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Comparative Studies on the Free Amino Acid Composition in the Total Body Homogenate of some Lepidopterous and Neuropterous Insects

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ABSTRACT

Three different insect species belonging to three families [i.e. *P. gossypiella* (PBW), *S. littoralis* (CLW) and *C. carnea* (APL)] were studied for their amino acid composition. Seventeen free amino acids (FAA) figured out in the total body homogenate of the tested insects. The essential AA were threonine (THR), valine (VAL), methionine (MET), isoleucine (ILE), leucine (LEU), phenylalanine (PHE), lysine (LYS). Semi-essential AA were histidine (HIS), arginine (ARG), cysteine (CYS), and Non-essential were aspartic acid (ASP), serine (SER), glutamic acid (GLU), glycine (GLY), alanine (ALA), tyrosine (TYR), and proline (PRO). Generally, results showed that non-essential AA were the most predominant fraction of FAA, following by essential then semi-essential AA, and the PBW had the highest average of AA (1.89, 1.32 & 2.41 for essential, semi-essential & non-essential AA, respectively), followed by APL (1.78, 1.22 & 2.34, respectively), then CLW (1.66, 1.16 & 2.17, respectively). In details, from the results it could be grouped AA in 3 groups as follows: 1- A high AA concentration (more than 3 mg/100mg). 2- A moderate AA concentration (more than 2 mg/100mg). 3- A low AA concentration (less than 2 mg/100mg).The results showed that the PBW characterized by a high AA concentration of ASP (3.19 mg/100mg) and GLU (4.24 mg/100mg), while CLW and APL had only a high AA concentration of GLU (3.98 & 5.24 mg/100mg, respectively), and a moderate concentration of ASP (2.46 & 2.90 mg/100mg, respectively). In addition, GLU had a highest concentration in APL. The obtained results indicated that glutamic acid and aspartic acid (non-essential AA) were the most predominant FAA. For moderate AA, PBW characterized by an amino acids VAL and TYR (2.28 mg/100mg; 17.26% & 2.53 mg/100mg; 13.88%, respectively). In contrast, CLW and APL had a low VAL and TYR concentration (1.73 & 1.78 mg/100mg; 14.91 & 14.25% & 1.63 & 1.87 mg/100mg; 9.96 & 10.61% for VAL & TYR, respectively). Also, ALA had a moderate concentration in PBW (2.28 mg/100mg) and CLW (2.50 mg/100mg), while had a low concentration in APL (1.83 mg/100mg). In addition, CYS had a least concentration of AA (0.55, 0.59 and 0.57 mg/100mg for PBW, CLW and APL, respectively.

INTRODUCTION

Insects are responsible for main crop losses globally. Understanding of their chemical composition of amino acids (AA) has potential practical significance. Amino acids are not only the building blocks of proteins but are also key regulators of various pathological and physiological processes (Sankar & Yogamoorthi, 2012); including immune responses (Yoneda *et al.*, 2009), isosmotic intracellular regulation (Weber & van Marrewijk, 1972), osmoregulation (Van Waarde, 1988) and neurotransmission (Anaya & D'Aniello, 2006). Also, it used as source of energy producing compounds (Van Marrewijk & Ravestein *et al*., 1974), as a taxonomic tool (Schaefer & Wallace, 1967), and for the determination of nutritional requirements (Kleiner & Peacock, 1971). Further, some insects can develop molecular strategies depended on the secretion amino acids as a neutralizer, to feed on the host plants which defended by accumulated PCs (Konno *et al.*, 2010 and Ahmed *et al*., 2014 & 2019).

In this study is a preliminary comparing study on the amino acids composition of three insects which belonging to three families. It includes the cotton bollworm, *Pectinophora gossypiella*, Sound (Lepidoptera: Gelechiidae); cotton leafworm, *Spodoptera littorallis*, Boisd (Lepidoptera: Noctuidae) and Green lacewings, *Chrysoperla carnea*, Steph (Neuroptera: Chrysopoidea).

MATERIALS AND METHODS

The present study has been carried out in the laboratory of Bollworms Department, Plant Protection Research Institute, ARC, Dokki, Giza.

Insects: three insects in 3 different families listed in Table 1 are maintained in our institute as experimental insects:

Table 1. Insects used in the present study

Common name	Scientific name	Order	Family
pink bollworm (PBW)	*Pectinophora gossypiella*	Lepidoptera	Gelichidiea
Cotton leafworm (CLW)	*Spodoptera littoralis*	Lepidoptera	Noctuidae
Aphid lion (APL)	*Chrysoperla carnea*	Neuroptera	Chrysopidae

This study was conducted in order to determine free amino acids (FAA) content in different insect families. Full-grown larvae of each insect were collected and refrigerated (at 5ºC) for few minutes and then dried in the oven overnight (at 60ºC) for FAA determination. Samples were homogenized to get homogenate dry powder. Homogenates were weighted and kept till the biochemical determinations.

Amino Acids Determination: Quantitative determination of amino acids was carried out at Regional Center for Food and Feed, ARC, Dokki, Giza, by high perform Amino Acid analyzer (Biochrom 30) and EZ chrome manual (software for data collection and processing), according to AOAC (2012).

RESULTS AND DISCUSSION

The amino acid composition of tested insects which belonging to different families (i.e. *P. gossypiella, S. littoralis and C. carnea*) are illustrated in Table (2) and Figure (1). The table exhibited that there were seventeen free amino acids (FAA) figured out in the total body homogenate of the studied insects. It illustrated that the FAA located under three groups as follows:

1. **Essential AA:** threonine (THR), valine (VAL), methionine (MET), isoleucine (ILE), leucine (LEU), phenylalanine (PHE), lysine (LYS).
2. **Semi-essential AA:** histidine (HIS), arginine (ARG), cysteine (CYS).

3. **Non-essential:** aspartic acid (ASP), serine (SER), glutamic acid (GLU), glycine (GLY), alanine (ALA), tyrosine (TYR), and proline (PRO).

Generally, results in Table (2) and Figure (2) showed that non-essential AA (insects can synthesize by themselves) were the most predominant fraction of FAA, following by essential then semi-essential AA. The percentage of average concentrations of non-essential AA were 43, 44 & 44% for PBW, CLW and APL, respectively. These percentages for essential AA were 34, 33 & 33% for PBW, CLW and APL, respectively, and for semi-essential AA were 23, 23 & 23% for PBW, CLW and APL, respectively. Sankar & Yogamoorthi (2012) mentioned that the non-essential amino acids play a more important part than the essential ones in the cellular osmotic pressure regulation.

According to the average of AA calculated in Table (2) it could be said that PBW had the highest average of AA (1.89, 1.32 & 2.41 for essential, semi-essential & non-essential AA, respectively), followed by APL (1.78, 1.22 & 2.34, respectively), then CLW (1.66, 1.16 & 2.17, respectively). Amino acids have long been recognized to differ in its composition according to studded insect (Haag & Sullivan, 1984 and Sankar & Yogamoorthi, 2012), so it could be used as a taxonomic tool (Schaefer & Wallace, 1967). In addition, it also differed according to the studied insect stage ((Haag & Sullivan, 1984 and our unpublished data on different stages of PBW); host plant (PAL *et al*., 1973) and detecting method (Kleiner & Peacock, 1971).

Table 2. Concentration of free amino acids (mg/100 mg) and its percent in the total body homogenate of full-grown larvae of *P. gossypiella* (PBW), *S. littoralis* (CLW) and *C. carnea* (APL).

Amino acids		PBW		CLW		APL	
		AA concentration	%	AA concentration	%	AA concentration	%
Essential	Threonine (THR)	1.66	12.57	1.36	11.72	1.52	12.17
	Valine (VAL)	2.28	17.26	1.73	14.91	1.78	14.25
	Methionine (MET)	1.03	7.80	0.88	7.59	1.15	9.21
	Isoleucine (ILE)	1.58	11.96	1.55	13.36	1.59	12.73
	Leucine (LEU)	2.55	19.30	2.31	19.91	2.45	19.62
	Phenylalanine (PHE)	1.82	13.78	1.52	13.10	1.44	11.53
	Lysine (LYS)	2.29	17.34	2.25	19.40	2.56	20.50
	Sum	13.21		11.60		12.49	
	Average	1.89		1.66		1.78	
Semi-essential	Histidine (HIS)	1.16	19.80	0.88	17.13	1.07	19.62
	Arginine (ARG)	2.26	38.59	2.01	39.13	2.03	37.22
	Cystine (CYS)	0.55	9.39	0.59	11.48	0.57	10.45
	Sum	5.86		5.14		5.45	
	Average	1.32		1.16		1.22	
Nonessential	Aspartic acid (ASP)	3.19	17.51	2.46	15.04	2.9·	16.46
	Serine (SER)	1.37	7.52	1.38	8.44	1.35	7.66
	Glutamic acid (GLU)	4.24	23.27	3.98	24.33	5.24	29.73
	Glycine (GLY)	1.47	8.07	1.52	9.29	1.50	8.51
	Alanine (ALA)	2.28	12.51	2.5·	15.28	1.83	10.38
	Tyrosin (TYR)	2.53	13.88	1.63	9.96	1.87	10.61
	Proline (PRO)	1.82	9.99	1.73	10.57	1.71	9.70
	Sum	18.22		16.36		17.62	
	Average	2.41		2.17		2.34	

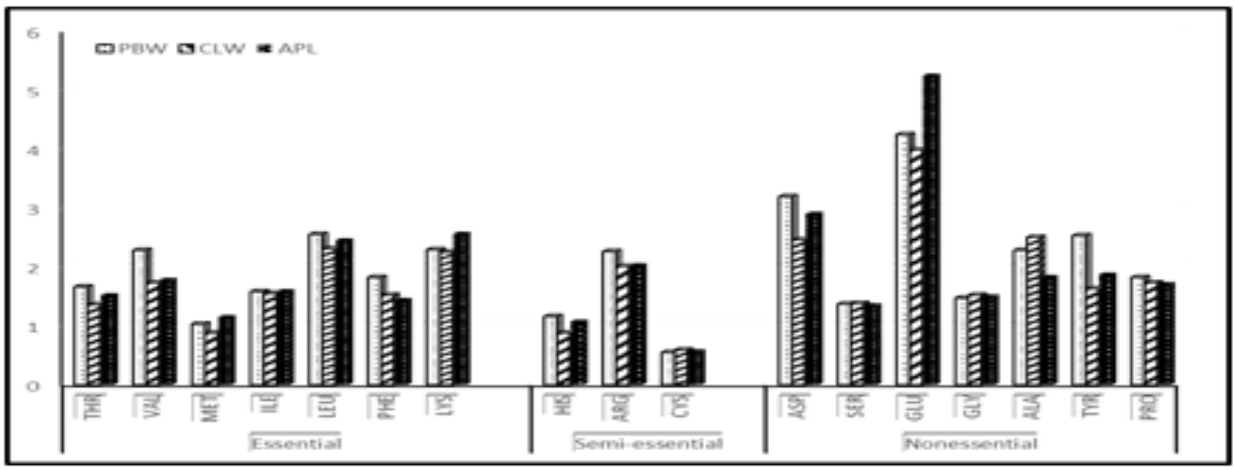

Figure 1. The composition of free amino acids in the total body homogenate of *P. gossypiella* (PBW) *S. littoralis* (CLW) *and C. carnea* (APL).

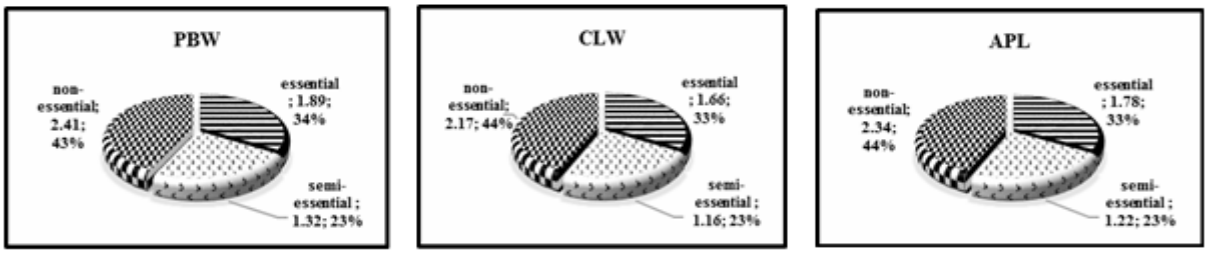

Figure 2. Average free amino acids in total body homogenate of *P. gossypiella* (PBW) *S. littoralis* (CLW) *and C. carnea* (APL).

In details, from the results in Table (2) it could be grouped AA in 3 groups (summarized in Table 3) as follows:

1. A high AA concentration (more than 3 mg/100mg).
2. A moderate AA concentration (more than 2 mg/100mg).
3. A low AA concentration (less than 2 mg/100mg).

The results summarized in Table (3) showed that some of AA were found at about the similar quantity in the examined species. However, some AA fluctuated considerably among examined species. These fluctuations may be taken as the characteristic of the species. From the Table it could be noted that PBW characterized by a high AA concentration of ASP (3.19 mg/100mg) and GLU (4.24 mg/100mg), while CLW and APL had only a high AA concentration of GLU (3.98 & 5.24 mg/100mg, respectively), and a moderate concentration of ASP (2.46 & 2.90 mg/100mg, respectively). In addition, GLU had a highest concentration in APL. The obtained results indicated that glutamic acid and aspartic acid (non-essential AA) were the most predominant FAA. In un published data we found that ASP increased from stage to other of PBW, while Dang & Doharey (1968) mentioned that ASP were absent in the larvae of *Chilo zonellus*, but appeared in the pupae. Thompson (1974) mentioned that the amino acid compositions of the total proteins of *E. roborator* were quantitatively dominated by glutamic acid, aspartic acid, and amides. Duchaterau & Florkin (1958) analysed AA in the haemolymph of 29 different species of Lepidoptera and found that particularly ARG, GLU, HIS, LYS and PRO were generally high in Lepidoptera. The highest concentration of GLU confirms its central role in AA metabolism (Wheatly, 1985). Also, Leaf & Usherwood (1973) proposed that high concentrations of glutamate had a role in transmission action of this AA at the neuromuscular junction. It activates the excitatory synapses and quickly desensitize the receptors on the post-synaptic membrane to the action of the transmitter released from the motor nerve terminals. In un published data we found that despite of AA increased when PBW morph from stage to other, some AA decreased like GLU.

Table 3. Summarized data for the concentration of free amino acids (mg/100 mg) in the total body homogenate of full-grown larvae of *P. gossypiella* (PBW), *S. littoralis* (CLW) and *C. carnea* (APL).

AA		PBW	CLW	APL
>3	essential	——	——	——
	Semi- essential	——	——	——
	Non- essential	ASP (3.19)	——	——
		GLU (4.24)	GLU (3.98)	GLU (5.24)
>2	essential	VAL (2.28)	——	——
		LEU (2.55)	LEU (2.31)	LEU (2.45)
		LYS (2.29)	LYS (2.25)	LYS (2.56)
	Semi- essential	ARG (2.26)	ARG (2.01)	ARG (2.03)
	Non- essential	——	ASP (2.46)	ASP (2.90)
		ALA (2.28)	ALA (2.50)	——
		TYR (2.53)	——	——
<2	essential	THR (1.66)	THR (1.36)	THR (1.52)
		——	VAL (1.73)	VAL (1.78)
		MET (1.03)	MET (0.88)	MET (1.15)
		ILE (1.58)	ILE (1.55)	ILE (1.59)
		PHE (1.82)	PHE (1.52)	PHE (1.44)
	Semi- essential	HIS (1.16)	HIS (0.88)	HIS (1.07)
		CYS (0.55)	CYS (0.59)	CYS (0.57)
	Non- essential	SER (1.37)	SER (1.38)	SER (1.35)
		GLY (1.47)	GLY (1.52)	GLY (1.50)
		——	——	ALA (1.83)
		——	TYR (1.63)	TYR (1.87)
		PRO (1.73)	PRO (1.82)	PRO (1.71)

Mansingh (1967) found that the levels of glutamine concentrations (can be synthesized from glutamic acid) increased in the pupal stage before decrease during adult development, while glutamic acid decreased at the larval-pupal transformation before rising during adult development. He stated that the variation in the concentration may be due to the role of this AA in nitrogen transport during morphing from pupae to adult. For moderate AA (more than 2 mg/100mg), PBW characterized by an amino acids VAL and TYR (2.28 mg/100mg; 17.26% & 2.53 mg/100mg; 13.88%, respectively). In contrast, CLW and APL had a low VAL

and TYR concentration (1.73 & 1.78 mg/100mg; 14.91 & 14.25% & 1.63 & 1.87 mg/100mg; 9.96 & 10.61% for VAL & TYR, respectively). Also, ALA had a moderate concentration in PBW (2.28 mg/100mg) and CLW (2.50 mg/100mg), while had a low concentration in APL (1.83 mg/100mg).Haemolymph amino acids play an important role in the synthesis of cuticle constituents and in silk production (Pant & Argawal, 1964 and Wyaat, 1961).

Proline and alanine are believed to be involved in cold hardness. All amino acids found are likely to contribute as a general pool for the synthesis of new proteins and for the products of protein breakdown and may also be involved in osmoregulation and buffering (Pant & Argawal, 1964). CYS, a semi-essential AA, had a least concentration of AA (0.55 mg/100mg, 9.39%; 0.59 mg/100mg, 11.48% and 0.57 mg/100mg, 10.45% for PBW, CLW and APL, respectively. CYS decreased when PBW morphed from egg to larvae, then increased when morphed from larvae to pupae but still under the value recorded in egg (un published data). The decreasing in CYS concentration during morphing from egg to larvae means it could be used in developing embryo in the egg stage (Fyhn & Serigstad, 1987), or assist as significant sources of substrate for the Krebs cycle as in the case of proline and glutamate particularly (Winteringham & Harrison, 1956 and Winteringham, 1958). Several researchers have reviewed the FAA composition for many insect species (Boctor, 1981; Duchateau & Florkin, 1958 and He & Zhang, 2017). Pant & Agrawal (1964) found that the haemolymph of *Attacus ricini* characteristic by presence of α-aminoisobutyric acid, homoarginine, hydroxyproline and more than one guanidine derivative besides arginine. He attributed that to the existence of a specific guanidine metabolism in this insect. Boctor (1981) where the results showed that asparagine (Asparagine can be synthesized from aspartic acid), glutamine (can be synthesized from glutamic acid), cystine, ornithine, histidine and valine were the prevailing FAA for all stages of *Heliothis armigera.* According to the Duchateau & Florkin (1958), concentrations of some AA particularly arginine, glutamic acid, histidine, lysine and proline were generally high in Lepidoptera. By no means generalization, Bursell (1980) mentioned that the only characteristic which highlight as a general characteristic of insect hemolymph is the prevalence of proline and/ or of glutamate and its amide glutamine. These in partially agreement with the obtained results whereas it stated the highly relevance of GLU (non-essential AA). But we can't generalize that where AA differ in its composition according to studded insect tissue (Sankar & Yogamoorthi, 2012); insect and the insect stage (Haag & Sullivan, 1984 and our un published data on PBW); host plant (PAL *et al*., 1973) and method used for detecting AA (Kleiner & Peacock, 1971).

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دراسة لمقارنة محتوى الأحماض الأمينية لبعض حشرات رتبتي حرشفية وشبكية الأجنحة

رانيا الشناوي ، دينا أحمد و منال الشرقاوى

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تم اختيار ثلاث حشرات منتمية لثلاث عائلات مختلفة وهي : دودة اللوز القرنفلية، دودة ورق القطن وأسد المن وذلك لدراسة ومقارنة محتواها من الأحماض الأمينية ، وقد ثبت وجود سبعة عشر حمضا أمينيا في الحشرات الثلاث محل الدراسة ، وتقع هذه الحماض تحت ثلاث مجموعات رئيسية هي :١- أحماض أمينية ضرورية : ثريونين ، فالين، مثيونين ، أيزوليوسين ، ليوسين ، فينايل الأنين ، ليسين. ٢- أحماض أمينية شبه ضرورية : هستدين ، أرجنين ، سيستين. ٣- أحماض أمينية غير ضرورية : أسبارتيك أسيد ، جلوتاميك أسيد ، جليسين ، ألانين ، تايروزين ، برولين. وقد أثبتت الدراسة بصورة عامة أن النسبة الأكبر كانت للأحماض الأمينية غير الضرورية ، يليها الأحماض الأمينية الضرورية ، فالشبه ضرورية؛ كما ثبت أن دودة اللوز القرنفلية كانت أعلى الحشرات من حيث متوسط محتوى الأحماض الأمينية (١.٨٩، ١.٣٣٢ و ٢.٤١ لكل من الأحماض الضرورية، الشبه والضرورية، على التوالي) ، يليها أسد المن (١.٧٨، ١.٢٢ و ٢.٣٤، على التوالي) ، وأخيرا دودة ورق القطن (١.٦٦، ١.١٦ و ٢.١٧، على التوالي). وبشيء من التفصيل فقد أمكن تقسيم الأحماض وفقا للتركيز لثلاث مجموعات كالتالي: ١- الأحماض الأمينية ذات التركيز العالي (التي تجاوز تركيزها ٣ مجم/ ١٠٠ مجم). ٢- الأحماض الأمينية متوسطة التركيز (التي تجاوز تركيزها ٢ مجم / ١٠٠ مجم). ٣- الأحماض الأمينية منخفضة التركيز (ذات التركيز الأقل من ٢ مجم / ١٠٠ مجم). ومن خلال هذه النتائج فقد وجد أن دودة اللوز القرنفلية تتميز بمحتوى عالي من الأحماض الأمينية الأسبارتيك (٣.١٩ مجم / ١٠٠ مجم)، والجلوتاميك (٤.٢٤ مجم / ١٠٠ مجم)، في حين امتلك كل من دودة ورق القطن وأسد المن محتوى مرتفع من الجلوتاميك (٣.٩٨ و ٥.٢٤ مجم / ١٠٠ مجم، على التوالي)، ومتوسط من الأسبارتيك (٢.٤٦ و ٢. ٩٠ مجم / ١٠٠ مجم، على التوالي)، ومن الملاحظ أن أسد المن كان أعلى الثلاث حشرات من حيث محتوى حمض الجلوتاميك. ومن جهة أخرى، فبالنسبة للأحماض الأمينية متوسطة التركيز فقد تميزت القرنفلية بوجود الفالين والتايروزين بتركيز (٢.٢٨ و ٢.٥٣ مجم / ١٠٠ مجم، على التوالي) ، وعلى العكس من ذلك فقد كان تركيز الأحماض منخفضا في دودة ورق القطن وأسد المن (١.٧٣ و ١.٧٨ مجم / ١٠٠ مجم للفالين؛ ١.٦٣ و ١.٨٧ مجم / ١٠٠ مجم للتايروزين، في دودة ورق القطن وأسد المن، على التوالي). كما تواجد الألانين بتركيز متوسط في دودة اللوز القرنفلية (٢.٢٨ مجم / ١٠٠ مجم) ودودة ورق القطن (٢.٥٠ مجم / ١٠٠ مجم) ؛ في حين تواجد بتركيز منخفض في أسد المن (١.٨٣ مجم / ١٠٠ مجم). أما أقل الأحماض الأمينية تواجدا فكان السيستين حيث كان تركيزه ٠.٥٥، ٠.٥٩ و ٠.٥٧ مجم /١٠٠ مجم في كل من دودة اللوز القرنفلية، دودة ورق القطن وأسد المن على التوالي.