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Original article (Orijinal araştırma)

Histochemical and ultrastructural analysis of macromolecules in trophocytes of the Oriental cockroach, *Blatta orientalis* (L., 1758) (Blattodea: Blattidae)[1]

Doğu hamam böceği *Blatta orientalis* (L., 1758) (Blattodea: Blattidae)'in trofositlerindeki makromoleküllerin histokimyasal ve ince yapı analizi

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Abstract

The fat body is a major storage area for glycogen, lipid and protein. The trophocyte is the main cell of fat body and stores these macromolecules. The fat body consists of two parts; peripheral and perivisceral. Peripheral fat body is located below the integument while perivisceral fat body is around the digestive tract. The study was conducted in EGEMIKAL Analysis Laboratory and Histology Laboratory of Ege University between 2018 and 2020. The fat body contents of insects at all stages were examined comparatively in three selected sections through histochemical and ultrastructural studies. We identified macromolecules stored in the trophocytes. Both the granular form of proteins and asterisk structure of glycogen localized around the lipid droplets were observed clearly. It was found that accumulation of protein continued in the trophocytes, but glycogen accumulation decreased considerably in adults compared to all nymphal stages. We also found that larger lipid droplets were stored in the PF fat body, while glycogen and protein accumulation was much higher in the PV fat body. These results may contribute to understanding of the mechanisms underlying activities such as amino acid, nitrogen, lipid and carbohydrate metabolism and protein synthesis in insects.

Keywords: *Blatta orientalis*, fat body, histochemistry, TEM, trophocyte

Öz

Böcek yağ dokusu glikojen, lipid ve protein için başlıca depolama alanıdır. Trofosit yağ dokusunun temel hücresidir ve bu makro molekülleri depolamaktadır. Yağ dokusu integümentin hemen altında yer alan periferal ve sindirim kanalının etrafında yer alan perivisseral yağ dokusu olmak üzere iki kısımdan oluşmaktadır. Bu çalışma 2018-2020 yılları arasında Ege Üniversitesi EGEMİKAL Analiz Laboratuvarı ve Histoloji Laboratuvarı'nda yürütülmüştür. Böceklerin tüm dönemlerine ait seçilen üç bölgesindeki yağ dokusu karşılaştırmalı olarak histokimyasal ve ince yapı çalışmalarıyla incelenmiştir. Trofositlerde depolanan makromoleküller belirlenmiştir. Hem granüler protein hem de lipid damlalarının etrafında yerleşim gösteren yıldız şeklindeki glikojen yapıları net bir şekilde gözlenmiştir. Tüm nimfal evrelerle erginler karşılaştırıldığında trofositlerde protein birikiminin devam ettiği, ancak glikojen birikiminin önemli ölçüde azaldığı belirlenmiştir. Ayrıca, periferal yağ dokusunda daha iri lipid damlaları depo edilirken, perivisseral yağ dokusunda ise daha fazla glikojen ve protein birikiminin olduğu tespit edilmiştir. Bu bulgular, böceklerde amino asit, nitrojen, lipid ve karbonhidrat metabolizmaları ve protein sentezi gibi faaliyetlerin altında yatan mekanizmaların anlaşılmasına katkı sağlayabilecektir.

Anahtar sözcükler: *Blatta orientalis*, böcek yağ dokusu, histokimya, TEM, trofosit

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Introduction

Insect fat body is considered as a tissue equivalent to that of the liver in vertebrates (Sobotnik et al., 2006; Liu et al., 2009; Gullan & Cransto, 2014). The fat body participates in numerous metabolic functions during the life cycle of insects. It is a major storage area for glycogen, lipid and protein (Haunerland & Shirk, 1995; Lipovsek & Novak, 2016) and the target organ of hormones such as neural, juvenile and ecdysone hormones (Hoshizaki, 2005; Roma et al., 2010; Lipovsek & Novak, 2016). It is also responsible for many activities such as amino acid, nitrogen, lipid and carbohydrate metabolism and protein synthesis (Liu et al., 2009; Arrese & Soulages, 2010; Li et al., 2019).

The fat body is divided into two regions in many insects. These are peripheral (PF) fat body located below the integument and perivisceral (PV) fat body, observed around the digestive tract (Resh & Carde, 2003; Lipovsek & Novak, 2016). These two regions can have different lipid, glycogen, and protein densities (Carvalho et al., 2013). It has been suggested that lipid synthesis and storage occur mainly in PF fat body, whereas PV fat body is associated with protein synthesis and storage in various insects (Dean et al., 1985; Roma et al., 2010).

The primary cell of fat body is the trophocyte. It differentiates to form other cell types; mycetocyte, urocyte, chromotocyte, and hemoglobin cells (Roma et al., 2010; Park et al., 2013; Toprak et al., 2020). A trophocyte is a major amorphous cell with a central nucleus. In its cytoplasm, there is an intense lipid, protein, and glycogen storage. In some species, lipids can be stored in the form of small droplets, while in others as large droplets that cover most of the cytoplasm. In some cases, proteins crystallize or form granules as they accumulate while carbohydrates are stored as glycogen (Paes-de-Oliveira & Cruz-Landim, 2003; Lipovsek et al., 2011; Vaca et al., 2019).

Cockroaches are thought to be harmful insects due to several diseases (dysentery, typhoid, cholera) they may transmit. Also, long-term contact with cockroaches may cause allergic rhinitis (Stankus et al., 1990) and asthma (Sohn & Kim, 2012; Bassirpour & Zoratti, 2014) in humans. Especially, *Blatta orientalis* (L., 1758) (Blattodea: Blattidae), *Blattella germanica* (L., 1767) (Blattodea: Blattellidae) and *Periplaneta americana* (L., 1758) (Blattodea: Blattidae) species are known as important household pests (Cornwell, 1968; Resh & Carde, 2003). Many forms of insecticides have been developed for these harmful insects, but due to their long-term use and toxicity to humans and animals, environmental contamination, and toxicity to non-target insects, these insects have started to show resistance to chemicals, like cyclodiene, deltamethrin, fipronil, imidacloprid, organophosphate, carbamate, pyrethroid insecticides (Thompson et al., 1993; Dehkordi et al., 2017; Nasirian & Salehzadeh, 2019). For this reason, newly created insecticides are focused on tissues such as fat body which was not considered as a convenient target before (Cornwell, 1968; Resh & Carde, 2003). For example, plants are of great interest for novel botanical insecticides (neem oils, pongom oils, essential oils from some aromatic plants) for insect pest- controlling agents without environmental contamination and toxicity (Pavela, 2007; Grdiša & Gršić, 2013). Therefore, it is very important to know the structure and contents of trophocytes in these insects throughout their development. Although trophocytes have been determined in the species of Blattodea orders (Park et al., 2013; Makki et al., 2014), there are not enough studies related to the *B. orientalis*. Our study focuses on macromolecules of trophocytes of cockroaches, *B. orientalis* (Oriental cockroach) at different developmental stages (6 nymphal stages and adult) under optimum conditions and employs different histochemical methods to analyze the macromolecules apart from light and electron microscopy observations.

Materials and Methods

Insect rearing and sampling

The study material, *B. orientalis* specimens were reared and monitored in Ege University, Faculty of Science-EGEMIKAL Analysis Laboratory, Insect Group Biological Activity Laboratory. The laboratory

conditions were kept constant at 25°C, 60-70% humidity, 12 hours night/12 hours day during the whole growing period. Further applications were carried out in the Histology Laboratory of Ege University between 2018 and 2020. Sampling was done at the beginning of each nymphal (6 in total) and adult stages as described by Zülfikaroğlu et al. (2022). Approximately 10 individuals at each stage were used for histology and histochemistry (240 in total) while 30 individuals were used for electron microscopy in total.

Histology and histochemistry

For histological analyses, the insects, which were divided into two parts, were fixed in Bouin's solution (saturated picric acid, formaldehyde, and acetic acid, 15:5:1) for 24 hours. Then, the fixative was washed away with 70% alcohol solution. After the dehydration (70-100%) and clarification (xylol), they were embedded in paraffin (Merck, 107337), and were cut into 5 µm thickness sections. Mayer's Hematoxylin-Eosin (H&E) (Merck, 109249; CI. 75290) was used for staining.

For histochemical analyses, two staining methods were applied: (1) Samples were fixed in Saint-Marie solution (95% ethanol, glacial acetic acid, 99:1) and dehydrated directly without washing. After the clarification, the tissues were embedded in paraffin and they were sectioned. These sections were stained with alcian blue 8GX (Merck, 105234; CI. 74240)-Periodic Acid Schiff (PAS) (Merck, 109033; CI 42500) for the determination of glycogen storages (2). They were fixed in 10% formalin solution and washed under running tap water for as long as the fixation period. After the routine histological preparation steps, the sections were stained with mercuric bromophenol blue (MBB) (saturated acetic acid, mercuric chloride, bromophenol blue (B8026, Sigma, 40:24:1) for the determination of protein storages (Humason, 1962; Presnell & Schreibman, 1997). Since the previously mentioned fixatives were insufficient for penetrating the enlarged body of the growing insects, the last nymphal and adult stages were fixed in Carnoy solution (60% ethanol, 30% chloroform, 10% glacial acetic acid) for both histological and histochemical analyses. This fixative was preferred because of its compatibility with the stains used in this study. All samples were kept in fixatives for at least 24 hours. Finally, the slides were photographed through ZEN image analysis software using a Zeiss Axio Scope A1 microscope.

Transmission electron microscopy

At the last nymphal stage, female and male cockroaches were dissected. The fat bodies obtained from the cockroaches were placed in Eppendorf tubes with a Karnovsky fixative (Karnovsky, 1965) (0.2M cacodyl tampon, 25% glutaraldehyde, 8% paraformaldehyde and distillated water). The samples were washed with buffer after the overnight incubation at +4ºC. Then, the second fixative, 1% osmium tetroxide (Millonig, 1961), was applied. After that, semi-thin and thin sections were cut and stained as in Zülfikaroğlu et al. (2022). Finally, both section types were examined and photographed under a Zeiss Axio Scope A1 light microscope and a Transmission Electron Microscope (TEM) Geol 100C accordingly.

Statistical analysis

The diameters of lipid droplets in semi-thin sections from the 6th nymph, female and male cockroaches, which showed the most difference between stages, were measured with the help of ZEN image analysis software. The measurements were carried out in three regions (thorax, beginning, and end of abdomen) in a selected area of 500 μm^2. Measurements were conducted 3 times for 10 individuals per stage (last nymph, female and male). Considering the diameter variable, the three stages were compared firstly in terms of each region, and then the three regions were compared across the stages. In order to statistically perform these comparisons, One Way ANOVA method was planned to be applied, but since the assumptions for normality and homogeneity of variances required for this method were not ensured, Kruskal-Wallis test was applied. All analyses were performed using the statistical software-IBM SPPS 25.

Results

To show the general structure and morphology of the trophocytes, H&E staining was used. Trophocytes are generally large and their nucleus is centrally located. In the study, the lipid droplets in the cytoplasm appeared on the foreground with their transparent appearance following the H&E staining. These cells are separated from one another by a basal lamina. Two types of fat body were detected for all the stages. While the cell boundaries of trophocytes were more distinct and compact for the early stages their boundaries were transformed into a spongy structure at the 6th nymphal and adult stages, especially in the PV fat body (Figure 1).

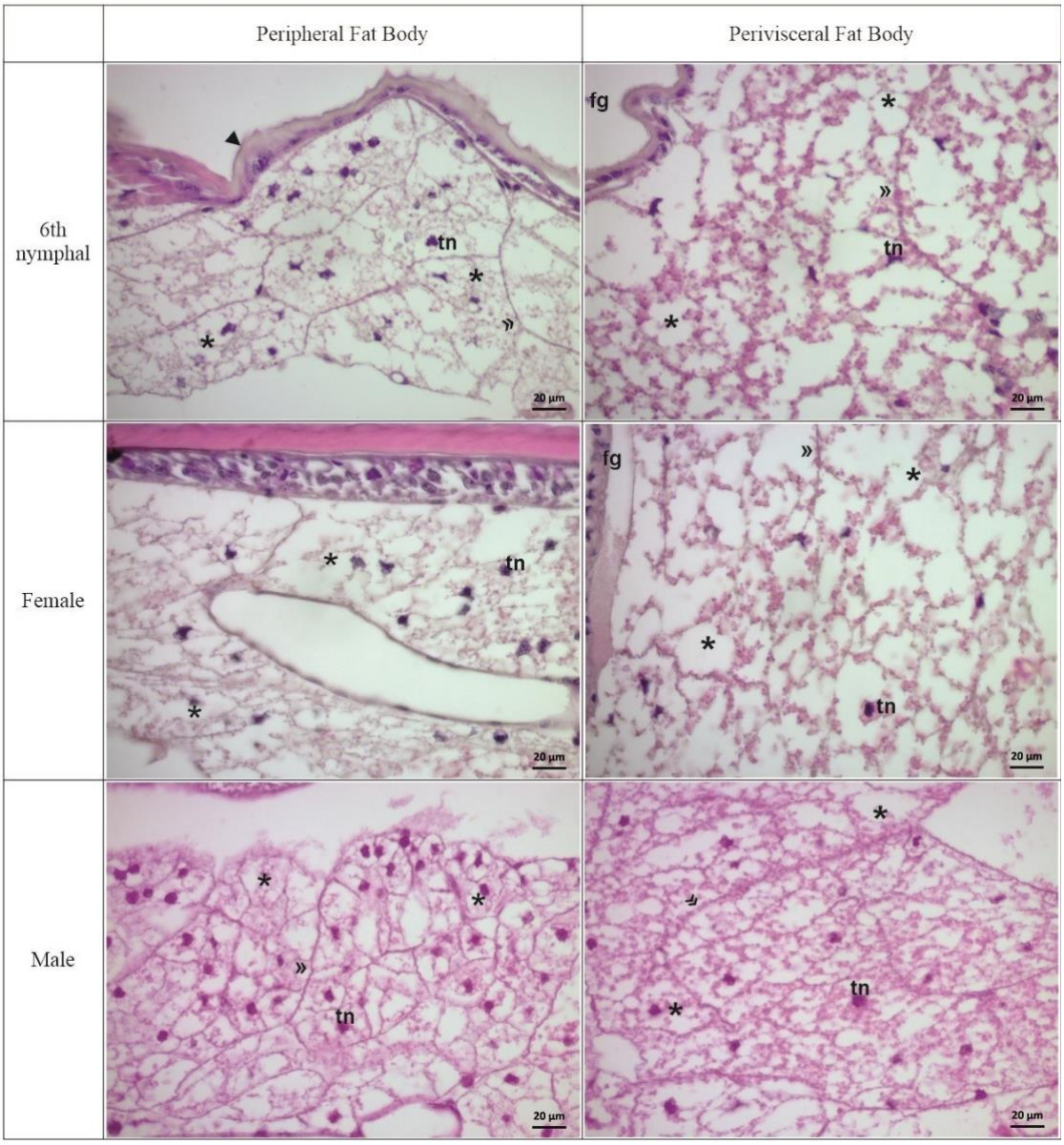

Figure 1. Peripheral and perivisceral fat bodies in the thorax regions of various stages stained with Mayer H&E. fg: foregut; tn: nucleus of trophocyte; *: trophocyte; ►: integument; »: basal lamina.

PAS staining was used to show glycogen accumulation in the fat bodies of *B. orientalis*. In the developmental stages, the thorax, the beginning and the end of the abdomen revealed lipid droplets in the middle of trophocytes, with pink-purple colored glycogen accumulation around them. In all three regions, it was observed that the density of glycogen deposits accumulated in the trophocytes of the PF fat body was less than that of the PV fat body (Figures 2&3). Additionally, the amount of glycogen stored in trophocytes decreased considerably in adults compared with all the nymphal stages (Figure 3).

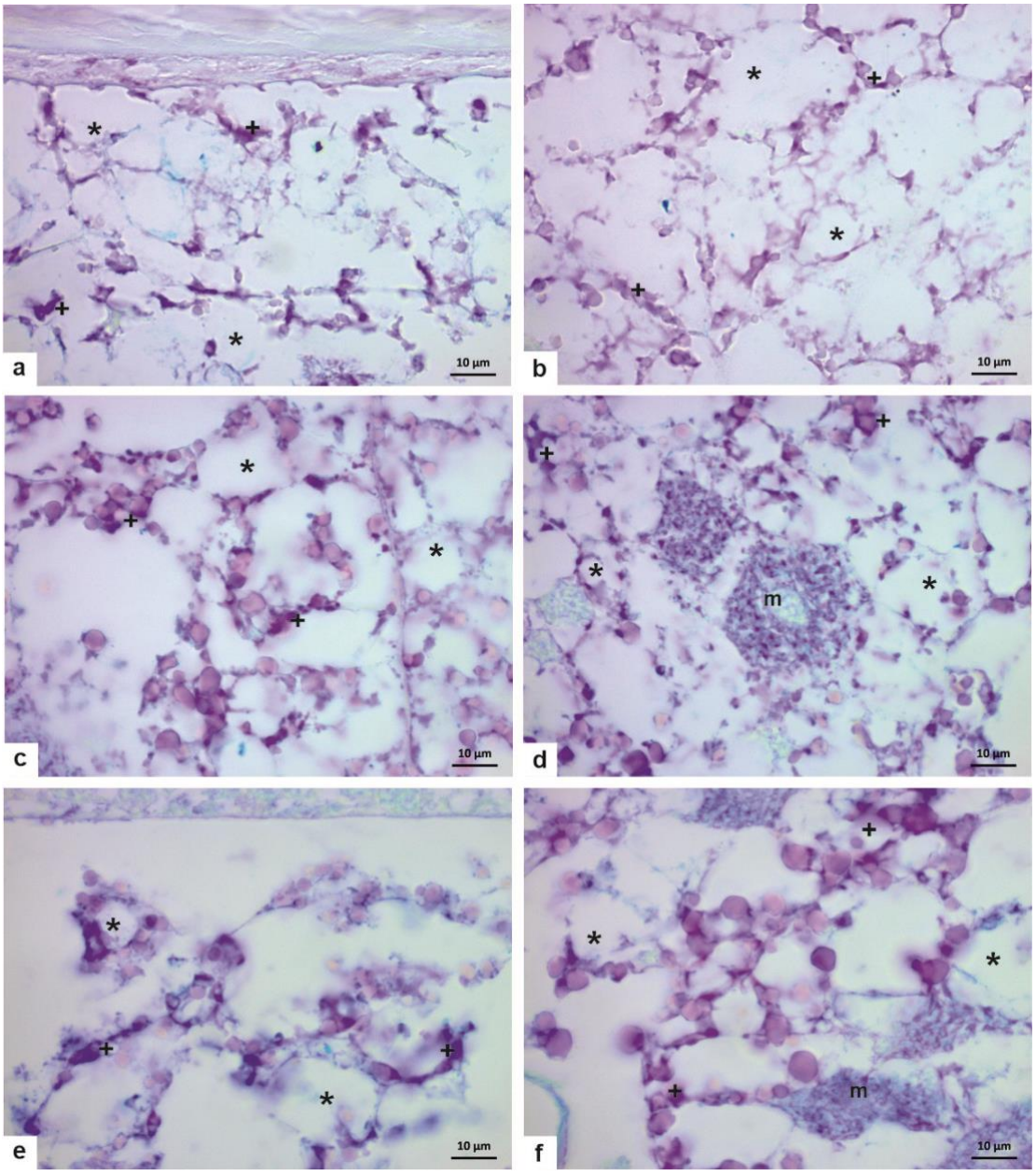

Figure 2. Accumulation of glycogens at the 6th nymphal stage stained with PAS: a) thorax PF fat body; b) thorax PV fat body; c) beginning of abdomen PF fat body; d) beginning of abdomen PV fat body; e) end of abdomen PF fat body; f) end of abdomen PV fat body. m: mycetocytes; *: trophocyte; +: glycogen.

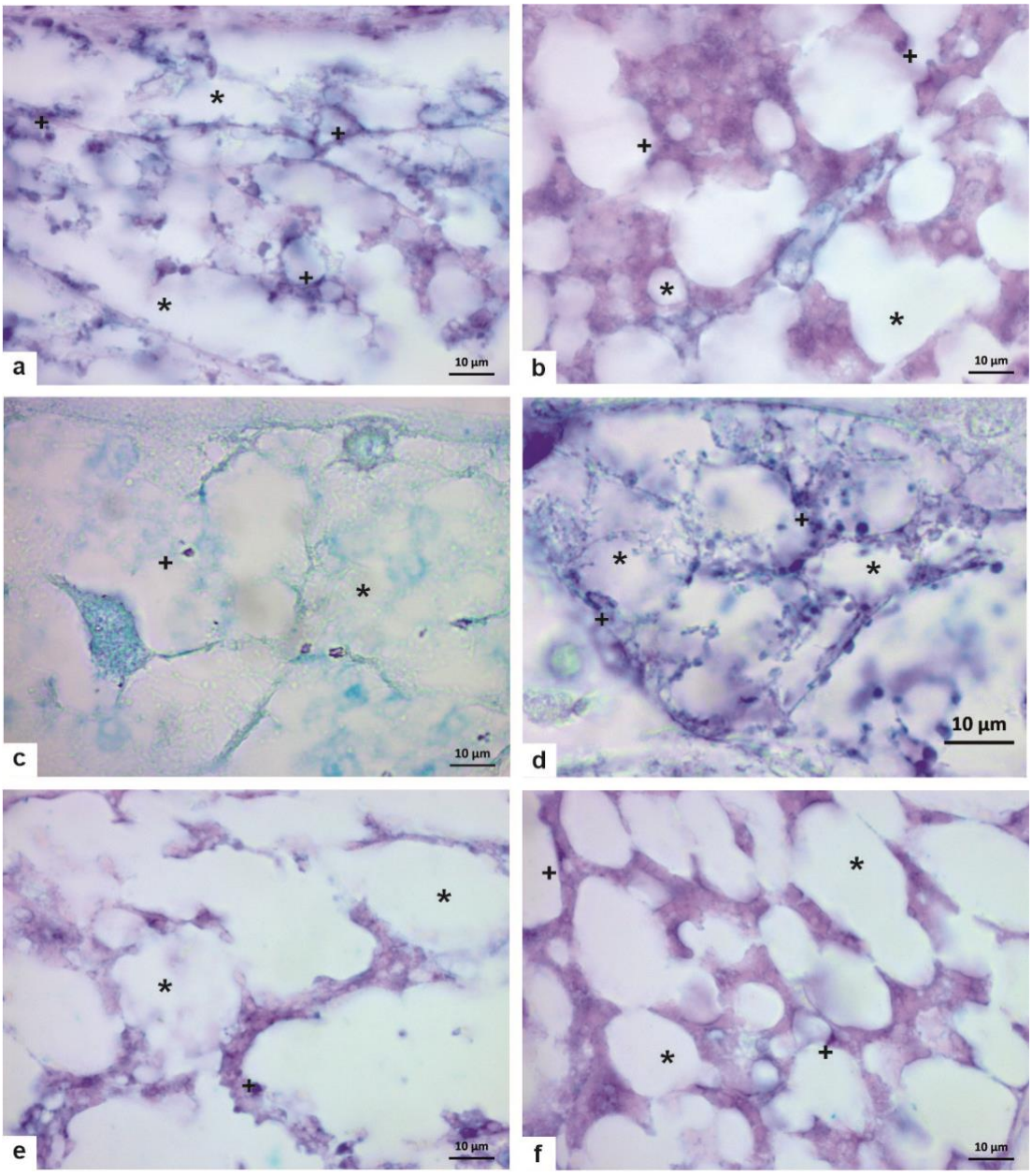

Figure 3. Accumulation of glycogens in the adult stage stained with PAS: a) thorax PF fat body; b) thorax PV fat body; c) beginning of abdomen PF fat body; d) beginning of abdomen PV fat body; e) end of abdomen PF fat body; f) end of abdomen PV fat body. *: trophocyte; +: glycogen.

Proteins accumulating in the trophocytes were stained with MBB. Protein granules were blue colored and like glycogen; they were positioned around lipid droplets (Figures 4&5). Throughout the development of the nymph, there was a notable increase in the accumulation of protein granules. Examination of the PF and PV fat bodies in each region revealed that the accumulation of protein granules in the PF fat body was less significant than in the PV fat body (Figures 4&5).

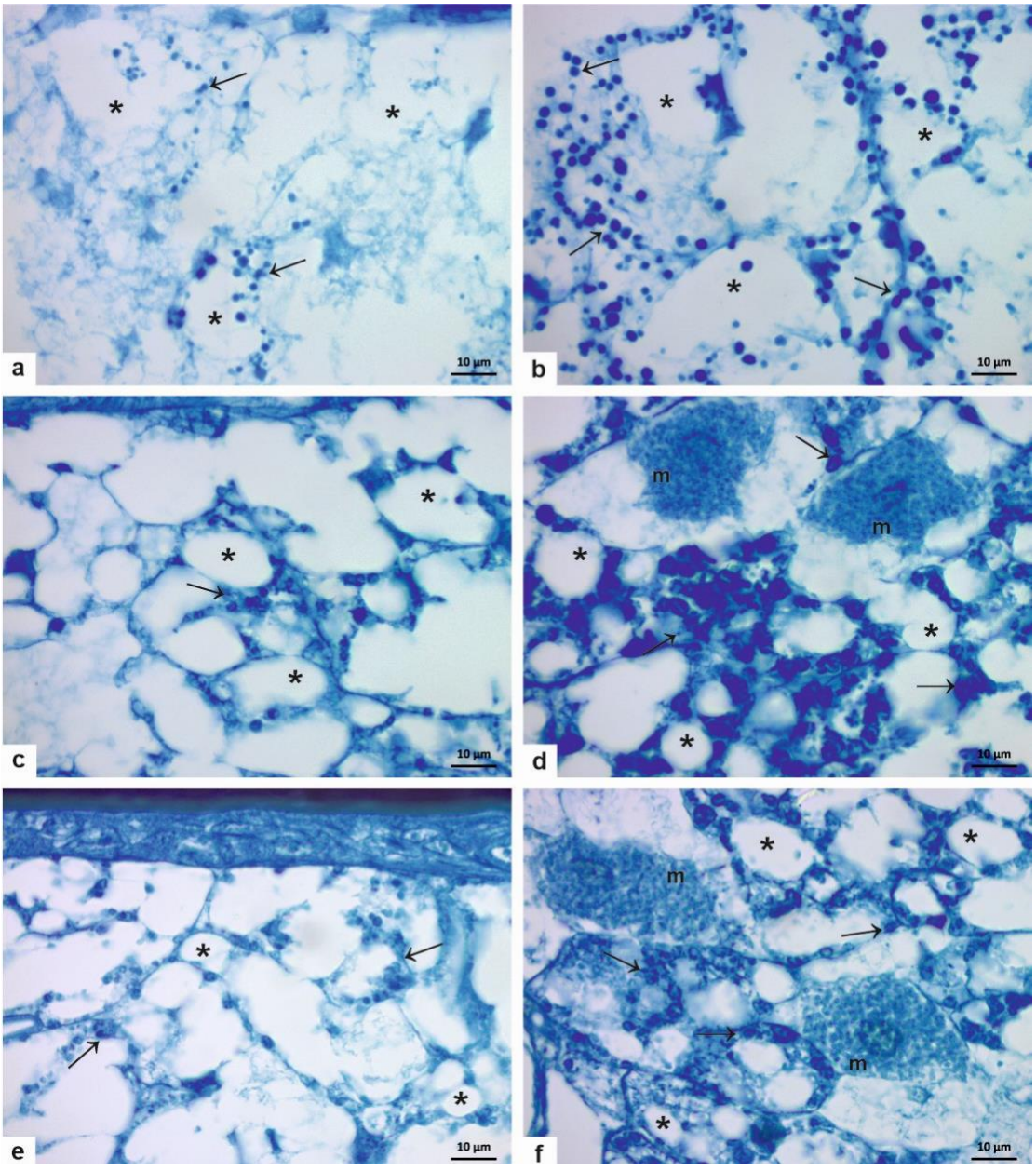

Figure 4. The accumulation of proteins at the 6th nymphal stage stained with MBB: a) thorax PF fat body; b) thorax PV fat body; c) beginning of abdomen PF fat body; d) beginning of abdomen PV fat body; e) end of abdomen PF fat body; f) end of abdomen PV fat body. m: mycetocytes; *: trophocyte; →: protein granules.

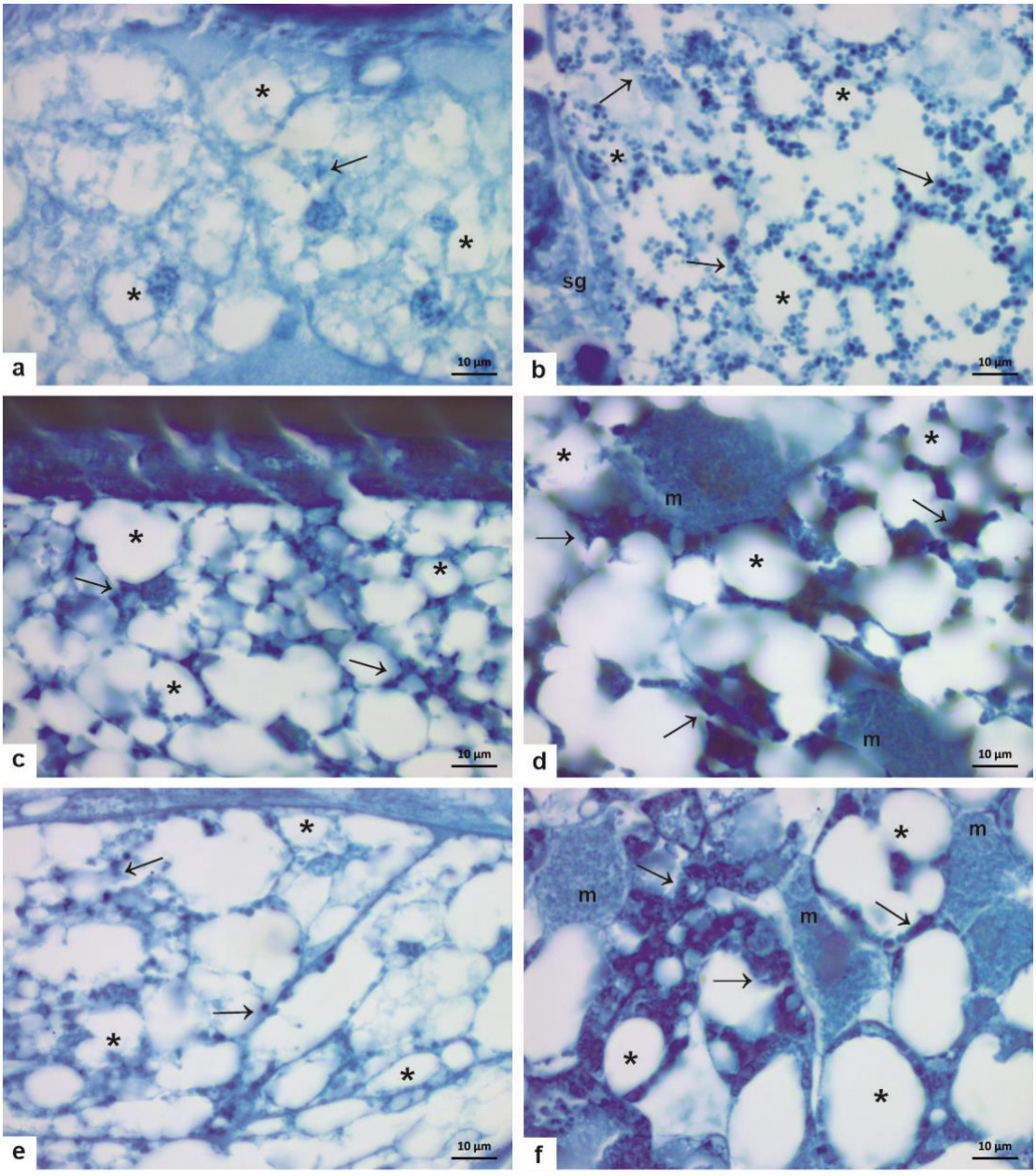

Figure 5. The accumulation of proteins in the adult stained with MBB: a) thorax PF fat body; b) thorax PV fat body; c) beginning of abdomen PF fat body; d) beginning of abdomen PV fat body; e) end of abdomen PF fat body; f) end of abdomen PV fat body. m: mycetocytes; sg: salivary gland; *: trophocyte; →: protein granules.

The semi-thin sections of the 6th nymphal stage and adult (female and male) cockroaches, stained with toluidine blue, showed the lipid droplet boundaries clearly in the cytoplasm of trophocytes (Figure 6). Thus, these sections were used for the statistical analyses. Furthermore, the thin sections of these stages revealed electron-lucent lipid droplets (Figure 7). Additionally, the other macromolecules were easily distinguished around the nucleus on electron microscopy images. While the glycogen deposits from the 6th nymphal and adult stages showed an asterisk shape structure scattered in the cytoplasm, the dark granular form of the storage proteins were also evident in these stages (Figure 7).

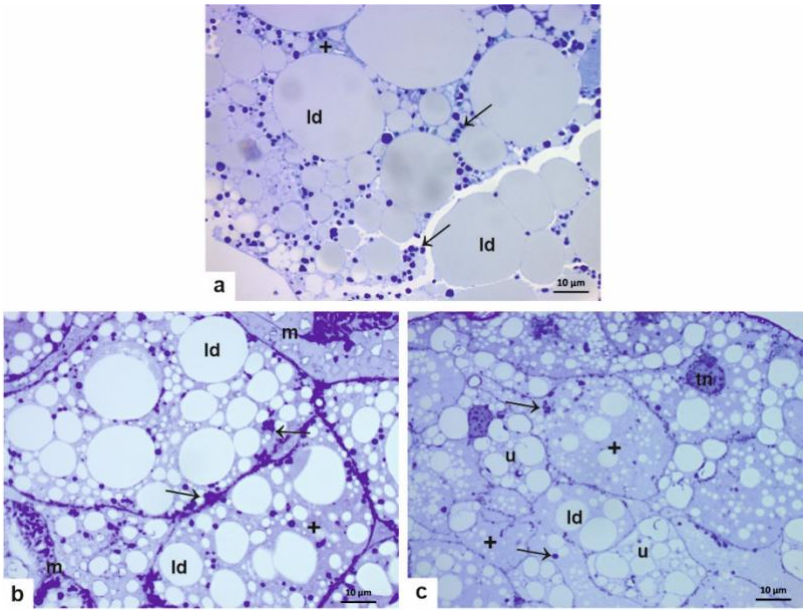

Figure 6. Demonstration of trophocytes stained with toluidine blue: a) 6th nymphal stage thorax; b) female end of abdomen; c) male end of abdomen. ld: lipid droplet; m: mycetocyte; tn: nucleus of trophocyte; u: urocyte; +: glycogen; →: protein granule.

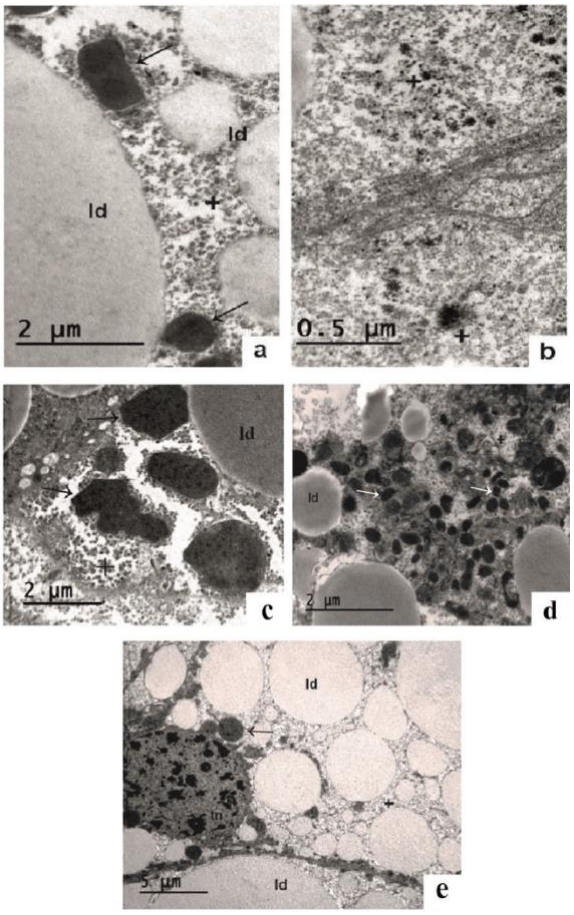

Figure 7. Ultrastructures of trophocytes: a) 6th nymphal thorax; b) Adult thorax; c) 6th nymphal stage, beginning of abdomen; d) adult, beginning of abdomen; e) adult, end of abdomen. ld: lipid droplet; tn: nucleus of trophocyte; +: glycogen; →: protein granule.

According to the Kruskal-Wallis test results, it can be said that there is statistically no significant difference in the diameters across all the three stages and three regions. p-Value>0.05 for all comparisons (Tables 1&2). Also, Figure 8 shows the mean diameter comparisons between stages and regions. Error bars are constructed using standard deviation.

Table 1. Kruskal-Wallis test results for comparisons between three stages while one region held constant

Null Hypothesis	Region	Kruskal-Wallis Test Statistic	p-Value
The distribution of "diameter" is the same across categories of "stage"	Thorax	1.910	0.385
	Beginning of abdomen	4.113	0.128
	End of abdomen	4.056	0.132

The significance level is 0.05.

Table 2. Kruskal-Wallis test results for comparisons between three regions while one stage held constant

Null Hypothesis	Stage	Kruskal-Wallis Test Statistic	p-Value
The distribution of "diameter" is the same across categories of "region"	6th nymph	0.923	0.630
	Female	0.534	0.766
	Male	0.083	0.959

The significance level is 0.05.

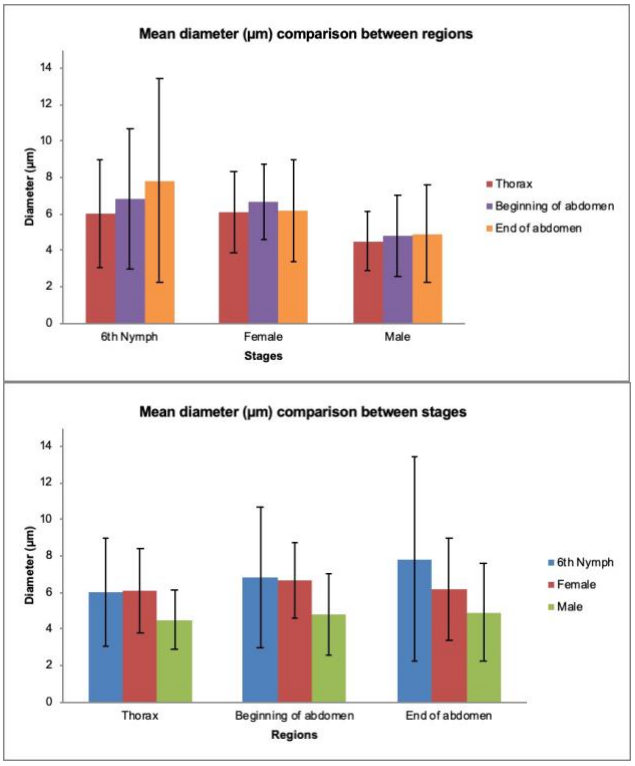

Figure 8. Mean diameter (µm) (±SD) comparisons. No significant differences observed among means ($p < 0.05$).

Discussion

There are numerous studies on the structure, composition, and function of fat body in insect species. For example, studies on *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae) and some Diptera species have shown that larger lipid droplets are stored in the PF fat body, while the PV fat body has smaller lipid droplets and contains more protein granules (Dean et al., 1985). The abundance of protein in the PV fat body of *Helicoverpa zea* Boddie, 1850 (Lepidoptera: Noctuidae) was also determined to be higher than that in the PF fat body (Haunerland & Shirk, 1995). In *Lutzomyia longipalpis* (Lutz & Neiva, 1912)

(Diptera: Psychodidae) and *Phlebotomus papatasi* (Scopoli, 1786) (Diptera: Psychodidae), it was observed that glycogen accumulation was higher in PV fat body cells after feeding the flies with sugar (Assis et al., 2014). Although the PF and PV fat bodies of the caterpillar *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Erebidae) have different roles, it has been stated that both of them were involved in the synthesis of lipids and other substances (Carvalho et al., 2013). In our study on *B. orientalis*, we observed that larger lipid droplets were stored in the PF fat body while more protein granules and glycogen were deposited in the PV fat body similar to the results reported by Dean et al. (1985) and Haunerland & Shirk (1995).

Trophocytes are the basic cells in the fat body and store lipids, proteins, and glycogens (Arrese & Soulages, 2010; Azeez et al., 2014; Lipovsek & Novak, 2016). We have not found any studies comparing the cell boundaries of trophocytes in PF and PV fat bodies in literature. there is a study on *A. gemmatalis*, in which cytoplasm of the trophocytes in the PF fat body was reported to be more acidophilic while that of PV was found to be more basophilic (Carvalho et al., 2013), which suggested that this situation was associated with trophocytes in PV containing a large amount of granular endoplasmic reticulum (GER). In our study on the Oriental cockroach, we have found that the cellular boundaries of both the PF and PV fat bodies were more distinct in the first nymphal stages, whereas in 6th nymphal and adult stages, especially in the PV fat body, their boundaries were deteriorated and transformed into a more reticular structure. This could be resulted from the increased accumulation of glycogen and protein in the PV fat body as the development proceeds.

Generally, studies show that trophocytes contain an irregular-shaped nucleus with dense chromatin and cytoplasm with various vesicular structures, mitochondria, GER and Golgi body (Paes-de-Oliveira & Cruz-Landim, 2003; Roma et al., 2010). These vesicular structures in the cytoplasm; are either digestive vacuoles (autophagic, heterophagic, multi-vesicular bodies) or storage vacuoles (protein, glycogen, lipid, urates) (Locke, 1984; Roma et al., 2010). In *B. orientalis*, we have also found that the trophocytes contained a nucleus with very dense chromatin and cytoplasm with accumulations of lipids, proteins and glycogens, as observed in *Aedes aegypti* (L., 1762) (Diptera: Culicidae) (Martins & Ramalho-Ortigao, 2012), *Melipona quadrifasciata* Lepeletier, 1836 (Hymenoptera: Apidae) (Furtado et al., 2013), *Pachycondyla villosa* (Fabricius, 1804) (Hymenoptera: Formicidae) (Zara & Caetano, 2004) and *P. americana* (Park et al., 2013).

Lipids, which constitute about 70% of the cytoplasm in trophocytes, are mostly in the form of triglycerides. When transported to hemolymph, they are converted into diglyceride. In fat bodies of different insects, lipids can be stored around the nucleus as small or large droplets (Paes-de-Oliveira & Cruz-Landim, 2003; Lipovsek et al., 2011). It has been shown that lipid accumulation is vital to meet the basic energy needs of flying muscles and other organs in the species of Diptera order (Assis et al., 2014), or during metamorphosis in *Calpodes ethlius* (Stoll, 1782) (Lepidoptera: Hesperiidae) (Haunerland & Shirk, 1995), and during foraging and oogenesis in the Attini tribe (Roma et al., 2010). While lipids needed in the cuticle are provided by oenocytes and trophocytes (Klowden, 2007), variations may be seen in the lipid storage capacity across males and females for some species due to different needs. For example, it has been stated that the lipid storage in male *Hyalophora cecropia* L., 1758 (Lepidoptera: Saturniidae) is higher compared to the female due to long flights during the search for mates (Dean et al., 1985). In our study on *B. orientalis*, the analysis of trophocytes' lipid content was done on individuals at the 6th nymphal stage and adults (female and male). The lipids were stored as small or large, white spherical droplets around the nucleus. We propose that the female stores lipid as an energy source at the 6th nymphal stage to create egg packages; whereas in males, lipid stores are used for foraging and mate-seeking. The analysis of the lipid droplet measurements revealed no statistically significant difference between individuals at the 6th nymphal and adult stages as well as among the regions (thorax, beginning, and end of abdomen). This may be due to the cockroach being a hemimetabolous insect, and it does not undergo a major change during metamorphosis.

Another macromolecule that meets the basic energy needs in addition to lipids in trophocytes is carbohydrate. Among insects, carbohydrates are stored as glycogen, similar to other animal groups (Lipovsek et al., 2011; Li et al., 2019; Vaca et al., 2019; Toprak et al., 2020). It was thought that the high number of glycogen stores detected in *Atta laevigata* (Smith, 1858) (Hymenoptera: Formicidae) and *Mycetarotes parallelus* (Emery, 1906) (Hymenoptera: Formicidae) are used for foraging and courtship display (Roma et al., 2010). Also, glycogen is mainly used in the formation of the cuticle (Klowden, 2007). In our study on *B. orientalis*, it has been determined that glycogen was deposited freely in the cytosol. In nymphal stages, glycogen accumulated intensely, especially in the PV fat body. However, after transitioning to the adult form, the amount of glycogen considerably decreased since it was used for cuticle formation and maturation of gonads. In the studies of other species, similar results have been found related to glycogen. For example, in *Rhodnius* genus, which is commonly known as kissing bugs, the glycogen stores are considerably reduced after the formation of the cuticle (Dean et al., 1985). In *Bombus terrestris* (L., 1758) (Hymenoptera: Apidae), *Calpodes ethlius* (Stoll, 1782) (Lepidoptera: Hesperiidae) and *P. villosa* (Zara & Caetano, 2004), it was observed that the trophocyte cytosol had glycogen stores accumulated freely as long as the feeding continued (Roma et al., 2010). Park et al. (2013) determined that lipid and glycogen stores were exhausted in *P. americana*, as in *Manduca sexta* L., 1763 (Lepidoptera: Sphingidae) in case of prolonged fasting. Then, they showed that the glycogen was converted rapidly into trehalose, the sugar form in the hemolymph, and the repositories were quickly filled after refeeding.

Storage of proteins in trophocytes may have various forms in different species. For example, in the ants of the Attini tribe, they are stored in granular form of various sizes; whereas in *Atta sexdens rubropilosa* Forel, 1908 (Hymenoptera: Formicidae) and *Monomorium pharaonis* (L., 1758) (Hymenoptera: Formicidae), they are accumulated in crystal form (Roma et al., 2010). Also, in some Lepidoptera, such as silkworm, s protein accumulation continues during the pupal stage and it is exhausted a few days after becoming an adult (Dean et al., 1985). In *H. zea*, when the larval stage is completed, the protein synthesis is stopped and they are stored in granular form in the trophocytes (Roma et al., 2010). However, in cockroaches such as *P. americana*, protein accumulation starts at the beginning of the nymphal stage and continues during adult life even if there are fluctuations due to metabolic functions. Furthermore, after the cuticle synthesis in *Rhodnius*, it was determined that the protein storage was not exhausted, unlike glycogen (Dean et al., 1985). As with *A. laevigata* and *M. parallelus* (Roma et al., 2010), intense protein accumulation was observed in *P. villosa* and *Scaptotrigona postica* (Latreille, 1807) (Hymenoptera: Apidae) (Zara & Caetano, 2004), and the irregular-shaped nucleus in their trophocytes was also thought to support the intense protein synthesis (Roma et al., 2010). In *B. orientalis*, we have observed a dense protein accumulation during the developmental stages, especially in the PV fat body. In parallel to the findings in the literature (Dean et al., 1985), the protein storage didn't decrease after the adult stage. We have demonstrated that proteins were stored in a granular form. The active protein synthesis and storage could be supported by the trophocytes with multilobed nuclei.

Literature information on the fat body structure in *B. orientalis* is currently inadequate. Majority of the studies used *Periplaneta* sp. as an organism, and some are focused on the effects of stress (Chowański et al., 2017) and starvation (Park et al., 2013). In studies using *B. germanica* (Patino-Navarrete et al., 2014) and *B. orientalis* species (Corsaro et al., 2007), only symbionts in mycetocytes were examined. In this study, histologically reliable comparisons were made by determining standard locations such as the thorax, the beginning of the abdomen and the end of the abdomen. Different fat body types in the first and last nymphal stages and adults were also included as peripheral and perivisceral fat body. Furthermore, the article was statistically enriched by measuring the diameters of lipid droplets in the trophocytes in the semi-thin sections of adults with the last nymphal stage. We also analyzed the macromolecules of trophocytes in *B. orientalis* at different developmental stages under optimum conditions for the insect, which can be used as a baseline for comparative analyses under stress conditions.

In conclusion; lipid, protein, and glycogen contents stored in the trophocytes of *B. orientalis* under optimum-rearing conditions were analyzed in this study. We hope that these results will contribute to understanding the mechanisms underlying the activities such as; amino acid, nitrogen, lipid and carbohydrate metabolism and protein synthesis in insects. We think that it would be more reasonable to apply the application in the nymphal stages of cockroaches in future insecticide studies. In this way, it will be more effective in insect control as there will be a decrease in the transition rate of the insect to adult. Both the PV fat body providing the energy required for the transition to adult, and the PF fat body being supportive in protecting against external factors (cold, impact, etc.) are affected. Hopefully, these data may provide new insights for pest control studies.

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