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Full Length Research Paper

Comparative effectiveness of some host plants on the general body metabolism of black blister beetle *Meloe proscarabaeus* (*Coleoptera: Meloidae)*

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The black blister beetle *Meloe proscarabaeus* is firstly recorded in Egypt during 1970s. The present work was carried out aiming to investigate the effects of feeding on different host plants, *viz. Vicia faba*, *Trifolium alexandrinum*, and *Lactuca sativa*, on the general body metabolism of adult females. Concerning the carbohydrate content (CC) in haemolymph, feeding on *V. faba* resulted in unchanged content during the ovarian maturation period and oviposition period but decreased during the post-oviposition period. By feeding on *T. alexandrinum* or *L. sativa*, CC gradually decreased through the successive time intervals. With regard to CC in the fat body, no change could be detected during the first two successive intervals while a slight increment was estimated during the post-oviposition period, after feeding on *V. faba*. Another fashion was determined by feeding on *T. alexandrinum* or *L. sativa*. The protein content (PC) gradually decreased in haemolymph by the adult age after feeding on *V. faba*. On the contrary, PC appeared in another course by feeding on *L. sativa* but no certain trend could be explored after feeding on *T. alexandrinum*. PC in the fat body turned down by age after feeding on *V. faba* or *L. sativa*, but another trend could be conceived by feeding on *T. alexandrinum* The lipid content (LC) in the haemolymph, after feeding on *V. faba*, was promoted during the oviposition period and then was prohibited during the last life interval. Feeding on *T. alexdandrinum* or *L. sativa* led to gradually increasing LC during the oviposition period and the next period. In the fat body, LC increased progressively by age after feeding on *V. faba* and a similar approach could be easily seen during the time intervals of adult females fed *L. sativa* but not fed *T. alexandrinum*

Keywords: Haemolymph, fat body, carbohydrate, protein, lipid, *Vicia faba*, *Trifolium alexandrinum*, *Lactuca sativa*

INTRODUCTION

Carbohydrates play an important role for the structure and functions of all tissues during metamorphosis as well as for the normal functioning of the male and female reproductive organs and embryonic development (*cf.* Chippendale, 1978). On the other hand, the carbohydrate content in the haemolymph is an important indicator of the level of metabolism in insects, and a dynamic balance of the absorption, metabolism, and utilization by different tissues (Zhu et al**.**, 2012**).** Protein synthesis is necessary for the maintenance of body growth and reproduction. Many factors had been implicated in the control of protein synthesis (Carlisle et al., 1987). Proteins enter in various reactions such as the hormonal regulation and they integrated in the cell as a structural element at the same time as the carbohydrates and the lipids (Cohen, 2010**;** Sugumaran, 2010). Quantity of lipids available for the reserves seems to be the result of a balance between the catch of food and the requests for reserves by processes such as maintenance, growth and reproduction, and this balance is disturbed by any toxic product (Canavoso et al., 2001). Lipids are important source of energy for insects. Lipid turnover in insects is regulated by neuroendocrine-controlled feed-back loops (Downer, 1985).

The haemolymph of insects resembles the blood of vertebrates in that it contains cellular components, lipid, proteins, salt, free amino acids, carbohydrates, water, hormones, etc. Food substances are transported and

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stored in the haemolymph, which also carries hormones through the body. The chemical composition of the haemolymph is nearly as diverse. Often there is variation in different stages of the same species and in individuals depending upon their nutritional background, environment, and physiological state. In locusts the amount of carbohydrate in the haemolymph is greater than that stored in other tissues (Goldsworthy, 1969), but the opposite is true for fat reserves (Jutsum et al., 1975). The haemolymph sometimes contains substances which, in the case of reflex bleeding, may protect the insect from attack (Mellanby, 1939). In insect haemolymph, lipid transport is accomplished mainly by lipophorin (Wang and Patton, 1969; Chino, 1985; Shapiro et al., 1988).

The insect fat body is an organ analogous which carries out a variety of different metabolic activities comparable to mammalian liver. It is the place of intense biosynthetic activity throughout the insect life and is the main source for the haemolymph proteins. Also, it is the storage site of food reserves (Staurengoda-Cunha, and Cruz-Landim, 1983). The insect fat body tissues are constituting mostly introphocyte, or adipocytes, of mesodermal with several origin capable of storing proteins, carbohydrates and lipids (Keeley, 1985; Dean et al., 1985), in addition to synthesizing lipids and proteins (Roma et al., 2008). It plays a vital role in reproduction, metabolizes hormones and other essential messenger molecules and also detoxifies wastes or harmful compounds (Denardi et al., 2008). The control of vitellogenin production and release from the fat body and the uptake of vitellogenin and lipids into the oocytes are primarily under the control of juvenile hormone (Engelmann, 1983).

Blister beetles (Meloidae) are of particular interest because of their importance to applied science (biological control of grasshoppers, pharmacology, veterinary and agricultural problems) as well as their distinctive biology (hypermetamorphic development, parasitoid larval habits, defensive attributes, and diverse courtship behaviour)(Bologna and Di Giulio, 2011). Meloid beetles with about 3000 species in 120 genera (Dettner, 1997) are widespread throughout the world except for New Zealand and the Antarctic and their diversity is greatest in arid or semiarid regions (Arnett et al., 2002). Adults of some meloid species are phytophagous and feed on leaves and flowers of several families of plants, particularly Compositae, Astraceae, Leguminosae, Solanaceae and Umbliferae, causing different economic losses (Ghoneim, 2013a) whereas larval instars are parasite or predator and feed on the provisions and immature stages of bees and in subfamilies Epicautini and Mylabrini, the eggs of grasshoppers (Nikbakhtzadeh and Tirgari, 2002, Ghoneim, 2013 b).

In Egypt, Alfieri (1976) recorded the black blister beetle *Meloe proscarabaeus* Linnaeus 1758 among Meloidae and other families of Coleoptera. The first reference reporting this beetle as an agricultural pest attacking faba bean, peas, alfalfa, onion and wild weeds was Ali et al. (2005 a). Ali et al. (2005 b) carried out a field study on this species in El-Farafra oasis (in western desert of Egypt). This paper is part of an extended study on this beetle in Egypt (Ghoneim et al., 2012 a, b). Objective of the present paper was to investigate the effect of food quality on the body main metabolites in haemolymph and fat body of *M. proscarabaeus* adult females.

MATERIALS AND METHODS

Experimental Beetle

Adults of the black blister (Oil) beetle, *Meloe proscarabaeus* Linnaeus 1758. (Coleoptera: Meloidae) had been collected just after the emergence using pit-fall traps in faba bean and Egyptian clover fields in El-Farfra oasis (in western desert of Egypt near latitude 27.06º North and longitude 27.97º East, at 580 km from Cairo) in order to maintain a continuous culture under the laboratory conditions (23±2ºC, 46±10% RH, 12L:12D photoperiod). Much effort was conducted for maintaining a culture but unfortunately failed (Ghoneim et al., 2012a). Therefore, newly emerged adult beetles of both sexes (of 0-day old) were collected from the field and directly transferred to the laboratory of Insect Physiology, Faculty of Science, Al-Azhar University, Cairo, Egypt. Under the previously mentioned laboratory conditions, these adults were fed on different host plants, separately, *viz.* faba bean *Vicia faba* (Fabaceae), Egyptian clover *Trifolium alexandrinum* (Leguminosae), and lettuce *Lactuca sativa* (Asteraceae).

Preparation of samples

Fat body

Fat body was collected from three dissected adult beetles, individually, during each of the ovarian maturation period, reproductive life time and post-oviposition period, the visceral and parietal fat bodies were collected by a fine forceps and weighed using 4 digital electronic balance. Then, the collected fat bodies were homogenized in a saline solution (the fat body of one insect /1 ml saline solution 0.7 %) using a fine electric homogenizer, tissue grinder for 2 min. Homogenates were centrifuged at 4000 r.p.m. for 15 min. The supernatant was used directly or frozen for a few days until the use. Three replicates were used, separately.

Haemolymph

The haemolymph was drawn from three adult beetles, individually, during each of the maturation period,

Table 1.Carbohydrate content in haemolymph and fat bodies of adult females black blister beetle, *Meloe proscarabaeus*, feeding on different plant hosts

Host plants	**Ovarian maturation (Pre-oviposition) period**		**Reproductive life time (Oviposition period)**		**Post-oviposition period**	
	Haemolymph (g/dL)	**Fat bodies (mg/g)**	**Haemolymph (g/dL)**	**Fat bodies (mg/g)**	**Haemolymph (g/dL)**	**Fat bodies (mg/g)**
Vicia faba	0.073 ±0.91	0.61 ±0.06	0.073 ±0.009	0.61 ±0.66	0.052 ±0.009	0.71 ±0.09
Trifolium alexandrinum	0.07 ±0.013 a	0.68 ±0.06 a	0.038 ±0.006 c	0.72±0.07 a	0.032±0.001 b	0.73 ±0.14 a
Lactuca sativa	0.067 ±0.12 a	1.01±0.22 b	0.034 ±0.003 c	0.62±0.09 a	0.061±0.004 a	0.53 ±0.09 b

Faba bean (*Vicia faba*) was used as standard host plant for the comparison purpose. No. of adult ♀♀ = 3 individually. Mean ± SD followed with the letter (a): non-significantly different ($P>0.05$), (b): significantly different ($P<0.05$), (c): highly significantly different ($P<0.01$), (d): very highly significantly different ($P<0.001$)

reproductive life time and post-oviposition period. Haemolymph was drawn from a cut of coxal joint into Eppendorff Pipetman containing 1-2 milligrams of phenoloxidase inhibitor (phenylthiourea) to prevent tanning or darkening and then diluted 5× with saline solution 0.7%. The diluted haemolymph was frozen for 20 second to rupture the haemocytes. The haemolymph samples were then centrifuged at 2000 r.p.m. for 5 min, and only the supernatant fractions were used for assay directly or frozen for a few days until use.

Determination of the main body metabolites

Total carbohydrate (as glycogen) content in haemolymph or fat body was quantitatively determined by using the anthrone reagent according to Singh and Sinha (1977) and utilizing the Spectrophotometer at 620 mµ.

Quantitative determination of the total protein content in haemolymph or fat body was conducted according to Doumas (1975) and using a kit of Bioadwic company. The method depends on the protein forms a violet complex with cupric ions in alkaline medium, and then measured the absorbance at 550 nm using a spectrophotometer.

Quantitative determination of the total lipid content in haemolymph or fat body was conducted according to the technique of Folch et al. (1957) and lipid estimation was taken place by phosphovanilin reagent depending on Knight et al. (1972) and using the Spectrophotometer at 520 mµ.

Statistical analysis of data

Data obtained were calculated as mean ±SD and analyzed using the Student *t*-distribution and were refined by Bessel's correction (Moroney, 1956) for testing the significance of difference between means at probability 0.05, 0.01 and 0.001.

Results and Discussion

The availability of different host plants plays an essential role in causing population outbreaks for polyphagous insects (Singh and Parihar, 1988) and the analysis of nutritional indices can lead to understanding of the behavioural and physiological basis of insect response to host plant (Lazarevic and Peric-Mataruga, 2003). Lower fitness of some lepidopterans on some host plants may be due to the presence of some secondary phytochemicals in these host plants, or absence of primary nutrients necessary for growth and development (Hemati et al., 2012).

Influenced carbohydrate content in *M. proscarabaeus* by food quality

During the ovarian maturation period of the present black blister beetle *Meloe proscarabaeus*, the haemolymph carbohydrate content of females fed on *V. faba* was determined in a value which did not change during the next period but decreased during the last time interval (Table 1). Feeding on *T. alexandrinum* resulted in gradually decreasing haemolymph carbohydrate content throughout the adult life. A similar trend could not be detected after feeding on *L. sativa* which may be due to the high carbohydrate content in lettuce since carbohydrate content of the diet is reported to directly influence the carbohydrate content in insects (Nettles et al., 1971). Concerning the carbohydrates in fat body, feeding of *M. proscarabaeus* adult females on *V. faba* resulted in no changed content along the first and second life intervals but a slight increment was estimated during the post-oviposition period (Table 1). Another fashion was explored by feeding on *T. alexandrinum* because the carbohydrates increased through the life intervals in spite of a reverse trend appreciated by feeding on *L. sativa*.

To our knowledge, no works had been reported on the effects of host plants on carbohydrate metabolism in the black blister beetle in spite of many works had been

Table 2. Protein content in haemolymph and fat bodies of adult females black blister beetle, *Meloe proscarabaeus*, feeding on different plant hosts

Host plants	**Ovarian maturation (Pre-oviposition) period**		**Reproductive life time (Oviposition period)**		**Post-oviposition period**	
	Haemolymph (g/dL)	**Fat bodies (mg/g)**	**Haemolymph (g/dL)**	**Fat bodies (mg/g)**	**Haemolymph (g/dL)**	**Fat bodies (mg/g)**
Vicia faba	6.11 ±0.86	60.03 ±9.11	4.83 ±0.60	25.31 ±4.11	3.50 ±0.26	34.81 ±5.12
Trifolium alexandrinum	7.48 ±0.46 a	59.79 ±6.14 a	2.50 ±0.27 c	74.7 ±3.1 d	3.2 ±0.27 a	34. 92 ±5.04 a
Lactuca sativa	5.38 ±0.41 a	52.34 ±2.03 a	6.00 ±0.63 a	33.84 ±3.77a	6.35 ±0.95 c	41.5 ±3.8 a

Faba bean (*Vicia faba*) was used as standard host plant for the comparison purpose. No. of adult ♀♀ = 3 individually. Mean ± SD followed with the letter (a): non-significantly different (P>0.05), (b): significantly different (P<0.05), (c): highly significantly different (P<0.01), (d): very highly significantly different (P<0.001)

reported for the effects of synthetic insect growth regulators (IGRs), chitin synthesis inhibitors (CSIs) or plant extracts (botanicals). As for examples, Tanani et al. (2012) recorded a disturbed carbohydrate content in haemolymph and fat body of adults of the desert locust *Schistocerca gregaria* by IGRs pyriproxyfen, tebufenozide and lufenuron. To some extent, similar results were obtained for the Lychee Shield bug *Chrysocoris stolli* (Saha et al., 1986) and the cotton leafworm *Spodoptera littoralis* (Abdel-Hafez et al., 1988) and for the mealy bug *Ferrisia virgata* (Ezz and Fahmy, 2009).

The decreasing carbohydrate content in haemolymph of adult females of *M. proscarabaeus*, in the current study, may be due to the accumulation or utilization of such metabolite during the reproductive cycle or may be attributed to unknown secondary chemicals in the tested host plants. Some of these phytochemicals may mimic a certain metabolizing hormones since the production or utilization of the main body metabolites, such as carbohydrates, are under control on juvenile hormone (Slama and Hodkova, 1975; Gade, 2004; Sugumarau, 2010). Some of the phytochemicals, in the present host plants, may affect the ability of *M. proscarabaeus* to modify the synthesis of carbohydrate and disrupt the functionality of this beetle (Rodriguez-Ortega et al., 2003). Some of the carbohydrates have been used to synthesize proteins or lipids since carbohydrates have been reported to contribute to the building up of protein in the blowfly *Phormia regina* (Diptera)(Tate and Wimer, 1974). Also, Ramdev and Rao (1979) suggested that carbohydrates are utilized by insects either for maintenance or for conversion to body lipid rather than being stored.

On the other hand, the decreased carbohydrate content can be understood in view of a reduced alkaline phosphatase (ALP) activity in the gut of adult *M. proscarabaeus* since the insect gut ALPs, generally, play a role in epithelial transport (Eguchi, 1995; Yan et al., 2009). For example, two silkworm gut isozymes, a membrane-bound form (m-ALP) and a soluble form (s-ALP), are believed to participate in the transport of glucose and fatty acids across intestinal wall membranes (Sridhara and Bhat, 1963). Also, the patterns of s-or m-ALP-specific activities were identified in the beetle mid-gut and were correlated with feeding activity of Colorado potato beetle, *Leptinotarsa decemlineata* (Yi and Adams, 2001). However, the increasing carbohydrate content in fat body during certain intervals of the adult life of *M. proscarabaeus* may be due to an increase in the gut ALPs activities.

Influenced protein content in *M. proscarabaeus* by food quality

In the present study, proteins gradually decreased in the haemolymph throughout the adult life by feeding on *V. faba* (Table 2). On contrast, haemolymph protein content appeared in another course by feeding on *L. sativa* because gradual increase was estimated throughout the three life intervals. No certain trend could be detected during these life intervals after feeding on *T. alexandrinum*. With regard to the protein content in fat body, feeding on *V. faba* resulted in a certain protein level during the ovarian maturation period which turned down by age (Table 2). To some extent, a similar fashion was appreciated for the proteins through the life periods by feeding on *L. sativa*. Different trend could be conceived by feeding on *T. alexandrinum* because elevated protein level (during the oviposition period) but declined level (during the post-oviposition period) was measured.

The decreasing content of proteins in haemolymph or fat body during the ovarian maturation period of the adult females of *M. proscarabaeus*, in the present study, can be understood in view of the transferring proteins from these tissues for the yolk deposition (vitellogenesis) while the decreasing content during the two successive life intervals or the exceptional increase in protein content in haemolymph or fat body after feeding on some of the tested plants cannot be exactly explained now. However, there is no direct correlation between the protein content

Table 3. Total lipid content in haemolymph and fat bodies of adult females black blister beetle, *Meloe proscarabaeus*, feeding on different plant hosts

Host plants	**Ovarian maturation (Pre-oviposition) period**		**Reproductive life time (Oviposition period)**		**Post-oviposition period**	
	Haemolymph (mg/dL)	**Fat bodies (mg/g)**	**Haemolymph (mg/dL)**	**Fat bodies (mg/g)**	**Haemolymph (mg/dL)**	**Fat bodies (mg/g)**
Vicia faba	672.88±25.19	6.40 ±0.38	1012.42±25.10	9.88 ±0.76	813.67±32.34	11.09 ±0.66
Trifolium alexandrinum	792.96±56.02 b	9.77 ±0.31 **c**	1113.87±50.84 b	9.8 ±0.16 a	1169.77±22.40 d	8.07 ±0.78 c
Lactuca sativa	714.29±68.42 a	7.16 ±0.59 a	718.45±68.92 c	8.86 ±0.34 a	1149.07±387.98 a	10.91 ±0.95 a

Faba bean (*Vicia faba*) was used as standard host plant for the comparison purpose. No. of adult ♀♀ = 3 individually. Mean ± SD followed with the letter (a): non-significantly different (P>0.05), (b): significantly different (P<0.05), (c): highly significantly different (P<0.01), (d): very highly significantly different (P<0.001)

in the host plant and the protein content in the beetle. Outside the study of host plant influences upon the protein content in haemolymph or fat body, several authors reported some increase or decrease in these tissues as responses to IGRs, CSIs or plant extracts. Ameri et al. (2010) estimated some effects of the host plants fed by nymphs of Sunn pest, *Eurygaster intedriceps* (Hemiptera), on the total body protein content of adults. Ghoneim et al. (2012c) determined tremendously declined proteins in haemolymph and fat body of adult *S. gregaria* by the nymphal treatments with pyriproxyfen, tebufenozide or lufenuron. More or less, similar results had been obtained for other insect species by certain IGRs (Ghoneim et al., 2005; Kebbeb et al., 2008; Ezz and Fahmy, 2009). The effect of abamectin on adults of insecticide-resistant and -susceptible strains of red flour beetle, *Tribolium castaneum* was estimated. In both resistant and susceptible strains, a depletion of total body protein content was determined (Hussain et al., 2012). However, the depleted proteins in haemolymph or fat body of adult females was interpreted by the action of IGRs on the major metabolization of this metabolite as well as reduction of its synthesis, or was attributed to a direct effect of IGRs on the nutritional requirements of egg maturation (Miranda et al., 2003), although Cloyd (2003) suggested no effects on such physiological process in the citrus mealy bug *Planococcus citri*.

Influenced lipid content in *M. proscarabaeus* by food quality

Some IGRs prohibited the lipid content in haemolymph and fat body or whole body of various insect species (Ghasemi et al., 2010; Zibaee et al., 2011; Hamadah et al., 2012) and azadirachtin (plant extract) exhibited a similar effect on lipids (Rharrabe et al., 2008). Instead of the inhibition by IGRs or plant extracts, activated lipids were reported by some authors using different IGRs against various insects (Ghoneim, 1994; Soltani-Mazouni et al., 1999; Bouaziz et al., 2011). Also, the adult females of Sunn pest, *Eurygaster intedriceps* (Hemiptera), produced from nymphs grown on wheat, had the highest total body lipid content followed by barley and rye (Ameri et al., 2010).

In the present study, the haemolymph lipid content of adult females of the black blister beetle was affected by the food quality (Table 3). After feeding on *V. faba*, the haemolymph lipid content was promoted during the oviposition period and then prohibited during the last period, when compared to the lipid content during the ovarian maturation period. Feeding on *T. alexandrinum* or *L. sativa* resulted in gradually increasing lipid content during the oviposition period and the last period. In connection with the lipids in fat body, feeding on *V. faba* led to progressively increasing content by age (Table 3). A similar tendency could be effortlessly observed throughout the adult life of adults fed on *L. sativa*. Such approach could not be detected for this metabolite during the adult life by feeding on *T. alexandrinum*. In the light of commonly increasing lipids in haemolymph and fat body, with a few exceptions, it can be concluded that the present host plants interfered with not only the synthesis of lipids but also their mobilization as enhanced to convert into other metabolites or fatty acids (Hamadah et al., 2012). Such suggestion may be supported by some works using IGRs against various insects (Leonardi et al., 2001; Etebari et al., 2007). Also, some substantiating results are found in the literature since lipid content in ovaries of the cotton leafworm *Spodoptera litura*, mealworm *Tenebrio molitor*, Mediterranean flour moth *Ephestia kuehniella* was depleted as a response to some IGRs (Hami et al., 2004; Perveen and Miyata, 2000; Perveen, 2012). Unfortunately, the exact explanation of the common tendency of increasing lipid content in haemolymph and fat body of adult females of *M. proscarabaeus* after feeding on the tested host plants could not be available now although we hypothesize the presence of IGR-mimicking substances in these food plants which affected the lipid content in these tissues. Frankly, transportation problems between different organs or tissues, such as haemolymph and fat body, are still controversial and need further studies to accurately describe the lipid mobilization!!

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