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LIPID AND FATTY ACID COMPOSITIONS OF FAT BODY AND HAEMOLYMPH IN PRE- AND POSTVITELLOGENIC PHASES OF CARAUSIUS MOROSUS BR. (ORTHOPTERA: PHASMATIDAE)

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ABSTRACT

Lipid and fatty acid compositions of fat body and haemolymph of young and mature females of parthenogenetic wingless stick insect *Carausius morosus* Br. (Orthoptera: Phasmatidae) in pre- and postvitellogenic phases were investigated.

Lipid composition of the tissues were determined qualitatively and quantitatively by Thin-Layer Chromatographic and Photodensitometric methods respectively. The total lipid content of the fat body of young females was about three times higher than that of mature females, of which 93 % was triacylglycerols. Highest proportions of free fatty acids and sterol esters were found in the haemolymph of young females, whereas diacylglycerols and sterols were higher in the same tissue of adult females. Phospholipids composed more than 50 % of the total lipids of haemolymph and fat body in adult females. Phosphatidylethanolamine and phosphatidylcholine were found as the main phospholipids.

Constituent fatty acids of the lipid classes and the composition of sterols were studied by Gas Chromatographic methods. Sterols composed mainly of cholesterol, highest proportions of which were found in the haemolymph of mature females. Twenty fatty acids with chains ranging between 14 and 20 carbons were identified and their distribution among the tissues and phases were evaluated according to the lipid classes.

INTRODUCTION

Vitellogenic phase of oogenesis is a key event in ovarian development in insects. It occurs by the synthesis of specific yolk precursors and a rapid accumulation of different storage materials in the oocytes takes place during vitellogenesis (Giorgi and Macchii, 1980). Almost all recent literature on the source of yolk components, are concerned with protein yolk (Kambysellis *et. a.l*, 1986).Insect eggs, however, include both protein and lipid yolk bodies as well as glycogen droplets (Wigglesworth, 1974). Almost all absorbed and synthetised lipids were used for somatic growth in nymphs and for ovarial growth in adults of orthopteroid insects (Woodring *et. al.*, 1979). Adult feeding provides raw material from

which specific oogenesis procursors are synthetised by the fat body (Hagedorn and Kunkel, 1979).

Studies dealing with lipid yolk formation rely entirely upon cytological and histochemical considerations of the ovary (Mohanty, 1982; Verma *et. al.*, 1982). The possibility of lipid originating outside the ovary, however, had not been taken into account in these studies. Results of various studies indicate that fat body lipids might be the extraovarian source of yolk lipids in insects (Dutkowski and Ziajka, 1970). Fat body does not undergo a net mobilisation of reserves as it does during yolk formation period when the ovaries are absent or inactive (Gilbert, 1967). Thus, ovaritectomized insects have larger fat body mass than ovulated females (Clifford and Woodring, 1986). The concomitant decrease in fat body lipids with maturation of lipid-rich eggs in various insects led several authors to suggest a transfer of lipids from fat body to ovaries.

Information on the lipid and fatty acid composition of different tissues in insects during vitellogenesis is rare (Nelson *et. al.*, 1967). Since, the most likely extra-ovarian source of lipids is fat body and the mobilisation medium is haemolymph in insects, lipid and fatty acid composition of fat body and haemolymph were examined to identify possible quantitative and qualitative differences between previtellogenic and postvitellogenic phases of parthenogenic stick insect *Carausius morosus*, which in turn reveal information on lipid and fatty acid utilisation from fat body by the developing oocytes during reproductive cycle.

MATERIALS AND METHODS

Laboratory cultures of *C. morosus* were held at approximately 22° C and 70 % r.h. under crowded conditions. Illumination periods of the rearing cages were 14 hrs light and 10 hrs dark. Animals were fed on corly kale. Selection of experimental insects were based on differences in their ovarium morphology. Fifty females were used for each of following two phsiological stages: i) Young female (YF) with ovaries in previtellogenic phase ii) Mature female (MF) with ovaries in postvitellogenic phase having fully mature oocytes, for each experiment.

Haemolymph was collected into a tube, which contained 20 ml chloroform-methanol mixture (2:1 v/v), by cutting the metathorasic leg from coxa. The mixture was homogenised in ultraturrax after collecting sufficient volume (1 ml), and total lipids were extracted according to the method described by Folch *et al.* (1957).

Fat body was removed after cutting the head and dissecting the insect dorsally. Dissection was made by careful severing the cuticula, tracheal connetctions and malphigian tubules in Wyatt's medium at – 5° C under a binocular microscope. Pooled fat bodies from 50 individuals were weighed and immediately homogenised by adding 10 parts of chloroform – methanol (2:1 v/v) mixture to one part of tissue. The lipids were then extracted according to Folch *et. al.* (1957).

Lipids obtained from the fat body and haemolymph were weighed, redissolved in a known volume of chloroform, BHT (Butylated hydroxytoluene, Merck, Darmstad, W. Germany) was added as antioxidant and stored at – 20° C until Thin-Layer Chromatographic analysis.

Total lipids were separated into their lipid fractions on precoated Silica Gel-G TLC plates (Merck), which are 0.25 mm in thickness and activated at 110° C for 2 hours beforehand. Plates were developed in a petroleum ether, diethylether and acetic acid (70; 30; 1 v/v/v) solvent mixture (Johnson and Davenport, 1974). Detection was made by spraying the developed plates with 3 % (CH3 COO)2 Cu solution in 7 % aqueous H3PO4, spots appeared sufficiently for densitometric detection by charring the plates at 180° C for 25 minutes. UV detection was also made when necessary by spraying the plates with 2', 7'–dichlorofluorescein (0.2 % in methanol). Separated lipid classes were identified by comparing their Rf values with those of authentic standarts !(Sigma, St. Louis, MO., USA). Human serum lipids were applied as control mixture to each plate. Diacylglycerols (DG), Sterols (S), Free Fatty Acids (FFA), Triacylglycerols (TG) and Sterol Esters (SE) were being separated without an overlap. As polar lipids, Phospholipids (PL) remained at the origin. They were scraped off and fractionated by TLC, using Silica Gel-G (Merck), 0.25 mm in thickness, as adsorbant and chloroform-methanol-water (60:20:2.5 v/v/v) as solvent mixture. Visualisation and identification of the phospholipid classes were done as for the lipid classes. Sterol samples, obtained from TLC separation were prepared for further Gas-Liquid Chromatographic (GLC) analysis as described in a previous study (Üner, 1988a).

Densitometry was applied for quantification of lipid and phospholipid classes (Fewster *et. al.*, 1969). Optical densities of the charred spots were measured with a TLC flying-spot densitometer, equipped with a linear recorder and an integrator (Vitatron, Model TLD 100, Dieren, The Netherlands).

Methylation, GLC separation, identification and quantification of fatty acid constituents of lipid classes and GLC analysis of sterols were performed as described previously (Üner, 1988b).

All the critical stages of the experiments were carried out and the samples were stored in an inert atmosphere of nitrogen. The solvents used for the analysis of lipids were reagent grade. Fatty acid shorthand used in this study was; Number of carbons: Number of double bonds.

RESULTS

Lipid content of the fat body was 22.93 % and 6.40 % in young and mature females respectively on fresh weight basis. While, haemolymph contained 1.5 % total lipid in young and 1.2 % in mature females.

Total lipid fractions identified by TLC analysis were PL, D.G, S, FFA, TG, and SE (Fig. 1). Quantification by densitometry showed that the predominant part of the lipids were formed of TG (Table 1)., accounting for about 93 % of them in fat body of the young females (YFB). Whereas, in the fat body of females bearing mature oocytes (MFB) TG accounted less than half of the above percentage (41 %). Principal lipid fraction of MFB was PL (51 %), which was a very high percentage than it was in YFB (4 %). Lipid fractions, such as DG, S, FFA and SE were all minor components of total lipids which were all found in higher percentages in MFB than in YFB with the expection of SE.

Principal lipid class both in YH (37 %) and MH (51 %) was PL, followed by TG in young and DG in mature haemolymph (Table 1). In haemolymph of young females (YH) PL and DG were in lower ratios than in mature haemolymph (MH), while the reverse was true for FFA, TG and SE fractions.

TLC separation of phospholipids in young and mature female tissues are shown in Figure 2. Lysophosphatidylcholine (LPC), Sphingomyelin (SM), Phosphatidylcholine (PC), Phosphatidylinositol (PI), Phosphatidylserine (PS), Phosphatidylethanolamine (PE) and Cardiolipin (CL) were identified and found in changing proportions in all the tissues and stages examined.

Principal phospholipids of the tissues were PC and PE. Young and mature fat bodies contained moderate amounts of LPC, SM, PI and PS fractions, while these were in small or trace amounts in haemolymph samples.

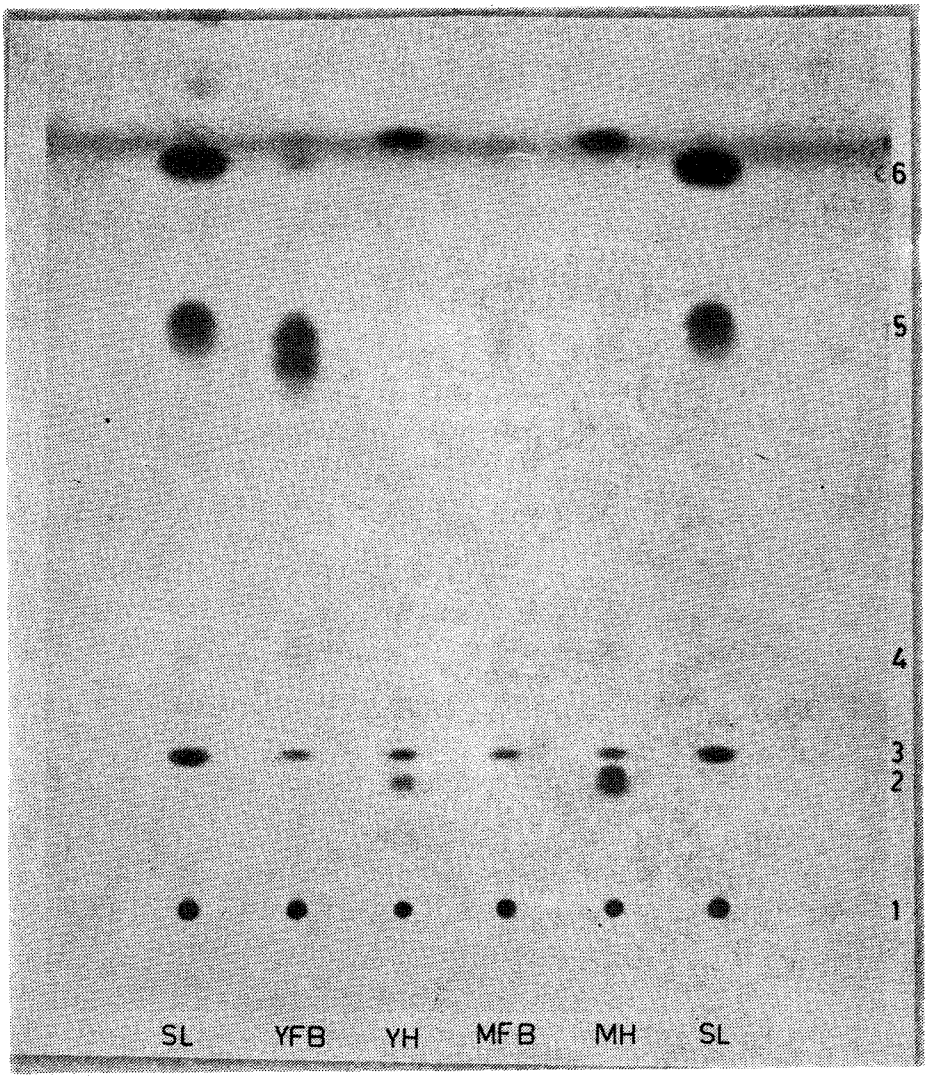

Figure 1. TLC chromatogram of lipid classes in haemolymph and fat body of young and mature females. Adsorban, Silica Gel-G (;.25 mm); Solvent system, Petroleum ether – Diethylether – Acetic acid (70:30:1 v/v/v*j*; Detection, 3 % (CH3 COO)2 Cu in 7 % aqueous H3PO4. SL, Human Serum Lipids; YFB, Young Fat Body; YH, Young Haemolymph; MFB, Mature Fat Body; MH, Mature Haemolymph; 1, PL; 2, DG; 3, S: 4, FFA; 5, TG; 6, SE (Abbreviations as in text).

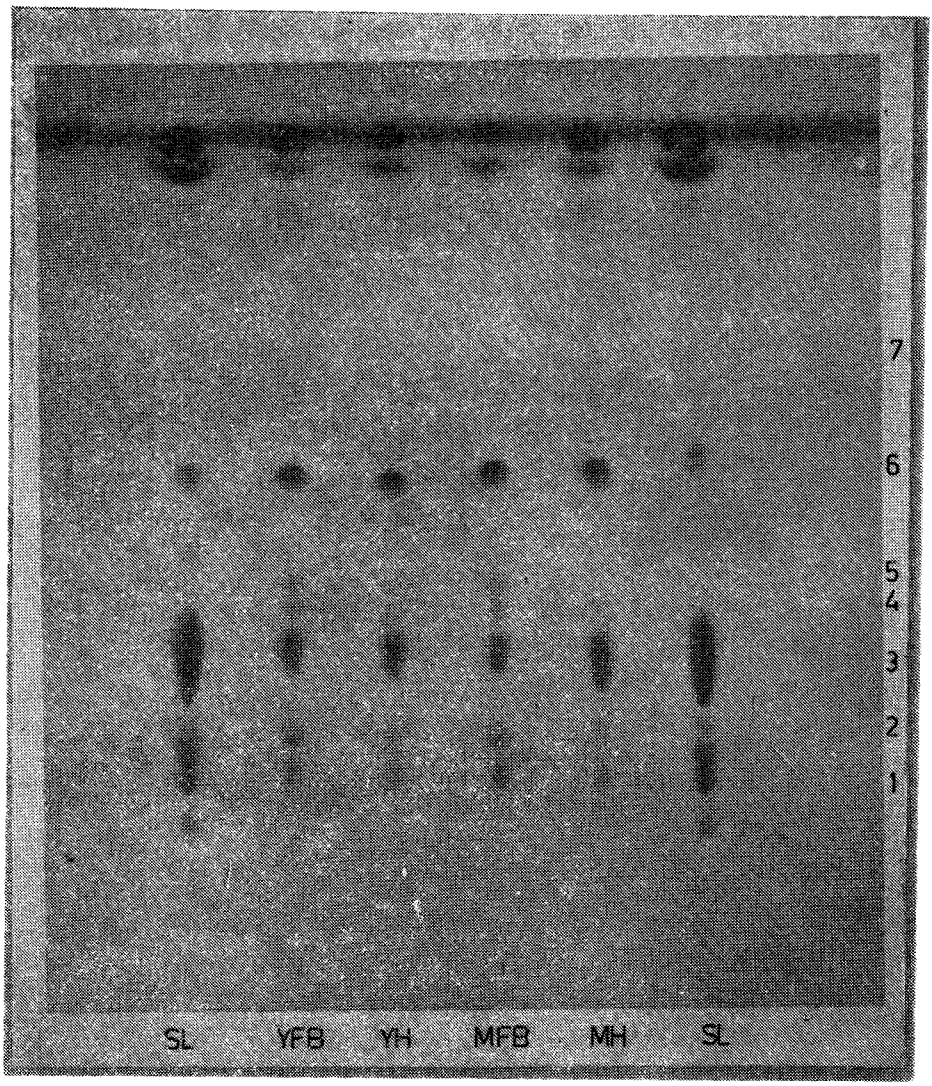

Figure 2. TLC chromatogram of Phospholipid classes in haemolymph and fat body of young and mature females. Adsorban, Silica Gel-G (0.25 mm); Solvent system, Chloroform – Methancl – Water (60:20:2.5 v/v/v); Detection, 3 % $(CH_3COO)_2$ Cu in 7 % aqueous H_3PO_4. SL, Human Serum Lipids; YFB, Young Fat Body; YH, Young Haemolymph; MFB, Mature Fat Body; MH, Mature Haemolymph; 1, LPC; 2, SM; 3, PC; 4, PI; 5, PS; 6, CL (Abbreviations as in text).

Table 1. Composition of haemolymph and fat body total lipids of young and mature females

Lipid Class	Percentage Composition			
	YH	MH	YFB	MFB
PL	36.57	50.67	4.01	51.36
DG	12.44	36.86	0.28	1.27
S	1.00	1.38	0.53	1.19
FFA	11.47	3.75	0.74	3.98
TG	24.90	3.38	93.23	41.18
SE	13.61	3.96	1.22	1.02

Densitometric quantification showed that total sterols were mainly concentrated in haemolymph (1.38 %), followed by fat body (1.19 %) in mature females. Total sterol percentages of young females, however, were rather low both in haemolymph (1 %) and in fat body (0.53 %).

GLC separation of total sterols revealed that cholesterol was the main sterol, ranging between 82–91 in percentage, together with β-sitosterol in changing ratios (Table II).

Table II. Composition of Sterols in haemolymph and fat body of young and mature females

Sterol	Percentage Composition			
	YH	MH	YFB	MFB
Cholesterol	86.00	81.59	9.58	84.03
β-sitosterol	14.00	18.40	9.42	15.97

YH, Young Haemolymph; MH, Mature Haemolymph; YFB, Young Fat Body; MFB, Mature Fat Body.

Presence of twenty fatty acids with carbon chains ranging between 14 and 22 were shown by GLC analysis in the lipid classes of young and mature female haemolymph and fat body. All the fatty acids examined had unbranched and evennumbered carbon chains with the exception of heptadecanoic acid (17:0). Although shorter chained acids containing less than 14 carbons, were also detected, their proportions were less than 0.01 per cent, hence did not taken into account. Fatty acids with chain lengths longer than 22 carbons were not detected. Members of the n3 and n6 series polyunsaturates were also identified.

Table III and IV show the percentage composition of fatty acids in lipid classes of young and mature haemolymph respectively. In general, higher saturation was observed in young and higher unsaturation was observed in mature haemolymph in the lipid classes examined. Main

Table III. Fatty acid composition of haemolymph lipid classes of young females

Fatty Acid	Percentage of Fatty Acids				
	PL	DG	FFA	TG	EC
14:0	9.29	21.66	26.16	10.55	65.48
16-0	9.59	14.76	30.32	20.60	3.15
16:1	0.30	1.90	3.12	3.96	5.41
16:2	0.82	0.79	0.72	0.76	0.59
17:0	0.37	0.61	0.82	0.74	1.00
18:0	18.60	2.30	14.56	10.51	2.19
18:1	18.02	8.49	14.24	28.43	3.33
18:2	8.52	6.32	1.50	2.25	0.59
18.3	26.67	35.82	1.17	4.10	0.59
20:0	3.03	2.33	0.40	0.28	0.59
20:1	2.38	1.11	–	0.10	–
20:2	0.39	0.68	1.28	0.76	0.63
20:3n6	0.45	–	–	–	2.66
22:1+20:4n6	0.75	1.59	1.63	0.94	4.22
20:4n3	0.16	–	–	–	–
20:5n3	0.37	1.31	2.85	1.03	7.24
22:4n6	0.30	0.45	1.23	15.00	2.35

Table IV. Fatty acid composition of haemolymph lipid classes of mature females

Fatty Acid	Percentage of Fatty Acids				
	PL	DG	FFA	TG	EC
14:0	3.09	3.46	21.31	11.42	48.82
16:0	11.89	19.31	24.40	21.48	4.40
16:1	4.60	5.00	4.78	8.75	7.93
16:2	0.29	0.86	0.88	1.19	0.74
17:0	0.30	0.55	0.54	1.09	1.55
18:0	6.27	1.76	8.79	3.24	1.19
18:1	32.55	14.31	17.06	23.67	6.00
18:2	14.74	7.94	3.06	2.85	1.78
18:3	24.14	45.31	5.93	15.87	3.11
20:0	–	–	0.74	0.46	2.08
20:1	0.76	–	0.49	0.19	0.10
20:2	0.18	0.18	2.12	2.17	1.58
20:3n6	0.21	–	–	–	6.23
22:1+20:4n6	0.25	0.24	2.14	1.95	7.11
20:4n3	0.44	0.15	–	–	–
20:5n3	0.19	0.93	5.99	1.95	7.39
22:4n6	0.10	–	1.15	3.72	–
22:5	–	–	0.61	–	–

saturates and unsaturates found in haemolymph were 14:0, 16:0 and 18:1, 18:3 respectively. Higly polyunsaturated fatty acis, with chain longer than 18 carbons were observed in trace or small amounts in all the lipid fractions of the tissues examined, with the exception of SE fractions.

Present data indicate that fatty acid constitution of the lipid fractions revealed qualitative and quantitative differences among each other as well as with the corresponding fractions of young and mature fat bodies (Table V and VI).

Table V. Fatty acid composition of fat body lipid classes of young females

Fatty Acid	Percentage of Fatty Acids				
	PL	DG	FFA	TG	EC
14:0	5.30	55.24	19.52	0.28	39.60
16:0	8.25	6.34	17.27	7.26	1.00
16:1	0.29	2.39	2.10	16.52	3.23
16:2	1.00	0.80	0.49	2.58	0.40
17:0	0.52	0.62	0.62	0.75	0.39
18:0	15.99	3.58	25.92	0.45	0.45
18:1	16.21	6.54	17.19	16.76	17.78
18:2	12.20	2.46	3.20	8.13	7.48
18:3	37.19	12.82	2.18	46.31	16.54
20:0	–	0.45	0.46	–	–
20:1	1.56	–	0.35	0.13	–
20:2	0.27	2.63	1.43	0.08	3.28
20:3n6	0.54	–	–	0.07	–
22:1+20:4n6	0.28	3.64	2.01	0.16	1.77
20:4n3	0.34	–	–	0.29	–
20:5n3	0.06	2.50	5.87	0.14	6.40
22:4n6	0.01	–	1.40	0.03	1.68
22:5n6	–	–	–	0.02	–
22:5n3	–	–	–	0.04	–

Table VI. Fatty acid composition of fat body lipid classes of mature females

Fatty Acid	Percentage of Fatty Acids				
	PL	DG	FFA	TG	EC
14:0	1.46	30.45	5.93	2.78	67.80
16:0	8.11	13.25	11.79	21.63	2.09
16:1	2.57	2.54	1.59	9.09	1.73
16:2	0.57	0.52	0.46	0.21	–
17:0	0.36	1.82	0.15	0.34	–
18:0	14.90	7.85	64.36	2.17	0.66
18:1	31.08	18.54	7.10	29.50	2.47
18:2	9.03	3.57	0.94	4.13	–
18:3	29.36	12.12	1.33	28.16	1.32
20:0	–	0.47	0.15	0.31	–
20:1	1.03	–	–	0.23	–
20:2	0.12	2.73	1.03	0.05	4.39
20:3n6	0.15	–	–	0.16	–
22:1+20:4n6	0.19	3.83	1.11	0.33	9.89
20:4n3	0.35	–	–	0.30	–
20:5n3	0.08	2.30	3.60	0.14	7.50
22:4n6	0.10	–	0.46	0.21	2.14
22:5n6	–	–	–	0.26	–

DISCUSSION

In addition to its role as the prime metabolic structure, fat body acts as a storage organ in insects. The funtion of fat body during vitellogenesis in egg vesicles seems to be determined by the increasing demand for those materials that had been produced and stored in the course of nymphal instars (Woodring *et. al.*, 1979). It seems likely that these materials could only be used by the developing oocytes after their release from the fat body and transference via haemolypmh.

Correlation between the ovarium development and the disappearance of fat body has shown to be true for many insects (Dutkowski and Ziajka, 1970). Coincident with the final stages of yolk deposition, weight of fat body decreases significantly to reach its minimum after ovulation (Gillott and Elliott, 1976), Most of the fat body mass was used during oogenesis in *Acheta domesticus* (Clifford and Woodring, 1986). The mass of ovulated eggs in this insect was about five times higher than the mass lost by the fat body, which clearly shows that these eggs were primarily produced from ingested materials that are processed by fat body. The amount of lipid released by fat body increased if the dietary supply was experimentally interrupted in *Pyrrhocoris apterus* (Martin, 1969a). Females, which were deprived of food for 2 or 3 days before oviposition, developed mature oocytes by the usage of fat body. Hill *et. al.* (1968) also showed a positive relationship between yolk deposition progress and fat body mass loss in *Schistocerca gregaria*. Total fat body mass loss was taken into account in the above mentioned studies, which clearly indicate an interdependance between fat body and developing ovaries. Despite the existance of a good deal of information concerning the protein metabolism associated with egg maturation (Zhai *et. al.*, 1987), related studies on lipid metabolism are limited (Mwagni and Goldsworthy, 1980). Data on the composition of fat body and haemolymph lipids during this period are especially rare (Nelson *et. al.*, 1967).

Changing functions of fat body, which is the main lipid storage site, according to pre- and postivitellogenic phases of *C. morosus* were investigated together with the differences in lipid composition of haemolymph and fat body between the above physiological stages. An important decrease (from 23 % to 6 %) was observed in the total lipid content of fat body as the insect passes to postvitellogenic phase, while the total lipid content haemolymph remained rather unchanged. A decrease in TG and increase in all the other, especially PL fractions, were observed

between the lipid classes of YFB and MFB. Corresponding changes between the YH and MH were a decrease in FFA and SE fractions and an increase in all the remaining fractions. Decreases found in haemolymph FFA (from 12 % to 4 %) and the main storage neutral lipid, fat body TG, (from 94 % to 41 %) during vitellogenic phase were probably due to the rising lypolytic activity of fat body as a result of increasing demand by developing oocytes. No production of FFA by fat body was observed after ovaritectomy in *Galleria mellonella* (Dutkowski and Ziajka, 1972) A rapid increase, with a maximum prior to ovulation was observed in the total lipid content of developing ovaries during oogenesis in *Leucophaea maderae* (Gilbert, 1967).

Changing patterns found in the lipid classes of fat body and haemolyph might be an indication of the dynamic states of these metabolites during ovarium development. A hormonal factor may also be engaged in this control. This is in agreement with the hypotesis put forward by Martin (1969b), that such a factor might be responsible for the effects of ovaries in release and uptake of fat body lipids. Considerable work has been done in order to explain the relationship between ovaries and fat body by ovaritectomy. Removal of ovaries caused a hypertrophy of fat body in most cases (Butterworth and Bodenstein, 1968). Ovariectomy increased the blood volume and total plasma protein (Elliott and Gillott, 1977), accumulated vitellogenin (Bradley and Edwards, 1978), increased the level of lipophorin (Mwagni and Goldswothy, 1980), A dramatic increase in lipid concentration with an increase in the volume of haemolymph also observed after ovaritectomy (Lee and Goldsworthy, 1976). Low levels of lypolytic activities of fat body of ovaritectomized insects at periods corresponding vitellogenesis in *G. mellonella* was explained by the lack of demand for fat body lipids, normally evoked by vitellogenesis (Dutkowski and Ziajka, 1972). High juvenile hormone titre is also essential in insects for the deposition of yolk in developing ovaries (Koeppe *et. al.*, 1985; Venkatesh *et.a.l.*, 1988). It directs vitellogenin production and release from fat body and controls the uptake of vitellogenin and lipids into oocytes (Engelman, 1983)

A high ecdysteroid peak was observed at the beginning of oocyte development of *Aedes detritus* and *A. caspius* females (Guilward *et. al.*, 1984). It is well known that the insect ovary contains a wide range of members of ecdysone family and most of these ecdysteroids are undoubtedly originated from ovaries (Hetru *et. al.*, 1978) Neurosecretory hormones from the brain stimulates ecdysone synthesis in the previtello-

genic ovary of dipters (Postlethwait *et. al.*, 1980; Huybrects and De Loof, 1982), Unusally high concentrations of SE (14 %) found in previtellogenic haemolymph of *C. morosus* might be due to the sterol need of ovaries for ecdysteroid synthesis. The role of ecdysone during vitellogenesis is suggested to be the stimulation of vitellogenin synthesis in the fat body of *A. eagyptil* (Hagedorn and Fallon, 1975). Ovaries convert labelled cholestrol into ecdysone in adult females of *Locusta migratoria* (Lagueux *et. al.*, 1977). Cholesterol also has been reported to function either directly or indirectly to stimulate the ovarian development (Robbins and Shortino, 1962).

As stated by Shapiro *et. al.*, (1988), one of the functions of female specific lipoprotein vitellogenin, 10 % of which are lipids, is to transport lipids from their synthesis sites (fat body) to the sites where they serve as energy stores in embryogenesis and early larval life (oocytes). The increase in 14 C-palmitat incorporation into haemolymph and ovarium lipids supports the influence of developing oocytes on lipid biosynthesis in *G. mellonella* (Dutkowski and Ziajka, 1972). Highest concentrations of palmitic acid (16:0) found in the FFA fraction of young haemolymph in the present study might be the result of the same need.

TG is the main storage fraction of the fat body, hence differences observed in the TG fatty acid composition between young and mature fat body of *C. morosus* might reflect the utilisation of fatty acids, such as 16:1, 18:2 and 18:3. Changes found in the fatty acid composition of lipid classes of haemolymph might be mainly due to their incorporation into the transport lipoproteins as a result of preferential uptake and utilisation by the developing ovaries as well as the dynamic process of their absorbtion and release from fat body.

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ÖZET

Partenogenetik bir tür olan *Carausius morosus* Br. (Orthoptera: Phasmatidae)'un dişilerinde yağ dokusu ve hemolenfin lipid ve yağ asidi bileşenleri genç ve olgun bireylerde vitellogenez öncesi ve sonrası evrelerde incelendi. Dokuların lipid kompozisyonu kalitatif olarak İnce – Tabaka Kromatografisi, kantitatif olarak Fotodensitometre yöntemleri ile belirlendi.

Genç dişilerin yağ dokularında olgun bireylerdekinin üç katından fazla total lipid bulunarak bunun % 93 ünü Triaçilgliserollerin oluşturduğu saptandı. Serbest yağ asitleri ile sterol esterleri en yüksek oranda genç dişilerin hemolenfinde, diasilgliserol ve steroller ise olgun evrenin hemolendinde saptandı.

Fosfolipidlerin olgun bireylerde hemolenf ve yağ dokusu total lipidlerinin yarıdan fazlasını oluşturduğu belirlendi. Fosfatidiletanolamin ve fosfatidilkolin başlıca fosfolipidler olarak bulundu.

Lipid sınıflarının yağ asidi bileşenleri ve sterollerin kompozisyonu Gaz-Likid Kromatografisi yöntemi ile incelendi. Steroller başlıca kolestrolden oluşup, en yüksek oranda olgun bireylerin hemolenfinde bulunduğu saptandı. Karbon zinciri uzunluğu 14 ile 22 arasında değişen yirmi yağ asidi tanımlanarak bunların dokular ve evreler arasında lipid sınıflarına göre dağılımları incelendi.

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