

Original Research Articles

Evaluation of earthworm meal (*Eudrilus eugeniae*) as fish meal replacement in juvenile Mangrove Crab (*Scylla serrata*) diet

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Two feeding trials were conducted to evaluate the performance of the earthworm (*Eudrilus eugeniae*) meal (vermimeal) as a replacement for fish meal in the diet of juvenile mangrove crab (*Scylla serrata*) in terms of growth, feed efficiency, intermolt duration, carapace width increment, molting success, survival, and incidence of cannibalism. Four experimental diets were formulated with 0, 25, 50, and 75% fish meal protein replaced with earthworm meal protein, designated as D0, D25, D50, and D75, respectively. In the first feeding trial, the experimental diets were fed to crablets with an initial average weight of 0.09 ± 0.007 g. Each replicate comprised ten (10) crablets individually stocked in 750ml-capacity plastic containers randomly distributed in a static water bath. Final weight gain, feed efficiency and survival were measured after the 5th molt. In the second feeding trial, 150 crablets with an average weight of 0.07 ± 0.002 g were stocked at 30 crablets each in five (5) replicate tanks. Survival and incidence of cannibalism were monitored daily until the end of the culture period of 30 days. The first study showed that the growth of mangrove crab juveniles fed the diet with up to 50% of the fish meal protein replaced by the earthworm meal protein was statistically comparable to the control group. The survival of crablets in the second feeding trial was highest in D25 (33%), followed by D50 (29%), D0 (18%), and lowest in the group fed with D75 (13%). Overall, earthworm meal (*Eudrilus eugeniae*) can replace as much as 50% of the fish meal protein in juvenile mangrove crab (*Scylla serrata*) diet without negative effects on growth, carapace width increment, feed efficiency, intermolt duration, molting success, survival, and cannibalism.

INTRODUCTION

The use of artificial feeds in commercial-scale aquaculture of mangrove crabs is relatively recent compared to other aquaculture species. It is regarded to be in the early stages of development.¹ However, the declining supply of fish meal worldwide threatens the anticipated eventual shift of feeding crabs and other crustaceans to formulated diets and the use of fish by-products and fish meal as main feed ingredients. Hence, the search for alternative protein sources for high-value aquaculture species, such as the mangrove crab, continues to be a major challenge.

While many studies have identified alternative protein sources for aquatic animals, supply sustainability has been a critical issue. One potential source of animal protein for fish meal replacement is earthworm meal. It has an amino

acid profile similar to fish meal² and contains lipids high in n-3 fatty acids, which can enhance immunity in animals.³ Earthworm meal has a protein content of 50-70% and lipid content of 5-10% on a dry weight basis, which are in the range of fish meal and fits well with the nutrient requirements of crustaceans such as prawns and crabs.^{4,5} Earthworms are among the least expensive animals to mass produce as they require comparably cheaper raw materials. Production of earthworms is relatively simple, with locally-sourced inputs that can easily be sustained. However, due to low demand, mass production of earthworms cannot be easily materialized unless processed as an earthworm meal and used as a feed ingredient for high-value aquatic species. Replacement of fish meal with earthworm meal in fish⁶⁻¹² and in *Penaeus monodon*¹³ diets had been shown to promote growth and survival in these species.

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Table 1. Proximate composition of fish meal and earthworm meal

Feed Ingredient	Moisture (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	Ash (%)	NFE (%)
Fish meal*	10.00	65.90	12.00	0.30	13.0	3.00
Earthworm meal**	6.58	60.40	15.36	2.44	11.58	3.64

*Data from SEAFDEC/AQD, Philippines

**Analyzed values

Survival rate and weight gain of tiger shrimp fed a formulated diet containing 10% dried earthworm (*Eudrilus eugeniae*) without fish meal was higher than in shrimp fed a diet containing 30% fish meal.¹³ To our knowledge, the use of earthworm meal in the culture of mangrove crabs has not been reported. Owing to its high protein content and amino acid profile, which is comparable to that of fishmeal, earthworm meal has the potential to be a fishmeal replacement in mangrove crab diets.

The objective of this study was to evaluate the performance of diets with different levels of earthworm meal as an alternative to fish meal protein in terms of growth, feed conversion ratio or feed efficiency, intermolt duration, carapace growth, molting success, survival, and incidence of cannibalism in mangrove crabs. This study focused on the juvenile stage of mangrove crab (0.5-1.0 cm carapace width or CW) since this stage is characterized by a high incidence of cannibalism, adversely affecting survival and hence a bottleneck in the mass production of mangrove crab seeds. Thus, suitably formulated diets that support good growth and high survival by reducing cannibalism will create a demand for such diets by mangrove crab hatchery operators. The earthworm meal can become economically viable if it supports significantly improved survival in mangrove crab hatchery seed production. Moreover, it could contribute to developing both the mangrove crab and vermiculture industries and further promote organic farming.

MATERIALS AND METHODS

TEST FEED INGREDIENT

Live earthworms (*Eudrilus eugeniae*) commonly called as African night crawlers were obtained from a local supplier. The earthworms were processed into a meal, first, by washing thoroughly to remove mud, silt and dirt followed by blanching in lukewarm water (60-65°C) and drying in an oven at a temperature not exceeding 80°C for 72 h. The dried worms were then ground and made to pass through a 60-micron mesh sieve. The sieved earthworm meal was mixed thoroughly and stored in a plastic container until use. A representative sample was taken for proximate analysis. The proximate composition of the earthworm meal and the fish meal used in this study is presented in [Table 1](#).

The mangrove crab feed formulation based on Catacutan¹⁴ was modified by including different levels of earthworm meal as a protein source ([Table 2](#)). Four (4) test diets

with 0, 25, 50, and 75% of fish meal protein substituted by earthworm meal protein were formulated and coded as D0, D25, D50, and D75, respectively. Samples of the formulated diets were taken for proximate analysis.

EXPERIMENTAL ANIMAL

The juvenile crabs were produced in the hatchery of the Southeast Asian Fisheries Development Center, Aquaculture Department (SEAFDEC, AQD), Tigbauan, Iloilo, Philippines, from wild-caught *Scylla serrata* breeders. The identity of the species was based on the description of Keenan et al.¹⁵

FEEDING TRIALS

The experiments were conducted at the hatchery facility of the Brackishwater Aquaculture Center, Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas, Leganes, Iloilo, Philippines.

FEEDING EXPERIMENT 1 (EVALUATION OF THE EXPERIMENTAL DIETS IN MANGROVE CRABS IN TERMS OF GROWTH, CARAPACE WIDTH INCREMENT, FEED EFFICIENCY, INTERMOLT DURATION, AND MOLTING SUCCESS)

Juvenile mangrove crabs with an average weight of 0.09±0.007g were reared individually in 750ml-capacity plastic containers placed inside a wooden trough measuring 16ft (L) x 4ft (W) x 0.5ft (H). The trough was half-filled with seawater, which served as a water bath to maintain the temperature in a static water-rearing system.

Each dietary treatment (D0, D25, D50, and D75) had 4 replicates of 10 crabs stocked individually in plastic containers per replicate or 40 crabs per treatment. The crablets were maintained and fed with the control diet at 3-5% body weight (BW) until they molted (M0). The crablets' initial weight and carapace width were measured individually using a caliper and an analytical balance (Mettler-Toledo), respectively, after their shell had hardened 36 to 42 hours after molting and feeding of the experimental diets started. The number of molts measured the growth of crabs, and each crab was monitored every day until the fifth molt (M5). The crablets' weight and carapace were measured a day after each molting. The crablets were also monitored daily for successful molting or unsuccessful molting that resulted in mortality. They were fed at satiation level (approximately 3-5 % BW) twice daily at 9 am and 4 pm. The culture water in individual containers was changed just

before feeding in the morning and afternoon. The water temperature and salinity of the containers were monitored once daily. Final weight gain, feed efficiency, and survival were measured after M5. Intermolt duration and molting success were likewise measured.

FEEDING TRIAL 2 (EVALUATION OF THE EXPERIMENTAL DIETS IN MANGROVE CRAB IN TERMS OF SURVIVAL AND INCIDENCE OF CANNIBALISM)

One hundred fifty (150) juvenile mangrove crabs with an average weight of 0.07 ± 0.002 g were stocked at 30 individuals per 100L-capacity plastic tanks with a dimension of 60cm (L) x 45cm (W) x 45cm (H) provided with sufficient aeration. The dietary treatments with five (5) replicates were assigned randomly to each tank. Three bunches of shelters with about 25 strips of 0.30m long plastic straw tied together in the mid portion were also provided in each tank to reduce the chance of encounter among crabs and to minimize cannibalism.

Each diet (D0, D25, D50 and D75) was fed to four (4) groups of crabs in five (5) replicate tanks. Each tank was supplied with flow-through seawater daily for one (1) hour before feeding in the morning and in the afternoon with a flow rate of 1 L min^{-1} . Survival and incidence of cannibalism (dead crabs and those with bite marks and missing body parts) were monitored daily. The crabs in each tank were weighed in bulk every week for 30 days for feed adjustment and growth monitoring.

Water parameters such as ammonia, dissolved oxygen, pH, salinity and temperature were monitored every five days.

The various parameters were computed as follows:

$$\text{Specific Growth Rate (SGR)} = \frac{(\ln W_f - \ln W_i)}{T}$$

where: $\ln W_f$ = natural log of final weight

$\ln W_i$ = natural log of initial weight

T = culture period (No. of days)

Weight Gain (g) = $W_f - W_i$

where: W_f = Final weight(g)

W_i = Initial weight (g)

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Feed consumed (g)}}{\text{Weight gain (g)}}$$

Intermolt Duration = no. of days between molts

Carapace Width Gain = $CW_f - CW_i$

where: CW_f = Final Carapace Width after nth molts(cm)

CW_i = Initial Carapace Width(cm)

Molting Success = Survival (%) after nth molts

$$\text{Survival Rate (\%)} = \frac{\text{Final count}}{\text{Initial count}} \times 100$$

Incidence of cannibalism = no. of dead crabs and those with bite marks and missing claws

STATISTICS

Data were analyzed using SPSS Version 15. Homogeneity and normality of the data were checked using Levene's test, and the Kolmogorov-Smirnov and the Shapiro-Wilk tests, respectively. Significant differences among treatment means were determined by Analysis of variance (ANOVA) followed by Tukey's test for post-hoc comparison of treatment means.

RESULTS

FEEDING TRIAL 1

The proximate compositions of the experimental diets are presented in [Table 2](#). Nutrient contents were similar for all the diets with 49.8-51% protein, 7.8-8.8% lipid, and 356.8-366.8 kcal 100g^{-1} diet energy levels. The growth performance and survival of the juvenile mangrove crab fed with the different diets are shown in [Table 3](#). Weight gain (D50-1.55g), specific growth rate (SGR) (D50-2.39% day^{-1}), and survival (D50-98%) of the crab fed diets with earthworm meal replacing fish meal protein up to 50% was not statistically different from the control group with weight gain, SGR, and survival rate of D0-1.71g, D0-2.61% day^{-1} , and D0-98%, respectively. Similarly, the carapace width increment (D0-0.89%/day) was not affected up to 50% (D50-0.86% day^{-1}) with earthworm replacement ([Table 4](#)). Growth (D75-2.23% day^{-1}) and survival (D75-78%) of the crabs fed with D75 were the lowest and significantly different from the control group (SGR, D0-2.61% day^{-1} ; Survival, D0-98%) ([Table 3](#)). Likewise, the feed conversion ratio (FCR) showed the same pattern with the growth of the crab. Replacement of fish meal with earthworm meal protein to as much as 50% had no remarkable adverse effects on the efficiency of the diet. Also, diets at any replacement levels did not influence the success of molting in the crabs. Among the four treatments, only one crab from the group fed D75 was observed to have exhibited unsuccessful molting during the period M2-M3.

[Table 5](#) shows a decreasing trend in crude protein, crude lipid, and nitrogen-free extract (NFE) values as earthworm incorporation increased. In contrast, the opposite trend was observed in their crude fiber and ash contents.

FEEDING TRIAL 2

Results of the monitoring of survival rate and incidence of cannibalism in each treatment are presented in [Figure 1](#). Survival was highest in D25 at 33% followed by D50 at 29%, D0 at 18% and lowest in D75 at 13%. Incidence of cannibalism equates to mortalities and therefore exhibited the opposite trend, with D25 having the lowest percentage of incidence, followed by D50, D0, and D75 with the highest incidence of cannibalism/mortality.

DISCUSSION

The study showed that replacing fish meal protein with earthworm meal protein by as much as 50% did not adversely affect growth and survival, feed efficiency, molting duration and success, and incidence of cannibalism in the mangrove crab *Scylla serrata*. The earthworm (*Eudrilus eugeniae*) meal used in the present study had an analyzed protein level of 60.4%, within the reported values for earthworm meals. Growth of crabs until the fifth molt (M5) demonstrated that such a high substitution of fish meal for earthworm meal is possible. Studies have shown the potential value of earthworms as a protein source for feeds.^{16,17}

Table 2. Composition (g/kg) and proximate analysis (%) of the experimental diets*

Ingredients	CP (%)	D0	D25	D50	D75
Fish meal (Danish) 65.9	69.5	470.0	352.5	235.0	117.5
Earthworm meal 60.4	60.4	0	135.0	270.0	406.0
Squid meal		20.0	20.0	20.0	20.0
Acetes sp.		85.0	85.0	85.0	85.0
Seaweed		10.0	10.0	10.0	10.0
Defatted soybean meal		90.0	90.0	90.0	90.0
Bread flour		150.0	150.0	150.0	150.0
Danish fish oil		20.0	20.0	20.0	20.0
Soybean oil		20.0	20.0	20.0	20.0
Lecithin		0.3	0.3	0.3	0.3
Vitamin C		0.5	0.5	0.5	0.5
Dicalcium phosphate		10.0	10.0	10.0	10.0
CarboxymethylCellulose		30.0	30.0	30.0	30.0
Mineral mix ^a		20.0	20.0	20.0	20.0
Vitamin mix (Tas Mix) ^b		10.0	10.0	10.0	10.0
Rice bran		63.8	45.3	29.2	10.7
Total(g)		1000.0	1000.0	1000.0	1000.0
Moisture (%)		6.3	6.4	6.5	6.1
Crude Protein (%)		50.8	51.0	49.8	50.6
Crude Lipid (%)		8.7	7.8	8.8	8.5
Crude Fiber (%)		2.2	2.6	2.5	2.5
Ash (%)		12.6	12.5	11.8	11.8
NFE (%)		19.4	19.7	20.6	20.5
Gross Energy (kcal/100g diet) ^c		366.8	356.8	362.4	363.6

*Modified from Catacutan.¹⁴^aMineral Mix (per 100g diet): Iron-40mg, Manganese-10mg, Zinc- 40mg, Copper- 4mg, Iodine-1.8mg, Cobalt-0.02mg, Selenium-0.2mg)^bVitamin mix (per 100gdiet): Vit A-1,200IU, Vit D3 – 200 IU, Vit E,20 IU, Vit B1-8mg, Vit B2-8mg, Vit B6-5mg, Vit B12- 2mg, Niacin- 40mg, Calcium Pantothenate-20mg, Biotin-4mg, Folic Acid-18mg, Ethoxyquin, 0.05mg.^cComputed based on the following physiological values: 4.5kcal/g protein, 8.0 kcal/g lipid and 3.3 kcal/g nitrogen-free extract**Table 3. Growth, feed efficiency, and survival in *Scylla serrata* crablets fed the experimental diets until the 5th molt (M0 to M5)***

Dietary Treatment	Initial Wt. (M0) (g)	Final Wt. (M5) (g)	Wt. gain (g)	SGR ¹ (%/day)	FCR ²	Survival (%)	ID ³ M0 to M5 (No. of Days)
D0	0.09	1.8	1.71±0.06 ^b	2.61±0.03 ^{bc}	1.77±0.08 ^{ab}	98±2.50 ^b	118±1.49 ^{ab}
D25	0.09	1.99	1.90±0.09 ^b	2.75±0.05 ^c	1.66±0.07 ^a	95±2.89 ^b	113±0.87 ^a
D50	0.09	1.64	1.55±0.04 ^{ab}	2.39±0.05 ^{ab}	2.19±1.07 ^{bc}	98±2.50 ^b	122±2.12 ^{ab}
D75	0.09	1.41	1.32±0.07 ^a	2.23±0.04 ^a	2.48±0.07 ^c	78±2.50 ^a	124±3.43 ^b

*Means±SD of 4 replicates. Means not sharing the same superscript letters are significantly different (P<0.05)

¹Specific Growth Rate²Feed Conversion Ratio³Intermolt Duration

Sabine¹⁸ and Lee¹⁹ determined the crude protein of earthworm *Eisenia foetida* to range from 60-70%. According to Paripuranam et al.,⁷ earthworm meal was a more suitable and acceptable feed ingredient than fish meal for *Labeo rohita* and *Hemigrammus caudovittatus* fingerlings. The study conducted by Tuan et al.¹² also revealed that protein di-

gestibility in earthworm meal-substituted diet for common carp (*Cyprinus carpio*, L.) was higher than the full fish meal-based protein diet. The amino acid profile of earthworms is very similar to that of fish meal, as analyzed in the present study (Table 6). A previous study conducted by Dedeke et al.² on four earthworm species (*Eudrilus eugeniae*, *Hy-*

Table 4. Carapace width increment in *Scylla serrata* crablets fed the experimental diets until the 5th molt (M0 to M5)*

Dietary treatment	Initial Width (M0) (cm)	Final width (M5) (cm)	Width increment (cm)	Width Increment (%/day)
D0	0.88±0.01	2.47±0.06 ^b	1.60±0.05 ^b	0.89±0.04 ^{ab}
D25	0.91±0.04	2.57±0.09 ^b	1.66±0.05 ^b	0.92±0.03 ^b
D50	0.89±0.04	2.42±0.07 ^{ab}	1.53±0.05 ^{ab}	0.86±0.10 ^{ab}
D75	0.89±0.02	2.30±0.09 ^a	1.41±0.08 ^a	0.77±0.03 ^a

*Means not sharing the same superscript letters are significantly different ($P<0.05$).

Table 5. Proximate composition (%) of the crablets fed the experimental diets until the 5th molt (M0 to M5)

Crab sample	Moisture (%)	Crude Protein (%)	Crude Lipid (%)	Crude Fiber (%)	Ash (%)	NFE (%)
Initial*	6.77	33.09	7.18	3.51	32.29	17.16
D0	7.06 ^a	35.98 ^d	4.50 ^d	5.95 ^a	28.99 ^a	17.52 ^c
D25	7.24 ^a	35.15 ^c	3.79 ^c	6.54 ^b	30.12 ^b	17.16 ^c
D50	8.82 ^b	34.23 ^b	2.85 ^b	6.47 ^b	31.52 ^c	16.11 ^b
D75	8.86 ^b	33.10 ^a	2.70 ^a	8.69 ^c	32.38 ^c	14.27 ^a

*Before administering the experimental diets.

Means not sharing the same superscript letters are significantly different ($P<0.05$).

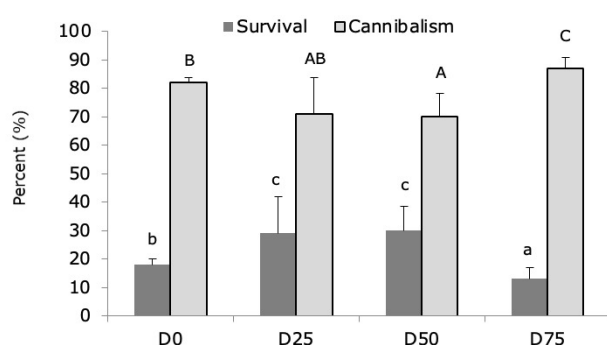


Figure 1. Survival and cannibalism in juvenile mangrove crab-fed diets with fish meal replaced with earthworm meal protein. Means $\pm$ SD (n=5 replicate tanks). The means that do not share the same superscript letters differ significantly ($P<0.05$).

riodrilus africanus, *Alma millsoni*, and *Libyodrilus violaceus*) revealed a total of 17 amino acids consisting of 9 essential (lysine, histidine, arginine, threonine, valine, methionine, isoleucine, leucine and phenylalanine) and 8 non-essential amino acids (aspartic acid, serine, glutamic acid, proline, glycine, alanine, cystine and tyrosine). Among the 4 species of earthworms studied, African nightcrawlers (*Eudrilus eugeniae*) showed the highest value of the essential amino acids such as lysine, arginine, valine, isoleucine and phenylalanine. Edwards²⁰ showed that earthworm protein contains a higher level of essential amino acids such as lysine and methionine than either meat or fish meal. Dynes²¹ also confirmed that the amino acid profile of earthworm such as

Eisenia foetida, *Lumbricus terriestris* and *Perionyx excavatus* were similar or even better than that of fish meal.

Studies conducted on different fish species revealed successful substitutions of fish meal with earthworm meal. Steamed earthworm-based pellets at 12.16% substitution level were proven to enhance catfish *Clarias gariepinus* growth.⁹ Moreover, 56% replacement of fish meal by whole earthworm meal in the diet of catfish *Clarias gariepinus* enhanced growth and survival of the fish.¹¹

Results of growth and survival in the present study were similar to the findings of Piedad-Pascual¹³ with 30 percent inclusion of *Eudrilus eugeniae* meal for *Penaeus monodon* resulted in higher weight gain and survival rate. A decreasing trend in crude protein, crude lipid, and nitrogen-free extract and an increasing trend the crude fiber and ash contents were shown in the proximate composition of juvenile crabs fed with increasing levels of earthworm meal in the diet in the present study. Nevertheless, the growth, feed efficiency, and survival were not adversely affected up to 50% replacement level; only the crablets fed at 75 % replacement level exhibited significantly inferior results when compared against the control. The results of this study are also comparable to the study done on milkfish fry where 55% earthworm (*Eudrilus eugeniae*) meal replacement for fish meal protein had no deleterious effects on the growth, survival, and FCR in the fish.⁶ In tilapia fry diet, 50% earthworm meal was found to be the optimum substitution level for Peruvian fish meal. Growth parameters (weight gain, and specific growth rate or SGR), feed utilization efficiency (FCR and FCE), protein efficiency ratio (PER) and % protein retention of tilapia fry did not vary significantly when up to 50% fish meal protein was substituted with earthworm meal.²² Olele and Okonkwo⁸ suggested the

Table 6. Amino acid compositions of earthworm meal, fish meal, experimental diets and juvenile mangrove crabs

	Protein sources		Experimental diets				Crablets				
	Earthworm meal	Fish meal	D0	D25	D50	D75	Initial	D0	D25	D50	D75
Protein (%)	60.4	65.9	50.8	51.0	49.8	50.6	33.1	35.9	35.2	34.2	33.1
Amino acids and composition (%AA)											
Aspartic acid	9.54	5.48	10.38	10.22	9.97	9.619	9.04	11.68	11.18	10.96	10.44
Threonine	5.43	2.61	2.70	3.56	3.91	4.291	3.11	4.25	4.27	4.14	4.28
Serine	5.93	2.58	5.80	5.89	5.71	5.65	4.52	3.82	3.47	3.24	3.33
Glutamic Acid	14.15	8.48	15.66	15.60	15.20	14.77	11.10	6.70	4.10	3.36	3.48
Glycine	8.37	3.88	13.89	12.37	11.03	9.95	18.66	14.16	15.55	16.33	16.36
Alanine	12.20	4.01	13.06	12.83	12.55	12.14	9.57	12.25	13.00	13.20	13.27
Cystine	2.00	0.72	2.02	2.26	2.86	4.49	8.73	1.81	2.41	3.97	2.68
Valine	6.74	3.07	4.13	4.80	5.35	5.58	4.40	6.52	6.86	6.86	7.02
Methionine	2.46	1.65	2.64	2.59	2.47	2.39	1.88	2.54	2.11	1.90	1.82
Iso-leucine	5.53	2.39	2.62	3.36	4.03	4.30	1.36	4.93	5.28	5.36	5.44
Leucine	9.65	4.69	7.32	7.82	8.39	8.44	3.76	9.13	9.51	9.59	9.90
Tyrosine	3.07	2.02	1.84	2.06	2.22	2.39	1.12	4.09	4.17	3.84	3.99
Phenylalanine	3.77	2.57	3.82	3.72	3.80	3.74	3.17	4.37	4.51	4.28	4.50
Histidine	1.86	2.00	3.52	2.98	2.82	2.63	6.88	2.68	2.26	2.05	2.05
Lysine	6.09	4.79	6.03	5.98	5.98	5.97	4.68	6.90	7.19	7.02	7.12
Tryptophan	0.33	3.70	0.38	0.43	0.42	0.46	0.54	0.59	0.63	0.70	0.72
Arginine	2.87	0.50	4.21	3.54	3.31	3.19	7.49	3.58	3.45	3.20	3.60

use of 50% earthworm meal in place of fish meal for formulation of diet for *Heteroclinus* fingerlings. For common carp, Pucher et al.¹⁰ found earthworms to be a suitable sole animal protein source in supplemental feeds under semi-intensive pond management, whereas Tuan et al.¹² found it to be suitable only as partial replacement.

It must be pointed out that the growth of crabs fed formulated diets in the present study was slower (1.4–1.9 g in 120 days) compared to the crabs fed either live or frozen *Artemia* diets (~3.7 g and ~2.03 g in 40 days) in the study of Anh et al.²³ However, growth was greatly reduced when they were fed *Artemia*-based formulated diet (~0.29 g in 40 days). This may suggest components in the live and frozen *Artemia* that are missing in the dried *Artemia*-based diet as well as in the diets used in this study. Moreover, individual rearing of mud crabs results in higher survival at the expense of growth compared to group or communal rearing even within the same type of diet.²⁴

Early reports showed that formulated diets were inferior feeds for crabs compared to natural feeds^{25–27} which may explain the poor growth of crabs in all treatments of this study. Unfortunately, natural feeds were not included as one of the control treatments. The control treatment used in this study was a diet patterned after the best mud crab diet tested at the Southeast Asian Fisheries Development Center by Catacutan.²⁸ However, the crabs used in the present study were very small (~0.07 g) while those used in the study of Catacutan²⁸ were way bigger (~9.15 g). Wilson²⁹ reported that bigger juvenile crabs such as those used in the soft-shell crab industry adapt easily to formulated diets as compared to the smaller juveniles. This may be related to the fact that the digestive capacity of smaller crabs is limited compared to that of adults.^{30,31}

On average, the total number of days for the juvenile crabs to molt from M0 to M5 in the present study was shortest (113 days) in those fed D25 than those fed the control (D0) diet (117 days) or D50 (122 days). Crabs fed D75 had the longest duration (124 days) to molt from M0–M5. Ecdysone acts as a molting hormone and regulates physiological functions such as growth, metamorphosis and reproduction.³² The success of molting is influenced by the level of molting hormone ecdysone in the body of mangrove crab larvae,³³ which is affected by many factors, including nutritional status. Certain amino acids can activate signaling pathways, such as the Target of Rapamycin (TOR) pathway,³⁴ to promote ecdysone synthesis. In *Eriocheir sinensis*, the addition of cottonseed meal hydrolysates above 4% increased the ecdysone receptor transcription, ecdysone concentration, and weight-to-growth ratio, resulting in improved growth and molting performance.³⁵ Hence, failed molting can be attributed to inadequate ecdysone hormones required for the crab to shed its shell and grow. Throughout the experiment in the present study, only one unsuccessful molting was observed during molting from M2–M3 in the group fed D75 and was insignificant. This suggested that earthworm meal can replace fish meal protein by up to 50 percent without negative effects on juvenile mangrove crabs' growth (as measured by molting duration). The molting of crabs in the study of Anh et al.²³ was more

frequent (at least 5 times in 40 days) compared to the results of this study (5 times in 118–124 days). This may be explained by the differences in the size of crabs at stocking (0.0079 g vs 0.07 g in the present study). Based on observations, the intermolt duration lengthens as the crabs grow, and the growth increment decreases with size. As in the case of growth, molting frequency is also affected by the kind of feed, e.g., live and frozen *Artemia* vs dried *Artemia*-based or earthworm-based diets. Until now, no artificial feed that can replace natural feed for mud crabs is commercially available.³⁶ Successful mud crab nursery culture employs natural feeds or a combination of natural and artificial diets.^{24,37} Other groups have reported the use of formulated diets that perform equally well as natural feeds,^{5,38} but these research findings need further testing to fully understand the factors that may promote or inhibit growth/molting and pave the way for the widespread use of commercially available formulated diets for all stages of mud crab.

Cannibalism, as evidenced by the high mortality of crablets stocked in the same tank, is a common problem among crustaceans. This can be minimized by lowering stocking densities, use of shelters, and by giving adequate good quality feed that provides for the crab's nutrient requirements. In this feeding experiment, crablets fed D25 and D50 gave a significantly higher survival than the control (D0), with only 18% survival. As crab feeding can respond to some chemical cues, earthworm meals might contain nutrients or substances that affected/lessened the cannibalistic behavior of crabs. The slower growth and lower molting frequency (compared to other studies) might be compensated by lesser cannibalism; thus, higher survival was obtained with earthworm meal-based diets in this study.

Overall, earthworm meal (*Eudrilus eugeniae*) is a promising alternative protein source for the formulated diet of juvenile mangrove crab (*Scylla serrata*). Under the conditions of the present study, earthworm meal can replace fish meal protein by as much as 50% in the juvenile mangrove crab diet without adversely affecting growth, feed efficiency, intermolt duration, carapace width increment, molting success, survival, and cannibalism. Notwithstanding, further improvement in the formulation is necessary to achieve growth and performance parameters comparable to feeding with natural feeds.

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REFERENCES

1. Shelley C, Lovatelli A. *Mud Crab Aquaculture- A Practical Manual*. FAO; 2011.
2. Dedek GA, Owa SO, Olurin KB. Amino acid profile of four earthworm species from Nigeria. *Agriculture and Biology Journal of North America*. 2010;1(2):97-102. <http://www.scihub.org/ABJNA>
3. Beg MM, Mandal B, Moulick S. Potential of earthworm meal as a replacement of fish meal for Indian major carps. *International Journal of Fisheries and Aquatic Sciences*. 2016;4(3):357-361. <http://www.fisheriesjournal.com>
4. Bhavani M, Hareesh K, Suneetha Y, Srinivasulu Reddy M. The effect of dietary protein on the growth potentials and nitrogen excretion in freshwater prawn *Macrobrachium rosenbergii*. *International Journal of Fisheries and Aquatic Studies*. 2014;2(2):243-248.
5. Zheng P, Han T, Li X, et al. Dietary protein requirement of juvenile mud crab *Scylla paramamosain*. *Aquaculture*. 2020;518:734852. doi:[10.1016/j.aquaculture.2019.734852](https://doi.org/10.1016/j.aquaculture.2019.734852)
6. Catacutan MR. *A Terminal Report: Vermimeal (African Nightcrawler, Eudrilus Eugeniae) Protein as Fishmeal Substitute in the Diet of Milkfish Fry (Chanos Chanos Forskal)*.; 2007.
7. Paripuranam TD, Divya V V, Ulaganathan P, Balamurugan V, Umamaheswari S. Replacing fishmeal with earthworm and mushroom meals in practical diets of *Labeorohita* and *Hemmigrammus caudovittatus* fingerlings. *Indian Journal of Animal Research*. 2011;45(2):119. <http://www.arccjournals.com>
8. Olele NF, Okonkwo JC. Replacement of fishmeal with graded levels of earthworm meal in the diet of fingerlings: Effect on feed and growth parameters. *Journal of Agricultural Science and Technology A*. 2012;2:901-908.
9. Zakaria Z, Liam K, Hajar Rakanudin I. Steaming process optimization, physical testing and analysis of growth performance parameters in earthworm-based pellets for African catfish (*Clarias gariepinus*). *Journal of Asian Scientific Research*. 2013;3(6):578-586. <http://acssweb.com/journal-detail.php?id=5003>
10. Pucher J, Ngoc TN, ThiHanhYen T, Mayrhofer R, El-Matbouli M, Focken U. Earthworm meal as fishmeal replacement in plant based feeds for common carp in semi-intensive aquaculture in rural Northern Vietnam. *Turk J Fish Aquat Sci*. 2014;14(2):557-565. doi:[10.4194/1303-2712-v14_2_27](https://doi.org/10.4194/1303-2712-v14_2_27)
11. Omeru ED, Solomon RJ. Comparative analysis on the growth performance of catfish (*Clarias gariepinus*) fed with earthworm as a replacement of fish meal. *American Journal of Research Communication*. 2016;4(6):89-125. <https://www.researchgate.net/publication/319162860>
12. Tuan NN, Pucher J, Becker K, Focken U. Earthworm powder as an alternative protein source in diets for common carp (*Cyprinus carpio* L.). *Aquac Res*. 2016;47(9):2917-2927. doi:[10.1111/are.12743](https://doi.org/10.1111/are.12743)
13. Piedad-Pascual F. An evaluation of three worms as feed ingredients in formulated diets for juvenile *Penaeus monodon* Fabricius. *Fish Res J Philippines*. 1985;10:9-15.
14. Catacutan MR. Biological evaluation of existing feed formulations for the grow-out culture of the mud crab, *Scylla serrata*. In: Quintio ET, Parado-Estepa FP, Coloso RM, eds. *Philippines: In the Forefront of the Mudcrab Industry Development: Proceedings of the 1st National Mud Crab Congress, 16-18 November 2015, Iloilo City, Philippines*. SEAFDEC Aquaculture Department; 2017:69-76.
15. Keenan CP, Davie PJF, Mann DL. A revision of the genus *Scylla* de Haan 1833 (Crustacea: Decapoda: Brachyura: Portunidae). *The Raffles Bulletin of Zoology*. 1998;46(1):217-245.
16. Stafford EA, Tacon AGJ. Nutritive value of the earthworm, *Dendrodrilus subrubicundus*, grown on domestic sewage, in trout diets. *Agricultural Wastes*. 1984;9(4):249-266. doi:[10.1016/0141-4607\(84\)90084-2](https://doi.org/10.1016/0141-4607(84)90084-2)
17. Vielma-Rondon R, Ovalles-Duran JF, Leon-Leal A, Medina A. Nutritional value of earthworm flour (*Eisenia foetida*) as a source of amino acids and its quantitative estimation through reversed phase chromatography (HPLC) and pre-column derivation with o-phthalaldehyde (OPA). *Pharmaceutica*. 2003;44(1):43-58.
18. Sabine JR. Earthworms as a source of food and drugs. In: Satchell JE, ed. *Earthworm Ecology: From Darwin to Vermiculture*. Chapman and Hall; 1983:285-296. doi:[10.1007/978-94-009-5965-1_24](https://doi.org/10.1007/978-94-009-5965-1_24)
19. Lee KG. *Earthworm: Their Ecology and Relationship with Soils and Land Use*. Academic Press; 1985.
20. Edwards CA. Production of feed protein from animal waste by earthworms. *Phil Trans R Soc Lond B*. 1985;310(1144):299-307. doi:[10.1098/rstb.1985.0120](https://doi.org/10.1098/rstb.1985.0120)

21. Dynes RA. *Earthworms Technology Information to Enable the Development of Earthworm Production: A Report for the Rural Industries Research and Development Corporation (RIRDC)*. Australia; 2003:1-33.
22. Papalid LV. *Replacement of Fishmeal by Vermimeal in the Diets for Sex Reversed Tilapia, Oreochromis Mossambicus Peters Fry Reared in a Closed Recirculating System*. M.S. Thesis. University of the Philippines Visayas; 2008.
23. Anh NTN, Ut VN, Wille M, Hoa NV, Sorgeloos P. Effect of different forms of Artemia biomass as a food source on survival, molting and growth rate of mud crab (*Scylla paramamosain*). *Aquaculture Nutrition*. 2011;17(2):e549-e558. doi:[10.1111/j.1365-2095.2010.00796.x](https://doi.org/10.1111/j.1365-2095.2010.00796.x)
24. Syafaat MN, Azra MN, Waiho K, et al. A review of the nursery culture of mud crabs Genus *Scylla*: current progress and future directions. *Animals*. 2021;11(7):2034. doi:[10.3390/ani11072034](https://doi.org/10.3390/ani11072034). PMID:34359163
25. Marasigan E. Development of practical diet for grow-out of mud crab species *Scylla serrata* and *Scylla tranquebarica*. In: Keenan C, Blackshaw A, eds. *Mud Crab Aquaculture and Biology*. Vol 78. ACIAR Proceedings; 1999:187-195.
26. Genodepa J, Failaman A. Evaluation of selected commercial feeds as substitute for natural feeds in rearing of mud crab (*Scylla serrata*) juveniles. *ACCL Bioflux*. 2016;9(5):993-1000.
27. Khan MSK, Haque MM, Islam MM, Hoq ME. Nursery rearing of wild seeds of mud crab (*Scylla* sp.) in saline water earthen pond. *The Journal of NOAMI*. 2017;34(1):33-38.
28. Catacutan MR. Growth and body composition of juvenile mud crab, *Scylla serrata*, fed different dietary protein and lipid levels and protein to energy ratios. *Aquaculture*. 2002;208(1-2):113-123. doi:[10.1016/S0044-8486\(01\)00709-8](https://doi.org/10.1016/S0044-8486(01)00709-8)
29. Wilson T. Development of extruded feeds for soft-shell crab production in Thailand. In: *Abstracts, International Workshop on Culture, Fisheries and Stock Enhancement of Portunid Crabs, Iloilo City, Philippines*. ; 2005:37.
30. Holme MH, Zeng C, Southgate PC. A review of recent progress toward development of a formulated microbound diet for mud crab, *Scylla serrata*, larvae and their nutritional requirements. *Aquaculture*. 2009;286(3-4):164-175. doi:[10.1016/j.aquaculture.2008.09.021](https://doi.org/10.1016/j.aquaculture.2008.09.021)
31. Genodepa J, Zeng C, Southgate PC. Changes in digestive enzyme activities and nutrient utilization during embryonic development and starvation of newly hatched larvae of the mud crab, *Scylla serrata*. *Aquaculture*. 2018;493:137-143. doi:[10.1016/j.aquaculture.2018.04.053](https://doi.org/10.1016/j.aquaculture.2018.04.053)
32. Gunamalai V, Kirubakaran R, Subramoniam T. Hormonal coordination of molting and female reproduction by ecdysteroids in the mole crab *Emerita asiatica* (Milne Edwards). *General and Comparative Endocrinology*. 2004;138(2):128-138. doi:[10.1016/j.ygcen.2004.06.002](https://doi.org/10.1016/j.ygcen.2004.06.002)
33. Imayavaramban L, Dhayaparan D, Devaraj H. Molecular mechanism of molt-inhibiting hormone (MIH) induced suppression of ecdysteroidogenesis in the Y-organ of mud crab: *Scylla serrata*. *Federation of European Biochemical Societies Letters*. 2007;581(27):5167-5172. doi:[10.1016/j.febslet.2007.09.029](https://doi.org/10.1016/j.febslet.2007.09.029)
34. Mykles DL. Signaling pathways that regulate the crustacean molting gland. A review. *Frontiers in Endocrinology*. 2021;12:1-21. doi:[10.3389/fendo.2021.674711](https://doi.org/10.3389/fendo.2021.674711)
35. He CF, Liu WB, Zhang L, Chen WL, Liu ZS, Li XF. Cottonseed meal protein hydrolysate improves the growth performance of Chinese mitten crab (*Eriocheir sinensis*) by promoting muscle growth and molting performance. *Aquaculture Nutrition*. 2023;2023:1-12. doi:[10.1155/2023/8347921](https://doi.org/10.1155/2023/8347921). PMID:37415969
36. Liew KS, Yong FKB, Lim LS. An overview of the major constraints in *Scylla* mud crabs grow-out culture and its mitigation methods. *Aquaculture Studies*. 2024;24(1):993. doi:[10.4194/aquast993](https://doi.org/10.4194/aquast993)
37. Paran BC, Jeyagobi B, Kizhakedath VK, et al. Production of juvenile mud crabs, *Scylla serrata*: captive breeding, larviculture and nursery production. *Aquaculture Reports*. 2022;22:101003. doi:[10.1016/j.aqrep.2021.101003](https://doi.org/10.1016/j.aqrep.2021.101003)
38. Gong YY, Lu JX, Wang XS, Huang YQ. Response of hatchery-cultured mud crab (*Scylla paramamosain*) instar fed natural preys and an artificial feed. *Israeli Journal of Aquaculture - Bamigdeh*. 2017;69(1). doi:[10.46989/001c.21058](https://doi.org/10.46989/001c.21058)