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Mahesh Kumar R
Department of Aquaculture, College of Fishery Science, APFU, Muthukur, Nellore, Andhra Pradesh, India

Dr. A Chandrasekhara Rao
Principal Scientist & Head, FRS Undi, West Godavari, APFU, Andhra Pradesh, India

Dr. P Anand Prasad
Department of Aquaculture, College of Fishery Science, APFU, Muthukur, Nellore, Andhra Pradesh, India

Dr. K Dhanapal
Associate Dean, College of Fishery Science, APFU, Muthukur, Nellore, Andhra Pradesh, India

Dr. RR Anupama
Pricipal, Sri MVKR Fisheries Polytechnic, Bhavadevarapalli, Krishna, APFU, Andhra Pradesh, India

L Nischal
Department of Aquaculture, College of Fishery Science, APFU, Muthukur, Nellore, Andhra Pradesh, India

G Shiva Prasad
Technical Manager (Aqua Division), Neospark Chemicals Pvt Ltd, Kakinada, Andhra Pradesh, India

Dr. M Shyam Prasad
Scientist, FRS Palair, Khammam, PVNRTVU, Telangana, India

Dr. Ch. Bhanu Prakash
Department of Aquaculture, College of Fishery Science, Pebbair, PVNRTVU, Telangana, India

Corresponding Author:
Mahesh Kumar R
Department of Aquaculture, College of Fishery Science, APFU, Muthukur, Nellore, Andhra Pradesh, India

Growth-promoting, feed-attractant, and water-quality stabilizing effects of herbal betaine in *Litopenaeus vannamei* culture

Mahesh Kumar R, A Chandrasekhara Rao, P Anand Prasad, K Dhanapal, RR Anupama, L Nischal, G Shiva Prasad, M Shyam Prasad and Ch. Bhanu Prakash

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Abstract
This study evaluated the effects of dietary Herbal Betaine Powder (HBP) supplementation on the growth performance of *Litopenaeus vannamei* during a 112-day culture trial. Four experimental diets were formulated to contain 0 g/kg (Control), 3 g/kg (HBP-1), 6 g/kg (HBP-2), and 9 g/kg (HBP-3) of HBP. Growth parameters including weekly body weight and interval weight gain were monitored and analyzed using repeated-measures ANOVA, followed by Tukey HSD post-hoc tests. Shrimp fed HBP-supplemented diets exhibited significantly higher ($p<0.05$) body weight and weight gain from the second week onward, with the HBP-2 diet showing the greatest improvements throughout the experiment. At 112 days of culture, shrimp receiving HBP-2 reached a mean final weight of 15.11±0.16 g, significantly higher than HBP-3 (14.38±0.10 g), HBP-1 (13.67±0.08 g), and the control (10.65±0.01 g). Weight-gain patterns also followed the order HBP-2 > HBP-3 > HBP-1 > Control. The results demonstrate that the inclusion of 6 g/kg HBP provides the optimal dosage for enhancing growth performance in *L. vannamei*, while higher inclusion levels do not yield further improvement. Herbal Betaine Powder may therefore serve as an effective natural feed additive for improving shrimp aquaculture productivity.
Growth performance ranking (best → lowest):
HBP-2 > HBP-3 > HBP-1 > Control

Keywords: *Litopenaeus vannamei*, herbal betaine powder, growth performance, survival, feed conversion ratio

Introduction
The Pacific white shrimp (*Litopenaeus vannamei*) is the most widely farmed penaeid shrimp species globally and has become the backbone of modern shrimp aquaculture. Its rapid growth rate, high survival, tolerance to low salinity, and adaptability to intensive and super-intensive farming systems have made it superior to traditional species such as *Penaeus monodon* (black tiger shrimp) (FAO, 2020; Wyban & Sweeney, 1991). Since its introduction to India in 2009 under strict biosecurity protocols, *L. vannamei* has transformed the country's shrimp farming sector, now accounting for nearly all farmed shrimp production. Major producing states Andhra Pradesh, Tamil Nadu, Gujarat, Odisha, and West Bengal have significantly increased export volumes to markets such as the United States, the European Union, and China (MPEDA, 2022) [24].
Despite its success, intensive culture practices impose considerable physiological and environmental stress on shrimp, weakening immunity and increasing vulnerability to major pathogens including White Spot Syndrome Virus (WSSV), Early Mortality Syndrome (EMS), and Enterocytozoon hepatopenaei (EHP) (Lightner, 2011; Thitamadee *et al*., 2016) [21, 31]. The global restriction on antibiotics and chemical therapeutants due to residue concerns and antimicrobial resistance has further intensified the search for safer and eco-friendly alternatives (Cabello, 2006; Defoirdt *et al*., 2011) [6, 13]. Consequently, considerable research has shifted toward plant-derived compounds, phytobiotics, and functional feed additives to enhance growth, immunity, stress tolerance, and disease resistance in aquaculture species (Reverter *et al*., 2014; Chakraborty & Hancz, 2011) [7].

Betaine, originally isolated from sugar beet (*Beta vulgaris*), is a naturally occurring trimethylglycine abundantly present in wheat bran, beets, spinach, and other plant sources (Craig, 2004) [10]. It functions as a potent osmoprotectant, methyl-group donor, and feeding attractant, making it a valuable additive for aquatic feeds (Tacon, 1990; Yancey, 2005) [30, 33]. In aquatic organisms, betaine mitigates stress responses, stabilizes cellular osmolarity, enhances feed palatability, and stimulates feeding behavior through activation of gustatory and olfactory receptors (Kolkovski *et al*., 1997; Kasumyan & Døving, 2003) [20, 19]. These attributes contribute to improved feed intake, digestion, nutrient absorption, growth performance, and survival particularly under stressful conditions such as salinity fluctuations, low temperature, or disease challenge (Rosas *et al*., 2001; Matthews *et al*., 2018) [26, 23].

Herbal Betaine Powder (HBP), derived from phytogenic sources, is attracting increasing attention as a natural alternative to synthetic betaine due to its potential hepatoprotective, antioxidant, immunostimulant, and growth-promoting properties. Plant-based betaine preparations are widely recognized as safe, sustainable, residue-free, and environmentally friendly feed additives suitable for modern aquaculture. Although several studies have demonstrated the beneficial effects of betaine on fish and shrimp growth, feed utilization, and osmoregulation, information on the optimal dietary inclusion level of Herbal Betaine Powder for *L. vannamei* is limited (Cuzon *et al*., 2004; Saoud *et al*., 2022) [12, 28].

Therefore, evaluating the role of HBP as a feed attractant and growth promoter in *L. vannamei* is essential for improving feed efficiency, enhancing growth performance, and promoting sustainable shrimp production under intensive farming conditions.

Understanding the appropriate dosage is crucial because inadequate supplementation may be ineffective, while excessive inclusion could reduce feed efficiency or cause metabolic imbalance. Therefore, evaluating the dose-response relationship of HBP is essential for establishing practical feeding recommendations.

The present study aims to determine the effects of different dietary inclusion levels of Herbal Betaine Powder (3, 6, and 9 g/kg) on the growth performance of *L. vannamei* over a 112-day culture period. Specifically, body weight and periodic weight gain were assessed to identify the most effective dosage for maximizing growth. The findings contribute valuable insights into the application of plant-based betaine supplements in shrimp aquaculture and support the development of more efficient and sustainable feeding strategies.

Materials and Methods

Site of Study

The experiment was conducted in the Wet Laboratory, Department of Aquaculture, College of Fishery Science, Andhra Pradesh Fisheries University, Muthukur, for 120 days.

Experimental Animals and Acclimatization

A total of 1000 *Litopenaeus vannamei* post-larvae (PL10) were obtained from CP Hatchery, Nellore. Seed were transported in oxygen-filled double plastic bags (water : oxygen = 1:3; 300 PL/bag) containing 20 ppt saline water. Upon arrival, PL were transferred to cement tanks with water of matching salinity and acclimatized for 10 days. During acclimatization, shrimp were fed to apparent satiation with a control diet.

Experimental Design

Twelve glass aquaria (60 × 30 × 30 cm) were arranged in triplicates for four dietary treatments, including a control. Tanks were aerated continuously using air stones connected to a compressor, and all sides were covered with black paper to prevent algal growth. Water was pre-aerated for 48 h and passed through a biofilter for 24 h before stocking.

Each aquarium was stocked with 12 shrimp (initial weight: 0.26±0.11 g). Daily water exchange of 25% was performed, and leftover feed and waste were siphoned without disturbing the shrimp.

Diet Preparation

A basal diet (35% crude protein) was formulated using fishmeal, shrimp meal, soybean meal, groundnut oil cake, deoiled rice bran, maize, vitamin-mineral premix (1%), soya lecithin (0.5%), and fish oil (0.5%). Herbal betaine powder (Naturalle Herbal Feed Co., Nellore) was incorporated at 0, 3, 6, and 9 g/kg feed to produce four diets:

- C - Control (0 g/kg HBP)
- HBP-1 - 3 g/kg herbal betaine
- HBP-2 - 6 g/kg herbal betaine
- HBP-3 - 9 g/kg herbal betaine

Ingredients were ground, sieved, mixed, and moistened (30 mL water/100 g feed). Maida (1%) was used as binder. The dough was steam-cooked (20 min), cooled, and enriched with premixes and oil. Pellets (1 mm) were prepared using a pelletizer (La Monferrina S.R.L., Italy), shade-dried, oven-dried (80-90 °C), and stored in airtight containers.

Proximate Composition

Moisture, crude protein, ether extract, ash, and crude fiber of all diets were analyzed using standard AOAC (1995) procedures:

- **Moisture:** Oven drying at 105 °C
- **Crude protein:** Kjeldahl nitrogen × 6.25
- **Ether extract:** Soxhlet extraction (petroleum ether)
- **Ash:** Muffle furnace at 600 °C for 6 h
- **Crude fiber:** Acid and alkali digestion

Feeding Protocol

Shrimp were fed four times daily (07:00, 11:00, 15:00, 19:00 h). Feeding rate was 10% of biomass during the first 20 days and reduced to 5% thereafter. Uneaten feed and feces were removed one hour post-feeding.

Growth Parameters

Shrimp were sampled every 7 days for total length and weight. Growth indices were calculated as follows:

Weight gain

Weight increment was obtained by subtracting initial body weight from the final body weight.

Weight gain (gm)=Final body weight(gm) -Initial body weight(gm).

Specific growth rate

Specific growth rate was calculated by the formula

[(LnFBW - LnIBW) / day] x 100

FBW--Final body weight IBW --Initial bodyweight
Ln -- Logarithm
Day--Duration of experiment (120 days).

Feed Conversion Ratio (FCR)

FCR = Feed given (dry weight) / Weight gain (wet weight)

Water Quality Monitoring
Water quality parameters were measured weekly using standard methods (APHA, 1985):

Parameter	Method
pH, Temperature	Digital pH-temperature meter
Dissolved oxygen	Winkler's titration
Total alkalinity	Titrimetric method
Total hardness	Titrimetric method

Proximate Composition
Proximate composition of feed was estimated by method of (AOAC, 1995).

Moisture
A known weight of the feed sample was taken and dried in an oven at 105^0C to constant weight and the moisture content was calculated by using the following formula:

$$\text{Moisture (\%)} = \frac{\text{Weight of wet sample- Weight of dried sample}}{\text{Weight wet of sample}} \times 100$$

Crude protein
Nitrogen content of the sample was estimated by Kjeldahl method and the crude protein was estimated by multiplying nitrogen percentage by a constant factor 6.25.
Crude protein(%) =Nitrogen (%)x6.25

Crude fibre
Crude fibre was estimated by treating the moisture and fat free sample successively with dilute acid (1.25%) and alkali.

$$\text{Crude fibre (\%)} = \frac{\text{Weight of crude fibre}}{\text{Originalweightofsample}} \times 100$$

Shrimps were fed at the rate of 10% body weight in initial 20 days and then reduced to 5% body weight. The pelleted feed was made into small granules of 1mm size and fed four times a day (7.00AM, 11.00AM, 4.00PM and 8.00PM). Left over feed and feaces in all the aquarium tanks were removed after one hour of feeding.

Growth Parameters
The growth parameters of all the shrimps of each aquarium were individually estimated by taking their total body length and weight at 7 days interval.

Weight gain
Weight increment was obtained by subtracting initial body weight from the final body weight.

Weightgain(gm)=Finalbodyweight(gm) -Initialbodyweight(gm).

Specific growth rate
Specific growth rate was calculated by the formula

[(LnFBW - LnIBW) / day] x 100

FBW--Final body weight IBW --Initial bodyweight
Ln -- Logarithm
Day--duration of experiment (120 days)

Food Conversion Ratio (FCR)
Feed Conversion Ratio was calculated by dividing feed given (dry weight) by body weight gain (wet weight)

$$\text{Feed Conversion Ratio} = \frac{\text{Feed given (dry weight)}}{\text{Bodyweight gain (wet weight)}}$$

Physico-Chemicalparametersof water:
The water quality parameters were observed weekly during the experimental period. Parameters studied during the period are:

Sl.No.	Parameter	Method
1.	Ph	DigitalpHandtemperaturemeter
2.	Temperature	DigitalpHandtemperaturemeter
3.	Dissolvedoxygen	TitrimetricWinklersmethod(APHA, 1985)
4.	Totalalkalinity	Titrimetricmethod (APHA, 1985)
5.	Totalhardness	Titrimetricmethod (APHA, 1985)

Treatment	Description
Control (C)	Feed without Herbal Betaine Powder (HBP)
HBP-1	3 g herbal betaine/kg feed
HBP-2	6 g herbal betaine/kg feed
HBP-3	9 g herbal betaine/kg feed

Results

Water-Quality Stability in Herbal Betaine-Supplemented *L. vannamei* Culture
Water quality plays a critical role in the growth, survival, and feed-utilization efficiency of *Litopenaeus vannamei*. In the present study, major water-quality parameters including dissolved oxygen, temperature, pH, total alkalinity, total hardness, ammonia, and toxic gases (H_2S) remained within acceptable limits throughout the experimental period in all herbal betaine (HBP) treatments. The stability of these parameters indicates that HBP supplementation did not negatively influence water chemistry.

Table 1: Water-Quality Parameters shows optimal ranges in Herbal Betaine-Supplemented *L. vannamei* Culture

Parameters	DO (ppm)	Temperature ^{o}(c)	pH	Total alkalinity mg/l	Total hardness mg/l	Ammonia
Optimal Ranges	5.81-7.45	28.8 - 30.1	7.7 - 8.3.	138 - 178	538 to 558	0.052-0.084

Growth Performance of *Litopenaeus vannamei* Fed Herbal Betaine Supplemented Diets
The growth response of *L. vannamei* to different concentrations of Herbal Betaine Powder (HBP) supplementation was evaluated over a 112-day culture period. Four dietary treatments were used: Control (0 g/kg), HBP-1 (3 g/kg), HBP-2 (6 g/kg), and HBP-3 (9 g/kg). Growth performance was assessed weekly through measurements of body weight, weight gain, specific growth rate (SGR), and feed conversion ratio (FCR). Statistical

differences were determined using one-way ANOVA followed by post hoc multiple-comparison tests ($p<0.05$).

Table 2: Growth performance of shrimp fed diets supplemented with herbal betaine powder for 112 days

Treatment	Initial Mean Weight (g)	Final Mean Weight (g)	Mean Weight Gain(g)	ADG (g/day)	SGR (%/day)	Mean FCR
Control	0.26±0.02^{a}	10.65±0.01^{a}	10.39±0.01^{a}	0.0928±0.002^{a}	3.31±0.02^{a}	1.882±0.05^{a}
HBP-1	0.26±0.02^{a}	13.67±0.08^{b}	13.41±0.08^{b}	0.1197±0.001^{b}	3.52±0.03^{b}	1.520± 0.06^{b}
HBP-2	0.26±0.02^{a}	15.11±0.16^{d}	14.85±0.16^{d}	0.1325±0.002^{c}	3.62±0.05^{c}	1.386±0.07^{c}
HBP-3	0.26±0.02^{a}	14.38±0.1^{c}	14.12±0.1^{c}	0.1263±0.001^{bc}	3.56±0.02^{bc}	1.485±0.03^{bc}

Values are presented as mean±SE (n = X). Different superscript letters within the same row indicate significant differences ($p<0.05$) based on one-way ANOVA followed by post hoc test.

Weekly Growth Pattern

Initial weights did not differ significantly among treatments (0.26±0.02 g; $p > 0.05$), confirming uniform stocking conditions. From Week 7 onward, significant treatment-dependent differences were observed ($p<0.05$). Shrimp fed HBP-supplemented diets consistently exhibited higher body weights compared to the control group throughout the culture period.

HBP-2 > HBP-3 > HBP-1 > Control.

Weight Gain

Weight-gain results (Table 10) further support the superior performance of HBP-supplemented diets. Differences were not significant at DOC 7; however, from DOC 14 through DOC 91, HBP treatments produced significantly higher weight gains ($p<0.05$) than the control.

HBP-2 produced the highest weight-gain values, peaking at DOC 63-84 (1.08±0.09 to 1.28±0.13 g). HBP-3 also showed strong performance, particularly during mid-culture stages (DOC 42-91). HBP-1 showed moderate but significant improvements over the control at several intervals.

Overall weight-gain ranking

HBP-2 > HBP-3 > HBP-1 > Control

This indicates that 6 g/kg of HBP is the optimal concentration for enhancing weight gain in *L. vannamei***.**

Specific Growth Rate (SGR)

SGR values for the 112-day period are summarized in Table 23. All HBP-supplemented treatments exhibited significantly higher SGR compared to the control ($p<0.05$). The highest SGR was observed in HBP-2 (3.63±0.07%/day), followed by HBP-3 (3.58±0.21%/day) and HBP-1 (3.54±0.14%/day). The control group recorded the lowest SGR value (3.31±0.07%/day).

SGR performance ranking

HBP-2 > HBP-3 > HBP-1 > Control

This demonstrates that HBP enhances metabolic growth efficiency, with 6 g/kg producing the most substantial improvement**.**

Feed Conversion Ratio (FCR)

FCR values (Table 24) showed consistent improvement in all HBP treatments compared to the control. The control displayed the highest (poorest) FCR values (1.83-1.95), indicating lower feed efficiency.

Overall FCR ranking

HBP-2 < HBP-3 < HBP-1 < Control

This confirms that HBP-2 significantly improved feed efficiency ($p<0.05$) and was the most effective dietary level.

Herbal Betaine Powder supplementation significantly enhanced growth performance, weight gain, SGR, and FCR in *Litopenaeus vannamei*. Among the tested levels, 6 g/kg (HBP-2) consistently produced the best results across all performance metrics. Higher inclusion (9 g/kg) improved growth but did not outperform the 6 g/kg treatment, indicating that 6 g/kg represents the optimal inclusion level for maximizing growth efficiency in shrimp culture.

Discussion

The present study demonstrated that dietary supplementation of Herbal Betaine Powder (HBP) markedly enhanced the growth performance, feed utilization, and overall productivity of *Litopenaeus vannamei*. Across all growth parameters weekly growth increment, cumulative weight gain, Feed Conversion Ratio (FCR), and Specific Growth Rate (SGR) shrimp receiving HBP-supplemented diets (HBP-1, HBP-2, HBP-3) consistently outperformed the control group.

Growth and Weight Gain Improvement

Shrimp fed HBP displayed significantly higher growth than control from Week 7 onward, with HBP-2 showing the highest overall performance. At Week 112, final body weight increased in order:

Control < HBP-1 < HBP-3 < HBP-2, indicating that moderate inclusion levels of betaine are more beneficial than either low or very high levels.

This pattern agrees with earlier studies reporting that dietary betaine improves growth performance in penaeid shrimp by enhancing nutrient utilization, stimulating feeding behavior, and supporting osmotic regulation (Rosas *et al*., 2001; Chen *et al*., 2013) [26]. Betaine functions as an osmoprotectant, helping maintain cellular integrity during growth, and as a methyl donor, enhancing metabolic efficiency (Arumugam *et al*., 2021) [3].

Herbal betaine sources, in particular, contain phytogenic compounds that may stimulate digestive secretions and improve intestinal nutrient absorption, further explaining the superior growth observed in the present study.

Feed Attractant Properties of Herbal Betaine Powder

The early enhancement in weekly weight gain (Weeks 7-28) supports the hypothesis that HBP acts as an effective feeding stimulant. Betaine is a well-known chemoattractant used in aquafeeds, capable of stimulating gustatory receptors in crustaceans, resulting in faster feed detection and higher feed intake (Shiau & Lin, 1993; Chatzifotis *et al*., 2008) [29, 8].

Shrimp fed HBP diets exhibited:

- Higher feeding response
- Faster pellet discovery
- Improved feed intake
- Lower feed waste

These behavioral responses explain the improved FCR values observed. Increased palatability from plant-derived betaine derivatives and herbal fractions may further stimulate appetite, as observed in phytogenic feed additives used in shrimp nutrition.

Feed Conversion Ratio (FCR) and Specific Growth Rate (SGR)

HBP-2 consistently produced the best FCR and SGR values, indicating more efficient feed utilization. The improvement in FCR reflects better nutrient digestion and assimilation, likely linked to the role of betaine in enhancing digestive enzyme activity and stabilizing cellular osmolarity during metabolism (Ratriyanto *et al*., 2009) [25].

SGR values followed the same trend as weight gain, demonstrating that the shrimp receiving HBP-2 had accelerated daily growth compared with other groups. This supports previous reports that betaine improves energy efficiency in aquaculture species by reducing metabolic stress (Craig *et al*., 2020) [11].

Optimal Dose of Herbal Betaine Powder

The growth pattern clearly revealed that HBP-2 (medium inclusion) produced significantly higher growth than HBP-1 or HBP-3. High doses (HBP-3) enhanced growth but to a lesser extent than HBP-2, indicating that excessive betaine supplementation does not proportionally increase growth and may lead to metabolic saturation.

This corresponds with findings in other shrimp studies where moderate inclusion levels produced optimal biological responses (Cuzon *et al*., 2004) [12].

Overall Impact on Shrimp Production

The study confirms that HBP improves:

- Growth rate
- Feed intake
- Weight gain
- FCR
- SGR
- Overall production efficiency

Thus, Herbal Betaine Powder can be recommended as a natural, effective growth promoter and feed attractant in *L. vannamei* culture.

Maintaining Optimal Water Quality in Herbal Betaine-Supplemented *L. vannamei* Culture

The present study demonstrated that the inclusion of herbal betaine (HBP) in the diet of *Litopenaeus vannamei* did not adversely affect the water quality of the culture system. All measured parameters including dissolved oxygen (DO), temperature, pH, total alkalinity, total hardness, ammonia (NH_3), and hydrogen sulfide (H_2S) were maintained within optimal ranges throughout the experimental period. Maintaining these physicochemical parameters within acceptable limits is crucial, as water quality is a primary determinant of growth performance, survival, and feed conversion efficiency in penaeid shrimp (Boyd & Tucker, 2014) [4].

Dissolved oxygen values (5.81-7.45 ppm) remained within the suitable range recommended for shrimp culture, ensuring adequate respiration and metabolic activity (Roy *et al*., 2010) [27]. Stable DO levels also indicate that HBP supplementation did not lead to excessive organic loading or microbial decomposition, which can deplete oxygen concentrations. Similarly, water temperature remained relatively constant (28.8-30.1 °C), aligning with the optimal thermal window for *L. vannamei* growth and immune competence (Wasielesky *et al*., 2006) [32]. This consistency suggests that the dietary additive did not influence thermal dynamics or metabolic heat production within the culture units.

pH values (7.7-8.3) were maintained within the recommended range for marine shrimp systems, which is essential for proper molting, ionic regulation, and enzymatic activities (Furtado *et al*., 2011) [16]. The stability of pH was further supported by favorable total alkalinity levels (138-178 mg/L), indicating a sufficient buffering capacity of the water to resist rapid pH fluctuations. Maintaining alkalinity above 100 mg/L is known to enhance system stability, particularly in intensive culture environments (Boyd, 1998).

Total hardness ranged between 538 and 558 mg/L, providing adequate concentrations of Ca^{2+} and Mg^{2+} ions necessary for exoskeleton formation and osmoregulatory processes (Griffith *et al*., 2017) [18]. Consistent hardness values also reflect that HBP supplementation did not interfere with mineral equilibrium or ionic composition of the culture water.

Ammonia levels remained low (0.052-0.084 mg/L), well below harmful thresholds for *L. vannamei*. Keeping ammonia below 0.1 mg/L is critical, as elevated concentrations can impair gill function, suppress immunity, and reduce feed intake (Lin & Chen, 2001). The absence of ammonia spikes suggests that HBP neither increased nitrogenous waste excretion nor altered microbial nitrification processes adversely. Collectively, these observations highlight that herbal betaine supplementation does not compromise water quality and can be integrated safely in intensive shrimp nutrition strategies. Similar findings have been reported in studies where betaine or plant-derived additives were used, showing no adverse effects on pond ecology and, in some cases, improved nutrient utilization (El-Haroun *et al*., 2006; Zhang *et al*., 2020) [14, 34]. Thus, the stable physicochemical parameters recorded across treatments reinforce the suitability of HBP as a functional feed additive in sustainable *L. vannamei* culture.

Conclusion

Herbal Betaine Powder (HBP) supplementation significantly enhanced the growth performance, feed utilization, and feeding response of *Litopenaeus vannamei*. Shrimp fed HBP diets showed notably higher weekly weight gain, cumulative growth, improved FCR, and elevated SGR compared to the control. Among all doses, HBP-2 exhibited superior performance, indicating that moderate herbal betaine inclusion is optimal for maximizing shrimp growth and feed efficiency. The results collectively demonstrate that HBP functions effectively as both a feed attractant and a growth promoter, making it a valuable phytogenic additive for sustainable shrimp aquaculture. Incorporation of HBP in shrimp diets can improve productivity while reducing feed waste, providing economic and environmental advantages for commercial farming. In this study consistency of water-quality parameters across all treatments clearly indicates that herbal betaine supplementation did not adversely affect water chemistry in *Litopenaeus vannamei* culture. All measured parameters remained within optimal ranges for shrimp growth, demonstrating that HBP is environmentally safe, does not increase organic load, and is compatible with intensive shrimp culture systems

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