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LIPID COMPOSITION OF THE FAT BODY, HAEMOLYMPH AND MUSCLE IN *TENEBRIO MOLITOR* L. (COLEOPTERA: TENEBRIONIDAE) LARVAE

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ABSTRACT

Total and neutral lipids of the yellow mealworm *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae, which is one of the main stored product pest, were studied in the fat body, haemolymph and muscle. The lipid content of the tissues was determined on the fresh and dry weight basis.

The lipid compositions of the tissues was examined by Thin-Layer chromatography and photodensitometry. Phospholipids were the prodominant lipids of the total lipids in haemolymph and muscle, while triacylglycerols accounted for the major lipids in the fat body.

In the neutral lipid fraction, diacylglycerols were the most abundant lipids of the haemolymph, whereas triacylylycerols were predominant in the fat body and muscle. Sterols, sterol esters and free faty acids were present as well as diacylgycerols and triacylglycerols in all the tissues examined. There were about two times more 1,2-diaclyglycerols than 1,3-diacylglycerols in haemolymph. Monoacylglycerols were not detected neither in fat bodynor muscle, while 1,3-diacylglycerols were not present in fat body.

INTRODUCTION

The importance of lipids in terms of metobolic and structural function in insects is well documented (Gilbert, 1967). Lipids are known to be utilized in various insects as the energy source during oogenesis (Dutkowski and Ziajka, 1972), metamorphosis (Henson et. al., 1972), starvation (Justem et. al. 1975), flight (Van der Horst, 1983) and diapause (Ito, 1986).

Although there have been several studies on the content and compositions of lipids in insects, majority of chemical analysis have been caried on lipids extracted from whole animals (Otto, 1974; Lee *et. al.*, 1975). Only a few studies have been conducted regarding the lipid composition of different tissues (Beenakkers and Scheres, 1971).

The data available on the individual lipid classes of haemolymph are particularly scarce (Stevenson, 1972; Thomas, 1974).

Since we do not yet know the qualitative and quantitative differances of the lipid classes in the Coleopteran *Tenebrio molitor* larvae and because of the obvious importance of the fat body as the organ of lipid biosynthesis and storage, haemolymph as the medium of lipid transport and the muscle as the utilization site, the lipid content and composition of these tissues were studied.

MATERIALS AND METHODS

Fully grown yellow mealworm *T. molitor* larvae were drown from a culture maintained, according to the procedure described by Murray (1960), in the laboratory. Larvae were bred on wheat bran and kept at 25°C and 70 % r.h.

After a cuticular puncture, haemolymph was collected in capillary tubes. The pooled haemolymph (1 ml.) was added to 10 ml. chloroform-methanol (2: 1 v/v) mixture and homogenized. The fat body and muscle were removed following bisectioning the animal under a binocular microscope in Wyatt's medium. Samples were held at –5°C on ice–salt mixture. The dry weight of the tissues were determined by drying and weighing an aliquot of the samples. The pooled tissues from 20 individuals were weighed and immidiately homogenized in Ultraturax by adding ten parts of chloroform–methanol (2:1 v/v) mixture to one part of tissue.

Lipids were extracted according to the method described by Folch *et. al.* (1957). Methanol and other water–soluable fractions were removed and the chloroform fraction was dried over Na_2SO_4. Chloroform was evaporated on rotary evoporator under N_2 streem. The lipids obtained were weighed, redissolved in a known volume of chloroform and stored at –20°C until Thin–Layer Chromatography (TLC) analysis.

Individual lipid fractions were separeted by TLC on 20 x 20 cm silica–gel G layers (Merck, Darmstad, W. Germany), 250 μm in thickness. Plates were activated and developed in hexan–ether–formic acid (80: 20: 2 v/v/v) solvent mixture (Christie *et. al.*, 1970). Detection was made by spraying the developed plates with a 3 % cupric acetate solution in 7 % aqueous H_3PO_4. The plates were beated at 180°C for 25 min. until the sports were sufficiently charred.

The lipid classes were identified by comparing the Rf values with reference standarts (Sigma, St. Louis, MO, California, USA). 2–5 mg of lipid mixture could be separeted from each application. Monoacylglycerols (MG), 1,2– and 1,4–diacylglycerols (1,2–DG; 1,3–DG), free fatty acids (FFA), sterols (S), esters of sterols (ES) and tiacylgycerols (TG) were separeted without an overlap at the concentrations tested. As polar lipids, phospholipids (PL) remain at the origin.

Densitometry was applied for quantification of the lipid classes (Fewster *et.al.*, 1969). Optical densities of the charred spots were measured with a TLC flying–spot densitiometer equipped with linear recorder and integrator (Vitatron, Model TLD 100, Dieren, The Netherlands). All the solvents used in the experiments were reagent grade.

RESULTS

Lipid Composition of the Fat Body

The lipid content of the fat body on fresh and dry weight basis was 18.97 and 41.62 per cent respectively. Polar lipids constituted 14.61 % of the total lipids.

TG, 1,2–DG, MG, FFA, S and ES were identified as the components of neutral lipid fraction by TLC analysis (Fig. 1)

Quantification by densitometry showed that TG formed the predominant part of the neutral lipids, accounting for about 82 % of them. S, ES and FFA, however, were all minor components, accounting for no more than 5 %, while MG and 1,2–DG were found in very small ratios. 1,3–DG were not detected in the neutral lipids of the fat body. Relative percentage compositions of total and neutral lipids are shown in Table I and Table II respectively.

Lipid Composition of the Haemolymph

Total lipids accounted 1.2 % of the haemolymph, of which polar and neutral lipids constituted 66.64 and 33.36 per cent respectively (Table I).

The neutral lipids consisted 1,2– and 1,3–DG, FFA, S, TG, ES and MG fractions in the haemolymph (Fig. 1).

DG, as 1,2– and 1,3–DG, accounted for about 38 % of the neutral lipids. The amount of 1,2–DG was about two times more than 1,3–DG, TG, FFA and S constituted about 20, 13 and 17 per cent of the neutral

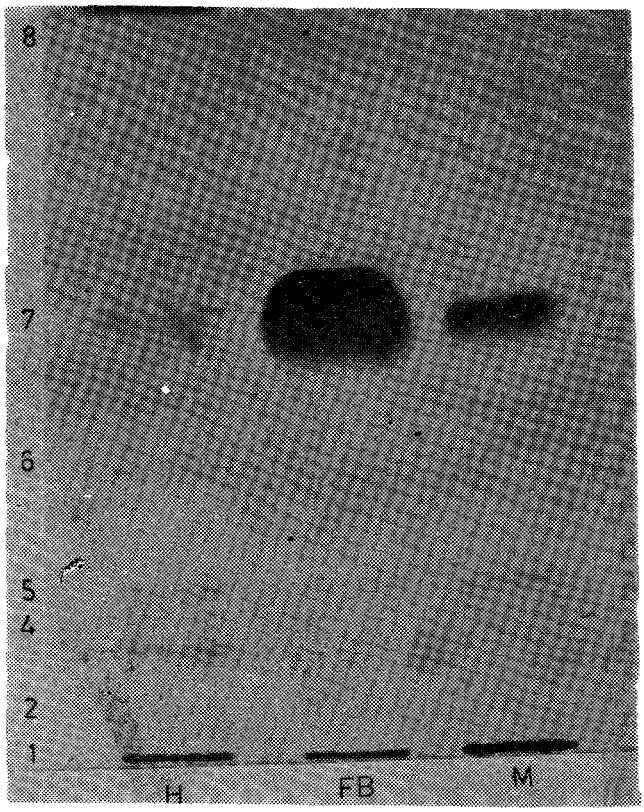

Figure 1. Thin-Layer chromatogram of the lipids in haemolymph, fat body and muscle of *Tenebrio molitor*.

W: Silica-gel : (50 μm); Solvent system, hexan ether-formic acid (80:20:2 v/v/v); detection, 3 % $(CH_2COO)_2Cu$ in 7 % aqueous H_2PO_2.

1. PL; 2. MG: 3. 1,2-DG; 4. 1,3-DG; 5. S; 6. FFA; 7. TG; 8. ES; H, haemolymph; FB, fat body; M, muscle.

lipids respectively. MG and ES were relatively minor components (Table II).

Lipid Composition of the Muscle

The lipid content of the muscle was 1.96 and 12.96 % on fresh and dry weight basis respectively. About 57 % of the total lipids was polar lipids (Table I).

TG was the major component of the neutral lipids, constituting about 68 %, while FFA and S were the secondary constituents. ES

Table I. Relative percentage composition of total lipids in haemolymph, fat body and muscle of *Tenebrio molitor*.

Lipid Fractions	Percentage Composition		
	H	FB	M
PL	66.64	14.61	57.28
MG	2.96	1.18	—.—
1,2-DG	8.76	2.01	2.39
1,3-DG	4.01	—.—	—.—
S	5.60	4.10	4.92
FFA	4.18	4.02	5.48
TG	6.53	70.16	28.96
ES	1.32	3.92	0.97

H, Haemolymph; FB, Fat body; M, Muscle; PL, Polar lipids; MG, Monocylglycerols; 1,2- and 1,2- and 1,3-DG, 1,2- and 1,3-Diacylglycerols; S, Sterols; FFA, Free fatty acids; TG, Triacylgylcerols; ES, Esters of sterols.

Table II: Relative percentage composition of neutral lipids in haemolymph, fat body and muscle of *Tenebrio molitor*

Lipid Fractions	Percentage Composition		
	H	FB	M
MG	8.87	1.38	—.—
1,2-DG	26.26	2.35	5.59
1,3-DG	12.02	—.—	—.—
S	16.79	4.80	11.52
FFA	12.53	4.71	12.83
TG	19.57	82.16	67.79
ES	3.96	4.59	2.27

H, Haemolymph; FB, Fat body; M, Muscle; MG, Monoacylglycerols; 1,2- and 1,3-DG, 1,2- of rols.

was a very minor component (Table II). MG and 1,3–DG were not detected in the neutral lipids of the haemolymph (Fig. 1).

DISCUSSION

Most holometabolous insects accumulate large quantities of fat during their larval life. In some insects lipid synthesis increases sharply in the middle of the Vth instar (Lawrence et. al., 1986), hence in the early pupal stage relative lipid content is about two or three times that of the early stages of the Vth instar (Gilbert and Schneiderman, 1961).

In the present study total lipid content in the fat body of mature *T. molitor* larvae was found 19 and 42 percent on fresh and dry weight basis respectively. Fast (1970) in his review on insect lipids concluded

that three-quarters of the insect species studied, larvae contain on a dry weight basis 30 % lipid. Since the fat body is the main lipid storage tissue in insects, the data on the lipid percentage of the fat body and on the total insects are comparable. Total lipid content of the Coleopteran larvae *Hylobius pales* was 34.1 % on dry weight basis (Richmond and Thomas, 1975) and was 3.6 % and 14.7 % on wet and dry weight basis respectively in *Anthonomus grandis* (Henson *et. al.*, 1972). The lipid content of the haemolymph was reported as 1.5 % in the mature larvae of *Acheta domesticus* (Wang and Patton, 1969) and 2.4 % in *Galleria melonella* (Wlodawer and Wisniewska, 1965). In the present study total lipids accounted 1.2 % of haemolymph of the mature larvae of *T. molitor*. .Phospholipids were the major fraction of the total lipids of haemolymph and muscle. The composition and the fatty acid constituents of these structural polar lipids have been discussed previously by Üner (1988a). The distribution of sterols and ester of sterols among the tissues also have importance in the last larval instar for restructuring larval tissues and as precursors of ecdysial hormons (Üner, 1988b).

In all the insect species investigated TG formed the major fraction of neutral lipids (Gilbert, 1967; Fast, 1970). TG are synthesized and accumulated in the fat body. It has been reported that no TG is synthesised in the haemolymph (Chino and Gilbert, 1965). The insect fat body is metabolically the most important organ in supplying lipid to haemolymph during metabolic needs. It combines some functions of vertebrate adipose tissue and liver.

The predominant lipid fraction of fat body of *T. molitor* larvae is TG, accounting for about 82 % of the neutral lipids. This is in complete agreement with the findings on other insects (Martin, 1969; Dutkowski and Ziajka, 1972). This might be the highest concentration value of larval development since as shown in *Trichoplusia ni*, the concentration of TG increases during larval development (Thompson, 1983) and is utilized during metamorphosis (Henson *et al.*, 1972). The high TG content of the fat body in *T. molitor* probably accounts for the higher rate of lipid biosynthesis in the last instar larvae which is preparing itself for pupation. The lipid reserves accumulated in the fat fody, must be mobilized, transported in the haemolymph and taken up by the tissues, including muscle.

The lipid content of the haemolymph may vary considerably among insect species (Bailey, 1975). However, in all but a few species

examined so far, DG constituted the dominant neutral lipid classes in the haemolymph (Downer and Matthews, 1976; Beenakkers *et. al.*, 1981, 1984a). Fat body is the main site for DG formation (Candy *et. al.*, 1976).

DG was mobilized from the fat body TG stores by the action of lipases (Van der Horst, 1982) under the control of adipokinethic hormone which is released by corpora cardiaca (Van der Horst, 1983). There are some differences in the release of lipids from the fat body in different species of insects. While the major form of lipid released is TG in *Phyrrocoris apterus* (Martin, 1969) and FFA in *G. melonella* (Wlodawer and Lagwinska, 1967), it is DG in *Manduca sexta* (Bhakthan and Gilbert, 1970), *Oncopeltus fasciatus* (Thomas, 1974) and *Locusta migratoria* (Beeakkers *et. al.*, 1984b). Present results showed that DG formed the principal lipids class, accounting for about 38 % of the neutral lipids in haemolymph. Such a high concentration of DG suggested that the release and transport of the neutral lipids mainly occurs in the form of DG in *T. molitor* larvae. DG consisted of about 27 % 1,2–DG and 12 % 1,3 DG in the haemolymph.

There is no report on the role of 1,3–DG in insects although their presence were reported by various authors (Kinsella and Symith, 1966; Agarwal and Rao, 1969; Lawrence *et. al.*, 1986). It seems possible that 1,3–DG have a spesific function in haemolymph because of their absence in neutral lipid fractions of the fat body and muscle.

As to the production of DG from TG in the fat body during flight two possible mechanisms have been proposed. Spencer and Candy (1976) suggested monoacyl cleavage of the stored TG to be the primary route of 1,2–DG production. This pathway may prevail in the fat body of the cockroach (Hoffman and Downer, 1979). An alternative mechanism is the degradation of TG to 2–monoacylglycerol (2–MG) followed by a reacylation to 1,2–DG (Tietz and Weintraub, 1980).

Uptake and transport of DG in haemolymph requires binding to specific lipoproteins, namely LPI and LPII (Chino *et. al.*, 1981), which carries DG to other tissues. Haemolymph lipoproteins have been identified in several insect species, particularly in silkmoth, locusts and cockroaches (Wyatt and Pan, 1978).

In insects, MG and FFA are present either as hydrolytic products of TG in the process of utilization for energy production or absorbed

from diets in such forms (Agrawal and Rao, 1969). The MG content in various life stages of insects was found to be lower than that of DG (Kinsella and Smyth, 1966). In the present study, DG and MG were the very minor components of neutral lipids in the fat body. The ratio of DG to MG was about 2:1.

The failure to detect MG in muscle suggested that catabolism progress was at a slower rate in the muscle. This might be due to the slow motion of mature larvae, which is accumulating its lipid sources for the following pupal stage in the form of TG. The high content of TG in the fat body (about 82 %) and muscle (about 68 %) found in the present study supports the above statements.

ÖZET

Önemli bir ambar zararlısı olan *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvalarında yağ dokusu, hemolenf ve kasın total ve nötral lipidleri incelendi. Total lipid miktarı kuru ve yaş ağırlığa göre belirlendi.

Dokuların lipid kompozisyonu İnce- Tabaka kromatografisi ve fotodansitometre yöntemleri ile tayin edildi. Fosfolipidler hemolenf ve kas lipidlerinin, triasilglyseroller ise yağ dokusu lipidilerinin başlıca komponentleridir.

Nötral lipid fraksiyonunda, diasilglyseroller hemolenfin başlıca lipidini oluştururken, triasilgliseroller yağ dokusu ve kasda en yüksek oranlardadır. İncelenen tüm dokularda diasilglıserol ve triasilgliserollerle birlikte steroller, sterol esterleri ve serbest yağ asitleri bulunmaktadır. Hemolenfte 1,2– diasilgliseroller, 1,3–diasilgliserollerin iki katı kadardır. Yağ dokusu ve kasta monoasilgliseroller ve yağ dokusunda ise 1,3–diasilgliseroller bulunmamaktadır.

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