Jurnal Medik Veteriner
DOI: 10.20473/jmv.vol8.iss2.2025.329–342

October 2025, Vol.8 No.2, 329–342
online at https://e-journal.unair.ac.id/JMV

Effects of Anchovy By-Product-Based Diets Supplemented with Soy Lecithin on the Reproductive Performance of Red Claw Crayfish (*Cherax quadricarinatus*)

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Abstract

The present study aimed to determine the growth and reproductive performances of red claw crayfish (*Cherax quadricarinatus*) fed formulated diets based on anchovy by-product meal, supplemented with varying levels of lecithin. The SL0 diet, containing 0% soy lecithin, was used as the control, and four experimental feeds were included with different lecithin levels: SL1: 1% soy lecithin, SL2: 2% soy lecithin, SL3: 3% soy lecithin and SL4: 4% soy lecithin. Five broodstock (four females and one male) were stocked in the square fiberglass tank (105 cm × 105 cm × 36 cm; 400L) and fed twice daily (0900h and 1600h) until satiation for 11 weeks. The data were analyzed using one-way ANOVA followed by Tukey's test at $p < 0.05$. Growth and survival rates showed no significant differences among treatments. Notably, SL4 exhibited the highest gonadosomatic index (GSI) at 2.50 ± 1.99%, while SL3 had the highest hepatosomatic index (HSI) at 6.20 ± 1.18%. The spawning rate peaked in SL1 (63.89%), followed by SL0 (47.22%), SL2 (41.67%), SL3 (41.67%), and SL4 (25.00%). SL4 also had the highest fecundity (14.94 eggs/g female), with SL3 (10.72 eggs/g female) and SL0 (9.50 eggs/g female) following. Lipid content in muscle varied from 1.14% (SL4) to 1.46% (SL3), with no significant differences. However, lecithin inclusion notably affected lipid content in hepatopancreas and gonad, with SL1 exhibiting the highest hepatopancreas lipid (61.04%) and SL2 the highest gonad lipid (27.78%). The data suggest that dietary lecithin plays an important role in the reproductive performance of red claw crayfish. Overall, anchovy by-product meal has high potential to serve as a dietary ingredient in the feed formulation for both juvenile and broodstock of red claw crayfish.

Keywords: Anchovy by-product, broodstock, juvenile, *Cherax quadricarinatus*, red claw crayfish, soy lecithin

Received: April 16, 2025 **Revised:** July 11, 2025 **Accepted:** August 13, 2025

INTRODUCTION

The reproductive performance of red claw crayfish, *Cherax quadricarinatus*, is significantly influenced by their diet. Providing the right balance of nutrients is crucial for supporting successful ovarian maturation, spawning, and the production of high-quality larvae. While there is considerable knowledge regarding the nutritional needs of juvenile crustaceans, specific dietary requirements for crayfish broodstock are still not well established (Saoud *et al.*, 2012). Understanding these requirements is essential to improve reproductive success in aquaculture operations involving this species.

At present, the aquafeed industry relies on alternative protein sources to reduce the use of expensive fish meal. Anchovy by-products (ABP) are among the promising candidates, though their potential has not been fully explored. Several studies have shown that ABP contains significant amounts of protein and lipids, despite being commonly regarded as waste. According to previous research, inclusion of ABP in the diet of juvenile red claw crayfish improves survival and growth rates, while providing calcium to support the molting process.

Soy lecithin is widely recognized as a major source of phospholipids (PL), an essential component in broodstock diets. During ovarian development, phospholipids play a critical role by aiding the absorption and transport of lipids from the hepatopancreas to the ovary via the haemolymph (Harrison, 1990; Hoa *et al.*, 2009; Corral-Rosales *et al.*, 2018; Song *et al.*, 2019). Since most crustaceans lack the ability to synthesize sufficient amounts of phospholipids on their own, especially during the early developmental stages (Tian *et al*., 2020), dietary supplementation becomes necessary. Although research has been done on the phospholipid requirements of larval and juvenile crustaceans—such as the American lobster (Conklin *et al*., 1980), penaeid shrimp (Chen *et al*., 1993; Coutteau *et al*., 1996; Thongrod and Boonyaratpulin, 1998), giant freshwater prawn (Briggs *et al*., 1988), mitten crab (Wang *et al*., 2004), and red claw crayfish (Thompson *et al*., 2003)—information for broodstock nutrition remains limited.

Evidence from previous studies on *Litopenaeus vannamei* suggested that broodstock diets containing more than 2% phospholipids improved spawning frequency and fecundity (Cahu *et al*., 1994). Similarly, dietary phospholipid supplementation for *E. sinensis* broodstock was found to enhance ovarian development and reproductive performance (Wang *et al*., 2004). Building on this knowledge, the present study aims to evaluate the effects of different lecithin inclusion levels in feeds formulated with anchovy by-products on the reproductive performance of red claw crayfish. The study focuses on assessing the biochemical composition of ingredients and feeds, growth performance, fecundity, and the whole-body proximate composition of the crayfish broodstock.

MATERIALS AND METHODS

Ethical Approval

This study was approved by Animal Ethic Research Committee, University Malaysia Sabah (AEC 0028/ 2022).

Study Period and Location

This study was conducted for 11 weeks (March 2023 to May 2023) at the Crustacean Hatchery, Universiti Malaysia Sabah. A proximate composition of the ingredients, feeds, and fish samples was conducted at the Biotechnology Laboratory, Borneo Marine Research Institute, Universiti Malaysia Sabah.

Feed Preparation

For the feed preparation, anchovy by-products (ABP) were ground into fine form using a grinder and sieved to produce a homogenous meal. Before the ABP is subjected to grinding, the samples were dried in an oven at 55°C for 5 hours to remove remaining moisture. Diet formulations were produced using Microsoft Excel. The experimental feeds were formulated using ABP with the combination of soybean meal by using ratio (3:1). A total of five feeds were evaluated with different ratios of soy lecithin added to SL0 (control), SL1 (1% soy lecithin), SL2 (2% soy lecithin), SL3 (3% soy lecithin), and SL4 (4% soy lecithin) (Table 1). Dissolved oxygen (DO), water temperature (°C), and pH were monitored weekly throughout the experiment using a multiparameter instrument (YSI ProQuatro Multiparameter Meter, USA).

Broodstock Rearing

Each treatment tank (105 cm × 105 cm × 36 cm; 400L) was stocked with 4 females (BW: 18.40 ± 0.39 g, BL: 9.73 ± 0.07 cm) and 1 male (BW: 22.70 ± 0.37 g, BL: 10.33 ± 0.04 cm) (Celada *et al*., 2005) and fed with experimental feeds twice a day (09.00h and 16.00h). The presence of males was essential, as they produced spermatozoa necessary for fertilizing the eggs released by females, thereby influencing reproductive outcomes. Each basket was equipped with a PVC pipe (3.2 cm in diameter and 9 cm in length) to serve as shelter for the berried females. Berried females were checked daily by observing the abdomen (Chowdhury *et al*., 1993).

Proximate Composition

The standard methods of AOAC (2003) were followed for analysis of ingredients, feeds, and fish samples. The moisture content was measured after the samples were dried in an oven at 105°C for 24 hours. The samples were incinerated in a muffle furnace at 550°C for 6 hours, allowed to cool, and then weighed to determine the crude ash content. Crude protein content was determined using a Kjeltec-Protein Analyzer (Kjeltec™ 2300, Foss, Sweden) after undergoing acid digestion. Crude lipid content was determined using a Soxtec-Lipid Analyzer (Soxtec™ 2043, Foss, Sweden), with petroleum ether (boiling point 40–60°C) employed as the extraction solvent.

Growth Performance and Somatic Indices

At the beginning and end of the feeding trial, the body weight and length of each red claw crayfish were recorded individually. This study focused exclusively on female red claw crayfish to assess the growth performance and reproductive potential of female broodstock.

Table 1. Ingredient composition of experimental feed (g/100g dry weight)

Ingredients	Treatments				
	SL0	SL1	SL2	SL3	SL4
Anchovy by-product	29.83	29.83	29.83	29.83	29.83
Soybean meal	10.81	10.81	10.81	10.81	10.81
Shrimp meal	6.81	6.81	6.81	6.81	6.81
Wheat gluten	6.04	6.04	6.04	6.04	6.04
Vitamin premix	2.00	2.00	2.00	2.00	2.00
Mineral premix	1.00	1.00	1.00	1.00	1.00
Soy lecithin	0	1.00	2.00	3.00	4.00
CMC binder	3.50	3.50	3.50	3.50	3.50
Cholesterol	0.5	0.5	0.5	0.5	0.5
Tapioca starch	33.32	33.21	33.11	33.00	32.89
Palm oil	6.19	5.30	4.40	3.51	2.62

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

Parameters	Treatments				
	SL0	SL1	SL2	SL3	SL4
Moisture	9.34 ± 0.16^{b}	10.27 ± 0.29^{c}	9.92 ± 0.41^{c}	8.64 ± 0.08^{a}	8.83 ± 0.21^{a}
Crude Protein	32.83 ± 0.07^{a}	32.29 ± 0.15^{a}	33.24 ± 0.47^{a}	33.55 ± 0.54^{ab}	34.70 ± 0.30^{b}
Crude lipid	10.61 ± 0.28^{b}	10.32 ± 0.15^{b}	9.06 ± 0.42^{a}	8.61 ± 0.20^{a}	10.33 ± 0.64^{b}
Dry matter	90.66 ± 0.16^{a}	89.73 ± 0.29^{a}	90.08 ± 0.41^{a}	91.36 ± 0.08^{a}	91.17 ± 0.21^{a}
Crude ash	15.12 ± 0.11^{b}	15.59 ± 0.16^{b}	14.43 ± 0.06^{a}	15.80 ± 0.04^{b}	15.30 ± 0.86^{b}

^{a,b,ab,c} Values bearing different superscripts in the same row indicate significant differences ($p < 0.05$); mean ± S.D.

Performance Evaluation and Statistical Analysis

Data for fecundity was collected 14 days after spawning (Carral *et al.*,1992). The eggs were manually removed from the abdomen of the female crayfish. The broodstock and its egg clutch were weighed separately. The egg clutch was carefully blotted with filter paper to remove excess water and weighed to the nearest 0.01 g. For each individual, 30 eggs sample were taken.

The number and size of eggs were counted and measured using a stereo microscope (Olympus, Japan). Fecundity was then calculated by determining the number of eggs per gram of female body weight, using the formula: fecundity = number of eggs / female weight (g).

Daily egg development was observed by naked eye and described as color changes following Yeh and Rouse (1994). The spawning rate, gonadosomatic index (GSI) and

hepatosomatic index (HSI) were determined and calculated using the following formula:
Spawning rate (%) = No.of berried females/Total no.of females ×100...(1)
Gonadosomatic index = Reproductive system weight/Female final body weight ×100 ....(2)
Hepatosomatic index = Hepatopancreas weight/Female final body weight ×100 ....(3)

Hepatosomatic index was measured to evaluate the changes with reproductive performance, which usually peak during vitellogenesis depends on the dietary composition.

Data Analysis

A one-way ANOVA was performed to assess the significant impact of different diets on weight gain, survival rate, feed efficiency, body indices, and whole-body proximate composition. Statistical significance was established at a probability level of $p < 0.05$. All data analyses were carried out using SPSS version 20 software. The outcomes were expressed as the mean values along with their corresponding standard deviations (SD).

RESULTS AND DISCUSSION

Proximate Composition of Experimental Diet

Table 2 presents the proximate composition of the experimental diets. The analyzed values for moisture, crude protein, crude lipid, crude ash, and dry matter content ranged between 8.83% and 10.27%, 32.29% and 34.70%, 8.61% and 10.61%, 14.43% and 15.80%, and 89.73% and 91.36%, respectively.

Table 3. Growth performance and body indices of female red claw crayfish fed different feeds

Parameters	Treatments				
	SL0	**SL1**	**SL2**	**SL3**	**SL4**
Initial female's weight, g	18.55 ± 2.27^{a}	18.99 ± 1.88^{a}	18.21 ± 1.79^{a}	17.96 ± 0.37^{a}	18.40 ± 0.39^{a}
Final female's weight, g	27.20 ± 3.77^{a}	22.10 ± 7.58^{a}	24.69 ± 4.19^{a}	26.44 ± 1.84^{a}	27.21 ± 2.32^{a}
Weight gain, %	49.39 ± 37.77^{a}	46.77 ± 4.03^{a}	50.71 ± 38.24^{a}	44.50 ± 5.90^{a}	51.36 ± 11.30^{a}
Specific growth rate (%/day)	0.50 ± 0.31^{a}	0.50 ± 0.04^{a}	0.51 ± 0.33^{a}	0.48 ± 0.05^{a}	0.54 ± 0.10^{a}
Survival rate, %	93.33 ± 11.55^{a}	80.00 ± 20.00^{a}	93.33 ± 11.55^{a}	100 ± 0.00^{a}	100 ± 0.00^{a}
Gonadosomatic Index (GSI), %	1.39 ± 0.23^{ab}	0.53 ± 0.15^{a}	1.81 ± 0.63^{ab}	1.41 ± 0.31^{ab}	2.50 ± 1.99^{b}
Hepatosomatic Index (HSI), %	3.79 ± 0.54^{a}	4.23 ± 0.36^{a}	5.95 ± 0.12^{b}	6.20 ± 1.18^{b}	6.06 ± 0.36^{b}

^{a,b,ab} Values bearing different superscripts in the same row indicate significant differences ($p < 0.05$); mean ± S.D.

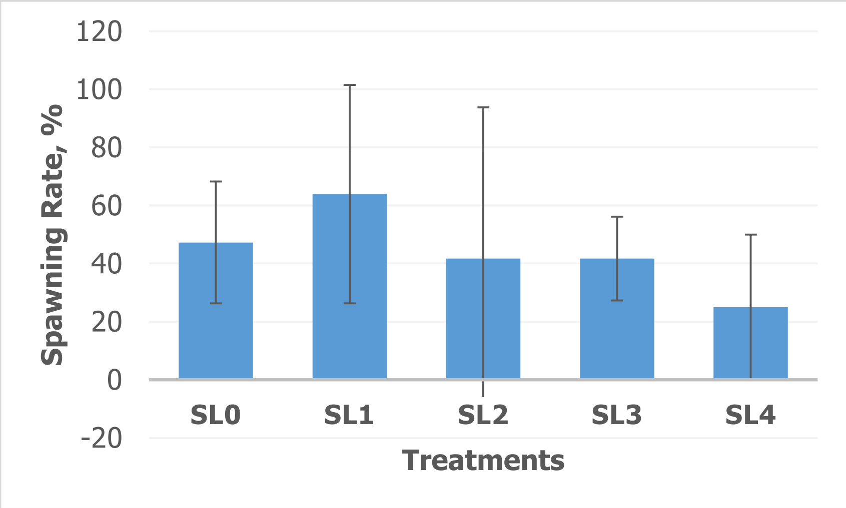

Figure 1. Spawning rate of female red claw crayfish.

Table 4. Fecundity and egg quality parameters of red claw crayfish

Parameters	Treatments				
	SL0	SL1	SL2	SL3	SL4
Total number of spawns	5	4	5	4	3
No. female drop their broods	3	2	3	2	2
Fecundity	9.50 ± 5.59	8.73 ± 3.58	9.30 ± 1.86	10.72 ± 2.72	14.943
Mean no. of egg	256 ± 73.00	203 ± 169.71	171 ± 9.19	298 ± 98.99	346
Mean weight of per egg, g	0.005 ± 0.0004	0.004 ± 0.0002	0.005 ± 0.001	0.004 ± 0.0002	0.004
Mean weight (egg clutch), g	1.17 ± 0.37	0.82 ± 0.65	0.79 ± 0.05	1.31 ± 0.39	1.369
Egg diameter, mm (n = 30)	1.87 ± 0.04	1.82 ± 0.06	1.89 ± 0.07	1.84 ± 0.04	1.74 ± 0.04

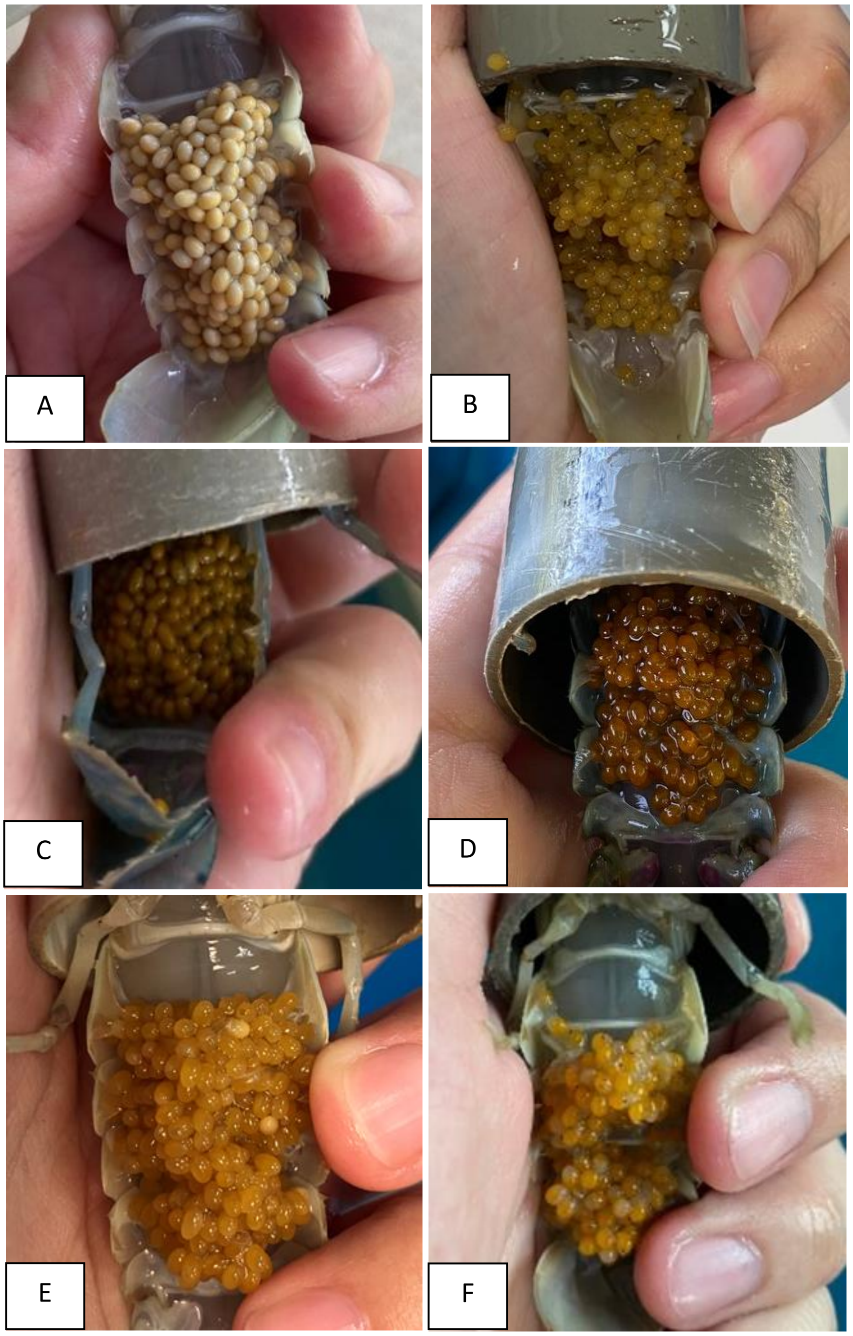

Figure 2. Egg color development of red claw crayfish, (A) Stage 1 (light yellow), (B) Stage 2 (Olive green), (C) Stage 3 (Khaki), (D) Stage 4 (Dark brown), (E) Stage 5 (Orange), (F) Stage 7 (Eye spot detected).

Table 5. Whole-body proximate composition (% wet weight basis) of red claw crayfish

Parameters	Treatments				
	SL0	SL1	SL2	SL3	SL4
Moisture	73.34 ± 1.45[a]	75.42 ± 2.24[a]	77.01 ± 3.79[a]	77.64 ± 2.57[a]	75.53 ± 0.59[a]
Crude Ash	8.80 ± 0.81[a]	6.11 ± 5.29[a]	8.33 ± 0.48[a]	7.43 ± 0.06[a]	5.59 ± 4.84[a]
Crude Protein	11.44 ± 0.16[b]	9.53 ± 0.88[a]	9.45 ± 0.70[a]	10.21 ± 0.32[a]	9.76 ± 0.79[a]
Crude Lipid	0.70 ± 0.14[c]	0.44 ± 0.06[b]	0.17 ± 0.16[a]	0.18 ± 0.02[ab]	0.13 ± 0.11[a]

[a,b,ab,c] Values bearing different superscripts in the same row indicate significant differences ($p < 0.05$); mean ± S.D.

Table 6. Lipid content (%) for muscle, hepatopancreas, and gonad of the red claw crayfish

Parameters	Treatments				
	SL0	SL1	SL2	SL3	SL4
Muscle	1.40 ± 0.18[a]	1.20 ± 0.42[a]	1.27 ± 0.13[a]	1.46 ± 0.22[a]	1.14 ± 0.21[a]
Hepatopancreas	51.20 ± 0.91[c]	61.04 ± 1.99[d]	39.41 ± 0.61[a]	44.21 ± 0.15[b]	51.58 ± 0.46[c]
Gonad	22.34 ± 0.48[a]	26.06 ± 0.10[ab]	27.78 ± 0.12[b]	24.07 ± 2.37[ab]	22.25 ± 2.69[a]

[a,b,ab,c] Values bearing different superscripts in the same row indicate significant differences ($p < 0.05$); mean ± S.D.

Growth Performance and Somatic Indices

The findings on growth performance, survival, gonadosomatic index (GSI), and hepatosomatic index (HSI) are summarized in Table 3. No significant differences were observed in growth performance and survival rates among the different treatments. Nevertheless, based on numerical values, the highest weight gain in female red claw crayfish was recorded in the SL4 group (51.36%), followed by SL2 (50.71%), SL0 (49.39%), SL1 (46.77%), and SL3 (44.50%). The specific growth rate (SGR) ranged between 0.48%/day in SL3 and 0.54%/day in SL4. Survival rates were consistently high across all treatments, ranging from 80% to 100%. Unlike growth and survival, the GSI and HSI showed significant variations among the treatments.

Spawning Rate of Female Red Claw Crayfish

The spawning rate of red claw crayfish is presented in Figure 1. The calculation of the spawning rate was also included for the crayfish that managed to spawn but dropped their eggs due to several factors. The spawning rate was recorded as the highest in treatment SL1 (63.89%), followed by SL0 (47.22%), SL2 (41.67%), SL3 (41.67%), and SL4 (25.00%). Nevertheless, no significant differences were detected between the treatments.

Fecundity and Egg Quality Parameters of Red Claw Crayfish

The results for fecundity are presented in Table 4. The females that had dropped their broods were not included in the calculations. The fecundity of red claw crayfish broodstock was highest in SL4 (14.94), followed by SL3 (10.72), SL0 (9.50), SL2 (9.30), and SL1 (8.73). Regarding the number of eggs, treatment SL4 recorded the highest count, while SL2 had the lowest. Egg clutch weight was heaviest in treatment SL4, followed by SL3, SL0, SL1, and SL2. The weight per egg ranged from 0.004 to 0.005 grams. The greatest egg diameter was recorded in treatment SL2 (1.89 ± 0.07 mm), whereas the smallest was observed in SL4 (1.74 ± 0.04 mm). Despite these variations, the differences among treatments were not statistically significant.

Egg Color Development of Red Claw Crayfish

Egg development stages in crayfish can be identified by changes in colour and the appearance of body structures in the developing embryo (Yeh and Rouse, 1994). In the present study, development stages from Stage 1, Stage 2, Stage 3, Stage 4, Stage 5, and Stage 7 were successfully captured (Figure 2). Stage 6 in the egg color development could not be obtained due to the unavailability of samples at that specific developmental stage during the observation period.

Whole-Body Proximate Composition

In Table 5, the result for proximate of the whole body is presented. Based on the result, crayfish fed with SL3 have higher moisture content, whereas SL0 has the lowest moisture content. Among dietary treatments, there were significant differences detected in crude protein and crude lipid contents in red claw crayfish.

Lipid Content in Muscle, Hepatopancreas and Gonad of Female Red Claw Crayfish

Table 6 presents the lipid content in the muscle, hepatopancreas, and gonads. Muscle lipid content ranged from 1.14% in SL4 to 1.46% in SL3, with no significant differences observed between treatments. However, lecithin inclusion had a significant effect on lipid content in the hepatopancreas and gonads. The highest lipid accumulation in the hepatopancreas was recorded in SL1 (61.04%), followed by SL4 (51.58%), SL0 (51.20%), SL3 (44.21%), and SL2 (39.41%). Meanwhile, the lipid content in the gonads was highest in SL2 (27.78%) and lowest in the control treatment (22.34%).

Discussion

Weight gain and survival of red claw crayfish fed the experimental diets showed no significant differences across treatments. However, there was an increasing growth trend with higher levels of soy lecithin in the feeds. It is unclear why SL3 exhibited the lowest growth while SL2 and SL4 showed an increase of growth. Possible factors could be the interference with nutrient absorption or metabolic disruptions. The SGR was around 0.5%/day, indicating that the experimental crayfish were slowing down their growth and approaching maturity. A similar result obtained by Sanchez *et al.* (2011) shows that *Penaeus vannamei* fed 4% soy-lecithin grew faster than those fed 1% soy-lecithin and stated this could be attributed to increased lipid deposition and greater energy availability for growth, potentially linked to improved lipid transport and mobilization from the hepatopancreas to the haemolymph and other body tissues. Soy lecithin has been observed to enhance growth performance across various larval and shrimp species, such as *P. japonicus* (Teshima *et al*., 1986; Kanazawa *et al*., 1985), *P. monodon* (Tan *et al*., 2022), *L. vannamei* (Gong *et al*., 2000; Hu *et al*., 2011; Liou *et al*., 2023), and *Macrobrachium rosenbergii* (Thanh Hien *et al*., 2005). Previous studies also showed that larval mud crab, *Scylla serrata* (Holme *et al*., 2007), juvenile swimming crab, *Portunus trituberculatus* (Li *et al*., 2014; Wang *et al*., 2016) and Chinese mitten crab, *Eriocheir sinensis* (Lin *et al*., 2020, Lin *et al*., 2021) also benefited from the inclusion of soy lecithin that leads to the improvement of feed conversion, better survival rate, growth rate and molting frequency.

Besides promoting growth performance, the benefits of dietary soy lecithin are further appreciated for its effects on gonadal development of various broodstock such as red claw crayfish, *C. quadricarinatus* (Wang *et al.*, 2013), Chinese mitten crab, *Eriocheir sinensis* (Sui *et al*., 2009), adult sea urchin, *Strongylocentrotus intermedius* (Zhang *et al*., 2022), and swimming crab, *Portunus trituberculatus* (Ding *et al*., 2017, Song *et al*., 2019). In this study, both the gonadosomatic index (GSI) and hepatosomatic index (HSI) showed an increasing trend with higher levels of dietary lecithin in the feeds. The result shows that female red claw crayfish fed with 4% phospholipid have significantly higher GSI than other treatments. Same results were obtained on broodstock *Marsupenaeus japonicus* (Alava *et al*., 1993), *E. sinensis* (Sui *et al*., 2009), adult *C. quadricarinatus* (Wang *et al*., 2013), and female juvenile crayfish, *Procambarus clarkii* (Hou *et* *al*., 2022), which suggested that dietary lecithin affect the mobilization of lipids from the hepatopancreas to the ovary, and higher dietary lecithin yield higher GSI. It is proven that an increased amount of soy lecithin improves ovarian development.

It is apparent that recent findings resemble to the study conducted by Wang *et al*. (2013) which also aims at investigating the impact of SL but focuses more on assessing vitellogenin mRNA expression levels in female *C. quadricarinatus.* However, the differences were in the type of ingredients incorporated in the diets, the condition that the organisms were cultured in and the size of the organisms. In summary, their findings show that higher SL levels also contributed to higher percentage of polyunsaturated and highly unsaturated fatty acids in ovaries specifically linoleic and alpha-Linolenic which are crucial in ovarian development. It has provided evidence that dietary SL can enhance ovarian maturation in crayfish fed 2% SL diet, based on the vitellogenin mRNA expression level.

The highest spawning rate was recorded in crayfish fed with diet SL1 (1% lecithin). Although spawning rate peaked at this level, increasing the amount of dietary soy lecithin in the feed also led to a higher total egg count and greater fecundity. These findings are consistent with previous research on *E. sinensis* (Sui *et al.*, 2009; Wu *et al*., 2007). Likewise, similar patterns were observed in Litopenaeus stylirostris broodstock fed diets containing 1.5% soy lecithin (Bray *et al.*, 1990). Furthermore, studies on *L. vannamei* broodstock showed that increasing dietary phospholipid levels improved hatching rates, larval output, and spawning frequency, supporting recommendations to include 2% phospholipids in broodstock diets (Cahu *et al*., 1994). A study by Cavalli *et al*. (2000) observed no detectable effect on reproductive performance when increasing the dietary PL inclusion in the diet, hence recommended that 0.79% which is the lowest dietary PL level should be sufficient for *Macrobrachium rosenbergii* broodstock. Previous studies have discussed the positive effects of soy lecithin in the diet of red claw crayfish on reproductive performance; however, they did not specifically address its impact on spawning rate. To our knowledge, this is the first study on ABP-based diet with the supplementation of soy lecithin for red claw crayfish broodstock.

Egg development of experimental crayfish in the present study followed the stages described in a previous study reported by Yeh and Rouse (1994). The red claw crayfish (*C. quadricarinatus*) exhibits distinct ovarian development stages, characterized by changes in egg color and number. Primary vitellogenesis involves milky white oocytes, while secondary vitellogenesis produces yellow to orange eggs (Abdu *et al.*, 2000). Ovarian maturation can be assessed using an in vivo staging technique, with fecundity ranging from 200 to 1000 eggs per female, increasing with female size (Jones, 1995). The ovary undergoes morphological changes, progressing from parallel strands to H-shaped and finally Y-shaped (Vazquez *et al.*, 2008). Hatching can occur within 30 days at 28°C but may take as many as 45 days at 26°C (Vogt *et al*., 2004). Changes in egg characteristics and ovarian development prepare the females for spawning and egg-holding during their reproductive cycle (Sagi *et al*., 1996). Despite this, more specific research is required to determine its precise influence on egg color in red claw crayfish, as current literature does not explicitly address this factor.

It has been noted that some of the female crayfish drop their broods during incubation, which is a common phenomenon across various crustacean species and is influenced by several factors. One of the possible reasons could be sperm limitation due to the high number of females compared to males present in the environment. This can result in few ovigerous females with a limited number or size of clutches or reduce fertilization rate as numbers of females have restricted choice of appropriately sized males for mating (Sato *et al*., 2010; Van Son and Thiel, 2007). Additionally, static water conditions may foster the fungal growth of eggs (Edgerton *et al*., 2002; Fikri *et al*., 2022). However, not all of the clutches of eggs were affected, which means that maternal care of the crustacean can help to minimize the occurrence of infections. In addition, young and inexperienced females are more likely to lose eggs, as it takes several broods for them to learn proper care techniques (Baeza *et al.*, 2016). Experienced females, conversely, exhibit higher egg retention rates. In addition, stress, such as being handled roughly by humans, can also prompt egg release, leading to the abandonment of the entire egg clutch. Furthermore, social interactions were observed to contribute to increased egg losses as well as egg cannibalism in freshwater crayfish (Mason, 1970).

The dietary treatments did not significantly affect the moisture and crude ash content in red claw crayfish. However, an increasing level of soy lecithin in the diets was associated with a gradual reduction in the crayfish's lipid content. A similar trend was previously reported in channel catfish, where whole-body lipid levels remained unchanged despite higher dietary lecithin supplementation. This outcome, however, differs from findings in other species such as rainbow trout (Poston, 1990), white sturgeon (Hung *et al.*, 1987), Atlantic salmon (Poston, 1991), Nile tilapia (Kasper and Brown, 2003), and red drum (Craig and Gatlin, 1997), where increased dietary lecithin led to higher body lipid content.

CONCLUSION

The present study demonstrated that dietary lecithin inclusion in ABP-based feeds has positive effects on red claw crayfish broodstock. Increasing dietary lecithin has a significant effect on the GSI, HSI, and fecundity of the red claw crayfish. However, the highest spawning rate was observed in the lowest dietary lecithin in the feed. Thus, it is recommended that at least 1% of lecithin should be supplemented in the feeds based on ABP. These data provide a useful starting point for understanding the role of lecithin in red claw crayfish broodstock and the requirement in broodstock feed for the commercial production of red claw crayfish.

ACKNOWLEDGEMENTS

We would like to express our gratitude to the Borneo Marine Research Institute for providing the research facilities to conduct this research. This research is supported by the Skim Dana Khas awarded by the Universiti Malaysia Sabah (SDK0127-2020).

AUTHORS' CONTRIBUTIONS

MM contributed to conceptualization, investigation, visualization, formal analysis, and writing of the original draft. RS was responsible for methodology, validation, visualization supervision, and writing – review and editing. ASKY, ADT, LLS, and ASA contributed to formal analysis, validation, and data curation. All authors have read and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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