



Effect of Black Soldier Fly and Bromelain Extracts supplementation on digestibility, growth performance in Giant Freshwater Prawn under a complete Recirculating Aquaculture System water system

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Abstract

Currently, farmers cultivating giant freshwater prawns (*Macrobrachium rosenbergii*) encounter challenges like low survival rates, slow growth, and high production costs. This research aims 1) to study the optimized conditions of bromelain to digest black soldier fly larvae - BFSL (*H. illucens*), which is included as a protein source in giant freshwater prawn feeds, 2) to study the suitable level of bromelain enzymes for growth enhancement of giant freshwater prawn, and 3) to study the pattern of using BFSL and bromelain for giant freshwater prawn production. The first experiment studied the digestibility of 2 enzymes: bromelain extracted from pineapples and protease extracted from the hepatopancreas. Results showed that bromelain digestion activities in BSFL were higher than those of protease at every pH at both 25°C and 30°C temperatures. In the second experiment, growth performance, feed efficiency, and cost-effectiveness were evaluated in four different treatments for 90 days. T1- control feeds without BFSL and bromelain, T2- control feeds supplemented with 250 ppt bromelain, T3- Feeds included 12% BFSL, and T4 - Feeds included 12% BFSL supplemented with 250 ppt bromelain. The growth performance in T3 and T4 outperformed other treatments. No disparities were detected in feeding efficiency and feed conversion ratio. Giant freshwater prawns fed with BSFL supplementation exhibited the highest observed values in the molecular structure of chitin content within their shells. The chitin structure, analyzed using FTIR and X-ray diffraction techniques, displayed features indicative of α -chitin, evidenced by a peak in the spectrum between 2,000 - 1,500 cm^{-1} . Consequently, this study suggests that supplementing BSFL and bromelain holds promise for reducing costs in aquaculture.

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1. Introduction

The primary challenges in giant freshwater prawn (*M. rosenbergii*) production include low survival rates, slow growth, and high cultivation costs attributed to expensive factors like feeds and seeds. These challenges are compounded by labour shortages and high Labour costs. The Department of Fisheries gave the giant freshwater prawn aquaculture production for 2020 at 22,257 metric tons worth 5,171 million Thai Baht. This is smaller by 1.0 per cent in quantity and 0.02 per cent in value than in 2019. Overcoming such challenges requires technological advancements and new techniques for culturing giant freshwater prawns, which are essential for lowering production costs, boosting profits, and promoting sustainable aquaculture.

Giant freshwater prawns are economically important in Thailand and in the world, and are quite popular (high demand) in tourist areas. The large size of these prawns adds an extra allure, positioning them as a key attraction for tourists and meeting the discerning preferences of consumers. The high cost of the giant freshwater prawn, plus the long time it takes to cultivate an adult, and the small number of prawns produced, make the prawn so valuable in the market. Its challenge is to keep meeting consumer expectations in size and quality. This challenge can yield profitable returns to the farmers, help to stabilize the prices of large freshwater prawn products, and make them more available to the consumers. Good nutrition – particularly protein and fat – encourages molting, which helps crustaceans make good strides toward growth and quality produce of meat.

The utilization of a high-protein diet results in increased protease activity because the enzyme production of aquatic animals changes to a certain extent according to the protein level in the feeds. If the prawn is unable to completely digest that protein. Remaining protein can lead to ammonia issues and nitrogenous waste in water. This may affect the health and growth of prawns and potentially cause mortality. Climate change is also creating scarcity and potential issues for open aquaculture systems. Northern Thailand has cool weather, especially during the winter, which affects feed consumption, growth rate and longer production period. Maintaining a closed recirculating water system, controlled environment aids in regulating water quality, mitigating issues related to excess ammonia levels and nitrogenous waste.

The black soldier fly (*Hermetia illucens*), found in both tropical and temperate climates, is a non-pathogenic insect and measures up to 20 millimetres in length [1,2]. Rich in protein (42-51%) and fat (35%), with an energy content of 2,900 kcal/kg, they efficiently remove 65.5-78.9% of total organic waste. Cultivating larvae on pineapple peels indicated that the larval stage, prepupa stage, and pupal stage were approximately 31.42 days, 17.66 days, and 6.3 days, respectively. Nutritional benefit 40.15% protein and 9.13% fiber. Fed with jackfruit and pineapple peels exhibits higher fat as 32.75% [3]. In the United States, the utilization of black soldier fly larvae (BSFL) is permitted specifically on salmonids exclusively. The dried whole larva, dried ground larva, and oil forms of black soldier fly are key products that are particularly beneficial when included in giant freshwater prawn diets. The diet advantage promotes the prawns growth and quickens the molting process. Thailand has applied black soldier fly larvae meal as part of the diverse types of aquafeeds, including the inclusion of 30% black soldier fly larvae meal in climbing perch (*Anabas testudineus* Bloch), which is recommended to reduce production costs [5]. The larval oil contains 35% fat and has a high saturated fatty acid (SFA) content, especially lauric acid as well as high levels of essential fatty acids such as linoleic acid and linolenic acid [6]. Black soldier fly has several properties that make it stand out: it has a well-balanced Ca:P ratio and a high level of a number of minerals. It is highly recommended for giant freshwater prawn and other crustaceans feed due to their characteristics. Furthermore, oil from the larva is biologically available for antioxidant activity, antimicrobial activity and boosting immune system of animals. Besides, black soldier fly larvae has been a positive influence in the production and growth of the Pacific White Shrimp (*Litopenaeus vannamei*) in the juvenile stage [7]. The larvae have been successfully used as an alternative to fish meal in the diets of salmon [8]. Moreover, larvae of black soldier fly can be used as a protein source in feed production of ornamental aquatic animals without any negative effects on aquatic animals as an inexpensive alternative in feed production [9].

Protein digestive enzyme (bromelain) can be efficiently extracted from waste (peel and core) of pineapples (*Ananas comosus*), which can be used in aquaculture foods. It can support the digestion of proteins in the digestive system and boost the efficiency of metabolism. Furthermore, bromelain is also anti-inflammatory and can help to keep shrimp healthy [10]. Recently, research was undertaken on the use of pineapple peels and other residues in diets of various kinds of aquatic animals, such as crude extract of pineapple residues in tilapia feeds that showed promising results [11] and similar uses with formulations of the feeds for white shrimp [12] and commercial bromelain powder in the feeds for white shrimp [13].

Bromelain supplementation resulted in positive effects on Juvenile Sterlet fish (*Acipenser ruthenus*) [14]. The beneficial effects of bromelain were also observed with grass carp (*Ctenopharyngodon idella*) [15] and cichlid fish (*Oreochromis mossambicus*) [16] where protein digestion was more efficient and growth enhanced. Notably, experiments have also been performed with white shrimp, and bromelain was found to be useful also in stimulating molting, thus coming up with a novel use in shrimp farming. To address this problem, integrating Black Soldier Fly (*H. illucens*) and bromelain enzyme extract into giant freshwater prawn feeds in a complete Recirculating Aquaculture System (RAS) water system offers a potential solution.

2. Materials and Methods

2.1. Experimental Giant Freshwater Prawns

Giant Freshwater Prawns (*M. rosenbergii*), initial weight 5 g/prawns, from a private farm, were randomly cultured in 0.25-m² plastic tanks at a density of 15 prawns per tank. No barriers were used to simulate a commercial farming

environment. They were acclimated for 1 week with a control diet (Regular feeds without BFSL and bromelain) before starting the experiment.

2.2. Raw Materials and Bromelain Extract Preparation

The black soldier fly larva from Siam Farm Services Co., Ltd. in Lampang, Thailand. The pineapple peels and cores were received from a local market in Lampang, Thailand. The pineapple peels and cores were blended, filtered through the filter cloth, and the crude enzyme was then collected and kept at 4 °C.

2.3. Experimental Diet

Giant Freshwater Prawns were fed with a pellet diet, 35% protein. The proximate composition of the diets was determined according to standard methods (AOAC, 2000) [17]. The dietary experimental produced into four treatments as T1- control feeds without BFSL and bromelain, T2- control feeds supplemented with 250 ppt bromelain, T3- Feeds included 12% BFSL, and T4 - Feeds included 12% BFSL supplemented with 250 ppt bromelain enzyme, referencing the application in white shrimp feed [18].

2.4. Experimental Design

2.4.1 Experiment 1

Examine the digestibility of both dry matter and chitin within the black soldier fly larva utilizing protease, amylase, lipase, and chitinase enzymes extracted from the hepatopancreas of giant freshwater prawns, following the Geminez method [19] and Chitinase Assay Kit, respectively. Additionally, employ bromelain enzyme extracted from pineapple using Ketnawa et al method [20]. The samples used in the study include black soldier fly larvae and giant freshwater prawn fed commercially. The following samples are carefully ground and screened through a 200-micron fine-mesh sieve. The material is then weighed and extracted for oil using solid-liquid extraction with organic solvent ether, and the Soxhlet extraction method [17] is used. The extracted samples are then treated with bromelain (from pineapple extract), protease, and chitinase (from hepatopancreas of giant freshwater prawns). The experimental conditions involve temperatures of 25 °C, 30 °C, and 40 °C, as well as a pH of 4–10, following [21]. The data obtained from these experiments are utilized to determine the efficacy of black soldier fly larvae and bromelain enzyme in the diet for giant freshwater prawns, with a specific focus on growth, health, feed utilization efficiency, and survival rates. The acquired data in each experimental group will be further analyzed for the differences in the data by analysis of variance (ANOVA) at a confidence level of 95% and compare the differences between the means of each set of experiments using Duncan New's Multiple Range Test.

2.4.2. Experiment 2

Releasing 15 post-larval freshwater prawns, with an initial weight of approximately 5 g. / prawn, from a private farm, were randomly cultured in 0.25-m² plastic tanks with a feeding regimen consisting of four treatments as T1- control feeds without BFSL and bromelain, T2- control feeds supplemented with spray coating 250 ppt bromelain, T3- Feeds included 12% BFSL and T4 - Feeds included 12% BFSL supplemented with spray coating 250 ppt bromelain enzyme. The experimental cultivation was conducted over a period of 90 days, feeding five times a day in a complete Recirculating Aquaculture System (RAS) water system.

2.5. Data collection

Conducting an experimental cultivation of freshwater prawns according to the experimental plan involves weighing and counting the number of individual freshwater prawns in each tank. Weighing is performed using a scale with measurements in grams every 30 days throughout the 90-day cultivation period. At the conclusion of the experiment, growth rates and the cost-effectiveness of freshwater prawns are calculated. Water quality is monitored by measuring pH, ammonia, and nitrite levels every 7 days. Additionally, the quality of freshwater prawn meat and feeds is analyzed using the proximate analysis method at the conclusion of the experiment.

$$1. \text{ Weight gain (WG, \%)} = \frac{\text{Final weight(g)} - \text{Initial weight(g)}}{\text{Initial weight(g)}} \times 100$$

$$2. \text{ Specific growth rate (SGR \% / day)} \\ = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Time (day)}} \times 100$$

$$3. \text{ Protein efficiency ratio (PER)} = \frac{\text{Body weight gain (g)}}{\text{Protein intake (g)}}$$

$$4. \text{ Apparent net protein retention (ANPR; percent)} \\ = \frac{[(W1 \times P1) - (W2 \times P2)]}{P} \times 100$$

W1 = Final average weight (grams)

W2 = Initial average weight (grams)

P = Protein intake

P1 = Final percentage of protein in the prawn

P2 = Initial percentage of protein in the prawn

5. Total Feeds Intake (TFI, grams/prawn) = $\frac{\text{Total number of feed intake}}{\text{Number of prawns}}$
6. Daily feed intake (DFI % / day) = $\frac{(\text{Average feeds intake per day} \times 100)}{[(\text{Initial weight} + \text{Final weight})/2]}$
7. Feed conversion ratio (FCR) = $\frac{\text{Feed consumed (g)}}{\text{Weight gain (g)}}$
8. Survival rate (SR, %) = $\frac{\text{Number of surviving prawns}}{\text{Total number of prawns}} \times 100$
9. Yield (grams) = Final weight in each cell x Total count (Total Biomass)

2.6. Statistical analysis

Collected and calculated growth information for statistical analysis by analyzing variance (ANOVA) to study the differences in each treatment. Compare the differences in treatment means using Duncan's New Multiple Range test at the 95% confidence level.

3. Results and Discussion

3.1. Experiment 1: Examined the digestibility efficiency of black soldier fly larva dry matter using protease, amylase, lipase and chitinase enzymes from hepatopancreas of giant freshwater prawns and bromelain enzyme from pineapple

Characterizing enzyme activities extracted from the giant freshwater prawn hepatopancreas (Table 1) revealed optimal pH and temperature conditions. Protease exhibited peak activity at pH 6 and 37°C, indicative of a mildly alkaline environment, as these enzymes are primarily serine proteinases produced in the hepatopancreas, although some metalloproteinases were also present. One of the serine proteinases has been characterized as a typical trypsin which is considered as one of the most important enzymes in decapods.[22] Amylase demonstrated optimal activity at neutral to mild pH, with the highest value at pH 5 and 30°C, while lipase most efficiently at pH 9 and 30°C in alkaline conditions. Enzyme activities (protease, amylase, and lipase) in size 0.5 g giant freshwater prawns were found to be 1.00 ± 0.12 , 0.75 ± 0.11 , and 0.22 ± 0.02 U/mg protein, respectively [23]. These values closely align with our findings using prawn of comparable sizes in this experiment. Chitinase activity were not statistically different at each temperature. ($P > 0.05$) Bromelain enzyme activity was compared during centrifugation before being used for activity analysis and without centrifugation. It was found that centrifugation clearly resulted in the enzyme working better (Table 2), and the optimum temperature for the bromelain enzyme to work is 25 °C.

Table 1 Hepatopancreas enzyme activities (U/min/mg protein) and chitinase (U/mg). of giant freshwater prawns at different pH and temperatures.

Protease enzyme	pH	Temperature (°C)		
		37	30	25
	4	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a	0.01 ± 0.00 ^a
	5	0.18 ± 0.01 ^a	0.08 ± 0.00 ^b	0.05 ± 0.00 ^c
	6	0.22 ± 0.01 ^a	0.15 ± 0.00 ^b	0.11 ± 0.00 ^c
	7	0.18 ± 0.00 ^a	0.06 ± 0.00 ^c	0.09 ± 0.00 ^b
	8	0.20 ± 0.00 ^a	0.04 ± 0.00 ^c	0.07 ± 0.00 ^b
	9	0.04 ± 0.00 ^a	0.00 ± 0.00 ^b	0.04 ± 0.00 ^a
	10	0.09 ± 0.00 ^a	0.03 ± 0.00 ^b	0.02 ± 0.00 ^b
Amylase enzyme	pH	Temperature (°C)		
		37	30	25
	4	0.38 ± 0.01 ^a	0.39 ± 0.00 ^a	0.07 ± 0.02 ^b
	5	0.39 ± 0.01 ^c	0.75 ± 0.04 ^a	0.48 ± 0.02 ^b
	6	0.43 ± 0.00 ^b	0.69 ± 0.01 ^a	0.34 ± 0.02 ^c
	7	0.38 ± 0.00 ^a	0.67 ± 0.00 ^a	0.46 ± 0.00 ^b
	8	0.21 ± 0.00 ^c	0.55 ± 0.02 ^a	0.48 ± 0.07 ^b
	9	0.17 ± 0.01 ^b	0.08 ± 0.01 ^c	0.23 ± 0.00 ^a
	10	0.18 ± 0.01 ^b	0.23 ± 0.06 ^a	0.06 ± 0.00 ^c
Lipase enzymes	pH	Temperature (°C)		
		37	30	25
	4	0.03 ± 0.00 ^a	0.01 ± 0.00 ^c	0.02 ± 0.00 ^b
	5	0.08 ± 0.02 ^a	0.06 ± 0.03 ^b	0.04 ± 0.00 ^c
	6	0.100 ± 0.00 ^a	0.08 ± 0.00 ^b	0.08 ± 0.00 ^b
	7	0.05 ± 0.02 ^b	0.10 ± 0.02 ^a	0.05 ± 0.00 ^b
	8	0.21 ± 0.02 ^b	0.24 ± 0.01 ^a	0.12 ± 0.01 ^c

	9	-0.02 ± 0.01 ^c	0.40 ± 0.00 ^a	0.09 ± 0.00 ^b
	10	0.11 ± 0.01 ^a	0.04 ± 0.00 ^b	0.05 ± 0.01 ^b
Chitinase enzymes	pH	Temperature (°C)		
		37	30	25
		0.21 ± 0.01	0.22 ± 0.01	0.23 ± 0.01

Remark: Mean values marked with different letters on the same line indicate a statistically significant difference ($P < 0.05$).

Table 2 Bromelain enzyme activity (U/min/mg protein) extracted from pineapple at different temperatures both centrifugal and not centrifuged

Temperature (°C)	Centrifugal	
	Centrifugal	Non centrifugal
25	4.14 ± 0.01 ^a	0.51 ± 0.01 ^a
30	4.11 ± 0.01 ^b	0.49 ± 0.01 ^a
37	4.11 ± 0.00 ^b	0.42 ± 0.00 ^b

Remark: Mean values marked with different letters on the same line indicate a statistically significant difference ($P < 0.05$).

Centrifugation purifies bromelain enzyme from the extract, enhancing its activity and substrate specificity. Bromelain from the fruit (peel and rhizome/stem) has a molecular weight range of 24.5-32.5 and 26-37 kDal, respectively [24]. This study investigated the *in vitro* protein digestibility efficiency (%) of black soldier fly larvae (BSFL) using bromelain enzyme extracted from pineapple and protease enzyme from the hepatopancreas of giant freshwater prawns (Table 3). The enzymes were evaluated at different pH levels and temperatures. Bromelain exhibited superior protein digestion compared to protease at 25°C and 30°C across most pH levels. However, at 37°C, digestibility fluctuated. Both enzymes achieved peak digestion efficiency at pH 4, indicating this as the optimal condition for protein extraction from BSFL. These findings suggest bromelain may be a more effective enzyme for BSFL protein extraction at moderate temperatures. Bromelain, a plant-derived enzyme, functions as a cysteine endopeptidase, specifically cutting peptide bonds within the peptide chain. [25] This enzyme type also contains glycosidases, phosphatases, ribonucleases, cellulases, peroxidases, and glycoproteases. Additionally, it has the capability to cut peptide bonds of alanyl, glycyl, and leucyl [26,27]. This may result in protein digestion efficiency of giant freshwater prawn appears promising, potentially surpassing the digestibility observed in white shrimp, size 0.17 g, found to be 85.1% [28]. When contrasted with an *in vitro* protein digestibility study on Trichoderma-fermented black soldier fly larva found 32.8 – 42.8% [29], Performing the *in vitro* digestibility of black soldier fly larvae meal for monogastric animals found 76% at 37 °C with pH of the stomach and intestine at 2 and 9 [30], a value comparable to giant freshwater prawn digestion at around pH 4 and 37 °C.

Table 3. Efficiency of protein digestibility (%) *in vitro* in black soldier fly larvae using the enzyme bromelain extracted from pineapple and protease enzyme from hepatopancreas of giant freshwater prawns.

Temperature (°C)	pH	Enzymes	
		Bromelain	Protease
25	4	88.28 ± 0.16 ^a	83.92 ± 1.79 ^b
	5	65.97 ± 0.78 ^a	34.78 ± 1.18 ^b
	6	46.31 ± 2.65 ^a	41.71 ± 0.94 ^b
	7	38.97 ± 0.78 ^a	35.75 ± 0.20 ^b
	8	40.92 ± 2.11 ^a	37.83 ± 0.92 ^a
	9	26.88 ± 2.42 ^b	34.54 ± 1.87 ^a
30	4	81.41 ± 0.11 ^a	72.09 ± 0.11 ^b
	5	53.72 ± 0.11 ^a	34.73 ± 0.99 ^b
	6	14.12 ± 2.25 ^a	3.86 ± 0.22 ^b
	7	13.07 ± 2.75 ^a	8.90 ± 0.80 ^b

	8	15.16 ± 1.10 ^a	-2.40 ± 0.11 ^b
	9	15.16 ± 1.10 ^a	-8.99 ± 0.55 ^b
37	4	94.00 ± 1.20 ^a	87.99 ± 0.51 ^b
	5	48.93 ± 0.83 ^a	48.52 ± 0.37 ^a
	6	28.33 ± 0.09 ^b	47.38 ± 0.09 ^a
	7	53.31 ± 0.24 ^a	45.63 ± 1.47 ^b
	8	28.76 ± 0.34 ^b	43.94 ± 0.64 ^a
	9	20.94 ± 0.12 ^b	25.48 ± 0.47 ^a

Remark: Mean values marked with different letters on the same line indicate a statistically significant difference ($P < 0.05$).

Apart from investigating the protein digestibility efficiency in black soldier fly larvae, there has been a comprehensive exploration of the nutritional composition of black soldier fly larvae compared to the meat of giant freshwater prawns (Table 4). BSFL exhibited a rich protein and fat content, while giant freshwater prawns' meat primarily contains protein, with the highest value.

Table 4 Chemical compositions of black soldier fly larvae, giant freshwater prawn meat and initial chitin in black soldier fly larva.

Chemical compositions (%)	Black soldier fly larva	Freshwater prawn meat
Moisture	7.50 ± 0.05 ^b	8.59 ± 0.07 ^a
Ash	11.62 ± 0.13 ^b	6.04 ± 0.09 ^b
Crude lipid	34.56 ± 0.09 ^a	3.85 ± 0.17 ^b
Crude protein	37.25 ± 0.43 ^b	82.74 ± 0.47 ^a
Crude fiber	7.15 ± 0.52 ^a	0.73 ± 0.00 ^b
Chitin	57.66 ± 2.25	

Remark: Mean values marked with different letters on the same line indicate a statistically significant difference ($P < 0.05$).

Black soldier fly larvae (BSFL) exhibit a variable nutritional composition depending on their developmental stage and the type of feed provided. Protein content ranges from 37% to 63% (dry weight), lipid levels range from 7% to 39%, and ash levels range from 9% to 28% [31]. As another reported showed protein levels between 29.9% - 44.6%, lipid levels between 9.5% - 49%, ash levels between 6.7% - 15.4%, and moisture levels ranging from 8.4% - 11%, also contingent upon the type of feed used during their cultivation [32]. The pupal stage of BSFL exhibits a dry weight nutrient profile of 32% protein, 37% fat, 19% minerals, and 9% chitin [33]. However, studies have shown higher chitin content (18%) in the pupae [34]. Furthermore, chitin levels progressively increase throughout development, with reported values of 33.09%, 35.14%, 68.44%, and 87.92% for larvae, pupae, adults, and emerging adults, respectively [35].

The giant freshwater prawn dietary experimental produced into four treatments (including control): T1- control feeds without BFSL and bromelain, T2- control feeds supplemented with 250 ppt bromelain, T3- Feeds included 12% BFSL, and T4 - Feeds included 12% BFSL supplemented with 250 ppt bromelain (Table 5). Following this, *in vitro* protein digestibility (%) in all four treatments was evaluated using bromelain and protease enzymes across different pH and temperature conditions (Table 6). Protease and bromelain exhibited temperature-dependent protein-digestion activity. At 37 °C, both enzymes showed similar activity across all pH conditions and feed formulations. However, at 25 °C and 30 °C, protease displayed greater digestion efficiency for treatments T3 and T4 compared to bromelain. Conversely, T2, control feeds supplemented with spray coating 250 ppt bromelain, showed effective digestion with bromelain.

Table 5: Giant freshwater prawn dietary supplemented with black soldier fly larvae (BSFL) and bromelain enzyme extract.

Ingredients	Diets (kg)
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4	67.46 ± 1.76 ^b	84.17 ± 1.85 ^a	67.77 ± 0.55 ^b	85.31 ± 0.17 ^a	62.74 ± 2.63 ^b	79.89 ± 4.34 ^a	68.42 ± 0.90 ^b	75.40 ± 1.97 ^a
5	58.12 ± 1.05 ^a	61.06 ± 3.87 ^a	62.40 ± 1.03 ^b	67.81 ± 0.00 ^a	58.88 ± 3.42 ^a	62.30 ± 0.62 ^a	44.71 ± 1.89 ^b	51.86 ± 0.24 ^a
6	40.80 ± 0.36 ^a	43.30 ± 0.79 ^a	47.95 ± 2.05 ^a	32.43 ± 0.62 ^b	27.72 ± 2.46 ^a	51.69 ± 3.31 ^a	55.11 ± 1.64 ^a	46.58 ± 2.13 ^b
7	18.43 ± 1.49 ^b	35.45 ± 2.02 ^a	57.83 ± 0.07 ^a	28.69 ± 0.11 ^b	32.93 ± 3.47 ^b	43.38 ± 3.54 ^a	36.14 ± 2.11 ^b	42.39 ± 1.80 ^a
8	21.15 ± 1.53 ^a	21.37 ± 3.25 ^a	44.99 ± 3.23 ^a	17.65 ± 0.50 ^b	28.60 ± 4.05 ^b	46.22 ± 1.26 ^a	38.01 ± 0.14 ^a	24.51 ± 1.17 ^b
9	25.85 ± 0.70 ^a	24.92 ± 3.69 ^a	40.52 ± 2.60 ^a	14.35 ± 0.63 ^b	16.30 ± 0.48 ^b	52.20 ± 3.71 ^a	40.11 ± 0.66 ^a	37.86 ± 2.46 ^a
37°C								
4	67.46 ± 1.76 ^b	84.17 ± 1.85 ^a	62.74 ± 2.63 ^b	85.31 ± 0.17 ^a	67.77 ± 0.55 ^b	79.89 ± 4.34 ^a	68.42 ± 0.90 ^b	75.40 ± 1.97 ^a
5	58.12 ± 1.05 ^a	61.06 ± 3.87 ^a	58.88 ± 3.42 ^b	67.81 ± 0.09 ^a	62.41 ± 1.03 ^a	62.30 ± 0.62 ^a	55.11 ± 1.64 ^a	51.86 ± 0.24 ^a
6	40.80 ± 0.32 ^a	43.30 ± 0.79 ^a	27.72 ± 2.46 ^a	32.43 ± 0.62 ^a	47.95 ± 2.05 ^a	51.69 ± 3.31 ^a	44.71 ± 1.89 ^a	46.58 ± 2.13 ^a
7	18.43 ± 1.49 ^b	35.45 ± 2.02 ^a	32.93 ± 3.47 ^a	28.69 ± 0.11 ^a	57.83 ± 0.07 ^a	43.38 ± 3.54 ^b	36.14 ± 2.11 ^a	42.39 ± 1.80 ^a
8	21.15 ± 1.53 ^a	21.37 ± 3.27 ^a	28.60 ± 4.05 ^a	17.65 ± 0.50 ^b	44.99 ± 3.23 ^a	46.22 ± 1.26 ^a	38.01 ± 0.14 ^a	24.51 ± 1.17 ^b
9	25.85 ± 0.70 ^a	24.92 ± 3.69 ^a	16.30 ± 0.48 ^a	14.35 ± 0.63 ^a	40.52 ± 2.60 ^b	52.20 ± 3.71 ^a	40.11 ± 0.66 ^a	37.86 ± 2.47 ^a

Remark: The average values marked with different letters on the same line for each formula in each pH and temperature indicate a statistically significant difference ($P < 0.05$)

Experiment 2: Growth, feed efficiency, and cost-effectiveness in giant freshwater prawn production using black soldier fly larvae and bromelain supplementation

A 90-day experiment raising giant freshwater prawns with four treatments, notable growth increases and varied survival rates ($P < 0.05$) were observed across all experimental groups (Table 7). In evaluating the growth of giant freshwater prawns on the experimental diet, those fed with a combination of BSFL (T3) and BFSL supplemented with bromelain (T4) exhibited superior growth compared to other groups ($P < 0.05$). This improvement was manifested in increased weight gain, percentage weight gain, average daily growth, and specific growth rate. In terms of production, control feeds supplemented with bromelain (T2) and those fed with BSFL (T3) showed higher production than other experimental groups ($P < 0.05$). However, Survival rates did not differ significantly ($P > 0.05$), but each experimental group experienced relatively low survival rates due to cannibalism during the molting period.

The growth of giant freshwater prawns or crustaceans occurs through the molting process and the availability of accumulated nutrients. Shrimp molting is triggered by both internal and external factors. External factors include food, size, temperature, minerals, salinity, and environment. Internal factors include weight and hormones [36]. Shrimp receiving adequate nutrients, including chitin and minerals, increase molting frequency, resulting in enhanced growth [37]. Giant freshwater prawns fed supplemented BSFL with or without bromelain had exhibited the highest growth and chitin levels in shells. Giant freshwater prawns fed with incorporated feeds mixed with BSFL had the highest blood osmolality, indicating higher protein, fat, and chitin intake than fishmeal. BFSL are rich in nutrients, especially fat (34.56%) and chitin (57.66%), which is higher than fishmeal. Chitin promotes better and more frequent molting, leading to improved growth in terms of weight gain, specific growth rate, and daily growth rate.

The growth performance of the group utilizing BSFL (T3) and BFSL supplemented with bromelain (T4), higher than both the control group (T1) and the group receiving only bromelain enzyme supplementation (T2). This enhanced growth is attributed to bromelain, which specifically cleaves peptide bonds within the peptide chain. Therefore, the supplementation of the bromelain enzyme enhances protein digestion, particularly in black soldier fly larvae, and also improves the overall efficiency of protein digestion from raw materials in giant freshwater prawn diets. In vitro protein digestibility studies revealed that the protein digestibility efficiency of enzymes extracted from hepatopancreas of giant freshwater prawns was comparable to that of bromelain enzymes in T4, which was previously supplemented with bromelain enzymes, at both 25°C and 37°C. However, in terms of yield, the group with the highest yield was the group fed with BSFL (T3), which was attributed to its correlation with the survival rate and exhibited a commendable survival rate comparable to that of the group receiving feeds supplemented with bromelain.

The key finding here is that giant freshwater prawns fed a diet containing BFSL showed superior growth performance, with increased weight gain compared to other groups. This is probably because there is a greater amount of chitin present in their body which is involved in molting and hardening the shell. This may, in turn, help to decrease post-molting cannibalism among prawns. The other group, comprising BSFL + bromelain (T4), had low survival rates. This effect may be due to an increase in cannibalism among the prawns, after they have molted, which may have been caused by increased protein utilization afforded by bromelain [36]. Using the BSFL diet, which is protein and fat-rich, seems to be more effective in molting under optimal conditions, while bromelain supplementation can help to shorten the molting process with efficient nutrient accumulation for molting. But, with some supplement of minerals, insufficient quantity, quality or water conditions can negatively affect proper shell development. This, in turn, can result in post-molting cannibalism because of the retarded shell growth.

It will negatively affect the survival rates and reduce yields from their group fed only BSFL (T3). This is in line with the efficient feed utilization found with feed intake and Feed Conversion Ratio (FCR) consistently. Studies have been done

on employing the BSFL and BFSL by-products in different aquafeeds. The same effect as this experiment was obtained, when these ingredients were replaced for fishmeal and added to fresh feeds, including Frass as a by-product from the BFSL industry to the feeds used for white shrimp, which increased growth rate of white shrimp [39]; also, using Frass as a raw material in white shrimp feed formulations [40], and replacing white shrimp feed in the farming with the same feed but with substituted raw materials (7).

Table 7 Growth performance of giant freshwater prawns fed diet supplemented with black soldier fly larva and bromelain extract for 90 days

	Treatments			
	T1	T2	T3	T4
Initial weight (g/prawn)	5.73 ± 0.33	5.38 ± 0.54	5.67 ± 0.19	5.49 ± 0.34
Final weight (g/prawn)	18.98 ± 2.26 ^b	18.41 ± 1.72 ^b	26.60 ± 1.12 ^a	25.29 ± 1.47 ^a
Weight Gain (g/prawn)	13.35 ± 1.86 ^b	13.03 ± 1.18 ^b	20.92 ± 0.93 ^a	19.80 ± 1.13 ^a
Percentage of Weight gain (%)	228.47 ± 5.11 ^b	242.29 ± 2.24 ^b	368.57 ± 4.05 ^a	360.66 ± 1.75 ^a
ADG (g/prawn/day)	0.16 ± 0.02 ^b	0.16 ± 0.01 ^b	0.25 ± 0.01 ^a	0.24 ± 0.01 ^a
SGR (%/day)	1.70 ± 0.42 ^b	1.46 ± 0.01 ^c	1.84 ± 0.01 ^a	1.81 ± 0.01 ^a
Survival Rate (%)	50.00 ± 14.14	60.00 ± 0.00	60.00 ± 0.00	35.00 ± 7.07
Yield (grams/ replication)	103.58 ± 0.95 ^b	127.65 ± 14.00 ^{ab}	145.88 ± 12.62 ^a	103.50 ± 2.55 ^b

Remark: Averages marked with different letters on the same line. Shows that there is a statistically significant difference ($P < 0.05$)

The feed efficiency assessment (Table 8) revealed that the intake of feeds, average daily intake (ADI), and feed conversion ratio (FCR) were not statistically different among the experimental groups ($P > 0.05$). Based on the statistical analysis, the nutrient levels, such as energy and amino acids, are comparable in various recipes, showing the consistency of the nutritional values. Even if the fish feed composition differed in fish meal and BFSL as the main protein sources, no difference could be detected in digestion/absorption. BFSL contains higher levels of amino acids such as arginine, methionine, isoleucine, phenylalanine, prolyl, tricline, and valine compared to fish meal [41]. However, fish meal exhibits elevated concentrations of other essential amino acids not as abundant in BSFL.

Despite these differences, the proportional levels of these amino acids may not significantly impact food intake and protein digestion. Moreover, since BSFL belong to the Arthropoda Phylum, the same as shrimp, the feed appeals more to palatability compared to fish meal. Consequently, shrimp exhibited similar feed consumption across all experimental groups, with no statistically significant differences observed. When assessing the Feed Conversion Ratio (FCR), no statistically significant differences were observed in the values across each experimental group ($P > 0.05$). However, there was a noticeable trend in both the BFSL group and the bromelain enzyme-supplemented group. During the larval stage, amino acids such as glutamic acid and phenylalanine (with cystine) exhibited higher concentrations in the range of 13.4–16.6% and 9.7–11.5%, respectively. Tryptophan, methionine, and histidine values were found in the range of 1.4–1.6%, 1.4–2.7%, and 2.9–4.2%, respectively.

Amino acids such as arginine, leucine, phenylalanine (plus cystine), threonine, tryptophan, and valine showed consistent levels during the larval growth stage [42]. The improved feed conversion efficiency observed in diets containing BFSL compared to those solely reliant on fish meal can likely be attributed to the optimal balance of amino acids found within BSFL. The amino acid composition of the test diet is close to the requirements of juvenile cauque river prawn (*Macrobrachium americanum*), with protein level of 37.2%, and is rich in essential amino acids such as valine, isoleucine, leucine, lysine and arginine. Notably, these amino acid values are comparable to that of BSFL [43]. Hence, the use of BSFL in feed formulations to replace fishmeal is a potential strategy to achieve an optimum amino acid balance that supports growth. Shrimp group fed BSFL showed higher growth rates than the control group. Feed efficiency did not differ but it is important to note that the feed efficiency of various fish species can be influenced by deficiencies or imbalances of the dietary amino acids, as reported by [44].

Moreover, studies have revealed that for the fishmeal replacement level of 50% and 70%, FCR of white shrimp was significantly higher than the FCR of fishmeal fed shrimp [7]. The substitution of fish meal with BSFL in yellow catfish [45], observed similar trends. Fish fed the BSFL diets instead of fish meal had similar or even better Feed Conversions Ratio (FCR) as the control fish. This result is in agreement with our current study, where 12% BSFL was included in the feed formulations of the GHP instead of the absence of fish meal, which led to better FCR than the control. From these results, it can be concluded that adding BSFL at this level is adequate in providing amino acids in the diet for optimal growth.

The use of BSFL and feeding the fish this with protease enzymes supplemented in the feed formulas for giant freshwater prawns was found to produce more proteases, which means there was a better digestion of protein. This indicates that the protein contained in BSFL might be more difficult to digest than the protein contained in fish meal or other similar

proteins, prompting the organism to produce more enzymes to process the protein. Interestingly, even when the protein-digesting enzyme bromelain was added to their diet, the giant freshwater prawns still increased their production of enzymes for protein digestion. The observed results correspond to experimental results and show that protein digestibility is efficient for BSFL when using bromelain enzyme alongside protease enzyme. Protease enzyme was observed to perform optimally at pH 6 and temperature 31°C, and shown to have a superior capability in the digestion of protein as compared to bromelain under the same conditions.

The study shows that, in the giant freshwater prawn feed formula containing BSFL, the enzyme from the giant freshwater prawn is the primary source of digestion, while a certain amount of digestion is provided by bromelain. The organism thus improves the quantity of protease enzymes to effectively break down the proteins of BSFL. The different protein digestibility and protease level in the prawns could be because the ratio of all amino acids present in the fish meal and BSFL is different. The enzyme bromelain, in particular exoprotease, was added, which is potentially a contributor to better protein digestibility of the BSFL and fish meal and other protein sources. The purpose of this supplementation is to improve the digestibility of nutrients and thereby enhance the quality and quantity of nutrients. These nutrients can further activate the secretion of the inner enzyme in the hepatopancreas of shrimp, in the process of digestion [46].

Therefore, the protease enzyme activity in the hepatopancreas was more pronounced in the giant freshwater prawn group, which was supplemented with bromelain and BSFL, than in the other experimental groups. This resulted in increased digestion efficiency of protein [47]. Protein digestion efficiency of the feed in this group was better than that of the other experimental groups, indicating that feeding BSFL was not only nutritionally complete but was also more beneficial than feeding a regular feed formula. Even with the enzyme bromelain in there, it may not be enough to jumpstart greater enzyme release. Likewise, a BSFL sole feeding system could not lead to the optimal efficiency of digestion of proteins. Hence, the use of BSFL along with bromelain enzyme supplementation is considered a viable solution as it will provide adequate nutritional supplementation for the growth of giant freshwater prawns, along with efficient digestion.

Table 8 Feeds efficiency performance of giant freshwater prawns fed diet supplemented with black soldier fly larva and bromelain extract for 90 days

Feeds Efficiency	Treatments			
	T1	T2	T3	T4
TFI (g/prawn)	30.14 ± 0.84	31.84 ± 5.67	36.08 ± 2.66	34.25 ± 2.33
DFI (% / day)	29.80 ± 3.48	35.29 ± 3.14	27.59 ± 2.78	26.10 ± 0.85
Feed intake (g/prawn/day)	0.67 ± 0.08	0.51 ± 0.01	0.81 ± 0.07	0.81 ± 0.08
Feed conversion ratio (FCR)	4.26 ± 0.15	3.31 ± 0.38	3.26 ± 0.08	3.41 ± 0.14
Feeds cost (THB)	10.15 ± 0.32 ^b	11.83 ± 0.08 ^b	10.96 ± 0.95 ^a	10.83 ± 1.49 ^a

Remark: Averages marked with different letters on the same line. Shows that there is a statistically significant difference ($P < 0.05$)

The chemical composition of giant freshwater prawn meat and chitin values from prawn shells across different experimental groups (Table 9). Statistically significant differences were observed in each group. Giant freshwater prawn meat in the group supplemented with bromelain enzyme at 250 ppt exhibited higher levels compared to the groups without bromelain ($P < 0.05$), including increased fat content. The percentage of chitin in giant freshwater prawn shells was notably high in all experimental groups, except for the control group, which had the lowest value ($P < 0.05$). Ash and protein percentages in shrimp meat did not show significant differences among the experimental groups ($P > 0.05$), with protein values ranging from 84.01% to 87.07% and ash values ranging from 5.84% to 6.40%, respectively.

Our study found that prawns fed a diet containing BSFL had the lowest moisture and fat content in the chemical composition of prawn meat. Interestingly, the group receiving bromelain enzyme supplements exhibited the best growth performance. This decrease in tissue water content may be associated with an increase in the concentration of free amino acids, as noted in other saltwater crustaceans [48-50], which investigated the replacement of fish meal by BSFL in white shrimp. They reported that higher protein and fat content in the whole-body protein and lipid content could be achieved when BSFL inclusion was restricted to < 29% and 15%, respectively. However, Fat and protein values did not differ significantly across experimental groups. Similarly, BSFL in turbot diets, our findings indicate that using BSFL has no significant impact on the ash and protein content in whole shrimp in each experimental group [51-52]. Furthermore, employing Frass, a by-product of BSFL production, at a 30% level in the white shrimp diet, revealed no significant impact on ash and protein content in in whole shrimp varied across experimental groups, indicating that the use of BSFL or its by-products does not affect ash accumulation and protein content in prawn meat [38].

Chitin was analyzed in the shells of prawns, and it was found that the prawns fed diets containing BSFL and those of prawns supplemented with both BSFL and bromelain had significantly higher chitin levels in their shells. This is consistent with the better growth and molting results seen in both of these groups in comparison to the other groups, probably because the BSFL is structurally very rich in chitin (57.66%) as found in their body. Dietary chitin from BSFL seems to have a direct effect on the chitin content of prawns.

Chitin is essential for crustaceans, such as giant freshwater prawns, in their growth process when they shed their exoskeletons. Increasing the chitin content can stimulate molting. With a proper diet, this may aid in the faster molting of prawns, resulting in strong growth. Although prawns and BSFL share the same phylum, Arthropoda, this study shows

that they can be effective in chitin accumulation, with the enhanced molting and growth rate of BSFL. During the investigation, it was focused on the chitin contents in the prawn shells, for diets T3 and T4 only. The observed growth difference in the BSFL-fed group is thought to be due to the molting, which supplies a source of nutrients, minerals, and chitin. The group that was not fed BSFL, however, had lower chitin content in its shell and slower-growth rates. Studies have been conducted on marine shrimp, and adequate chitinase enzyme activity has been found to digest the chitin content of feeds that contain BSFL, which results in efficient chitin utilization. mRNA Chi isoenzymes 1, 2, and 3 and LvChi1 and LvChi3 are present in the hepatopancreas cDNA of white shrimp, indicating their role in the digestion of chitin originating from crustacean feed sources [54]. In shrimp shell chitin, the main chain is composed of N-acetylglucosamine units linked together by N-acetyl groups, which is a similar structure to cellulose. Bromelain is a member of the cellulase enzyme complex that can help to further enhance chitin digestion and improve utilization efficiency. The results of this experiment proved that chitin obtained from BSFL can be effectively used by shrimp even when supplemented with bromelain. Importantly, shrimp raised in a diet containing high chitin had higher growth performance than the control shrimp.

Table 9 Chemical composition of giant freshwater prawn meat and chitin from prawn shells across different experimental groups for 90 days.

Chemical composition (%)	Treatments			
	T1	T2	T3	T4
Moisture	80.55 ± 0.10 ^b	81.45 ± 0.09 ^a	78.73 ± 0.09 ^d	80.24 ± 0.01 ^c
Ash	6.40 ± 0.21	5.85 ± 0.07	5.84 ± 0.46	6.08 ± 0.06
Crude lipid	1.55 ± 0.41 ^b	2.23 ± 0.09 ^a	1.11 ± 0.40 ^b	1.52 ± 0.04 ^b
Crude protein	87.07 ± 1.48	84.34 ± 0.81	85.10 ± 0.62	84.01 ± 1.63
Chitin	22.50 ± 0.70 ^b	25.50 ± 1.41 ^{ab}	26.82 ± 0.45 ^a	28.00 ± 2.12 ^a

Remark: Averages marked with different letters on the same line. Shows that there is a statistically significant difference ($P < 0.05$)

In the present study, it is observed that digestive enzyme activities such as protease, amylase, and lipase show statistically significant ($P < 0.05$) differences among experimental groups (Table 10) when fed to giant freshwater prawns (*G. donax*) using different diets. In particular, the faecal protease enzyme activity of prawns that were fed BSFL with the addition of bromelain was higher ($P < 0.05$). Further, the prawns fed the black soldier fly larvae meal had an increase in amylase and lipase activities.

A significant increase in enzyme activity was observed in the hepatopancreas, where the supplementation of white shrimp feed with protease and amylase contributed to an elevation in protease activity [47]. Interestingly, lipase activity showed no variance, as the formula did not include lipase enzyme. Similarly, our study revealed that introducing bromelain, an exoprotease enzyme, impacted the augmentation of endoprotease but did not influence the rise of amylase and lipase produced by shrimp. Replacing the feed with fresh black soldier fly larvae exhibited no effect on amylase and lipase enzyme activities from the hepatopancreas in the experimental groups. However, a significant increase in protease enzyme synthesis occurred when feeds were replaced with 25% or more of fresh BSFL. In addition, our results are similar to those reported in enzyme supplementation studies using white shrimp [55] and fishmeal replacement studies using soybean meal for white shrimp [56], supporting the consistent effect of enzyme supplementation on enzyme levels in aquaculture. Amylase and lipase activities from giant freshwater prawns were increased when they were fed a diet containing black soldier fly larvae (BSFL). This increased enzyme production may be explained by the rich fat content of BSFL, which is one of the main nutrients. Interestingly, in terms of the level of the NPF (non-fiber carbohydrate), BSFL is not only higher than fishmeal, but it is significantly higher. The amount of fat obtained was 34.56%, which fell within the range of 28.43% to 38.36% reported in previous studies. Likewise, the levels of NFE, which are found to be between 6.45 – 7.59% in previous studies [57], have been observed in the present study. Comparative analysis showed that shrimp with high-fat feed, especially with a high carbohydrate content, had higher enzyme activity and synthesis of the enzymes lipase and amylase. This effect was more pronounced in the absence of bromelain enzyme supplementation and showed lower protease enzyme activity than in the bromelain-supplemented group. An enzyme activity relationship with nutrient content corroborates observations that enzyme activities in crustaceans digestive system are a response to nutrient content variations, which can be affected by feeding behaviour and feed completeness [58].

Table 10 Activities of digestive enzymes extracted from the hepatopancreas of giant freshwater prawn.

Treatments	Enzyme activity (U/min/mg protein)		
	Protease	Amylase	Lipase
T1	0.09 ± 0.00 ^b	0.45 ± 0.01 ^c	0.12 ± 0.01 ^c

T2	0.10 ± 0.02 ^{ab}	0.37 ± 0.03 ^d	0.14 ± 0.02 ^c
T3	0.07 ± 0.00 ^b	0.64 ± 0.00 ^a	0.46 ± 0.04 ^a
T4	0.13 ± 0.00 ^a	0.51 ± 0.00 ^b	0.26 ± 0.00 ^b

Remark: Averages marked with different letters on the same column. Shows that there is a statistically significant difference ($P < 0.05$)

Important for evaluating mineral homeostasis in the blood of aquatic animals, especially those that depend on mineral for molting and growth, is osmolality which is the total concentration of dissolved particles in a solution. The present work showed that the supplemented giant freshwater prawns with BSFL had the highest osmolality (Table 11). This discovery indicates a possible relationship between plasma osmolality, molting capacity and the faster growth rate in this group. Crustaceans, which are known to be very tolerant towards salinity changes (euryhalin), have various shrimp osmotic systems; they are categorized as sea-euryhaline organisms (hyper-regulators and hypo-regulators) when considering osmolality, an important parameter showing the function of the osmoregulatory system, which is essential for maintaining fluid balance with the environment through osmosis. Giant freshwater prawns (GWP) exhibit hemolymph osmolality between 453 and 489 mOsm/L, which are quite high for organisms that are primarily found in freshwater, such as Blue and Chinese crabs [59]. The moderate osmoregulation crayfish has an osmolal value of hemolymph of 370–450 mOsm [60]. These results demonstrate the good osmoregulation ability for freshwater organisms. The hemolymph Osm values in these prawns are also similar to that reported for giant freshwater prawns, between 416 and 437 mOsm kg⁻¹, in another study with a mean value of 424 mOsm kg⁻¹ for the males and females [61].

Notably, when raised at higher salinity levels (from 7% to 14%), their blood osmolality increased significantly, reaching 465 mOsm kg⁻¹ for males and 454 mOsm kg⁻¹ for females. Furthermore, other research agrees with these findings, showing that giant freshwater prawns in 18% saltwater could maintain hemolymph osmolality at approximately 420 mOsm/kg-1 [48]. The adjustment of salinity led to corresponding changes in osmolality, with values of 433.3, 452.4, and 640.6 mOsm kg-1 for hemolymph in water with 0%, 15%, and 25% salinity, respectively [48]. This observation contrasts with the osmolality values in *Macrobrachium tenellum* (Smith, 1871) raised at salinity levels of 0–10 psu, which exhibited blood osmolality values ranging from 426 to 504 mmol kg⁻¹ [62].

Table 11 Osmolality of giant freshwater prawns fed across different experimental groups for 90 days.

Treatments	Osmolality (mOsm/L)
Initial	339 ± 5.69 ^d
1	453 ± 4.00 ^c
2	475 ± 6.36 ^b
3	489 ± 4.51 ^a
4	472 ± 3.46 ^b

Remark: Averages marked with different letters on the same column. Shows that there is a statistically significant difference ($P < 0$).

Characterization of chitin by FTIR analysis

Chitin characterization using FTIR spectroscopy (Figure 1). Each organic bond corresponds to a specific frequency in the spectrum, resulting in a unique reflection pattern in the infrared region. FTIR is a valuable tool for studying molecular structures by generating spectra that reveal how the sample absorbs infrared wavelengths [63]. In the case of chitin, distinctive wavelengths include 3480–3450 cm⁻¹ (O\H stretch); 1660, 1627 cm⁻¹ or 1656 cm⁻¹ (C_O stretch); 1560 cm⁻¹ (N\H bend, C\N stretch); 1420 cm⁻¹ (CH₂ ending and CH₃ deformation); 1380 cm⁻¹ (CH bend, CH₃ symmetrical deformation); 1020 cm⁻¹ (C\O\C asymmetric stretch in phase ring) and 890 cm⁻¹ (CH ring stretch). These characteristics of chitin structure have been identified in various organisms, including shrimps, prawns, crabs, giant freshwater prawns, silkworms, and grasshoppers [64-66]

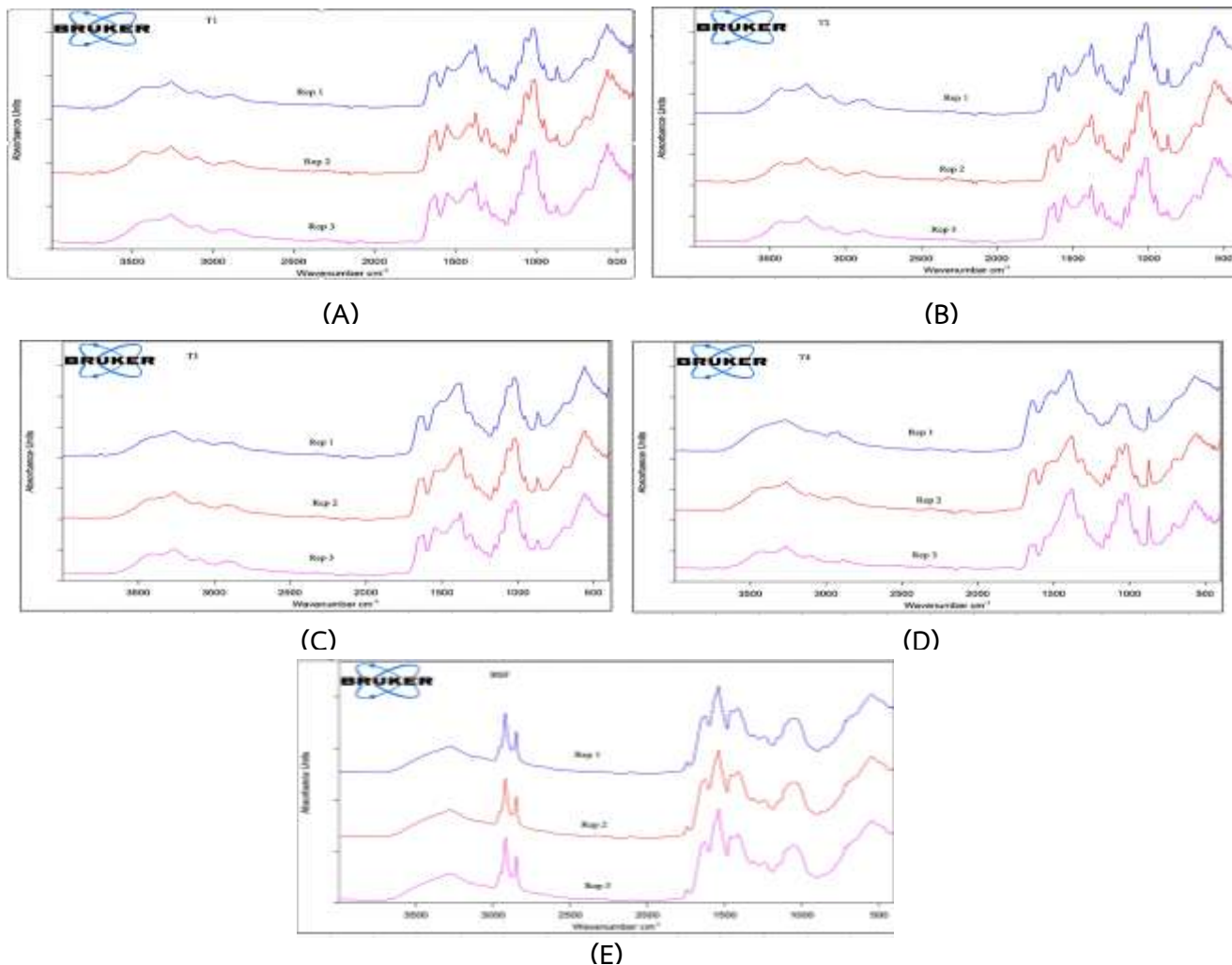


Figure 1 FTIR spectra of chitin extracted from giant freshwater prawn shells were analyzed across different dietary treatments: (A) control diet, (B) diet supplemented with bromelain enzyme, (C) diet with black soldier fly larvae, (D) diet with black soldier fly larvae and bromelain enzyme and (E) chitin extracted from black soldier fly larvae

FTIR spectra of giant freshwater prawn shells and black soldier fly larvae samples revealed slight differences, particularly in two regions: $3,500\text{--}3,000\text{ cm}^{-1}$ and $3,000\text{--}2,800\text{ cm}^{-1}$. Values within the range $1,700\text{--}500\text{ cm}^{-1}$ were found to be similar in all giant freshwater prawn samples among the experimental groups, which suggested that the chitin in the prawn was of consistent quality. Interestingly, the chitin structure of black soldier fly larvae and giant freshwater prawn shells was the same. This was, however, not the case for the black soldier fly larvae spectra as a higher peak was observed in the $3000\text{--}2500\text{ cm}^{-1}$ range, which may suggest impurities or coloured compounds. However, there were still absorptive bands in the spectra of black soldier fly larvae samples that were representative of chitin.

FTIR spectroscopy can be used as a useful diagnostic tool to help determine the molecular structure of a material, including the crystalline form (α , β or γ) of chitin. In α -chitin, two hydrogen bonds are detected [68]: an intramolecular bond (between the carbonyl group and CH_2OH) with an absorbance at 1620 cm^{-1} and an intermolecular bond (between NH and the carbonyl group) with an absorbance at 1660 cm^{-1} . β -chitin exhibits a single signal at 1650 cm^{-1} , reflecting weakened intramolecular hydrogen bonds [69, 70]. γ -chitin displays split amide I bands, with a less pronounced band at 1660 cm^{-1} and a clear, sharp band at 1620 cm^{-1} [68]. Analysis of commercial giant freshwater prawn using FTIR spectroscopy indicates two absorption bands, consistent with the known presence of α -chitin in these prawns. Black soldier fly larvae and giant freshwater prawn shells fed a diet containing black soldier fly larvae or supplemented with bromelain enzyme exhibited similar strong bands in the $2000\text{--}1500\text{ cm}^{-1}$ range, confirming the presence of α -chitin in both samples.

Chitin characterization using X-ray diffraction

X-ray diffraction (XRD) analysis is a technique used to study the atomic and molecular arrangement of crystalline materials. It utilizes the principle of X-ray scattering to determine the crystal structure. Figure 2 presents the results of X-ray diffraction (XRD) analysis of chitin indicates higher chitin content in samples from giant freshwater prawn shells fed with T4 supplemented with black soldier fly larvae (BSFL) and bromelain enzyme. Peak intensity decreases with decreasing BSFL usage in the treatment, with the lowest chitin content observed in samples extracted from BSFL themselves.

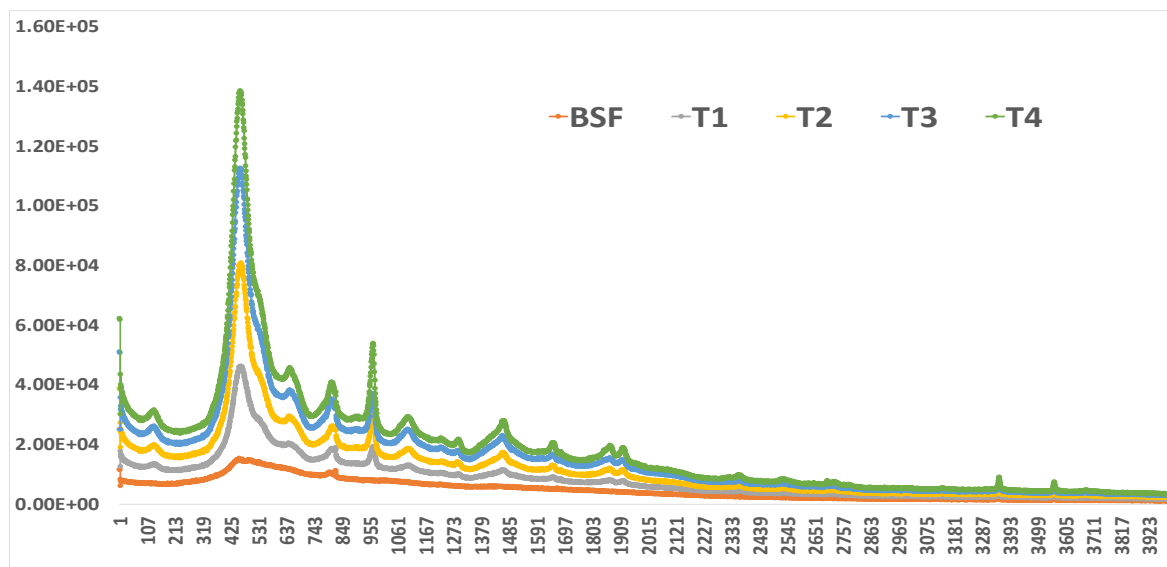


Figure 2 XRD spectrum of chitin extracted from black soldier fly (BSF) and giant freshwater prawn shells fed with T1-control feeds without BSFL and bromelain, T2- control feeds supplemented with 250 ppt bromelain, T3- Feeds included 12% BSFL and T4 - Feeds included 12% BSFL supplemented with 250 ppt bromelain.

The feeds supplemented with BSFL had the highest values, due to the high (up to 9%) mineral components the feeds contained, namely ash. This caused higher osmolarity values in the shrimp's blood because the minerals were absorbed into the shrimp's blood. Increased mineral concentrations in the blood cause more rapid molting, which is related to increased growth rate. However, T4, which included BSFL in the feed formula and was supplemented with bromelain enzyme, had lower Osmolarity, which meant that blood mineral content was reduced. The ash content of this group was found to be more than that of the group that consumed only BSFL in their diet (T3); however, the muscle tissue of this group showed lower ash content than that of the other group with LBAM (T2). While these differences were noted, both groups had strong growth rates and increased molt, which was likely due to mineral and nutrient accumulation obtained as a result of feeding BSFL.

4. Conclusion

This study showed that dried, ground black soldier fly larvae (BSFL, *H. illucens*) meal at 12% inclusion rate can be used to replace fishmeal in diets for giant freshwater prawn (*M. rosenbergii*) in a recirculating aquaculture system. The significant improvements in weight gain, average daily gain, and specific growth rate with BSFL-based diets with no increase in feed intake and no significant reduction in feed conversion ratio, signifying better nutrient use efficiency. Protein digestion was further improved by providing bromelain (250 ppm) from pineapple waste as supplementation, which also demonstrated potential to enhance the growth performance. Overall, the BSFL-only diet resulted in the highest yield, however, due to its better growth/survival ratio. These findings suggest that BSFL and bromelain can enhance the digestion of nutrients, mineral status, and other physiological processes associated with moult. However, molting-related cannibalism is still a significant production challenge, especially in BSFL–bromelain treatments, which should be mitigated by providing better shelters, stocking, mineral utilization, and feeding practices. In conclusion, the integration of BSFL and bromelain sourced from pineapple into prawn feeds is a promising approach that offers sustainability, cost savings, and better production yield of the giant freshwater prawn.

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