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Alteration of protein and amino acids at different developmental stages of *Schistocerca gregaria* (Orthoptera: Acrididae)

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Abstract:

The composition changes in total protein and total amino acids (AA) of *Schistocerca gregaria* Forsskål (Orthoptera: Acrididae) were estimated according to stages and gender. The concentrations of protein and amino acids in samples showed wide range variations. Results showed that *S. gregaria* contained high amount of total protein contents (49.64 – 67.9% of dry matter). Seventeen amino acids were detected as the basic components of proteins, which play an important role in insect metabolism they were; threonine (Thr), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), lysine (Lys), histidine (His), arginine (Arg), cysteine (Cys), aspartic acid (Asp), serine (Ser), glutamic acid (Glu), glycine (Gly), alanine (Ala), tyrosine (Tyr) and proline (Pro). The results revealed the AA possibly divided based on essentiality: essential, semi-essential and non-essential; which average concentrations in males and females were 1.94-2.42 and 2.19-2.44 mg/100 mg, respectively. Semi-essential with the average concentrations in both sex's males and females were 1.5- 1.71 and 1.46-1.7 mg/100 mg. And non-essential AA its average concentrations in males and females were 3.24-3.89 and 3.53-4.04 mg/100 mg respectively. Also, another possibly divided based on the concentrations as high >3, moderate >2 and low <2 mg/100 mg. Differences of amino acids were found during developmental stages and sexual maturation. The average concentrations of AA showed detraction of the immature and mature stages of two sexes comparing with the first nymphal stages. The present investigations could give a sight about the physiology of total protein and AA in *S. gregaria*.

Introduction

The desert locust *Schistocerca gregaria* Forsskål (Orthoptera: Acrididae) is an economically damaging pest of a wide range of crops, mainly cereals in the Sahel region of Africa. It occurs in many parts of Africa and Asia. *S. gregaria* plagues can be an important contributing factor to famines and a threat to food security in many regions of the world (FAO, 2012). The most important functions of amino acids, the building blocks of the proteins that are derived from the insect diet, include the synthesis of structural

proteins of the integument and the synthesis of hormones and enzymes that participate in the synthesis nucleic acids (Klowden, 2007). Amino acids are also required by insects for transport and storage and as receptor molecules. Besides, some amino acids are involved in morphogenesis (Chapman, 2002). The pattern of hemolymph amino acids can be modified by development, oogenesis, feeding, cuticular tanning, silk production, or flight activity (Blum, 1985).

The present study was designed to evaluate total protein and total amino acids which play an important role in physiology, metabolism and development at different developmental stages in *S. gregaria* at both sexes.

Materials and methods

1. Mass rearing of insects:

This study has been executed in the laboratory of locust and Grasshopper Department, Plant Protection Research Institute, ARC, Dokki, Giza. The insects were maintained in the laboratory under crowded and controlled conditions at 30±2°C and 30-65% RH and reared according to Robert *et al.* (2002).

2. Biochemical analysis:

2.1. Sample preparation for the assay of total protein and amino acids:

This study was conducted to estimate protein and amino acids (AA) content in different insect stages and sexes. Groups of 50 hoppers of 1st instar to 4th nymphal instars were used as one sample but separation according to gender started with 5th instar. The whole insects of each stage were collected individually, then refrigerated and stored at -5ºC and then dehydrated in the oven 48-72h at 55-60ºC. Samples were homogenized to get homogenate dry powder. Homogenates were weighted and kept until the biochemical determinations (Ahmed *et al*., 2019 and Abd-El Wahed and Ahmad, 2019).

2.2. Analysis of total protein content and amino acids:

Determination of proteins and amino acids was carried out at Regional Center for Food and Feed, ARC, Giza, according to AOAC (2012). The Kjeldahl method was used for protein determination. Amino acids were determined by high perform Amino Acid analyzer (Biochrom 30) and EZ chrome manual (software for data collection and processing).

Results and discussion

1.Total protein in body homogenate of *Schistocerca gregaria* :

The values of total protein in the *S. gregaria* of males and females of different ages are shown in Figure (1) . In general, the percentage of total protein content was higher in nymphal instar compared with adults stages. Data presented in Figure (1) shows that the content of total protein was 66.63% of dry matter in the interval from 1st to 4th instar. At 5th instar this value decreased in the males to 61.06% of dry matter and increased in the female to 67.9% of dry matter. This decreasing continued until immature stage where the total protein recorded 61 and 60.27% % of dry matter in the adults immature in both males and females, respectively. Also found sharply diminishing of protein content of mature adult males and females 49.64 and 56.24%, respectively.

The increasing at 5th nymphal females in protein content of *S. gregaria* may be due to proteins represent the main component of the nutrient composition and development of insects, overall, that showed solid agreement with Abd-El Wahed and Ahmad (2019), they reported, the protein percentages in both adults and nymphs of *S. gregaria* contained more than 55%, but in nymphs were higher than adults the values were 65.92 and 56.79%, respectively. The highest percentage of protein 76 and 70% were

found in *S. gregaria* and the cricket *Gryllodes sigillatus* (tropical house cricket), respectively (Ewelina *et al.*, 2015). Similar value (77%) was shown by Ramos-Elorduy *et al.* (1997) for *Sphenarium histrio* Gerstaecker (Orthoptera: Pyrgomorphidae) and, slightly lower (61%) for *Schistocerca* sp. The content of protein in *Tenebrio molitor* (Orthoptera: Tenebrionidae) (mealworm beetle) was 52.35% and this value is comparable with the results of Rumpold and Schlüter (2013).

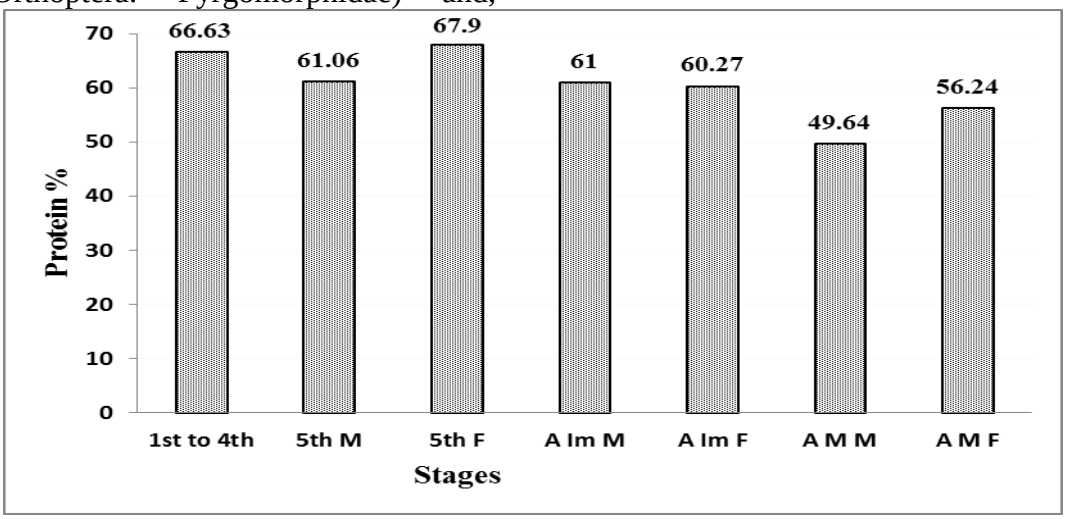

Figure (1): The percentage of total protein in total body homogenate of *Schistocerca gregaria*. 1st to 4th nymphal instar, 5th nymphal instar males (5th M), 5th nymphal instar females (5th F), Adult immature males (AImM), Adult immature females (AImF), Adult mature males (AMM), Adult mature females (AMF) based on dry matter.

2. Total amino acids of *Schistocerca gregaria* at different stages:

According to the obtained data in Tables (1 and 2) and Figure (2), seventeen amino acids were detected; threonine (Thr), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), lysine (Lys), histidine (His), arginine (Arg), cysteine (Cys), aspartic acid (Asp), serine (Ser), glutamic acid (Glu), glycine (Gly), alanine (Ala), tyrosine (Tyr) and proline (Pro), seven are essential consisted of Thr, Val, Met, Ile, Leu, Phe and Lys; three are semi-essential: His, Arg and Cys, while seven are non-essential: Asp, Ser, Glu, Gly, Ala, Tyr & Pro, this results in full agreement with (El-Shennawy, *et al.* 2019 and Abd-El Wahed and Ahmad, 2019). The seventeen AA were identified in all stages of *S. gregaria.* AA is not only the building blocks of proteins but also key regulators of various pathological and physiological processes **(**Sankar and Yogamoorthi, 2012) and including immune responses (Yoneda *et al.*, 2009). From the results, the variations of AA composition in all developmental stages of *S. gregaria*. The AA composition was detected by the changes in the percentages. The essential AA showed that Met was the smallest value in 1st to 4th instar (5.12%) while the highest value was for Leu in adult males mature and adult females immature, were 23.12% and 23.47%, respectively. Whereas in the semi-essential AA represent Cys was represented the lowest 16.02% while Arg was the highest value at 58.23% for 1st to 4th instar. But in the non-essential AA, the lowest and highest values were Ser and Ala 5.64 and 28.47%, respectively in adult males mature whilst adult females immature were 6.83 and 29.70%, respectively.

Table (1):The composition of amino acids in the total body homogenate of *Schistocerca gregaria* of 1st - 4th to immature and mature adult male.

Amino acids AA	1st to 4th instar		5th instar			Adult (immature and mature)					
	AA con.	%	AA con.	%	% Change than 1st to 4th instar	AA con.	%	% Change than 5th instar	AA con.	%	% Change than adult immature
Essential											
Threonine (Thr)	2.09	12.74	1.91	12.16	8.61	2.1	12.40	-9.95	1.49	10.97	29.05
Valine (Val)	3.27	19.93	3.3	21.01	-0.92	3.27	19.30	0.91	2.74	20.18	16.21
Methionine (Met)	0.84	5.12	0.81	5.16	3.57	1.25	7.38	-54.32	0.84	6.19	32.80
Isoleucine (Ile)	2.11	12.86	2.08	13.24	1.42	2.2	12.99	-5.77	1.92	14.14	12.73
Leucine (Leu)	3.56	21.69	3.47	22.09	2.53	3.71	21.90	-6.92	3.14	23.12	15.36
Phenylalanine (Phe)	1.61	9.81	1.55	9.87	3.73	1.59	9.37	-2.58	1.29	9.50	18.88
Lysine (Lys)	2.93	17.86	2.59	16.49	11.60	2.82	16.65	-8.88	2.16	15.91	23.40
Sum	16.41		15.71			16.94			13.58		
Average	2.34		2.24			2.42			1.94		
Semi-essential											
Histidine (His)	1.19	25.76	1.17	22.81	1.68	1.17	24.63	0	0.98	21.78	16.24
Arginine (Arg)	2.69	58.23	2.66	51.85	1.12	2.69	56.63	-1.13	2.33	51.78	13.38
Cystine (Cys)	0.74	16.02	1.3	25.34	-75.68	0.89	18.74	31.54	1.19	26.44	-33.71
Sum	4.62		5.13			4.75			4.5		
Average	1.54		1.71			1.58			1.5		
Non-essential											
Aspartic acid (Asp)	3.93	14.74	3.73	14.26	5.09	3.92	14.41	-5.09	3.05	13.44	22.19
Serine (Ser)	2.18	8.18	1.83	7.01	16.06	2.07	7.61	-13.11	1.28	5.64	38.16
Glutamic acid (Glu)	6	22.51	5.42	20.76	9.67	5.86	21.54	-8.12	4.47	19.70	23.72
Glycine (Gly)	2.64	9.90	2.75	10.53	-4.17	2.96	10.88	-7.64	2.49	10.97	15.88
Alanine (Ala)	6.13	22.99	6.69	25.62	-9.14	7.04	25.87	-5.23	6.46	28.47	8.24
Tyrosin (Tyr)	2.75	10.32	2.7	10.34	1.82	2.11	7.76		2	8.82	5.21
Proline (Pro)	3.03	11.37	2.99	11.45	1.32	3.25	11.94		2.94	12.96	9.54
Sum	26.66		26.11			27.21			22.69		
Average	3.81		3.73			3.89			3.24		

***Con.=Concentration**

$$\%\text{Change} = \frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100$$

Data expressed as mg/100mg of dry matter.

Table (2) The composition of amino acids in the total body homogenate of *Schistocerca gregaria* of 1st - 4th to immature and mature adult females

Amino Acids AA	1st to 4th Instar		5th instar			Adult (immature and mature)					
	AA con.	%	AA con.	%	% Change than 1st to 4th nymphal instar	AA con.	%	% Change than 5th instar	AA con.	%	% Change than adult immature
Essential											
Threonine (Thr)	2.09	12.74	2.13	12.77	-1.91	1.79	11.39	15.96	1.83	11.92	-2.24
Valine (Val)	3.27	19.93	3.38	20.26	-3.36	3.33	21.20	1.479	3.02	19.67	9.31
Methionine (Met)	0.84	5.12	0.9	5.40	-7.14	0.78	4.97	13.33	0.9	5.86	-15.39
Isoleucine (Ile)	2.11	12.86	2.21	13.25	-4.74	2.16	13.75	2.26	2.07	13.49	4.17
Leucine (Leu)	3.56	21.69	3.6	21.58	-1.12	3.73	23.74	-3.61	3.55	23.13	4.83
Phenylalanine (Phe)	1.61	9.81	1.63	9.77	-1.24	1.46	9.29	10.43	1.46	9.51	0
Lysine (Lys)	2.93	17.86	2.83	16.97	3.41	2.46	15.66	13.07	2.52	16.42	-2.44
Sum	16.41		16.68			15.71			15.35		
Average	2.34		2.38			2.44			2.19		
Semi-essential											
Histidine (His)	1.19	25.76	1.26	24.66	-5.88	1.16	24.22	7.94	1.13	25.86	2.59
Arginine (Arg)	2.69	58.23	2.89	56.56	-7.43	2.67	55.74	7.61	2.49	56.98	6.74
Cystine (Cys)	0.74	16.02	0.96	18.79	-29.73	0.96	20.04	0	0.75	17.16	21.88
Sum	4.62		5.11			4.79			4.37		
Average	1.54		1.7			1.6			1.46		
Non-essential											
Aspartic acid (Asp)	3.93	14.74	4.05	14.34	-3.05	3.44	13.2	15.06	3.46	14.01	-0.58
Serine (Ser)	2.18	8.18	2.21	7.82	-1.38	1.78	6.83	19.46	1.88	7.61	-5.62
Glutamic acid (Glu)	6	22.51	6.14	21.74	-2.33	5.12	19.65	16.61	5.07	20.53	0.98
Glycine (Gly)	2.64	9.90	2.92	10.34	-10.61	2.28	8.75	21.92	2.62	10.61	-14.91
Alanine (Ala)	6.13	22.99	7.22	25.56	-17.78	7.74	29.70	-7.20	6.88	27.85	11.11
Tyrosin (Tyr)	2.75	10.36	2.32	8.21	15.64	2.49	9.56	-7.33	2.03	8.22	18.47
Proline (Pro)	3.03	11.37	3.39	12	-11.88	3.21	12.32	5.31	2.76	11.17	14.02
Sum	26.66		28.25			26.06			24.7		
Average	3.81		4.04			3.72			3.53		

***Con.=concentration**

$$\%\text{Change} = \frac{\text{Treatment - Control}}{\text{Control}} \times 100$$

Data expressed as mg/100mg of dry matter.

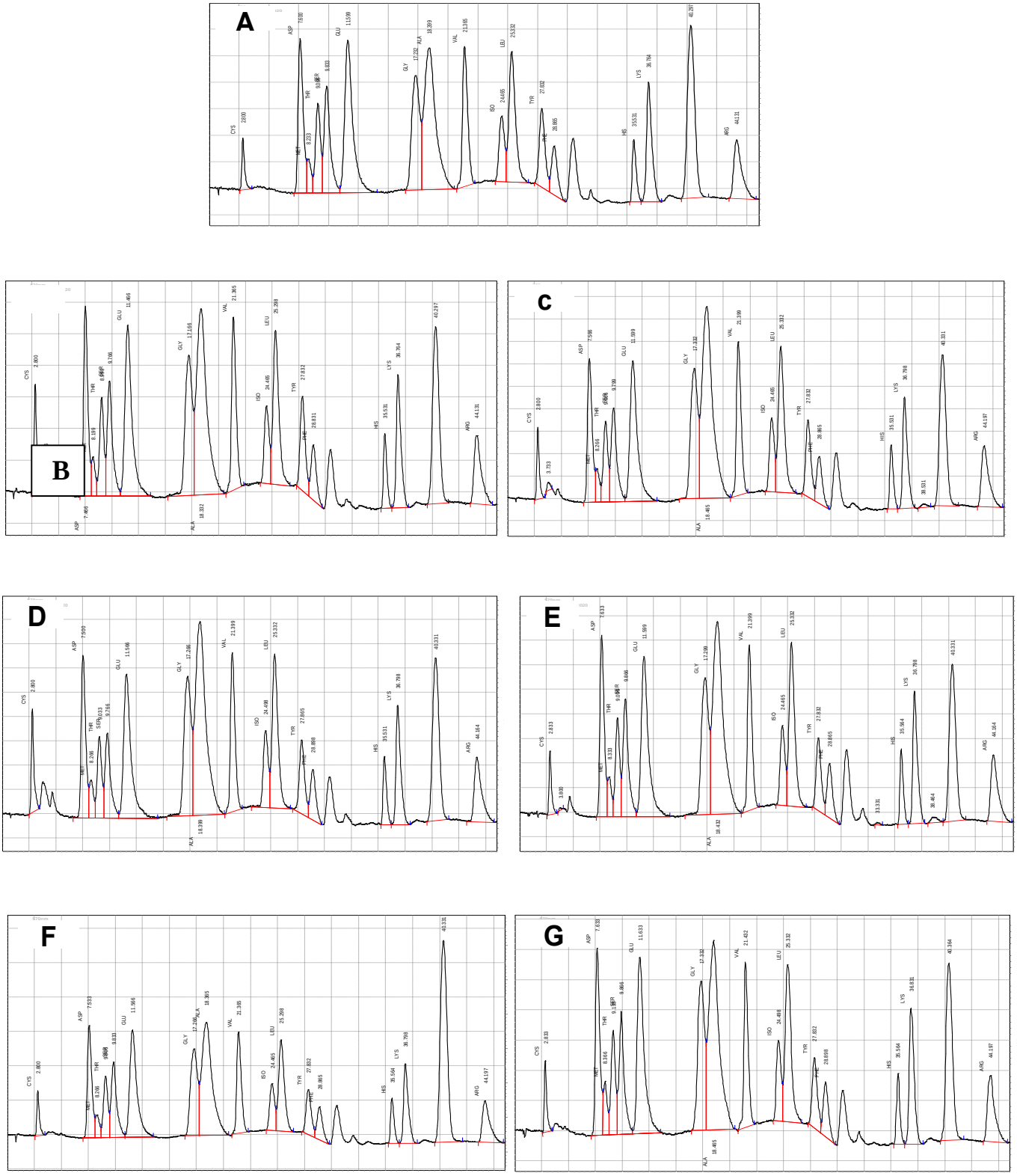

Figure (2): The composition of amino acids in the total body homogenate of *Schistocerca gregaria*. (A) 1st to 4th nymphal instar, (B) 5th nymphal instar male, (C) 5th nymphal instar female, (D) Adult immature male, (E) Adult immature female, (F) Adult mature male, (G) Adult mature female.

The results in Table (3) revealed that in examined samples, the AA possibly divided based on the concentrations as high, moderate, and low, which were >3, >2, and <2 mg/100 mg, respectively. In examined samples, some of the AA was present in approximately the same amount, while others fluctuated; these fluctuations may be because of different stages of development, the same results found by (El-Shennawy *et al.,* 2019; Ahmed *et al.* , 2019 and Abd-El Wahed and Ahmad, 2019) or different sex (Kulkarni and Mehrotra, 1970). From Table (3) the high concentrations of AA (more than 3 mg/100 mg) in an essential AA fluctuated in Val between 3.38 and 3.02 mg/100 mg at the females of 5th nymphal instar and in adult mature females, respectively; also in Leu between 3.73 and 3.14 mg/100 mg at adult immature females and adult mature males, respectively. Semi-essential AA was not detected. Moreover, non-essential AA of Asp, Glu and Pro they recorded in females 5th nymphal instar were 4.05, 6.14 and 3.39 mg/100 mg, respectively, but Ala was 7.74 mg/100 mg in adult immature females; these AA fluctuated until reach low values, in Asp and Glu were 3.05 and 5.07 mg/100 mg at adult mature males and females respectively, and both of AA Ala and Pro were 6.13 and 3.03 mg/100 mg respectively, in 1st to 4th nymphal instar.

The moderate concentrations of AA (more than 2 mg/100 mg), in an essential AA of Thr and Ile their values in females of 5th nymphal instar were 2.13 and 2.21 mg/100 mg respectively; but fluctuated between the previous values and 2.09 & 2.16 in 1st to 4th nymphal instar and in adult mature females, respectively, also the values were fluctuated in Lys AA between 2.93 and 2.46 mg/100 mg in 1st to 4th nymphal instar and an adult mature females, respectively. There was only one of AA in semi-essential it was Arg it has values between 2.89 and 2.33 mg/100 mg in females of 5th nymphal instar and adult mature males, respectively. In non-essential AA there were Ser, Gly, Tyr, and Pro with values between (2.21 and 2.07), (2.96 and 2.28), (2.75 and 2), and (2.99 and 2.76) mg/100 mg in (females of 5th nymphal instar and adult immature males), (adult immature males and females), (1st to 4th nymphal instar and adult mature males) and (males of 5th nymphal instar and adult mature females), respectively.

The low concentrations of AA (less than 2 mg/100 mg) in an essential were at Thr, Met and Phe with values (1.91 and 1.49), (1.25 and 0.78) and (1.63 and 1.29) mg/100 mg in (males of 5th nymphal instar and adult mature males), (adult immature males and females) and (females of 5th nymphal and adult immature males), respectively. However, the values of AA in semi-essential were at His and Cys their between (1.26 and 0.98) and (1.3 and 0.74) mg/100 mg in (5th nymphal instar females and adult mature males) and (5th nymphal instar males and 1st to 4th nymphal instar), respectively. As a non-essential AA Ser was 1.88 and 1.28 mg/100 mg in adult mature females and males, respectively.

Table (3): Summarized data for the concentration of amino acids (mg/100mg) in the total body homogenate of *Schistocerca gregaria*.

Amino acids			1st to 4th nymphal instar	5th nymphal instar		Adult immature		Adult mature	
				Males	Females	Males	Females	males	Females
>3	Essential	Val	3.27	3.3	3.38	3.27	3.33	-----	3.02
		Leu	3.56	3.47	3.6	3.71	3.73	3.14	3.55
	Semi-essential		-----	-----	-----	-----	-----	-----	-----
	Non-essential	Asp	3.93	3.73	4.05	3.92	3.44	3.05	3.46
		Glu	6	5.42	6.14	5.86	5.12	4.47	5.07
		Ala	6.13	6.69	7.22	7.04	7.74	6.46	6.88
		Pro	3.03	-----	3.39	3.25	3.21	-----	-----
>2	Essential	Val	-----	-----	-----	-----	-----	2.74	-----
		Thr	2.09	-----	2.13	2.1	-----	-----	-----
		Ile	2.11	2.08	2.21	2.2	2.16	-----	2.07
		Lys	2.93	2.59	2.83	2.82	2.46	2.16	2.52
	Semi-essential	Arg	2.69	2.66	2.89	2.69	2.67	2.33	2.49
	Non-essential	Ser	2.18	-----	2.21	2.07	-----	-----	-----
		Gly	2.64	2.75	2.92	2.96	2.28	2.49	2.62
		Tyr	2.75	2.7	2.32	2.11	2.49	2	2.03
		Pro	-----	2.99	-----	-----	-----	2.94	2.76
<2	Essential	Thr	-----	1.91	-----	-----	1.79	1.49	1.83
		Met	0.84	0.81	0.9	1.25	0.78	0.84	0.9
		Ile	-----	-----	-----	-----	-----	1.92	-----
		Phe	1.61	1.55	1.63	1.59	1.46	1.29	1.46
	Semi-essential	His	1.19	1.17	1.26	1.17	1.16	0.98	1.13
		Cys	0.74	1.3	0.96	0.89	0.96	1.19	0.75
	Non-essential	Ser	-----	1.83	-----	-----	1.78	1.28	1.88

Several studies have shown that; the concentration of some AA varied during metamorphosis (Mansingh, 1967 and Mitsuhashi, 1978). Many of AA increased of immature and mature stages this observed results partially agreed with Pal *et al.* (1973) who stated that; the AA in general are more concentrated in the pupal stage, of *Euproctis fraterna* Moore (Lepidoptera: Lymantriidae) this could be due to increased proteins breakdown. The variations in the pattern of AA at various insect developmental stages revealed a shift of AA requirements during growth and development (Rock and King, 1966). Amino acid levels can also be affected by changes in the levels of other substances such as carbohydrates and their metabolic intermediates and derivatives, which also change during metamorphosis (Somme, 1967 and Chino, 1958). Abd-El Wahed and Ahmad (2019) found that the total AA concentration in *S. gregaria* were higher in nymphs than adults, essential, semi-essential and nonessential were (16.27 and 15.40), (4.95 and 4.60), and (27.01 and 25.17) mg/100g, respectively. Dang and Doharey (1968) studied the AA compositions variations in the larval and pupal stage of *Chilo zonellus* (Swinhoe) (Lepidoptera: Crambidae) (maize borer) and have shown that free Pro, Tyr and Asp acid were absent in the larvae, but appeared in the pupae. PAL *et al*. (1973) reported that AA fluctuating between accession and detraction during metamorphosis in *E. fraterna* . These are partially agreement with this study whereas AA fluctuating

between increasing and decreasing during developmental stages of *S. gregaria* except decreasing for Met and Cys of first nympal stages. The sulfur containing amino acids Met and Cys play important roles in cell metabolism for instance, Met initiates the protein synthesis in all eukaryotic cells (Brosnan and Brosnan, 2006). Protein synthesis is believed to be initiated with the AA Met because the Aug translation initiation codon of mRNAs is recognized by the anticodon of initiator Met transfer RNA (Sasaki and Nakashima, 2000). Furthermore, Met is a methyl and sulfur donor and an important factor for antibody response (Swain and Johri, 2000 and Bunchasak, 2009). Cys is directly involved in body sulfur transport processes, active sulfate biosynthesis, and the synthesis of taurine, coenzyme A, and Glu (Wu, 2009; Baker *et al*., 1996 and Baker, 2006). The results showed that, the increasing in Ala concentration of mature males and immature females are believed to be involved in cuticle hardness or metamorphosis this is in agreement with Pant and Agrawal (1964), who mentioned that; the variation in AA concentration may be due to the role of transport during morphing from pupae to adult. Also, AA in hemolymph plays an important role in the synthesis of cuticle constituents and silk production. The decrease in Cys concentration during morphing from 1^{st} to 4^{th} instar means it could be used in developing embryo in the egg stage (Fyhn and Serigstad, 1987). Results represented in Table 2 and 3 showed increasing in Leu value of mature males and immature females of essential group. Also observed increasing in Arg concentration of 1^{st} and 4^{th} nymphal instar of semi-essential group. Whereas in non-essential group found decreasing in Ser concentration of mature males and immature females. The fact that Leu and Arg increased during different developmental stages was related to its proposed function in the metabolic process in the healthy nymph or to its accumulation as guanidine derivative (Pant and Agrawal, 1964). It was observed that the decrease of Ser value of mature males and immature females is partially in agreement with Blum (1985), who reported that; the use of AA for osmoregulation may at times be passive process in which all AA increase or decrease proportionally, but the change in specific AA titers in some insects indicates active regulated processes. Amino acids have long been recognized to differ in its composition according to studded insect tissue so it could be used as a taxonomic tool (Sankar and Yogamoorthi, 2012 and Schaefer and Wallace, 1967). Besides, it also differed according to the studied insect stage and host plant (Haag and Sullivan, 1984 and Pal *et al*., 1973), detecting method as well as insect and the insect stage (Kleiner and Peacock, 1971; Ahmed, 2020 and Abd-El Wahed and Ahmad, 2019).

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References

Abd-El Wahed, S. M.N. and Ahmad, A. F. (2019): Variations in chemical composition value of adults and nymphs desert locust, *Schistocerca gregaria* Forsskål (Orthoptera: Acrididae). Journal of Plant Protection and Pathology, Mansoura Univ., 10(12): 677-681.

Ahmed, D. A. (2020): Free amino acids composition and its changes at developmental stages of pink

bollworm, *Pectinophora gossypiella*. Egypt. J. Agric. Res., 98 (1): 1-10.

Ahmed, D. A.; El-Sharkawy, M. A.A. and ELShennawy, R. M. (2019): Role of amino acids in protecting *Pectinophora gossypiella* (Saund.) against strong protein-denaturing activity of phenolic compounds in cotton plants. Egypt, J. Agric. Res., 97 (2): 579-585.

AOAC (2012): Official method of analysis AOAC 19th international No. 994.12 chapter 4: 18-19 Edition.

Baker, D.H. (2006): Comparative species utilization and toxicity of sulfur amino acids. J. Nutr. , 136: 1670–1675.

Baker, D.H.; Fernandez, S.R. and Webel, D.M. (1996): Parsons C.M. Sulfur amino acid requirement and cystine replacement value of broiler chicks during the period three to six weeks posthatching. Poult. Sci., 75: 737–742.

Blum, M.S. (1985): Fundamentals of insect physiology. John Wiley and sons. Inc. New York.

Brosnan, J.T. and Brosnan, M.E. (2006): The sulfur-containing amino acids: An overview. J. Nutr.136: 1636S–1640S.

Bunchasak, C. (2009): Role of dietary methionine in poultry production. J. Poult. Sci., 46:169–179.

Chapman, R. F. (2002): The Insects: Structure and Function. 4th Edition, Cambridge University Press, United Kingdom, pp 69-108.

Chino, H. (1958): Carbohydrate metabolism in the diapause egg of the silkworm *Bombyx mori*. II. Conversion of glycogen into sorbitol and glycerol during diapause. J. Insect Physiol., 2: 1–12.

Dang, K. and Doharey, K.L. (1968): Variations in free amino acids in the larvae and pupae of *Chilo zonellus*. Indian J. Ent., 30: 246.

El-Shennawy R. M.; Ahmed, D. A. and El-Sharkawy, M. A.A. (2019): Comparative studies on the free amino acid composition in the total body homogenate of some lepidopterous and neuropterous insects. J. Plant Prot. and Path., Mansoura Univ., 10 (8): 391 – 395.

Ewelina, Z.; Barbara, B.; Monika, K.; Kamila, R. and Anna, J. (2015): Selected species of edible insects as a source of nutrient composition. Food Research International FRIN-06011; No of Pages 7

FAO (2012): Desert Locust threat in the Sahel. p. 4–19. Available: http://www.fao.org/ag/ locusts/common/ecg/2002/en/121 005SahelDLthreatIDMpackageE. pdf.Accessed 2014 Dec 7.

Fyhn, H.J. and Serigstad, B. (1987): Free amino acids as energy substrate in developing eggs and larvae of the cod *Gadus morhua.* Marine Biology, 96: 335-341.

Haag, K.H. and Sullivan, D.M. (1984): Free amino acid concentrations in plasma of larval and adult hydropsychidae (Trichoptera). Insect Biochemistry, 14 (3): 353-358.

Kleiner, G.F. and Peacock, J.W. (1971): Amino acids in the haemolymph of smaller European elm bark beetle larvae, *Scolytus multistriatus* (Marsham) (Coleoptera: Scolytidae). The Ohio Journal of Science, 71 (1): 36-43.

Klowden, M.J. (2007): Physiological Systems in Insects. Second Edition, Academic Press, U.S.A.

Kulkarni, A. P. and Mehrotra, K. N. (1970): Amino acid nitrogen and

proteins in the haemolymph of adult desert locusts, *Schistocerca gregaria*. J. lnsect Physiol., 16: 2181-2199.

Mansingh, A. (1967): Changes in the free amino acids of the haemolymp of *Antheraea pernyi* during induction and termination of diapause. J. lnsect Physiol., 13 (11): 1645-1655.

Mitsuhashi, J. (1978): Free amino acids in the haemolymph of some Lepidopterous insects. Appl. Ent. Zool., 13 (4): 318-320.

Pal, R.N.; Batra, R.C. ; Munshi, S.K. ; Mehrotra, N.K. and Singh, R. (1973): Free amino-acids composition of larvae and pupae of *Euproctis fraterna* moore (Lepidoptera: Lymantriidae) reared on the leaves of different host plants. *Experientia*. 29 (1): 34–36.

Pant R. and Agrawal, H.C. (1964): Free amino acids of the haemolymph of some insects. J. Insect Physiology, 10 (3): 443-446.

Ramos-Elorduy, J.; Moreno, J. M. P.; Prado, E. E.; Perez, M. A.; Otero, J. L., and De Guevara, O. L. (1997): Nutritional value of edible insects from the state of Oaxaca, Mexico. Journal of Food Composition and Analysis, 10(2): 142–157.

Robert, M.O.; Andrena, k.; Gottel, M.S.; Jacques, B. and Micheal, J.B. (2002). Attenuation of fungal infection in thermo regulating *Locusta migratoria* is accompanied by changes in heamolymph proteins. Journal of Invertebrate pathology, 81: 19-24.

Rock, G.C. and King, K.W. (1966): Amino acid composition in hydrolysates of the red-banded leaf roller, *Argyrotaenia velutinana* (Lepidoptera: Tortricidae) during development. Annals of the Entomological Society of America, 59 (2): 273–277.

Rumpold, B. A., and Schlüter, O. K. (2013): Nutritional composition and safety aspects of edible insects. Molecular Nutrition and Food Research, 57(3): 802–823.

Sankar, R.S. and Yogamoorthi, A. (2012): Free amino acid composition in hemolymph and muscle of the ghost crab, *Ocypode platytarsis*. Pakistan Journal of Biological Sciences, 15: 490-495.

Sasaki, J. and N. Nakashima (2000). Methionine-independent initiation of translation in the capsid protein of an insect RNA virus. PNAS. 97 (4): 1512-1515.

Schaefer, C.H. and Wallace, D.R. (1967): Evaluation of larval haemolymph amino acids as a taxonomic character for Neodiprion sawflies (Hymenoptera: Diprionidae). Can. Entomol., 99: 574-578.

Somme, L. (1967): The effect of temperature and anoxia on haemolymph composition and supercooling in three overwintering insects. J. Insect Physiol., 13: 805-814.

Swain, B.K. and Johri, T.S. (2000): Effect of supplemental methionine, choline and their combinations on the performance and immune response of broilers. Br. Poult. Sci., 41:83–88.

Wu, G. (2009): Amino acids: Metabolism, functions, and nutrition. Amino Acids, 37: 1–17.

Yoneda, J.; Ayatoshi, A. and Takehana, K. (2009): Regulatory roles of amino acids in immune response. Current Rheumatology Reviews, 5 (4): 252 – 258.