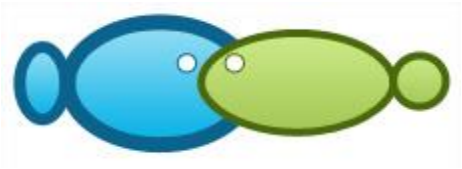

# Effect of dietary betaine hydrochloride and probiotic supplementation on enzymatic activity, intestinal microbiota, and growth in *Panulirus ornatus* (Fabricius, 1798)

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**Abstract**. This study aimed to analyze the effects of dietary betaine hydrochloride (BHCl) and probiotic supplementation on digestive enzyme activities, total bacterial count (TBC), and growth performance of the ornate spiny lobster, *Panulirus ornatus* (Fabricius, 1798). Four experimental diets were formulated, namely 0% BHCl without probiotic (Diet A /Control diet), 1% BHCl + 20 mL probiotic $kg^{-1}$ diet (Diet B), 3% BHCl + 20 mL probiotic $kg^{-1}$ diet (Diet C), and 5% BHCl + 20 mL probiotic $kg^{-1}$ diet (Diet D). A total of 36 lobsters were distributed into 12 tanks, 3 individuals per tank, for 50 days of feeding trial, and feed was administered twice daily (08:00 a.m and 08.00 p.m). The results showed that the inclusion of 3% BHCl + 20 mL probiotic $kg^{-1}$ diet significantly enhanced digestive enzyme activities (amylase, protease, and lipase), feed intake, weight gain (WG), feed efficiency (FE), and total bacterial in lobster intestine compared to the control. This suggests that dietary inclusion of 3% BHCl combined with probiotics can improve digestive function, microbial abundance, and growth performance of *P. ornatus*, thereby contributing to more efficient and sustainable lobster aquaculture.
**Key Words**: attractant BHCl, probiotic, digestive enzyme activity, *Panulirus ornatus*, growth performance.

**Introduction**. The ornate spiny lobster, *Panulirus ornatus* (Fabricius, 1798 is a high-value crustacean species with strong market demand and significant aquaculture potential in Indonesia. The export price ranges from 50-70 USD $kg^{-1}$, making it one of the most valuable marine commodities (Jones et al 2019). Indonesia is recognized as one of the major producers of *P. ornatus*, with suitable farming sites distributed across Nusa Tenggara, Sulawesi, and Papua (Priyambodo et al 2015). Despite this potential, productivity remains relatively low compared to the available natural resources.

One of the major constraints in *P. ornatus* aquaculture is the low feed intake of formulated diets, resulting in slow growth and poor feed efficiency (FE) (Jones et al 2019; Astuti et al 2023; Kurnia et al 2025). *P. ornatus* generally prefer natural feeds such as mussels, squid, and trash fish, which have a stronger odor and softer texture compared to commercial pellets (Smith et al 2005; Sudewi et al 2021; Rivaie et al 2023). Therefore, feed attractants are considered a promising approach to enhance the palatability and consumption of artificial diets. Attractants act by stimulating the chemosensory organs of slipper lobster through compounds such as free amino acids, peptides, and trimethylamine oxide (Teoh et al 2023). Previous studies on *Litopenaeus vannamei* showed that supplementation of attractants significantly improved feed intake, specific growth rate (SGR), and FE (He et al 2022; Saridu et al 2025). Moreover, Kurnia

et al (2025) stated that supplementation of betaine hydrochloride (BHCl) as an attractant in the diet could improve feed consumption and growth performance of *P. ornatus*.

Apart from feed palatability, microbiological and physiological factors play a crucial role in white shrimp growth performance (Wang et al 2020). Probiotics have been widely used in aquaculture to improve gut microbial balance, suppress pathogenic bacteria, and stimulate digestive enzyme activities such as protease, amylase, and lipase (Newaj-Fyzul & Austin 2015). Supplementation of *Bacillus subtilis* and *Lactobacillus plantarum* has been reported to enhance digestive enzyme activity and growth in shrimp species (Hoseinifar et al 2018; Tamilarasu et al 2021). In *Panulirus homarus*, probiotic supplementation improved immune responses and reduced mortality against *Vibrio harveyi* infection (Haryanti et al 2017).

Digestive enzyme activity serves as an important indicator of feed utilization efficiency in crustaceans. Increased protease and amylase activities indicate enhanced digestive capacity for protein and carbohydrate metabolism in the crustacean (Johnston et al 2004). A balanced gut bacteria population also supports nutrient absorption and maintains intestinal homeostasis (Chaudhary et al 2024). Therefore, the interaction between attractants, probiotics, enzyme activity, and gut microbiota is expected to produce synergistic effects on lobster growth and health.

Presently, limited information is available regarding the combined effects of attractant BHCL and probiotic supplementation on digestive enzyme activity, bacterial abundance, and growth performance in *P. ornatus*. Therefore, this study aimed to evaluate the influence of dietary BHCl and probiotic supplementation on the parameters. The outcomes are expected to contribute to the development of more efficient and sustainable formulated feeds for *P. ornatus* aquaculture.

## Material and Method

***Experimental feed***. Four experimental diets were formulated to contain approximately 40% crude protein, corresponding to the nutritional requirement of *P. ornatus*. These diets include 0% BHCl without probiotic (Diet A/Control diet), 1% BHCl + 20 mL probiotic $kg^{-1}$ diet (Diet B), 3% BHCl + 20 mL probiotic $kg^{-1}$ diet (Diet C), and 5% BHCl + 20 mL probiotic $kg^{-1}$ diet (Diet D). All other ingredients were identical across treatments and included molluscan (mangrove snail, golden apple snail, and clam), shrimp head diet, soybean diet, corn diet, rice bran, and sago starch as the carbohydrate binder. Corn oil and fish oil were added as lipid sources, while a vitamin-mineral premix was included to meet micronutrient requirements (Table 1).

All ingredients were finely ground, weighed, and thoroughly mixed according to formulation ratios. Warm water, approximately 30% of the total ingredient weight, was added to form a uniform dough, which was then pelleted using a mechanical extruder. The pellets were sun-dried until completely hard, packed in airtight containers, and stored under refrigeration until use.

Table 1

Formulation of experimental diets and results of proximate analysis

| *Feed ingredients* | *Experimental diets (g 100 $g^{-1}$ diet)* | | | |
|---|---|---|---|---|
| | *0% BHCl, NPr* | *1% BHCl, NPr* | *3% BHCl, NPr* | *5% BHCl, NPr* |
| Telescopium muscle meal | 15 | 15 | 15 | 15 |
| Gold snail meal | 15 | 15 | 15 | 15 |
| Mud scallops meal | 10 | 10 | 10 | 10 |
| Shrimp head meal | 25 | 25 | 25 | 25 |
| Soybean meal | 15 | 15 | 15 | 15 |
| Corn meal | 5 | 3 | 3 | 3 |
| Fine bran meal | 5 | 5 | 4 | 3 |
| Sago meal | 5 | 6 | 5 | 4 |
| BHCl | 0 | 1 | 3 | 5 |
| Corn oil | 1 | 1 | 1 | 1 |
| Fish oil | 2 | 2 | 2 | 2 |
| Vitamin and mineral mix.* | 2 | 2 | 2 | 2 |
| *Proximate composition (%)* | | | | |
| Moisture | 8.57 | 12.01 | 13.87 | 13.91 |
| Crude protein | 40.00 | 39.75 | 40.89 | 39.89 |
| Crude fat | 12.17 | 12.36 | 12.38 | 12.98 |
| Crude ash | 15.72 | 14.33 | 15.33 | 15.33 |
| Crude fibre | 6.95 | 6.06 | 5.51 | 6.45 |
| NFE ** | 16.60 | 15.50 | 12.02 | 8.44 |
| GE (kal $g^{-1}$) *** | 3,990.15 | 3,949.23 | 3,860.22 | 4,010.12 |

*Vitamin A 12,000. IU, Vitamin D3 2,000. IU, Vitamin E 8,000 IU, Vitamin K3 2,000 mg, Vitamin B1 2,000 mg, Vitamin B2 5,000 mg, Vitamin B6 500 mg, Vitamin B12 12,000 ug, Vitamin C 25,000 mg, Calcium-D-pantothenate 6,000 mg, Niacin 40,000 mg, Cholin chloride 10,000 mg, Methionine 30,000 mg, Lysine 30,000 mg, Manganese 120,000 mg, Iron 20,000 mg, Iodine 200 mg, Zinc 100,000 mg, Cobalt 200 mg, Copper 4,000 mg.
**NFE: nitrogen-free extract was calculated according to Jiang et al (2015); NFE = 100 - (protein + fat + ash);
***GE: gross energy.

***Probiotic preparation***. Probiotic culture was prepared following the method of Widanarni & Jusadi (2015) with slight modifications. In this experiment, the probiotic used was Probio-7, containing various microorganisms, including *Saccharomyces cerevisiae*, *Lactobacillus acidophilus, Bacillus subtilis, Aspergillus oryzae, Rhodopseudomonas, Actinomycetes, and Nitrobacter*. A stock culture of Probio-7 was activated using molasses and water in a 1:1:1 ratio (probiotic:molasses: water). The mixture was incubated for 24 hours at room temperature (28-32°C) to stimulate bacterial proliferation. The resulting suspension was sprayed evenly on experimental feeds at a concentration of 20 mL per kg of diet (except for the control diet). After spraying, feeds were air-dried for 30 minutes and stored at room temperature for 24 hours before feeding (Gruber et al 2023).

***Feeding trial***. A total of 36 *P. ornatus* juveniles were obtained from the wild and acclimated for one week in fiberglass tanks, initially given natural feed (trash fish and clams) before gradual adaptation to the experimental diets. *P. ornatus* were divided into three weight classes, namely 90-120 g, 121-150 g, and 151-180 g, with three individuals per tank (total 12 tanks).

Each plastic rearing tank (61 cm × 43 cm × 38 cm) was connected to a seawater recirculation system and supplied with continuous aeration through air stones. Tanks were filled with 70 L of filtered seawater previously aerated for 24 hours. Three PVC pipe shelters were provided in each tank to reduce stress and aggression.

*P. ornatus* were fed twice daily at 08.00 a.m and 05.00 p.m at a feeding rate of 3% of the body weight. Uneaten feed and feces were siphoned before each morning feeding and used for the measurement of total feed consumption (TFC). The rearing

period lasted for 50 days, and individual weights were recorded at the 0-day, 25-day, and 50-day points of the trial using a digital balance (precision 0.01 g).

***Proximate analysis of test feed***. Proximate composition of the diets, including moisture, crude protein, lipid, ash, and crude fiber, was determined using standard methods. Moisture was measured by oven drying at 110°C (SNI 01-2891-1992). Crude protein was analyzed using titrimetric Kjeldahl methods (18-8-31/MU/SMM-SIG). Total lipid content was determined using Soxhlet extraction (18-8-5/MU/SMM/SIG), and ash content was obtained by incineration at 600°C. Crude fiber was measured by sequential acid and alkali digestion (gravimetric method). In general, all analyses were performed in triplicate.

***Assay of digestive enzyme activity***. Activities of amylase, lipase, and protease were assayed from the hepatopancreas and intestine of *P. ornatus* at the end of the experiment. Amylase activity was determined using soluble starch (1%) in 20 mM sodium phosphate buffer (pH 6.9) containing 6.0 mM NaCl as substrate. The reaction was terminated by dinitrosalicylic acid (DNS) reagent, and absorbance was measured at 540 nm (Worthington 1993). Lipase activity was analyzed using olive oil emulsion as substrate and Tris-HCl buffer (0.1 M, pH 8.0). The reaction was terminated with 95% ethanol, and the released fatty acids were titrated with NaOH 0.01 N and read at 715 nm (Borlongan 1990). Protease activity was determined with casein as substrate in phosphate buffer (0.05 M, pH 7). The reaction product (tyrosine equivalent) was quantified colorimetrically using Folin-Ciocalteu reagent and measured at 578 nm (Bergmeyer 1982).

***Total bacterial count***. The total viable bacterial count in *P. ornatus* digestive tracts was determined using the Total Plate Count (TPC) method. Serial dilutions ranging from $10^{-1}$ to $10^{-8}$ CFU $mL^{-1}$ were prepared by homogenizing 1 g of gut tissue in sterile seawater and diluting in 0.9% NaCl solution. Aliquots (0.1 mL) from appropriate dilutions were spread-plated onto Sea Water Complex agar medium composed of 5 g Bacto Peptone, 1 g yeast extract, 3 mL glycerol, 15 g agar, 750 mL seawater, and 250 mL distilled water (Widanarni et al 2003). Plates were incubated at 30°C for 24-48 hours, then colonies in the range of 30-300 CFU per plate were counted using a colony counter and expressed as CFU per gram of gut sample (Reonanda et al 2023).

***Growth performance and data analysis***. Growth performance was evaluated using the following parameters: weight gain (WG), daily growth rate (DGR), SGR, feed conversion ratio (FCR), TFC, FE, protein retention (PR), protein efficiency ratio (PER), molting frequency (MF), and survival rate (SR). Growth performance data and digestive enzyme activity were analyzed by one-way analysis of variance (ANOVA). Significant differences among treatments were determined using Duncan's Multiple Range Test (DMRT) in SPSS (Version 16.0). Statistical significance was set at $p < 0.05$.

## Results

***Growth performance and feed utilization***. The addition of varying levels of betaine attractant combined with probiotics in the diet produced a significantly different ($p < 0.05$) effect in the WG, DGR, SGR, TFC, FE, protein retention (PR), PER, molting activity, and survival of *P. ornatus*, as shown in Table 2. *P. ornatus* given the 3% BHCl and Probiotic diet showed the best performance, achieving WG, DGR, and SGR values of 35.35±4.78 g, 0.71±0.10 g $day^{-1}$, and 0.39±0.09%, respectively.

Feed utilization variables included TFC, FCR, FE, PR, and PER. *P. ornatus* given the 3% BHCl and Probiotic diet treatment, had the most favorable outcomes for these parameters, namely TFC of 402.17±14.12 g, FCR of 11.47±2.53, FE of 10.04±2.37%, PR of 10.43±0.11%, and PER of 19.91±0.74%. Furthermore, the samples showed the highest SR (100 ± 0.00%) and the second-highest MF (0.89±0.19 times $individual^{-1}$). In

contrast, the control group without BHCL and probiotic supplementation consistently demonstrated the poorest growth response, feed utilization, molting rate, and survival.

Table 2

Parameters of growth performance, feed efficiency, and molting frequency of *Panulirus ornatus* during the feeding trial

| *Parameter* | *Treatments* | | | |
|---|---|---|---|---|
| | *0% BHCl, NPr* | *1% BHCl, NPr* | *3% BHCl, NPr* | *5% BHCl, NPr* |
| WG (g) | 10.13±2.37$^{a}$ | 16.067±3.78$^{b}$ | 35.35±4.78$^{c}$ | 16.47±0.41$^{b}$ |
| DGR (g day$^{-1}$) | 0.20±0.05$^{a}$ | 0.32±0.08$^{a}$ | 0.71±0.10$^{b}$ | 0.33±0.01$^{a}$ |
| SGR (%) | 0.14±0.07$^{a}$ | 0.20±0.07$^{b}$ | 0.39±0.09$^{c}$ | 0.22±0.08$^{b}$ |
| TFC (g) | 239.36±44.88$^{a}$ | 332.96±4.08$^{b}$ | 402.17±14.12$^{c}$ | 321.68±17.05$^{b}$ |
| FCR | 21.21±5.87$^{c}$ | 22.75±7.87$^{ab}$ | 11.47±2.53$^{a}$ | 19.50±0.35$^{bc}$ |
| FE (%) | 3.97±1.87$^{a}$ | 5.58±1.1.94$^{a}$ | 10.04±2.37$^{b}$ | 5.42±0.51$^{a}$ |
| PR (%) | 3.32±4.21$^{a}$ | 5.49±2.22$^{ab}$ | 10.43±0.11$^{b}$ | 4.88±0.67$^{ab}$ |
| PER (%) | 12.26±3.40$^{a}$ | 14.63±0.00$^{a}$ | 19.91±0.74$^{b}$ | 12.69±0.87$^{a}$ |
| MF (Time ind$^{-1}$) | 0.33±0.00$^{a}$ | 1.11±0.38$^{c}$ | 0.89±0.19$^{bc}$ | 0.44±0.19$^{ab}$ |
| SR (%) | 55.56±19.25$^{a*}$ | 77.78±19.25$^{ab}$ | 100±0.00$^{b}$ | 77.78±19.25$^{ab}$ |

Note: Means in the same row with different superscript letters are significantly different ($p < 0.05$) based on One-Way ANOVA and Duncan's Multiple Range Test (DMRT); NPr: Non-Probiotic, Pr: Probiotic, WG: Weight gain (g), DGR: Daily growth rate (g day$^{-1}$), SGR: Specific growth rate (%), TFC: Total feed consumption (g), FCR: Feed consumption ratio, FE: Feed efficiency (%), PR: Protein retention (%), PER: Protein efficiency ratio (%), MF: Molting frequency (Time ind$^{-1}$), and SR: Survival rate (%).

***Digestive enzyme activities in the intestine***. Intestinal digestive enzyme profiles of *P. ornatus* are summarized in Table 3. Statistical analysis showed that different inclusion levels of BHCL combined with probiotic supplementation significantly influenced enzyme activity in the intestine ($p < 0.05$). In general, amylase activity at the beginning of the trial was lower than at the end, except for the group given 1% BHCL plus probiotic. By the end of the rearing period, the highest amylase level was detected in *P. ornatus* fed the diet containing 3% BHCl and probiotic (6.09±0.29 IU mL$^{-1}$), followed in descending order by the 5% BHCl plus probiotic treatment (5.78±0.35 IU mL$^{-1}$), the control diet without BHCl or probiotic (5.26±0.33 IU mL$^{-1}$), and the 1% BHCL plus probiotic group (4.14±0.19 IU mL$^{-1}$).

A similar trend was observed for protease activity. Protease activity was consistently higher at the end of the experiment compared to the initial measurement, with the exception of the 1% BHCL + probiotic treatment. The 3% BHCL + probiotic group recorded the greatest protease activity (0.35±0.02 IU mL$^{-1}$), followed by the 5% BHCL + probiotic treatment (0.31±0.02 IU mL$^{-1}$), the control group (0.29±0.01 IU mL$^{-1}$), and the 1% BHCL + probiotic group (0.23±0.01 IU mL$^{-1}$).

Lipase activity increased from the initial to the final sampling point in most treatments. The highest activity at the end of the trial was recorded in *P. ornatus* given 3% BHCL with probiotic supplementation (0.22±0.02 IU mL$^{-1}$), followed closely by the 5% BHCL + probiotic treatment (0.22±0.04 IU mL$^{-1}$). The control group showed a value of 0.20±0.01 IU mL$^{-1}$, while the lowest activity was observed in the 1% BHCL + probiotic group (0.17±0.02 IU mL$^{-1}$).

Table 3

Enzyme activities in the intestine of *Panulirus ornatus* at the initial and the end of the experiment

| *Enzyme activity* | *Initial* | *0% BHCl, NPr* | *1% BHCl, NPr* | *3% BHCl, NPr* | *5% BHCl, NPr* |
|---|---|---|---|---|---|
| Amylase (IU $mL^{-1}$) | 5.24±0.12 | 5.26±0.33$^{b}$ | 4.14±0.19$^{a}$ | 6.09±0.29$^{d}$ | 5.78±0.35$^{c}$ |
| Protease (IU $mL^{-1}$ ) | 0.29±0.02 | 0.29±0,01$^{a}$ | 0.23±0.01$^{a}$ | 0.35±0.02$^{d}$ | 0.31±0.02$^{c}$ |
| Lipase (IU $mL^{-1}$) | 0.18±0.01 | 0.20±0.01$^{a}$ | 0.17±0.02$^{b}$ | 0.22±0.02$^{b}$ | 0.22±0.04$^{b}$ |

Note: Means in the same row with different superscript letters are significantly different ($p < 0.05$) based on One-Way ANOVA and Duncan's Multiple Range Test (DMRT); NPr: Non-Probiotic, Pr: Probiotic.

***Digestive enzyme activities in the hepatopancreas***. The hepatopancreatic activities of amylase, protease, and lipase in *P. ornatus* are shown in Table 4. Statistical evaluation indicated that dietary supplementation with varying levels of BHCl and probiotics significantly influenced digestive enzyme activity in this tissue ($p < 0.05$). Overall, *P. ornatus* given diets containing BHCl showed greater enzyme activity compared to those offered diets lacking this attractant ingredient.

Amylase activity at the beginning of the trial was generally higher than at the end across treatments. By the end of the experiment, the greatest amylase activity was recorded in samples fed 3% BHCl + probiotic (4.92±0.30 IU $mL^{-1}$), followed sequentially by the 5% BHCl + probiotic group (4.66±0.23 IU $mL^{-1}$), the 1% BHCl + probiotic treatment (4.03±0.32 IU $mL^{-1}$), and the control (3.64±0.41 IU $mL^{-1}$).

Protease activity at the initial sampling point exceeded values measured at the end of the feeding period. At the final sampling, the highest protease level was observed in the 3% BHCl + probiotic group (0.27±0.02 IU $mL^{-1}$), with slightly lower activities recorded in the 5% BHCl + probiotic (0.26±0.02 IU $mL^{-1}$), 1% BHCl + probiotic (0.23±0.02 IU $mL^{-1}$), and the control group (0.20±0.02 IU $mL^{-1}$).

Lipase activity demonstrated a comparable pattern, with higher initial values compared to final activity measurements. At the end of the experiment, *P. ornatus* given 3% BHCl with probiotic showed the highest lipase activity (0.17±0.02 IU $mL^{-1}$), closely followed by those fed 5% BHCl + probiotic (0.17±0.01 IU $mL^{-1}$). The 1% BHCl + probiotic treatment and the control recorded lipase activities of 0.15±0.02 IU $mL^{-1}$ and 0.15±0.02 IU $mL^{-1}$, respectively.

Table 4

Enzyme activities in the hepatopancreas of *Panulirus ornatus* at the initial and the end of the experiment

| *Enzyme activity* | *Initial* | *0% BHCL, NPr* | *1% BHCl, Pr* | *3% BHCl, Pr* | *5% BHCl, Pr* |
|---|---|---|---|---|---|
| Amylase (IU $mL^{-1}$) | 5.16±0.46 | 3.64±0.41$^{a}$ | 4.03±0.32$^{b}$ | 4.92±0.30$^{d}$ | 4.66±0.23$^{c}$ |
| Protease (IU $mL^{-1}$) | 0.29±0.03 | 0.20±0.02$^{a}$ | 0.23±0.02$^{b}$ | 0.27±0.02$^{d}$ | 0.26±0.02$^{c}$ |
| Lipase (IU $mL^{-1}$) | 0.19±0.02 | 0.15±0.02$^{a}$ | 0.15±0.02$^{a}$ | 0.17±0.02$^{b}$ | 0.17±0.01$^{b}$ |

Note: Means in the same row with different superscript letters are significantly different ($p < 0.05$) based on One-Way ANOVA and Duncan's Multiple Range Test (DMRT); NPr: non-probiotic, Pr: probiotic.

***Total bacteria in the midgut***. Total bacterial count (TBC) in the gut of *P. ornatus* varied among dietary treatments (Table 5). Lobster fed the control diet without BHCl and probiotic showed the lowest microbial abundance, reaching $8.3\times10^{9}$ CFU $mL^{-1}$. Supplementation with BHCl in combination with probiotics led to a progressive increase in total bacterial population. The group given 1% BHCl + probiotic demonstrated a count of $1.44\times10^{10}$ CFU $mL^{-1}$, while higher inclusion levels of 3% and 5% BHCl + probiotic further

elevated bacterial populations to $1.65\times10^{10}$ and $1.82\times10^{10}$ CFU $mL^{-1}$, respectively. Description analysis confirmed that treatments supplemented with BHCl and probiotics had significantly greater gut bacterial counts compared to the control, indicating that combined supplementation effectively enhanced the microbial community in the digestive tract.

Table 5

Total bacteria in the gut of *Panulirus ornatus* fed with different dosages of BHCl and probiotic

| *Treatments* | *Total bacteria (CFU $mL^{-1}$)* |
|---|---|
| 0%BHCl, NPr | $8.3 \times 10^5$ |
| 1%BHCl, NPr | $1.44 \times 10^{10}$ |
| 3%BHCl, NPr | $1.65 \times 10^{10}$ |
| 5%BHCl, NPr | $1.82 \times 10^{10}$ |

**Discussion**. This study shows that dietary supplementation of BHCl combined with probiotic on optimum dosage significantly enhanced TFC of *P. ornatus*. Increasing the consumption of feed containing probiotics directly caused improvements in feed utilization, digestive enzyme activity, and total bacterial abundance in the intestine.

*P. ornatus* given 3% BHCl combined with probiotics in the diet showed significantly higher WG, SGR, and survival compared to the control group. These improvements were accompanied by higher TFC and superior FE indices, including PR and PER. The positive growth response is consistent with a previous study showing that betaine enhances feed palatability, stimulates feeding behavior, and improves nutrient utilization in aquatic animals (Jiang et al 2015; Muhamad et al 2021). The enhanced feed intake observed supports the established role of betaine as a highly water-soluble chemoattractant that rapidly diffuses and activates chemosensory receptors in crustaceans, thereby stimulating feeding consumption (Harpaz & Steiner 1990; Heinen 2009; Teoh et al 2023).

The superior FCR and nutrient retention in the 3% BHCl treatment are consistent with previous results in shrimp, where dietary betaine supplementation improved digestive use of protein and energy. The improved protein utilization observed probably reflects the protein-sparing effects of betaine, which has been associated with enhanced lipid and carbohydrate metabolism and reduced catabolism of dietary protein for energy (Saoud & Davis 2005).

Probiotic application further strengthened growth performance and survival in *P. ornatus*, possibly by improving gut microbial balance, stimulating digestive physiology, and supporting immune competence, as commonly reported in crustaceans (Hai 2015; Goh et al 2023; Yang et al 2024). Activities of amylase, protease, and lipase increased significantly in samples given BHCl and probiotic, particularly at 3% level. These results indicate enhanced digestive capacity and are consistent with previous observations that digestive enzyme activity increases with age and with improved diet quality in crustaceans (Farnés et al 2007; Rodríguez-Viera et al 2021). The elevated protease and amylase activities observed may partially explain the higher FE and PR. Increased digestive enzyme activity in response to probiotically-enriched diets has been widely reported in shrimp and lobster species (Gómez et al 2008; Hoseinifar et al 2018; Yang et al 2024).

Diet composition is a known regulator of enzyme synthesis in crustaceans, where elevated carbohydrate intake typically up-regulates amylase expression while high protein content increases protease secretion (Silva et al 2020; Nazir et al 2023). These results suggest that BHCl not only stimulates feeding but may also amplify digestive responses to dietary substrates.

TBC increased progressively with BHCl and probiotic inclusion, indicating enhanced microbial colonization in the gut. Higher bacterial loads in well-fed crustaceans typically reflect greater substrate availability and optimized digestive environments. The increase in beneficial microbiota may also contribute to improved nutrient assimilation, immune stimulation, and epithelial protection, as previously suggested for crustaceans

supplemented with probiotic or attractants (Duan et al 2018; Ringø et al 2020; Mirbakhsh et al 2023).

In addition to improving feed attractiveness, this study reinforces the crucial role of probiotics in enhancing digestive physiology and nutrient assimilation in *P. ornatus*. The substantial improvement in WG, FE, and digestive enzyme activities observed in probiotic-supplemented groups suggests that beneficial bacteria promoted a healthier intestinal microenvironment, stimulated digestive enzyme secretion, and facilitated nutrient hydrolysis and absorption. These mechanisms are consistent with the widely accepted modes of probiotic action in crustaceans, including competitive exclusion of pathogens, modulation of gut microbiota composition, and production of extracellular enzymes such as proteases, amylases, and lipases (Hoseinifar et al 2018; Amenyogbe et al 2024). Similar enhancements in growth performance and digestive capacity have been reported in *Litopenaeus vannamei* following dietary supplementation with *Bacillus* spp. and *Lactobacillus plantarum*, which resulted in higher amylase, protease, and lipase activities, improved FCR, and increased SGR (Zheng et al 2018; El-Raghi et al 2024). Comparable effects have also been documented in *Panulirus homarus*, where probiotic inclusion enhanced digestive enzyme profiles and conferred resistance against *Vibrio* infection (Faturrahman et al 2025). Collectively, these results indicate that probiotics act synergistically with attractants such as betaine to support efficient feed utilization, digestive competence, and intestinal health in spiny lobster. Therefore, the integration of targeted chemoattractants and functional microbial additives represents a promising feeding strategy to accelerate the domestication and sustainable culture of high-value spiny lobster species.

**Conclusions**. In conclusion, this study demonstrates that dietary supplementation with BHCl in combination with probiotics is associated with improvements in growth performance, feed utilization, digestive enzyme activities, and gut bacteria abundance in *P. ornatus* under the experimental conditions applied. Among the tested treatments, the diet containing 3% BHCl combined with probiotics consistently resulted in higher feed intake, WG, SGR, FE, and enhanced activities of amylase, protease, and lipase in both the intestine and hepatopancreas compared to the control diet. Further studies employing factorial experimental designs, larger sample sizes, and molecular analyses of microbial communities are needed to clarify the individual contributions of BHCl and probiotics and to confirm the broader applicability of these results in aquaculture.

**Conflict of interest**. The authors declare that there is no conflict of interest.

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