



Growth Performance of Smooth Marron (*Cherax cainii*) Fed Different Dietary Protein Sources

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Abstract

Fish farming including freshwater crayfish still has a dependency on the availability of fish meal as the main source of protein in feed. The purpose of this study was to evaluate alternative protein sources including poultry by-products, feather meal, lupine flour, soybean meal and meat, and bone meal for smooth marron (*Cherax cainii*) freshwater crayfish feed. A total of 150 juvenile marrons (9.09 ± 0.21 g) were kept for 90 days and distributed into 15 rearing tanks consisting of five treatments with three replications. The results indicated that there was no significant difference in the increase in biomass, survival rate, molting rate (weight), feed efficiency ratio, and feed digestibility ($P > 0.05$). However, marron juvenile fed with a protein source of poultry by-products meal had the highest specific growth rate (0.31 ± 0.05 g/day). In addition, marron fed with fish meal protein resulted in a significant increase in carapace length ($P < 0.05$). Based on the digestibility test, it was found that the digestibility level of the feed ranged from $76.39 \pm 0.01 - 79.11 \pm 0.01\%$ and replacement of fish meal had no significant effect on dry matter digestibility ($P > 0.05$). Overall, the results of this study indicate that the general growth performance of marron is not affected by the replacement of fish meal in the feed. Alternative protein materials can be used as a protein source to replace fish meal in marron feed so that the use of fish meal can be reduced.

INTRODUCTION

The massive use of fish meal as an aquafeed protein resource increase with the expansion of global aquaculture production in the last decades. Attempts to identify fish meal replacement ingredients have become a major concern in the aquaculture industry, including freshwater crayfish (crawfish). Studies on the use of animal-derived protein including poultry by-products, feather

meal, meat and bone meal, black soldier fly meal for crawfish diets have been reported (Foyosal *et al.*, 2019; Fuertes *et al.*, 2013; 2014). A recent experiment of inclusion poultry by-product meal in the diet of marron crayfish resulted in comparable growth (0.49 ± 0.12 %/day) have been reported (Foyosal *et al.*, 2019). Apart from animal protein, plant-derived protein such as soybean meal and lupin

meal have also been considered as alternative dietary protein resources for freshwater crayfish. In *C. quadricarinatus*, the performance of a juvenile fed diet containing soybean meal as the protein resources was reported to be resulting an acceptable specific growth rate of 4.12 ± 0.3 %/day (Thompson *et al.*, 2005). Besides, a specific growth rate of 1.12 ± 0.15 %/day in marron was reported after the fish meal substitution using lupin meal in the diet under field experimental (Fotedar, 2004).

Smooth marron (*Cherax cainii*) is one of the eight species within the *Cherax* genus which native to the Southwestern region of Western Australia and the Southeastern part of Australia. Begun in 1970, marron aquaculture production grows steadily and reach 63.8 tons of its production in the 2017/2018 period with a total value of \$AUD 2.1 million (Steven *et al.*, 2020). Current marron production is focusing on recreational purposes. However, potential aquaculture of this species also needs to be considered. In the Western Australian aquaculture industry, marron has the highest productive licenses compared to other eight cultured species including barramundi, mussels, yabbies, silver perch, goldfish, ornamental invertebrates, ornamental fish and rainbow trout (Steven *et al.*, 2020). Thus, indicated that marron is an important aquaculture commodity. Future marron aquaculture will be affected by the fact that the major protein sources of aquafeed are from the fish meal which is predicted to be limited in the future. To anticipate the problem of fish meal supply, further action is required including finding alternative protein resources for aquaculture diets including those for marron. Although fish meal replacement studies have been conducted on crustacean species, studies of fish meal replacement on the diet of marron are very limited. The present study was designed to evaluate total fish meal replacement by four dietary protein sources, including poultry by-product meal, meat and bone meal, lupin meal and feather meal to the

growth performance of juvenile marron and to assess the digestibility of each of these ingredients.

METHODOLOGY

Place and Time

The study was conducted at the Curtin University Aquatic Research Laboratory facility, Perth (Western Australia) from June to September 2016.

Research Material

The material used in the study were marron juvenile weighing 9.09 ± 0.21 grams, chromium oxidase, fish meal, feather meal, lupin meal, poultry by-product meal, meat and bone meal, soybean meal, wheat, corn/wheat starch, betacaine, cod liver oil, cholesterol and casein. The research equipment used were cylindrical fiberglass tanks, pellet extruder, oven, digital water quality checker, analytical balance and digital caliper.

Research Design

This study used five treatments with three replications. The diet containing fish meal was used as the marron basal feed (K). In the other four diets, fish meal was replaced by feather meal (D1), lupin meal (D2), poultry by-product meal (D3), and meat and bone meal (D4).

Work Procedures

Marron Juveniles Preparation

In this study, the marron juveniles (9.09 ± 0.21 g) were sourced from Aquatic Resource Management Pty. Ltd. in Manjimup, Western Australia and transported to Curtin University Aquaculture Research Laboratory. Around 200 marron juveniles were acclimated for two weeks in four cylindrical tanks (500 L volume) and fed with a commercial marron diet at 3% of body weight rate per day. At the end of acclimation, a total of 150 survived and healthy marron juveniles were selected and distributed into 15 cylindrical experimental fiberglass tanks

(10 animal per tank containing 100 L freshwater and continuous aeration system. Marron was separated individually using clear 750 mL containers. During the feeding trial, marron juvenile fed five formulated diet at 3% of body ratio once per day for 90 days of feeding experiment. All uneaten feed and fecal matter were siphoned from the tanks twice a day.

Diets Preparation

In this study, five different protein sources were used as protein sources of isoenergetic and isonitrogenous marron diets as previously described by Saputra *et al.* (2019). The quantity of fish meal replacer was adjusted until the final crude protein content around 30 g.kg⁻¹ dry weight basis for each diet (Table 1).

Table 1. Weight (gram dry weight) and proximate analysis of four experimental diets and a reference diet used to evaluate fish meal replacement in marron diets.

Ingredients	Diets				
	K	D1	D2	D3	D4
Fish meal ^a	301.5	0	0	0	0
Feather meal ^a	0	220	0	0	0
Lupinmeal ^a	0	0	364	0	0
Poultry by-product meal ^a	0	0	0	295	0
Meat and bone meal ^a	0	0	0	0	243
Soybean meal ^b	108	156.5	210	108	166
Wheat	481.3	483.1	286.7	508.8	431.3
Corn/wheat starch ^b	48	55	40.1	48	55
Betacaine ^b	12	10.2	35	12	35
Cod liver oil ^b	42	48	34.5	21	40
Cholesterol ^b	2.5	2.5	2.5	2.5	2.5
Casein ^b	2.5	22.5	25	2.5	25

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Before the diet production, the protein sources ingredients, soybean meal, and wheat for each diet were sieved using 100 µm mesh screens. The mixture was then added with the rest of the ingredients and mixed thoroughly with the addition of a small amount of distilled water. Additional calcium carbonate (0.2 g), ascorbic acid (0.5 g), and vitamin premix (1.5 g) was added to each tested diet prior

to pellet production. The dough was then processed with a pellet extruder to produce pellets (3 mm diameter) that were then dried in the oven at 60 °C for approximately 24 hours. Following diet production completion, the chemical parameter of the diet including ash content, gross energy and crude protein content were analyzed.

Table 1. Proximate Composition of Tested diets (g.kg⁻¹ dry weight basis).

Parameters	Diet				
	K	D1	D2	D3	D4
DM%	89.07	87.85	88.46	87.86	87.26
Ash%	8.24	3.18	6.21	4.05	10.13
GE MJ/kg	18.63	18.39	18.59	18.57	18.34
CP%	30.13	30.46	30.84	30.88	30.18

Data Collection

Growth

All marron juveniles were measured and weighed at the commencement of the

feeding trial, at day 30, day 60, and day 90. Percentage weight gain and specific growth rate (SGR) were determined using the formulae (Tan *et al.*, 2018) as follows:

$$\text{Weight Gain (\%)} = \frac{100 \times (W_t - W_0)}{W_0}$$

$$\text{SGR/day (\%)} = \frac{100 \times (\ln W_t - \ln W_0)}{t}$$

Note:

W_t = weight at the time intervals sampled
 W_0 = weight at the commencement of the experiment
 t = experiment period (days)

The feed efficiency ratio (FER) was calculated following equation:

$$\text{FER} = \frac{\text{weight gain}}{\text{feed mass consumed}}$$

The molting rate was calculated as follows:

$$\text{MR (\%)} = 100 \times \frac{\Sigma \text{moulting marron}}{\Sigma \text{marron}}$$

$$\text{MI (\%)} = 100 \times \frac{\text{weight after moult} - \text{initial weight}}{\text{weight before moult}}$$

$$\text{BI (\%)} = 100 \times \frac{\text{Final biomass} - \text{Initial biomass}}{\text{Initial biomass}}$$

Note:

MI = molt increment
 BI = biomass increment

Pellet Stability and Digestibility

Tested feeds from the original studies were used in subsequent digestibility study by incorporating 1% of chromium oxide (Cr_2O_3). The diet was reproduced with the addition of 1% Cr_2O_3 . The digestibility study was carried out for 10 days using seven marron juveniles in a similar experimental system to that of the first diet study. The fecal matter was monitored and collected twice per day after feeding and stored in a beaker glass until ready for analysis. The digestibility was measured in terms of the percentage of total dry matter digested.

To assess the quality of the tested diet, pellet water stability, and digestibility study was performed. Pellet stability was determined based on the leaching rate of the diets in the water at 10 minutes and 60 minutes. Six replicates of two grams of each diet were prepared by transfer it into 150 mL glass beakers containing 50 ml of distilled water. After the water immersion time was completed, water in the beaker glass was then siphoned off until the minimum level was reached. Feed samples were then dried at 60°C for 48 hours and then the percent dry matter loss was calculated using the following equation:

$$\text{DM loss (\%)} = \frac{\text{dry weight}}{\text{wet weight}} \times 100$$

To assess the Cr_2O_3 concentration in each sample, approximately 0.1 g of each sample was transferred to an assigned digestion tube and 5 mL of 70% HNO_3 was added. All tubes were then placed in the digester (Foss Tecator Digestor 8, Sweden) for 20 minutes at 300°C. Three mL of 70% perchloric acid was added to each sample after the digestion process. The reheating process changed the sample color from green to yellow or orange. The samples were then diluted into 50 mL distilled water and transferred into 100 mL flasks. Distilled water was added to each flask to obtain 100 mL total volume. The amount of Cr_2O_3 both in the diet and feces were measured photometrically (S20 spectrophotometer, Boeco, Germany) by comparing its absorbance with the standard curve at 350 nm of wavelength. A standard curve was made by diluting a series of 0.2, 0.4, 0.8, 1, 1.4, 1.8, and 2 mg of chromium oxide into 100 mL. The concentration of chromium oxide (mg/100 mL) both in feed and feces samples were determined by plotting the stock solution concentration against the average absorbance reading as described by Jones and De Silva (1998) using the equation:

$$\text{Cr}_2\text{O}_3 \text{ Concentration (\%)} = \frac{C}{W}$$

Note:

C = concentration (mg/100 mL)
 W = weight of the original sample (mg)

Apparent digestibility dry matter (ADMD) of each feed types was measured using the following equation:

$$\text{TD (\%)} = \frac{100 - (1\% \text{ marker in feed})}{\% \text{ marker in faeces}}$$

Note:

TD = total digestibility

Data Analysis

Statistical analyses were performed to determine the effect of feeding treatment on the physiological responses and diet performance of marron. All data were analyzed using parametric analysis in SPSS version 25.0 software. Results are presented as mean and standard error

values. The significant difference from each treatment was evaluated using randomized block design and analysis of variance ANOVA. The least significant difference posts hoc test tests were performed to determine significant differences amongst treatment groups and results were determined as significant at $P < 0.05$.

RESULTS AND DISCUSSION

Attempts on the evaluation of fish meal replacement in freshwater crayfish

diets have been reported with various results (Fotedar, 2004; Foysal *et al.*, 2019; Fuertes *et al.*, 2012, 2013; Tan *et al.*, 2018). As shown in Table 3, after 90 days of the feeding trial, the mean of the specific growth rate of marron juvenile fed with different dietary protein sources was not significantly different ($P > 0.05$). The mean of the specific growth rate is ranging from 0.21 to 0.31 % per day (Figure 1). Similar results were also observed in survival rate, feed conversion rate and feed efficiency rate among any marron juveniles fed with the test diets (Table 3).

Table 3. Specific growth rate, survival rate, FCR, and FER (mean \pm SE values). Data with different superscript (^{a,b}) in the same row indicate significantly different at $P < 0.05$ over periods.

Aspects	Diets Treatment				
	K	D1	D2	D3	D4
SGR (% day ⁻¹)	0.27 \pm 0.02 ^a	0.29 \pm 0.01 ^a	0.21 \pm 0.02 ^a	0.31 \pm 0.05 ^a	0.30 \pm 0.05 ^a
SR (%) [*]	90.00 \pm 0.58	83.33 \pm 1.20	100.00 \pm 00	90.00 \pm 0.58	96.67 \pm 0.33
FCR [*]	2.94 \pm 0.26 ^a	2.47 \pm 0.31 ^a	3.79 \pm 0.46 ^a	2.78 \pm 0.62 ^a	2.26 \pm 0.45 ^a
FER	11.40 \pm 0.98 ^a	13.72 \pm 1.60 ^a	9.01 \pm 1.22 ^a	13.37 \pm 3.44 ^a	15.65 \pm 2.62 ^a

There were also no significant differences ($P > 0.05$) in the molting rate and weight molt increment of the marron juveniles fed without fish meal in the diet.

However, marron fed with diet containing K showed a significantly longer ($P < 0.05$) molt increment compared with tested diets (Table 4).

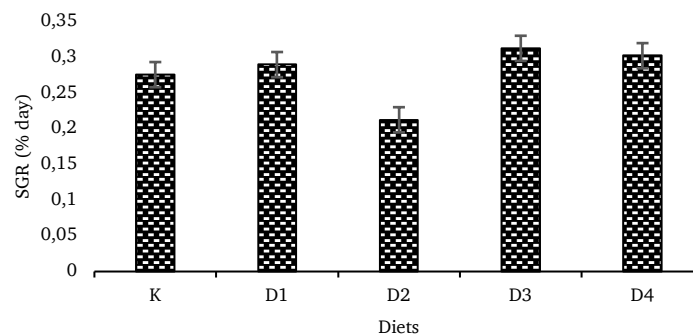


Figure 1. The specific growth rate of marron fed with different dietary protein sources. Bar with different superscript (^{a,b}) indicates significantly different at $P < 0.05$ over time periods.

The specific growth rate in the present study was comparable to previous experiments on marron (Ambas *et al.*, 2013; Foysal *et al.*, 2019). The probiotic supplementation on marron diets resulted in an SGR value of 0.27 to 0.51%/day (Ambas *et al.* 2013), while Foysal *et al.* (2019) reported the SGR value of marron

was 0.49 to 0.53 %/day after 60 days of feeding trial. In contrast, growth rates obtained in this study were lower than the results from previous studies using earthen pond culture systems (Fotedar, 2004). The lower growth rate obtained in the present study can be attributed to the shorten feeding trial period. The feeding

trial conducted by Fotedar (2004) was lasted for 10 months and under a semi-intensive culture system rather than under laboratory conditions. Another factor that may affect the low SGR value may be attributed to characteristics of the species. The consistent higher SGR value of other crayfish species has been reported. In *C. quadricarinatus* the SGR was reported between 3.43 – 3.95 %/day (Thompson *et al.*, 2010) while the SGR in *P. clarkii* was reported to be 1.02 - 2.43 %/day (Hua *et al.*, 2015; Tan *et al.*, 2018).

The inclusion of any types of protein resources in the diets did not affect the apparent digestibility of dry matter of the diets (Table 4). However, at both periods of water immersion, the mean of total dry matter loss of diet containing fish meal was significantly higher ($P < 0.05$) than the other diets (Figure 3). In contrast, diet D3 was significantly low ($P < 0.05$) at 10 minutes of water exposure. Each type of feed was not significantly affecting the water quality during the experiment period.

Table 4. Molting rate, molt weight increment, molt length increment, apparent digestibility of dry matter for marron fed four test diets and a control diet (K) after 90 days of culture (mean \pm SE values). Data with different superscripts (1,2) in the same row indicate significantly different at $P < 0.05$ overtime periods.

Aspects	Diets Treatment				
	K	D1	D2	D3	D4
MR	50.00 \pm 5.77 ^a	43.33 \pm 6.67 ^a	66.67 \pm 13.33 ^a	53.33 \pm 3.33 ^a	73.33 \pm 8.82 ^a
MI weight	30.90 \pm 5.98 ^a	19.54 \pm 4.51 ^a	19.71 \pm 1.78 ^a	25.30 \pm 3.75 ^a	26.74 \pm 3.2 ^a
MI length	11.39 \pm 1.21 ^a	6.24 \pm 0.66 ^b	6.77 \pm 0.47 ^b	9.77 \pm 1.48 ^b	7.32 \pm 1.38 ^b
ADMD	76.96 \pm 0.02 ^a	77.89 \pm 0.02 ^a	77.33 \pm 0.02 ^a	76.39 \pm 0.01 ^a	79.11 \pm 0.01 ^a

Previous fish meal replacement experiment using similar diets ingredients was reported by Saputra *et al.* (2019) with emphasis on the immunological aspects. Results indicated that immunological conditions of marron were affected by the total inclusion of fish meals. In contrast, the substitution of fish meal with several other dietary protein sources did not affect the survival rate of marron juvenile and feed conversion ratio. A similar high survival rate from equivalent feeding trials was also reported in other crawfish species (Fuertes *et al.*, 2012; 2013). It is difficult to link the direct impact of the protein sources in the diets with the survival rate, however, the isolation system as used in that study had a positive effect on the survival rate. That method can reduce the cannibalism behavior of marron especially during the molting period as reported by Fuertes *et al.* (2013). Feed conversion ratios obtained in the study were slightly lower than data reported (Hua *et al.*, 2015) and higher than *P. clarkii* (Tan *et al.*, 2018). Although the results were not

significantly different between the treatments, diets containing meat and bone meal exhibited the same FCR as all the other treatments. This similar FCR reflects the same diet utilization. Similarly, the diets containing meat and bone meal had the same ADMD value as all the other diets. Association of the ADMD value and FCR does not warrant further investigation based on these results.

Biomass increment is strongly associated with the growth of marron and has been used to determine the growth of crayfish in feeding trial experiments (Ambas *et al.*, 2013). In the present study, different protein sources had no effect on the BI of marron fed different test diets. The biomass increment varied from 21.54% to 33.71% at the end of the experiment. Although other attempts to increase BI have been conducted by the administration of customized probiotics into the marron, the result was not positive (Ambas *et al.*, 2013).

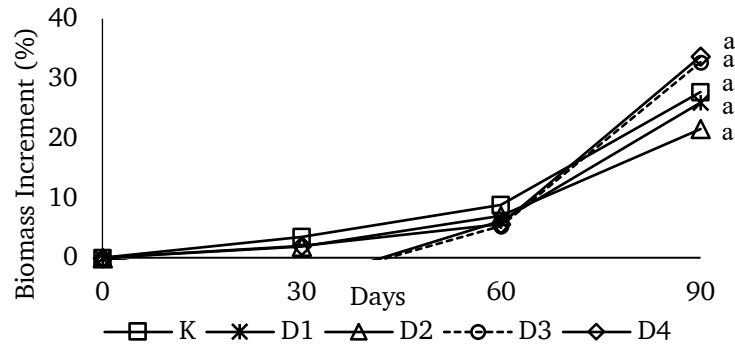


Figure 2. Biomass increment of marron juveniles fed with test diets with different protein sources. Data with different superscript (^{a,b}) on the top symbol indicate significantly different at P<0.05 overtime periods.

The molt increment can be determined using the increment of the bodyweight (Jones *et al.*, 1996) and carapace length (Salama and Hartnoll, 1992). The weight increment after molting of marron in the present study ranged from 19.54 to 30.90% which is in line with the results from Jussila and Evans (1996). The findings of the incorporation of fish meal protein effectively increase the carapace length increment of marron juveniles in the present study is a significant result. Evaluation of the effect of protein sources

in diets on the molting rate of freshwater crayfish is very limited. However, the fish meal inclusion combined with the protein levels in the diets of yabby has been reported to be strongly correlated with its molting rate (Jones *et al.*, 1996). In the present study, results varying among the treatments and the average molting rate is 57.33%. The average molting rate of the current study is comparable to a previous study molting rate of marron which was only $44.1 \pm 13.7\%$ (Rouse and Kartamulia, 1992).

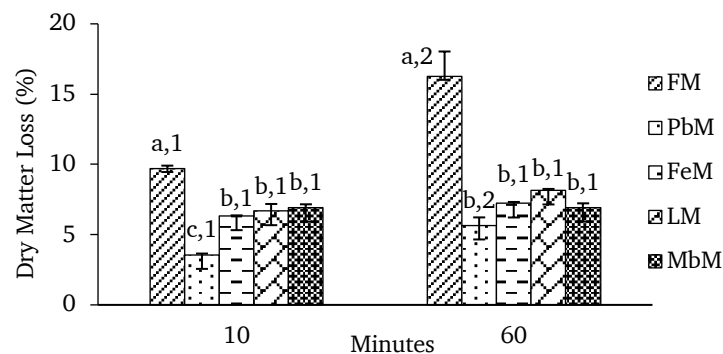


Figure 1. Total dry matter loss (%) test diets after 10 minutes and 60 minutes of water exposure. Data with different superscripts (^{a,b}) on the top bar indicate significantly different at P<0.05 within periods. Data with different superscript (^{1,2}) on the top bar indicate significantly difference at P<0.05 between periods.

Apparent dry matter digestibility of diets in the present study was not significantly influenced by the dietary protein source, which ranged from 76.29% to 79.11%. Similar to our data results, two previous digestibility studies

performed on crayfish (Jones and De Silva, 1998) resulted in high ADMD. Furthermore, according to the present dietary experiments, high diet stability in water was achieved in all diets tested and the leaching rate off all diets at 60 minutes

of water immersion was very low. That results were reflected by the optimum

condition of water quality during the experiment period (Table 5).

Table 5. Water quality of treatment tanks. Data with different superscript (^{a,b}) in the same row indicate significantly different at $P < 0.05$ overtime periods.

Water Quality	Treatments				
	K	D1	D2	D3	D4
Temperature	20.1 ± 0.68 ^a	19.7 ± 0.28 ^a	19.4 ± 0.58 ^a	19.6 ± 0.27 ^a	19.1 ± 0.34 ^a
DO (mg/L)	8.51 ± 0.18 ^a	8.54 ± 0.11 ^a	8.65 ± 0.07 ^a	8.77 ± 0.04 ^a	8.65 ± 0.07 ^a
pH	8.07 ± 0.01 ^a	8.09 ± 0.01 ^a	8.04 ± 0.02 ^a	7.95 ± 0.02 ^a	8.08 ± 0.01 ^a
Ammonia	< 0.05	< 0.05	< 0.05	< 0.01	< 0.05

Feed quality is one of several potential constraints in aquaculture activity. Poor feed quality will increase the ammonia level in the water and can be exacerbated by improper feed management. Improving FCR and stability of pelleted diet are possible mitigation strategies to reduce the adverse impact of poor-quality feed. Using optimum nutrient requirements and higher ingredients digestibility are also can be considered to maintain the water quality. The current study demonstrated that fish meal ingredients can be replaced completely by using other protein sources without negative effects on the general growth and other biological condition on marron juveniles. In addition, the total fish meal inclusion as protein resources in the diets did not affect the quality of the diets. Results of this study contribute to the body of knowledge of marron nutrition, as there was a limited record of the study that had been undertaken on the substitution of alternative protein resources in marron juvenile diets.

CONCLUSION

In conclusion, the fish meal replacement in marron juvenile diets did not impair the growth, feed efficiency ratio, molt increment weight, and molting rate. However, a noticeable effect on total fish meal inclusion was observed in molt increment length of marron. In terms of feed quality, the digestibility and diet stability study indicated that the diets are acceptable for marron culture. Therefore, the use of alternative protein sources

might be considered in commercial marron farming in the future.

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