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Effect of Betaine Hydrochloride as an Attractant on Feed Consumption, Growth Performance, and Digestive Enzyme Activity of Ornate Spiny Lobsters (*Panulirus ornatus*)

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ABSTRACT

The addition of attractant is highly essential to increase feed consumption and growth of ornate spiny lobsters. Therefore, this study aimed to evaluate the effects of different doses of betaine hydrochloride (BeHCl) as an attractant on feed consumption, growth, and enzyme activity of ornate spiny lobsters. Four types of feed containing 0, 1, 3, and 5% BeHCl were prepared and designed as test Feed A, B, C, and D, respectively. A total of 36 juvenile ornate spiny lobsters were distributed into 12 plastic tanks (three juveniles/tank) with a size of 61 × 43 × 38cm^3 and were reared in a recirculating system for a 50-day period. Lobsters were fed twice daily at 09.00 am and 05.00 pm at a dose of 3% of their biomass weight. The results showed that lobsters fed diets containing BeHCl exhibited higher feed consumption levels than those fed diets without attractant supplementation. Incorporating 3% BeHCl in feed resulted in the highest feed consumption, feed efficiency, and growth performance. Statistical analysis indicated that the activity of digestive enzymes, including amylase, protease, and lipase, in ornate spiny lobsters did not differ significantly among the different BeHCl doses. However, a tendency was observed associated with an increased digestive enzyme activity with higher BeHCl doses. In conclusion, supplementing diet with 3% BeHCl in feed can enhance feed consumption, growth, and digestive enzyme activity of ornate spiny lobsters.

INTRODUCTION

Marine lobsters (*Panulirus* spp.) are a fishery commodity that has high economic value in both domestic and international markets. Demand for marine lobsters increases yearly to reach 15% worldwide because of international market needs (**Achmad *et al.*,**

2021). **Thesiana *et al*. (2020)** reported a continual increase in demand and lobster prices in world trade due to supply scarcity in the wild.

Lobsters are generally exported from wild catches, showing that massive exploitation tends to threaten the availability and population. Therefore, maintaining continuity and meeting the global lobster needs can be achieved through cultivation **(Jones *et al*., 2019; Maskun *et al*., 2020; Lubis *et al*., 2023)**. The factor determining the success of lobster cultivation is feed management because 60-70% of production costs are allocated to fish feed management **(Cahyo *et al*., 2022; Okta *et al*., 2023; Giri *et al*., 2023; Budiyati *et al*., 2024)**. Lobster farmers still provide fresh seafood such as trash fish, molluscs, and crustaceans in the maintenance and rearing of ornate spiny lobsters **(Okta *et al*., 2023)**. However, providing fresh trash fish does not support the sustainability of lobster aquaculture due to the lack of nutritional content, waste residue polluting the cultivation environment, the presence of disease-carrying agents, and competition with humans **(Giri *et al*., 2023; Okta *et al*., 2023; Rivaie *et al*., 2023; Astuti *et al*., 2024)**. This shows a need to replace fresh seafood with artificial feed **(**pellets**)** to ensure aquaculture sustainability. Providing formulated feed has been confirmed to increase growth of tropical lobsters **(Kurnia *et al*., 2018; Nankervis & Jones, 2022; Kurnia *et al*., 2024)**. For aquaculture sustainability, the reared lobsters should be provided with pelleted feed **(Gora *et al*., 2018; Sudewi *et al*., 2021; Okta *et al*., 2023)**. Previous studies showed that lobsters mostly preferred to consume fresh seafood than artificial feed because of the attractant content capable of stimulating higher consumption **(Astuti *et al*., 2022; Rivaie *et al*., 2023)**.

Attractant is an addictive substance added to feed to increase consumption, which is used to positively increase growth and eating habits among farmed fish **(Syanya *et al*., 2023)**. Generally, attractant ingredients for fish feed are obtained from natural sources such as fish paste, shrimp paste, squid meat paste, squid oil, and clam paste. The chemical sources include trimethyl amine oxide **(**TMO**)**, dimethyl sulphone **(**DMS**)**, trimethyl amine, krill meals, fish and krill hydrolysates, squid meal, betaine, amino acids, AMP, and other animal-based meals **(Carr *et al*., 1996; Derby *et al*., 2016; Fang *et al*., 2019; Mohamad *et al*., 2021)**.

The use of attractant in feed industry is currently crucial due to providing appropriate signals for fish to recognize pellets as a food source **(Ndobe *et al*., 2022)**. This ingredient is implemented in lobster aquaculture as feed ingredients to increase consumption. An example of feed ingredients is the use of squid meal addition in feed of red claw, *Cherax quadricarinatus* **(Sacristan *et al*., 2014)**, and fish and shrimp paste as attractant for ornate spiny lobsters **(***Panulirus homarus***) (Astuti *et al*., 2023)**. Supplementation of attractant was applied to increase feed consumption of white shrimp, *Litopenaeus vannamei* **(He *et al*., 2022)**, and the black tiger shrimp, *Penaues monodon* **(Qu *et al*., 2024)**. Except crustaceans, attractant has been used in feed of other species including hybrid tilapia fish **(El-Dakar *et al*., 2008)**, and butter catfish, *Ompok*

bimaculatus **(Biswas *et al*., 2018)**. Additionally, there are five compound attractant in high plant-based feed to enhance intake and growth performance of juvenile turbot, *Scophthalmus maximus* L. **(Jiang *et al*., 2019)**, three attractant types in feed of largemouth bass fish **(Yue *et al*., 2022)**, as well as supplementation of shrimp head protein hydrolysate for the Asian red tail catfish, *Hemibagrus nemurus* **(Hadi *et al*., 2024)**. Other forms of supplementation include providing squid meal to improve feed intake of freshwater eel, *Anguilla marmorata* **(Ndobe, *et al*., 2021)**, and using three attractant types to increase consumption in the Chinese perch, *Siniperca chuatsi* **(Peng *et al*., 2022)**.

The addition of attractants to fish feed increases consumption and directly enhance digestive enzyme activity and growth **(Wang *et al*., 2019; Klahan *et al*., 2023)**. A previous study stated that the use of attractant could increase fish growth and feed efficiency **(**FE**) (Hansc, 2020)**. Meanwhile, factors influencing performance of digestive enzyme activity in the fish intestine include age, pH, seasonality, physiological state, temperature, and feed composition **(Garcia-Meilan, 2023)**. The ability to obtain nutritional compounds absorbed in the intestine largely depends on performance capacity of digestive enzymes of the fish and shrimp **(Fang *et al*., 2019; Muttharassi *et al*., 2021)**. Therefore, knowledge of digestive enzyme activity is crucial for ensuring the digestibility and absorption of feed intake as well as determining suitable feed to improve growth **(Candiotto *et al*., 2018; Navarro-Guillén *et al.*, 2022)**.

Previous investigations observed the relationship between attractant supplementation, growth performance, and digestive enzyme activity of some crustaceans and fish species. Furthermore, the addition of 3% fish paste as attractant did not initiate significant difference in growth and consumption level of spiny lobsters compared to counterparts offered standard feed without attractant **(Astuti *et al*., 2020)**. A study evaluating crystalline amino acids found that L-glutamic acid monosodium salt monohydrate, betaine, and glycine could be used as potent stimulatory chemoattractants to improve feed intake in the slipper lobster, *Thenus orientalis* **(Teoh *et al*., 2023)**. Similarly, **Hajirezaee *et al*. (2022)** reported that the efficacy of 2% betaine as a feed attractant that increased feed intake, growth, and enzyme activity of trypsin, chymotrypsin, and lipase in the common carp. Related results showed that the supplementation of three amino acids including 0.4% alanine, 0.5% arginine, and 0.7% glycine improved feed intake and growth performance of yellow river carp **(Li *et al*., 2022)**. The use of 1% citric acid in feed could increase weight gain (WG) and specific growth rate (SGR) of grand sturgeon, *Huso huso* fry **(Sudagar *et al*., 2010)**. Supplementation with 0.05% nucleotides was the most effective as a feed attractant to enhance activity of digestive and absorption enzymes, improve the use and deposition of nutrients, facilitate the digestion and absorption of ingested nutrients, as well as increasing the growth performance of the juvenile Chinese mitten crab, *Eriocheir sinensis* **(Li *et al*., 2025)**.

The chemoattractant added to lobster test feed in this study is betaine hydrochloride (BeHCl), a water-soluble compound with a significant effect on stimulating the olfactory bulb glands of fish. This compound is obtained in large quantities from the extraction of marine invertebrates, micro-organisms, and several plants to be used as an effective attractant for various species of aquatic animals **(Meyers, 1987)**. Additionally, the main physiological and metabolic function of BeHCl is related to osmoregulation, and the contribution of methyl to fish can protect cells from dramatic changes caused by osmotic pressure. BeHCl prevents enzyme inhibition and performs an important function in tissue formation, serving as a methyl donor for protein and energy metabolism. Several investigations related to the use of betaine in fish feed were conducted on Indian major carp (*Labeo Rohita*) **(Shankars *et al*., 2008)**, common carp (*Cyprinus carpio*) **(Murthy *et al*., 2016)**, and the Nile tilapia (*Oreochromis niloticus*) **(Ismail *et al*., 2020)**. Information regarding the use of BeHCL as attractant in feed to increase intake in the ornate spiny lobsters is currently unavailable. Therefore, this study aimed to evaluate the effect of BeHCl supplementation as an attractant on feed consumption, growth performance, and digestive enzyme activity of the ornate spiny lobsters, *Panulirus ornatus*.

MATERIALS AND METHODS

The feeding trial was conducted at the Laboratory of Fish Culture, Breeding, and Fish Production, Faculty of Fisheries and Marine Science Halu Oleo University Kendari Southeast Sulawesi, Indonesia for three months from September to November 2024. The steps performed in this experiment included the preparation of test lobsters and experimental feed, feeding trial, and measurement of parameters, as well as data analysis.

Experimental lobsters and the acclimatization period

Ornate spiny lobsters used as experimental animals were purchased from local fishermen in Southeast Sulawesi Indonesia, and all wild caught was acclimatized for a week. The juveniles were obtained from traders collecting lobsters in the Kendari City which were caught in the sea waters of Southeast Sulawesi. Subsequently, the spiny lobsters were brought to the seawater cultivation laboratory Faculty of Fisheries and Marine Science, Halu Oleo University and temporarily stored in a 1-ton fiber tank for one week to reduce stress. These were acclimatized in two rectangular fiber plastic tanks (190cm L × 103cm W × 100cm H, surface area: 10m^2, 50cm seawater depth, 1-ton water volume) with well-aerated water. During the acclimatizing period, all lobsters were fed with fresh trash fish at 20% of the total biomass daily. After 1 hour, uneaten food and waste particles were removed daily through syphonation. Water temperature was maintained at 28±1°C and salinity was at 34ppt during the experiment.

Experimental feed

Four experimental feeds were formulated to contain 0, 1.0, 3.0, and 5.0% BeHCl with isonitrogenous (40%) and isoenergetic (4.000 kcal/kg) properties. Basal feed ingredients consisting of Telescopium muscle meal, gold snail meal, mud scallops meal, shrimp head meal, and soybean meal were used as protein sources, while corn meal, fine bran meal, and sago meal served as carbohydrate sources. Corn and fish oil were used as lipid sources for the experimental feed, which also included vitamin and mineral mixtures. The method implemented for experimental feed preparation was carried out according to the following steps. Firstly, all the ingredients except minerals, vitamins, and lipid sources were ground and filtered through a 60-mesh sieve. Secondly, all ingredients were weighed in accordance with feed formulation and mixed starting from the smallest to the largest percentage. The entire ingredients were mixed uniformly, then the combination of fish and corn oil as well as water were added and stirred to achieve a homogeneous state. Thirdly, the mixture was molded using a pelletizer machine with diameter sizes of 1.0 and 1.5mm and dried under the sun for two to three days. All the dried experimental feed was put into a plastic bag and stored at room temperature in a ready-to-use form. Experimental feed formulation and the results of proximate composition analysis are presented in Table (1).

Table 1. Formulation of experimental feed and proximate analysis results

Feed ingredients	Experimental feed (g/100 g feed)			
	0%BHC	1%BHC	3%BHC	5%BHC
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
BeHCl	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	15.98
Crude ash	15.72	14.33	15.33	15.33
Crude fiber	6.95	6.06	5.51	6.45
NFE **	16.60	15.50	12.02	8.44
GE (kal/g) ***	3990.15	3949.23	3860.22	4010.12

*Vitamin A 12.000.000 IU, Vitamin D3 2.000.000 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 the procedure by **Jiang *et al*. (2015); NFE = 100 – **(**protein + fat + ash**)**; and
***GE: gross energy

Feeding trial

Post acclimatization, the test lobsters were weighed and categorized into Group I (90-120g), Group II (121-150g), and Group III (151-180g) based on body weight. The test lobsters were reared in plastic tanks sized 61cm ×43cm ×38cm, previously filled with 70L of filtered seawater. In addition to being provided with aeration stones, all maintenance containers were equipped with three shelters prepared from PVC pipes (Length: 20cm) as hiding areas for the test lobsters. A total of 36 juveniles were placed into twelve plastic boxes (3 lobsters per plastic tank) and were reared in a recirculation water system for 50 days. The test lobsters were fed twice daily (09.00 am and 05.00 pm) with a dose of 3% biomass weight. The seawater was changed once a week to replace approximately 30% of the total volume of the container water. Siphoning was carried out every morning to remove feces while feed remained in the containers. The unconsumed feed was dried in the sun, then weighed to be used as a reduction from the total amount of feed given in calculating consumption level. Lobster weighing was carried out three times on days 0, 25, and 50 to measure growth performance. Three lobsters were collected from each treatment for proximate analysis and assessment of digestive enzyme activity. Water parameters consisting of temperature (thermometer), salinity (refractometer), pH (pH meter PH-009(1)), and dissolved oxygen (PDO- 519, Japan) were measured periodically during the experiment.

Proximate analysis of experimental feeds and body carcass

Proximate analysis of test feed included moisture, crude protein, crude lipid, crude ash, and fiber examination. This was carried out by analyzing water content using the heating method in an oven at 110℃ (SNI 01-2891-1992 point 5.1 method). Furthermore, protein levels were tested using the Titrimetry method (18-8-31/MU/SMM-SIG method). Total fat content was measured using the extraction method with a Soxhlet apparatus (method 18-8-5/MU/SMM/SIG point 3.2.1). Ash content was determined by drying the material in a heating oven at 600℃ (SNI method 01-2891-1992 point 6.1). Crude fiber was evaluated using the sample dissolution method with strong acids and bases (18-11-111/MU/SMM-SIG gravimetry method).

Samples preparation for enzyme assay

Enzyme assay included checking for protease, lipase, and amylase in digestive tract of spiny lobsters and the hepatopancreatic organ at the start (day 0) and the completion of feeding trial (day 50). Some steps were conducted during the sample preparation for enzyme assay. Firstly, the hepatopancreas and intestine were collected through dissection of the organs after anesthetizing lobsters by positioning on ice for

10min. Secondly, the intestine and hepatopancreas were weighed to measure the intesntine somatic index and hepatosomatic index. Finally, the samples were put into a 1.5mL microtube, which has previously been filled with 10% formalin solution, and the samples tube were placed in freezer before being sent to the Laboratory of Animal Husbandry, IPB University.

The measurement of digestive enzyme activities (Amylase, Lipase and Protease)

The measurement of amylase activity were performed by using the method of **Worthington (1993)**. This method determines applying some steps. 0.5mL of substrate solution was added to 0.5mL supernatant solution (sample), then incubated for three min at 95^{o}C (water bath). Approximately 0.5mL dinitrosalicylic acid (DNSA) was added to the sample solution and incubated at 95^{o}C (water bath) for 5min. The absorbance was measured using a spectrophotometer at a wavelength of 540nm.

Lipase activity was measured according to the procedure described by **Borlongan (1990)**. A total of 1ml of lipase enzyme substrate in 1.5ml of buffer containing 0.1 M Tris-HCl at pH 8.0 were mixture with 1ml of enzyme crude extract and the mixture were incubated for 6 hours at 37^{o}C, and before ending the incubation process, the mixture was added with 3ml of 95% ethyl alcohol. The mixture was titrated with 0.01 N NaOH using 0.9% thymolphthalein in ethanol as an indicator.

Protease activity was determined according to the method of **Bergmeyer and Grassi (1983)**. Firstly, some tubes were prepared for samples, standards, and blanks. Subsequently, all test tubes were filled with 1ml of 0.05 M phosphate buffer pH 7, and 1 mL of casein substrate solution of 20mg/ mL pH 7 was added. A total of 0.2mL of sample was put into the test tubes containing the sample. The standard tubes were filled with 0.2mL of 5mmol/ L Tyrosine standard solution. The blank tubes were filled with 0.2ml of distilled water and incubated at 37^{o}C for 10 minutes. Approximately 2ml of 0.1 M TCA solution was added to all tubes, 0.2mL of 2mmol/ L CaCl2 solution was filled into the blank and standard tubes, while 0.2mL of aquadest was added to the sample tubes only. All mixtures in the tubes were allowed to stand at 37^{o}C for 10 minutes and were centrifuged for 10 minutes at 3.500rpm. The filtrate measuring 1.5mL was collected from each tube, then 1ml of Folin Ciaocalteau's solution and 5mL of 0.4 M Na_2CO_3 were added to all the tubes and the absorbance of mixture was determined by using a spectrophotometer in wavelength of 578nm.

Growth performance and data analysis

Growth performance was observed by evaluating initial weight, final weight, Weight gain (WG = Wt – Wo), Specific growth rate (SGR = Ln Wt – Ln Wo/t × 100), Feed conversion ratio (FCR) = Feed Intake/ Wt-Wo, Total feed consumption (TFC) = dry diet given – dry diet remained) , Feed efficiency (FE = final body weight – initial body weight/ feed intake) , protein retention (PR= Amount of fish body protein at the end -

Amount of fish body protein at the beginning/ Amount of protein consumed during maintenance), net protein utilization (NPU= WG/ Total protein intake), Protein efficiency ratio (PER= WG/Dry weight of protein), and molting frequency. All data of growth performance (WG, SGR, FCR, TFC, FE, PR, NPU, PER) and molting frequency were determined. All data of growth performance (WG, SGR, FCR, TFC, FE, PR, PHR, NPU, PER) and digestive enzyme activity were analyzed through one-way analysis of variance (ANOVA). The significant difference between the treatments was determined by Duncan's multiple range test (DMRT) using SPSS (Version 20.0), and the significance level used was 0.05.

RESULTS

Experimental feed

The proximate analysis results of experimental feed (% dry matter basis) are presented in Table (1). The crude protein and fat levels of the experimental feed ranged between 39.75 – 40.89% and 12.17 – 15.98%, respectively. Meanwhile, the crude ash and fiber ranged between 14.33 – 15.72% and 5.51 – 6.95%, respectively. The NFE and GE were 8.44 – 16.60% and 3860.72 – 4010.12 cal./g, respectively. All the parameters of water quality, including temperature, pH, dissolved oxygen, and salinity had optimum values. During feeding trial, the water temperature and salinity ranged between 25.0 – 27.0ºC and 32.0 – 34.0g/ Kg, while dissolved oxygen content and pH were 6.0 – 8.0mg.L^{-1} and 7.2 – 7.6, respectively.

Growth performance and feed utilization

Supplementation of different doses of betaine attractant in feed significantly (P<0.05) affected growth performance, feed utilization, molting frequency, and survival rate of ornate spiny lobsters, as presented in Table (2). Among growth performance parameters, the highest of WG, DGR, and SGR were observed in lobsters given feed containing 3% BeHCL with values of 35.35±4.78g, 0.71±0.10g.day^{-1}, and 0.39±0.09%, respectively. Similarly, supplementation of 3% BeHCl in feed produced the highest feed utilization parameters, including TFC, FCR, FE, PR, and PER of the ornate spiny lobsters with values of 402.17±14.12g, 11.47±2.53, 10.04±2.37%, 10.43±0.11%, and 19.91±0.74%, respectively. The inclusion of 3% BeHCl generated the highest SR and the second highest level of molting frequency with values of 100 ± 0.00% and 0.89±0.19 time/ind, respectively. However, the lowest growth performance, feed utilization, molting frequency, and SR were found in lobsters fed without BeHCl supplementation.

Table 2. Parameters of growth performance, feed utilization, and molting frequency of ornate spiny lobsters during feeding trial

Parameter	Treatments			
	A	B	C	D
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]
Molting Freq. (Time/ind.)	0.33±0.00[a]	1.11±0.38[c]	0.89±0.19[bc]	0.44±0.19[ab]
Survival rate (%)	55.56±19.25[a]	77.78±19.25[ab]	100±0.00[b]	77.78±19.25[ab]

Digestive enzyme activity in intestine

The results of digestive enzyme activity of amylase, protease, and lipase in the intestine of ornate spiny lobsters are shown in Table (3). Statistically, feeding with various BeHCl doses generated significantly different digestive enzyme activity. The amylase activity in the intestine of the ornate spiny lobsters at the onset was lower than after the experiment, except for treatment B. At the completion of the experiment, the highest amylase activity was observed in lobsters given Feed C (6.09±0.29 IU/mL) and followed by the group offered Feed D (5.78±0.35 IU/mL), A (5.26±0.33), and B (4.14±0.19 IU/mL), respectively. Similarly, protease activity in the intestine during the initial experiment was lower than at the completion, except in treatment B. At the completion of the experiment, the protease activity of lobsters given Feed C (0.35±0.02 IU/mL) was higher when compared to the group offered Feed D (0.31±0.02 IU/mL), A (0.29±0,01 IU.mL), and B (.0.23±0.01 IU/mL), respectively. The lipase activity during the initial experiment was generally lower than at the completion. The highest lipase activity at the completion of the experiment was found in lobsters given Feed C

(0.22±0.02 IU/mL) and D (0.22±0.04 IU/mL), followed by those offered Feed A (0.20±0.01IU/mL) and B (0.17±0.02 IU/mL), respectively.

Table 3. Enzyme activity in the intestine of ornate spiny lobsters at the onset and the completion of the experiment

Enzyme activity	Initial	Experiment completion			
		A	B	C	D
Amylase (IU/mL)	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)	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)	0.18±0.01	0.20±0.01^{a}	0.17±0.02^{b}	0.22±0.02^{b}	0.22±0.04 ^{b}

Digestive enzyme activity in hepatopacreas

The results of digestive enzyme activity in the hepatopancreas of the ornate spiny lobsters are presented in Table (4). Statistical analysis showed that feeding with various BeHCl doses generated significantly different digestive enzyme activity in the hepatopancreas. Lobsters given feed containing BeHCl (attractant) commonly had higher digestive enzyme activity than the counterparts fed without BeHCl. Moreover, activity of amylase in the initial period of the experiment was higher. At the completion of the experiment, the highest amylase activity was observed in lobsters given Feed C (4.92±0.30 IU/mL), followed by the group offered Feed D (4.66±0.23 IU/mL), B (4.03±0.32 IU/mL), and A (3,64±0.41 IU/mL), respectively. Meanwhile, activity of the protease enzyme in the hepatopancreas was higher than in the initial experiment. At the completion, the protease activity of lobsters given Feed C (0.27±0.02 IU/mL) was higher than in the groups offered Feed D (0.26±0.02 IU/mL), B (0.23±0,02 IU/mL), and A (.0.20±0.02 IU/mL), respectively. The lipase activity was higher during the initial experiment, but the highest activity at the completion was found in lobsters given Feed C (0.17±0.02 IU/mL) and D (0.17±0.01 IU/mL), followed by B (0.15±0.02 IU/mL) and A (0.15±0.02 IU/mL), respectively.

Table 4. Enzyme activity in the hepatopancreas of ornate spiny lobsters at the initial and the completion of the experiment

Enzyme activity	Initial	Experiment completion			
		A	B	C	D
Amylase (IU/mL)	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)	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)	0.19±0.02	0.15±0.02[a]	0.15±0.02[a]	0.17±0.02[b]	0.17±0.01[b]

DISCUSSION

The results showed that feed consumption level and growth of lobsters given feed containing BeHCl were higher than in counterparts fed without attractant. The highest consumption level was obtained in lobsters given feed containing 3% BeHCl (402.17 ± 14.12g) and the lowest was found in the group lacking attractant supplementation (239.36 ± 44.88g). BeHCl can help increase feed consumption level in different fishes by stimulating the olfactory bulb due to being a highly water soluble and diffusible compound commonly present in marine invertebrates, micro-organisms, and some plants at high quantities **(Polat & Beklvek, 1999; Ajiboyeo *et al*., 2012)**. **Jiang *et al.* (2019)** stated that betaine supplementation could enhance growth performance and FE of juvenile Turbot because of adequate nutrition and higher absorption. Similarly, **Muhammad *et al.* (2021)** reported that betaine supplementation of 0.5% could increase feed utilization efficiency and growth performance of hybrid grouper. The addition of BeHCl to feed can increase consumption in the vannamei shrimp **(Saoud & Davis, 2005)**, the goldfish **(Murthy *et al*., 2016; Othman *et al*., 2023)**, the Nile tilapia **(Ismail *et al*., 2020)**, the Salmo truta juvenile **(Mohseni, *et al*., 2020)**, the largemouth bass **(Yue *et al*., 2022)**, and abalone **(Ma *et al*., 2024)**. Among feed treatments B, C, and D containing attractant, the best dose that produced the highest growth and consumption levels was the group of lobsters supplemented with 3% BeHCl. This result is different from the study by **Dong *et al.* (2018)** showing that a dose of 0.4% betaine was the best for growth and FE of gourami fish. Meanwhile, the optimum dose of betaine to support

growth of the blunt snout bream is 1.2% or 0.8% **(Djoumani *et al*., 2019)**. **Hadjirezaee *et al*. (2024)** reported that the highest growth of the goldfish was obtained when provided feed containing 1-2% betaine.

The addition of betaine as an attractant in feed increases growth and feed consumption levels as well as FE, PR, PER, molting frequency, and survival rate, but reduces the FCR of ornate spiny lobsters. Previous studies showed that betaine addition to feed can increase the FE of white shrimp, and the Nile tilapia **(Ismail *et al*., 2020)**, hybrid grouper fish **(Idul *et al*., 2021)**, and common carp **(Othman *et al*., 2023; Hadjirezaee *et al*., 2024)**. High values of FE, PR, and PER, as well as low FCR, were found in the group of lobsters given feed containing attractant because betaine functions to increase protein digestibility **(Eklund *et al*., 2005)**. The fish PER value is used as an indicator of protein utilization and growth **(Saade *et al*., 2021)**. The addition of betaine to feed can increase growth, as well as protein and body fat retention in animals **(Abdelsattar *et al*., 2019)**. The high PER and PR values in the group of lobsters given feed containing 1, 3, and 5% BeHCl show that only a small amount of protein is needed to increase fish body weight **(Marzuqi *et al*., 2012)**. This is because betaine can enhance fat and carbohydrate metabolism **(Figueroa-Soto & Valenzuela-Soto, 2018)** which has a protein-sparing effect to ensure using protein for growth only and not energy.

BeHCl inclusion in feed could improve the molting frequency and survival rate of ornate spiny lobsters. The highest molting frequency and survival were found in the groups given feed containing 3% BeHCl. These two parameters are closely related to the high value of consumption level and FE. Except for the optimum quality of the cultivation environment, other factors influencing the occurrence of molting are feed consumption level and eating habits **(Satrio *et al*., 2017)**. The high feed consumption level has a direct effect on the molting frequency. Meanwhile, the survival rate of lobsters offered feed containing BeHCl was higher than in counterparts lacking attractant supplementation because survival is closely related to both consumption level and the optimum quality of the cultivation environment **(Akbarurrasyid *et al*., 2023)**. Llobsters with a low level of feed consumption are weak and very susceptible to cannibalism, leading to high mortality.

Activity values of digestive enzymes including amylase, lipase, and protease in the hepatopancreas or intestine during the initial experiment were lower than at the completion. This is because enzyme activity of ornate spiny lobsters is influenced by age and digestive enzyme activity increases with increasing larval age **(Farnes *et al*., 2007; Rodriguez *et al*., 2021)**. During the juvenile stage, digestive system and enzymatic functions are still very simple and have not developed completely, potentially leading to limited food digestion ability **(Jiao *et al*., 2023; Susanto, 2023)**. According to **Qian *et al*. (2022)**, enzyme activity is influenced by life stage, feed, and feeding management. Changes in the composition level can alter digestive enzyme activity and nutrient

absorption capacity, initiating greater feed utilization and ensuring increased growth of gilthead sea bream **(Meilan *et al*., 2023)**.

Digestive enzymes in both the hepatopancreas and intestines of ornate spiny lobsters given feed containing BeHCl had higher activity than in counterparts lacking attractant supplementation. This is because BeHCl compound stimulates lobsters through chemoreceptor organs in the nose to detect feed thereby increasing total consumption in lobsters **(Vital *et al*., 2018)**. Activity of digestive enzymes is influenced by dietary nutrients consumed by fish **(Nurhayati *et al*., 2014)**. The high total feed consumption resulting from the supplementation of BeHCl in the feed directly effect enhances the absolute growth of ornate spiny lobsters. Growth performance is closely affected by activity of digestive enzymes which break down macromolecules into micromolecules to increase nutrient absorption during the metabolic process and FE in fish or shrimp **(Kumar *et al*., 2022; Quintino-Rivera *et al*., 2023)**.

In this study, the highest digestive enzyme activity of the ornate spiny lobsters was observed for amylase, followed by protease, while the lowest activity was recorded for lipase. This corresponds with the proximate analysis results showing that the carbohydrate content of the test feed was the highest (35.42-43.8%), followed by protein (33.76-38.52%), and fat was the lowest (1.11-2.96%). **Meilan *et al.* (2023)** reported that digestive enzyme activity was closely related to the value of the proximate analysis results. Some studies stated that protease, lipase, and amylase enzyme activity were significantly affected by the level of protein, lipid, and carbohydrate content in feed **(Silva *et al*., 2020; Gomez *et al*., 2023; Nazir *et al*., 2023)**. The function of amylase is related to the utilization of carbohydrates produced in the pancreas, stomach, and intestines. Hydrolysis of carbohydrates by amylase produces simple molecules in the form of mono/disaccharides, showing a tendency to increase enzyme activity **(Krogdahl *et al*., 2005; Rodriguez-Viera *et al*., 2021)**. Additionally, enzyme activity can be a comparative indicator of feed utilization and digestive capacity of fish **(Abbas *et al.*, 2021; Melianawati *et al*., 2023)**. Among the three types of digestive enzymes, lipase functions to break down fats into smaller molecules including glycerol and three fatty acids. Protease breaks down proteins into amino acids and peptides, while amylase degrades carbohydrates into simple sugars and glucose **(Rodriguez *et al*., 2017; Romero *et al*., 2021; Amenyogbe *et al*., 2024)**. Digestive enzyme activity values for the ornate spiny lobsters in this study are lower than the values reported previously **(Gora *et al*., 2018; Lubis *et al*., 2023)**.

CONCLUSION

In conclusion, the results showed that the total feed consumption, growth performance, and digestive enzyme activity of ornate spiny lobsters given feed containing BeHCl were higher than those of counterparts lacking attractant supplementation. Additionally, the supplementing with 3% BeHCl was found to be the optimum dose for

enhancing the total feed consumption, growth performance, and digestive enzyme activity.

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