Effect of feeding black soldier fly larvae diets on growth and culture condition of kuruma prawn (Penaeus japonicus)

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Effect of feeding black soldier fly larvae diets on growth and culture condition of kuruma prawn (*Penaeus japonicus*)

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Abstract: Insect-based feeds are attracting considerable attention in aquaculture industries as alternatives to traditional fish-based feeds. Insect-based ingredients could be potential replacements for fishmeal in aquaculture feeds due to their implication in enhancing fish growth and disease resistance. However, their environmental impact should be fully assessed before insect-based feeds are used more frequently and on a larger scale. Although several attempts have been made to examine the utilization of insect-based diets in aquaculture species, the experimental conditions are limited. As a result, the comprehensive understanding of their impacts on environments remain elusive. To fill the knowledge gap, this research introduces the utilization of black soldier fly *Hermetia illucens* larvae (BSFL) as an alternative to fishmeal in diet for Kuruma prawn, *Penaeus japonicus,* focusing on the effects on growth and culture condition against a traditional fishmeal-based diet. A feeding experiments was conducted using juvenile Kuruma prawns (body weight, 106 ± 9.5 mg) and experimental diets with 0% BSFL (100% fishmeal), 50% BSFL (50% of fishmeal was substituted with BSFL), and 80% BSFL (80% of fishmeal was substituted with BSFL). The prawn was individually kept in a glass container and fed respective diets once every two days. The feeding trial was conducted at 20°C. The body weight of prawn in the 50% BSFL treatment was significantly higher than that in the 0 % BSFL treatment after one week, while the body weights were not significantly different between the 0% BSFL and 80% BSFL treatments. The concentrations of dissolved inorganic nutrients in the rearing water were measured periodically during the one-week feeding period. The concentration of dissolved ammonium nitrogen became lower with the increase of BSFL content ($p < 0.05$). On the other hand, phosphate concentrations tended to be higher in the BSFL-supplemented treatments. However, no significant difference was found between the 0% (100% fish meal) and 50% BSFL treatments, the latter of which noted a higher prawn growth. Oxidative conditions of the bottom sediment are important for monitoring the health of benthic organisms such as Kuruma prawn. Therefore, we monitored the redox potential of the bottom sediment in real time. The redox potential was found to be highest in the 80% BSFL treatment, lowest in the 0% BSFL treatment, and at an intermediate level in the 50% BSFL treatment (80% BSFL > 50% BSFL > 0% BSFL). Overall, our results indicate that for prawn culture, 50% of dietary fishmeal could potentially be replaced by BSFL with growth promotion and a lower environmental impact.

Key words: aquaculture, insect-based diets, dissolved inorganic nutrients, redox potential

Introduction

With the increasing demands, technological advancements, and advanced efforts, the global production of shrimp has been increasing rapidly. Aquaculture has been contributing substantially to maintaining the sustainable use of marine resources and is essential to ensure the food security (Costello *et al*. 2020). Most of fish and crustacean aquaculture practices are sometimes criticized for the disruption of the food web, resulting in an ecological imbalance. As a result of using wild pelagic fish (e.g., sardines and anchovies) for the production of fishmeal that have been used as a feed ingredient,

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particularly for farming marine carnivorous fish at higher inclusion levels (Naylor *et al*. 2000), the disruption tends to be more serious. Despite not being commonly used, insect-based ingredients are attracting significant attention as alternative sources relative to traditional fishmeal in aquaculture industries (Henry *et al*. 2015). The insect-based ingredients could be potential replacements for fishmeal in aquaculture feeds due to their implication in enhancing fish growth and disease resistance (Ido *et al*. 2019). The effects of different feeds used in aquaculture, including insect-based feeds, on the rearing environment should be assessed because the knowledge is limited.

To further improve the aquaculture production in terms of growth and profitability, the improvements in feed formulation and quality are greatly needed. Black soldier fly *Hermetia illucens*, the larvae of which (BSFL) could grow on low-value organic matters and are able to supply a protein-rich and high-value biomass (Diener *et al*. 2009), is a promising species and commercially exploited in various countries. Thus, the use of BSFL as an alternative protein source in aquaculture industries has been rising because BSFL is considered to supply required nutrients needed in aquaculture production (Bruni *et al*. 2018; Foysal *et al*. 2019). Kuruma prawn (*Penaeus japonicus*) is distributed in the areas of Indo-Pacific regions including Japan except for Hokkaido Island (Hayashi 1992), and has been an important commercial aquaculture species. Diet quality is always a vital factor affecting the shrimp aquaculture practices and BSFL could provide nutrients required for maintaining and improving the health of aquaculture species. However, utilization of BSFL in Kuruma prawn diet has been unknown in terms of the effects on their growth, survival, and aquaculture environment. Especially, the environmental impact assessment should be addressed in advance to practical use of insect-based feeds at a larger scale. To fill the knowledge gap, this research introduces the results of our study examining the effects of dietary inclusion of BSFL as an insect-based ingredient on the growth and environmental condition of Kuruma prawn against a traditional fishmeal-based diet. Since Kuruma prawn are sensitive to the condition of the bottom sediment, maintaining the bottom sediment environment in a healthy condition is extremely important in their aquaculture. In this study, we also report the results of measurements of the growth of Kuruma prawn fed BSFL supplemented diets and a fish meal-based diet, and the impact on the bottom sediment environment using a newly developed bottom sediment monitoring system (Shono *et al*. 2022).

Short Materials and Methods

Feeding performance of kuruma prawn using BSFL-containing diets

A feeding experiment was conducted using juvenile Kuruma prawn (body weight, 106 ± 9.5 mg) and experimental diets with 0 % BSFL (100 % fishmeal), 50 % BSFL (50 % of fishmeal was substituted with BSFL), and 80 % BSFL (80 % of fishmeal was substituted with BSFL). Individual prawn was kept in a glass container and fed one of the diets once every two days. The feeding trial was conducted at 20°C for one week. The feed conversion ratio (FCR) was calculated for individuals using the following formula.

$$\mathrm{FCR} = \frac{\text{Total feed consumed (g)}}{\text{Weight gain (g)}}$$

Evaluation of rearing environment using BSFL-containing diets

For dissolved inorganic nutrient analysis, seawater samples were collected from each experimental breeding container, frozen and stored until analysis. Concentrations of dissolved inorganic nitrogen (NH_4-N, NO_2-N, and NO_3-N), dissolved inorganic phosphate (PO_4-P) and dissolved silicate (DSi) were measured by an autoanalyzer QuAAtro 39 (BL-TEC, Osaka, Japan), following the standard methods detailed in Strickland and Parsons (1972).

We also measured the redox potential of the bottom sediment. A two-electrode electrochemical cell (EC, 160 ml capacity) was assembled using an Ag/AgCl/saturated KCl as reference electrodes. A fluorine-doped tin oxide (FTO)-coated glass electrode (surface area of 28.26 cm^2, SPD Laboratory, Shizuoka, Japan) was used as a working electrode and was placed on the bottom of the EC reactor (Fig.1). Twenty grams of wet sediments and 145 ml of sea water were added to the EC reactor. One reactor was placed in each experimental container by inserting the receptor end inside the shallow benthic sedimentary layer (Fig.2) and was maintained at 20°C. Redox potential measurements were continuously conducted using open circuit potential measurements performed with an automatic polarization system HAL-3001A (Meiden Hokuto, Tokyo, Japan).

Statistical analysis

All data were expressed as mean ± standard deviation. Statistical analysis was performed using Microsoft Excel Touki 2010 for Windows version 1.03 (Esumi, Tokyo, Japan). The

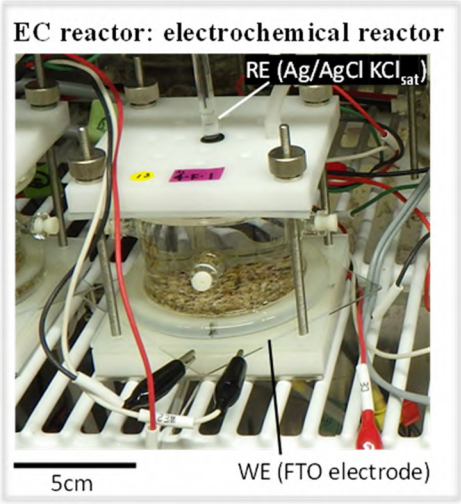

Fig.1 (A) the model benthic ecosystem constructed in an electrochemical (EC) reactor

RE, and WE denote the reference and working electrodes, respectively.

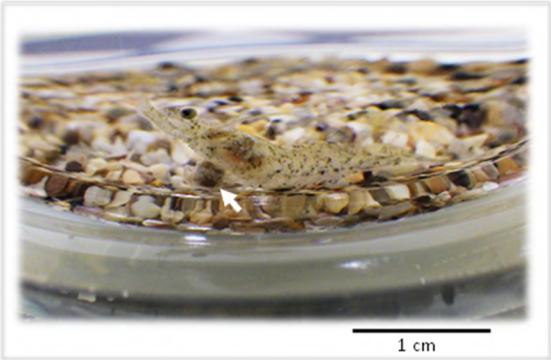

Fig.2 Feeding behavior of Kuruma prawn for a BSFL diet (white arrow) in the EC reactor

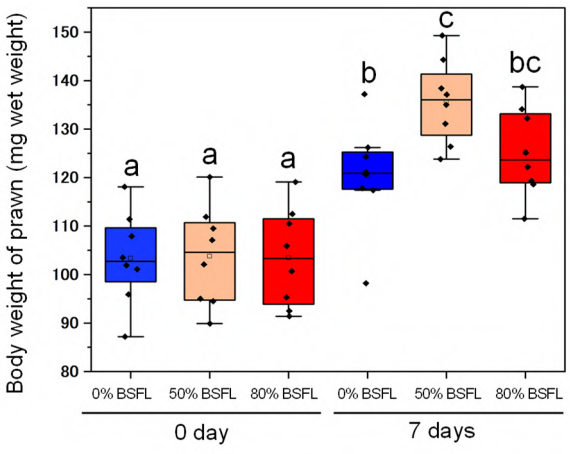

Fig.3 The body weights of Kuruma prawns before and after one week of feeding experiment

Blue, orange, and red indicate the results of 0%, 50%, and 80% BSFL treatments, respectively. Values with different letters are significantly different from each other ($p < 0.05$).

differences between treatment means were tested using Tukey's multiple comparison test and were considered significant at $p < 0.05$.

Results and Discussion

Growth performance

Survival rate was more than 90% across the experimental treatments and no significant difference was observed during the experimental period. The Kuruma prawn actively foraged within each test container and actively consumed the diets during the experimental period (Fig.2). The feeding behavior of Kuruma prawn for a BSFL-based diet is shown in Fig.2. An improved growth performance was observed in prawn fed the BSFL-containing diets compared to that of the control diet (Fig. 3). The final body weight of prawn in the 50% BSFL treatment was significantly higher than that in the 0% BSFL treatment while the weight in the 80% BSFL treatment was intermediate between the values of the other two treatments (Fig.3). The utilization of insect larvae has been extensively studied in aquaculture fish while that in crustaceans has rarely been studied. Different metamorphic stages (i.e., larvae or pupae) of a given insect could bias the growth performances of aquaculture shrimp, due to the variabilities in their nutritional compositions (Huyben *et al*. 2019). The FCR in the 50% BSFL treatment was the lowest value of 2.1, indicating that nutrients in the BSFL diet were efficiently converted into prawn body (Table 1). Kuruma prawn is omnivorous and feeds on certain invertebrates such as bivalves, polychaetes and crustaceans, and other fauna in the wild. Therefore, the prawn could be able to efficiently assimilate BSFL.

Previous studies reported the growth promotion due to the substitution of fishmeal with BSFL (Renna *et al*. 2017; Wang *et al*. 2019), which fully agrees with the finding of the present study. The composition of the fatty acids in BSFL could trigger the growth performance compared to that of fishmeal. Wang *et al*. (2021) demonstrated that dietary inclusion of BSFL initiated the hepatocyte interruption and lowered the unsaturated fatty acids, leading to the increase in growth

Table 1 The feed conversion ratio of Kuruma prawn fed each diet

	Feed conversion ratio
0% BSFL	4.0 ± 1.8
50% BSFL	2.1 ± 0.7
80% BSFL	3.0 ± 0.7

performance in Pacific white shrimp *Litopenaeus vannamei*. The excessive substitution of fishmeal with alternative ingredients does not always improve the growth performance as it might affect the metabolism, intestinal histology, and digestion of shrimps (Rahimnejad *et al*. 2019; Shao *et al*. 2020). These observations support the finding of our study that 80 % substitution negatively affected the growth performance relative to that of the 50 % BSFL treatment (Fig.3). Ling *et al*. (2025) comprehensively investigated the multifaceted impacts of BSFL on shrimp aquaculture and recommended its potential as a suitable and sustainable alternative protein source relative to traditional fishmeal. They concluded that BSFL is a promising ingredient that enhances the growth performance and feed efficiency in certain conditions, contributing to the economic viability of shrimp aquaculture practices. On the other hand, it has been stated that substitution of 6-8 % fishmeal with BSFL is an appropriate level in a diet for *L. vannamei*, a species closely related to Kuruma prawn, and that 10 % substitution with BSFL may induce pathological abnormality in the intestine and decrease the shrimp's immunity (Chen *et al*. 2023). Although the species are different, our study suggests that substitution of fishmeal up to 50 % with BSFL might enhance the immunity of Kuruma prawn. In a future study, the effects of long-term culture of Kuruma prawn with a BSFL diet not only on their growth but on their gut flora that is known to be involved in the immune system, should be addressed.

Rearing environment for Kuruma prawn due to dietary BSFL inclusion

The concentrations of dissolved inorganic nutrients in the rearing water were measured periodically in each experimental container for one week (Fig.4). The concentration of dissolved ammonium nitrogen became lower with the increase of BSFL content ($p < 0.05$). On the other hand, phosphate concentrations tended to be higher in the BSFL-supplemented treatments, but no significant difference was found between the 0 % BSFL (100 % fish meal) and 50% BSFL treatments, the latter of which noted a higher prawn growth. The redox potential in the sediment during the prawn culture was generally higher in the order of 80 % BSFL > 50 % BSFL > 0 % BSFL. These results indicate that 50 % substitution of fishmeal with BSFL could be favorable for prawn culture, increasing the growth with a lower environmental impact.

Traditionally, the environmental assessment of aquaculture fields uses various indicators, such as chemical and biological ones (e.g., benthic fauna; Holmer *et al*. 2008). However, these approaches are not suitable for assessing short-term environmental changes, such as those that fluctuate on hourly and daily basis. Although chemical indicators, such as oxygen, nitrogen, phosphorus, and sulfur compounds, could instantly respond to the changes caused by feeding, enormous efforts are required for on-site water and sediment samplings and comprehensive analysis of complex chemical compounds.

In the present experiment, the sediment of the 0 % BSFL treatment with a higher dissolved ammonium nitrogen concentration (Fig.4A, blue bar) had lower redox potential levels and was in a reduced environment (Fig.5, blue line). On the other hand, the sediment of the 80 % BSFL treatment with a lower dissolved ammonium nitrogen concentration (Fig.4, red bar) had higher redox potential levels, indicating the sediment was in an oxidizing environment (Fig.5, red line). The dissolved inorganic phosphate (PO4-P) in the 50 % BSFL treatment was not statistically different from that of the control treatment in the current study (Fig.4D). The water quality is known to be negatively influenced by the feeding activities, leading to growth retardation of aquatic animals. He *et al*. (2022) reported the negative correlation between the concentrations of NH_4-N and NO_3-N and the inclusion levels of BSFL, which partially supports the finding of the current study (consistent decrease in NH_4-N with BSFL; see Fig.4A). Generally, 20-30 % of dietary nutrients are efficiently utilized by the animals in shrimp aquaculture, while the others are accumulated in the bottom environment as leftovers that are often responsible for the deterioration of sediment conditions in aquaculture farms (Paez-Osuna 2001). Our results indicate that the BSFL diets were possibly utilized more efficiently by Kuruma prawns compared to the fishmeal-based diet. By the measurement of redox potential which is a physicochemical value determined by the integration of various factors such as biological, chemical, and physical parameters, we have recently successfully implemented a real-time monitoring system at an aquaculture site (Ito *et al.*, in preparation). In the future, it is expected that the sediment environment which has been adversely impacted by aquaculture could be monitored and evaluated by the real-time redox potential monitoring of the sedimentary environment. In conclusion, 50 % replacement of fishmeal by BSFL was found to be ideal in terms of limited feed loss, higher growth performance, survival, and quality of the environmental characteristics. Especially, the introduction of unique redox potential monitoring system that integrates various physicochemical measurements in the future aquaculture designs could lead to the improvement in monitoring the aquaculture environment.

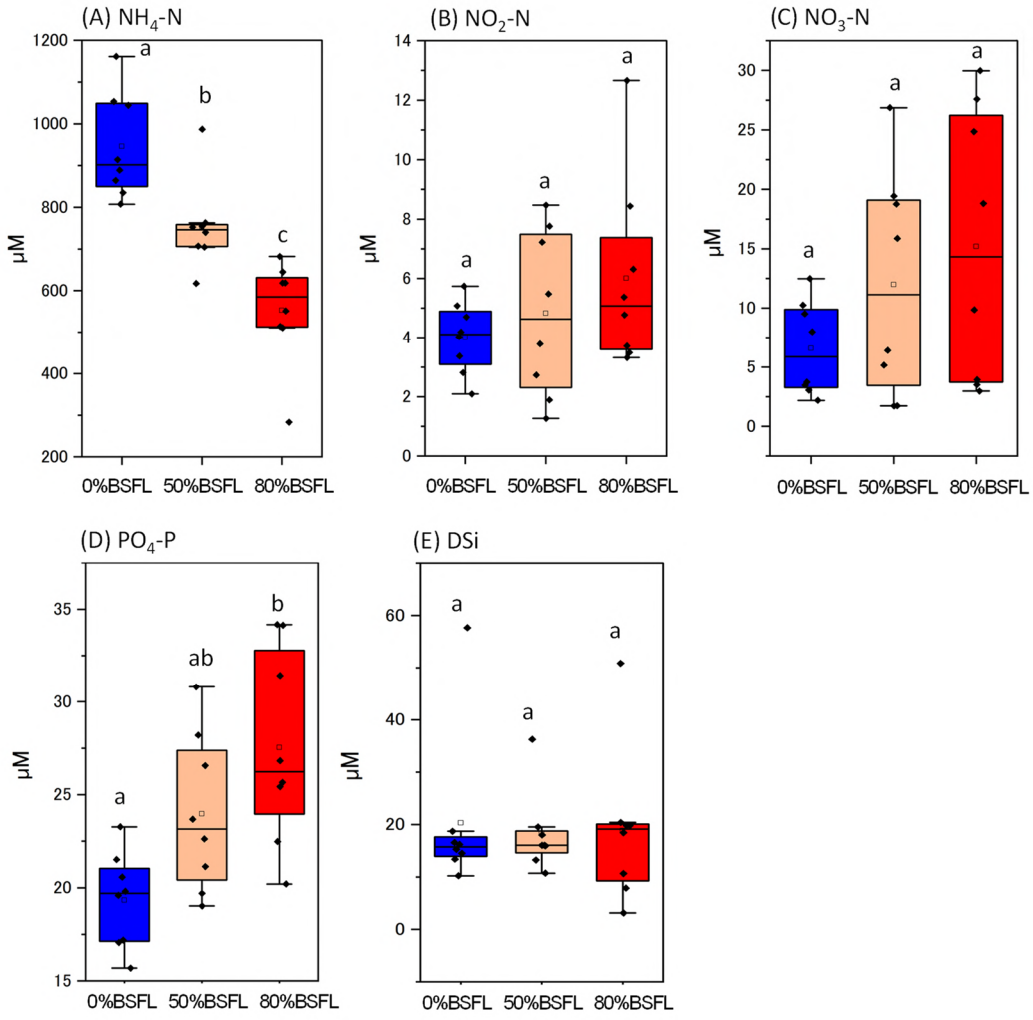

Fig.4 The concentrations of dissolved nutrients in rearing seawater after one week of feeding experiment in Kuruma prawn fed BSFL diets

The concentrations of dissolved inorganic nitrogen, NH_4-N (A), NO_2-N (B), and NO_3-N (C), dissolved inorganic phosphorus (PO_4-P) (D), and dissolved silicate DSi (E) are presented. Values with different letters are significantly different from each other ($p < 0.05$).

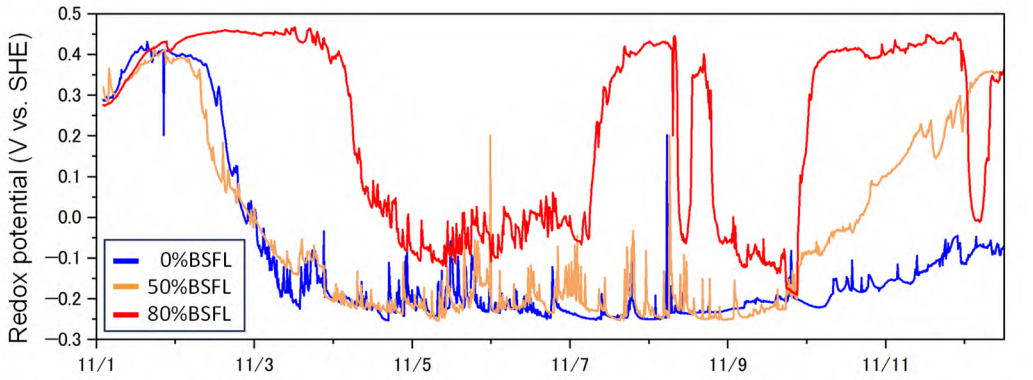

Fig.5 The fluctuations in the redox potential of the bottom sediments in each rearing container during one week feeding experimet in Kuruma prawn

The blue, orange, and red lines represent 0%, 50%, and 80% BSFL treatments, respectively.

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Annotated Bibliography of Key Works

(1) Shono N, Ito M, Umezawa A, Sakata K, Li A, Kikuchi J, Ito K, Nakamura R (2022) Tracing and regulating redox homeostasis of model benthic ecosystems for sustainable aquaculture in coastal environments. *Front. Microbiol*., **13**, 907703. https://doi.org/10.3389/fmicb.2022.907703

Aquaculture practiced in coastal environments has an increasingly important role in the world's food supply; however, the accumulation of organic compounds on the seafloors due to overfeeding harms benthic ecosystems. To assess the ecological resilience of aquafarms to nutrient influx, they investigated the redox homeostasis of benthic ecosystems using a marine oligochaete as a model benthic organism in aquaculture fields. Real-time monitoring of the redox potential of a model benthic ecosystem constructed in an electrochemical reactor allowed evaluation of the homeostatic response of the system to nutrient addition. Although the detrimental effects of overfeeding were confirmed by irreversible potential changes in the sediment, redox homeostasis was reinforced through a cooperative relationship between oligochaetes and sediment microorganisms. Specifically, the oligochaetes exhibited reversible changes in the metabolism and body position in response to dynamic changes in the sediment potential between -300 and 500 mV, thereby promoting the decomposition of organic compounds. The potential-dependent changes in the metabolism and body position were reproduced by artificially manipulating the sediment potential in the electrochemical reactors. Given the importance of benthic animals in sustaining coastal ecosystems, the electrochemical monitoring and physiologic regulation of marine oligochaetes could offer an intriguing approach toward sustainable aquaculture.