

Replacement of Fish Meal Protein with Giant Worm (*Zophobas morio*) and Black Cricket (*Gryllus bimaculatus*) in Diet of Cobia (*Rachycentron canadum*)

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ABSTRACT

Replacement of fish meal with alternative protein ingredients is becoming increasingly important in aquaculture diets. In the present study, giant worm (*Zophobas morio*) and black cricket (*Gryllus bimaculatus*) were used to replace fish meal in diets of cobia (*Rachycentron canadum*). Cobia fingerlings, with average initial weight 80.84 ± 0.65 g, were fed for eight weeks with three diets: basal diet (100% fish meal as the main protein ingredient), 30 % replacement of fish meal with giant worm meal, and 30 % replacement with black cricket meal. The results showed that the apparent digestibility coefficients of dry matter (63.2-69.1 %) and crude protein (83.9-88.1 %) of cobia were not significantly different ($p > 0.05$) among the three diets. No significant difference in growth was observed among treatments ($p > 0.05$); the average final weight of the experimental fish ranged between 113.11 ± 3.67 and 120.01 ± 2.89 g, and average specific growth rate ranged between 0.53 ± 0.06 and 0.61 ± 0.04 %. Similar results were revealed for feed conversion ratio (1.16 ± 0.07 - 1.21 ± 0.05) and survival rate (100 %), wherein no significant ($p > 0.05$) differences were observed among treatments. Therefore, the present results clearly demonstrate that giant worm meal and black cricket meal can be used to replace fish meal in cobia diets, at least at 30 % replacement.

Keywords: Aquaculture, Digestibility coefficient, Feed conversion ratio, Feeding trial

INTRODUCTION

The expansion of aquaculture globally has increased the demand for aquaculture feeds, and fish meal is commonly used as main source of protein due to its high nutritional value for fish. Currently, increasing demand for fish meal has led to a global price rise, which partly accounts for the increase in cost of aquaculture feed. The replacement of fish meal in aquatic diets with alternative protein ingredients is becoming increasingly important (Jabir *et al.*, 2012). Some alternative protein sources from animal by-products have insufficient essential

amino acids, e.g., poultry by-product meal (Shapawi *et al.*, 2007) and blood meal (Hussain *et al.*, 2011), while plant-based protein sources contain anti-nutrition factors (Akande *et al.*, 2010). In recent decades, protein from commercially raised insects has been accepted even for human consumption (Finke, 2002). Not surprisingly, then, insects have also received attention as an alternative protein source in animal diets.

Insect meals are becoming increasingly popular as an alternative protein source in place of fish meal due to their high protein content. In

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addition, insects can be produced in large quantities in a short period of time because of their short life cycle. However, many insects are mainly composed of chitin (in the exoskeleton) which may reduce their digestibility by fish, resulting in reduced feed utilization efficiency. The amount of chitin varies by species and stage of development, while the ability of fish to digest chitin also varies by fish species (Barroso *et al.*, 2014). The giant worm (*Zophobas morio*) is an insect that is popularly cultured in Thailand. Although this species has high protein content (Jabir *et al.*, 2012), there has been little study on its use as a protein source in aquatic diets despite the potential demonstrated in similar worm species such as the mealworm (*Tenebrio molitor*) (Ng *et al.*, 2001). Among other popular cultured insects, black cricket (*Gryllus bimaculatus*) is notable for its high protein and essential amino acid content (Finke, 2002). The black cricket is abundant in Thailand and has been studied as poultry feedstuff and as a freshwater fish food. Taufek *et al.* (2016) reported that ground cricket can replace up to 100% of fish meal without impairing growth in African catfish (*Clarias gariepinus*). Therefore, the giant worm and black cricket should be evaluated for their suitability to replace fish meal in other fish diets.

Cobia (*Rachycentron canadum*) is a large carnivorous pelagic fish cultured in Southeast Asian countries including Thailand (Jeffrey and Holt, 2005). This fish can gain up to 6 kg in weight during a single year of cultivation in offshore cages (Chou *et al.*, 2001). Recently, Phuket Coastal Aquaculture Research and Development Center has been successful in breeding cobia in Thailand, and as a consequence, cobia farming in Thailand has expanded. This has prompted research in lower-cost feeds, especially by replacing the protein from fish meal without compromising digestive efficiency by the fish.

Studies have examined fish meal replacement in fish diets, e.g., poultry by-product meal in *Nibea miichthioides* (Wang, 2006), meat and bone meal in *Pseudosciaena crocea* (Ai, 2006), soybean meal in *Perca flavescens* (Kasper *et al.*, 2007), and insects in *Oreochromis niloticus* diets (Jabir *et al.*, 2014). However, research is lacking

on the use of insects in carnivorous marine fish diets, especially a determination of digestibility of insects, which would reflect their potential for fish growth (Koprucu and Ozdemir, 2005). Therefore, the present study was conducted to evaluate the potential of the giant worm and black cricket to replace fish meal in cobia diets.

MATERIALS AND METHODS

Insect preparation

Fresh giant worms and fresh black crickets were obtained from an insect farm in Phuket Province, operated under the “Insects as Marine Fish Feed Ingredients” project of King Mongkut's Institute of Technology, Ladkrabang and Phuket Coastal Aquaculture and Development Center (Phuket CARD C). They were transferred alive to Phuket CARD C and then freeze dried for 1 h followed by drying in an oven at 60 °C overnight. The dried insects were minced and kept at 4 °C until used for analysis and experiments.

Before commencing the feeding trials, the three protein sources (fish meal, prepared giant worm meal, and black cricket meal) were analyzed for chemical composition as follows: crude protein using Truspec CN Carbon/Nitrogen Determination (LECO); crude lipid using Fat Extraction TFE2000 (LECO); ash, moisture and fiber based on AOAC methods (AOAC, 2005). Amino acid profiles were analyzed using HPLC Agilent 1100 series by using Post column method (Bidleymeyer *et al.*, 1987). Tables 1 and 2 show the chemical composition and amino acid profiles of experimental feed ingredients.

Experimental diets

The diets in this experiment were formulated to have approximately 40-42 % protein and 380-390 kJ·g⁻¹ energy, which satisfies the nutrient requirements of cobia (Lovell, 1989; Zhou *et al.*, 2006; 2007). Each test diet contained 70 % reference diet and 30 % test ingredient (Cho and Linger, 1979). The reference diet was formulated using 30% fish meal. The test diets were formulated using either 30% giant worm meal or 30% black cricket meal.

Chromic oxide (Cr_2O_3) was used as an inert marker at a concentration of 1% in the reference diet. Dry ingredients were combined with liquid ingredients and water ($30 \text{ g} \cdot 100 \text{ g}^{-1}$ diet) in a mixer. Then, the well-mixed ingredients were pelletized by extruder machine and subsequently dried in a rotary dryer at 80°C for 1 h. After that, the pelleted diets were passed through a 3-5 mm pore sieve, and stored in polythene bags at 4°C until used. The chemical composition and amino acid profiles of the experimental diets were analyzed using the same methods described for the analysis of feed ingredients. The formulation and chemical composition of the experimental diets are shown Table 3. The amino acid profile is shown in Table 4.

Fish and experimental set-up

The feeding experiment was conducted at Phuket CARDIC, Thailand. The cobia fingerlings used for this study were produced at Phuket CARDIC hatchery. The fingerlings ($80.84 \pm 0.65 \text{ g}$) had been acclimatized for two weeks prior to stocking in the experimental tanks. Two hundred seventy cobia were randomly divided into nine groups of 30 fish each, and the three treatments were each randomly assigned to three fish groups. Tanks (500 L), which contained 400 L of seawater in a closed recirculation system, were used for the

feeding trials. The seawater in the tanks was maintained at $28-29^\circ\text{C}$, and the flow rate of seawater was a constant $250 \text{ mL} \cdot \text{min}^{-1}$ for the circulation of dissolved oxygen. Approximately 20-30 % of the seawater was replaced once each day to maintain water quality.

Feeding and fecal collection

Before starting the experiment, the fingerlings in each experimental tank were acclimatized for seven days. The feeding trial lasted eight weeks. Fecal collection started after five days of feeding, and continued throughout the experiment. During the experiment, the fingerlings were fed to satiation twice a day (at 8.30 a.m. and 16.30 p.m.). Three hours after feeding, the uneaten feed was drained out from each tank by opening the valves of the tanks. Then, the tanks were siphoned thoroughly to remove any remaining feed particles and refilled with water. The feces were collected from the fecal collection tube of the tank twice a day. Care was taken to avoid breaking the thin fecal strings in order to minimize nutrient leaching. Fecal material from each tank was dried and stored in polythene bags at -20°C for chemical analysis. The eight-week experiment resulted in 4-5 g of fecal material collected from each tank. The feces were freeze dried, ground, and then analyzed for the estimation of nutrient values.

Table 1. Chemical composition of fish meal, giant worm meal and cricket meal.

Ingredients	Moisture (%)	Proximate composition (% dry matter)				
		Protein	Lipid	Ash	Fiber	NFE
Fish meal	8.44	58.08	9.81	20.65	1.59	9.86
Giant worm meal	8.52	40.42	9.94	2.01	1.48	46.15
Black cricket meal	8.70	42.50	8.98	1.68	1.84	45.01

Note: Nitrogen free extract (NFE) = $100 - (\% \text{ protein} + \% \text{ fat} + \% \text{ ash} + \% \text{ fiber})$

Table 2. Essential amino acid profile in fish meal, giant worm meal and cricket meal ($\text{g} \cdot 100 \text{ g}^{-1}$ crude protein).

Amino acid	Fish meal	Giant worm meal	Black cricket meal
Methionine	2.12 ± 0.07	1.05 ± 0.05	2.08 ± 0.06
Lysine	4.12 ± 0.03	3.42 ± 0.20	4.05 ± 0.10
Arginine	6.16 ± 0.16	2.19 ± 0.21	6.20 ± 0.16

Table 3. Formulation of experimental diets ($\text{g}\cdot\text{kg}^{-1}$).

Diet ingredients ($\text{g}\cdot\text{kg}^{-1}$)	Fish meal diet	Giant worm meal diet	Black cricket meal diet
Fish meal	300	210	210
Giant worm meal	-	300	-
Black cricket meal	-	-	300
Defatted soybean meal	180	126	126
Fermented soybean meal	80	56	56
Corn gluten meal	60	42	42
Wheat flour	200	140	140
broken milled rice	60	42	42
Vitamin premix ¹	20	14	14
Mineral premix ²	15	10.5	10.5
Sodium ascorbic phosphate	1	0.7	0.7
Fish oil	20	14	14
Palm oil	20	14	14
Choline chloride	5	3.5	3.5
Carboxyl methyl cellulose	29	20.3	20.3
Cr_2O_3	10	7	7
Proximate composition of experimental diets (% dry matter basis)			
Dry matter	10.84	10.11	10.64
Crude protein	38.09	34.49	35.25
Crude lipid	8.14	8.75	8.16
Crude ash	9.56	7.16	7.24
Crude fiber	2.07	1.89	2.06
NFE ³	42.14	47.71	47.29

Note: ¹Vitamin premix ($\text{mg}\cdot\text{kg}^{-1}$ diet): Thiamine (B1) : Riboflavin (B2) : Pyridoxine HCl (B6) : Panthothenic acid (B5) : Niacin : Folic acid : Inositol : Cyanocobalamin (B12) : Choline Chloride 50% : Biotin : AD3 (IU) : E (IU) : K3-0.05 : 0.1 : 0.07 : 0.167 : 0.67 : 0.03 : 133.33 : 0.40 : 500 : 0.4 : 8.3 : 0.33 : 0.167; ²Mineral premix ($\text{g}\cdot\text{kg}^{-1}$ diet): CoCl_2 : CuSO_4 : FeSO_4 : MnSO_4 : NaSeO_3 : ZnSO_4 -0.0125 : 0.13 : 1 : 0.5 : 0.0025 : 1; ³NFE = 100-(% protein+% fat+% ash+% fiber)

Table 4. Essential amino acid profiles of diets ($\text{g}\cdot 100\text{ g}^{-1}$ crude protein).

Amino acid	Fish meal diet	Giant worm meal diet	Black cricket meal diet
Methionine	1.41 \pm 0.13	1.34 \pm 0.17	2.01 \pm 0.12
Lysine	2.47 \pm 0.13	2.75 \pm 0.11	3.02 \pm 0.14
Arginine	2.95 \pm 0.15	2.72 \pm 0.10	3.24 \pm 0.10

Data collection

The experimental diets and feces were analyzed in three replicates for proximate composition as follows: crude protein using Truspec CN Carbon / Nitrogen Determination (LECO); crude lipid using Fat Extraction TFE2000 (LECO); ash, moisture and fiber based AOAC methods (AOAC, 2005). The chromic oxide content was analyzed by using the Furukawa and Tsukahara (1966) method. The apparent digestibility coefficients (ADC) of dry matter and protein were calculated as follows:

$$\text{ADC of dry matter (\%)} = 100 \times [1 - (\text{dietary Cr}_2\text{O}_3 / \text{fecal Cr}_2\text{O}_3)]$$

$$\text{ADC of protein (\%)} = 100 \times [1 - (\text{dietary Cr}_2\text{O}_3 / \text{fecal Cr}_2\text{O}_3) \times (\text{fecal protein concentration} / \text{dietary protein})]$$

At the end of the experimental period, all fish in each tank were weighed to assess growth. The specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR) were measured and calculated for each treatment as follows:

$$\text{SGR} = [\ln \text{ final weight of fish (g)} - \ln \text{ initial weight of fish (g)}] / \text{days of experiment}$$

$$\text{FCR} = \text{Food fed (g)} / \text{Live weight gain (g)}$$

$$\text{SR (\%)} = (\text{Final number of fish} / \text{Initial number of fish}) \times 100$$

Statistical analysis

All ADC values and growth data were subjected to one way analysis of variance (ANOVA) using SPSS. Means between treatments were compared using Tukey's test and regarded as significantly different at $p < 0.05$. Descriptive statistics were presented as mean \pm SD.

RESULTS AND DISCUSSION

Apparent digestibility of the three diets (fish meal, giant worm replacement, and black cricket replacement) by cobia was not significantly different ($p > 0.05$), either in terms of dry matter or protein. The ADC values of dry matter ranged from 63 to 69 %, while ADC of protein ranged from 83 to 88 % (Table 5). These results indicate that cobia is able to digest the dry matter and protein in giant worm meal and black cricket meal equally well as fish meal (Table 5), despite the presence of chitin in the two insects. Notably, black cricket has lower chitin than other cricket species such as *Gryllus testaceus* (Wang *et al.*, 2005).

The digestibility of protein was relatively high for all diets (Table 5), and ADC values of both dry matter and protein were in line with the same measurements for a cricket diet fed to *Clarias gariepinus* (73.9 and 81.2 %) (Taufek *et al.*, 2016). High ADC of protein can be explained by the fact that high protein content of the feed ingredients stimulates the activity of proteases (Eusebio and Coloso, 2002). In addition, the extruded pellet production process increases the digestibility of aquatic diets (Hilton *et al.*, 1981).

Table 5. Apparent digestibility coefficients in experimental diets of cobia.

Test diets	Apparent digestibility coefficients (%)	
	ADC of dry matter	ADC of protein
Fish meal	69.1 \pm 1.7 ^a	88.1 \pm 1.2 ^a
Giant worm meal	63.2 \pm 1.0 ^a	83.9 \pm 1.1 ^a
Black cricket meal	68.9 \pm 0.5 ^a	85.8 \pm 0.4 ^a

Note: Means \pm SD in a column superscripted with the same lowercase letter are not significantly ($p > 0.05$) different.

At harvest the experimental fish were 113-120 g in body weight. None of the measured parameters (final weight, specific growth rate, feed conversion ratio) of cobia fed the three test diets were significantly different ($p>0.05$). The results are shown in Table 6.

Cobia fed diets partially replaced by giant worm meal and black cricket meal retained comparable growth rates and feed conversion ratios to cobia fed with fish meal as the sole protein source. This was not unexpected, as the test diets had comparable crude protein, and the amounts of essential amino acids such as methionine, lysine and arginine in the three diets were similar (Table 2, 3). In particular, the test and reference diets contained sufficient amounts of these essential amino acids required by cobia (1.19, 2.33, and 2.82 g·100g⁻¹ crude protein of methionine, lysine and arginine, respectively) (Zhou *et al.*, 2006; 2007; Ren *et al.*, 2014). This is consistent with Taufek *et al.* (2016) who reported that alternative insect proteins, such as *G. bimaculatus*, provide adequate amounts of essential amino acids for fish. These amino acids play crucial roles in the growth of aquatic animals. Methionine promotes fish growth (Ahmed *et al.*, 2003) by supporting protein synthesis and other physiological functions (Zhou *et al.*, 2006). In addition, fish have been shown to improve growth efficiency, feed consumption and immune response when fed a diet supplemented with methionine (Rolland *et al.*, 2015). Lysine is high in fish muscle tissue, which is involved in growth and nitrogen balance, and used in protein cross-linking, especially

in collagen (Marcouli *et al.*, 2006). Arginine promotes fish growth and fish health (Buentello and Gatlin, 2001).

The feed conversion ratios of cobia were not different in this study because the digestibility of dietary proteins in the reference and test diets was similar. The present results on growth and feed conversion ratios support those by Jabir *et al.* (2014), who reported that 25-50% giant worm meal in *Oreochromis niloticus* diet produced suitable growth and feed efficiency.

The present study did not show an advantage in fish health from supplementing insect meals in the diets, as survival rates were all 100 %. However, there are reports suggesting that chitin in insects does not decrease fish innate immunity (Vahedi and Ghodrati-zadeh, 2011), and in fact shows antioxidant properties (Khoushab and Yamabhai, 2010).

It may be possible to replace fish meal with giant worm meal or black cricket meal at rates higher than 30 % in cobia diets. Taufek *et al.* (2013) reported that crickets can replace fish meal up to 100 % in feeds of freshwater fish such African catfish. Jabir *et al.* (2012) reported that 50 % super worm (*Z. morio*) meal can be included in the diet of *O. niloticus*. The giant worm and black cricket are locally available in Thailand and are well adapted to the tropical climate. Currently, live giant worms and black crickets are commercially available in pet stores, as fish bait, and as supplementary feed for ornamental fish.

Table 6. Growth and feed performance of cobia fed experimental diets for eight weeks.

Parameter	Fish meal	Giant worm meal	Black cricket meal
Initial weight (g)	81.11±1.92 ^a	80.78±0.84 ^a	80.67±2.00 ^a
Final weight (g)	120.01±2.89 ^a	113.11±3.67 ^a	117.13±3.74 ^a
Specific growth rate (%)	0.61±0.04 ^a	0.53±0.06 ^a	0.58±0.08 ^a
Feed conversion ratio	1.16±0.07 ^a	1.21±0.05 ^a	1.19±0.02 ^a
Survival rate (%)	100±0.00 ^a	100±0.00 ^a	100±0.00 ^a

Note: Means±SD in a row superscripted with the same lowercase letter are not significantly ($p>0.05$) different.

CONCLUSION

The apparent digestibility coefficients of dry matter and protein for cobia fed giant worm meal and black cricket meal diets were not different from those of cobia fed fish meal as the main protein source. The cobia fed with the two insect meal diets had growth, feed conversion ratios and survival rates comparable to the group fed with fish meal. In conclusion, both giant worm meal and black cricket meal are suitable alternative protein sources to replace fish meal in cobia diets, at least at 30 % replacement.

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