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Comparative Amino Acid Profiling of Arthropod Haemolymph in Zaria, Nigeria

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Abstract
This study investigates the amino acid profile of Haemolymph in eight arthropod species from Zaria, Nigeria, representing the classes Insecta, Arachnida, and Crustacea. Haemolymph samples (1 mL) were collected using the novel Antennae method from anesthetized and cleaned arthropods. Amino acid analysis was conducted using the Applied Biosystems PTH Amino Acid Analyzer following hydrolysis and defatting. A total of 17 amino acids were detected in the Haemolymph, including leucine, lysine, isoleucine, phenylalanine, serine, aspartic acid, valine, methionine, proline, arginine, tyrosine, histidine, cystine, alanine, glutamic acid, glycine, and threonine. Notably, Glutamic acid was found to be the most abundant in spider (5.08g/100g) while cystine was the least abundant in cricket (0.02 g/100g). Statistical analysis revealed significant differences ($P<0.05$) in amino acid composition between arthropod species and compared to RPMI 1640 control. These results suggest that amino acids play a critical role in cellular processes, including protein synthesis, energy production, and immunity, aiding in the arthropods' defense against infections. This finding aligns with previous studies, highlighting the importance of these compounds in arthropod physiology and their potential for further studies on metabolic processes and immune responses in invertebrates.

Keywords: Arthropod, Arthropod Haemolymph, Amino Acid, Kaduna State, Nigeria

Introduction

The ability of arthropods to resist infection and also protect themselves against infection is as a result of the presence of protein in the Haemolymph (Aboul-Naga et al., 1991). When these proteins are digested or broken down, amino acids are left; these amino acids are used by the body to make proteins and eventually help the body to break down food. Amino acids in Haemolymph play an important role in the osmoregulation in insects (Drilhon et al., 1951; Narayanan, 1969; Slama et al., 1983). Amino acids are fundamental building blocks supporting life. In arthropod Haemolymph, the high levels of free amino acid concentrations vary from species to species and also vary according to the arthropod developmental stage (Chapman, 1998). These amino acids are used by the cells to build up or replace enzymes and hormones as well as proteins needed for muscles, egg yolk, ribosomes, cuticle, and many other purposes. Out of the twenty (or twenty-one) naturally occurring amino acids, at least 10 must be present in diet. These 10 amino acids are called essential amino acids namely: lysine, tryptophan, histidine, phenylalanine, leucine, isoleucine, threonine, methionine, valine, and arginine. The other 10 amino acids are considered non-essential because they can be synthesized from other amino acids or similar chemical building blocks. Selenocysteine is derived from serine during protein synthesis. It is the 21st amino acids recognized by some authorities. Human diets and insect diets require the same 10 essential amino acids (Ma et al., 2016), hence the importance of this study.

Materials and Methods

Eight arthropods were selected for this study belonging to the class; insecta, Arachnida and Crustacean. These arthropods were collected from Zaria using different sampling methods of arthropod collection (Adenusi et al., 2018; Schauff, 2001; Gibb & Oseto, 2006; Mora-Aguilar et al., 2023). Haemolymph (1ml) was collected from each arthropod sampled using novel Antennae method of Haemolymph Sampling (Borsuk, 2017). This method involves the collection of Haemolymph from selected arthropods that have been anesthetized on ice and carefully cleaned with 70% ethanol after excising the metathoracic legs and gently pressing the abdomen. Collection of

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Haemolymph was carried out with micropipettes and drops collected was transferred to Eppendorf tubes (on ice) and kept in freezing (-11°C) compartment (Duman & Horwath, 1983) of ordinary refrigerator prior to use. Amino acid profile of Haemolymph was determined as described by AOAC (2005) with slight modification. Each arthropod Haemolymph sampled was dried at 70°C to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into Applied Biosystems PTH Amino Acid Analyzer (model 120A)to calculate the peak area that is proportional to the concentration of each of the amino acid. All arthropod Haemolymph samples were tested for the 19 known amino acids (leucine, lycine, isoleucine, phenylalanine, serine, aspartic acid, valine, methionine, proline, arginine, tyrosine, histidine, cystine, alanine, glutamic acid, glycine and threonine).

Results

Amino acid analysis of sampled arthropod Haemolymph reveals the presence of seventeen (17) amino acids expressed in g/100g protein (Table 1). Out of nineteen (19) amino acids tested, 17 were detected while two (2) amino acids were not detected. Amino acids detected were leucine, lysine, isoleucine, phenylalanine, valine, methionine, proline, arginne, tyrosine, histidine, cystine, alanine, glutamic acid, glycine, threonine, serine and aspartic acid. Norleucine and tryptophan were not detected. The sampled arthropod Haemolymph showed varied values as compared to RPMI1640. Amino acid with the highest values per gram of protein detected in arthropod Haemolymph were as follows: leucine (2.09g/100g) in scorpion; lysine (1.30g/100g), isoleucine (1.72g/100g), phenylalanine (0.89g/100g), valine (1.52g/100g), proline (1.93g/100g), argine (3.96g/100g), tyrosine (0.68g/100g) and glycine (1.56g/100g) in beetle; histidine (1.47g/100g) in grasshopper; alanine (1.23g/100g), glutamic acid (5.08g/100g) in butterfly; methionine (0.57g/100g) in cricket; phenylalanine (0.89g/100g), serine (3.25g/100g), aspartic acid (4.05g/100g) and threonine (0.79g/100g) in cockroach and Cystein (0.50g/100g) was higher in RPMI 1640 than all Haemolymph of sampled arthropods. However, lower values of amino acid per gram of protein were recorded in cricket with the Haemolymph recording the lowest values in 12 amino acids (leucine, lycine, isoleucine, phenylalanine, serine, aspartic acid, valine, proline, arginine, tyrosine, histidine, cystine and glutamic acid). The control (RPMI 1640) had the lowest values in 5 amino acids (proline, histidine, alaine, glycine and threonine) when compared to sampled Haemolymph while crab had the lowest methionine content per gram of protein. Statistical analysis shows a significant difference ($p<0.05$) between amino acid compositions of arthropod Haemolymph and between Haemolymphs and RPMI 1640.

Table 1: Amino Acid Concentration of Haemolymph of Arthropods and RPMI 1640

	Mean Amino acid concentration in Haemolymph of arthropods and RPMI 1640								
Haemolymph	**Beetle**	**Butterfly**	**Cockroach**	**Crab**	**Cricket**	**Grasshopper**	**Scorpion**	**Spider**	**Control (RPMI 1640)**
Leucine	1.67 ± 0.03^{b}	0.81 ± 0.01^{e}	1.19 ± 0.01^{c}	0.94 ± 0.01^{de}	0.08 ± 0.01^{g}	1.00 ± 0.01^{cde}	2.12 ± 0.05^{a}	1.06 ± 0.14^{cd}	0.50 ± 0.00^{f}
Lysine	1.31 ± 0.01^{a}	0.88 ± 0.04^{abc}	1.10 ± 0.01^{ab}	0.85 ± 0.01^{abc}	0.08 ± 0.01^{d}	0.88 ± 0.01^{abc}	0.88 ± 0.01^{abc}	0.49 ± 0.51^{bcd}	0.40 ± 0.00^{cd}
Isoleucine	1.71 ± 0.01^{a}	1.04 ± 0.03^{cd}	1.46 ± 0.02^{ab}	0.82 ± 0.02^{de}	0.04 ± 0.01^{f}	1.40 ± 0.01^{abc}	1.1 ± 0.02^{bcd}	1.02 ± 0.28^{d}	0.50 ± 0.00^{e}
Phenylalanine	0.89 ± 0.00^{a}	0.33 ± 0.02^{a}	0.89 ± 0.00^{a}	0.44 ± 0.01^{a}	0.08 ± 0.01^{a}	0.64 ± 0.04^{a}	0.53 ± 0.00^{a}	1.34 ± 1.27^{a}	0.15 ± 0.00^{a}
Serine	2.95 ± 0.01^{ab}	1.73 ± 0.04^{c}	3.15 ± 0.14^{a}	2.04 ± 0.01^{bc}	0.07 ± 0.01^{d}	2.06 ± 0.03^{bc}	2.25 ± 0.35^{abc}	1.61 ± 0.62^{c}	
Aspartic acid	3.84 ± 0.01^{ab}	1.83 ± 0.02^{cd}	4.07 ± 0.03^{a}	1.98 ± 0.01^{c}	0.23 ± 0.03^{d}	2.15 ± 0.01^{bc}	1.94 ± 0.05^{cd}	1.03 ± 1.30^{cd}	0.50 ± 0.00^{cd}
Valine	1.5 ± 0.02^{a}	0.94 ± 0.02^{e}	1.23 ± 0.01^{c}	1.15 ± 0.02^{d}	0.02 ± 0.01^{h}	1.33 ± 0.01^{b}	0.79 ± 0.01^{f}	1.17 ± 0.01^{d}	0.20 ± 0.00^{g}
Methionine	0.27 ± 0.01^{c}	0.58 ± 0.02^{a}	0.17 ± 0.01^{d}	0.11 ± 0.01^{e}	0.16 ± 0.01^{de}	0.16 ± 0.01^{de}	0.36 ± 0.01^{b}	0.12 ± 0.01^{de}	0.15 ± 0.00^{de}
Proline	1.98 ± 0.07^{a}	1.23 ± 0.02^{c}	1.32 ± 0.00^{bc}	1.28 ± 0.01^{c}	0.15 ± 0.01^{d}	1.42 ± 0.00^{b}	1.22 ± 0.00^{c}	1.27 ± 0.07^{c}	0.20 ± 0.00^{d}
Arginine	3.96 ± 0.01^{a}	2.05 ± 0.01^{f}	2.83 ± 0.00^{c}	2.22 ± 0.02^{e}	0.05 ± 0.01^{h}	3.01 ± 0.00^{b}	2.75 ± 0.00^{d}	2.24 ± 0.00^{e}	2.00 ± 0.00^{g}
Tyrosine	0.68 ± 0.00^{a}	0.28 ± 0.01^{cde}	0.48 ± 0.06^{b}	0.34 ± 0.01^{cd}	0.03 ± 0.01^{f}	0.22 ± 0.06^{de}	0.17 ± 0.00^{e}	0.34 ± 0.00^{c}	0.20 ± 0.00^{e}
Histidine	1.14 ± 0.02^{a}	0.48 ± 0.03^{ab}	1.15 ± 0.01^{a}	0.51 ± 0.03^{ab}	0.15 ± 0.01^{b}	1.08 ± 0.54^{a}	0.86 ± 0.01^{ab}	0.52 ± 0.02^{ab}	0.15 ± 0.00^{b}
Cystine	0.29 ± 0.02^{b}	0.16 ± 0.01^{cd}	0.24 ± 0.00^{bc}	0.14 ± 0.01^{d}	0.02 ± 0.01^{e}	0.26 ± 0.02^{b}	0.27 ± 0.04^{b}	0.15 ± 0.04^{cd}	0.50 ± 0.00^{a}
Alanine	1.17 ± 0.01^{b}	0.78 ± 0.02^{e}	1.03 ± 0.01^{d}	1.24 ± 0.01^{a}	0.32 ± 0.01^{f}	1.02 ± 0.01^{d}	1.09 ± 0.01^{c}	1.23 ± 0.03^{a}	0.20 ± 0.00^{g}
Glutamic acid	4.71 ± 0.03^{b}	2.42 ± 0.04^{f}	3.39 ± 0.03^{d}	5.04 ± 0.06^{a}	0.32 ± 0.03^{g}	3.03 ± 0^{e}	4.09 ± 0.01^{c}	5.08 ± 0.11^{a}	3.00 ± 0.00^{e}
Glycine	1.55 ± 0.01^{a}	1.12 ± 0.00^{c}	1.26 ± 0.00^{b}	1.04 ± 0.01^{d}	0.12 ± 0.00^{f}	1.06 ± 0.02^{cd}	0.8 ± 0.01^{e}	1.04 ± 0.05^{d}	0.10 ± 0.00^{f}
Threonine	0.78 ± 0.01^{a}	0.42 ± 0.01^{d}	0.78 ± 0.01^{a}	0.50 ± 0.01^{c}	0.23 ± 0.03^{e}	0.52 ± 0.01^{c}	0.68 ± 0.01^{b}	0.51 ± 0.06^{c}	0.20 ± 0.00^{e}

Note: rows with same alphabets as superscript are not significantly different at $p > 0.05$

Discussion

In this study, the 17 amino acids; leucine, lysine, isoleucine, phenylalanine, serine, aspartic acid, valine, methionine, proline, arginine, tyrosine, histidine, cystine, alanine, glutamic acid, glycine and threonine present in the Haemolymph of sampled arthropod could be due to their importance in cellular processes which include; protein synthesis, energy production, nucleotide metabolism and cell signaling, this makes arthropod to fight against infection. This finding is in line with the works of (Wyatt et al., 1956; Sowa & Keeley, 1996; Huong et al., 2001; Consoli& Vinson, 2002; Tillinghast & Townley, 2008; Sankar &Yogamoorthi, 2012). Amino acids play important role in animals especially in the fight against infection where it helps provide protection against evading pathogens. Wyatt et al. (1956) reported the presence of 19 amino acids in Haemolymph of silkworm Bombyx. Sankar and Yogamoorthi (2012) also reported the presence of 14 amino acid in the Haemolymph and

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muscles of both sexes in *Ocypodeplatytarsis* with glutamic acid, histidine, threonine, serine and leucine found to be highest in both sexes. 18 out of 20 standard amino acids were found in the Haemolymph of *Blaberusdiscoidalis* cockroach (Sowa & Keeley, 1996). They also reported hydroxyproline and ornithine which they termed to be non standard amino acid in the Haemolymph of the cockroach studied. In line with presence of amino acid in Haemolymph, Huong et al. (2001) also had a similar work that reported the presence of glycine, arginine, alanine, proline and lysine in the giant fresh prawn. In this study, the high glutamic acid, a non-essential amino acid observed in the arthropod Haemolymph is similar to the works of Wyatt et al. (1956), Consoli and Vinson (2002), Edwards et al. (2008), and Sankar and Yogamoorthi(2012), its abundance could be as a result of its importance in survival, proliferation and metabolism of the arthropods cells (Wu et al., 2023). Although, Huong et al. (2001) reported alanine to be the highest in giant fresh prawn, its alanine value was increased after the prawn was exposed to varying salinity for a week. The high glutamic acid content found in beetle in this study is similar to those reported in the study carried out by Ademola et al. (2017) who reported glutamic acid to be the most abundant in all the five samples (bee brood, soldier termite, snout beetle larva and pupa) studied. Similarly, Yi and Adams (2000) also reported proline, glutamine and valine to be most abundant amino acid in both sexes of beetle *Leptinotarsadecemlineata*. In the study of the chemical composition and amino acid content of five species of edible grasshoppers from Mexico, the authors reported glutamic acid to be abundant in all five species (Melo-Ruiz et al., 2015). In contrast to the above reports, Kohler et al. (2019) reported Leucine to have the highest concentration in all the insects sampled and the lowest amino acid was reported to be methionine.

Cockroach Haemolymph showed high values in aspartic acid, glutamic acid and serine which could be because of their importance in survival of the arthropod, proliferation and metabolism.This finding is in contrast with the work of Sowa and Keeley (1996) who reported the absence of aspartic acid and cystine in the Haemolymph of cockroach (*Bladerusdiscoidalis*), although, the species of the cockroach they worked on is different from the species of cockroach studied in this work. The similarity in the findings of this present work as compared to the work of Osborne and Neuhoff (1974) who reported proline, alanine, glycine, glutamic acid and glutamine to be present in the nervous tissue muscle and blood of cockroach (*Periplanetaamericana*) could be as a result of similar species of cockroach used in both study. Ping et al. (2017) reported a high value of arginine in the muscle and Haemolymph of crab *Portunustrituberulata*, this is in agreement with the present study where crab Haemolymph was found to have the highest amino acid value in glutamic acid and arginine while its lowest value in methionine. Sankar and Yogamoorthi also reported the presence of 14 amino acid in the Haemolymph and muscles of both sexes in *Ocypodeplatytarsis*(ghost crab), the amino acids reported to be present in their work is similar to the present work.Previous studies have shown the presence of amino acid in grasshopper (Shaw, 1955; Melo-Ruiz et al., 2015; Finke, 2015; Zamudio-Flores et al., 2019).Which is in line with the report from this present study. Glutamic acid which was found to be the most abundant in the work of Melo-Ruizet al. (2015), is in agreement with the present study which reveals glutamic acid, arginine and aspartic acid to be abundant in grasshopper Haemolymph. Similarly, the eggs of grasshopper was studied by Shaw (1955) and reported the presence of 17 amino acids while Buszewska-Floajta et al. (2014) also reported 7 amino acids present in grasshopper.

The presence of amino acids in scorpion have been worked on by several researchers (Lee et al., 2013; Kaya &Kocatepe, 2014; Sai'd et al., 2021; Aslant et al., 2024). The work of Kaya and Kocatepe (2014) is in agreement with this present work; lycine and glutamic acid were observed to be the most abundant in *Scorpaenaporcus*, Linnaeus. While other researchers revealed the presence of several amino acids found in the venom of scorpion, Lee et al. (2013) in another dimension reported the presence of aspartic acid, histidine, alanine, tyrosine and cystine in a hot water extraction of amino acid of scorpion Haemolymph as compared to 70% ethanol extracts. Glutamic acid is a very important amino acid to arthropods. Among all arthropod Haemolymph studied in this work, the highest value of glutamic acid was found in scorpion belonging to the class arachinida and the second highest was found in spider belonging to the same class. This finding is in line with the work of Tillinghast and Townley (2008) who confirmed glutamine to be the most abundant in their work. While researchers Punzo (1983), Tillinghast and Townley (2008), and Kuhn-Nentwig and Nentwig (2012) confirmed the presence of proline in high amount in the Haemolymph of spider. Stratakis **e**t al. (1993) confirmed the abundance of tyrosine and phenylalanine in spider of *Eurypemacalifornicum*.

Conclusion

The results of the study revealed the presence of leucine, lysine, isoleucine, phenylalanine, serine, aspartic acid, valine, methionine, proline, arginine, histidine, cystine, alanine, glutamic acid, glycine and threonine in the arthropod's Haemolymph studied. Glutamic acid was found to be the most abundant in spider (5.08 ± 0.11^{a}) while cystine was the least abundant in cricket (0.02 ± 0.01^{e}). Arthropod used in this study could serve to provide rich source of amino acid such as glutamic acid, arginine and aspartic acid in human and animal feeds. Butterfly, beetle, scorpion, cricket and spider Haemolymph can be exploited for culture media for the propagation of *T. b. brucei in-vitro* to conserve foreign exchange.

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