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FATTY ACID COMPOSITION OF ACYLGLYCEROL AND FREE FATTY ACID FRACTIONS OF FAT BODY, HAEMOLYMPH AND MUSCLE LIPIDS IN *TENEBRIO MOLITOR L.* (COLEOPTERA: TENEBRIONIDAE) LARVAE

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ABSTRACT

The fatty acid compositions of lipid classes in non-esterified fatty acid and in acylglycerol fractions of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae were identified in fat body, haemolymph and muscle by Gas-Liquid Chromatography.

The identified 18 fatty acids had straight, mostly odd numbered carbon chains with C numbers ranging from 14 to 22. Some of the n3 and n6 members of polyenoics were also separated. Quantitative and some qualitative differences were observed in fatty acid compositions between the lipid classes of the same tissue and between a given lipid fraction of all tissues.

The main saturated fatty acid was C 16:0 in all the lipid classes. All the other saturates, namely C 14:0, C 17:0, C 18:0 and C 20:0, were also existed in various proportions. In FFA fractions C 18:0 was distinctively higher than in acylglycerol fractions.

High unsaturation was observed primarily as a result of high proportions of C 18: 1 and C 20:4 n3 in 1,2-DG, TG and FFA fractions and in MG and 1,3-DG fractions respectively.

INTRODUCTION

Several factors have been reported regarding the changes in the fatty acid patterns of insects. Some characteristics of the fatty acid composition appear to be taxonomically related (Thompson, 1973). Characteristic fatty acid composition, reported by Thompson (1973) for terrestrial insect orders, was shown to be true for the related aquatic species (Hansen *et al.*, 1985).

Insects show some changes in fatty acid compositions during metamorphosis (Richeson, *et al.*, 1971; Cookman *et. al.*, 1984) and in larval stage (Lee *et al.*, 1975; Dwyer, *et. al.*, 1986). Factors such as rearing conditions (Grau and Terriere, 1971), diet, sex and extend of activity (Nation and Bowers, 1982) were also found to be effective on the fatty acid composition of insects.

Thomas (1974) suggested that variations in the composition of fatty acids, which might occur even in closely related species, could be due to the metabolic differences between individuals.

Analysis of fatty acids in total body lipids would only reflect the fatty acid composition of major lipid fraction of the fat body, namely triacylglycerol. Moreover, fatty acid composition of individual lipid classes in several tissues have to be known for a better understanding of metabolic procedures. The above mentioned studies were performed on the whole body extraction of total lipids, hence little information is available on the fatty acid composition of individual lipid fractions of whole insects or insect tissues (Thomas, 1974; Hoppe *et. al.*, 1975; Stanley–Samuelson and Dadd, 1981).

Reviews on fatty acid analysis of insect tissues (Fast, 1964 and 1970; Dadd, 1981) reveal a general absence of records on long–chained polyenoic fatty acids which were presented as structurally and functionally very important components in the following recent studies. Polyenoic unsaturates are essential components for proper membrane functions (Hanson *et. al.*, 1985). They are also important as necessary procursors for 1 and 2 series prostaglandins (Loher *et. al.*, 1981; Stanley –Samuelson and Loher, 1983) and as being aessential nutrients for several mosquito species (Stanley–Samuelson and Dadd. 1981).

Present study was carried out to identify the fatty acid composition, including some members of n3 and n6 series of polyenoics, mono–, di– and triacylglycerol and free fatty acid fractions of fat body, haemolymph and muscle of *Tenebrio molitor* larvae in order to obtain information on the fatty acid moieties of different lipid fractions of these tissues.

MATERIALS AND METHODS

Rearing conditions of the larvae, preparation of tissues and extraction of total lipids were performed as described previously by Üner (1988a).

Lipid classes were separated by Thin-Layer Chromatography (TLC) using sillica-gel G (Merck, Darmsadt, W. Germany) coated plates, 500 μm in thickness. The plates were developed in hexane-ether-formic acid (80: 20:2 v/v/v) solvent mixture. After development, regions containing the separated lipids were located by visualition ender UV light, of authentic standarts (Sigma, St. Louis, MO., USA) in reference lanes, which were sprayed with 2,7-dichlorofluorescein. The desired unsprayed bands of mono-, di- and triacylglycerols (MG, DG and TG) and free fatty acids (FFA) were scraped off and transferred into the test tubes.

Fatty acid moieties of lipid fractions were methylated according to the method described by Christie (1972) for gas-liquid chromatogdarhy (GLC). One ml pentadecanoic acid (C 15: 0, 3 mg/ml)was added, as internal standart to each fraction. 1 ml dichloromethane (BDH Poole, UK) and 2 ml 2N sodium methoxide were added to TG fraction, 2ml boron trifluoride methanol complex (BDH) to FFA fraction and 1 ml 2N sodium methoxide (BDH) to MG and DG fractions for methylation. The mixtures were allowed to methylate at 50°C for 30 min. after vigorous shaking. The upper phase was transferred into a tube which contained potassium bicarbonate and water and then 0.3 ml acetic acid, 3-4 ml water and 5-6 ml diethyl ether were added. The resulting methyl esters were mixed with 5 ml of hexan and dried over anhydrous sodium sulphate. Esters were ready for GLC analysis by making the necessary volume adjustments. All the critical stages of the experiments were carried out in an inert atmosphere of nitrogen. The solvents used were reagent grade.

Analysis of fatty acid methyl esters were performed on Varian Aerograph (1400 and 1700 series) gas chromatographs equipped with hidrogen flame ionization detector (HFID). The glass couled columns 2m in lenght and 1.8 mm in inner diameter were packed with 10 % ethylene glycol succinate on 125/150 mesh (EGSS-X) and 100/120 mesh (EGSS-Y) Gaschrom P. Column temperature was maintained isothermically at 185°C; injector and detector temperatures were 210°C and 220°C respectively. Nitrogen was the carrier gas at a flow rate of 40 ml/min. 2 μl aliquot of sample was injected for each seperation.

Identification of methyl esters was achieved by comparing their relative retention times with those of authentic standarts (Sigma) which were analysed daily to evaluate the efficiency of the chromatog-

raphic seperation. Peaks were quantified using an authomatic chart integrator (Varian Aerograph) and relative quantities of individual fatty acid methyl esters were recorded as percentages of the total peak area.

The fatty acid shorthand used is; Number of carbons: Number of double bonds. EGSS–Y was used for seperation of antioxidant BHT (Butylated hydroxytoluen, Merck) from C 14:0, C 18:3 from C 20:1 and C 22:1 from C 20:4 n6.

RESULTS

GLC analysis revealed the presence of at least 15 fatty acids with carbon numbers ranging between 14 and 22 in the acylygcerol and free fatty acid fractions of the neutral lipids of fat body, haemolymph and muscle. All the fatty acids examined had unbranched and even numbered carbon chains with the exception of heptadecanoic acid (C: 17: 0). Although shorter chained (C<14:0) acids were detected, their proportions were lower than 0.01 per cent, hence, did not taken into acound. Members of the n3 and n6 (ω3 and ω6) series polyunsaturates were also identified. Fatty acids with chain lengths longer than C 22 were not detected.

Table I, II and III show the percentage composition of fatty acids in neutral acyl and free fatty acid fractions of fat body, haemolymph and muscle respectively. The fatty acids given in these tables are in the order of increasing retention times. Examples of GLC separation of each lipid fraction are given in Figures 1–6.

Table I: Relative percentage composition of fatty acids in acylglycerol and free fatty acid fractions of fat body

Fatty Acid	Per cent fatty acid in			
	MG	1.2-DG	TG	FFA
14: 0	—	—	3.72	0.20
16: 0	4.06	22.18	17.62	20.42
16: 1	0.45	2.03	3.49	2.56
17: 0	0.45	—	—	—
18: 0	0.48	2.37	2.64	10.96
18: 1	0.73	26.10	51.81	20.49
18: 2	2.42	9.64	16.75	9.26
20: 0	—	—	0.07	5.42
18: 3 + 20: 1	1.27	4.15	0.57	0.05
20: 2	3.99	13.73	0.13	5.83
20: 3	—	10.68	0.10	1.38
22: 1 + 20: 4 n6	4.36	0.64	0.90	6.74
20: 4 n3	81.79	8.48	2.20	6.76
20: 5 n3	—	—	—	1.84
22: 4 n6	—	—	—	6.09

Table II: Relative percentage composition of fatty acids in acylglycerol and free fatty acid fractions of haemolymph

Fatty Acid	Per cent fatty acid in				
	MG	1,2-DG	1,3-DG	TG	FFA
14: 0	—	—	—	0.62	6.99
16: 0	7.56	30.81	15.38	20.37	31.60
16: 1	1.72	1.74	—	4.48	4.46
17: 0	—	—	—	0.55	1.60
18: 0	0.67	0.87	0.95	2.78	10.58
18: 1	1.49	31.08	22.47	50.12	23.05
18: 2	2.41	14.93	8.38	14.57	5.47
20: 0	—	—	—	3.85	2.34
18: 3 + 20: 1	—	—	—	0.59	—
20: 2	0.32	—	1.89	0.82	2.39
20: 3	—	—	—	—	1.32
22: 1 + 20: 4 n6	0.38	0.58	1.76	0.94	4.29
20: 4 n3	85.45	19.99	49.17	0.31	5.90

MG, monoacylglycerols: 1,2-DG, 1,2-diacylglycerols: 1,3-DG, 1,3-diacylglycerols; TG, triacylglycerols; FFA, free fatty acids,

MG, Monoacylglycerols: 1,2-DG, 1,2-diacylglycerols; TG, triacylglycerols; FFA, free fatty acids.

Table III: Relative percentage composition of fatty acids in acylglycerol and free fatty acid fractions of muscle

Fatty Acid	Per cent fatty acid in		
	1,2-DG	TG	FFA
14: 0	—	4.10	6.38
16:0	16.60	18.76	23.17
16: 1	3.55	3.76	2.86
18: 0	11.83	2.85	7.81
18: 1	26.64	50.41	24.29
18.2	24.46	19.02	16.55
20: 0	1.85	0.25	—
18: 3 + 20: 1	0.59	0.37	4.45
18· 4	—	0.02	—
20: 2	4.14	0.11	1.27
20: 3	4.43	0.04	1.11
22: 1 + 20: 4 n6	5.91	0.27	4.67
20: 4 n3	—	0.04	7.44

1,2-DG, 1,2- diacylglycerols; TG, triacylglycerols; FFA, free fatty acids.

Although the mixtures of C 18:3 + C 20:1 and C 22:1 + C 20::4 n6 could be separated by EGSS–Y column, the values for these were given in summation because of the very low percentage of C 20:1 and C 22:1 in the mixtures.

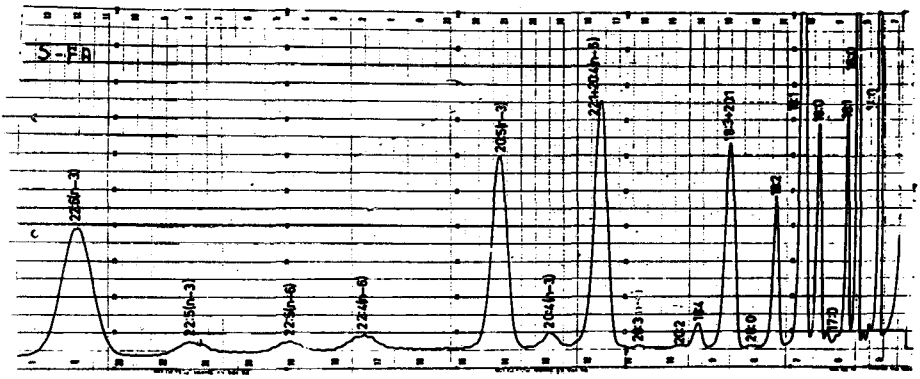


Figure 1. Separation of the standard fatty acid mixture by GLC. Column EGSS-X on Gas Chrom P, 125–15; mesh; Column temp., 185°C; Carrier ges, N_2; Flow rate, 40 ml / min; De tector, HFID; Attenuation, X4; sample volume, 2 μl.

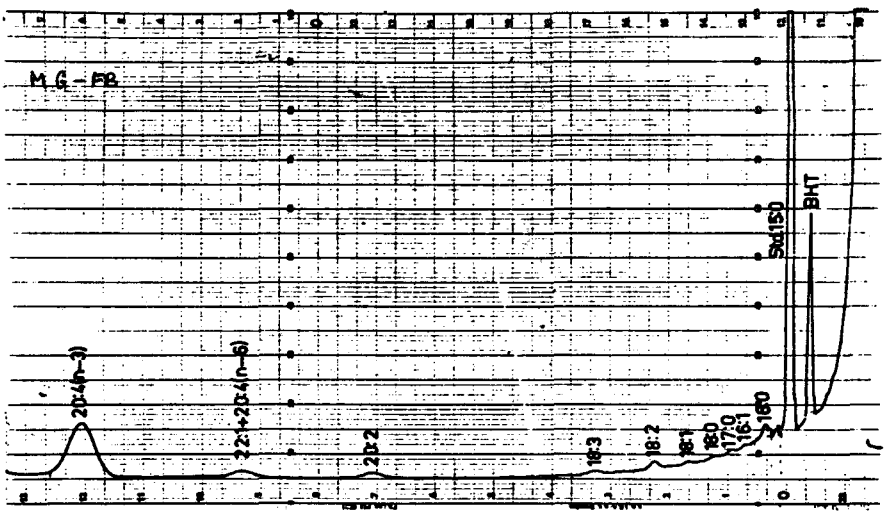


Figure 2. GLC separation of monoglyceride fatty acids in fat body. Column EGSS-X on Gas Chrom P, 125–150 mesh; Column temp., 185°C; Carrier gas, N_2; Flow rate, 40 ml / min; Detector, HFID: Attenuation, X4, X16; sample volume, 2 μl.

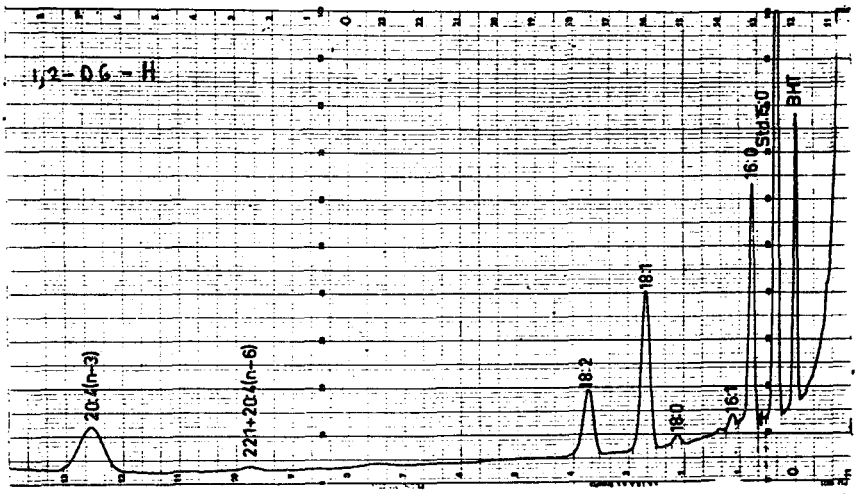
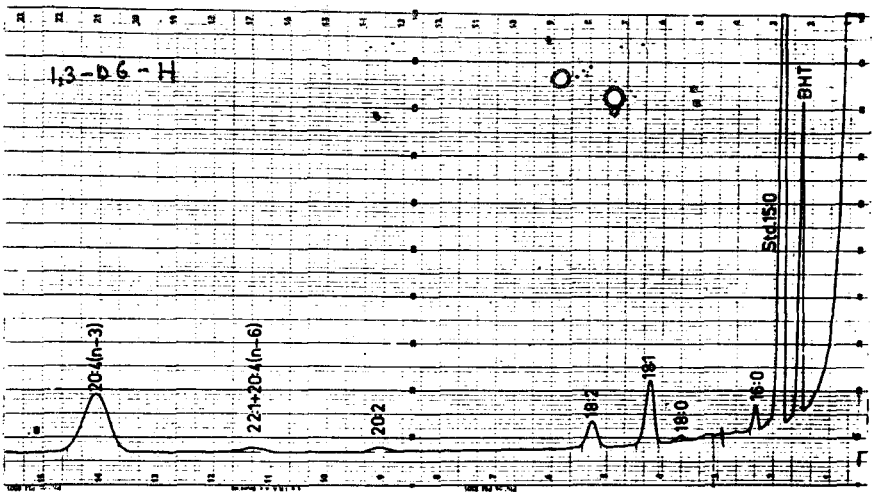


Figure 3. GLC separation of 1,2-diglyceride fatty acids in haemolymph. Columm EGSS-X on Gas Chrom P, 125–150 mesh· Column temp., 185°C; Carrier gas, N_2; Flow rate, 40 ml/min; Detector, HFID; Attenuation, X4, X16; sample volume, 2 µl.



Fugure 4. GLC separation of 1,3-diglyceride fatty acids in haemolymph. Column EGSS-X on Gas Chrom P, 125–150 mesh; Column temp., 185°C; Carrier gas, N_2; Flow rate, 40 ml/min; Detector, HFID; Attenuation, X4, X16; sample volume, 2 µl.

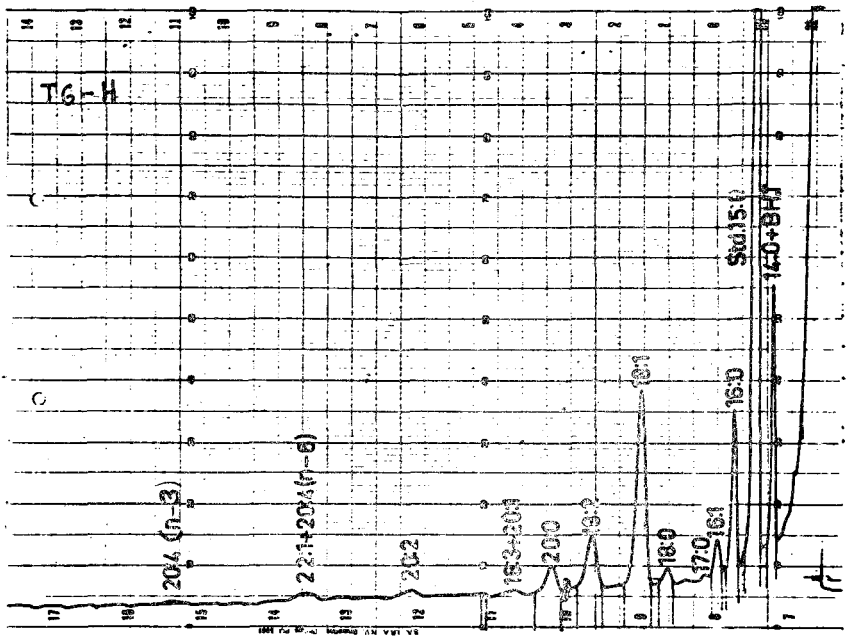


Figure 5· GLC separation of triglyceride fatty acids in haemolymph. Column EGSS-X on Gas Chrom P, 125–150 mesh; Column temp., 185°C; Carrier gas, N_2; Flow rate, 40 ml/min; Detector, HFID; Attenuation, X4, X16; sample volume, 2 µl.

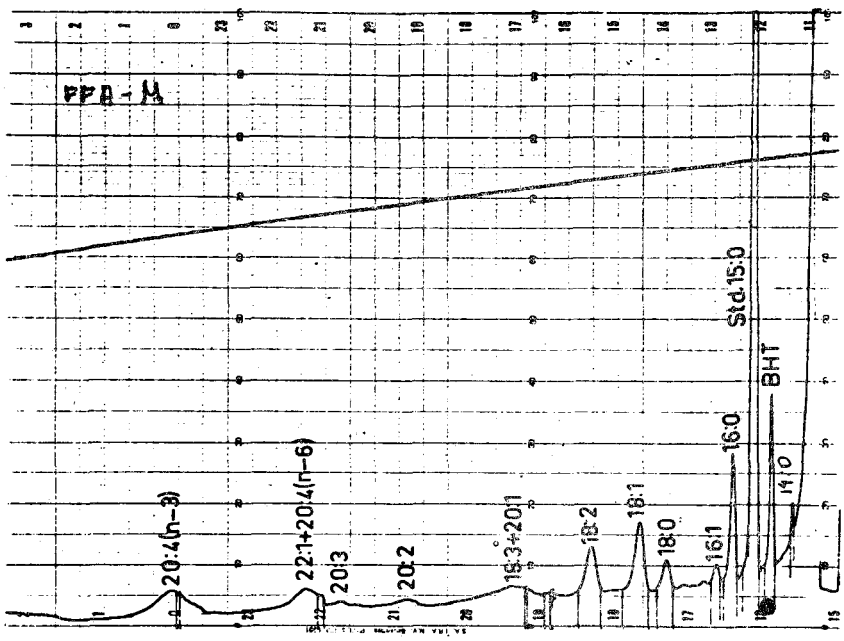


Figure 6. GLC separation of free fatty acids in muscle. Column EGSS)X on Gas Chrom P, 125–150 mesh; Column temp., 185°C; Carrier gas, N_2; Flow rate, 40 ml/min; Detector, HFID; Attenuation, X_{2}, X16; sample volume, 2 µl.

Present data incate that fatty acid profiles of the lipid fractions reveal qualitative and quantitative differences among themselves as well as with the corresponding fractions of the other tissues.

MG, by containing the highest percentages of C20:4 n3, in fact about 82 and 86 per cent in fat body and haemolymph respectively, differed from all the other lipid fractions, except 1,3–DG which also contained a very high percentage of C 20: 4 n3 and only existed in haemolymh.

Palmitic (C 16:0), linoleic (C 18:2) and linolenic (C 18:3) acids were the major fatty acids in the 1,2–DG, TG and FFA fractions of all the tissues examined. Oleic acid (C: 18:1), which constituted upto 52 per cent of the total fatty acids, had the highest percentage of fatty acids present in the above mentioned fractions. Linoleic acid was the second unsaturated (15–19 %), while palmitic acid was the most common saturated fatty acid, the highest percentage (32 %) of which was found in the FFA fraction of haemolymph.

The fatty acid composition of TG differed markedly among lipid classes of the tissues in constituting very small proportions of fatty acids with chain lengthes longer than 18 carbons.

FFA fractions of the tissues generally were distinctive in their higher percentae of steaic acid (C 18:0) content than acylyglycerol fractions, except 1,2–DG of muscle. FFA of the fat body was the only fraction which comprised C 20:5 n3 and C 22:4 n6 polyenoics.

In general, unsaturates were the predominant fatty acids, being over 60 per cent of almost all the lipid fractions of the tissues except the FFA of haemolymph in which 53 per cent of the total fatty acids was saturates (Table IV).

Table IV: Proportions of saturated and unsaturated fatty acids as percentage of total fatty acids ib acylglycerol and free fatty acid fractions of the tissues.

	Per cent saturates and unsaturates in									
	MG		1,2–DG		1,3–DG		TG		FFA	
Tissue	S	VS	S	VS	S	VS	S	VS	S	VS
Fat Body	4.99	95.01	24.55	75.45	—	—	24.05	75.95	37.00	63.00
Haemolymph	8.23	91.77	31.68	68.32	16.33	83.67	28.17	71.83	53.12	46.88
Muscle	—	—	30.28	69.72	—	—	25.96	74.04	37.36	62.64

MG, monoacylglycerols; 1,2-DG, 1,2–diacylglycerols; 1,3–DG, 1,3–diacylglycerols; TG, triacylglycerols; FFA, free fatty acids, S, saturated; VS, unsaturated.

Unsaturation was primarily due to the presence of high percentages of oleic acid in 1,2–DG, TG and FFA fractions. The reason for unsaturation in 1,3–DG and MG fractions, however, was the presence of C 20:4 n3, comprising over 50 per cent of the total fatty acids.

Polyenoic fatty acids, constituted high percentages of unsaturated faty acids ranging from about 52 to 99 per cent in all the lipid fractions examined, excep TG and haemolymph FFA fractions in which monousaturates were predominant.

DISCUSSION

The fatty acid composition of *Tenebrio molitor* larvae was generally in agreement with whole–body fatty acid analysis of other coleopterans, with the exception of long–chained polyunsaturates which were found in high proportions in MG and DG fractions. Oleic acid is the common fatty acid in coleopteran lipids and is followed by palmitic and linoleic acids (Fast, 1964 and 1966). Kok and Norris (1972), however, found palmitic acid to be slightly higher in concentration that oleic acid in neutral lipid fraction of *Xyloborus ferrigineus* female. Richeson *et al.* (1971) examined the fatty acids of *Ipps calligraphus* (Germar) in five developmental stages, and found the predomination of oleic and palmitic acids. In newly emerged male and female of *I. paraconfusus* (Lanier), oleic was the highest in concentration. followed by plamitic (Penner and Barlow (1972). This was also true for *Dendroctonus frontalis* (Hodges and Barras, 1974), in whic oleic was the most abundant fatty acid.

All the above whole–extraction data is only comparable with present TG data. Hence fatty acid composition determined for whole–body extracts would generally do not represent the fatty acid composition of individual tissues, except fat body or of lipids, except TG. Fatty acids of TG may dilute the minor fatty acids of other fractions below the level of detection in whole–body or in unfractioned lipids. Therefore, quantitatively minor fatty acids might be more apperent in specific tissues or fractions, such as C 22:4 n3 in the MG of haemolymph and fat body and in the 1,2–DG and 1,3–DG of haemolymph in *T. molitor*.

Percentage composition of fatty acids in fat body TG of *T.molitor* was in complete agreement with the above mentioned reports in having highest proportions of oleic, followed by palmitic and linoleic acids.

This was also the general pattern for haemolymph and muscle TG fractions, which contained very small proportions of fatty acids with carbon chains longer than 18 and differed from MG, DG and FFA fractions in this respect. Large increase in the proportions of monoenoic fatty acids in animal tissues, as found in present TG fractions of *T. molitor*, was reported as a characteristic of polyenoic defficiency (Holman, 1968). In contrast with the high C 18:1 in TG, C 18:2 was the highest unsaturate in phospholipids of *T. molitor* (Stanley–Samuelson and Dadd, 1983; Üner 1988b) and of other insects (Thomas, 1974; Hoppe *et al.*, 1975; Downer, 1978; Stanley–Samuelson and Dadd, 1981).

FFA fractions comprised high proportions of C 18: 0 and relatively high C 22:1 and C 22:4 n3 in the tissues examined. This fraction contained the longest (C 22:4 n6) and the highest polyenoics (C 20: 5 n3 in the fat body.

In most of the insects examined so far, unsaturates accounted a greater proportion of the fatty acids (Beenakkers and Gilbert, 1968; Thomas, 1974). In approximately two–thirds of the listed species in Fast's review (1970), which were primarily phytophagous, 60 to 80 per cent of the fatty acids were unsaturated. Coleopteran species *Anthonomus grandis* (Lambremont and Blum, 1963), *I. calligraphus* (Richeson *et al.*, 1971) and *I. paraconfusus* (Penner and Barlow, 1972) were shown to contain 62, 62 and 72 % total unsaturates respectively in their fatty acids.

Monoenoic and C 14–18 saturated fatty acids can ve synthesized from two carbon units which are metabolized from food constituents, such as carbohydrates and amino acids (Downer, 1978). Insects, however, do not have the ability of biosynthesizing either linoleic or linolenic acids or both (Downer, 1978; Blomquist *et. al.*, 1982; Dwyer *et. al.*, 1986). It was shown by nutritional studies, on the other hand, linoleic or linolenic (Turunen, 1974; Dadd, 1983) or both acids (Sivapalan and Gnanapagasam, 1979; Dadd, 1981) are essential nutrients for many insects. Although it is generally accepted that linoleic and linolenic acids are direct procursors of higher polyunsaturates, which can be derived by elongation and further desaturation from their procursors (Stanley–Samuelson, and Loher, 1983), arachidonic and structurally related long chained polyenoics were recently reported as essential nutrients for mosquitoes (Stanley–Samuelson and Dadd, 1981).

It was demonstrated that *T. molitor* needs one per cent of linoleic acid in its synthetic diet for optimal growth (Davis and Sousolski, 1973). High percentages of linoleic acid were observed in neutral lipids of phytophagous insects whose food lipids contain predominant amount of this acid (Fast, 1970). This generalization was also true for *E. molitor* larvae, whose food was reported to contain 56 % of linoleic acid (Nelson *et. al.*, 1963). The fatty acid compositions of insects is known to be restricted by their taxonomic characters, (Thompson, 1973; Hanson et al., 1985) since lipogenesis is primarly under the control of the genes. Among the hundreds of fatty acid analysis reported, a very few of them record long-chained polyunsaturates beyond C 18:3 (Fast, 1964 and 1970; Jenkin *et. al.*, 1976; Stanley-Samuelson, and Dadd 1981), despite the fact that C 20 polyunsaturates are necessary procursors of prostaglandins in insects (Destephano and Brady, 1977; Setty and Ramajah, 1979), The results of fatty acid analysis of lipid fractions, reported for various insect species of different orders, included phospholipids and triacylglycerols from the total-body extracts of *T. molitor*, although the developmental stage of the insect was not noted (Stanley -Samuelson and Dadd, 1983). When compared with the present results, the amount of C 18: 2 in TG was in accordance in both studies, but in the former research no polyunsaturates was recorded below C 18:2, except C 20:3 n6. Whereas the results of the present study showed that TG contained C 18:3, C 20:2, C 22:1, C 20:4 n6 and C 20:4 n3 in changing proportions.

Percentage of fatty acids with chain lengths shorter than 16 carbons were rather high in the aggs of *D. frontalis* and decreased markedly in the larvae (Hodges and Barras, 1974). Fatty acid sythesis in young larvae of *Trichoplusia ni* is relatively low, particularly in regard to unsaturated components, while synthesis increased and unsaturates accumulated towards the end of the last larval instar (Stephen and Gibbert, 1969). Based on this finding, Stephen and Gilbert (1970) suggsted an inverse correlation between the rates of the fatty acid synthesis, desaturation and the levels of juvenile hormone present. General lipogenesis observed in *T.ni* during larval development also appeared to be related with hormone levels (Dwyer, 1986).

In a number of studies on lepidopteran species, fluctuations was shown to occur in the levels of juvenile hormone, juvenile hormone esterase and ecdysone during late larval development (Spark *et al.*, 1979; Riddiford, 1980; Wing *et. al.*, 1981). High proportions of unsa-

turates found in the last larval stage of *T. molitor* (Table IV) might be attributed to their functions as structural components and as procurs of ecdysial hormones in restructuring the larval tissues. Phospholipids (Stanley–Samuelson and Dadd, 1983; Üner, 1988b) and sterol ester fractions (Üner, 1988c) were also shown to contain exceeding amounts of unsaturates.

Variations observed in fatty acid composition of neutral acyl fractions of fat body and haemolymph suggest that the release of lipid classes do not occur at random, but some specific fatty acids are preferentially included in these lipid classes, such as the high amounts of C 20:4 n3 in MG and DG fractions of *T. molitor*. Specific activity differences of an enzyme in the regulation of fatty acid turnover for a given lipid class between the tissues (Linsday and Barlow, 1970), may suggest that various fatty acid components of lipid fractions of different tissues are arranged according to a specific physiological role.

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ÖZET

Tenebrio molitor L. (Coleoptera: Tenebrionidae) larvalarında asilgliserol ve esterleşmemiş yağ asidi fraksiyonlarının yağ asidi bileşenleri yağ dokusu, hemolenf ve kasta gaz–likit kromatografisi yöntemi ile belirlenmiştir.

Tanımlanan yağ asitleri genellikle çift karbon sayılı düz zincirli ve zincir uzunlukları 14–22 karbon arasında olan asitlerdir. Uzun zincirli doymamış yağ asitlerinden n3 ve n6 serilerine ait bazı asitler de ayrılmışlardır. Aynı dokunun lipit sınıfları arasında veya farklı dokulardaki aynı lipit fraksiyonları arasında yağ asiti bileşiminde kantitatif ve bazı kalitatif farklar bulunmuştur.

Bütün lipit sınıflarında C 16:0 başlıca doymuş yağ asitidir. Diğer doymuş yağ asitleri olan C 14:0, C 17:0, C 18:0 ve C 20:0 lipit sınıfları sırasında değişen oranlarda bulunmaktadır. C 18:0 serbest yağ asiti fraksiyonunda asilgliserol fraksiyonlarında olduğundan oldukça fazla bulunmuştur.

Lipit sınıflarında genellikle ortaya çıkan yüksek derecedeki doymamışlık 1,2–diasilgliserol, triasilgliserol ve serbest yağ asidi fraksiyonlarında başlıca C 18:1 nedeniyle, monoasilgliserol ve 1,3–diasilgliserol fraksiyonlarında ise başlıca C 20:4 n3 nedeniyle oluşmaktadır.

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