

Effects of sugar feeding on lipid, glycogen, and total sugar levels of a female parasitoid, *Bracon hebetor* (Say) (Hymenoptera: Braconidae)

Eylem AKMAN GÜNDÜZ*, Adem GÜLEL, Özgür VARER İŞİTAN, Ali BOZ, Özlem CESUR
Ondokuz Mayıs University, Faculty of Science and Arts, Department of Biology, 55139 Kurupelit, Samsun - TURKEY

Received: 15.04.2009

Abstract: The goal of this study was to compare the lipid, glycogen, and total sugar levels in completely starved (no water or sugar provided), honey-fed, sucrose-fed, or sugar-starved (only water provided) females of the parasitoid *Bracon hebetor* (Say) (Hymenoptera: Braconidae). We also quantified the lipid, glycogen, and total sugar levels in newly emerged unfed (day 0) female (n = 30) wasps. The mean amounts of reserves obtained from these females were regarded as a standard for the initial amount of reserves. The glycogen level of emerging females was $17.99 \pm 2.02 \mu\text{g}$. As expected, sucrose- or honey-fed females had significantly higher glycogen levels than emergent, completely starved, or sugar-starved females. There was no significant difference between completely starved and sugar-starved females for glycogen levels. Emerging females had significantly lower total sugar levels ($10.38 \pm 0.88 \mu\text{g}$) than those receiving other diet treatments. Total sugar levels did not significantly differ between completely starved and sugar-starved females. Similarly, honey-fed or sucrose-fed females had similar levels of total sugars. Female *B. hebetor* emerged with the highest amounts of lipid ($261.07 \pm 16.57 \mu\text{g}$). Lipid levels in females of all diet treatments declined significantly from emergence to the tenth day of adult life. The decline in lipid levels was slower in honey-fed or sucrose-fed females than in those of completely starved or sugar-starved females.

Key words: *Bracon hebetor*, glycogen, lipid, sugar feeding, total sugar

Şekerle beslenmenin, parazitoit *Bracon hebetor* (Say) (Hymenoptera: Braconidae) dişilerinin lipid, glikojen ve toplam şeker miktarına etkisi

Özet: Bu çalışmanın amacı, tamamen aç bırakılan (ne su ne de şeker verilmiş), bal ile beslenen, sukroz ile beslenen ya da şeker yönünden aç bırakılan (sadece su verilen) parazitoit *Bracon hebetor* (Say) (Hymenoptera: Braconidae) dişilerindeki lipid, glikojen ve toplam şeker miktarlarını karşılaştırmaktır. Aynı zamanda, yeni ergin beslenmemiş (0 günlük) dişi (n = 30) parazitoitlerdeki lipid, glikojen ve toplam şeker miktarları da belirlenmiştir. Bu dişilerden elde edilen maddelerin ortalama miktarları, söz konusu maddelerin başlangıç miktarları olarak kabul edilmiştir. Yeni ergin dişilerin glikojen miktarı $17.99 \pm 2.02 \mu\text{g}$ olarak belirlendi. Beklenildiği gibi, sukroz ya da bal ile beslenen dişiler, yeni ergin, tamamen aç ya da şeker verilmeyen dişilerden daha fazla glikojene sahip oldu. Tamamen aç bırakılan ya da şeker verilmeyen dişilerin glikojen miktarları birbirinden farklı değildi. Yeni ergin dişilerin toplam şeker miktarı, diğer besin gruplarında bulunanlardan daha düşüktü. Tamamen aç bırakılan ve şeker verilmeyen dişilerin toplam şeker miktarları birbirinden farklı değildi. Benzer şekilde, bal ya da sukroz ile beslenenlerin toplam şeker seviyeleri aynıydı. Dişi *B. hebetor* ergin olduğunda en yüksek lipid oranına ($261.07 \pm 16.57 \mu\text{g}$) sahipti. Tüm besin gruplarındaki dişilerin lipid miktarları, yeni erginleştikleri günden ergin hayatın onuncu gününe kadar önemli ölçüde azaldı. Lipid seviyelerindeki bu düşüş, bal ya da sukroz ile beslenen dişilerde, tamamen aç bırakılan ya da şeker verilmeyen dişilere göre daha yavaş gerçekleşti.

Anahtar sözcükler: *Bracon hebetor*, glikojen, lipid, şekerle beslenme, toplam şeker

* E-mail: eakman@omu.edu.tr

Introduction

The adults of many parasitoid species depend entirely or primarily on carbohydrates as energy resources. Carbohydrate feeding optimizes parasitoid effectiveness by increasing longevity, fecundity, and/or parasitism rates (Godfray 1994; Olson and Andow 1998; Olson et al. 2000; Fadamiro and Heimpel 2001; Gündüz and Gülel 2004; Lee et al. 2004; Chen and Fadamiro 2006).

Sugar sources for parasitoids in the field include floral and extrafloral nectar as well as honeydew excreted by homopteran insects (Jervis and Kidd 1986; Hagley and Barber 1992; England and Evans 1997). Sucrose and its 2 monosaccharide components, glucose and fructose, are key components of nectar and honeydew. In addition to these key components, honeydew also contains several disaccharides, such as maltose and melibiose, and trisaccharides, such as melezitose, raffinose, and erlose (Wäckers 2001). These sugars can be used immediately to generate energy metabolic purposes or can be stored for later use through conversion to trehalose or glycogen (Rivero and Casas 1999; Fadamiro et al. 2005). The fat body is an organ equivalent in function to the human liver and stores carbohydrates in the form of glycogen and lipids in the form of triglycerides (Rivero and Casas 1999).

Bracon hebetor (Say) (Hymenoptera: Braconidae) is a gregarious, idiobiont, synovigenic larval parasitoid of pyralid moths. The biology of *B. hebetor* has been intensively studied because of both its suitability as a model organism, since it is easy to rear in the laboratory, and its potential as a biological control agent of stored product moths (Benson 1973; Taylor 1988; Brower and Press 1990; Gündüz and Gülel 2005; Milonas 2005; Magro et al. 2006).

The host moth, *Ephesia kuehniella* Zeller (Lepidoptera: Pyralidae) (Mediterranean flour moth), is a common pest of stored products; it is also used as a laboratory host or prey for various parasites and predators reared for release as biological control of other pests (Corbet 1968; Momen and El-Laithy 2007). It is possible that quantitative and qualitative differences exist among different insect groups and species in terms of the effect of sugar feeding on their fitness (Wäckers 2001; Chen and Fadamiro 2006).

Several studies have indicated that sugar feeding substantially changes total sugar, glycogen, and lipid levels of parasitoids (Olson et al. 2000; Lee et al. 2004; Chen and Fadamiro 2006). However, little is known about the impact of different factors on glycogen, lipid, and total sugar reserves of female *B. hebetor* (Gündüz et al. 2008). Accordingly, here we investigated, under laboratory conditions, the effect of sugar feeding upon glycogen, lipid, and total sugar reserves of female parasitoids.

Materials and methods

B. hebetor females used in this study were reared on late-stage larvae of the Mediterranean flour moth, *E. kuehniella*, at the Department of Biology, Ondokuz Mayıs University, Samsun, as described by Gündüz and Gülel (2004). Culturing and all experimental procedures were carried out in a room with a 16:8 L:D photoperiod, at a temperature of 26 ± 2 °C and 60 ± 5 % RH.

Newly emerged females were placed individually into glass tubes (15 × 100 mm) and provided with one of the following feeding treatments: (1) completely starved (no sugar or water provided); (2) sugar-starved (only water provided); (3) honey-fed (50% honey solution in water provided); and (4) sucrose-fed (50% sucrose solution in water provided). For the sugar-starved (water) treatment, a piece of cotton soaked in water was placed inside the tube; for the other 2 treatments females were given a similar piece of cotton soaked in a solution of honey or sucrose in water.

After the 10 days had elapsed, live females were frozen at -20 °C for biochemical analysis. Each treatment included at least 30 female wasps. We also collected 30 newly emerged females for baseline comparisons.

Lipid, glycogen, and total sugar levels were quantified using a series of biochemical tests originally developed by van Handel (1985a, 1985b) for mosquitoes, and adapted for parasitoids (Olson et al. 2000; Fadamiro and Heimpel 2001; Lee et al. 2004). Briefly, a female parasitoid was crushed with a plastic pestle in a 1.5 mL microcentrifuge tube containing 50 µL of 2% sodium sulfate in distilled water and then placed on ice. The solved nutrient was then extracted with 450 µL of chloroform-methanol (1:2), after which

the tube was vortexed. The tube was then centrifuged at $14,000 \times g$ for 2 min and 200 μL of the resulting supernatant was transferred to a glass test tube (12×75 mm) for the sugar assay and another 200 μL was transferred to a similar glass tube for the lipid assay. The precipitate was left in the microcentrifuge tube for the glycogen assay. All tubes were heated at 90°C until all of the solution had evaporated from the lipid and glycogen tubes and approximately 50 μL of solution remained in the sugar tubes.

Lipid

To analyze lipids, 40 μL of sulfuric acid was added to the tubes containing the lipid precipitate. The tubes were then heated at 90°C for 2 min. The tubes were cooled on ice and 960 μL of a vanillin-phosphoric acid reagent was added. The solution in each tube was left to react at room temperature for 30 min. They were then mixed and the absorbance read at 525 nm. Lipid concentrations were obtained from a standard curve using corn oil.

Glycogen

For glycogen analysis, 1 mL of anthrone reagent was added to the tube containing the precipitate and heated at 90°C for 15 min. The tube was cooled on ice and the absorbance read at 625 nm. Because all glycogen in the sample is presumed to have precipitated to the bottom of the tube, the absorbance amount is considered representative of the whole insect. Glycogen concentrations were obtained from a standard curve using glucose.

Total sugars

A hot anthrone test was used to estimate the amount of total sugars in each female. We added 950 μL of anthrone reagent to the sugar tube, heated at 90°C for 10 min and then cooled on ice. The absorbance was read at 625 nm. As for the glycogen, concentrations were obtained from a standard glucose curve.

Statistical analysis

Statistical analysis were executed using SPSS (Landau and Everitt 2004). Differences in lipid, glycogen, and total sugar levels of parasitoids were compared using one-way analysis of variance (ANOVA). Where the differences were significant, means were separated using the Student-Newman-Keuls (SNK) multiple range test at a probability level of $P \leq 0.05$.

Results

The mean lipid levels of emerging females was $261.07 \pm 16.57 \mu\text{g}$ ($n = 30$). It then declined significantly in females of all diet treatments ($P \leq 0.05$) (Table 1). The rate of lipid decline was slower in honey- or sucrose-fed females than in completely starved or sugar-starved females. The lipid levels of sucrose-fed wasps did not differ from those of honey-fed wasps. Moreover, completely starved and sugar-starved females contained similar lipid levels (Table 1).

Table 1. Amounts (mean \pm S.E.) of glycogen, lipid, and total sugars in female *Bracon hebetor* that were either newly emerged, completely starved, sugar-starved (only water provided), fed 50% honey, or fed 50% sucrose over the ten days of their lifespan.

	Lipid (μg) (Mean \pm S.E.) n = 30	Glycogen (μg) (Mean \pm S.E.) n = 30	Total Sugar (μg) (Mean \pm S.E.) n = 30
Newly emerged	$261.07 \pm 16.57\text{a}$	$17.99 \pm 2.02\text{b}$	$10.38 \pm 0.88\text{c}$
Completely starved	$49.49 \pm 8.90\text{c}$	$8.90 \pm 0.86\text{c}$	$27.28 \pm 3.94\text{b}$
Sugar-starved	$65.06 \pm 8.54\text{c}$	$9.39 \pm 1.13\text{c}$	$25.81 \pm 6.29\text{b}$
Honey-fed	$147.20 \pm 9.38\text{b}$	$24.91 \pm 1.59\text{a}$	$58.39 \pm 6.33\text{a}$
Sucrose-fed	$130.57 \pm 8.15\text{b}$	$22.87 \pm 1.97\text{a}$	$51.49 \pm 5.33\text{a}$

n indicates the number of females used in analysis. Values within the same column having different letters are significant ($P \leq 0.05$) (Student-Newman Keuls test).

The mean estimated amount of glycogen present in newly emerged female *B. hebetor* was 17.99 ± 2.02 μg . There was a significant effect from the diet treatments on the glycogen levels of female wasps (Table 1). The mean glycogen levels of honey- and sucrose-fed wasps were considerably higher than those of completely starved and sugar-starved wasps. However, there was no significant difference between honey- and sucrose-fed females ($P > 0.05$). Similar results were obtained for completely starved and sugar-starved females; the mean glycogen levels of these wasps were not significantly different (Table 1) ($P > 0.05$).

B. hebetor females emerged with lower levels of total sugar (10.38 ± 0.88 μg) when compared to older females as measured with the hot anthrone test. The sugar levels of females that had fed on sucrose and honey were significantly higher than those of the newly emerged, completely starved, and sugar-starved wasps (Table 1) ($P \leq 0.05$). Furthermore, completely starved and sugar-starved wasps had significantly higher sugar levels than newly emerged females ($P \leq 0.05$).

Discussion

We found that the lipid, glycogen, and total sugar levels of female *B. hebetor* were affected by sugar feeding.

B. hebetor females emerged with high reserves of lipids. In our study, lipid reserves appear irreplaceable through feeding; they decrease with time even in females fed ad libitum. In previous studies, similar results have been observed for *Macrocentrus grandii* (Goidanich) (Hymenoptera: Braconidae), *Nasonia vitripennis* (Walker) (Hymenoptera: Braconidae), and *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) (Olson et al. 2000; Rivero and West 2002; Lee et al. 2004).

While in sucrose- or honey-fed females lipid reserves decrease progressively with time, completely starved and sugar-starved females appear to make use of a disproportionate amount of their lipid reserves. This result indicates that a strong relationship exists between the parasitoid's nutritional state and their utilization of metabolic reserves.

We did not investigate the extent of lipogenesis in the parasitoid *B. hebetor* by conducting radiotracer studies. As the lipid composition of parasitoids decreased over their lifetime even if food was available, we can suggest that lipids are synthesized only during the pre-adult stages and that adults of this species have no lipogenic capabilities. Surprisingly, all parasitoid species studied so far seem unable to synthesize lipids from sugars (Olson et al. 2000; Rivero and West 2002; Lee et al. 2004). This is in contrast to de novo lipid synthesis in a number of other insect species fed only sugar (Walker et al. 1970; Warburg and Yuval 1996).

Anthrone tests of whole females showed that total sugar and glycogen levels of completely starved and sugar-starved individuals were significantly lower than those fed honey and sucrose. Likewise, in a recent study, Wyckhuys et al. (2008) compared the effects of honey, 50% sucrose solution, and honeydew produced by the soybean aphid, *Aphis glycines*, on nutrient levels and longevity of the parasitoid *Binodoxys communis*. They reported that total sugar and glycogen levels of honey- or sucrose-fed individuals were consistently higher than those fed honeydew or water. Similar results have also been recorded for several other parasitoids including *M. grandii* (Olson et al. 2000) and *D. insulare* (Lee et al. 2004). By contrast, Rivero and West (2002) reported that the glycogen content of honey-fed *N. vitripennis* females did not change with time.

We also noted that the emergence levels of glycogen were decreased in completely starved and sugar-starved females. The decrease in glycogen reserves in starved females appears to be associated with an increase in sugar reserves. This pattern suggests the mobilization of glycogen to body sugars. Indeed, it is known that glycogen can be stored only when sugar concentration in hemolymph reaches a certain threshold. In contrast, glycogen reserves are mobilized when the sugar concentration of the hemolymph has declined below a certain value (Ziegler and Schultz 1986; Rivero and Casas 1999).

In summary, our results provide insights into the temporal patterns of nutrient metabolism in the braconid wasp *B. hebetor*. Thus, it is hoped that this work will contribute to the growing body of literature on the nutritional ecology of this important parasitoid.

References

- Benson JF (1973) Intraspecific competition in the population dynamics of *Bracon hebetor* Say (Hymenoptera: Braconidae). *J Anim Ecol* 42: 105-124.
- Brower JH, Press JW (1990) Interaction of *Bracon hebetor* (Hymenoptera: Braconidae) and *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) in suppressing stored-product moth populations in small inshell peanut storages. *J Econ Entomol* 83: 1096-1101.
- Chen L, Fadamiro HY (2006) Comparing the effects of five naturally occurring monosaccharide and oligosaccharide sugars on longevity and carbohydrate nutrient levels of a parasitic phorid fly, *Pseudacteon tricuspis*. *Physiol Entomol* 31: 46-56.
- Corbet SA (1968) The influence of *Ephestia kuehniella* on the development of its parasite *Nemeritis canescens*. *J Exp Biol* 48: 291-304.
- England S, Evans EW (1997) Effects of pea aphid (Homoptera: Aphididae) honeydew on longevity and fecundity of the alfalfa weevil (Coleoptera: Curculionidae) parasitoid *Bathyplectes curculionis* (Hymenoptera: Ichneumonidae). *Environ Entomol* 26: 1437-1441.
- Fadamiro HY, Heimpel GE (2001) Effects of partial sugar deprivation on lifespan and carbohydrate mobilization in the parasitoid *Macrocentrus grandii* (Hymenoptera: Braconidae). *Ann Entomol Soc Am* 94: 909-916.
- Fadamiro HY, Chen L, Onagbola EO, 'Fudd' Graham LC (2005) Lifespan and patterns of accumulation and mobilization of nutrients in the sugar-fed phorid fly, *Pseudacteon tricuspis*. *Physiol Entomol* 30: 212-224.
- Godfray HJC (1994) Parasitoids. Behavioral and Evolutionary Ecology. Princeton University Press, Princeton, New Jersey.
- Gündüz EA, Gülel A (2004) *Bracon hebetor* (Say) (Hymenoptera: Braconidae) erginlerinde konukçu türünün ve besin tipinin ömür uzunluğuna etkisi. *Türk Entomol Derg* 28: 275-282.
- Gündüz EA, Gülel A (2005) Investigation of fecundity and sex ratio in the parasitoid *Bracon hebetor* (Say) (Hymenoptera: Braconidae) in relation to parasitoid age. *Türk J Zool* 29: 291-294.
- Gündüz EA, Gülel A, İşitan ÖV (2008) İki konukçu türünün, larva ektoparazitoiti *Bracon hebetor* (Say, 1836) (Hymenoptera: Braconidae)' da protein, lipit ve glikojen miktarlarına etkisi. *Türk Entomol Derg* 32: 33-42.
- Hagley EAC, Barber DR (1992) Effect of food sources on the longevity and fecundity of *Pholetesor ornigis* (Weed) (Hymenoptera: Braconidae). *Can Entomol* 124: 241-246.
- Jervis MA, Kidd NAC (1986) Host-feeding strategies in Hymenopteran parasitoids. *Biol Rev* 61: 395-434.
- Landau S, Everitt, BS (2004) A Handbook of Statistical Analyses using SPSS. Chapman & Hall, CRC, London, Boca Raton, FL.
- Lee JC, Heimpel GE, Leibe GL (2004) Comparing floral nectar and aphid honeydew diets on the longevity and nutrient levels of a parasitoid wasp. *Entomol Exp Appl* 111: 189-199.
- Magro SR, Dias AB, Terra WR, Parra JRP (2006) Biological, nutritional, and histochemical basis for improving an artificial diet for *Bracon hebetor* (Say) (Hymenoptera: Braconidae). *Neotrop Entomol* 35: 215-222.
- Milonas PG (2005) Influence of initial egg density and host size on the development of the gregarious parasitoid *Bracon hebetor* on three different host species. *Bio Control* 50: 415-428.
- Momen FM, El-Laithy AY (2007) Suitability of the flour moth *Ephestia kuehniella* (Lepidoptera: Pyralidae) for three predatory phytosemites (Acari:Phytoseiidae) in Egypt. *Int J Trop Insect Sci* 7: 102-107.
- Olson DM, Andow DA (1998) Larval crowding and adult nutrition effects on longevity and fecundity of female *Trichogramma nubilale* Ertle and Davis (Hymenoptera: Trichogrammatidae). *Environ Entomol* 27: 508-514.
- Olson DM, Fadamiro H, Lundgren JG, Heimpel GE (2000) Effects of sugar feeding on carbohydrate and lipid metabolism in a parasitoid wasp. *Physiol Entomol* 25: 17-26.
- Rivero A, Casas J (1999) Incorporating physiology into parasitoid behavioral ecology: the allocation of nutritional resources. *Res Popul Ecol* 41: 39-45.
- Rivero A, West SA (2002) The physiological costs of being small in a parasitic wasp. *Evol Ecol Res* 4: 407-420.
- Taylor AD (1988) Host effects on larval competition in the gregarious parasitoid *Bracon hebetor*. *J Anim Ecol* 57: 163-172.
- Van Handel E (1985a) Rapid determination of glycogen and sugars in mosquitoes. *J Am Mosq Control Assoc* 1: 299-301.
- Van Handel E (1985b) Rapid determination of total lipids in mosquitoes. *J Am Mosq Control Assoc* 1: 302-304.
- Wäckers FL (2001) A comparison of nectar- and honeydew sugars with respect to their utilization by the hymenopteran parasitoid *Cotesia glomerata*. *J Insect Physiol* 47: 1077-1084.
- Walker PR, Hill L, Bailey E (1970) Feeding activity, respiration, and lipid and carbohydrate content of the male desert locust during adult development. *J Insect Physiol* 16: 1001-1015.
- Warburg MS, Yuval B (1996) Effect of diet and activity on lipid levels of adult Mediterranean fruit flies. *Physiol Entomol* 21: 151-158.
- Wyckhuys KAG, Strange-George JE, Kulhanek CA, Wäckers FL, Heimpel GE (2008) Sugar feeding by the aphid parasitoid *Binodoxys communis*: How does honeydew compare with other sugar sources? *J Insect Physiol* 54: 481-491.
- Ziegler R, Schultz M (1986) Regulation of carbohydrate metabolism during flight in *Manduca sexta*. *J Insect Physiol* 32: 997-1001.