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Abstract: Phospholipid and triacylglycerol fatty acid compositions from eggs; seventh, eighth and last nymphal stages; and 1, 20- and 40-day-old adult females of the black cricket, *Melanogryllus desertus*, grown in stock–culture medium under laboratory conditions were analyzed by gas chromatographic methods.

A large amount of the fatty acid components at all the development stages of the insects analyzed comprised oleic, linoleic, palmitic and stearic acids. However, palmitoleic, myristic and linolenic acids were also found, at lower levels.

There were fluctuations in fatty acid levels at different development stages, such as nymphs and adults. Oleic acid was lower in 1-day-old and older adults compared to nymphal stages, whereas linoleic acid was higher.

Key Words: Phospholipid, Triacylglycerol, Fatty Acid Compositions, Development Stages, *Melanogryllus desertus*

Melanogryllus desertus (Orthoptera: Gryllidae)'un Çeşitli Gelişim Safhalarında Fosfolipit ve Triaçilgliserol Yağ Asiti İçeriği

Özet: Bu çalışmada, laboratuvar koşullarında stok kültür ortamında yetiştirilen Karaçekirge *Melanogryllus desertus* (Orthoptera: Gryllidae)'un yumurta, yedinci, sekizinci ve son nimf safhası, bir günlük, yirmi günlük ve kırk günlük ergin dişilerinin fosfolipit ve triaçilgliseroldeki yağ asiti bileşenleri, gaz kromatoğrafisi yöntemiyle analiz edilmiştir.

Böceklerin tüm gelişim safhalarında, yağ asitlerinin büyük bir kısmını oleik, linoleik, palmitik ve stearik asitler oluşturdu. Palmitoleik, miristik ve linolenik asitler ise daha düşük oranda bulundu.

Nimf ve ergin gibi farklı gelişim evrelerinde , yağ asitlerinde artma ve azalmalar kaydedildi. Bir günlük ve daha yaşlı erginlerde, nimflere oranla oleik asit azalırken, linoleik asit arttı.

Anahtar Sözcükler: Fosfolipit, Triaçilgliserol, Yağ Asiti İçeriği, Gelişim Safhaları, *Melanogryllus desertus*

Introduction

Fatty acids serve various functions in insects. They are the primary energy source during periods of nonfeeding, such as diapause (1) and long migratory flights (2), and during nonfeeding stages of development (3). Fatty acids serve as precursors in the biosynthesis of pheromones (4), waxes and eicosanoids (5) and as structural components of membranes and defensive secretions (5), and they are essential components in the function of the cuticle (6). Because the relative importance of each of these functions varies throughout development, the rate of fatty acid biosynthesis also would be expected to fluctuate according to physiological need.

In previous studies about fatty acid compositions of the black cricket *Melanogryllus desertus*, linoleic acid biosynthesis in the black cricket (7), the effect of various diets on the total lipid composition of the black cricket (8), PL and TG fatty acid compositions of *M. desertus* (9) and the distribution in lipid classes of fatty acids biosynthesized by the black cricket (10) were

investigated. These studies have focused primarily on fatty acid composition, which often was determined only for a single instar.

The purpose of this study was to investigate changes in PL and TG fatty acid compositions of black cricket females depending on various development stages of the insect such as eggs; seventh, eighth and last nymphal stages; one-, 20- and 40-day-old adults; and its stock-culture medium. The last three nymphal stages and adults crickets were used because the sex of the insect is difficult to determine before ovipositor buds have formed. The results are compared with reported changes in fatty acid composition and with physiological and biochemical events.

Materials and Methods

Rearing of Insects

The eggs obtained from stock-culture medium (11) were sterilized for 30 min in 0.025% sodium hypochlorite solution containing Triton X, washed with sterile distilled water and 70% ethanol (12) and then transferred to moist, sterile sand in a dish and incubated at 30 °C for 11 days to obtain first instar nymphs. The nymphs were transferred to sterile plastic cups containing stock-culture medium and held at 30 ± 1 °C in darkness. Relative humidity was 55 ± 5%. Nymphs were fed on the stock-culture medium during the experiment. Eggs; seventh and last nymphal stages; and 1-, 20- and 40-day-old adult females of *M. desertus* were used for fatty acid analyses of PLs and TGs. Since the fatty acid profiles of both sexes are similar, males were not used.

Analysis of Insects

Total lipids of the insects were extracted by the method of Bligh and Dyer (13). Four-gram samples of stock culture medium were also extracted for their lipid contents. Autoxidation of unsaturated fatty acid was minimized by the addition of 50 µl of butylated hydroxytoluene (2% in chloroform, w/v). PL and TG fractions were prepared from total lipid extracts by developing Silica Gel G thin-layer chromatography plates (20 x 20 cm, 0.25 mm thick on glass plates) in petroleum ether:diethyl ether:acetic acid (80:20:1, v/v) (12). Fractions were visualized under UV light after spraying with 2´7´ - dichlorofluorescein and identified by comparison with authentic standards. PL and TG fractions were scraped from the plates, and transmethylated by refluxing the silica gel in acidified methanol for 90 min at 85 °C (14). Fatty acid methyl esters were extracted from the acidic methanol three times with hexane, concentrated and analyzed by gas chromatography as described below.

Gas Chromatography

The fatty acid methyl esters were concentrated, and then analyzed by gas chromatography. The methyl esters were chromatographed using an Ati Unicam 610 series equipped with a SP-2330 capillary column [(0.25 mm x 30 m, 0.2 µm film thickness (Supelco, Supelco Park PA)], a flame ionization detector and a Unicam 4815 recording integrator. All GC runs used used temperature programming from 180 to 200 °C at 5 °C/min, with an initial 2 min hold period. Injections were made in split mode (25:1) and separations were carried out with N_2 carrier gas. Flow rates of gases: N_2 + make up, 30 ml/min; hydrogen, 33 ml/min; dry air, 330 ml/min. Components were identified by comparisons of retention times with authentic standards.

Gas chromatographic analyses of fatty acid compositions in PL and TG were evaluated statistically using variance analysis (15). Duncan's (16) Multiple Range test was used to determine the significance of the difference between means.

Results

The fatty acid compositions of PLs and TAGs prepared from eggs; seventh, eighth and last nymphal stages; 1-, 20- and 40-day-old adult females of the black cricket *M. desertus* are set forth in Tables 1 and 2. The major fatty acid components of PLs are palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linolenic (C18:2n-6) acids. In addition to these components, small proportions of myristic (C14:0) and linolenic (C18:3n-3) acids occurred in most preparations.

There were increases and decreases in some fatty acids at different development stages such as nymphal stages and female adults. Proportions of palmitic acid decreased in 1-day old adult and older adult in females. Compared to nymphal stages, proportions of oleic acid decreased in 1-, 20- and 40-day-old insects. However, proportions of linoleic acid increased in 1-, 20- and 40-day-old females compared to other nymphal stages examined.

Table 1. Proportions of fatty acids, as percentage of total fatty acids, in the phospholipids of total lipid extracts from various development stages of females of *M. desertus**.

Fatty Acids	Eggs	7th Nymphal Stage	8th Nymphal Stage	Last Nymphal Stage	1-day-old Adult	20-day-old Adult	40-day-old Adult
12:0**	–	–	–	–	–	–	–
14:0	1.48 ± 0.09ab	1.60 ± 0.08b	1.14 ± 0.04a	1.82 ± 0.07b	1.23 ± 0.08a	1.13 ± 0.05a	1.30 ± 0.06a
16:0	26.11 ± 0.20a	18.09 ± 0.24b	24.32 ± 0.19a	17.52 ± 0.25b	19.92 ± 0.18b	18.15 ± 0.24b	16.32 ± 0.21c
16:1	3.46 ± 0.22a	4.41 ± 0.21b	3.19 ± 0.10a	3.82 ± 0.09ab	2.44 ± 0.07c	3.24 ± 0.11a	2.11 ± 0.04d
18:0	8.30 ± 0.31a	12.13 ± 0.45b	11.08 ± 0.22bc	12.73 ± 0.23b	13.38 ± 0.25b	11.66 ± 0.21bc	10.70 ± 0.22c
18:1	19.09 ± 0.33a	22.69 ± 0.38b	21.01 ± 0.33b	23.12 ± 0.41b	19.12 ± 0.16a	17.36 ± 0.25c	18.97 ± 0.18a
18:2n-6	40.35 ± 0.79a	33.42 ± 0.43b	30.66 ± 0.40d	32.18 ± 0.45b	36.12 ± 0.38d	40.16 ± 0.42a	43.24 ± 0.82e
18:3n-3	1.18 ± 0.06a	7.68 ± 0.17b	8.61 ± 0.14d	8.82 ± 0.15b	7.89 ± 0.13b	8.92 ± 0.17b	7.62 ± 0.13b

* Percent given as the mean ± SD (n: 3 analyses of individual each) -: Not detected.

** Means followed by the same letter are not significantly different (P > 0.05)

Table 2. Proportions of fatty acid, as percentage of total fatty acids, in the triacylglycerols of total lipid extracts from various development stages of females of *M. desertus**.

Fatty Acids	Eggs	7th Nymphal Stage	8th Nymphal Stage	Last Nymphal Stage	1-day-old Adult	20-day-old Adult	40-day-old Adult
12:0**	0.32 ± 0,02a	0.38 ± 0.03a	0.23 ± 0.02b	1.16 ± 0.02c	0.19 ± 0.03b	0.24 ± 0.04b	0.22 ± 0.03b
14:0	3.31 ± 0.21a	2.16 ± 0.16bb	1.93 ± 0.11ab	1.18 ± 0.12c	1.94 ± 0.11c	1.19 ± 0.13c	1.26 ± 0.09c
16:0	24.42 ± 0.25a	24.44 ± 0.30ba	22.16 ± 0.32ab	26.18 ± 0.42c	20.42 ± 0.20b	26.08 ± 0.52b	23.0.8 ± 0.43a
16:1	3.73 ± 0.22a	5.18 ± 0.22b	4.16 ± 0.18c	3.78 ± 0.25a	4.69 ± 0.21bc	5.69 ± 0.26b	4.13 ± 0.17a
18:0	3.25 ± 0.20a	4.73 ± 0.23b	2.89 ± 0.16a	2.44 ± 0.19a	1.46 ± 0.11c	3.12 ± 0.21a	3.42 ± 0.20a
18:1	35.52 ± 0.62a	33.75 ± 0.28a	39.63 ± 0.52b	36.54 ± 0.33a	39.02 ± 0.38b	30.00 ± 0.24c	32.79 ± 0.38a
18:2n-6	24.99 ± 0.42a	24.40 ± 0.53a	23.82 ± 0.36a	24.90 ± 0.52a	26.90 ± 0.42b	28.73 ± 0.53c	28.91 ± 0.63c
18:3n-3	4.56 ± 0.15a	4.70 ± 0.17a	5.16 ± 0.13ab	4.83 ± 0.21a	5.38 ± 0.25c	4.95 ± 0.24a	6.13 ± 0.26c

* Percent given as the mean ± SD (n: 3 analyses of individual each).

** Means followed by the same letter are not significantly different (P > 0.05)

Compared with PLs, the fatty acid profiles of TGs prepared from whole insects had higher proportions of C16:0 and C18:1, and lower proportions of two polyunsaturated fatty acids (PUFAs), C18:2n-6 and C18:3n-3. Palmitic acid comprised about 16-26% of PLs and about 23-31% of TGs. Oleic acid made up about about 17-23% of PLs and 30-35% of TGs. Linoleic acid comprised about 30-43% of PLs and about 20-29% of TGs.

There were increases and decreases in fatty acids in TG as a PL fraction. TG fatty acid profiles of nymphs were different from those of 20- and 40-day-old adults. For example, compared to older adults, the nymphs were higher in TG C18:1 and lower in C18:2n-6 (Table 2).

In the another experiment, the effect of stock-culture food on the fatty acid profiles of insects was investigated. The fatty acid compositions of PLs and TGs prepared from 1-day-old adult females and the total fatty acid composition of stock-culture food on which the insects were reared are presented in Table 3. Some differences were found between the fatty acid profiles of 1-day-old adults and their dietary profiles. In particular, the stock-

Table 3. Proportions of fatty acids, as percentage of total fatty acids, in the phospholipid and triacylglycerol fractions of total lipid extracts from 1-day old adult females of *M. desertus* and their respective stock-culture media.*

Fatty Acids	Total Fatty Acids of Stock-culture Media	1-day-old Adult (Phospholipid)	1-day-old Adult (Triacylglycerol)
12:0**	_	_	0.19 ± 0.03
14:0	_	1.23 ± 0.08 a	1.94 ± 0.11 a
16:0	21.39 ± 0.26 a	19.92 ± 0.18 a	20.42 ± 0.20 a
16:1	_	2.44 ± 0.07 a	4.96 ± 0.21 b
18:0	2.15 ± 0.12 a	13.38 ± 0.25 b	1.46 ± 0.11 c
18:1	19.92 ± 0.21 a	19.12 ± 0.16 a	39.02 ± 0.38 b
18:2n-6	42.44 ± 0.35 a	36.12 ± 0.38 b	26.90 ± 0.42 c
18:3n-3	14.03 ± 0.18 a	7.89 ± 0.13 b	5.38 ± 0.25 c

* Percent given as the mean ± SD (n: 3 analyses of individual each)
** Means followed by the same letter are not significantly different (P > 0.05)

culture food had higher proportions of C18:2n-6 (42 vs. 36%) and C18:3n-3 (14 vs. 7%) compared to PL fatty acid composition.

Discussion

The data presented here indicate that the quantitatively major fatty acids associated with PLs and TGs prepared from *M. desertus* include C16:0, C18:0, C18:1 and C18:2n-6, as described for other Gryllidae (17,18) and most other insect orders (14,19,20). No fatty acids beyond C18:3n-3 were detected.

Certain groups of insects stand out because they exhibit unusual and characteristic fatty acid profiles (5). Dipterans are characteristically high in 16:1 (19). Chinch bugs, members of the Lygaeidae (21), and the sunn pest, *Eurygaster integriceps*, (22) are also unusually high in 16:1. Some aphids have up to 80% 14:0 (23).

We did not detect eicosanoid–precursor C20 PUFAs or the odd–chain fatty acids C15:0, C17:0 and C21:0. These fatty acids occur in low proportions, and are reported in only a few instances. They are probably routinely present in the lipids of most insects, but are often overlooked because they are not present in easily detectable quantities. Odd–chain fatty acids were among the components reported from exocrine tissues of the cockroach *Periplaneta americana* (24), from the PLs of the dipteran *Microdon albicomatus* and its prey *Myrmica incompleta* (25), from selected tissues of the yellow mealworm beetle *Tenebrio molitor* (26), and from the desert cicada *Tibicen dealbatus* (27).

Fatty acid compositions are not fixed in insects. In particular, development and diet exert strong influences on the shape of fatty acid profiles. This is the case for the mosquito *Culex torsalis*, where larvae appear to be far lower than adults in 18:1 (28). The fatty acid profiles of 20-day-old larvae of the beetle *Lyctus planicollis* are very different from those of 60-day-old larvae in terms of 18:1 (29). PL fatty acid profiles differ among eggs, larvae, pupae and adults of the pine beetle *Dendroctonus frontalis* (30). Ogg and Stanley-Samuelson (31) indicate that fatty acid profiles of PLs and TGs from *Manduca sexta* are different in each stage of development.

The data reported here similarly indicate that the fatty acid profiles of PLs and TAGs from whole insects of *M. desertus* are different at each stage of development. For example, compared to adults, the nymphs were higher in C18:1 and lower in C18:2n-6. Lipids are stored in the fat body in preparation for the nonfeeding stage before ecdysis and for use as energy during ecdysis (32). Female crickets have an additional storage site for lipids in general and for TG in particular, the ovaries. During ovarial development, lipids synthesized in the fat body are transported to the developing ovary and stored for use in embryogenesis. Proportions of C18:2n-6 increased in adult black cricket females, which probably reflects increased synthesis of this component. Decreased proportions of C18:1 would seem most likely to be associated with the processes of egg development and oviposition. These findings indicate that there may be developmental changes in organismal fatty acid profiles.

In general, the large changes in lipid synthesis correlate closely with the physiological needs of the insect associated with its development. Changes in the fatty acid profiles in the PL fraction indicate that insect tissues are able to modify their fatty acid compositions, probably to suit local physiological requirements. Because, as indicated by Stanley-Samuelson and Pipa (24), tissue fatty acid compositions can be modified by the hydrolysis of some PL fatty acids, coupled with selective reacylation of others, and also by altering existing components. The ability to elongate and desaturate tissue polyunsaturated fatty acids (PUFAs) is one of the mechanisms of changing fatty acid profiles; such metabolic abilities are linked to physiological needs by providing C20 PUFAs.

One of the prominent patterns in insect fatty acid compositions is that PUFAs are generally found in higher proportions of PLs, as opposed to neutral lipids (14). Our

findings from *M. desertus* also agree on this point. C18 PUFAs, especially linoleic acid, tend to occur preferentially in insect PLs (25,26) and our data accord with this general situation, since C18 PUFAs, C18:2n-6 and C18:3n-3 are in considerably higher proportions for the PL fatty acids than for those of the TGs. High levels of these C18 PUFAs in the PL could be based on a number of physiological factors. One of the major functions of linoleic acid and polyunsaturated fatty acids in general is a structural component of membranes to maintain proper fluidity and permeability. Linoleic acid is also a precursor to arachidonic acid (20:4), a PUFA precursor to the eicosanoids, including prostaglandins, leukotrienes and thromboxanes. These components of the arachidonic acid cascade play an essential role in the regulation of physiological processes ranging from smooth muscle contraction to mediating the inflammation response. In insects, the eicosanoids have been shown to mediate such diverse activities as the stimulation of oviposition and the development of immunity to various antigens (34).

Linoleic acid has been determined to be essential to many insects belonging to various orders (35). However, in a previous study, it was shown that the black cricket *M. desertus* was able to biosynthesize linoleic acid by the introduction of a second double bond into the most common monounsaturated fatty acid, oleic acid (7), as only 15 insect species have been found to synthesize this fatty acid (5). In this single step, previously believed not to occur in any animal cell, a monounsaturated fatty acid is converted into a polyunsaturated one.

Aside from the effects of development, many studies have shown that dietary parameters may influence the fatty acid profiles of many insects. This is true for the red-bonded leaf roller *Argyrotaenia velutinana* (36), the butterfly *Pieris brassicae* (37), the bollworm *Heliothis zea* (38), the cabbage looper *Trichoplusia ni* (39), the boll weevil *Anthonomus grandis* (40), the wax-moth *Galleria mellonella* (41), and the mosquito *C. pipiens* (42). In this study, a comparison of the total fatty acid composition of the food and PL and TAG fatty acid compositions of 1-day-old adult females indicates that the fatty acid composition of the *M. desertus* lipids is not identical to the fatty acids of the dietary lipids. The black cricket does seem to store oleic acid or utilize two PUFAs, linoleic and linolenic acids, preferentially. Since the cricket lipids, generally TGs, are higher and lower respectively in these acids than is the cricket food. Dietary fatty acids are incorporated into tissues without modifications; also sugars and certain amino acids are converted into fatty acids, and then incorporated into tissues. Tissue fatty acids may be modified by a number of endogenous enzyme systems, including desaturases. We suggest that *M. desertus* is able to modify its fatty acid compositions, probably to suit physiological requirements.

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