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Protein and free amino acid analysis in the haemolymph of invertebrate food

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Abstract
Protein and amino acids are essential components of our lives since they help to maintain and grow our bodies. The goal of this investigation was to assess the protein content of certain invertebrate diet in Assam and Meghalaya's haemolymph and muscle tissue. Entomophagy is the key to meeting the world's expanding nutrient demands since edible insects may deliver high levels of proteins, lipids, vitamins, and minerals with significant economic and environmental benefits.

Keywords: Protein, amino acids, hemolymph, entommophgy, nutrient, minerals

Introduction

One of the most abundant and adaptable animal kingdoms, insects may be found in almost every ecosystem on the planet (Ratcliffe, 1985; Vilmos and Kurucz, 1998). Studies on insect haemolymph are of special importance because they provide us the context we need to evaluate the synthetic activity linked to the various stages of development. Abnormal changes in the content of amino acids in the haemolymph due to the application of microbial pesticides may have lethal effects (9, 19, 20, 29).

Due to their involvement in insect neurophysiology, the inorganic contents of haemolymph are also crucial. The correct concentration of these components within and outside of nerve membranes is necessary for the transmission of impulses. In 1991, a group of researchers led by ABOUL-ELA. *et al*.

First, haemolymph proteins were identified in lepidopteron insects, and research into their innate immune responses might be useful in a variety of contexts (Jiang *et al*., 2010).

Insects rely on haemolymph proteins for both their transport abilities and their enzyme activity. Hereditary and hormonal variables regulate haemolymph protein production and use (Hurliman and Chen, 1974). Although our understanding of insect haemolymph proteins has expanded substantially over the last decade, only the origin and function of a select few key proteins are now understood (Riddiford an Lawaw, 1983). Dean *et al*. (1985) and others may comfromrm haemocytes (Hughes and Prie (1976) and Katagiri (1977), however it is generally accepted that adipose tissue is a major source of haemolymph proteins.

Insect physiologists have focused most of their research on the hemolymph or blood of insects because of its unique features (M. George cheriah, 1968). The high amino acid composition is the characteristics of class insects which in some species may be more than 60 times higher than that in human blood. The work was reviewed by Maluf (939a). Wiggles worth (1939, 1953), Rapp (1947), Chauvin (1949a, 1956), Buck (1953), Wyatt (1961) and Chen (1962). The aliphatic amino acids play a dominant part in chemical composition of the haemolymph. (M. George Cherian, 1968). The high amino acid in the haemolymph is suggested to help in the buffering and in the osmotic regulations of the haemolymph by Florkin and Morgulis (1949), Buck (1953), Florkin (1960), Wyatt (1961), Gilmour (1961), Chen (1966). It has also been observed that the amounts of individual amino acid exhibit greatest variations (Gilmour, 1961). The free amino acids in insect blood are yet to be fully explored for their biological significance.

Therefore five invertebrate food are taken here for the investigation of the protein present in the haemolymph and muscle tissue and also to detect the total free amino acid present in the haemolymph. Insects taken for investigation are crab, water bettle, silkworm, prawn, water bug. The insects are collected from Assam and Meghalaya and are good source of nutrients. The insects are commonly found in the pond, paddy field, river, b etc. In order to make an educated guess as to the amount of protein in the haemolymph and to identify the total number of free amino acids in the haemolymph, the current study was conducted.

The Following goals were considered while designing the current study in few selected invertebrates:

1. To estimate protein in the haemolymph
2. To estimate protein in the muscle tissue
3. To detect free amino acids in the haemolymph

1. Classification of *Macrobrachium rosenbergii*

Kingdom	Metazoa
Phylum	Arthropoda
Class	Malacostraca
Order	Decapoda
Family	Palaemonidae
Genus	*Macrobrachium*
Species	*Macrobrachium rosenbergii*

2. Classification of *Paratelphusa hydrodomous*

Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Lepidoter
Family	Glechiide
Genus	*Paratelphusa*
Species	*Paratelphusa hydrodomous*

3. Classification of *Lethocerus americanus*

Kingdom	**Animalia**
Phylum	Arthropoda
Class	Insecta
Order	Hemiptera
Family	Belostomatidae
Genus	*Lethocerus*
Species	*L.americanus*

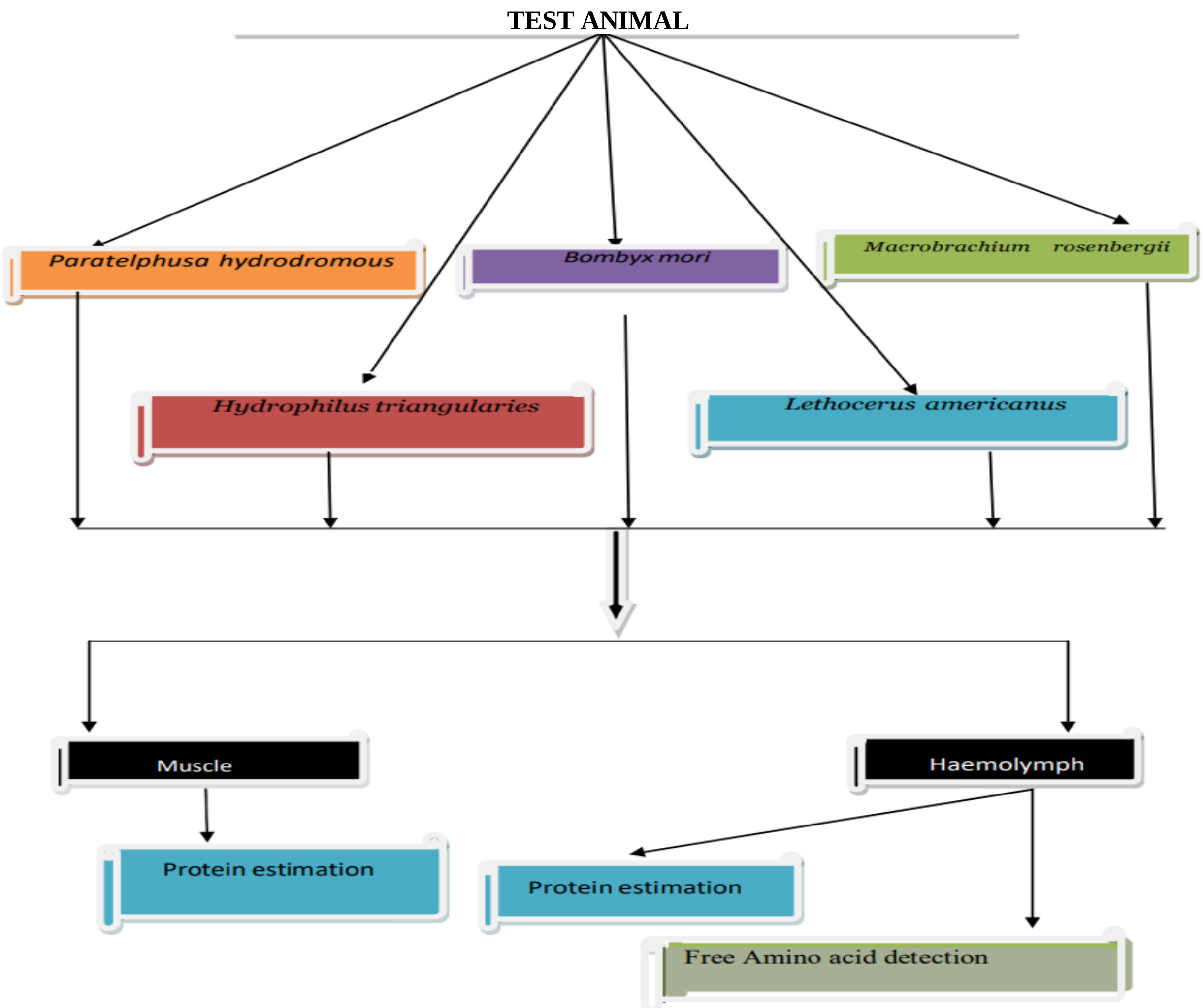
4. Classification of *Bombyx mori*

Kingdom	**Animalia**
Phylum	Arthropoda
Class	Insecta
Order	Lepidoptera
Family	Bombycidae
Genus	*Bombyx*
Species	*Bombyx mori*

5. Classification of *Hydrophilus triangularie*

Kingdom	**Animalia**
Phylum	Arthropoda
Class	Insecta
Order	Coloptera
Family	Hydrophilidae
Genus	*Hydrophilus*
Species	*Hydrophilus triangularie*

Materials and methods
Experimental design



Sample preparation

Within few hour of collection all the 5 edible invertebrates were brought to the laboratory and are kept in separate tanks in such a condition that is similar to their natural habitats. A day preceding examinations the insects were sterved so that there will be no variations in the protein and free amino acid if any.

Haemolymph collection

To the next first decimal place, we weighed animals that had been blotted and cleaned with a brush to remove any excess water or clinging sand or dirt from their tanks. (Sundara Rajulu & Kulasekarap Andian, 1971) [22, 23]. After inserting a syringe needle beneath the median frontal border of the carapace, close to the posterior gastric mill, to collect haemolymph from Macrobrachium Rosenbergii (DALL, 1964) [6]. Similar samples of haemolymph were obtained from the Paratelhphus hydrodromous by making a tiny incision in the arthrodial membrane at the base of the massive chela. From Bombyx mori the haemolymph was collected by clipping the abdomenal legs. From *Lethocerus americanus* and *Hydrophilus triangulares* the haemolymph is collected by inserting the needle into the mesothorax below the wing hinge and kept just under the cuticle.

Procedure for chemical extraction of protein I) for haemolymph

1. 50µl of haemolymph is added to 950 µl of distilled water and then precipitateby 10% and 5%.TCA sequentially inside the ice bucket.
2. Centrifuge at 4000rpm for 10 minutes.
3. Supernatant is discarded and precipitation is successively centrifuged byethanol followed by ether-ethanol (1:3) at same RPH.
4. Resultant supernatant is discarded and final amount of precipitation is dissolved in 0.1NaOH.

For muscle tissue

1. 1gm of muscle tissue is taken and 30ml of distilled water is added to it. Tissue is homogenated using homogenous or mortar and pestle.
2. Take 1 ml of tissue homogenate in a centrifuge tube and add 10% TCA in the same amount, mixing well.
3. The mixture is centrifuged for 5 minutes at 5000 rpm.
4. the surplus liquid is poured down the drain.
5. precipitate is successively centrifused first by 2ml of ethanol and then byether ethanol (1;3) at 5000rpm for 5 minutes.
6. supernatant is discarded again and a final amount of precipitate is dissolved in 1ml of 0.1NaOH. This will be the aliquot sample for estimation of tissue protein.

After that the sample become became ready for the further process of protein analysis

1. **Protein analysis:** The protein estimation has been done was followed by Lowry"s method (Lowry *et al*, 1951).
2. **Working period:** All the works has been done between 4th April 2022 to 27th May 2022.

Procedure for chemical extraction of free amino acid for haemolymph

1. 5-100µl of haemolymph is collected in a centrifuge tube containing 300-400µl of cold 80% ethanol and kept in ice bucket for 10 minutes.
2. Centrifuge at 3000 RPM for 10 minutes.
3. Supernatant is collected in a small beaker and the residue is washed with same grade of ethanol followed by centrifugation.
4. The process is repeated for 3 times and the final amount of supernatant was evaporated to dryness at 40-50ºC.
5. After complete drying the residue is washed thrice with solvent ether doremove lipid part.
6. Residue was then diluted with 100µl of distilled water and stored in icecondition.

Free amino acid analysis

The detection of free amino acid has been done by thin layer chromatography technique.

Statistical analysis

The table of total protein concentration(mg/gm) in dissending order of the protein concentration. Bar graph has been used to compare the protein concentration in muscle tissue and haemolymph (mg/gm) among the selected invertebrate food of assam and Meghalaya. Table is done to determine the presence or absence of various amino acids in the haemolymph. Bar graph has been use to compare the free amino acid content among the selected edible invertebrates taken for investigation.

Result

Table 1: The total amount of protein in the haemolymph of some invertebrate food in Assam and Meghalaya (Mean±SE).

S. No.	Species	Protein amount(mg/gm)
1	*Hydrophilus triangularies*	2.25±0.03 mg/gm
2	*Bombyx mori*	1.52±0.02mg/gm
3	*Paratelphusa hydrodromous*	1.45±0.01mg/gm
4	*Lethocerus americanus*	1.38±0.02 mg/gm
5	*Macrobrachium rosenbergii*	1.37±0.03mg/gm

Table 1 demonstrates that the greatest protein content may be found in the haemolymphof some invertebrate food of Assam and Meghalaya in *Hydrophilus triangularis* (2.25mg/gm) and the lowest protein content in the haemolymph is in *Macrobrachium rosenbergii* (1.37mg/gm).

Table 2: Total protein content in the muscle of some invertebrate food inAssam and Meghalaya (Mean ± SE)

S. No.	Species	Protein amount(mg/gm)
1	*Paratelphusa hydrodromous*	4.47±0.03mg/gm
2	*Lethocerus americanus*	1.70±0.03mg/gm
3	*Hydrophilus triangulares*	1.20±0.02mg/gm
4	*Bombyx mori*	0.90±0.04mg/gm
5	*Macrobrachium rosenbergii*	0.87±0.03mg/gm

As can be seen in Table 2, the maximum protein concentration is found in the muscle of some invertebrate food of Assam and Meghalaya in *Paratelphusa hydrodromous* (4.47mg/gm) and the lowest protein content in the muscle is in *Macrobrachium rosenbergii* (0.87mg/gm).

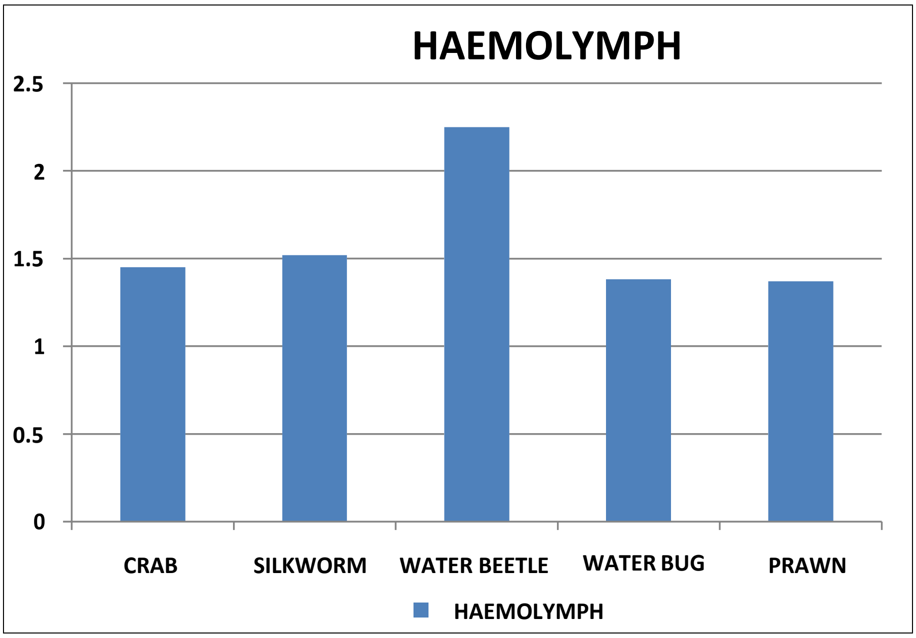


Fig 1: Graphical representation of total protein content in haemolymph.

Paratelphusa hydrodromous (crab)-1.37mg/gm
Bombyx mori (Silkworm)-1.42mg/gm
Hydrophilus triangularies (water beetle)-2.16mg/gm *Lethocerus americanus* (water bug)-1.31mg/gm
Macrobrachium rosenbergii (Prawn)-1.27mg/gm

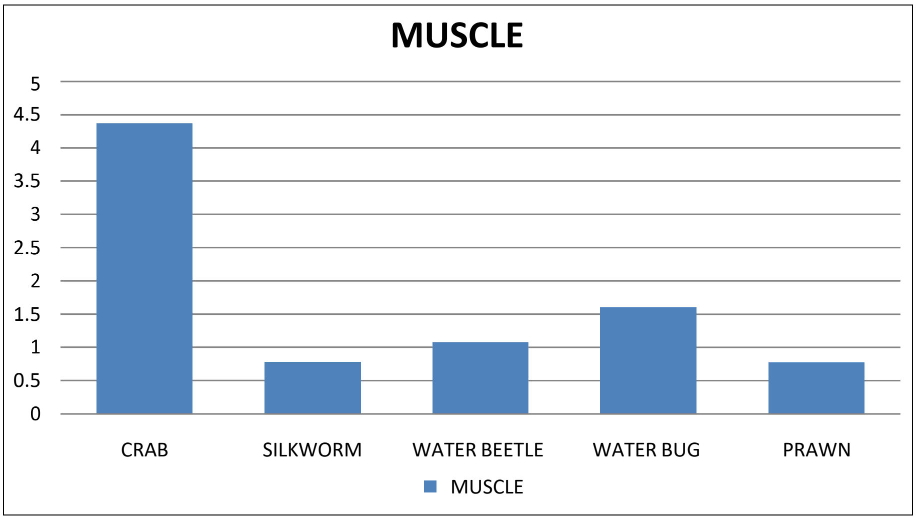


Fig 2: Graphical presentation of total protein content in muscle.

Paratelphusa hydrodromous (Crab)-4.37mg/gm
Bombyx mori (Silkworm)-0.78mg/gm
Hydrophilus triangularies (Water beetle)-1.08mg/gm *Lethocerus americanus* (Water bug)-1.60mg/gm
Macrobrachium rosenbergii (Prawn)- 0.77mg/gm

Table 3: The presence of free amino acid in the hemolymph of several Assam and Meghalaya invertebrate foods.

Amino acids	Silk worm	Water bettle	Crab	Prawn	Water bug
Alanine	—	—	—	—	—
Glutamine	—	+	+	—	+
Glycine	—	—	—	—	—
Leucine	—	—	—	—	—
Isoleucin	—	—	—	—	—
Valine	+	+	+	+	+
Serine	—	—	—	—	—

Threonine	—	+	+	—	—
Methionine	—	—	—	—	—
Cysteine	—	+	—	—	—
Aspartic acid	—	—	—	—	—
Glutanic acid	—	—	—	—	—
Arginine	+	+	+	+	+
Tyrosine	+	—	—	+	—
Tryptophan	+	+	—	—	+
Lysine	—	—	—	+	+
Histidine	—	+	—	—	—
Asparagine	—	—	—	—	—
Proline	+	+	+	+	+
Phenylalanine	+	—	—	+	+

+ = Present - = Absent

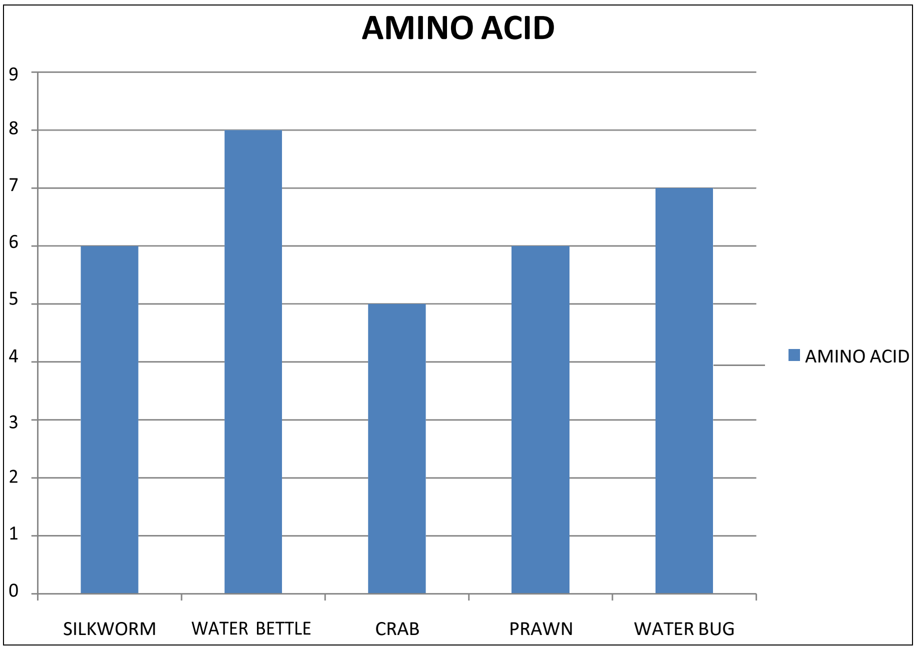


Fig 3: Total number of free amino acids detected or Chromatogram in thehaemolymph of some invertebrate food of Assam and Meghalaya.

Bombyx mori (Silkworm)-6
Hydrophilus triangularies (water beetle)-8 *Paratelphusa hydrodromous* (Crab)-5 *Macrobrachium rosenbergii* (Prawn)-6 *Lethocerus americanus* (Water bug)-7

Discussion

The data obtained from the present piece of work indicates a variable range of protein content in the different group of insect samples.

Table 1 and 2 show the total protein of each insect. The table 2 show the highest protein content in the haemolymph of some invertebrate food in Assam and Meghalaya in *hydrophilus triangularies* (2.25mg/gm) and the lowest protein content is in the haemolymph is in *Macrobrachium rosenbergii* (1.37mg/gm). Table 3 show the highest protein content in the muscle of some invertebrate food in Assam and Meghalaya in *Paratelphusa hydrodromous* (4.47mg/gm) and the lowest protein content in the muscle is in *Macrobrachium rosenbergii* which do not conform to the result of Belluco *et al*, 2013 [4].

There was an evaluation of research done on the crude protein content of several insect species that was commissioned by FAO in 2013 (Van Huis *et al*., 2013).

The chemical makeup of both insects is shown in Figures 1 and 2. Hydrophilus triangularies, a species of coleopterans, had the greatest haemolymph protein (2.25mg/gm) while Paratelphusa hydrodromous, a species of lepidopterans, had the highest muscle protein (4.47mg/gm) (Tables 1 and 2).

Insects used as a source of nutrition for other organisms have been shown, via proximate analysis, to be particularly nutrient-dense in the Indian states of Assam and Meghalaya. It was found that the protein content of edible insects was comparable to that of meat and fish, suggesting that they may be used as a cheaper and more accessible option to help fight poverty (Kariuki and White, 1991) [13].

Table 3 shows the presence and absence of free amino acids in the haemolymph of some invertebrate food in Assam and

Meghalaya. Proline, Arginine and valine are the most common as they are found in all the five invertebrate food of Assam and Meghalaya. Glutamine, Tryptopham, Phenylalanine are present in almost three to four edible invertebrate food of Assam and Meghalaya. Histidine, Lysine, Cysteine, Threonine least common ones as they are found only in one or two invertebrate food of Assam and Meghalaya and it do not confirm to the result of (Rajulu and Kulasekarapandia, 1971) Since the extracts were prepared at random from both male and female there is apparently no difference in the pools on the basis of sex.

Figure 2 show the chemical analysis of each insects. The highest number offree amino acids was detected in haemolymph of coleoptera order species *Hydrophilus triangularies.*

We demonstrated that that the edible insects were good source of protein due to their high protein content. Palatable bugs have been utilized as food in tropical and sub tropical countries to provide significant nutritional benefits. With the increase humane population, global food demand is growing rapidly and therefore insects may become valuable new source of protein.

Due to the lack of resources, insects are the main source of nutrition. Wild food resources are abundant, and the cost of gathering them is little. Despite their modest production costs, they are often sold at high prices in the market, providing excellent worldwide revenue potential (Munthali & Mughogho 1992; Payne 2014; Sribandit *et al*, 2008).

So, there is a scope for popularization and commercialization of edible insects species of Assam and Meghalaya as they contain high protein and also acceptable as indigenous people eat them from ages. It is popularized that it can replace costly protein sources with very low cost and can fulfill protein requirement of the people.

Conclusion

Protein and amino acid serves as an important component in our life as it contributes to maintenance and proper development of our body. The objective of this experiment was to estimate protein in the haemolymph and muscle tissue of some invertebrate food in Assam and Meghalaya. Edible insects supply abundant protein, lipid, vitamin, and mineral components, making them a viable economic and ecological solution to the world's expanding dietary demands. Insects have several uses besides just being tasty treats and tasty meals. Insects serve as a source of food and entertainment for humans. There is a great diversity of bug species that may be eaten, and these insects constitute a significant food source because of the high nutrient value they provide. To help manage vitamin deficiencies and improve overall food security, insects are increasingly being employed as a viable alternative food source. However, there is still apprehension that using edible insects may lead to safety and health problems. If the available insect can be commercialization can fulfill protein requirement and amino acid in cheap cost and also it can be a source of income for local inhabitants. The present study revealed that the *Hydrophilus triangularies* has the highest haemolymph protein, *Paratelphusa hydrodromous* has the highest muscle protein and the biggest number of amino corrosive was recognized in the haemolymph of *Hydrophilus triangularies.*

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