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Free amino acids composition and its changes at developmental stages of pink bollworm, *Pectinophora gossypiella*

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Abstract

Free amino acid has an important role in physiological functions. Pink bollworm (PBW), *Pectinophora gossypiella*, was investigated for the free amino acid (FAAs) composition at immature stages (*i.e.,* egg, larvae and pupae). Seventeen AAs were distinguished in the hole body of the different immature PBW stages. Essential AAs of tested stages of PBW consisted of threonine (THR), valine (VAL), methionine (MET), isoleucine (ILE), leucine (LEU), phenylalanine (PHE), lysine (LYS). Semi-essential AAs were histidine (HIS), arginine (ARG), cysteine (CYS), while non-essential AAs were aspartic acid (ASP), serine (SER), glutamic acid (GLU), glycine (GLY), alanine (ALA), tyrosine (TYR), and proline (PRO).

The most prevailing FAAs, which exceeded 2 mg/100 mg, were LEU, LYS, ARG, ASP, GLU, ALA and TYR in all stages. Another AAs joined this group: VAL in larval stage, whereas its concentration was 2.28 mg/100 mg and exceeded that in egg by 16.92% and PHE & PRO in pupal stage, where it increased to 2.19 and 2.07 mg/100 mg, respectively. Also, in other words GLU was the most abundant FAAs in the egg stage (4.46%), while ASP and GLU were the most abundant FAAs in the larval and pupal stages 3.19 & 3.35% for ASP and 4.24 and 4.14% for GLU, respectively. On the other hand, CYS is the lowest abundant one recorded 0.68, 0.55 and 0.64% for egg, larvae and pupae, respectively).

Furthermore, no qualitative changes in the FAAs composition of different immature stages of PBW, but there were quantitative changes. All FAAs increased from stage to other except for MET, CYS, SER, GLU and GLY, and the decreasing continued until pupal stage in the case of GLU, where it recorded 4.46, 4.24 and 4.14 mg/100mg in egg, larval and pupal stage, respectively. FAAs concentrations in egg were 1.21, 0.68, 1.78 and 1.82 mg/100mg for MET, CYS, SER, and GLY, respectively. These values decreased when PBW morphing from egg to larvae by 14.88, 19.12, 23.03 and 19.23%, respectively. After that, the change in FAAs concentration switch to increasing but still low from that recorded in egg in the case of CYS, SER & GLY represented 0.64, 1.52 & 1.50 mg/100mg, respectively. While, in MET the increasing in FAAs concentration when PBW morphing from egg to larvae continues until pupal stage and exceeded the value recorded in egg by 5.79% and 24.27% in larvae.

Key words: *Pectinophora gossypiella*, Pink bollworm, and free amino acid.

INTRODUCTION

Amino acids are vital component for insects. It occurs in the hemolymph and tissues in high concentration as free amino acids (FAAs) (**Sankar & Yogamoorthi, 2012**). FAAs play an important role in physiological functions: osmoregulation **(Beadle & Shaw, 1950)**; energy production for flight and cocoon construction **(Beadle & Shaw, 1950);** protein synthesis **(Buck, 1953)** and accelerating molting in insects **(Pandey e*t al.,* 1986)**. Also, it has been established as a biochemical characteristic of insect classes and taxonomic tool (**Schoffeniels & Gilles, 1970**) and for the nutritional requirements determination (**Kastings & McGinnis, 1962**).

Furthermore, the concentration of some of these FAAs change during metamorphosis (**Mansingh, 1967)**.

The present investigation was undertaken to study the FAAs composition of egg, larvae and pupae of pink bollworm (PBW), *Pectinophora gossypiella* (Lepidoptera: Gelichidiea), and it could give a sight about the physiology of FAAs in PBW.

MATERIALS AND METHODS

Pectinophora gossypiella were reared on a modified artificial diet according to **Abd El-Hafez *et al.* (1982)** at the laboratory of Bollworms Department, Plant Protection Research Institute, ARC, Dokki, Giza.

This study was conducted in order to determine free amino acids (FAAs) content in different stages of PBW (*i.e.* egg, larvae and pupae). Insect in different stages were collected and refrigerated (at 5ºC) for few minutes and then dried in the oven overnight (at 60ºC) for FAAs 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

1. Free amino acid content of egg, larvae, and pupae of *P. gossypiella*

The amino acid composition of different stages of PBW (egg, larvae, and pupae) are listed in Table (1). The table showed that there were seventeen free amino acids (FAAs) detected in the total body homogenate of the studied stages. As shown in Table (1), essential AAs of tested stages of PBW consisted of threonine (THR), valine (VAL), methionine (MET), isoleucine (ILE), leucine (LEU), phenylalanine (PHE), lysine (LYS). Semi-essential AAs were histidine (HIS), arginine (ARG), cysteine (CYS), while non-essential AAs were aspartic acid (ASP), serine (SER), glutamic acid (GLU), glycine (GLY), alanine (ALA), tyrosine (TYR), and proline (PRO).

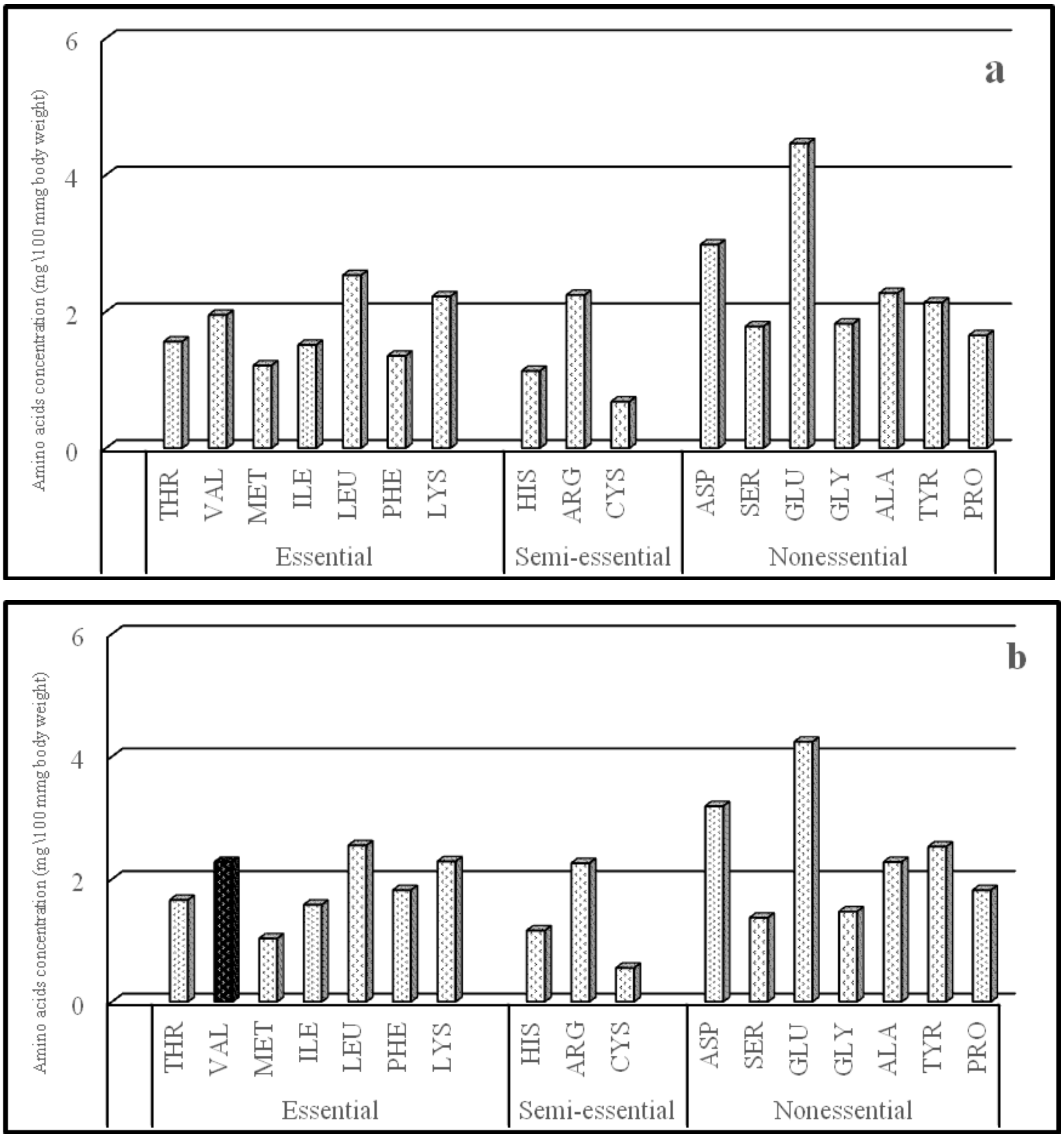
Also, the table results that LEU, LYS (essential AAs); ARG (semi-essential AAs); ASP, GLU, ALA and TYR (non-essential AAs) were the most prevailing FAAs (more than 2 mg/100 mg) in all tested stages (Figure 1). Another AAs joined this group: VAL in larval stage (black column, Fig 1b), whereas its concentration was 2.28 mg/100 mg and exceeded that in egg by 16.92% and PHE & PRO in pupal stage (black column, Fig 1c), where it increased to 2.19 and 2.07 mg/100 mg, respectively. Also, in other words GLU was the most abundant FAAs in the egg stage (4.46%), while ASP and GLU were the most abundant FAAs in the larval and pupal stage presented 3.19 & 3.35% for ASP and 4.24 and 4.14% for GLU, respectively. On the other hand, CYS is the lowest abundant one recorded 0.68, 0.55 and 0.64% for egg, larvae and pupae, respectively. In this respect, according our unpublished data, it is interesting to note that these findings extended to some different insect species; i.e. *Spodoptera littoralis* (Lepidoptera: Noctuidae) and *Chrysoperla carnea* (Neuroptera: Chrysopidae).

Free amino acids composition for many species have been reviewed (**Duchateau & Florkin, 1958; and He & Zhang, 2017)**. Amino acids have long been recognized to have a high concentration in insects, but differ in its composition according to studied insect tissue (**Sankar & Yogamoorthi, 2012)**; insect and the insect stage (**Haag & Sullivan, 1984** and our un published data on *P. gossypiella, S. littoralis* and *C. carnea* PBW); host plant (**PAL *et al.*, 1973)** and method used for detecting AAs **(Kleiner & Peacock, 1971)**.

Table (1): Concentration of free amino acids (mg/100mg) and % change in the total body homogenate of eggs, full gown larvae and pupae of *P. gossypiella*.

Amino acids		Egg	Larvae		Pupae		
		AAs concentration	AAs concentration	% change from egg	AAs concentration	% change from egg	% change from larvae
Essential	Threonine (THR)	1.56	1.66	**6.41**	1.87	**19.87**	**12.65**
	Valine (VAL)	1.95	2.28	**16.92**	2.44	**25.13**	**7.02**
	Methionine (MET)	1.21	1.03	**-14.88**	1.28	**5.79**	**24.27**
	Isoleucine (ILE)	1.51	1.58	**4.64**	1.76	**16.56**	**11.39**
	Leucine (LEU)	2.53	2.55	**0.79**	3.04	**20.16**	**19.22**
	Phenylalanine (PHE)	1.35	1.82	**34.81**	2.19	**62.22**	**20.33**
	Lysine (LYS)	2.22	2.29	**3.15**	2.60	**17.12**	**13.54**
Semi-essential	Histidine (HIS)	1.13	1.16	**2.65**	1.57	**38.94**	**35.34**
	Arginine (ARG)	2.24	2.26	**0.89**	2.65	**18.30**	**17.26**
	Cystine (CYS)	0.68	0.55	**-19.12**	0.64	**-5.88**	**16.36**
Non-essential	Aspartic acid (ASP)	2.98	3.19	**7.05**	3.35	**12.42**	**5.02**
	Serine (SER)	1.78	1.37	**-23.03**	1.52	**-14.61**	**10.95**
	Glutamic acid (GLU)	4.46	4.24	**-4.93**	4.14	**-7.17**	**-2.36**
	Glycine (GLY)	1.82	1.47	**-19.23**	1.50	**-17.58**	**2.04**
	Alanine (ALA)	2.27	2.28	**0.44**	2.71	**19.38**	**18.86**
	Tyrosin (TYR)	2.13	2.53	**18.78**	2.84	**33.33**	**12.25**
	Proline (PRO)	1.65	1.82	**10.30**	2.07	**25.45**	**13.74**

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


a
Amino acids concentration (mg \100 mmg body weight)
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THR
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PRO
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Amino acids concentration (mg \100 mmg body weight)
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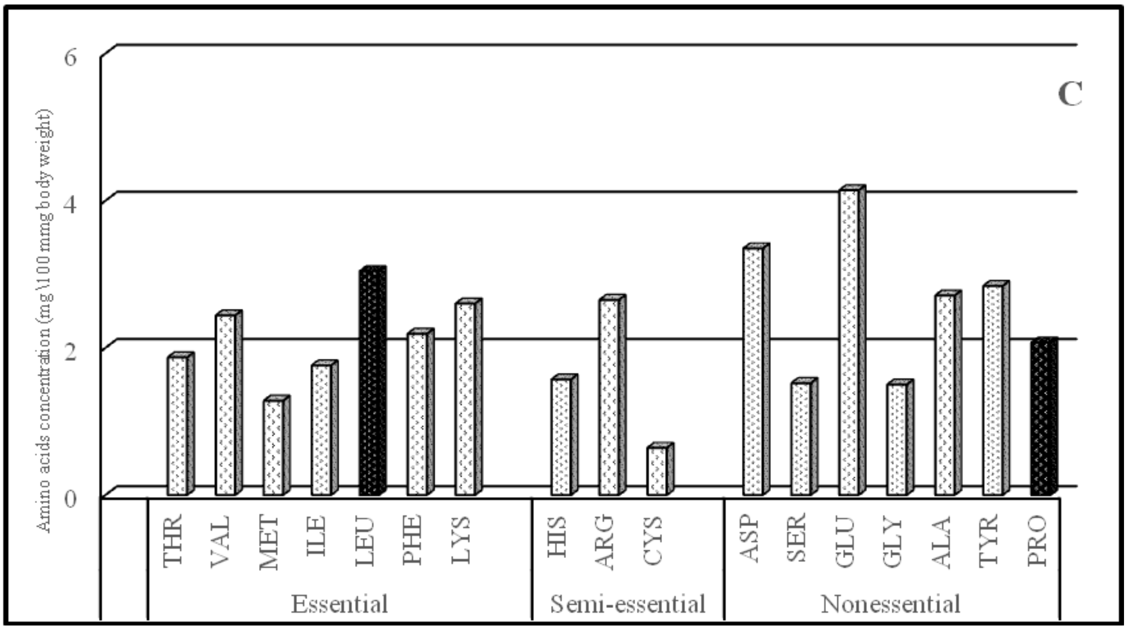


Figure (1): The composition of free amino acids in the total body homogenate of *P. gossypiella*: (a) egg, (b) 4th instar larvae, (c) pupae.

The obtained results also indicated that glutamic acid and aspartic acid were the most predominant FAAs. These data agree to some extent with those obtained for all stages of *Heliothis armigera* (**Boctor, 1981)** where the results showed that asparagine (can be synthesized from aspartic acid), glutamine (can be synthesized from glutamic acid), cystine, ornithine, histidine and valine were the prevailing FAAs. **Duchateau & Florkin (1958)** analyzed amino acids in the heamolymph of 29 different species of Lepidoptera. According to these authors the concentrations of some AAs particularly arginine, glutamic acid, histidine, lysine and proline were generally high in Lepidoptera. Also**, Bursell (1980)** mentioned that, by no means generalization, the only characteristic that highlight as a general feature of insect hemolymph is the prevalence of proline and/or of glutamate and its amide glutamine. So, the results suggested that GLU and ASP had an important role in protein composition. The highest concentration of GLU may be due to its central role in AAs metabolism (**Wheatly, 1985**), transmission action of this AAs at the neuromuscular junction (**Leaf & Usherwood, 1973)** and its role in nitrogen transport during morphing (**Mansingh, 1967)** or in Krebs cycle (**Winteringham, 1958)**.

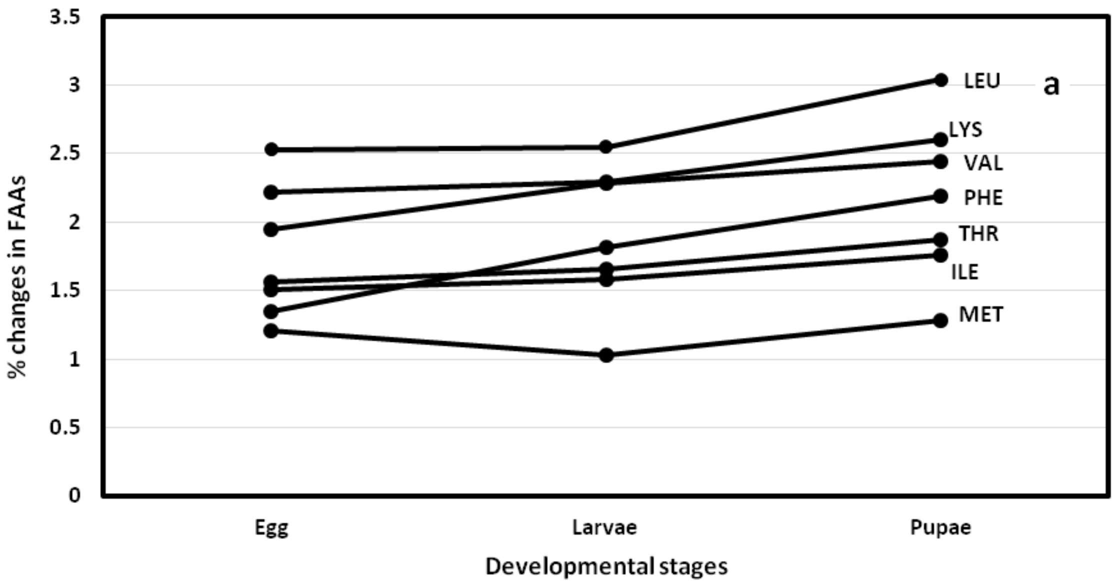
2. **Changes in the free amino acids content in the total body homogenate of different stage of *P. gossypiella*.**

There were no qualitative changes in the FAAs composition in PBW different stages, but there were quantitative changes (Figure 2). All FAAs increased from stage to other except for MET (essential AAs; dotted line Fig 2a); CYS (semi-essential AAs; dotted line Fig 2b); SER, GLU and GLY (non-essential AAs; dotted line Fig 2c). This decreasing continued until pupal stage in the case of GLU, where it recorded 4.46, 4.24 and 4.14 mg/100mg in egg, larval and pupal stage, respectively. **Mansingh** (**1967)** found that GLU during morphing from larvae to pupae, then increasing during development of adult. He stated that may be due to the role of this AAs in nitrogen transport during morphing from pupae to adult.

FAAs concentrations in egg were 1.21, 0.68, 1.78 and 1.82 mg/100mg for MET, CYS (the smallest abundant AAs), SER and GLY, respectively. These values decreased when PBW morphing from egg to larvae by 14.88, 19.12, 23.03 and 19.23%, respectively. Decreasing in AAs when insect morphing from egg to larvae means that these AAs could be used in developing embryo during the egg stage (**Fyhn & Serigstad, 1987)**.

After the decreasing these AAs during morphing from egg to larvae, the change in FAAs concentration switch to increasing but still low from that recorded in egg in the case of CYS, SER & GLY (0.64, 1.52 & 1.50 mg/100mg, respectively. While, in MET the increasing continues until pupal stage and exceeded the value recorded in egg by 5.79% and that recorded in larvae by 24.27%. The AAs containing sulfur (MET & CYS) play vital roles in the cell metabolism; i.e., MET initiates the eukaryotic cells protein synthesis (**Brosnan & Brosnan, 2006**). Additionally, MET is a donor of methyl and sulfur, and a significant factor for antibody response (**Bunchasak, 2009**). CYS is involved directly in processes of sulfur transport, biosynthesis of active sulfate, and taurine, coenzyme A, and glutathione synthesis (**Wu, 2009**).

Several studies have shown that the concentration of some FAAs change during metamorphosis. **Dang & Doharey (1968)** studied the amino acid compositions variations in the larval and pupal stage of *Chilo zonellus* and they found that PRO, TYR and ASP were indeterminate in larvae, but appeared in the pupae. Also, **Pal *et al.* (1973)** mentioned that, in general, AAs increased from larval to pupal stage, but some AAs decreased.



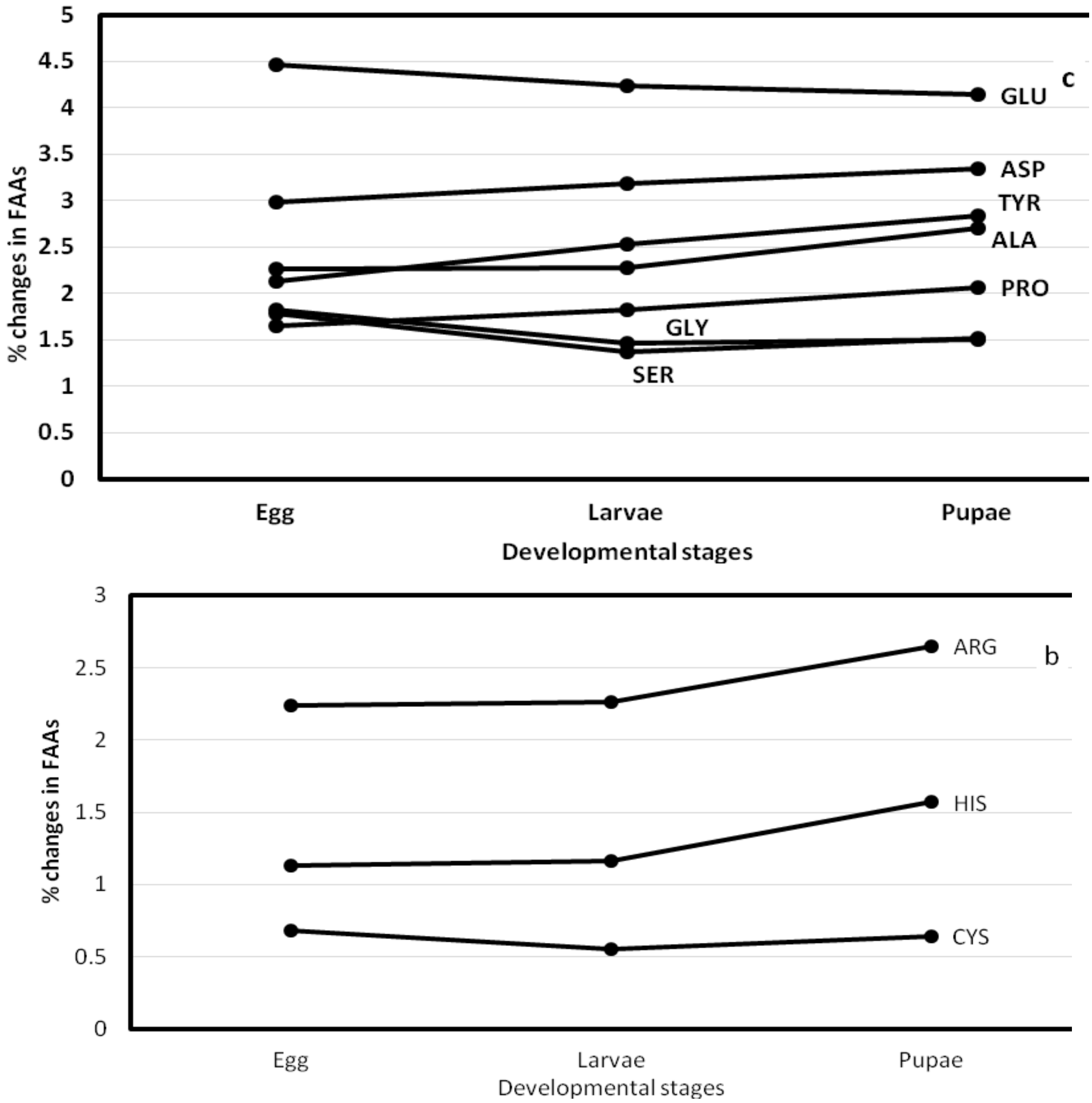


Figure (2): Changes in the content of free amino acids in the total body homogenate of different stage of *P. gossypiella*: (a) essential AAs, (b) semi-essential AAs, (c) non-essential AAs.

These are in agreement with our results whereas FAAs increased when PBW morph from stage to other except for MET, CYS, SER, GLU and GLY, and thy also mentioned that AAs generally had the highest concentration in the pupal stage, this could be because of increased proteins breakdown. The variations in the pattern of AAs at various insect developmental stages revealed a shift of amino acid requirements during growth and development (**Rock & King, 1966).** Additionally, changes in other substances contents during metamorphosis, like carbohydrates and its metabolic intermediates, can also affect AAs levels (**Somme, 1967)**.

Since very little is known about the physiology PBW, the current studies were done to investigate the changes in FAAs composition in egg, larvae, and pupae of PBW. Investigations on the PBW could contributed significantly to the present knowledge of insect physiology and genetics. Also, it has been established as a biochemical characteristic of insect classification.

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محتوى الأحماض الأمينية والتغيرات الحادثة فيها أثناء نمو الأطوار المختلفة لدودة اللوز القرنفلية

دينا أحمد

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للأحماض الأمينية العديد من الوظائف الفسيولوجية، لذا تم دراسة محتوى الأحماض الأمينية في أطوار دودة اللوز القرنفلية (البيض، اليرقات والعذارى)، وقد تم التعرف على وجود 17 حامض أميني، منها ما هو ضروري مثل الثريونين، الفالين، المثيونين، الأيزوليوسين، الليوسين، الفينايل الأنين، الليسن؛ ومنها ما هو شبه ضروري كالهيستدين، الأرجنين والسيستين؛ ومنها غير الضروري كالأسبارتيك، السيرين، الجلوتاميك، الجليسين، الألانين، التايروزين والبرولين.

وقد وجد أن أكثر الأحماض الأمينية تركيزا في كل الأطوار (تلك التي تجاوزت 2 مجم/ 100مجم) كانت الليوسين، الليسين، الأرجنين، اسبارتيك، الألانين والتايروزين. ينضم لهذه المجموعة الفالين في الطور اليرقي حيث يصل تركيزه لـ 2.28 مجم / 100 مجم بنسبة زيادة تتجاوز القيمة المسجلة في البيض بمقدار 16.92%؛ الفينايل الأنين والبرولين في الطور اليرقي حيث سجلا 3.19 و4.24 مجم / 100 مجم بنسبة زيادة 3.35% و4.14% لكل من الفينايل الأنين والبرولين، على التوالي؛ في حين كان السيستين هو الحامض الأميني الأقل تركيزا في الأطوار الثلاث موضع الدراسة حيث سجل 0.67، 0.55 و0.64 مجم /100 مجم في كل من البيض، اليرقات والعذارى على التوالي.

وبالإضافة لذلك لم يحدث تغير نوعي في محتوى الأحماض الأمينية في الأطوار المختلفة في حين لوحظت بعض التغيرات الكمية، فقد لوحظ ارتفاع محتوى الأحماض الأمينية بتطور الحشرة فيما عدا في حالة المثيونين، السيستين، السيرين، الجلوتاميك والجليسين، وقد استمر هذا التناقص حتى طور العذراء في حالة الجلوتاميك حيث كانت القيم المسجلة له كالأتي: 4.46، 4.24 و4.14 مجم / 100 مجم في حالة البيض، اليرقات والعذارى على التوالي.

كذلك كانت القيم المسجلة في طور البيضة للأحماض ميثيونين، سيستين، سيرين، والجليسين كانت كالتالي: 1.21، 0.68، 1.78 و 1.82 مجم / 100 مجم، على التوالي، وهذه القيم يحدث لها تناقص عند تحول الحشرة من طور البيضة لليرقة بمقدار 14.88، 19.12، 23.03 و19.23%، على التوالي؛ ثم يلي ذلك حدوث زيادة في التركيز عند التحول للعذراء، وإن كانت القيمة المسجلة ما زالت أقل من تلك المسجلة في طور البيضة، وكانت هذا القيم كالتالي: 0.64، 1.52، 1.50 مجم / 100 مجم لكل من السيستين، السرين و الجليسين، على التوالي، بينما في حالة الميثيونين فإن الزيادة في التركيز تستمر حتى عند التحول للعذراء بقيمة تتجاوز تلك المسجلة في طور البيضة بـ 5.79%، وتلك المسجلة في طور اليرقة بمقدار 24.27%.