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Short Communication

Evaluation of Carboxymethyl Cellulose (CMC) Binder and Squid Oil Attractant in the Formulation of *Litopenaeus vannamei* Diet

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Abstract

Pellet water stability and feeding attractant are the crucial factors to be considered in the formulation of shrimp feed to minimize nutrient leaching and improve food palatability, respectively. The aim of this study was to determine the binding effect of supplementation of carboxymethyl cellulose (CMC) during pellet manufacturing, and feeding responses with the inclusion of squid oil in pellet. Both experiments were conducted separately in five feed formulations ranged from 0.00% to 3.00% of CMC and squid oil. All T_0 was referred as control with no CMC or squid oil. Experiment was conducted using 30 shrimps/treatment with triplicates. The dry matter retention up to 120 min of immersion and the feeding response test were evaluated in pellet supplemented with CMC and squid oil, respectively. Results showed the percentage of dry matter retentions increased with the increase of CMC, over time. The CMC containing pellets at 2.00% and 3.00% had minimal disintegration in water and possessed high water stability after 120 min immersion. The addition of 3.00% squid oil in feed exhibited a significant result ($p < 0.05$) in the time taken for shrimp to access the pellet, also increased the time starts for ingestion. No significant change was observed in water quality indicated no water contamination occurred throughout the study. Results demonstrated that the addition of CMC binder at 2.00% and 3.00% improved pellet water stability, whilst 3.00% squid oil was recommended to enhance feed palatability. However, future studies on the activity of enzymes in shrimp bodies after treatment would be an advantage.

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1. Introduction

The aquaculture industry plays an important sector nowadays, with around 76.6 million metric tons production in 2016, and half of them come from the farmed industries (FAO, 2016). Among them, shrimp culture is the fastest growing industry and is developing rapidly compared to any other aquaculture industry. In order to ensure the continued sustainability with high quality of cultured shrimp, nutritive diets are essential while maintaining low-cost expenses for feeds. Indeed, true nutritional value of feeds does not only depend on their nutrient contents but also the availability of nutrients to be consumed by the animals. Thus, shrimp pellets with high water stability, palatability, and optimum digestibility are crucial.

High water stability is defined as the ability of feeds to retain water at a certain time with minimal leaching before consumption. Pellet water stability is necessary for shrimps as they are slow-eater aquatic life and require to nibble the feeds before ingestion which might induce pellet dispersion into water (Arguello-Guevara and Molina-Poveda, 2012). Pellet water stability is highly influenced by the method used in pelleting process, the particle size of the ingredients, raw materials, and type of binders used (Obaldo et al., 2002). In addition, water temperature and salinity also affect pellet water stability during feeding time (Arguello-Guevara and Molina-Poveda, 2012). Binders are used in pellet manufacturing to retain nutrients macromolecular in feeds like pectin, alginate, chitosan, starch, cellulose, and polymethylolcarbamide (PMC) (Ali et al., 2010; Paolucci et al., 2012). A good binder is a binder that can enhance gelling power and improve water stability once immersed in water during feeding time. All these effects able to minimize feed wastage and minimize water pollution which may affect animal survivability in ponds (Cruz-Suarez et al., 2007). PMC binder have been reported previously to promote good water stability and economic safe (Ali et al., 2010). However, their inclusion in shrimp diets must be lower than 0.5% to avoid any toxicity effects in shrimps. In fact, their long term use may result in accumulation and formation of toxic-sediment thus affects benthic invertebrates' community. Therefore the choice to CMC binder is more convincing due to their natural based products and efficiency (Obaldo et al., 2002). The carboxymethyl cellulose (CMC) binder is derived from natural gums, which is typically been used in the food industry as binders and texturizing agents in feed diet formulation (Obaldo et al., 2002). Previous studies have reported the effectiveness of gelatin, CMC, and guar gum binders sup-

plemented in feeds on their water stability and growth performances of given animals (Johnston and Johnston, 2007; Liu et al., 2008; Gao et al., 2019). Most of them resulted with dose-dependent on the water stability and growth performances (Volpe et al., 2012; Fabbrocini et al., 2012). However, all binders promote different binding affinity depending on the nutrient components used and manufacturing practices applied, as well as the tested aquatic organisms. In all these cases, hence make it impossible to nominate which type of binder is better than another binder (Volpe et al., 2012).

Other than feed water stability, feed with feeding attractant is also essential during feed formulation particularly in shrimp, as they can detect food easily just by their chemo-attractant senses located all over their body (Smith et al., 2005). Feeding attractant varies from chemical to natural, such as dimethyl sulphone (DMS), trimethyl amine oxide (TMO), trimethyl amine (TMA) and fish meat paste, clam meat paste, squid meat paste, respectively (Ali et al., 2007). All of them provided various degree of attractions depending on the target animals, also the availability of free amino acids and peptides (Ali et al., 2007). For instance, Ali et al. (2007) reported the inclusion of fish meat, clam meat, and squid meat as food attractant in diet formulation increased time taken for shrimp to approach and pick up the feed which consequently enhanced their growth. In another study reported by El-Sayed (2016), he established the use of soybean meal based shrimp feed to improve feed palatability and attractability in *L. vannamei*. Meanwhile, current study by Li et al. (2020) reported that the inclusion of lactic acid bacterium in feed could improve feed attractant, feed intake and weight gain in shrimp.

Due to various binders and feed attractants with multiple formulations provided by the feed manufacturer, it is very hard to make a conclusion which formulation and types of binders/attractant are better among others (Paolucci et al., 2012). Therefore, a study was carried out to determine the effect of CMC binder at varying concentrations in pellet water stability, also to evaluate the effectiveness of squid oil added into the formulated feed to improve feed intake in *L. vannamei*. Findings from this study may help the feed manufacturers to have a view for making a comparison for choosing binders and feed attractants for feed formulations during pelleting.

2. Materials and Methods

2.1 Experimental Animals and Acclimatization Period

Sub-adult shrimp, *L. vannamei* ranging in the size of 14.01 ± 1.04 g in body weight were employed. Animals were collected from Setiu, Terengganu and transported alive to Marine Hatchery, Universiti Malaysia Terengganu for acclimatization, prior to any test. During this period, animals were placed in a 60-L aquarium with continuous aeration and fed *ad libitum* commercial diet with a 45% crude protein diet (Soon Soon Oilmills Sdn. Bhd. Malaysia), twice daily. Water temperature was maintained at $28 \pm 1^\circ\text{C}$ and salinity was at 34ppt throughout the experiment.

2.2 Pellet Preparation

Pellets containing 40% protein and 8% lipid content were prepared in the following way: all major components

were ground to $425\mu\text{m}$ until homogenized. Then, fish oil, vitamin and minerals were added; CMC at varying percentage: 0.00% (control), 0.50%, 1.00%, 2.00% and 3.00%) was included in diet A, meanwhile squid oil: 0.00% (control), 0.50%, 1.00%, 2.00% and 3.00% was added in diet B. Both pellet formulations were prepared and tested separately. Finally, water was added gradually in the proportion of 1:2 (w/v) to accomplish the agglutination process. The mixture was pelleted using a 3-mm mesh sieve (Kingsmart Brand, Hong Kong) and dried in an oven (Mettler, Germany) at 75°C for 2h, followed by fan-ventilated oven drying (Mettler, Germany) at 60°C to reach a moisture content at below 10% in diets. All the dried pellets were packed in a sealed plastic bag, labeled and stored at room temperature until further use.

Table 1. Determination of crude protein content

Ingredients	Average Nitrogen in sample (%)	Average crude protein (%)
Fish meal	11.58	72.37
Soy bean meal	7.50	42.74
Wheat flour	2.82	16.06
Rice bran	21.2	12.09

Table 2. Determination of lipid content

Ingredients	Average of the crude lipid in sample (%)
Fish meal	9.67
Soy bean meal	1.77
Wheat flour	3.56
Rice bran	12.34

Table 3. Formulation and proximate analysis of the test diets (Diet A)

Treatment / Feed Component (%)	T ₀ (control)	T ₁	T ₂	T ₃	T ₄
Fish meal	48.61	48.61	48.61	48.61	48.61
Soy bean meal	11.63	11.63	11.63	11.63	11.63
Wheat flour	33.26	32.76	32.26	31.26	30.26
Fish oil	4.00	3.00	2.00	1.00	0.00
Vitamin	1.00	1.00	1.00	1.00	1.00
Mineral	1.50	1.50	1.50	1.50	1.50
CMC binders (%)	0.00	0.50	1.00	2.00	3.00

T₀/control: (contain 0.00% CMC); T₁: contain 0.50% CMC; T₂: contain 1.00% CMC; T₃: contain 2.00% CMC; T₄: contain 3.00% CMC

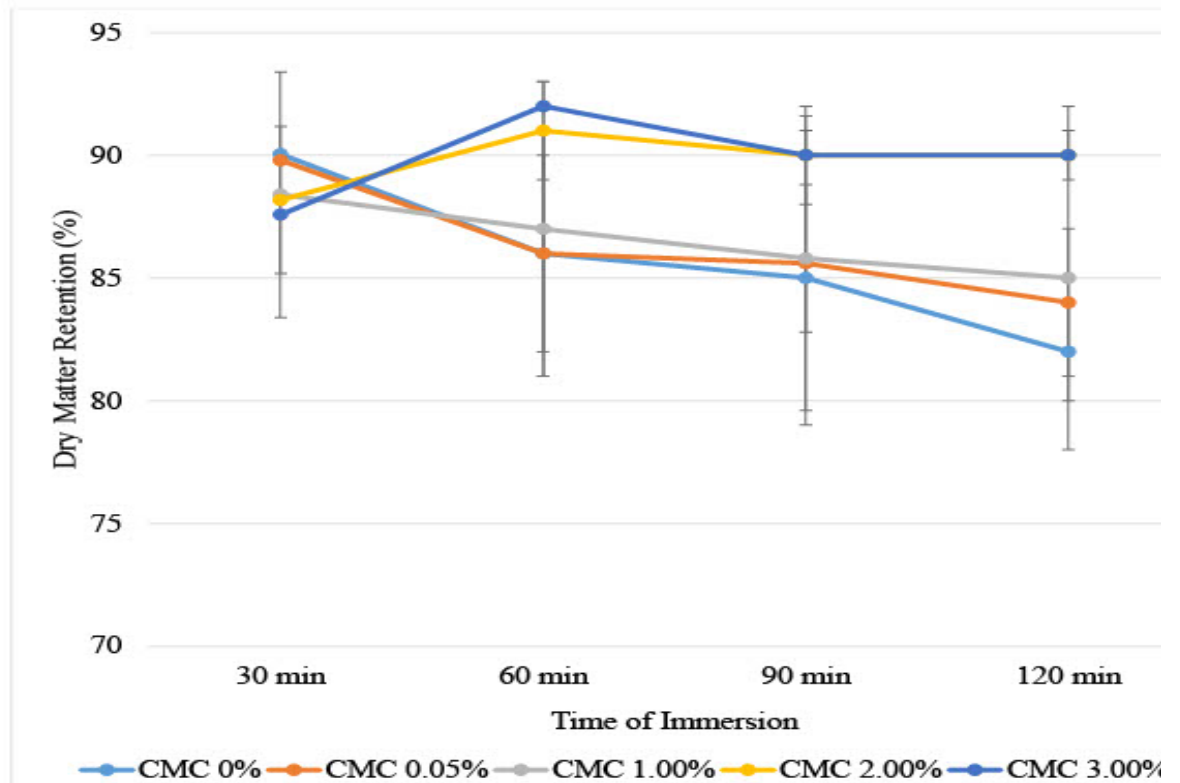


Figure 1. Effect of CMC concentration on dry matter retention over water immersion times.

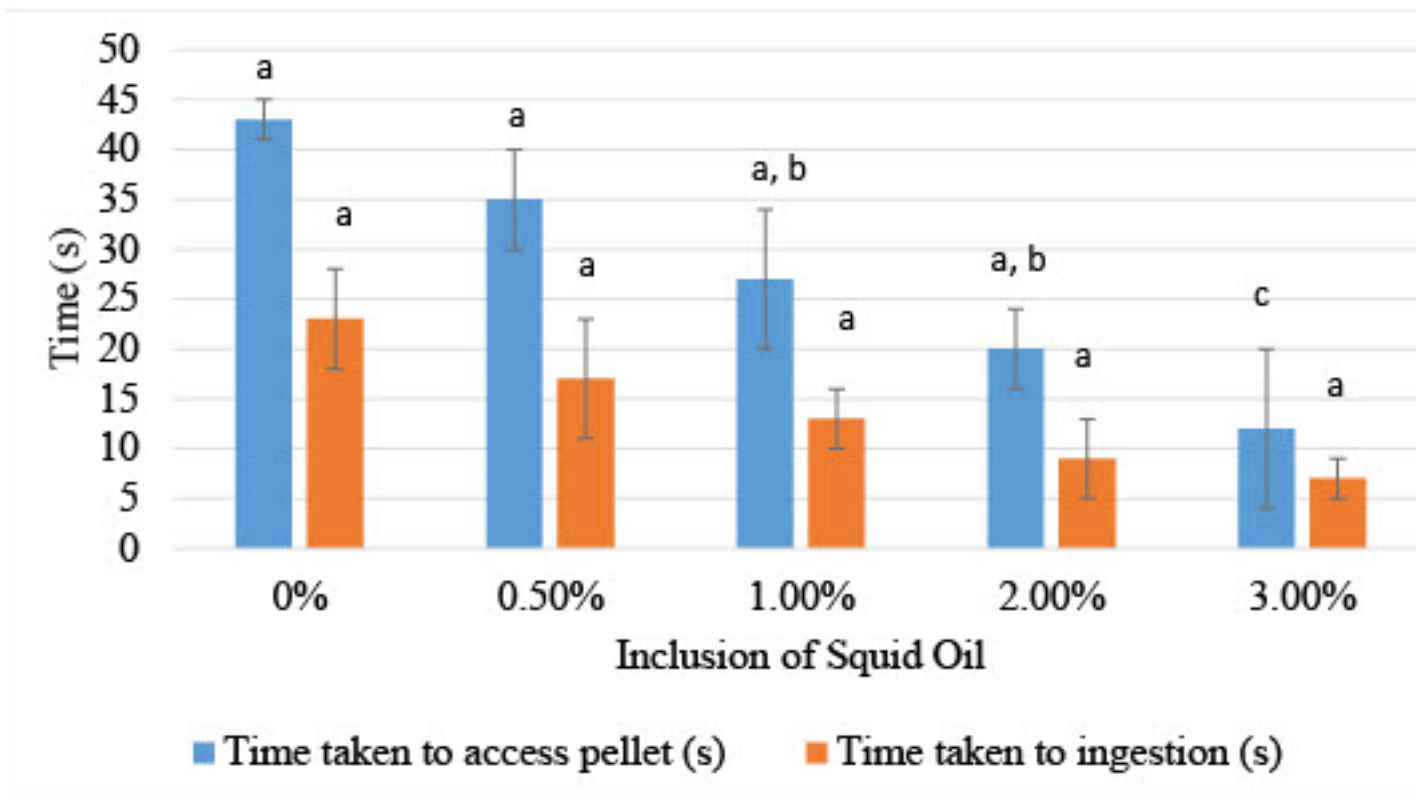


Figure 2. Feeding behavioral responses of shrimps at different inclusion of squid oil.

Table 4. Formulation and proximate analysis of the experimental diets (Diet B)

Treatment / Feed Component (%)	T ₀ (control)	T ₁	T ₂	T ₃	T ₄
Fish meal	48.61	48.61	48.61	48.61	48.61
Soy bean meal	11.63	11.63	11.63	11.63	11.63
Wheat flour	33.26	32.76	32.26	31.26	30.26
Fish oil	0.00	0.00	0.00	0.00	0.00
Squid oil	0.00	0.50	1.00	2.00	3.00
Vitamin	1.00	1.00	1.00	1.00	1.00
Mineral	1.50	1.50	1.50	1.50	1.50

T₀/control: (contain 0.00% squid oil); T₁: contain 0.50% squid oil; T₂: contain 1.00% squid oil; T₃: contain 2.00% squid oil; T₄: contain 3.00% squid oil

Table 5. Water quality parameters during experimental periods

Water parameters / Day	Temperature (°C)	Dissolved oxygen (mg/L)	pH	Salinity (ppt)
1	27.52 ± 0.30	6.98 ± 0.11	7.90 ± 0.44	32.60 ± 0.74
2	28.16 ± 0.53	7.01 ± 0.56	7.88 ± 0.27	33.13 ± 0.83
3	28.11 ± 0.44	7.04 ± 0.12	7.85 ± 0.40	33.07 ± 0.80
4	28.18 ± 0.53	6.95 ± 0.11	7.91 ± 0.40	33.13 ± 0.64
5	28.16 ± 0.63	7.00 ± 0.12	7.81 ± 0.36	33.07 ± 0.96

No significant difference in water quality parameters during treatment

2.3 Proximate Analysis Determination

The protein and lipid content in raw materials used in feed before the formulation of new pellet including fish meal, soybean meal, wheat flour, and rice bran were determined (Table 1 and 2). Then, the detailed analysis of the formulated diet; Diet A and Diet B were performed to determine the content of fish meal, soybean meal, wheat flour, fish oil, vitamin and mineral (Table 3 and 4). All the proximate analysis method was carried out according to the method in AOAC (2007).

2.4 Diet A: CMC Inclusion for The determination of Pellet Water Stability

Five grams of pellets were made with three replicates for each formulation type. Pellets were filled in a stability box in the size of (L: 5.08cm, W: 5.08cm, H: 5.08cm), which was built from a mosquito net, and soaked in an aerated water for up to 24 h. After the required immersion time (30, 60, 90, and 120 min), pellets were recovered, dried for 24 h and reweighted to get the percentage of dry matter retention (DMR), as follows:

$$\text{DMR (\%)} = 100 - [(DW_{bi} - DW_{ad}) / DW_{bi} * 100]$$

DW_{bi}: dry weight of diet before immersion

DW_{ad}: dry weight of diet after drying

2.5 Diet B: Squid Oil Inclusion for The Determination of Feeding Response Test

In order to start the feeding response test, shrimps were starved for 24 h prior to the experiment, and were randomly selected for each formulation tests. Briefly, a single animal was placed in the aquarium and 2g of the formulated pellet were offered followed by the observation of feeding response; (1) the time taken for the animal to start access towards the pellet and (2) the time taken for the animal to start ingesting the pellet. In case the food was not detected by the animal within a 7-min time, the observation was interrupted and the shrimp was replaced with another new acclimated shrimp. Aeration was only applied after the delivery of the pellet into water. Feeding observation was done for five days, from 7:30 to 9:00 pm with different pellet formulations

each day and water was replaced by new filtered sea-water to avoid any errors. The observation was done through naked eyes. Similar procedure were performed in triplicates, (n=30).

2.6 Water Quality Analysis

Water quality parameters were monitored daily to maintain the optimum conditions for *L. vannamei*. Water temperature and pH were checked daily at 9:00 in the morning by using a pH meter (Hanna Instrument). The dissolved oxygen (DO) was measured using a Hanna Instrument DO meter, whilst salinity was measured once a week as according to APHA (1989).

2.7 Statistical Analysis

A total of 30 shrimps (n=30) were used in each formulations and in triplicates. All data were presented in the value of the standard error of the mean (SEM) and were analyzed using the analysis of variance (ANOVA) with consideration at $p < 0.05$ as having a significant difference, by SPSS software (SPSS version 21.0, Armonk, NY, IBM Corp.).

3. Results and Discussion

3.1 Diet A: CMC Inclusion for The Determination of Pellet Water Stability

Binders often account as a chemical part in pellet manufacturing to minimize nutrient leaching by strengthening the pellet structure, particularly in shrimp diets which tend to manipulate the pellet using their mouth appendages first before ingestion (Volpe et al., 2012). Generally, the percentage of dry matter retentions were increased with an increased amount of CMC binders in the shrimp diets over the immersion times (Figure 1). Diet with 2.00% and 3.00% of CMC binders produced higher stability with the increased dry matter retention at an average of 88-90% up to 120 h of immersion time. Meanwhile, a diet with no CMC binder (control) resulted in the lowest dry retention time which was at 82%. Other diets with CMC binders (0.05% and 1.00%) were classified as having moderate water stability. The increase of CMC inclusion level from 0.05-3.00% improved pellet water stability, parallel with the immersion time.

All these situations occurred due to no presence of gelling formation to bind the pellet molecules (Volpe et al., 2012). Similar results were found by Brown et al. (2015) and Lwin (2018). Besides, Palma et al. (2008) had reported a decrease in food consumption among *Pa-*

laemonetes varians and *P. elegans*, as the pellets easily absorbed water and become soft, thus created difficulties for the shrimps to manipulate the pellet for ingestion. Also, the increased of dried matter loss would increase the loss of nutrient content in pellets, which after then issued on water quality; e.g. increasing the phosphorus and nitrogen content which then lead to rapid growth of algae and affects phytoplankton and benthic life communities (Cruz-Suarez et al., 2007).

However, a limitation baseline for pellet hardness is crucial to avoid pellets become too hard to ingest. Theoretically, the inclusion of binder is only necessary when the loss of dry matter reached more than 10% after 60 minutes of immersion (Cuzon et al., 1994). Despite the formulated diets being good, nutrients also must be able to be digested by shrimp for their growth advantage (Nunes et al., 2006). In some other cases, pellet loss into the water was induced as the high inclusion of binders reduced pellet digestibility, thus resulted in poor acceptance by shrimps (Patridge and Southgate, 1999).

3.2 Diet B: Squid Oil Inclusion for The Determination of Feeding Response Test

Besides the pellet water stability, feed palatability also plays an important role to increase attraction to shrimp for ingestion. The degree of pellet water stability could be lower somehow, but the presence of suitable feed attractants will enhance feed consumption (Obaldo et al., 2002). In the feeding behavioral responses test, the time taken by shrimp to access and ingest the formulated pellet was vastly detected at 3.00% of squid oil inclusion level, with less than 10 seconds (Figure 2). On the other hand, there was a significant difference ($p < 0.05$) on the time taken to access the pellet at 0.50-3.00% of squid oil level. On contrary, no significant difference ($p > 0.05$) in the time taken for the pellet to be ingested in 0.00% feed attractant. Generally, a diet containing squid oil was mostly chosen by shrimp as compared to the diet with 0.00% squid oil (control). In this case, higher responsiveness with shorter ingestion time in the formulated pellet containing squid oil indicated a better attractiveness to *L. vannamei* with the addition of high palatability.

The inclusion of squid oil in the shrimp formulated diet exhibited attractions to *L. vannamei* compared with the control group. These performances are due to the presence of protein-rich peptides, nucleotides, and nucleosides which act as feeding effectors for shrimp. All these small protein molecules in the formulated pellets demonstrated a huge impact in stimulating shrimp chemoreceptors (Smith et al., 2005). A study conducted

by Nunes *et al.* (2006) resulted in the ability of squid liver meal and whole squid protein hydrolysate to stimulate feeding response in *L. vannamei*. Positive observations were also supported by other studies related to squid oil (Fenucci *et al.*, 1980; Cordova-Murueta and Garcia-Carreno, 2002). Despite the high-protein contents in squid meal, the increased amount of squid oil also improved feeding attractants in *L. vannamei*. Previously, the inclusion of squid meal in feed diet increased the growth of shrimps due to high consumption of nutritive feeds (Smith *et al.*, 2005). However, high inclusion of squid oil in the feed is not always improved shrimp attractants (Smith *et al.*, 2005; Cordova-Murueta and Garcia-Carreno, 2002). To save the cost, the inclusion of feeding attractant at 0.5-5.0% in the shrimp formulated diet would be recommended for the optimum growth, depending on their attractant types, compositions, and shrimp species (Cordova-Murueta and Garcia-Carreno, 2002). Nevertheless, the lower inclusion with high growth performance is preferred as it can save cost and feeding time.

3.3 Water Quality Analysis

The water temperature ranged from 27.52-28.16°C, dissolved oxygen from 6.98-7.00 mg/L, pH from 7.90-7.81 and salinity below 35.00ppt were all within the satisfactory range for the optimal growth of shrimp (Table 5) (Ariadi *et al.*, 2019). Results indicated that the new formulation diets maintained the optimal condition for shrimps' growth. These values are important particularly for shrimp anaerobic respiration and oxidation-reduction process occurred in water or ponds (Boyd, 2000).

4. Conclusion

In conclusion, this study shows that the addition of 2.00% and 3.00% CMC binder in shrimp pellets were recommended, as it provides good water stability compared to other treatments. Meanwhile, the inclusion of squid oil at 3.00% was markedly improved the feed attraction in cultured *L. vannamei*; which might affect growth performance through their high-protein properties. Both of these criteria are important to meet shrimp physiological requirements and to avoid nutrient leaching into the aquatic environment which might create other water quality issues and stunted growth among cultured *L. vannamei*.

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Authors' Contributions

All authors have contributed to the final manuscript. The contribution of each author as follows, Siti Jalilah Mohamad; the first author, conducted the experiments, data analysis, and wrote the manuscript. Ahmad Farhan Zailudin; helped in conducted the experiments. Nor Asma Husna Yusoff and Mohd Ihwan Zakariah; involved in data analysis and wrote the manuscript. Marina Hassan; initiated the project and was greatly contributed in data analysis and manuscripts' writing.

Conflict of Interest

The authors declare that there is no conflict of interest present in this study.

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