



Special Issue (UniSZA), Malaysia:

ICAFT 2023: 3rd International Conference on Agriculture, Animal Sciences & Food Technology

Effects of Crustacean Waste as Feed on Growth Performances of Siamese Fighting Fish (*Betta splendens*)

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Abstract | The Siamese fighting fish (*Betta splendens*) are widely known as essential and demanded ornamental fish in Malaysia. This fish is attractive and elegant that it is being cultured domestically. Meanwhile, crustacean crab is produced about 40 % and it being discharged about 60 % to 80 % as biomass into the environment. As this waste contains protein that could enhance growth rate, it could be a good choice to be used as alternate source of feed for fighting fish. The objectives of this study are to determine proximate composition in formulated fish feed using crustacean waste and to examine effect of crab waste in fish feed on growth performances on *Betta splendens*. A total of 54 fishes with average weight (± 0.035 g) was used for 20-days feeding trial using six treatments in triplicates namely TC (100% krill; 0% crab waste), T1 (80% krill; 20% crab waste), T2 (60% krill; 40% crab waste), T3 (40% krill; 60% crab waste), T4 (20% krill; 80% crab waste) and T5 (0% krill; 100% crab waste) respectively. Growth indices such as Body Weight Gain (BWG), Feed Conversion Ratio (FCR), Feed Intake (FI), and Survival Rate (SR) were observed every 10 days while water quality parameters like pH, dissolved oxygen, and temperature were taken every 4 days for monitoring purpose. It can be concluded that T1 (80 % krill 20 % crab waste) with high protein (30.02 %) and low lipid (5.43%) is best treatment that led to good FCR and BWG for small fish.

Received | February 22, 2024; **Accepted** | August 06, 2024; **Published** | November 01, 2024

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Citation | Ramasamy, S.N.R., A. Kamu and C.F. Komilus. 2024. Effects of crustacean waste as feed on growth performances of Siamese fighting fish (*Betta splendens*). *Sarhad Journal of Agriculture*, 40(Special issue 1): 161-172.

DOI | <https://dx.doi.org/10.17582/journal.sja/2024/40/s1.161.172>

Keywords | *Betta splendens*, Crab waste, Proximate composition, Growth performances



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Introduction

The ornamental fish industry has emerged as a specialized market both nationally and globally. This worldwide sector is marked by the amalgamation of numerous species sourced from various developing

countries in the tropics, contributing to a lucrative demand on wholesale platforms such as Singapore and Hong Kong. Notably, Brazil, Colombia, Indonesia, Malaysia, Nigeria, Peru, Sri Lanka, and several European countries are recognized as key wholesale market regions, serving as primary suppliers of

freshwater ornamental species (Dey, 2016). According to a report in the Journal of New Straits Times on 1 July 2022, the export value of Siamese fighting fish has reached an impressive RM 370 million annually. Given the escalating demand for *Betta splendens* over time, the continued production of these ornamental fish has become imperative and holds the potential to serve as an alternative income source within the aquaculture industry (Gonzales *et al.*, 2022). Imported fish feeds which are commonly used as pose challenges due to their high cost and varying nutritional components across different brands. This has resulted in breeders expressing uncertainty about the ornamental fish feed brands, leading to a heightened demand for feeds in the market.

Crustaceans are a popular seafood choice in Malaysia, particularly in the peninsular region where locals have increasing access to seafood markets. A growing interest among scientists revolves around repurposing crustacean waste as an alternative source of both feed and income in the aquaculture and fish industry. According to Lanka (2021), millions of crustaceans are harvested annually for consumption, providing a protein source for the local population. However, more than half of the total mass of crustaceans including non-edible elements such as shells is discarded as waste. The challenge of managing seafood waste is especially significant in coastal regions where large quantities of waste are generated. Disposal of this waste poses a crucial issue for both local communities and the seafood processing industry (Zhao *et al.*, 2022). Often, leftover seafood or crustacean waste is either discarded on land, dumped into the ocean, or left untreated posing a substantial threat to the environment and human health. Despite the biodegradability of biological waste, excessive dumping leads to unpleasant odors and a prolonged decomposition process. A more effective and sustainable solution to this problem involves utilizing discarded crustacean shell waste to create value-added products (Premasudha *et al.*, 2017). In Malaysia, there is a prevalent practice among locals to dispose of crustacean waste without realizing its potential for reuse. The limited exposure and knowledge about the beneficial reuse of crustacean waste contribute to the prevailing trend of discarding this valuable resource.

In a biochemical context, crustacean waste is rich in chitin which identified as the most abundant molecular polymer compound (Muthu *et al.*, 2021).

The presence of biopolymers particularly substantial amounts of chitin, fosters internal biological activities such as immune responses and antioxidant functions (Hamed *et al.*, 2016). Various studies have demonstrated the inclusion of chitin in fish feed to achieve consistent growth performance in fish (Wang *et al.*, 2020). Notably, crustacean waste such as that from mud crab (*Scylla serrata*) and marine crab (*Portunus sanguinolentus*), contains a noteworthy concentration of carotenoids (Hooshmand *et al.*, 2017). Carotenoids, with biological properties like astaxanthin (ASX) play a role in enhancing physiological changes, particularly in growth metabolism (Nakano and Wiegertjes, 2020). This research represents a significant stride towards utilizing crustacean waste as a component in the feed for ornamental fish especially Siamese fighting fish (*Betta splendens*). Repurposing crustacean waste for fish feed not only minimizes the disposal of seafood waste but also holds promise in enhancing growth performances in Betta fish. This study aims to reduce global crab waste disposal by approximately 40 percent. The primary focus is on elevating the growth performances of *Betta splendens* because of protein content in crustacean crab waste from 15 percent to 50 percent can be utilized firmly. This incorporation of crab waste in fish feed has the potential to augment the growth metabolism reactions of *Betta splendens*.

Materials and Methods

Siamese fighting fish

A regional fighting fish breeder and supplier in Perak, Malaysia provided about 200 tails of Siamese fighting fish juvenile. Fish were carefully enclosed within a plastic bag comprising a gas mixture consisting of 25 % oxygen and 75 % percent water. They were then transported by car to Aquatic II Laboratory at Universiti Sultan Zainal Abidin (UNISZA), Besut, Terengganu. Fish were placed into rectangular aquarium tank measuring about (61.5cm x 32cm x 33.5cm) with half-filled dechlorinated tap water from storage tank. Those fish underwent a two-week acclimatization period under laboratory conditions with room temperature (12 light:12 Dark). Throughout this period, they were nourished with a commercial pelleted diet (Corporate Social Responsibility Fish Feed Sdn. Bhd). The feed contains 30% crude protein, 3 percent crude fat, 2% crude fibre and 10% moisture. Subsequently, after two weeks, 54 tails of betta fish were separately placed individually into a pet cup of 300 ml. The pet cup has a height of 4 inches, an opening diameter of 8 cm, and

a bottom diameter of 7 centimeter.

Crab shell preparation

Mud crabs (*Scylla serrata*) with total weight of 2 kg were purchased from Malaysian Fisheries Development Authority (LKIM) Fisheries Complex, Kuala Besut, Terengganu. Crabs that were purchased were brought to UniSZA's Feeding and Nutrition Laboratory and was placed in freezer at 3°C. The crabs were then intensively boiled until the crab flesh were thoroughly cooked. The following procedure to take out shell meat was according to Pandharipande and Prakash (2016). Crab meat was taken out of the shell and placed into oven about 50°C for an hour and a half until it was completely dried. Then, dried crab waste was blended into small pieces and followed by milling them using dry mills into fabric powder before sieved thoroughly to get even size powder about 0.30 – 0.35 nm. Fabric powder was filled into container and placed in chiller to avoid rancidity.

Fish pelleting

Fish pellet was prepared using Krill, Mud Crab Waste, Wheat Flour, Vitamin Mix, Mineral Mix, Sago Powder, Vitamin C, Choline and Palm Oil (Table 1). Preparation was carried out using grate technique according to Nik-Sin and Shapawi (2017). After the grinded, the mixture was clumped together as a dough to form a ball and wrapped with aluminium foil. The dough was then exposed to steam by using streamer machine for 15 minutes and was placed inside oven for one hour at 40° C. After steaming, it was immediately stored in freezer until it was grated using normal grater for further use.

Feeding trial

These 6 treatments have total fish about 54 tails. Fish was placed individually in each triplicate's treatment. Fishes were fed twice daily at 7 to 8 am in the morning

and 4 to 5 pm in the evening. About 3 percent of the fish's total weight was fed during the feeding study. Feeding experiment was carried out for 20 days. There were 2 phases of feeding treatments; phase 1 is starter diet feed (0–10 days) and phase 2 is finisher diet (0 – 20 days). Data was collected every 10 days. Water quality was also taken every four days by using YSI ProQuatro Multiparameter that includes dissolve oxygen (DO), pH, temperature (°C) and ammonia.

Proximate analysis

Proximate composition of fish diets was analysed according to Association of Official Analytical Chemists' guidelines (AOAC) 1997 as clarified by Asaduzzaman *et al.* (2018) and Arshad *et al.* (2021). Proximate analysis includes crude protein, crude lipid, crude fibre, moisture and ash.

Growth performances

This experiment is revised according to the research that have been previously done by Sankian *et al.* (2017). The period of this experiment was about 3 weeks (20 Days). The fishes were given diets at different percentage of crab shell waste component which initially start from 0%, 20%, 40%, 65%, 80% and 100%. Betta fish was taken out from the treatment cup before length and weight were measured. During the experiment period, the water quality such as dissolve oxygen (DO), pH, temperature (°C), and Ammonia (NH₃) was checked thoroughly every 4 days to ensure good water quality. When the treatment cup was found to be cloudy, it was immediately filtered out about 10 % of water and replaced with clean water. Feed was given twice per day at 9.00 am and 4.00 pm. Finally, fish was weighed, and the required growth variable was recorded at the end of this experimental study. Food conversion ratio (FCR), feed intake (FI), survival rate (SR), and body weight gain (BWG) were calculated and measured.

Table 1: Amount of fish feed formulation for Siamese fighting fish (*Betta splendens*).

Ingredients (g)	TC (0% cw)	T1 (20% cw)	T2 (40% cw)	T3 (60% cw)	T4 (80% cw)	T5 (100% cw)
Mud crab shell waste	0.00	10.0	20.0s	30.0	40.0	50.0
Krill	50.0	40.0	30.0	20.0	10.0	0.00
Wheat flour	15.0	15.0	15.0	15.0	15.0	15.0
Vitamin mix	7.0	7.0	7.0	7.0	7.0	7.0
Mineral mix	7.0	7.0	7.0	7.0	7.0	7.0
Vitamin C	0.5	0.5	0.5	0.5	0.5	0.5
Sago powder	15.0	15.0	15.0	15.0	15.0	15.0
Choline	0.5	0.5	0.5	0.5	0.5	0.5
Palm oil	5.0	5.0	5.0	5.0	5.0	5.0
Total	100.0	100.0	100.0	100.0	100.0	100.0

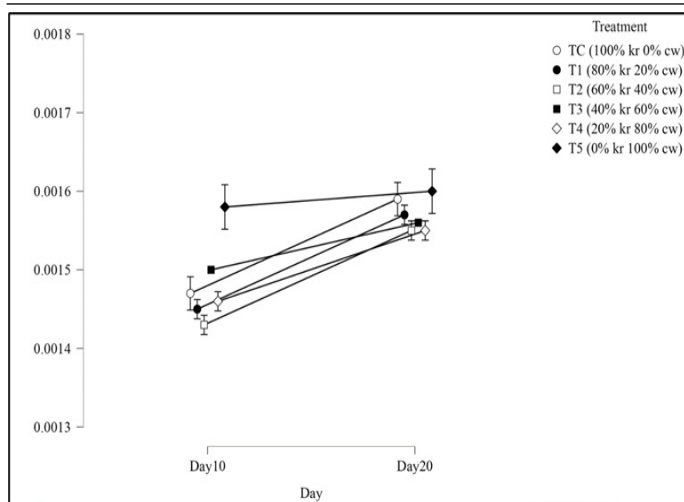


Figure 1: The Body Weight Gain (BWG) of Siamese fighting fish for overall treatments throughout 20 days between 0.001 g - 0.011 g.

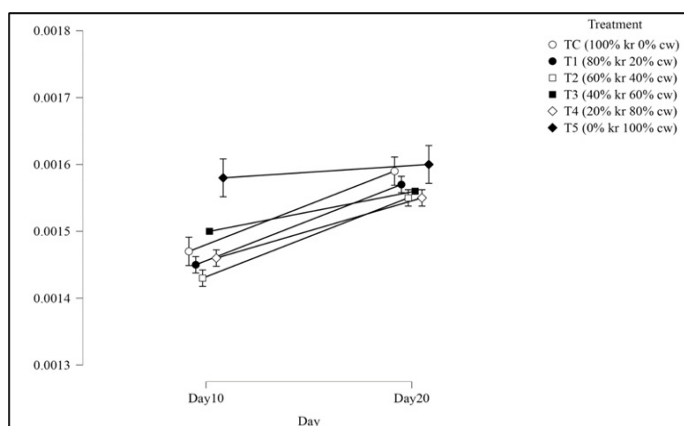


Figure 2: Feed Intake (FI) of Siamese fighting fish for overall treatments throughout 20 days between 0.0014 g - 0.0016 g.

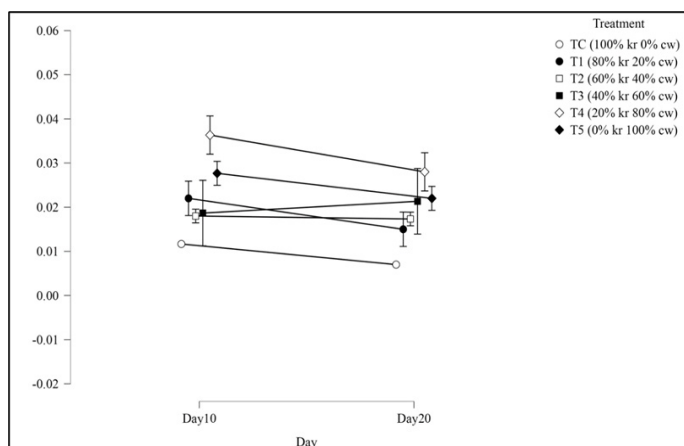


Figure 3: Feed Conversion Ratio (FCR) of Siamese fighting fish for overall treatments throughout 20 days is between 0.01 g - 0.04.

Analysis data

The substantial difference ($P < 0.05$) between each treatment was ascertained using one-way analysis of variance (ANOVA) and the statistical t-test (Hamdan *et al.*, 2016). The growth performances are a continuous variable thus the measurement of size is used to investigate them which for each trial, the

difference between the control and treatment means are normalized by the standard deviations and the effect size also determined using Cohen's d (CD) (Fagnon *et al.*, 2020). In this study, the statistical analysis was performed using SPSS Windows 27 software package to examine the differences between the control and the treatment groups at a 5% significance level. All experiments treatment were conducted in triplicates. Data were subjected to one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison tests to compare the mean values. Statistical significance was determined at a p-value threshold of less than 0.05. Results were presented as mean values $\pm$ standard deviation (S.D) (Kamarudin *et al.*, 2018).

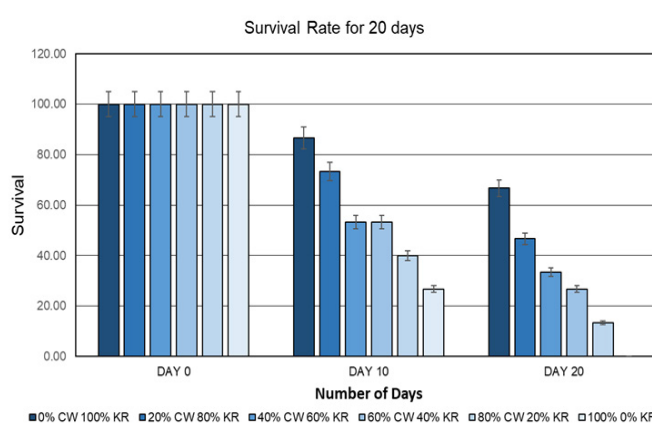


Figure 4: Survival Rate (SR) of Siamese fighting fish (Day 0; All treatments is 100%, Day 10; TC:86.67%, T1:73.33%, T2:53.33%, T3:53.33%, T4:40.00%, T5:26.67% and Day 20; TC:66.67%, T1:46.67%, T2:33.33%, T3:26.67%, T4:13.33% and T5:0.00%).

Results and Discussion

Proximate analysis

During the experiment period, Siamese fighting fish were exposed to 6 different treatment of diets which were TC (100% kr 0% cw), T1 (80% kr 20% cw), T2 (60% kr 40% cw), T3 (40% kr 60% cw), T4 (20% kr 80% cw) and T5 (0% kr 100% cw). The Kruskal-Wallis test (Table 4) demonstrated no statistically significant difference in moisture levels between the treatment groups ($H(5) = 10.479$, $p > 0.05$). Conversely, significant differences were observed in protein ($H(5) = 16.579$, $p < 0.05$), fibre ($H(5) = 16.392$, $p < 0.01$), and ash ($H(5) = 16.579$, $p < 0.01$) among the treatment groups. Table 3 shows that crude protein, moisture, ash and fibre showed significant differences among treatments while crude lipid did not show any significant differences among treatments. Crude protein for treatment control showed highest value

which was 35.02 % while treatment 5 with 100 % crab waste had only 13.55 %. Krill has high quality protein composition which includes all the essential amino acids (EAAs) and its lipid composition where it is rich in omega-3 long-chain poly unsaturated fatty acids (Thøgersen *et al.*, 2021). The percentage of protein composition reduces from treatment control to treatment 5 due to its combination value of krill and mud crab waste. From the experiment, the initial protein content of Krill was about 63.82 % and Mangrove Crab was 18.39% (Table 2). The utilization of 20 % crab waste (T1) still increased the protein content which was about 30.02%. It was found that a diet with 35% crude protein is ideal for improving growth rates and feeding efficiency in these fish in terms of their protein requirement. From the experiment, treatment 1 to treatment 5 do not achieved the necessary protein requirement of Siamese fighting fish and the closest to 35 % is treatment 1 with 20 % of crab waste. Thus, all treatments except control could not fulfil the requirements. This indicates the lower protein content influences growth factor of fish (Mignani, 2022).

The results of the one-way ANOVA indicated no statistically significant difference in lipid levels among the treatment groups ($F(5, 12) = 2.893$, $p > 0.05$). However, due to violations in the homogeneity of variance for protein, moisture, fibre, and ash, non-parametric Kruskal-Wallis tests were conducted.

Lipids are potential high energy nutrient that can be utilized as partial substitution of protein in fish feed. Lipids also supply about twice as energy content like protein and carbohydrate. The result shows that krill and crab waste did not show any differences which suggest that crab waste also can be used as lipid source to replace krill in market trend. As 100 % crab waste treatment shows higher content of lipid compared to treatment control, it can be used as a potential replacement for lipid source. Lv *et al.* (2022) has stated that there was a significant amount of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) in the lipids taken out of crab shells which are linked to reduced inflammation and enhanced cognitive function. Crab shell can also be intensively used or approached as a modern sustainable resource for lipid source replacement and catalyst materials (Yuliana *et al.*, 2021). There are significant differences ($p < 0.05$) of moisture between treatment control (5.31 ± 0.04) and other treatments T1 (6.43 ± 0.04), T2 (6.43 ± 0.26), T3 (6.53 ± 0.12), T4 (6.59 ± 0.37) and

Table 2: Proximate composition of mud crab waste and krill.

Proximate (%)	Mud crab (<i>Scylla serrata</i>)	Krill
Crude lipid (CL)	0.04 ± 0.03^a	3.18 ± 0.14^a
Crude protein (CP)	18.39 ± 0.09^a	63.82 ± 0.88^a
Moisture	24.04 ± 0.36^a	24.98 ± 3.38^a
Ash	1.17 ± 0.31^a	0.66 ± 0.23^a
Fibre	6.94 ± 0.34^a	3.35 ± 0.83^a

Table 3: Proximate composition of feed treatments. All the data are a mean $\pm$ SD of replicates.

Treatment	100% kr 0% cw	80 % kr 20 % cw	60 % kr 40 % cw	40 % kr 60% cw	20 % kr 80% cw	0 % kr 100% cw
CL	6.41 ± 1.44^a	5.43 ± 0.66^a	5.97 ± 1.37^a	7.49 ± 1.28^a	8.53 ± 0.59^a	7.82 ± 1.57^a
CP	35.02 ± 0.40^a	30.02 ± 0.04^b	27.00 ± 0.79^c	22.86 ± 0.05^d	17.76 ± 0.02^e	13.55 ± 0.28^f
Moisture	5.31 ± 0.04^a	6.43 ± 0.04^b	6.43 ± 0.26^b	6.53 ± 0.12^b	6.59 ± 0.37^b	6.73 ± 0.07^b
Ash	15.76 ± 0.18^a	19.21 ± 0.08^b	21.74 ± 0.46^c	26.50 ± 0.17^d	31.08 ± 0.19^e	35.32 ± 0.09^f
Fibre	3.12 ± 0.24^a	3.42 ± 0.09^a	4.48 ± 0.41^b	5.40 ± 0.23^b	7.12 ± 0.67^c	8.12 ± 0.15^d

Values are means $\pm$ SD of triplicates measurements. Treatments sample followed by a, b, c, e and f in the same column represent the significant difference at 5% level according to Tukey HSD test at $\alpha = 0.05$.

Table 4: Comparison test results.

Treatment	Protein	Moisture	Lipid	Fibre	Ash
Levene's test result	$F(5,12) = 6.197^*$	$F(5,12) = 5.668^*$	$F(5,12) = 1.243$	$F(5,12) = 4.639^*$	$F(5,12) = 4.293^*$
One-way ANOVA's result	NP	NP	$F(5,12) = 2.893$	NP	NP
Kruskal-Wallis test's result	$H(5) = 16.579^*$	$H(5) = 10.479$	-	$H(5) = 16.392^{**}$	$H(5) = 16.579^{**}$

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. NP: Because the homogeneity of variance was violated, one-way ANOVA was not performed. As a result, the Kruskal-Wallis test, a non-parametric test, was carried out.

Table 5: *Post Hoc comparisons – Treatment x Time.*

		Mean difference	SE	t	P _{bonf}	
TC, (100%, kr, 0%, cw), Day10	T1, (80%, kr, 20%, cw), Day10	0.002	0.001	2.214	1.000	
	T2, (60%, kr, 40%, cw), Day10	0.002	0.001	2.214	1.000	
	T3, (40%, kr, 60%, cw), Day10	0.002	0.001	2.214	1.000	
	T4, (20%, kr, 80%, cw), Day10	0.004	0.001	4.111	0.033	*
	T5, (0%, kr, 100%, cw), Day10	0.005	0.001	4.427	0.016	*
	TC, (100%, kr, 0%, cw), Day20	-0.005	8.278×10 ⁻⁴	-5.638	0.007	**
	T1, (80%, kr, 20%, cw), Day20	1.000×10 ⁻³	0.001	0.949	1.000	
	T2, (60%, kr, 40%, cw), Day20	0.002	0.001	1.581	1.000	
	T3, (40%, kr, 60%, cw), Day20	0.002	0.001	1.897	1.000	
	T4, (20%, kr, 80%, cw), Day20	0.003	0.001	3.162	0.311	
	T5, (0%, kr, 100%, cw), Day20	0.005	0.001	4.427	0.016	*
T1, (80%, kr, 20%, cw), Day10	T2, (60%, kr, 40%, cw), Day10	1.473×10 ⁻¹⁷	0.001	1.397×10 ⁻¹⁴	1.000	
	T3, (40%, kr, 60%, cw), Day10	9.695×10 ⁻¹⁸	0.001	9.197×10 ⁻¹⁵	1.000	
	T4, (20%, kr, 80%, cw), Day10	0.002	0.001	1.897	1.000	
	T5, (0%, kr, 100%, cw), Day10	0.002	0.001	2.214	1.000	
	TC, (100%, kr, 0%, cw), Day20	-0.007	0.001	-6.641	< .001	***
	T1, (80%, kr, 20%, cw), Day20	-0.001	8.278×10 ⁻⁴	-1.611	1.000	
	T2, (60%, kr, 40%, cw), Day20	-6.667×10 ⁻⁴	0.001	-0.632	1.000	
	T3, (40%, kr, 60%, cw), Day20	-3.333×10 ⁻⁴	0.001	-0.316	1.000	
	T4, (20%, kr, 80%, cw), Day20	0.001	0.001	0.949	1.000	
	T5, (0%, kr, 100%, cw), Day20	0.002	0.001	2.214	1.000	
T2, (60%, kr, 40%, cw), Day10	T3, (40%, kr, 60%, cw), Day10	-5.036×10 ⁻¹⁸	0.001	-4.777×10 ⁻¹⁵	1.000	
	T4, (20%, kr, 80%, cw), Day10	0.002	0.001	1.897	1.000	
	T5, (0%, kr, 100%, cw), Day10	0.002	0.001	2.214	1.000	
	TC, (100%, kr, 0%, cw), Day20	-0.007	0.001	-6.641	< .001	***
	T1, (80%, kr, 20%, cw), Day20	-0.001	0.001	-1.265	1.000	
	T2, (60%, kr, 40%, cw), Day20	-6.667×10 ⁻⁴	8.278×10 ⁻⁴	-0.805	1.000	
	T3, (40%, kr, 60%, cw), Day20	-3.333×10 ⁻⁴	0.001	-0.316	1.000	
	T4, (20%, kr, 80%, cw), Day20	1.000×10 ⁻³	0.001	0.949	1.000	
	T5, (0%, kr, 100%, cw), Day20	0.002	0.001	2.214	1.000	
T3, (40%, kr, 60%, cw), Day10	T4, (20%, kr, 80%, cw), Day10	0.002	0.001	1.897	1.000	
	T5, (0%, kr, 100%, cw), Day10	0.002	0.001	2.214	1.000	
	TC, (100%, kr, 0%, cw), Day20	-0.007	0.001	-6.641	< .001	***
	T1, (80%, kr, 20%, cw), Day20	-0.001	0.001	-1.265	1.000	
	T2, (60%, kr, 40%, cw), Day20	-6.667×10 ⁻⁴	0.001	-0.632	1.000	
	T3, (40%, kr, 60%, cw), Day20	-3.333×10 ⁻⁴	8.278×10 ⁻⁴	-0.403	1.000	
	T4, (20%, kr, 80%, cw), Day20	0.001	0.001	0.949	1.000	
	T5, (0%, kr, 100%, cw), Day20	0.002	0.001	2.214	1.000	
T4, (20%, kr, 80%, cw), Day10	T5, (0%, kr, 100%, cw), Day10	3.333×10 ⁻⁴	0.001	0.316	1.000	
	TC, (100%, kr, 0%, cw), Day20	-0.009	0.001	-8.538	< .001	***
	T1, (80%, kr, 20%, cw), Day20	-0.003	0.001	-3.162	0.311	
	T2, (60%, kr, 40%, cw), Day20	-0.003	0.001	-2.530	1.000	
	T3, (40%, kr, 60%, cw), Day20	-0.002	0.001	-2.214	1.000	
	T4, (20%, kr, 80%, cw), Day20	-0.001	8.278×10 ⁻⁴	-1.208	1.000	
	T5, (0%, kr, 100%, cw), Day20	3.333×10 ⁻⁴	0.001	0.316	1.000	

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		Mean difference	SE	t	P _{bonf}	
T5, (0%, kr, 100%, cw), Day10	TC, (100%, kr, 0%, cw), Day20	-0.009	0.001	-8.854	< .001	***
	T1, (80%, kr, 20%, cw), Day20	-0.004	0.001	-3.479	0.149	
	T2, (60%, kr, 40%, cw), Day20	-0.003	0.001	-2.846	0.640	
	T3, (40%, kr, 60%, cw), Day20	-0.003	0.001	-2.530	1.000	
	T4, (20%, kr, 80%, cw), Day20	-0.001	0.001	-1.265	1.000	
	T5, (0%, kr, 100%, cw), Day20	-6.325×10 ⁻¹⁹	8.278×10 ⁻⁴	-7.641×10 ⁻¹⁶	1.000	
TC, (100%, kr, 0%, cw), Day20	T1, (80%, kr, 20%, cw), Day20	0.006	0.001	5.376	0.002	**
	T2, (60%, kr, 40%, cw), Day20	0.006	0.001	6.008	< .001	***
	T3, (40%, kr, 60%, cw), Day20	0.007	0.001	6.325	< .001	***
	T4, (20%, kr, 80%, cw), Day20	0.008	0.001	7.589	< .001	***
	T5, (0%, kr, 100%, cw), Day20	0.009	0.001	8.854	< .001	***
T1, (80%, kr, 20%, cw), Day20	T2, (60%, kr, 40%, cw), Day20	6.667×10 ⁻⁴	0.001	0.632	1.000	
	T3, (40%, kr, 60%, cw), Day20	1.000×10 ⁻³	0.001	0.949	1.000	
	T4, (20%, kr, 80%, cw), Day20	0.002	0.001	2.214	1.000	
	T5, (0%, kr, 100%, cw), Day20	0.004	0.001	3.479	0.149	
T2, (60%, kr, 40%, cw), Day20	T3, (40%, kr, 60%, cw), Day20	3.333×10 ⁻⁴	0.001	0.316	1.000	
	T4, (20%, kr, 80%, cw), Day20	0.002	0.001	1.581	1.000	
	T5, (0%, kr, 100%, cw), Day20	0.003	0.001	2.846	0.640	
T3, (40%, kr, 60%, cw), Day20	T4, (20%, kr, 80%, cw), Day20	0.001	0.001	1.265	1.000	
	T5, (0%, kr, 100%, cw), Day20	0.003	0.001	2.530	1.000	
T4, (20%, kr, 80%, cw), Day20	T5, (0%, kr, 100%, cw), Day20	0.001	0.001	1.265	1.000	

Post hoc comparison with Bonferroni correction was performed on the interaction effect between respective treatments.

T5 (6.73±0.07). Treatment from 20 % to 100 % crab waste shows no significant difference indicating that optimized moisture content can be in range of 6.40 % to 6.73%. From this experiment, the dry feed was made for Siamese fighting fish. Floatability of feed also directly proportional with the composition of moisture content (Khater *et al.*, 2014). Ash content shows that treatment control has the lowest value compared to other treatments indicating significant differences among treatments. The result shows high ash content in treatments especially in treatment 5. The crude fibre also shows there are significant differences among treatments. Fish grow poorly because other elements in the diet are less digestible due to high fibre and ash content. Sun *et al.* (2019) stated that fish may benefit from modest dietary fibre concentrations (3% to 5%), whereas too much fibre may lower the apparent digestibility of dry matter and the efficacy of other nutrients. Results show that treatment 3 to treatment 5 have more than 5 % crude fibre indicating fibre content from mixture or combination of two sources of protein-based ingredient which are krill and mud crab waste. Bhilave *et al.* (2010) confirmed that fish feeds typically contain less crude fibre than 7% of the diet to reduce the quantity of undigested

material entering the culture system.

Post hoc comparisons (Treatment × time)

Bodyweight gain (BWG): The results of the between-within repeated ANOVA revealed that there was a significant interaction effect between the treatment and the time, $F(5, 12) = 4.216$, $p < 0.05$., on the body weight gain (BWG) (Table 5). The BWG of Siamese fighting fish shows significant differences ($p < 0.05$) between treatment control (100 % krill and 0 % crab waste) and other respective treatments comparatively. The BWG until Day 10 shows treatment 1 (80 % krill and 20 % crab waste) until treatment 3 (40 % krill and 60 % crab waste) are partially significantly difference with control (Figure 1). This indicate that weight gain until day 10 is partially proportional with growth stimulation. Treatment control shows highest BWG at day 10 and followed by treatment 1 until 3. The lowest BWG shows Treatment 5 (0 % krill and 100 % crab waste). At day 20, the BWG has changed massively in which treatment 1 started to show significant differences between treatment controls. T1 shows slight growth at day 20 which justifies the addition of 20 % crab waste is functional and inhibition for medium growth. In fact, previously

the proximate composition has indicated that protein content for TC (35 %) and T5 (13%) is influenced with the growth rate. Radhakrishnan and Mannur (2020) had illustrated that only protein is a key nutrient that will accurately reflect the anabolic process (growth) of the fish's body. Fish utilizes amino acids as energy substrates which is one of their key properties. Thus, in fish, amino acids are preferred as an energy source over glucose. However, an excessive amount of protein will be used to create new tissues with the leftover protein being turned into energy. This explains on the other treatments; T2 to T5 having less growth indicator and 100 % crab waste shows there is no growth of BWG until Day 20. This justifies less protein value affects the hormonal growth. James and Sampath (2012) have illustrated that protein levels over the ideal range resulted in slower growth in *B. splendens* and 35% protein is thought to be the ideal range for this fish too. The BWG percentage is not very steep for all treatments as this could be influenced by the ash content to especially for T5 which had about 35.32 % of ash level. A study by Keremah (2013) has stated reduction on growth performances could be brought on by lower feed consumption which intuitively brought stress on by high ash levels, and somewhat reduces fish survival in diets that containing crab waste. Quantity and quality of dietary protein affects the response to growth. The initial value of fish weight is about 0.030 g to 0.040 g. This shows *Betta splendens* is extremely small in weight size which they do required minimum dietary protein. Craig *et al.* (2017) have pointed out that small fish or juveniles require regular feedings or typically more than a high-protein diet. Small fish must be fed almost continuously and every hour due to their high energy requirements. Overfeeding them is not as problematic compared to overfeeding larger fish because small juvenile fish only need a modest amount of feed in relation to the amount of water in the culture system. This explains that BWG growth on smallest fish is distinct compared to big fishes.

Feed intake (FI)

The outcomes of the repeated measures ANOVA revealed a noteworthy primary influence of time on feed intake (FI) ($F(1, 12) = 82.618, p < 0.001$). However, there were no significant effects observed for treatment ($F(5, 12) = 2.880, p > 0.05$) or the interaction between treatment and time ($F(5, 12) = 2.994, p > 0.05$). These findings suggest that variations in feed intake (FI) were predominantly influenced by time alone. Feed intake shows there are no significant

differences among treatments. Feed intake as for day 20 is same about 0.002 g from Treatment control to Treatment 5 (Figure 2). The fish is small thus the feed intake value is really small. This lowest feed intake values indicates and relevant to weight gain by respective treatments. Lemos *et al.* (2014) has illustrated that fish feed intake is primarily regulated by energy concentration or if specific nutrients such as macronutrients (proteins, lipids, and carbohydrates) and micronutrients (vitamins and minerals) are to imperatively understand the mechanisms underlying fish feed intake regulation. It seems that *Betta splendens* which regulates its feed intake based on the type of protein it consumes rather than just the amount of protein, indicating a greater propensity towards food consumption driven by organoleptic qualities. The rise in feed intake seen in this fish fed diets lends support to this notion. It was observed that there was no food left in treatment cup which indicate the feed is fully consumed by Siamese fighting fish. In this experiment, overfeeding was avoided to ensure fish health is secured and remained at optimum level. The waste which is accumulated in treatment pet cup is known as overfeeding. Additionally, it causes bacterial loads to grow, low dissolved oxygen (DO) levels, low biological oxygen demand (BOD), and water pollution. This fish is only given the amount of food which they can swiftly ingest about in less than five to ten minutes. The feeding method also adhered to a sound general rule of thumb which is to give the fish around 80% of what they desire to eat until they are satisfied. During this experiment, this fish was closely observed as it was consuming the given feed. Contrary to the findings of Kumaran *et al.* (2021) which suggested that dietary supplementation with chitosan nanoparticles derived from crab waste has led to a notable improvement in the growth performance of Nile tilapia. This supports the crab waste is globally being approached as sustainable way of growth inhibitor. *Betta splendens* may not possess the ability to fully digest crab waste as a protein source because the crab shell size is distinctive. In this experiment, crab waste was grinded in fabric size which the result shows no differences in terms of feed intake.

Feed conversion ratio (FCR)

All the treatments show that the FCR value is between the ranges of 0.01 to 0.04 (Figure 3). TC (100 % krill and 0 % crab waste), T1 (80 % krill and 20 % crab waste), T2 (60 % krill and 40 % crab waste), T3 (40 % krill and 60 % crab waste) and T5 (0 %

krill and 100 % crab waste) has lowest FCR value at the end of Day 20. The development of suitable feeds depends critically on obtaining accurate FCR information regarding locally accessible ingredients (Tun and Swe, 2019). However, due to their distinct eating habits of fishes, it might be difficult to formulate suitable artificial feed for key fish species which further complicates the task. Craig and Kuhn (2017) have illustrated that high value on evaluating feed efficiency or feed conversion ratio occurs when given cost of feed are high. It also needs to be considered that the species, size, activity level, activity level of the fish, ambient circumstances, and the culture system used can all have an impact on the feed conversion ratio. This can be the reason for variation FCR readings in this study. Another reason for the smallest FCR in all treatment's fishes are due to size of fish which is incredibly small. The fish completely consumed the given feed which indicates that betta does like formulated feed. In fact, high consumption of feed intake has influenced the Feed Conversion Ratio (FCR) in which directly related to the growth of betta fish of each respective treatments. With higher FCR, the effect on protein content towards fish growth is comparatively involved. FCR is also affected by overfeeding. Heriansah *et al.* (2022) has confirmed that consuming too much feed increases the amount of energy required for digestion which might decrease the amount of nutrients available for growth.

Survival rate (SR)

At the beginning of experiment, all treatments from 0 % crab waste until 100 % crab waste have shown 100 % survival rate (Figure 4). On Day 10, survival rate has reduced linearly from TC (86.7%), T1 (73.3 %), T2 (53.3%), T3 (53.3%), T4 (40.0%) and T5 (26.7%). Even though small fish are very sensitive to protein requirements which highly signifies outcome result of survival. At Day 20, treatment 2 (60 % krill 40 % crab waste), treatment 3 (40 % krill 60 % crab waste), treatment 4 (20 % krill 80 % crab waste) and treatment 5 (0 % krill 100 % crab waste) shows survival rate less than 40%. There are several studies which support this hypothesis. Fadel *et al.* (2021) has conducted a study on influence of varied dietary protein levels on survival rate and growth performance of Guppies (*Poecilia reticulata*). It shows that nutrients present in a fish food with a high protein level satisfy the guppy's dietary needs and help it grow more quickly. High level condition factor

suggested that synthetic peptide amino acids can be used to enhance the healthy growth of fish, which is ideal for decorative fish farms. Even with highest protein content of TC (100 % krill) experimental Siamese fighting fish has shown linear mortality. This could be due to environmental factor or fish early habitat distribution. Crab waste only contained small amounts of protein and lipid source which highlights the effect on survival. In addition, Lichak *et al.* (2022) has mentioned that there is minimal success with attempted treatments with small size of fish in his experiment. The high mortality of fish observed in this study confirms to Lichak *et al.* (2022) that size of fish matters in influencing high survival rate. Smallest fish are more prone to mortality factors. Some waste products were viewed as growth inhibitors and unfavorable for the multiplication of immature *Betta splendens* (Pleeging and Moons, 2017). This could be another reason for the mortality of betta fish. The ash content was found to be above 15 % for treatment 1 (100% krill) and about 35 % for treatment 5 (100% crab waste). This could be another reason that had affected the survival rate of fish. This could be due to lowering the digestion of other elements in the food which causes the fish to grow poorly (Ahmed *et al.*, 2022). From the result, treatment of more than 60 % crab waste shows better and good survival rate and could replace usage of krill in future.

Conclusions and Recommendations

This study shows that treatment 1 which consists of 80% krill and 20% crab waste, was the most effective in promoting growth, achieving a growth index of 0.002 g within 20 days. It had a high protein content of 30.02% and a low lipid content of 5.43%, leading to better body weight gain than Treatments 2 to 5 and only being outperformed by the control. The feed conversion ratio (FCR) for Treatments 1 to 3 was consistently efficient at 0.02, and feed intake was similar across all treatments indicating complete consumption of the feed by the fish. Moreover, Treatment 1 maintained a survival rate above 40%, which was higher than other treatments except for the control, making it a promising feed formulation for enhancing fish growth and survival in aquaculture. This study achieved objectives and it can be concluded that crab waste cannot fully replaced krill as protein source for *Betta splendens* but it can used as additional source of protein in fish feed. For future research, it is suggested to change size of fish to bigger ones because

small sized fish are more vulnerable to mortality in feeding trials.

Acknowledgements

This project is approved by Universiti Sultan Zainal Abidin Terengganu Animal and Plant Research Ethics Committee UAPREC/07/014. This project is supported by Ministry of High Education Malaysia under Fundamental Research Grant FRGS RR400 1/2021 (FRGS/1/2021/WAB04/UNISZA/02/2).

Novelty Statement

This research provides essential information on utilizing mud crab waste (*Scylla serrata*) as potential feed ingredient for *Betta splendens* as following sustainable approaches.

Author's Contribution

All authors contributed to the conceptualisation, methodology, data collection and analysis, interpretation of results and writing of the original draft.

Conflicts of interest

The authors have declared no conflict of interest.

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