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Research Article

Black cricket (*Gryllus bimaculatus*) meal as a protein source in the practical diets for juvenile whiteleg shrimp (*Litopenaeus vannamei*)

Peh K.L.[1]; Shapawi R.[1]; Lim L.S.[1*]

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Abstract

The present study was conducted to evaluate the potential of black cricket (*Gryllus bimaculatus*) meal (BCM) as an alternative protein source in the practical diets for the whiteleg shrimp, *Litopenaeus vannamei* juveniles. Six dietary treatments with different levels of BCM at 0, 10, 20, 30, 40, and 50% (namely BCM 0–BCM 50, respectively) were prepared, and each diet was fed to triplicate tanks of *L. vannamei* juveniles (initial body weight 0.73±0.01 g) for 40 days. At the end of the experiment, shrimps fed the BCM-included diets (BCM10, 20, 30, 40 or 50) generally attained the higher weight gain (WG), specific growth rate (SGR), survival percentage, and the better feed conversion ratio (FCR) than that fed the control diet (BCM0). Nevertheless, the shrimps fed BCM50 attained the highest WG (1124.6%), SGR (2.71%/ day), survival percentage (88.3%), and the best FCR value (1.83) among the BCM-included dietary treatments, and these results were significantly higher ($p<0.05$) than those from the control group. In conclusion, the BCM protein can replace 50% of fish meal protein in the practical diets for *L. vannamei* juveniles without any adverse effects on the shrimp growth performance and survival.

Keywords: Insect meal, Two-spotted cricket, Alternative protein source, Pacific white shrimp, Feeding

1-Borneo Marine Research Institute, Universiti Malaysia Sabah. Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia
*Corresponding author's Email: leongsen@ums.edu.my

Introduction

The whiteleg shrimp (*Litopenaeus vannamei*) is an important aquaculture species in many parts of the world. In 2010, 72% (about 2.7 million tonnes) of global shrimp production was provided by *L. vannamei*, and its value was estimated at US$ 11.23 billion (Tacon *et al*., 2013). Shrimp feeds have been conventionally relying on fish meal (FM) as the main dietary protein source (Tacon *et al*., 2013). However, the price of FM is increasing (Tacon and Metian, 2008). As the feed cost comprises almost 40% to 60% of the total production cost of *L. vannamei* (Sookying *et al*., 2013), it is critical to find the suitable alternative protein source to replace the FM protein in the shrimp feeds (Amaya *et al*., 2007).

In recent years, insects have been known as one of the most potential alternative protein sources for animal feeds, mainly due to their high nutritional aspects, low production cost, and many other advantages (Rumpold and Schlüter, 2013; Makkar *et al*., 2014; Stamer, 2015). Many studies also have been conducted to exploit the potential of insects as a protein source in the diets of farmed fishes and crustaceans (Barroso *et al*., 2014; Riddick, 2014; Henry *et al*., 2015).

The black cricket (*Gryllus bimaculatus*), also commonly known as the two-spotted cricket is one of the most abundance cricket species which can be found in the tropical and subtropical regions of Asia, Africa, and Europe (Mito and Noji, 2008). It is easy to be bred and commonly supplied as the bait or food for pets (van Huis *et al*., 2013). Although there is still not many published information on the potential use of black cricket for aquafeeds production, the black cricket meal (BCM) was reported to contain high crude protein (~57%) and lipid (~25%) contents, and also a good essential amino acids profile, which is comparable to the FM in the study by Taufek *et al*. (2013). With these aspects, BCM is highly potential to be used as an alternative protein source to replace the FM protein in aquafeeds. Indeed, BCM can replace up to 100% of FM protein in the diet of African catfish (*Clarias gariepinus*) fingerlings, without adverse effects to the fish antioxidant enzyme activities, haematological response, nutrients and amino acids digestibility, and growth performance (Taufek *et al*., 2013, 2016a, b).

Based on the dietary protein (40%) (Nasir and Davis, 2002) and essential amino acids requirements (Huang *et al*., 2003) of *L. vannamei* juveniles (see Table 1), BCM seemed to be an excellent alternative dietary protein source for shrimps. However, there is still no study on the feasibility of replacing the FM protein in the diets for *L. vannamei* juveniles with BCM protein. It is unknown that how much FM protein in the *L. vannamei* juveniles diets can be replaced with the BCM without adverse effect on animal growth performance. Therefore, the present study aimed to determine the optimum inclusion level of BCM protein to replace the FM protein in the

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diet of *L. vannamei* juveniles.

Table 1: Ingredients, compositions and proximate of the experimental diets.

	Experimental diets					
	BCM0	**BCM10**	**BCM20**	**BCM30**	**BCM40**	**BCM50**
Ingredients (g per 100g diet)						
Fish meal [a]	42.61	38.36	34.10	29.83	25.57	21.31
Soybean meal	10.00	10.00	10.00	10.00	10.00	10.00
Shrimp meal	5.00	5.00	5.00	5.00	5.00	5.00
Fish oil [b]	4.72	3.94	3.16	2.38	1.59	0.81
Black cricket meal	0	5.21	10.44	15.86	20.89	27.6
Vitamin premix [c]	3.00	3.00	3.00	3.00	3.00	3.00
Mineral premix [d]	2.00	2.00	2.00	2.00	2.00	2.00
CMC [e]	5.00	5.00	5.00	5.00	5.00	5.00
Tapioca starch [f]	22.87	22.34	22.18	21.27	20.74	20.21
α-cellulose [g]	4.80	5.15	5.50	5.85	6.21	6.55
Proximate (% Dry matter basis - Mean±SD)						
Protein	42.2 ± 0.1	41.8 ± 0.6	41.9 ± 0.3	42.6 ± 0.3	42.7 ± 2.6	41.1 ± 0.2
Lipid	7.7 ± 0.1	8.1 ± 0.1	8.0 ± 1.0	7.9 ± 0	9.6 ± 0.8	6.8 ± 1.0
Moisture	7.3 ± 0.1	7.3 ± 0.1	8.1 ± 0.2	7.6 ± 0.2	10.3 ± 5.2	8.3 ± 0.3
Ash	11.2 ± 0.1	10.7 ± 0.1	10.3 ± 0.1	10.0 ± 0.2	8.9 ± 0.8	8.8 ± 1.1
Gross energy (Kcal/ 100g)	360	360	360	360	360	360

[a] Danish fish meal, Denmark
[b] Dexchem Industries Sdn. Bhd. , Malaysia
[c] Dexchem Industries Sdn. Bhd. , Malaysia. Contained (g/ kg): ascorbic acid, 300; inositol, 125; niacin, 50; rinoflavin, 15; pyridoxine, 12; thiamin mononitrite, 15; retiny acetate, 1.72; cholecalceriferol, 0.025; menadione sodium bisuphate, 5; niotin, 0.5; folic acid, 2.5; DL- α-tocopheryl acetate, 50; vitamin B12, 0.025; caplan, 25.
[d] Dexchem Industries Sdn. Bhd. , Malaysia. Contained (g/ kg): calcium phosphate • H_20 (MDCP), 397.65; calcium lactate, 327; ferrous sulphate • H_20, 25; magnesium sulphate • $7H_20$, 137; potassium chloride, 50; sodium chloride, 60; potassium iodine, 0.15; copper sulphate • $5H_20$, 0.785; manganese oxide, 0.8; cobalt carbonate, 0.1; zinc oxide, 1.5; sodium selenite • $5H_20$, 0.02.
[e] Carboxy-methyl-cellulose, EMD Chemicals, Inc. San Diego, CA.
[f] AAA Brand, Bake with Me Sdn. Bhd., Malaysia
[g] Sigma-Aldrich Brand, USA

Materials and methods

Preparation of black cricket meal

Black crickets were purchased from a local pet shop and instantly killed by immersing them into the ice water. The black crickets were then dried in an oven at 55°C for 24 hours and ground into powder form. Subsequently, the BCM was kept in a zip-lock bag and stored in a refrigerator at 4°C until use. Proximate composition of the BCM was then determined following the methods by AOAC (2000), while its amino acid profile was determined using a high performance liquid chromatography (HPLC) device (LC-10AVP, Shimadzu Brand, Japan) with a post-column derivation instrument (PCX5200, Picking Laboratories Brand, Mountain View, CA) (Lim *et al*., 2015).

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Formulation and proximate composition of the experimental diets

Following the protein and lipid requirements of *L. vannamei* juveniles which is reported by Nasir and Davis (2002), 6 isoproteic (40% crude protein) and isolipidic (10% crude lipid) diets were prepared. These diets contained different inclusion levels of the BCM at 0, 10, 20, 30, 40, and 50% to replace its FM content, and were named as the BCM0 (control, without BCM), BCM10, BCM20, BCM30, BCM40, and BCM50, respectively. Calculation for the diets formulation was done using Microsoft Office Excel Sheet software, and proximate composition of these diets was determined following the methods by AOAC (2000). Table 1shows the formulation and proximate composition of the experimental diets prepared in the present study.

Experimental animal, rearing condition and feeding protocols

The feeding trial was conducted at the Shrimp Hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah. A total of 360 *L. vannamei* at juvenile stage (body weight 0.73±0.01 g) were obtained from a local shrimp farm and evenly distributed into 18 fiberglass tanks (tank capacity 50 L; 20 shrimps per tank). These tanks were provided with constant flow through seawater (about 0.5 L/min) and aeration. Another 50 specimens from the same group of shrimp were sampled for proximate analysis (AOAC, 2000).

A 40-day feeding trial was conducted in the present study. Each dietary treatment was hand fed to triplicate tanks of shrimp 3 times (0800, 1200, and 1600) daily at 15% of the shrimp’s total body weight. In a 2-week interval, the total body weight of the shrimp in each tank was measured to adjust the amount of feed given. Shrimp mortality was recorded daily if any. At the end of the experiment, the body weight of all shrimp was individually measured. Ten shrimp were randomly sampled from each tank for whole body proximate analysis. Throughout the feeding trial, the water temperature, dissolved oxygen level, and salinity ranged from 27–31°C, 5–6 mg/L, and 30–32‰, respectively.

All experimental animals in the present study (including the black crickets) were cared and handled following the Researchers’ Guidelines on Code of Practice for the Care and Use of Animals for Scientific Purposes, provided by Universiti Malaysia Sabah.

Data and statistical analyses

The growth performance, feed utilization efficiency and survival of the shrimp were evaluated at the end of the experiment, based on the calculation using the formulas listed below:

Weight gain (WG, %) = 100% × (Final weight – Initial weight)/ Initial weight

Specific growth rate (SGR, %/day)=100% × [ln (final weight) – ln (initial weight)]/ day

Feed conversion ratio (FCR) = Total feed consumed/ weight gain

Survival (%) = 100% × (Final number of shrimp)/ (Initial number of shrimp)

All data including the whole body proximate of the shrimp were analyzed with the One-way ANOVA and Turkey HSD post-hoc test using the SPSS v21.0 software. Significant difference was assumed when $p<0.05$.

Results

Table 2 shows the proximate composition and amino acids profile of the BCM used in the present study. The BCM used in this study contained 59.5% crude protein, 24.4% lipid, 5.4% ash, and 5.3% moisture, and these aspects were similar with those of BCM (protein 57%, lipid 25.8%, both moisture and ash 4.8%) reported in Taufek *et al.* (2013). In term of essential amino acids profile, the BCM used in the present study possessed the lower methionine content (1.0%) than that prepared by Taufek *et al.* (2013) (3.8%). Nevertheless, both BCMs contained the same amount of lysine content at 2.9%.

Table 3 shows the growth performance and feed utilization of *L. vannamei* fed with the experimental diets containing different levels of BCM. The weight gain (WG) of shrimps fed BCM10 (mean WG, 829.9%), 20 (866.4%), 30 (856.8%), 40 (956.3%), and 50 (1124.6%) were higher than that of the BCM0 treatment (control, 796.4%). The WG of shrimps from the BCM50 treatment was significantly higher ($p<0.05$) than those from the BCM0 treatment but those from the BCM10, 20, 30, and 40 treatments were not significant ($p>0.05$).

Similar trend was also noticed in the SGR, FCR, and survival rate. The SGR of shrimps from the BCM40 (2.56%/d) and BCM50 (2.71%/d) treatments were significantly higher ($p<0.05$) than that from the BCM0 treatment (2.38%/ d). Shrimps fed BCM20 (2.46%/day) or BCM30 (2.45%/day) also attained the higher SGR than those fed BCM0 but no significant difference ($p>0.05$) was detected among them.

Among all dietary treatments, shrimps fed BCM50 attained the best FCR (1.83) and this result was significantly different ($p<0.05$) from those fed BCM0 (2.25), BCM10 (2.22) or BCM30 (2.17). No significant difference ($p>0.05$) was found among the FCRs of shrimps fed BCM20 (2.10), BCM40 (2.05) or BCM50.

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Table 2: Proximate and amino acid compositions of the black cricket meal prepared in the present study, and those prepared by Taufek *et al*. (2013). The essential amino acids requirement of the *L. vannamei* by Huang *et al*. (2013) was included.

	Black cricket [1] (Present study)	Black cricket [1] (Taufek *et al*., 2013)	Essential amino acids requirement of *L. vannamei* (Huang *et al*., 2003)
Proximate (%, dry matter basis)			
Protein	59.5	57.0	-
Lipid	24.4	25.8	-
Moisture	5.3	4.8	-
Ash	5.4	4.8	-
Amino acid profile (g/ 100g)			**g/ 100g body weight /day**
Aspartic acid	5.2	-	-
Threonine [2]	2.4	2.2	0.046
Serine	2.8	-	-
Glutamic Acid	7.7	-	-
Glycine	3.4	-	-
Alanine	6.6	-	-
Cystine	4.0	-	-
Methionine [3]	1.0	3.8	0.029
Isoleucine [4]	2.3	2.1	0.069
Leucine [5]	4.3	3.8	0.087
Tyrosine	3.5	-	-
Phenylalanine [6]	2.1	2.2	0.051
Lysine [7]	2.9	2.9	0.086
Arginine [8]	4.1	3.5	0.097

[1] *Gryllus bimaculatus*
[2-8] Essential amino acids

Table 3: Growth performance, feed utilization and survival of the shrimps fed with the experimental diets. Data was expressed in mean±SD. Different superscript alphabets indicate the significant difference (p<0.05).

	Experimental diets					
	BCM0	**BCM10**	**BCM20**	**BCM30**	**BCM40**	**BCM50**
WG (%)	796.4 ± 72.6^{a}	829.9 ± 81.5^{a}	866.4 ± 23.4^{a}	856.8 ± 4.8^{a}	956.3 ± 65.7^{ab}	1124.6 ± 201.5^{b}
SGR (%/ day)	2.38 ± 0.07^{a}	2.37 ± 0.08^{ab}	2.46 ± 0.02^{ab}	2.45^{ab}	2.56 ± 0.05^{bc}	2.71 ± 0.14^{c}
FCR	2.25 ± 0.19^{a}	2.22 ± 0.13^{a}	2.10 ± 0.09^{ab}	2.17 ± 0.11^{a}	2.05 ± 0.05^{ab}	1.83 ± 0.23^{b}
Survival (%)	61.7 ± 8.5^{a}	71.7 ± 10.3^{ab}	71.7 ± 6.2^{ab}	80.0^{b}	81.7 ± 2.4^{b}	88.3 ± 6.2^{b}

WG = Weight gain
SGR = Specific growth rate
FCR = Feed conversion ratio

The shrimp's survival percentage increased when the dietary inclusion levels of BCM increased. The highest survival percentage was observed in the BCM50 (88.3%), followed by the BCM40 (81.7%), BCM30 (80.0%), BCM20 and BCM10 (71.7%), and the BCM0 treatments (61.7%). Survival percentage from the BCM50, BCM40, and BCM30 treatments were significantly higher (p<0.05) than that of BCM0, while those from the BCM20 and BCM10 treatments were not significant (p>0.05).

The whole-body proximate compositions of shrimps fed with the

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experimental diets are shown in Table 4. No significant difference (*p*>0.05) was detected in the moisture, protein, lipid, and ash contents of shrimps among all dietary treatments. In general, the moisture was ranged from 69.1% to 76.6%, protein was 14.3% – 21.8%, lipid was 1.6% to 2.0%, and ash was 10.4% to 13.2%.

Table 4: Whole body proximate composition of the shrimp fed with the experimental diets. Data was expressed in mean±SD.

Whole body proximate (%, dry matter basis)	Initial	Experimental diets					
		BCM0	BCM10	BCM20	BCM30	BCM40	BCM50
Moisture	78.6 ± 2.1	69.1 ± 5.0	75.4 ± 2.9	76.6 ± 4.3	72.0 ± 1.8	74.9 ± 6.3	71.1 ± 1.0
Protein	14.3 ± 0.5	21.8 ± 3.9	17.1 ± 1.7	16.0 ± 4.3	19.1 ± 1.6	15.6 ± 2.2	14.3 ± 0.5
Lipid	3.5 ± 0.1	1.9 ± 0.5	1.6 ± 0.2	1.6 ± 0.3	2.1 ± 0.1	1.9 ± 0.1	2.0
Ash	15.8 ± 0.2	12.5 ± 0.3	10.4 ± 3.1	13.2 ± 1.0	11.5 ± 3.0	11.6 ± 0.6	12.2 ± 0.7

Discussion

The BCM used in the present study contained the similar crude protein and lipid levels but the lower methionine content than that prepared by Taufek *et al.* (2013). Such result can be due to the differences in the rearing (especially type of food fed to the insect) and processing methods of the insects between these two studies (Rumpold and Schlüter, 2013). Although the methionine content of BCM used in this study was rather low, it was sufficient to fulfill the requirement and support the growth of shrimp (see Table 2). Indeed, the WG and SGR of the shrimps significantly increased; the FCR and survival percentage were also significantly improved when the FM protein replacement by the BCM protein was increased from 0 to 50%. Although WG, SGR, FCR and survival percentage of the shrimps fed BCM40 showed no significant difference with those fed BCM50, the latter showed the better results than the former one. These results confirmed that the BCM is a good alternative protein sources to replace the FM protein in the diets of *L. vannamei* juveniles, and the optimum BCM dietary inclusion level was 50%. In fact, Taufek *et al.* (2013) also reported that BCM protein can fully (100%) replace FM protein in the diet of *C. gariepinus* fingerlings without any adverse effects on the fish growth performance and feed utilization. Apparently, higher replacement levels of dietary FM protein with BCM protein (>50%) can be practiced to further enhance the *L. vannamei* growth performance, and this hypothesis should be elucidated in the future.

The high efficiency of BCM in promoting the growth of *L. vannamei* was suspected to be contributed by the chitin, which existed in the BCM but not in the FM. According to Taufek *et al.* (2016a, b), the raw BCM contained approximately 7.15% of chitin while none was detected in the FM. In fact, chitin was reported to have protective effect against *Vibrio alginolyticus* in the *L. vannamei* by enhancing the immune ability (Wang *et al*., 2005). Similar results were also reported in freshwater prawn (*Macrobrachium rosenbergii*) (Kumar *et al*., 2015). Other studies also reported that dietary chitin supplementation can generally improve the growth performance and survival of the grass shrimp (*Penaeus monodon*) (Shiau and Yu, 1998) and *Macrobrachium tenellum* (Santos-Romero *et al*., 2017), while Fox (1993) reported the opposite results on the *P. monodon*. Up to now, there is still no information on the effects of dietary chitin supplementation on growth performance of *L. vannamei*; further study on this topic is necessary to confirm that the advantage of BCM over FM is contributed by its chitin content.

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