



Exploring Arthropods as Sustainable Protein Source and the Effect of Cockroach Meal Supplemented Diet on the Growth of Fish

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This comprehensive study centres on exploring the arthropods as sustainable protein sources, addressing both human dietary needs and livestock feed requirements. A diverse set of twenty arthropod species were collected from different locations of Kanyakumari District and the species were identified based on the identification keys pertaining to morphological characters. The surveyed animals comprises of Insects: Dragon Fly (*Pantala* sp.), Western subterranean termite (*Reticulitermes* sp.), Longhorn beetle (*Callipogon* sp.), Asiatic rhinoceros beetle (*Oryctes*

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rhinoceros), Brown grasshopper (*Melanoplus* sp.), Bush cricket (*Tettigonia* sp.), Grasshopper (*Diabolocatanotops* sp.), Housefly (*Musca domestica*), Cockroach (*Periplaneta americana*), Termite (*Macrotermes* sp.), Black carpenter ant (*Camponotus* sp.), Red carpenter ant (*Camponotus* sp.); Crabs: Mottled light footed crab (*Grapsus albolineatus*), *Sartoriana* sp., Asian paddle crab (*Charybdis* sp.), Box crab (*Calappa* sp.), Asian shore crab (*Hemigrapsus* sp.); Millipedes: Yellow spotted millipede (*Harpaphe haydeniana*), Pill millipede (*Arthrosphaera disticta*) and African giant millipede (*Thyropygus descriptus*) were analyzed for the protein content. The samples were tested for protein using Biuret's method. Among the different arthropods tested insects- *Callipogon* sp. and *Periplaneta americana*, crabs - *Calappa* sp. and *Charybdis* sp. and Myriapod - *Harpaphe haydeniana*, exhibited the highest protein content. Crustaceans demonstrated the highest protein content when compared to insects and millipedes. However, since cockroaches are generally considered as a pest and repugnant in human dwelling, it would be ideal to convert cockroach as a sustainable protein source in aquaculture. Moreover, since cockroach showed a higher protein content of 6.146 ± 2.024 mg/ml it was tried as a supplement feed for fish. Cockroach meal was prepared in two different proportions (2% and 3%) to evaluate its impact on the growth of Koi carp fish in comparison with cockroach free meal as control. The inclusion of 3% cockroach powder in the fish meal augmented the growth of Koi carp when compared to the control during a fifteen-day study period. Thus, this study has tapped a potential protein source from insects. Cockroach can be used as an ideal fish feed as it contributes to the pursuit of sustainable and environmentally friendly animal feed source.

Keywords: Animal protein; dietary supplement; protein content; sustainable feed.

1. INTRODUCTION

Animal products constitute 31% of the dietary protein and 75% of all species on earth constitute insects which are one of the most sustainable source of protein that can solve the problem of animal feed production [1]. Edible insects are a sustainable and natural feed source that offers a range of nutritional, economic and ecological benefits [2]. They boast a high feed-conversion efficiency due to their cold-blooded nature, enabling them to transfer low value organic waste into valuable protein [3]. As per the Food and Agricultural Organization of the United Nations, insects constitute a staple in the diets of at least two billion individuals worldwide [4]. A theoretical study conducted by Veldkemp et al. (2012) highlighted the technical feasibility of utilizing large insects as environmentally sustainable ingredients in high protein diets of livestock. For centuries, insects have been a part of traditional cuisine across Asia, including countries such as China, Japan and Thailand. Edible insect consumption is widespread among ethnic communities in Northeast India, particularly among the tribes residing in Arunachal Pradesh, Assam, Manipur, Nagaland, Meghalaya and Mizoram [5]. The tribe called the 'Irumbars' in the North Arcot district of Tamilnadu, along with tribes in Karnataka, consume winged termites, locally known as 'Eesal,' as a food sources [6].

In terms of nutrition, insects have a substantial protein content varying between 20% to and 76% of the dry matter, depending upon the species and development phase of the insect [7]. Limited studies have compared insects with other reference feeds, and the results showed that insects exhibit similar protein digestibility to fish meal [8]. In addition to protein and amino acids, insect-based foods offer a rich reservoir of vitamins, minerals, fats and trace elements [9]. Das [10] concluded from his analysis of the locust *Schistocerca gregaria* that locusts had high levels of crude protein and fat, rendering them suitable for consumption as food and utilization as fertilizer in India. Among insects Coleopterans are used as feed (34%) followed by Orthoptera (24%), Hemiptera (17%), Hymenoptera (10%), Odonata (8%), and Lepidoptera (4%) and Optra (2%) [11].

With the global fish catch declining, aquaculture is growing at an annual rate of approximately 5.6% (2004-2009) to meet the demand, necessitating the search for alternative animal protein sources. Barroso et al. [12] addressed the potential of replacing traditional fish meal in aquaculture with insect meals. Using mealworms (*Tenebrio molitor*) as a substitute protein source in poultry feeds highlights their favourable influence on broiler performance and their capacity to convert low-nutrient waste materials into high-protein diets [13]. Black soldier fly and housefly could potentially serve as a suitable diet for rainbow trout [14]. Henry et al., [15] discussed

the composition of insects in relation to the nutritional requirements of fish, including proteins/amino acids, lipids/fatty acids, minerals, and vitamins. In this context, the authors investigated the nutritional characteristics of 16 different insect species from the orders Coleoptera, Diptera and Orthoptera to determine their suitability as substitutes for fish meal in aquaculture diets.

The main objective of the study is to analyze the protein content of selected arthropod samples as prospective protein source and to study the effect of cockroach meal on the growth of Koi carp fish.

2. MATERIALS AND METHODS

2.1 Collection of Arthropods










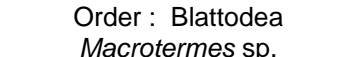
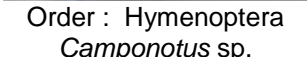
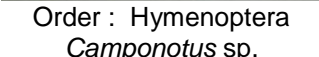
Twenty distinct types of arthropods (Table 1) representing different taxonomic groups were

used for the analysis of protein content. Insects and myriapods were collected from Holy Cross College campus, Nagercoil and crabs from the coastal regions of Kanyakumari Sea of Kanyakumari district, Tamilnadu, India. The arthropods were identified using the taxonomic keys available in manuals, books, research articles, thesis and websites.

2.2 Preparation of Sample

The whole body of insects and myriapods and muscle of crab were thoroughly dried and ground into a fine powder. 50 mg of the sample was taken and homogenized by adding 1 ml of 10% TCA and was centrifuged at 500 rpm for 5 minutes. The supernatant was discarded and the precipitate was dissolved by adding 1 ml of 0.1N sodium hydroxide.

Table 1. Arthropods collected and tested for protein

Class: Insecta		
Order : Odonata <i>Pantala</i> sp. Dragon Fly 	Order : Isoptera <i>Reticulitermes</i> sp. Western subterranean termite 	Order : Coleoptera <i>Callipogon</i> sp Longhorn beetle 
Order : Orthoptera Brown grasshopper <i>Melanoplus</i> sps. 	Order : Orthoptera <i>Tettigonia</i> sp. Bush cricket 	Order : Orthoptera <i>Diabolocatantops</i> sps. Grasshopper 
Order : Coleoptera <i>Oryctes rhinoceros</i> Asiatic rhinoceros beetle 	Order : Diptera <i>Musca domestica</i> House fly 	Order: Blattodea <i>Periplaneta americana</i> Cockroach 
Order : Blattodea <i>Macrotermes</i> sp. 	Order : Hymenoptera <i>Camponotus</i> sp. 	Order : Hymenoptera <i>Camponotus</i> sp. 

Class: Insecta

Termite



Black carpenter ant



Red carpenter ant



SUBPHYLUM : CRUSTACEA
CLASS : MALACOSTRACA

ORDER : DECAPODA

Grapsus albolineatus
Mottled lightfoot crab



Sartoriana sp.



Charybdis sp.
Asian paddle crab



Calappa sp.
Box crab



Hemigrapsus sp.
Asian shore crab



SUBPHYLUM : MYRIAPODA
CLASS : DIPLOPODA

Order : Polydesmida
Harpaphe haydeniana
Yellow spotted millipede



Order : Glomerida
Arthrosphaera disticta
Pill millipede



Order : Spirostreptida
Thyropygus descriptus
African giant millipede



2.3 Estimation of Protein

Protein analysis was performed in the selected arthropod samples by following the Biuret method. All the samples were taken in triplicates

and the concentrations were given as the percentage of the weight of the tissue.

Procedure: 1 ml each of sample, standard Bovine Serum Albumin and blank was taken in

separate tubes. In all three test tubes, the volume was made up to 5 ml by using 0.1N sodium hydroxide and 5 ml of Biuret reagent. After 30 minutes the optical density was read at 620 nm. The amount of protein in the given sample was calculated by using the formula,

Amount of protein in the sample =

$$\frac{\text{OD of the sample} \times \text{Conc. of standard}}{\text{OD of the standard}}$$

2.4 Dried Cockroach Powder (Cockroach Meal) as Fish Feed Supplement

Estimation of protein in arthropods showed high protein content in cockroach. Since cockroach is easily available and has high protein content, dried cockroach powder was incorporated as a supplementary food source by blending it with

the koi carp fish feed. The ingredients of the koi carp fish feed (control) are given in Table 2. Cockroach meal (i.e. the experimental diet) was prepared in two different proportions of 2 % and 3 % which served as Experimental I and Experimental II diet. Fish meal devoid of cockroach powder served as the control.

Three separate tubs were used for the experiment, each containing five Koi carp of uniform size fishes (Plate 1). The experiment spanned a duration of 15 days during which we closely monitored and recorded the growth of the fish in each tub.

Percent change in growth both in terms of length and weight is calculated using the formula:

$$(\text{Final value} - \text{Initial value}) / \text{Initial value} \times 100$$

Table 2. Ingredients of prepared fish feed

Ingredients	Quantity
Rice bran	400 g
Boiled peanuts	300 g
Dry fishes	100 g
Carrot	100 g
Beetroot	100 g
Spleen amaranth (Arai keerai)	100 g
Egg	1
Vitamin, mineral mix	10 g
Fish oil	10 ml
Sago pearls (Javvarisi)	100 g
Cockroach meal	2 % of cockroach powder (Experimental diet I) 3% of cockroach powder (Experimental diet II)



Plate 1. Experimental fish -Koi carp (*Cyprinus rubrofasciatus*)

3. RESULTS

3.1 Quantitative Analysis of Protein Content in Selected Arthropods

The protein content of dried form of selected samples of class Insecta is represented in Table 3. The maximum level of protein was observed in *Callipogon* sp. and *Periplaneta americana* (6.25 ± 0.25 and 6.146 ± 2.024 mg/ml), moderate amount in *Diabolocatantops* sp. and *Pantala* sp (5.623 ± 0.228 and 4.93 ± 0.4503 mg/ml) and low protein content in the ants *Camponotus* sp. and *Camponotus* sp. (2.413 ± 0.0850 and 2.58 ± 0.08 mg/ml).

The protein content of dried powders of selected crustacean samples are represented in Table 4. Maximum protein content was observed in *Calappa* sp. and *Charybdis* sp. (35.46 ± 0.321 and 30.45 ± 0.233 mg/ml). The protein content of *Grapsus albolineatus* though slightly lower than *Calappa* sp. and *Charybdis* sp. is relatively high (26.93 ± 5.571 mg/ml). *Sartoriana* sp. had the lowest protein content (11.43 ± 0.503 mg/ml) among the crustaceans tested.

The protein content of dried powders of selected myriapod samples are represented in Table 5. Among the three selected myriapods, the yellow spotted millipede *Harpaphe*

haydeniana has the highest protein content (4.46 ± 0.450 mg/ml) followed by the pill millipede *Zephronia* sps. (3.99 ± 0.417 mg/ml) and *Archispirostreptus gigas* (3.13 ± 0.152 mg/ml) (Table 3).

3.2 Integration of Dried Cockroach Powder as a Protein Source in Koi Carp Fish Feed

The effect of Cockroach meal on the growth of Koi carp (*Cyprinus rubrofusculus*) in terms of length is represented in Table 6. The control group showed a small increase in length over the fifteen-day period (0.10 cm). The Experimental group 1 also exhibited growth, although the increase in length (0.04 cm) is less compared to the control group. The experimental group 2 also experienced growth with a change in length of (0.07 cm) which is slightly higher compared to the experimental group-1.

The effect of Cockroach meal on the growth of *C. rubrofusculus* in terms of weight is represented in Table 7. The control group and experimental group 1 showed an increase in weight of 0.07 gm during the fifteen-day period while the experimental group 2 displayed a weight gain of 0.14 gm.

Table 3. Protein content of dried samples of class Insecta

Insects	Order	Protein content (mg/ml)
<i>Pantala</i> sp - Dragonfly	Odonata	4.93 ± 0.4503
<i>Reticulitermes</i> sp. - Western subterranean termite	Isoptera	3.88 ± 1.3
<i>Callipogon</i> sp. - Long horned beetle	Coleoptera	6.25 ± 0.25
<i>Oryctes rhinoceros</i> – Rhinoceros beetle		3.95 ± 3.97
<i>Melanoplus</i> sp. - Brown grasshopper	Orthoptera	2.596 ± 0.769
<i>Tettigonia</i> sp. - Bush cricket		3.69 ± 0.137
<i>Diabolocatantops</i> - Grasshopper		5.623 ± 0.228
<i>Musca domestica</i> - Housefly	Diptera	2.746 ± 0.217
<i>Periplaneta americana</i> - Cockroach	Blattodea	6.146 ± 2.024
<i>Macrotermus</i> sp. - Termite		3.763 ± 0.075
<i>Camponotus</i> sp. - Black carpenter ant	Hymenoptera	2.413 ± 0.0850
<i>Camponotus</i> sp. - Red carpenter ant		2.58 ± 0.08

Table 4. Protein content of dried samples of Crustaceans

Order Decapoda	Protein content (mg/ml)
<i>Grapsus albolineatus</i> - Mottled light foot crab	26.93 ± 5.571
<i>Sartoriana</i> sp.	11.43 ± 0.503
<i>Charybdis</i> sp. - Asian paddle crab	30.45 ± 0.233
<i>Calappa</i> sp. - Box crab	35.46 ± 0.321
<i>Hemigrapsus</i> sp. - Asian shore crab	15.79 ± 0.257

Table 5. Protein content of dried samples of myriapoda

Myriapods	Order	Protein content (mg/ml)
<i>Harpaphe haydeniana</i> Yellow spotted millipede	Polydesmida	4.46± 0.450
<i>Arthrosphaera disticta</i> Pill millipede	Glomerida	3.99± 0.417
<i>Thyropygus descriptus</i> African giant millipede	Spirostrepida	3.13± 0.152

Table 6. Effect of Cockroach meal on the growth of *C. rubrofuscus* in terms of length

Diet	Length (cm)		Percent change
	Initial	Final	
Control	5.16±0.152	5.26± 0.152	+ 1.93
Experimental-1	5.8± 0.4	5.84± 0.45	+ 0.7
Experimental-2	5.53±0.115	5.6± 0.1	+ 1.2

Table 7. Effect of Cockroach blended diet on the growth of *Cyprinus rubrofuscus* in terms of weight

Diet	Weight(gm)		Percent change
	Initial	Final	
Control	3.76± 0.568	3.83±0.513	+ 6.11
Experimental 1	4.33±0.208	4.4±0.173	+ 1.61
Experimental 2	4.1±0.692	4.24±0.635	+ 3.41

4. DISCUSSION

Arthropods, especially edible insects are highly nutritious and the cheapest source of animal protein and fat comparable to milk and meat. Insect proteins are highly digestible (between 77% and 98%) with high lysine and threonine content and limited tryptophan and methionine content [16]. A surge of interest in edible insects has been sparked by FAO efforts to promote edible insects as viable dietary alternatives [3,17].

Insects have high nutritional value, minimal space requirements, and low environmental impact, making them an attractive option for animal feed. However, the insects used as animal feed need to be farmed rather than harvesting from nature [18]. A major advantage is that insects are already found in many animal diets naturally [19]. In Asia and Africa, insects are frequently employed as feed for fish on small farms [20]. Without any negative consequences, Nile tilapia diets can substitute up to 75% of fishmeal with housefly-larvae meal [21]. The aroma, taste, and consistency of Atlantic salmon (*Salmo salar*) remain unaffected when fish meal is substituted with black-soldier-fly meal in their diets [22]. Silkworm pupa is considered as a viable alternative to fish meal for African catfish (*Clarius gariepinus*) fingerling diets [23].

The protein content of edible insects varies based on various factors, such as the species, sex, raising environment and development stages [24]. Insects have higher protein content because they are more efficient at converting food into protein and they have different sets of enzymes that help them to break down and digest food more efficiently which allows them to extract more nutrients from the same amount of food, which in turn increases their protein content.

In the present study, analysis of protein content of selected arthropod samples documented high protein content in Longhorn beetles (*Callipogon* sp.) of the order Coleoptera and Cockroach (*Periplaneta americana*) of the order Blattodea. Beetles (Coleoptera) are the most common insects eaten worldwide (Tuhumury, 2021). Although the creditability of the studies on *O. rhinoceros* is doubtful, coleoptera succeeds as partial replacement of fish meal in the fish diets [15]. Among most cockroach species, *Periplaneta americana*, is considered the most edible [25]. Among the selected crustacean samples, highest protein content was observed in *Calappa* sps. and *Charybdis* sp.. Crustaceans are a valuable protein source due to their high protein content and essential amino acid profile [26]. Among the selected myriapods, highest protein content was observed in the yellow spotted millipede *Harpaphe haydeniana*.

Myriapods are edible and nutritious sources of protein [27]. Myriapods are eaten in many cultures for their protein content [28].

Based on the results of the present study, incorporating 3% dried cockroach powder as a protein source in Koi Carp fish feed, appears to enhance the growth of Koi carp fish during the specified timeframe. The increase in weight may be due to the protein content and n-6 polyunsaturated fatty acids in the cockroach meal as reported by Xiaowen Long et al. [29]. Cockroaches (*Periplaneta americana*) are bred in captivity, sold in large quantities, and sold as feed for livestock by farmers, as well as being eaten by the general public for food, medicinal purposes, or simply as tonics [25]. Processed cockroach and meat powder can be incorporated into children, pregnant and lactating mother's diets or even adults who are malnourished [30]. Cockroach powder and processed meat can be adequately incorporated in the feeds of livestock especially poultry for faster growth [31]. The potential nutrient deficiencies of aqua feed incorporating insect feed could be reduced by the use of a package of different protein sources (variety of insects or insects with prebiotics with animal proteins or plant proteins).

Addition of 13% of varied grasshopper meal in the diets of Catfish, showed significant increase in the growth of the catfish [32]. A variety of strategies such as drying, defatting, ensilage, manipulating the insect diet, addition of antioxidants and rearing conditions could be used to get a higher percentage [33]. The information generated through this analysis will contribute significantly to the goal of embracing a more sustainable and environmentally friendly source of animal feed [34]. There is a great deal of future research potential on this exciting subject, with entomologists and fish nutritionists joining forces to explore the variety of insect species available [15].

5. CONCLUSION

Arthropods have been identified as a rich source of protein and are included in the diet of both human beings and livestock. Exploring the nutritive value of available arthropods in the locality has helped to identify few insects and crustaceans as a rich source of protein. Inclusion of powdered cockroach in the fish diet has enhanced the growth of fish thus suggesting its efficiency as a supplement diet for fish.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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