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EFFECTS OF DIFFERENT MINERAL INCLUSIONS IN ANCHOVY BY-PRODUCT BASED FEEDS ON GROWTH PERFORMANCE OF RED CLAW CRAYFISH, *Cherax quadricarinatus*

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ABSTRACT

The purpose of this study was to evaluate the effects of different mineral inclusion in anchovy by-product (ABP) based feeds on the growth performance of red claw crayfish juveniles, *Cherax quadricarinatus*. A total of five experimental feeds with the inclusion of different mineral levels from 0-2.0% of the diet (M0, M0.5, M1.0, M1.5, & M2.0%) were fed to triplicate groups of 12 juvenile red claw crayfish with an average initial body weight of 2.20±0.10 g. There was no significant difference in terms of final body weight (g), final length (cm), weight gain (%), length gain (%), specific growth rate (%/d), and survival rate (%) of the juvenile red claw crayfish at the end of the feeding trial. Feed conversion ratio ranged from 1.60 (M0.5) to 1.76 (M1.0). Similar to the growth performance, the molting frequency was not affected by the different mineral inclusions in the feeds. Mineral inclusion in the feeds based on ABP can be reduced up to 0%, at least in a short culture period, and higher possibility of including minerals less than 2% in the feeds for juvenile red claw crayfish in a long-term culture period. Considering the good growth and survival of juvenile red claw crayfish in all treatments, the use of ABP as a source of protein and mineral in feeds for juvenile red claw crayfish is highly recommended as this will reduce the feed cost due to the much lower price of ABP. It can be concluded that the anchovy by-product meal used in the present study is a high potential ingredient to supply dietary protein and minerals in the formulated feeds for juvenile red claw crayfish.

Key words: *Cherax quadricarinatus,* crayfish, fish by-product, formulated feeds, minerals

INTRODUCTION

Cherax quadricarinatus (von Martens, 1868) or commonly known as the red claw crayfish, is a native species in the water bodies of northern Australia and Papua New Guinea (Lawrence & Jones 2002). Red claw crayfish was introduced in Malaysia during the 1980s through the ornamental aquarium trade before the commercial culture activity was observed in the southern part of the Malaysian Peninsula in 2003 (Naqiuddin *et al*., 2016; Alimon, 2003). Among the crayfish species, red claw crayfish is recognized as a high-class food with a high market price, especially in the western market (Gherardi, 2011). Apart from that, aquaculture of the red claw crayfish also benefits from its physical and biological attributes such as a simple life cycle, fast growth, omnivorous feeding behavior, and demand as an aquaria species making it an excellent aquaculture species. These attributes have prompted increasing interest in the commercial culture of crayfish in Malaysia (Naqiuddin *et al*., 2016).

Feeds are a crucial component in crayfish culture. In most cases, the formulated feed given for red claw crayfish is those formulated for other aquatic species such as penaeid shrimp, fish, and other prawn species (Manuel *et al*. 2003; Thompson *et al*. 2003a, 2003b; Kiriyakit & Suwannagate 2018). The use of raw food such as vegetables is also practiced in many crayfish farms, including in Malaysia. Therefore, efforts to develop specifically formulated feeds for red claw crayfish should be intensified. In developing formulated feed, knowledge of the nutritional requirement of the target species is required. In general, quantitative major nutrient requirements have been determined in crayfish. The requirement for micronutrients such as vitamins and minerals was assumed to follow the level required in feeds for other crustacean species which requires more in-depth research in this aspect. Crustaceans, including crayfish, go through the molting process to grow, thus requiring a sufficient amount of minerals, especially calcium in the feeds. Crayfish stores calcium ions in the form of a calcium carbonate gastrolith for the calcification of its exoskeleton after molting. They can absorb minerals from the feeds and surrounding water. Mineral requirements in an aquatic organism's

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body are influenced by the food source, environment, species, developmental stages, and physiological status. In the effort to produce cost-effective feeds, a mineral supplement was generally added at 2% of the feeds in most aquatic species. The utilization efficiency of dietary mineral elements depends on the element's availability from a feed ingredient or complete diet.

Fishery by-products are used as alternative feed ingredients in formulated animal feeds to replace fish meals. Pelagic fishes make up more than 30% of the total marine fish landings in Malaysia, with anchovies contributing 1.2% of the total marine annual capture fisheries. For many years, anchovy has been traded in Malaysia as fresh or dried products with stock available all year round in almost all fish markets, either with or without the head (Noor & Zulkepli, 2018). The unwanted heads usually end up with no specific use and reduce the profit of the anchovy suppliers. At present, the anchovy head is only considered a waste. More scientific research is needed to investigate the full potential of this by-product as an ingredient in aquaculture feed. A recent study to investigate the acceptance of tilapia juveniles on feeds formulated with the combination of anchovy by-product (ABP) meal and soybean meal revealed that the combination of anchovy head meal and soybean meal at a ratio of 1:1 produced the best growth of tilapia (Majiun, 2020). This study also revealed that anchovy by-product meal is characterized by a high mineral content of up to 50%. It is interesting to determine if the mineral supplement is still needed in the diet for red claw crayfish when using an anchovy by-product as an ingredient. No study using anchovy by-products was done in the past involving red claw crayfish to unravel the full potential of this fishery by-product. Specifically, the present study aims to investigate if mineral inclusion below recommended levels can be achieved when using ABP as a major ingredient.

Fig. 1. Experimental feed formulated based on anchovy by-products

MATERIALS AND METHODS

Diet formulation and preparation

Anchovy by-product, ABP (consisting of the head, gut, fins, bones, & some flesh) was grounded using a grinder and sieved through into fine powder form. The experimental feeds were formulated to contain different mineral premix inclusion: 0% mineral (M0), 0.5% mineral (M0.5), 1% mineral (M1.0), 1.5% mineral (M1.5) and 2% mineral (M2.0) (Figure 1). Soybean meal (SBM) was included together with ABP at a ratio of 1:1. Minerals were added at the expense of tapioca starch. Proximate analyses of the ingredients and feeds were made in triplicates according to AOAC (1999) methods. Table 1 shows the proximate composition of ABP & SBM, and feed composition. Meanwhile, the proximate composition of the experimental feeds is shown in Table 2.

Table 1. Proximate composition of ABP & SBM, and experimental feed composition (%)

Ingredients	Proximate Composition				
	Moisture	Ash	Crude protein	Crude Lipid	Dry matter
ABP	11.34±0.49	35.43±0.32	50.54±0.28	4.36±0.17	88.66±0.49
SBM	9.90±0.06	6.22±0.20	48.28±0.10	1.48±0.05	90.10±0.06
Ingredients (g/100 g)	Feed composition				
	M0	M0.5	M1.0	M1.5	M2.0
Anchovies by-products	39.05	39.05	39.05	39.05	39.05
Soybean meal	40.23	40.23	40.23	40.23	40.23
Tapioca Starch	18.48	17.91	17.34	16.77	16.19
Carboxymethylcellulose	5.65	5.65	5.65	5.65	5.65
Vitamin[a]	3.00	3.00	3.00	3.00	3.00
Mineral[b]	0.00	0.50	1.00	1.50	2.00

ABP, Anchovy by-product; SBM, Soybean meal

[a]Vitamin mixture (g/kg mixture): Ascorbic acid, 45.0; Inositol, 5.0; Choline chloride, 75.0; Niacin, 4.5; Riboflavin, 1.0; Pyridoxine HCL, 1.0; Thiamine HCL, 0.92; D-calcium panothenate, 3.0; Retinyl acetate, 0.60; Vitamin D3, 0.083; Menadione, 1.67; DL alpha-tocopherol acetate, 8.0; D-biotin, 0.02; Folic acid, 0.09; Vitamin B12, 0.00135. All ingredients were diluted with alpha-cellulose to 1 kg

[b]Mineral mixture (g/kg mixture): Calcium phosphate monobasic, 270.98; Calcium lactate, 327.0; Ferrous sulphate, 25.0; Magnesium sulphate, 132.0; Potassium chloride, 50.0; Sodium chloride, 60.0; Potassium iodide, 0.15; Copper sulphate, 0.785; Manganese oxide, 0.8; Cobalt carbonate, 1.0; Zinc oxide, 3.0; Sodium salenite, 0.011; Calcium carbonate, 129.274

Table 2. Proximate composition (% as dry matter) of experimental feeds

Proximate analysis (%)	Dietary treatments				
	M0	M0.5	M1.0	M1.5	M2.0
Crude protein	39.84±0.10	39.59±0.12	39.18±0.09	39.25±0.21	39.28±0.06
Crude lipid	7.46±0.02	7.29±0.27	7.11±0.05	7.12±0.03	7.57±0.08
Dry matter	92.00±0.11	91.09±0.16	91.48±0.10	91.81±0.34	91.68±0.14
Ash	19.70±0.10	20.95±0.24	20.87±0.11	20.87±0.15	21.62±0.04
Moisture	8.00±0.11	8.91±0.16	8.52±0.10	8.19±0.34	8.32±0.14
NFE	32.99±0.14^{d}	32.17±0.29^{b}	32.84±0.03^{d}	32.28±0.39^{c}	31.52±0.10^{a}
Gross energy (kJ/g)	18.10±0.02^{c}	17.83±0.08^{b}	17.78±0.04^{b}	17.70±0.01^{a}	17.75±0.03^{b}

NFE, nitrogen-free extract. Values with different superscripts within a row are significantly different ($p<0.05$)

Water stability test

In the present study, experimental feeds were evaluated for water stability to determine the physical quality of the formulated feeds. The water stability test was done by applying the static water method described in Obaldo *et al*. (2002) with some modifications to suit the culture system for crayfish used in the present study. Six replicates of feed from each treatment with weights ranging from 0–2 g were taken (W0) and immersed in an 800 mL beaker filled with freshwater at six intervals of immersion time (30, 60, 120, 240, & 360 min). The feeds were maintained under 29 °C water temperature. After the desired leaching time, the feeds were taken out while the remaining solids suspended were recovered using Buchner filtration apparatus with Whatman glass microfiber filters (diameter 47 mm). The feeds were oven dried at 105 °C for 24 h and were cooled in a desiccator before weighing. The dry samples were weighed (W1) and water stability in each feed was calculated in terms of dry matter retention.

Experimental tank

The feeding trial was conducted at the Shrimp Hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah. A total of 15 fiberglass tanks with a volume of 100 L were used and the water level was maintained at 45 L. A closed static system was used in this experiment and crayfish shelters were set up by using 24 pieces of PVC pipes (10 cm in length and 15 mm in diameter) and 3 pieces of agriculture sunshade mesh (30 cm × 30 cm) in each experimental tank. For the duration of the feeding trial period, all experimental tanks were fully covered using agriculture sunshade mesh to prevent escapes and to shield experimental animals from predators.

Crayfish rearing and feeding trial

A total of 12 juvenile red claw crayfish, with a size range from 1-2 inches were stocked in each tank. All treatments were triplicated. All experimental animals were acclimatized for five days and fed with commercial pellets. Feed was given ad-libitum twice daily until apparent satiation was achieved. The feeding trial was conducted for 30 days. For maintenance, tank cleaning was done every day to remove the uneaten feed and feces. Uneaten feed was siphoned out and the weight was recorded. Throughout the feeding trial, the water quality such as temperature (26-29 °C), pH (7 – 8), dissolved oxygen (5.0 - 7.2 mg/L), ammonia (0 ppm), nitrate (0 ppm), and nitrite (0 ppm) were monitored and maintained by using a YSI multiparameter and API Freshwater Master test kit.

Molting frequency

The evaluation of total molting frequency was calculated from the total number of moltings recorded. The molting occurrence was based on the presence of old skeletons in each replication which was recorded twice daily, in the morning (at 0700 h) and evening (at 1700 h) before feeding throughout the feeding trial. The old skeletons were collected after each molt and refrigerated at 4 °C until further evaluation (Siti Nor Fatihah *et al*., 2017).

Data collection and analysis

The body weight and the length of the crayfish were measured individually at the start and end of the feeding trial. Bulk weight was premeasured once every two weeks for growth monitoring. Molting occurrences were observed and recorded daily for the duration of the feeding trial. Any mortality was also recorded. At the end of the feeding trial, all the remaining crayfish were sampled for measurement of growth performance. Subsequently, the weight gain (WG), length gain (LG), specific growth rate (SGR), survival rate (SR), molting frequency (MF), total feed intake (TFI), feed conversion ratio (FCR), and condition factor (CF) for each dietary treatment was calculated using the formula below:

$$\text{WG (\%)} = \frac{\text{Final crayfish weight} - \text{Initial crayfish weight}}{\text{Initial weight}} \times 100$$

$$\text{LG (\%)} = \frac{\text{Final crayfish length} - \text{Initial crayfish length}}{\text{Initial length}} \times 100$$

$$\text{SGR (\% d}^{-1}) = \frac{\text{Ln (Final crayfish weight)} - \text{Ln (Initial crayfish weight)}}{\text{Number of days}} \times 100$$

$$\text{SR (\%)} = \frac{\text{The final number of crayfish}}{\text{The initial number of crayfish}} \times 100$$

$$MF = \frac{\text{Total number of molts}}{\text{Total number of crayfish}}$$

$$TFI(g/crayfish) = \frac{(\text{The total feed given} - \text{Total uneaten feed})}{(\text{Number of crayfish})}$$

$$FCR = \frac{\text{Dry feed consumed (g)}}{\text{Wet weight gained (g)}} \times 100$$

$$CF = \frac{\text{Final crayfish weight}}{\text{Final crayfish length}^3} \times 100$$

$$\text{Dry matter retention } (\%) = \frac{(W1)}{(W0)} \times 100$$

W0 = Gram initial feed
W1 = Gram feed remaining

Statistical analysis and summary of the experimental design

All of the calculated data was compared using One-way Analysis of Variance (ANOVA) using SPSS software (version 28) for determining the significant differences among the treatment. The significant levels of all analyses were set at $p<0.05$. The differences between means for each group (individual means) were determined by the post hoc Tukey test when the interaction between both independent variables is significant. The summary of the experimental design is presented in Figure 2.

RESULTS

Water stability of formulated feeds

The results of the water stability test of formulated feeds are presented in Table 3. There was a significant difference in percent dry matter retention in all experimental feeds ($p<0.05$). During the initial 30 min of soaking, all feed recovery was stable at more than 80%. The percentage of dry matter retention was found to decrease with the increase of the percentage of minerals in feeds. The mean % water stability of experimental feeds ranged from 80.03–84.7% at 30 min before reducing to 61.64–67.65% at 360 min of immersion.

Growth performances of juvenile red claw crayfish

The growth performances of juvenile red claw crayfish fed experimental feeds are presented in Table 4. The data shows that different mineral inclusion in experimental feeds based on anchovy by-products did not significantly influence ($p>0.05$) the growth performance of *Cherax quadricarinatus* juveniles. There was no significant difference ($p>0.05$) in final body weight (g), final length (cm), weight gain (%), length gain (%), and specific growth rate (%/d) of the juvenile red claw crayfish at the end of the trial. M1.5 diet had the highest weight gain value (88.66±7.96%), followed by M0 (86.01±5.79), M0.5 (84.73±21.19), M2.0 (82.92±24.63) and M1.0 (66.52±12.18%), although the differences were statistically insignificant. A similar trend was observed for the length gain where the M1.5 diet had the highest value (25.36±2.48) and crayfish fed the M1.0 diet had the least length gain (19.97±3.21). Similarly, the specific growth rate followed the trend of weight and length gain where M1.5 recorded the highest value (2.11% d^{-1}) and M1.0 showed the lowest (1.69% d^{-1}).

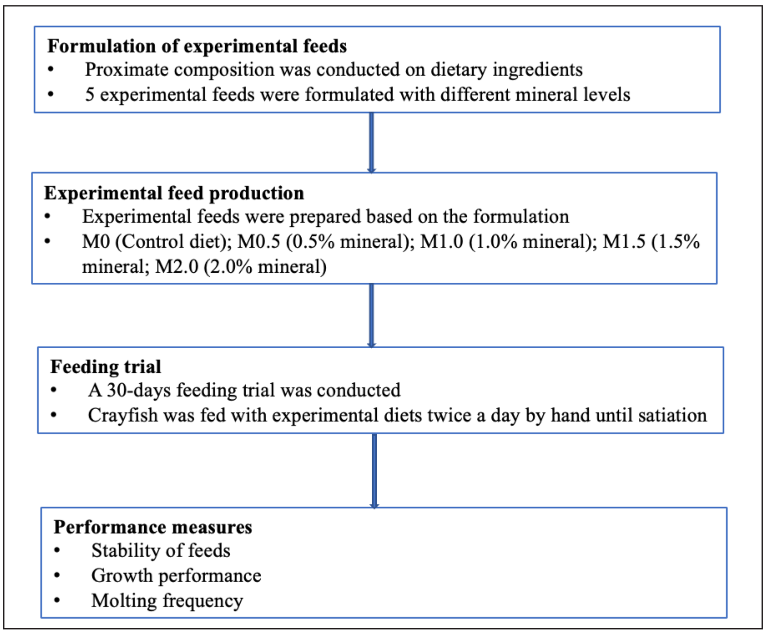

Fig. 2. Flow-chart of experimental design adopted in the present study

The survival rates ranged from 77.78% to 86.11%. Crayfish fed with experimental feeds M0.5, M1.0, and M2.0 showed relatively higher survival than crayfish in other experimental feeds although differences were not statistically significant. Crayfish fed M0 yielded the lowest survival at 77.78%. The condition factor values of the juvenile red claw crayfish were not significantly different from each other (p>0.05) and ranged from 2.03±0.09 (M0.5) to 2.10±0.02 (M2.0

There was no significant difference (p>0.05) in feed conversion ratio and total feed intake of crayfish among all feeds. Feed conversion ratio ranged from 1.60 (M0.5) to 1.76 (M1.0). The total feed intake for experimental diet M1.5 had the highest value of 6.31±0.23 g/crayfish followed by 6.17±0.57 (M2.0), 6.16±0.62 (M1.0), 6.16±0.17 (M0) and 6.08±0.25 (M0.5) g/crayfish.

Molting frequency of juvenile red claw crayfish

Figure 3 shows the molting frequency of juvenile red claw crayfish within the 30 days experimental period. Different levels of mineral inclusion in experimental feeds did not significantly affect (p>0.05) the molting frequency of *Cherax quadricarinatus* juveniles. In terms of values, red claw crayfish juveniles that were fed an M1.5 diet had the highest molting frequency with 0.51±0.08 molting. Whereas, red claw crayfish juveniles fed with an M1.0 diet had the lowest total frequency of molting with 0.29±0.19 molting within the 30-day experimental period.

Table 3. Mean percentage water stability of formulated feeds

Time (min)	Dietary treatments				
	M0	M0.5	M1.0	M1.5	M2.0
30	84.07±1.14^{b}	80.76±0.36^{a}	80.55±0.14^{a}	80.03±0.81^{a}	80.31±0.80^{a}
60	74.82±1.25^{b}	69.34±0.90^{a}	70.04±1.64^{a}	72.52±2.14^{b}	71.02±2.23^{b}
120	80.63±1.86^{a}	77.24±1.47^{a}	76.76±2.60^{a}	76.02±2.33^{a}	77.99±1.11^{a}
240	71.33±0.28^{b}	63.70±2.31^{a}	64.37±2.09^{a}	65.70±1.98^{a}	66.22±2.22^{b}
360	67.65±1.71^{b}	61.64±1.58^{a}	62.00±1.50^{a}	64.08±1.00^{b}	63.66±1.64^{b}

Values with different superscripts within the row are significantly different (p<0.05)

Table 4. Growth performance and feed utilization efficiency of juvenile red claw crayfish, *Cherax quadricarinatus*

Parameters	Dietary treatments				
	M0	M0.5	M1.0	M1.5	M2.0
Initial body weight (g)	2.19±0.04	2.20±0.01	2.22±0.03	2.20±0.04	2.17±0.01
Final body weight (g)	4.07±0.09	4.06±0.45	3.69±0.22	4.15±0.15	3.97±0.54
Initial body length (cm)	4.72±0.03	4.66±0.02	4.69±0.06	4.69±0.03	4.62±0.04
Final body length (cm)	5.85±0.11	5.84±0.19	5.63±0.11	5.88±0.11	5.73±0.27
Weight gain (%)	86.01±5.79	84.73±21.19	66.52±12.18	88.66±7.96	82.92±24.63
Length gain (%)	23.76±2.54	25.35±3.98	19.97±3.21	25.36±2.48	23.88±4.98
SGR (% d^{-1})	2.07±0.10	2.03±0.38	1.69±0.24	2.11±0.14	1.99±0.46
Survival (%)	77.78±4.81	86.11±4.81	86.11±9.62	80.55±4.81	86.11±12.73
K	2.04±0.10	2.03±0.09	2.07±0.02	2.04±0.06	2.10±0.02
FCR	1.67±0.10	1.60±0.13	1.76±0.11	1.67±0.11	1.65±0.25
Total feed intake (g $crayfish^{-1}$)	6.16±0.17	6.08±0.25	6.16±0.62	6.31±0.23	6.17±0.57

K, Condition Factor; FCR, Feed Conversion Ratio

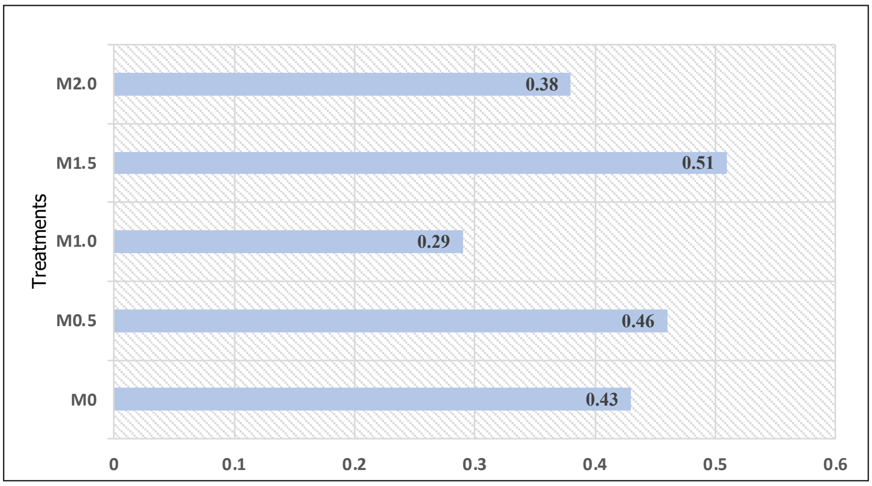

Fig. 3. Molting frequency of juvenile red claw crayfish, *Cherax quadricarinatus* fed experimental feeds containing different mineral inclusion.

DISCUSSION

In crustacean feed, water stability and nutrient leaching are crucial issues to be taken into consideration. In general, the water stability of the experimental feeds in the present study is similar to those reported on other crustacean experimental feeds with the rate of dry matter loss decreasing temporally (Ruscoe *et al*., 2005). Bortone (2005) suggested that shrimp feeds with 40 percent crude protein and 6 to 8 percent fat should have water stability of 75 percent or higher, similar to the values observed in the present study. In particular, the experimental diet M0 had the highest water stability compared to all other treatment feeds. This is attributable to the higher amount of tapioca starch as a binder used in the M0 formulation compared to other feeds. The feeds containing different levels of mineral inclusion were found to have relatively similar values of dry matter retention. According to a previous study by Kumar *et al*. (2018), the water stability of fish feed increased by up to 50% when corn starch was replaced with tapioca starch. Ahamed Ali (1998) in his work stated that tapioca starch was found to be a more suitable binder for prawn feed and the pellets prepared with these binders were shown to possess good water stability for a period of up to 8 hr. In this experiment, it was also observed that all experimental feeds were stable enough in the water for the crayfish to hold the feeds with their maxillipeds before entering the mouthparts.

The results of the present study demonstrated that different levels of mineral inclusion in anchovy by-product-based feeds for the red claw crayfish have no adverse effects on growth performance. Although no significant difference was observed, the growth performance of 0% mineral level in the diet (M0) was found to be marginally better than the feed containing 2% mineral level in the diet (M2.0), which suggests that a diet without any inclusion of mineral in anchovy by-product-based feeds can support the normal growth of crayfish juvenile, at least within a short term period. Therefore, the use of anchovy by-products has good potential to reduce crayfish feed costs, especially in terms of reducing mineral inclusion. It was also reported that common anchovy species found in Malaysian waters (eg. *Stolephorus indicus* & *S. commersonii*) are a rich source of lysine, leucine, and several essential amino acids, polyunsaturated fatty acids, n-3 fatty acids, and various minerals such as Na, K, Ca, Mn and Zn (Sankar *et al*., 2013; Ahmad *et al*., 2018).

The findings from the present study are considered important considering the importance of sufficient minerals in the diet of red claw crayfish, especially for the molting process. Deficiencies of vitamins and minerals can lead to slower growth, negatively impact reproduction, and can also cause mortality in crustaceans (Conklin, 1997; Davis & Lawrence, 1997). In narrow-clawed crayfish, *Astacus leptodactylus*, dietary supplementation of calcium chloride significantly influenced the survival rate of the crayfish (Sirin & Mazlum, 2017). Anchovy by-products contain high levels of nutrients especially minerals, which can serve as an alternative source of minerals in the feeds, apart from other nutrients such as protein. Knowledge of the mineral requirement of crayfish is rather limited because minerals can be obtained from the surrounding water. Seven minerals (calcium, copper, magnesium, phosphorus, potassium, selenium, and zinc) have been recommended for inclusion in penaeid shrimp and lobster feeds (Davis *et al*., 1996). In the present study, the individual mineral presented in ABP was not analyzed but based on the previous study on fish by-product, scales, fins, and bones are good source of calcium, magnesium, potassium, sodium, zinc, copper, manganese, iron, chromium and selenium (Jaziri *et al*., 2021). Thus, it is suggested that the mineral contents of the experimental feeds were able to support the normal growth of juvenile red claw crayfish for the duration of this 30-day feeding trial.

The total feed intake was not significantly different among all experimental feeds, indicating the good acceptance by the red claw crayfish. Ruscoe *et al*. (2002) and Loya-Javellana *et al*. (1993) stated that crayfish would move out of their shelter when food was offered and frantically search out new food. Similarly, this behavior was also observed during the present study, both during the morning and afternoon feeding sessions. The red claw crayfish in the present study searched for food and quickly ate the feeds offered to them.

The feed conversion ratio (FCR) is used to ascertain the amount of feed required per unit weight gain of the species grown for use as a measure of production efficiency in aquaculture. FCRs vary according to several factors such as species, feed type, and quality, production system, feeding technique, and water quality conditions. Crustacean species typically achieve FCR values of 1.5 to 2.5. In the present study, the FCR values of all experimental feeds ranged from 1.60 – 1.76 which can be considered efficient feed utilization.

Nutritional status is a good health indicator of crustaceans; inadequate levels of nutrients may result in reduced growth rates, poor feed conversion ratios, decreased resistance to stress, and reduced capability to heal wounds (López *et al*., 2003; Sánchez *et al*., 2005). In the present study, all experimental feeds were prepared using the same proportions of protein and lipid contents, this indicates that the nutritional value in anchovy by-product-based diet was adequate to support optimum growth and was directly proportional given the low feed conversion ratio value based on all the experimental diet tested.

As for the condition factor of red claw crayfish observed in this experiment, the value is relatively similar to those reported by Okayi and Iorkyaa (2004) for vampire shrimp, *Atya gabonensis,* and giant freshwater prawn, *Macrobrachium felicinium* in the Mu River. The condition factor reflects current biological and physical interactions and variations, particularly regarding feeding conditions, parasite illnesses, physiological parameters, food reserves, and overall fish health. The condition factor values of the juvenile red claw crayfish in the present study fall within the normal value of condition factor for aquatic organisms (Le Cren, 1951).

Following the growth trend, the molting frequency was not significantly affected by the inclusion of different mineral levels in the feeds. It is well documented that minerals are not only important to support normal growth but are also needed in the molting process. In particular, calcium is the most important mineral for any crustacean because this is the main component of their shell. For example, crayfish exoskeleton comprises 50% of dry weight and is mineralized with calcium carbonates and magnesium. *C. quadricarinatus* stores calcium as amorphous calcium carbonate during premolt in a pair of gastroliths synthesized in the stomach wall (Luquet *et al*., 2016). The anchovy by-product meal used in the present study contained about 35% ash. Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the muffle furnace, which provides a measure of the total amount of minerals within a food (Soren *et al*. 2020). This indicates that the mineral contents of experimental feeds including the control diet (without mineral inclusion) were able to support the molting process of juvenile red claw crayfish, apart from those present in the water. Siti Nor Fatihah *et al*. (2017) suggested that the usage of black netting as shelter tends to increase the molting frequency of mud crabs. In the present study, the experimental tank was provided with a black sheet net as a shelter for the crayfish to reduce cannibalism.

CONCLUSION

The stability of experimental feeds in terms of dry matter retention was affected by the different levels of dietary minerals but these values were still within the normal values for aquaculture feed stability in crustacean species. Throughout the 30-day feeding trial, there was no significant difference detected in final weight (g), final length (cm), weight gain %, length gain %, and specific growth rate (%/d) of red claw juveniles. The inclusion of different minerals in the feeds did not significantly influence the feed intake and feed conversion ratio. In general, the findings from the present study indicate that all experimental feeds including those without mineral inclusion (control diet) contained sufficient minerals required to support the normal growth of the juvenile red claw crayfish, at least for a short-term basis. Thus the inclusion of minerals below the de-facto 2% inclusion level is highly possible in a long-term culture period.

It can be concluded that the anchovy by-product meal used in the present study is a potential ingredient to supply dietary protein and minerals in formulated feeds for juvenile red claw crayfish. Considering the very low cost of anchovy by-products, the inclusion of this ingredient would be able to reduce the feed cost and be used as an alternative ingredient in the aquaculture feed industry. It is highly recommended to further evaluate the full potential of this product in a long-term feeding trial using different aquaculture species with an in-depth study looking at the complete nutritional profile and digestibility of this ingredient.

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ETHICAL STATEMENT

This study was conducted following the Researcher’s Guidelines on Code of Practice for the Care and Use of Animals for Scientific Purposes, Universiti Malaysia Sabah.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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