supplemented diets on the proximate whole-body composition and feed utilization parameters (FCR, PER and PPV) are illustrated in Table (5). Data in Table (1) show no significant differences in the chemical composition (protein, ether extract and ash) in either diet supplemented with or without different levels of Bacti-nil®Aqua.

Table 4. Means ± SE of some growth performance parameters of the shrimp fed on different levels of dietary Bacti-nil®Aqua during different intervals

	T1	T2	T3	T4
Initial body weight at 0 day	2.96 ± 0.07	2.96±0.07	3.03±0.07	2.96±0.07
Weight at the 15th day	4.36 ^{b}±0.08	4.80^{a}±0.08	5.03^{a}±0.08	5.10^{a}±0.08
Weight at the 30th	6.26^{b}±0.21	7.06^{ab} ±0.21	7.40^{a} ±0.21	7.46^{a} ±0.21
Weight at the 45th	9.06^{b}±0.28	10.83^{a}±0.28	10.96^{a}±0.28	11.06^{a}±0.28
Final weight at the 60th day	12.53^{c}±0.36	15.43^{b} ±0.36	17.43^{a} ±0.36	17.43^{a} ±0.36
Weight gain(WG)				
WG at 2 weeks	1.40^{b}±0.12	1.83^{ab} ±0.12	2.00^{a}±0.12	2.13^{a}±0.12
WG at 4 weeks	1.90^{b} ± 0.14	2.26^{a}±0.14	2.36^{a}±0.14	2.36^{a}±0.14
WG at 30-45 day	2.80^{b}±0.13	3.76^{a}±0.13	3.56^{a}±0.13	3.60^{a}±0.13
WG at 8 weeks	3.46^{c}± 0.14	4.60^{b}± 0.14	6.46^{a}± 0.14	6.36^{a}± 0.14
WG from 0 to 60th day	9.56 ^{c} ±0.4	12.46 ^{b}±0.4	14.36^{a} ±0.40	14.46^{a} ±0.40
Condition factor (CF)				
Initial CF	0.58 ±0.01	0.59 ±0.01	0.58 ±0.01	0.57 ±0.01
Final CF	0.64a ±0.01	0.62^{b}±0.01	0.63^{ab}±0.01	0.63^{ab} ±0.01
Average daily gain (ADG)				
ADG 0-15 day	0.10^{b}±0.01	0.13^{ab}±0.01	0.14^{a}±0.01	0.15^{a}±0.01
ADG 15-30 day	0.13^{b}±0.01	0.16^{a}±0.01	0.16^{a}±0.01	0.16^{a}±0.01
ADG30-45 day	0.20^{b}±0.01	0.27^{a}±0.01	0.25^{a}±0.01	0.25^{a}±0.01
ADG45-60 day	0.25^{c}±0.01	0.33^{b}±0.01	0.46^{a}±0.01	0.45^{a}±0.01
ADG from 0 to 60th day	0.16 ^{c} ±0.01	0.21^{b} ± 0.01	0.24^{a} ±0.01	0.24^{a} ±0.01
Relative growth rate (RGR%)				
RGR0-15 day	47.2^{b}±1.13	61.92^{b}±1.13	66.32^{a}±1.13	72·14^{a}±1.13
RGR15-30 day	43.5^{b}±154.9	46.5^{a}±154.9	46.96^{a}±154.9	46.3 ^{a}±154.9
RGR30-45 day	44.6^{b}±3.5	53.2^{a}±3.5	48.01^{a}±3.5	47.8^{a}±3.5
RGR45-60 day	8.01^{b}±218.0	42.3^{b}±218.0	59·02^{a}±218.0	58.2^{a}±218.0
RGRfrom 0 to 60th day	322.6^{b}±0.8	421.1^{ab}±0.8	475.1^{a}±0.8	488.7^{a}±0.8
Specific growth rate (SGR)%				
SGR0-15 day	2.76^{b}±0.23	3.43^{ab}±0.23	3.62^{ab}±0.23	3.8^{a}±0.23
SGR 15-30 day	2.58^{a}±0.12	2.75^{a}±0.12	2.74^{a}±0.12	2.72^{a}±0.12
SGR 30-45 day	2.63 ±0.09	3.05±0.09	2.81±0.09	2.81±0.09
SGR45-60 day	2.31^{b}±0.06	2.53^{b}±0.06	3.31^{a}±0.06	3.24^{a}±0.06
SGR from 0 to 60th day	2.57 ^{b}±0.06	2.94^{a} ±0.07	3.12 ^{a}± 0.7	3.16^{a} ± 0.07

*T1 = Control; T2, T3 and T4 are treatments supplemented with Bacti-nil®Aquaat 2, 3, and 4 g/kg diet; SR = survival rate; means within the same row with different superscript letters (a–c) are significantly different ($P < 0.05$).

The nutrient utilization parameters were significantly enhanced with the Bacti-nil®Aqua dietary supplementation. Shrimp that received 3 and 4 g/kg the Bacti-nil®Aqua (T3 and T4) presented significantly higher FCR, PER, and PPV values than those in T1 and T2. The best values of FCR, PER, and PPV were recorded in T4 (1.14, 2.7, and 89.43) and T3 (1.14, 2.7, and 88.51), respectively, with no significant differences

between both treatments. While, the worst values were recorded in the control treatment T1 (1.48, 2.09, and 60.35, respectively).

The chemical composition of *L. vannamei* exhibited no significant differences in dry matter and ash. The protein content of *L. vannamei* increased significantly when fed Bacti-nil®Aqua, compared to T1. In contrast, the highest values of lipid were observed in the control treatment (T1). The growth-promoting effect of Bacti-nil®Aqua on the healthy digestive system can be interpreted by a stable gut microbiota, and the effective digestion and absorption of nutrients **(Nuez-Ortin *et al.*, 2020)**.

Table 5. Chemical body composition of Pacific white shrimp *L. vannamei* and feed utilization

Parameter	**T1**	**T2**	**T3**	**T4**
moisture %	79.17± 0.22	79.04±0.41	79.00±0.28	79.4±0.24
Dry matter	20.83± 0.2	20.96±0.21	21.00±0.21	20.6±0.12
Protein	15.85^{b} ±0.18	16.17^{a}±0.22	16.23a±0.25	16.17a±0.12
Lipid	1.98a±0.28	1.53c±0.17	1.94a±0.08	1.77b±0.20
Ash	3.05±0.08	3.09±0.069	2.95±0.40	3.09±0.05
FCR	1.48^{a}±0.02	1.27b±0.02	1.14c±0.02	1.14c±0.02
PER	2.09 ± 0.01	2.41 ±0.01	2.7 ±0.01	2.7 ±0.01
PPV	60.35± 0.21	77.09±0.21	88.51±0.22	89.43±0.22

T1 = Control; T2 = 2 mg /kg diet; T3 = 3 mg/kg -; T4 = 4 mg Bacti-nil®Aqua /kg; means in the same row with different superscript letters (a–c) are significantly different ($P < 0.05$). FCR: Feed conversion ratio; (PER): Protein efficiency ratio; PPV: Protein productive value

It is worth mentioning that, there was a direct and indirect benefit of the supplementation of Bacti-nil®Aqua. The direct benefit involved the synergetic blend of short and medium chain of organic acids, while the indirect ones included better immune responses, reduced liver damage, and improved growth promotion **(Nuez Ortin *et al.*, 2020)**.

5- Nonspecific immune responses and antioxidant status

The nonspecific immune parameters, including total hematocytes count, phagocytic Index, lysozyme activity, and phenoloxidase activity, and antioxidant superoxide (dismutase activity) of shrimp are illustrated in Table (6).

Bacti-nil®Aqua supplemented diets (T2,T3 and T4) exhibited significant increase in nonspecific immune responses, THC, phagocytosis, lysozyme activity, phenoloxidase activity and superoxide dismutase activity compared to the control diet (T1), with no significant differences among T2-T4 in all the studied parameters, except for the phagocytic index. The current study evaluated the health benefits of Bacti-nil®Aqua by assaying the hemolymph, which provides information closely related to the health and immune status of shrimp, and it plays an essential role in the physiological, nutrition, and immune processes **(Huberman, 2000; Bachère, 2003; Ji *et al.*, 2009)**.

Litopenaeus vannamei fed Bacti-nil®Aqua-supplemented diets showed enhanced nonspecific immune responses, which is very interesting as invertebrates depend entirely on innate immunity for internal defense against diseases **(Rowley & Powell, 2007)** through phagocytosis, which is one of the main biological defense mechanisms that characterizes invertebrates.

Table 6. Immune and antioxidants parameters in *L. vannamei* fed different levels of Bacti-nil®Aqua-supplemented diets

Parameter in hemolymph	**T1**	**T2**	**T3**	**T4**
THC ($\times 10^4$ cells/ml)	$20.3^{b} \pm 0.6$	$23.33^{a} \pm 0.6$	$24.6^{a} \pm 0.6$	$24.33^{a} \pm 0.6$
Phagocytosis (%)	$22.23^{b} \pm 0.27$	$24.73^{a} \pm 0.27$	$24.6^{a} \pm 0.27$	$25.03^{a} \pm 0.27$
Phagocytic index	$3.12^{b} \pm 0.06$	$3.20^{b} \pm 0.06$	$3.76^{a} \pm 0.06$	$3.76^{a} \pm 0.06$
Lysosomal enzyme activity (U l^{-1})	$58.03^{b} \pm 0.79$	$65.13^{a} \pm 0.79$	$65.6^{a} \pm 0.79$	$66.6^{a} \pm 0.79$
Phenoloxidase activity (U/min/mg)	$18.13^{b} \pm 0.29$	$23.73^{a} \pm 0.29$	$24.7^{a} \pm 0.29$	$23.60^{a} \pm 0.29$
Superoxide dismutase activity (SOD) (U/min/mg)	$0.36^{b} \pm 0.01$	$0.56^{a} \pm 0.01$	$0.56^{a} \pm 0.01$	$0.56^{a} \pm 0.01$

*T1 = Control; T2, T3 and T4 are treatments supplemented with Bacti-nil®Aquaat 2, 3, and 4 g/kg diet; THC: Total hemolymph count. Means within the same row with different superscript letters (a–c) are significantly different ($P < 0.05$).

The role of phagocytes is not only to attack foreign agents as pathogens, but also activate T cells **(Parham, 2015)** and produce antibody signals, associated with the activation of acquired immunity. Furthermore, the production of haemocytes, which are defensive cells present in the haemolymph, is one of the defense mechanisms against foreign particles that is commonly found in invertebrates. These haemocytes are responsible for sucking pathogens and foreign substances through phagocytosis and the coagulation process, as well as encapsulation and enzymes secretion process that are responsible for the destruction of the invading elements. Data in the current study reflected the significant improvement in immune parameters, compared to the control treatment. This may be due to the immunity efficacy of Bacti-nil® Aqua that was likely to reduce the presence of pathogens in *L. vannamei* haemolymph.

CONCLUSION

The results of the current study demonstrated the potential use of Bacti-nil® Aqua as a feed additive in the *Litopenaeus vannamei* diet. The survival rate, growth performance, feed utilization, immune responses and body composition of *L. vannamei* with different levels of Bacti-nil® Aqua were higher than those recorded in the control.

Different concentrations of Bacti-nil® Aqua led to significant improvement in PER and PPV%, compared to the control treatment (T1). In addition, *L. vannamei* fed on Bacti-nil® Aqua-supplemented diets at 3 and4 g/kg had the best FCR, compared to the control group. The chemical composition of *L. vannamei* exhibited that, the protein content increased significantly in T2 to T4 with the increasing levels of Bacti-nil® Aqua, compared to T1. While, the highest values of lipid among all treatments were in T1. Bacti-nil®Aqua-supplemented diets (T2-T4) exhibited significant increase in nonspecific immune responses and antioxidants criteria, THC, phagocytosis, lysozyme activity, phenoloxidase activity and superoxide dismutase activity in respect to the control (T1). Overall, the current study concluded that the diet supplemented with the highest inclusion concentrations, 3 and 4g/kg of Bacti-nil®Aqua, resulted in enhanced growth parameters, survival rate, feed utilization, chemical composition, nonspecific immune responses and antioxidants status of *L. vannamei.*

REFERENCES

Aguirre-Guzmán, G.; Sanchez Martinez, J.G.; Pérez-Castañeda, R.; Palacios Monzón, A.; Trujillo Rodríguez, T. and Cruz-Hernández, N.I. (2009). Penaeid Shrimp Immune System. Thai J. Vet. Med., 39(3): 205-215.

AOAC (1995). Official method of analysis, 16th ed. Arlington, VA: AOAC.

AOAC (2000). Official Methods of Analysis, 17th Edition, The Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Bachère, E. (2003). Anti-infectious immune effectors in marine invertebrates: Potential tools for disease control in larviculture. Aquaculture, 227: 427– 438.

Beschin, A.; Bilej, M.; Torreele, E. and De Baetselier, P. (2001). On the existence of cytokines in invertebrates. Cell. Mol. Life Sci. CMLS, 58: 801–814.

Blaxhall, P. and Daisley, K. (1973). Routine haematological methods for use with fish blood. J. Fish Biol., 5:771–781.

Boyd, C. E. (1998). Water quality for pond aquaculture. Research and development series No. 43. International center for aquaculture and aquatic environments, Alabama: Agriculture Experiment Station, Auburn University. Fish-culture, 37 pp.

Brett, J. (1979). Environmental factors and growth. Fish Physiology, 8:599–675.

Canesi, L. and Procházková, P.(2014). Chapter 7—The Invertebrate Immune System as a Model for Investigating the Environmental Impact of Nanoparticles. In Nanoparticles and the Immune System; Boraschi, D., Duschl, A., Eds.; Academic Press: San Diego, CA, USA, pp.91–112.

Chiu, C.-H.; Guu, Y.-K.; Liu, C.-H; Pan,T.-M. and Cheng, W. (2007). Immune responses and gene expression in white shrimp, *Litopenaeus vannamei*, induced by *Lactobacillus plantarum.* Fish Shellfish Immunol., 23: 364–377.

Dash, G.; Raman, R.P.; Pani Prasad, K.; Makesh, M.; Pradeep, M.A. and Sen, S. (2014). Evaluation of *Lactobacillus plantarum* as feed supplement on host associated microflora, growth, feed efficiency, carcass biochemical composition and immune response of giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879). Aquaculture, 432: 225–236.

Davies, S. J.; Gouveia, J.; Laporte, S. L.; Woodgate, J., and El-Haroun, E. R. (2019). Validation of novel processed animal proteins (mono-PAPS) within experimental diets for juvenile gilthead sea bream (*Sparus aurata* L.) as primary fish meal replacers. Aquac. Nutr., 25(1):225–238.

Dong, B. and Sun, C. (2021). Production of an invertebrate lysozyme of *Scylla paramamosain* in *E.coli* and evaluation of its antibacterial, antioxidant and anti-inflammatory effects. Protein Expr. Purif., 177: 105745.

Duan, Y.; Wang, Y.; Dong, H.; Li, H.; Liu, Q.; Zhang, J. and Xiong, D. (2018). Physiological and immune response in the gills of *Litopenaeus vannamei* exposed to acute sulfide stress. Fish Shellfish Immunol., 81:161–167.

Eissa, E. Hemdan; Che-Zulkifli, C.I.; El-Badawi, A.A.; Ali, M.A.M.; Baghdady, E.S.; Abd Al-Kareem, O.M. and Ahmed, R.A. (2021b). Growth-Promoting and Immunomodulatory Impacts of Commercial Stimulants on Kuruma Shrimp, *Penaeus japonicus* (Bate, 1888) Juveniles. Egypt. Aquat. Biol. Fish., 25(3): 607 – 617

Eissa, E. Hemdan; Mohamed H. Yassien; Salah E. Sakr; Mohamed S. Ayyat (2021a). Effect of different salinity levels on egg hatching and survival rate of differentmetamorphosis larval stages of Kuruma shrimp, *Penaeus japonicus* (Bate, 1888). Egypt. Aquat. Biol. Fish., 25(6): 984 – 502

Engstad, R. E.; Robertson, B. and Frivold, E. (1992). Yeast glucan induces increase in activity of lysozyme and complement mediated haemolytic activity in Atlantic salmon blood. Fish and Shellfish Immunol. 2:287–297.

FAO (2018) The State of World Fisheries and Aquaculture 2018—Meeting the Sustainable Development Goals; FAO: Rome, Italy.

Goda, A.; Srour, T.; Omar, E.; Mansour, A.; Baromh, M.; Mohamed, S.; El-Haroun, E. and Davies, S J. (2019). Appraisal of a high protein distiller's dried grain (DDG) in diets for European sea bass, *Dicentrarchus labrax* fingerlings on growth performance, haematological status and related gut histology. Aquac. Nutr., 25:808–816

Goda, A. M. A-S.; Saad, A.; Hanafy, M.; Sharawy, Z., & El-Haroun, E. (2018). Dietary effects of *Azolla pinnata* combined with exogenous digestive enzyme (Digestin™) on growth and nutrients utilization of freshwater prawn, *Macrobrachium rosenbergii* (de Man 1879). J. Oceanol. Limnol.., 36(4): 1434–1441.

Gowda, N.M.; Goswami, U. and Islam Khan, M.(2008). T-antigen binding lectin with antibacterial activity from marine invertebrate, sea cucumber (*Holothuria scabra*): Possible involvement in differential recognition of bacteria. J. Invertebr. Pathol., 99: 141–145.

Green, B.W. (2008). Stocking strategies for production of *Litopenaeus vannamei* (Boone) in amended freshwater in inland ponds. Aquac. Res., 39(1): 10–17.

Han, S.Y.; Wang, M.Q.; Liu, M.; Wang, B.J.; Jiang, K.Y. and Wang, L. (2018). Comparative sensitivity of the hepatopancreas and midgut in the white shrimp *Litopenaeus vannamei* to oxidative stress under cyclic serious/medium hypoxia. Aquaculture, 490: 44–52.

Harun, A.A.C. ; Mohammad, N.A.H. ; Ikhwanuddin, M.; Jauhari, I. ; Sohaili, J.and Kasan, N.A. (2019). Effect of different aeration units, nitrogen types and inoculum on biofloc formation for improvement of Pacific White leg shrimp production. Egypt. J. Aquat. Res., 45 (3) : 287-292

Huang, W.; Li, H.; Cheng, C.; Ren, C.; Chen, T.; Jiang, X., … Hu, C. (2018). Analysis of the transcriptome data in *Litopenaeus vannamei* reveals the immune basis and predicts the hub regulation-genes in response to high-pH stress. PLoS One. ; 13(12): e0207771.

Huberman, A. (2000). Shrimp endocrinology. Areview. Aquaculture, 191: 191–208.

Itami, Y.; Takahashi, Y.; Tsuchihira, E., Igusa, H. and Kondo, M. (1994). Enhancement of disease resistance of kuruma prawn *Penaeus japonicus* and increase in phagocytic activity of prawn hemocytes after oral administration of β-1,3-glucan (Schizophyllan). In: β-Glucan in invertebrates. Proceedings of the 3rd Asian Fisheries Forum, October 26-30, 1992, Asian Fisheries Society, Manila, Philippines, pp. 375-378.

Ji, P.; Yao, C. and Wang, Z. (2009). Immune response and gene expression in shrimp *(Litopenaeus vannamei*) hemocytes and hepatopancreas against some pathogen-associated molecular patterns. Fish Shellfish Immunol.,.27: 563–570.

Jobling, M. (1983). A short review and critique of methodology used in fish growth and nutrition studies. J. Fish Biol., 83: 685 – 691.

Kesselring, J.; Gruber, C.; Standen, B. and Wein, S. (2021). Effect of a phytogenic feed additive on the growth performance and immunity of Pacific white leg shrimp, *Litopenaeus vannamei*, fed a low fishmeal diet. J World Aquacult Soc.; 52:303–315.

Labrador, J.R.P.; Recamar, C.; Guiñares, R.C. and Hontiveros, G.J.S. (2016). Effect of garlic powder-supplemented diets on the growth and survival of Pacific white leg shrimp (*Litopenaeus vannamei*). Cogent food agric., 2: 1210066.

Liu, M. S. and Manacebo, V. J. (1983). Pond culture of *Penaeus monodon* in the Philippines: Survival, growth and yield using commercially formulated feed. J. World Maric. Soc, 14: 75–85.

Loker, E.S.; Adema, C.M.; Zhang, S. M. and Kepler, T.B. (2004). Invertebrate immune systems—Not homogeneous, not simple, not well understood. Immunol. Rev. 198: 10–24.

Lukwambe, B.; Nicholaus, R.; Zhang, D.; Yang, W.; Zhu, J. and Zheng, Z. (2019). Successional changes of microalgae community in response to commercial probiotics in the intensive shrimp *(Litopenaeus vannamei* Boone) culture systems. Aquaculture 511:734257.

Madeira, D.; Mendonça, V.; Dias, M.; Roma, J.; Costa, P.M.; Larguinho, M.; Vinagre, C.; and Diniz, M.S. (2015) Physiological, cellular and biochemical thermal stress response of intertidal shrimps with different vertical distributions: *Palaemon elegans* and *Palaemon serratus*. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol., 183: 107–115.

Nasmia; Natsir, S.; Rusaini ; Tahya, A. M.; Nilawati, J., Ismail, S.N. (2022). Utilization of Caulerpa sp. as a feed ingredient for growth and survival of whiteleg shrimp and Chanos chanos in polyculture. Egypt. J. Aquat. Res., 48, (2): 175-180

Nishikimi, M.; Rao, N.A. and Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazinemethosulfate and molecular oxygen. Biochem.Biophys. Res. Commun., 46: 849–854.

Nonwachai, T.; Purivirojkul, W.; Limsuwan, C.; Chuchird, N.; Velasco, M. and Dhar, A.K. (2010). Growth, nonspecific immune characteristics,and survival upon challenge with *Vibrio harveyi* in Pacific white shrimp (*Litopenaeus vannamei*) raised on dietscontaining algal meal. Fish Shellfish Immunol., 29(2):298–304

Nuez-Ortín, W. G.; Isern-Subich, M. and Guérin, M. (2020). Organic acids - synergy at work to prevent vibriosis and promote growth in shrimp May/June 2020 AQUA Culture Asia Pacific

Parham, P. (2015). Chapter 5 Antigen Recognition by T Lymphocytes. In The Immune System, 4th ed.; Taylor & Francis Groups: New York, NY, USA.

Rowley, A.F. and Powell, A. (2007). Invertebrate immune systems–specific, quasi-specific, or nonspecific? J Immunol.;79:7209–7214.

Schleder, D. D.; da Rosa, J. R.; Guimarães, A. M.; Ramlov, F.; Maraschin, M.; Seiffert, W. Q., et al. (2017). Brown seaweeds as feed additive for white-leg shrimp: effects on thermal stress resistance, midgut microbiology, and immunology. J. Appl. Phycol. 29: 2471–2477.

Sharawy, Z. Z.; Thiele, R.; Abbas, E. M.; El-Magd, M. A.; Hassaan, M. S.; Peter, C., … Slater, M. J. (2017). Antioxidant response and body composition of whiteleg shrimp co-cultured with Nile tilapia in recirculating aquaculture. Aquac. Environ. Interact., 9:257–268.

Sharawy, Z.; Goda, A. M. A.S., and Hassaan, M. S. (2016). Partial or total replacement of fish meal by solid state fermented soybean meal with *Saccharomyces cerevisiae*

in diets for Indian prawn shrimp, *Fenneropenaeus indicus*, Postlarvae. Anim. Feed Sci. Technol. 212: 90–99.

Sirirustananun, N.; Chen, J.C.; Lin, Y.C.; Yeh, S.T.; Liou, C.H.; Chen, L.L.; Sim, S.S. and Chiew, S.L. (2011). Dietary administration of a *Gracilaria tenuistipitata* extract enhances the immune response and resistance against *Vibrio alginolyticus* and white spot syndrome virus in the white shrimp *Litopenaeus vannamei*. Fish Shellfish Immunol.,31: 848–855.

Supamattaya, K.; Pongmaneerat, J. and Klowklieng, T. (2000). The effect of β-glucan (MacroGard®) on growth performance,immune response and disease resistance in black tiger shrimp, *Penaeus monodon* Fabricius. Songklanakarin J Sci Technol., 22:677–688

Thornber, K., Huso, D., Rahman, M.M.; Biswas, H., Rahman, M.H., Brum, E. and Tyler, C.R. (2019). Raising awareness of antimicrobial resistance in rural aquaculture practice in Bangladesh through digital communications: a pilot study, Global Health Action, 12:sup1, 1734735.

Wang, X.; Li, E.; Xu, C.; Qin, J.G.; Wang, S.; Chen, X.; Cai, Y.; Chen, K.; Gan, L. and Yu, N. (2016). Growth, body composition, ammonia tolerance and hepatopancreas histology of white shrimp *Litopenaeus vannamei* fed diets containing different carbohydrate sources at low salinity. Aquac. Res. 47: 1932–1943.

Wang, Z.; Qu, Y.; Yan, M.; Li, J.; Zou, J. and Fan, L. (2019). Physiological responses of pacific white shrimp *Litopenaeus vannamei* to temperature fluctuation in low-salinity water. Front. Physiol. 10: 1025.

Wyban, J.; Walsh, W.A. and Godin, D.M. (1995). Temperature effects on growth, feeding rate and feed conversion of the Pacific white shrimp (*Penaeus vannamei*). Aquaculture, 138(1-4): 267-279.