

Protein, lipid, and glycogen levels in the parasitoid *Bracon hebetor* Say (Hymenoptera: Braconidae)

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Abstract: This study compared the protein, lipid, and glycogen reserves in recently emerged unfed female and male *Bracon hebetor* Say (Hymenoptera: Braconidae) to those in 5- and 10-day-old wasps fed honey. *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae) and *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) were used as host species. The mean level of protein in both sexes increased significantly from emergence to day 5, and then returned to emergence levels on day 10 in females, but remained constant in males during the same period. In honey-fed females glycogen levels increased significantly during the first 10 days of life; however, there was no significant increase in males. High levels of lipid were observed in 1-day-old females and males, and levels declined with age in wasps that were fed; the decrease in lipid levels was more pronounced in females than in males.

Key words: Age, lipid, protein, glycogen, *Bracon hebetor*, *Galleria mellonella*, *Ephestia kuehniella*

Parazitoit *Bracon hebetor* Say (Hymenoptera: Braconidae)'da protein, lipit ve glikojen miktarları

Özet: Bu çalışmada, yeni ergin ve beslenmemiş dişi ve erkek *Bracon hebetor* Say (Hymenoptera: Braconidae) bireyleri ile bal ile beslenen 5 gün ve 10 gün yaşlı bireylerin protein, lipit ve glikojen miktarları karşılaştırılmıştır. Konak olarak *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae) and *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) kullanılmıştır. Her iki eşeydeki ortalama protein miktarı yumurtadan çıkıştan, ergin hayatın onuncu gününe kadar arttı. Daha sonra, dişilerdeki protein miktarı başlangıçtaki seviyeye dönerken, erkeklerde aynı zaman süresince sabit kaldı. Balla beslenen dişilerin glikojen seviyeleri ergin hayatın ilk on günü süresince artarken, erkeklerde önemli bir artış olmadı. 1-gün-yaşlı erkek ve dişilerde fazla miktarda lipit belirlendi. Her iki eşeyde de ergin bireyler yaşlandıkça, lipit seviyesi azaldı. Lipit seviyesindeki azalma dişilerde, erkeklerden daha belirgin oldu.

Anahtar sözcükler: Yaş, lipit, protein, glikojen, *Bracon hebetor*, *Galleria mellonella*, *Ephestia kuehniella*

Introduction

Most adult parasitoids require considerable amounts of protein, lipid, and carbohydrate for

survival and reproduction (Morales-Ramos et al., 1996; England and Evans, 1997; Heimpel et al., 1997; Olson and Andow, 1998; Rivero and Casas, 1999;

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Olson et al., 2000; Fadamiro and Heimpel, 2001; Lee et al., 2004; Hogervorst et al., 2007). These nutrients are either carried over from the larval stage or may be synthesized de novo by adults after ingesting the relevant precursors (van Lenteren et al., 1987; Waldbauer and Friedman, 1991; Jervis et al., 1993).

Bracon hebetor is a gregarious, larval ectoparasitoid of many lepidopteran species. Females of this parasitoid are synovigenic, that is, they emerge with a very limited number of mature eggs. Egg production and maturation are continuous throughout the life of the female (Jervis and Kidd, 1986; Godfray, 1994). In synovigenic species, females consume host materials (host feeding) and/or other nutrient sources (e.g. nectar, pollen, plant exudates, or honeydew) for metabolic maintenance and egg production (Jervis and Kidd, 1986; Rivero and Casas, 1999).

Sugar is the main energy source for adult parasitoids and some species forage actively for sources of sugar in the field (e.g. nectar, pollen, plant exudates, or honeydew) (Jervis and Kidd, 1986; Heimpel et al., 1997; Lee et al., 2006; Wäckers et al., 2006). Ingested sugars can be used immediately for energy production or stored for later use following conversion to trehalose and glycogen (Rivero and Casas, 1999). In synovigenic species sugar feeding may help increase the number of mature eggs (Olson and Andow, 1998) and can prevent resorption of mature eggs (Heimpel et al., 1997). A number of authors have reported that feeding on sugar has a positive effect on longevity and/or fecundity in parasitoids (Godfray, 1994; Heimpel et al., 1997; Olson et al., 2000; Lee et al., 2004; Chen and Fadamiro, 2006). In our previous studies we showed that food types affect longevity and that parasitoid age affects fecundity and the sex ratio in *B. hebetor* (Gündüz and Gülel, 2004, 2005).

Although various studies have demonstrated the effects of age and diet on nutrient levels in different parasitoid species (Olson et al., 2000; Rivero and West, 2002; Giron and Casas, 2003; Lee et al., 2004), protein, lipid, and glycogen levels in *B. hebetor* adults are not well known. Accordingly, the objective of the present study was to compare these nutrient levels in recently emerged unfed *B. hebetor* to those in aged and fed parasitoids.

Materials and methods

Insects

We studied *B. hebetor*, a common parasitoid of pyralid moths in stored products (Taylor, 1988; Brower and Press, 1990; Milonas, 2005). Late-stage larvae of the Mediterranean flour moth (*Ephestia kuehniella*) and greater wax moth (*Galleria mellonella*) were used as hosts. All cultures were maintained at 26 ± 2 °C and $60 \pm 5\%$ RH under continuously illuminated laboratory conditions. Different wasps were used for the analysis of each nutrient.

Nutrient levels

In order to compare protein, lipid, and glycogen reserves in recently emerged unfed *B. hebetor* to those in aged and fed parasitoids, adults were analyzed at 1, 5, and 10 days of age. Unfed 1-day-old adults were used to determine initial levels of protein, lipid, and glycogen in adult females and males, whereas 5- and 10-day-old parasitoids were individually placed into a test tube and fed a honey solution. For this purpose a piece of cotton soaked in a solution of honey in water (1:1, v:v) was placed inside the tube and was changed every other day. After 5 or 10 days had elapsed, parasitoids were weighed in groups of the same sex, killed, and stored at -50 °C until biochemical analysis.

Protein

Protein extraction was carried out according to Plummer (1971). Parasitoids, containing 10 females or males, were homogenized in 500 μ L of 10% trichloroacetic acid solution and centrifuged at 3500 rpm at room temperature for 15 min. The supernatant was discarded and 500 μ L of 96% ethyl alcohol was added to the tubes. They were then mixed and centrifuged at 3500 rpm for 15 min. After centrifugation the supernatant was discarded and the pellet was redissolved in distilled water. The quantity of protein was determined using Folin-phenol reagent, as described by Lowry et al. (1951). Absorbance at 695 nm was read with a spectrophotometer. Bovine serum albumin was used as the standard protein.

Lipid

Lipid content was estimated according to Olson et al. (2000), but female parasitoid ovaries were not removed from the body. Parasitoids were transferred

one at a time to 1.5-mL microcentrifuge tubes and were crushed with a plastic pestle in 50 μ L of 2% sodium sulphate. Then 450 μ L of chloroform-methanol (1:2) was added to wash the pestle. The tubes were then vortexed and centrifuged at 16,000 rpm for 2 min, and then 200 μ L of the supernatant was transferred to a glass tube. Tubes were heated at 90 °C until the solution completely evaporated. Samples were then dissolved in 40 μ L of sulfuric acid and heated at 90 °C for 2 min. The tubes were cooled on ice and 960 μ L of vanillin-phosphoric acid reagent (van Handel, 1985) was added, the sample in each tube was left at room temperature for 30 min, and then absorbance was read at 525 nm with a spectrophotometer. Corn oil was used as the standard lipid.

Glycogen

Glycogen was extracted from insects using the method described by Joseph et al. (1961). Samples were homogenized in 10% trichloroacetic acid solution at 15,000 rpm for 5 min. During the homogenization period, the homogenizing flask was placed in an ice-water bath to maintain the temperature of the contents below 15 °C. The homogenate was passed through filter paper and the total volume of the filtered fluid was measured. Aliquots were pipetted into centrifuge tubes and 5 volumes of ethyl alcohol were added to precipitate glycogen. After the solution was kept overnight at 35-

40 °C, glycogen was precipitated at 3500 rpm for 15 min. The fluid was decanted and drained. Pellets were resuspended in 2 mL of distilled water and the proportion of glycogen was determined using the anthrone method, as described by Nicholas et al. (1956). Absorbance was measured at 620 nm with a spectrophotometer. Glucose solution was used as the standard.

Statistical analysis

For data analysis we used the mean quantity of nutrient per 100 mg of parasitoid mass. Differences in the protein, lipid, and glycogen levels between recently emerged unfed parasitoids, and aged and fed parasitoids were compared using one-way analysis of variance (ANOVA). When 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

Table 1 shows the comparison of the mean protein, lipid, and glycogen reserves in 1-, 5-, and 10-day-old *B. hebetor* females and males maintained on *G. mellonella*. The mean quantity of each nutrient obtained from 1-day-old unfed parasitoids was regarded as the standard for the initial reserve in *B. hebetor* females and males (Table 1).

Table 1. Protein, lipid, and glycogen contents in female and male *Bracon hebetor* reared on *Galleria mellonella*.

Age (Days)	Protein (mg 100 mg ⁻¹) (Mean \pm SE) (n)		Lipid (mg 100 mg ⁻¹) (Mean \pm SE) (n)		Glycogen (mg 100 mg ⁻¹) (Mean \pm SE) (n)	
	Female	Male	Female	Male	Female	Male
1	4.97 \pm 0.17a (200)	4.89 \pm 0.18a (200)	10.85 \pm 0.42a (100)	7.88 \pm 0.47a (100)	0.13 \pm 0.02a (300)	0.35 \pm 0.07a (300)
5	6.38 \pm 0.29b (200)	5.89 \pm 0.22b (200)	7.80 \pm 0.39b (100)	6.79 \pm 0.42a (100)	0.28 \pm 0.03b (360)	0.48 \pm 0.08a (320)
10	5.23 \pm 0.29a (200)	6.07 \pm 0.29b (200)	6.25 \pm 0.25c (100)	5.22 \pm 0.31b (100)	0.48 \pm 0.03c (320)	0.54 \pm 0.06a (360)

Numbers in parentheses indicate number of adults used in the analysis. Means within columns followed by the same letter are not significantly different at $P \leq 0.05$ (Student-Newman Keuls test).

The mean protein level in 1-day-old *B. hebetor* females and males was 4.97 ± 0.17 mg 100 mg⁻¹ and 4.89 ± 0.18 mg 100 mg⁻¹, respectively. Mean protein level increased in 5-day-old adults of both sexes and this level was maintained over time in honey-fed male parasitoids, but decreased to initial levels in females (Table 1) (females: $F_{2,57} = 8.341$, $P < 0.005$; males: $F_{2,57} = 7.357$, $P < 0.005$).

The highest lipid levels were observed in 1-day-old *B. hebetor* females and males; lipid levels then decreased in 5- and 10-day-old parasitoids (Table 1) (females: $F_{2,27} = 41.990$, $P < 0.005$; males: $F_{2,27} = 11.008$, $P < 0.005$). Lipid levels in honey-fed females were higher than in honey-fed males.

The mean quantity of glycogen in *B. hebetor* females and males upon emergence was 0.13 ± 0.02 mg 100 mg⁻¹ and 0.35 ± 0.07 mg 100 mg⁻¹, respectively (Table 1). The glycogen level then significantly increased in honey-fed females ($F_{2,15} = 43.562$, $P < 0.005$), whereas honey-fed males had a virtually stable level of glycogen ($F_{2,15} = 1.962$, $P = 0.175$).

Protein, lipid, and glycogen reserves in *B. hebetor* females and males maintained on *E. kuehniella* are shown in Table 2.

Protein levels in 1-day-old females and males were 7.30 ± 0.35 mg 100 mg⁻¹ and 6.66 ± 0.29 mg 100 mg⁻¹, respectively (Table 2). Protein levels then

significantly increased in 5-day-old female and male parasitoids (females: $F_{2,57} = 8.876$, $P < 0.005$; males: $F_{2,57} = 4.100$, $P < 0.005$). Protein levels dropped to initial levels in 10-day-old females, but not in 10-day-old males.

Lipid levels in emerging *B. hebetor* females and males were 7.68 ± 0.22 mg 100 mg⁻¹ and 4.65 ± 0.32 mg 100 mg⁻¹, respectively. They then decreased in 5- and 10-day-old individuals of both sexes (Table 2) (females: $F_{2,27} = 113.037$, $P < 0.005$; males: $F_{2,27} = 13.196$, $P < 0.005$). The decrease in lipid reserves was more pronounced in females than in males.

Low levels of glycogen were observed in 1-day-old females and males (Table 2). Honey-fed 1- and 10-day-old females had significantly higher glycogen levels ($F_{2,15} = 24.067$, $P < 0.005$); however, there was not a significant increase in the glycogen level in males ($F_{2,15} = 2.853$, $P = 0.089$).

Discussion

B. hebetor is a synovigenic autogenous parasitoid that produces yolk-rich (anhydropic) eggs during its imaginal stage. Protein is one of the main components of insect egg yolk (Nijhout, 1994; Rivero and Casas, 1999). In the present study protein levels in females and males increased significantly from emergence to the fifth day of adult life. In females it then decreased

Table 2. Protein, lipid and glycogen contents in female and male *Bracon hebetor* reared on *Ephestia kuehniella*.

Age (Days)	Protein (mg 100 mg ⁻¹) (Mean \pm SE) (n)		Lipid (mg 100 mg ⁻¹) (Mean \pm SE) (n)		Glycogen (mg 100 mg ⁻¹) (Mean \pm SE) (n)	
	Female	Male	Female	Male	Female	Male
1	$7.30 \pm 0.35a$ (200)	$6.66 \pm 0.29a$ (200)	$7.68 \pm 0.22a$ (100)	$4.65 \pm 0.32a$ (100)	$0.30 \pm 0.03a$ (300)	$0.57 \pm 0.06a$ (300)
5	$8.38 \pm 0.23b$ (200)	$0.57 \pm 0.06a$ (200)	$4.48 \pm 0.21b$ (100)	$3.97 \pm 0.23a$ (100)	$0.45 \pm 0.06b$ (300)	$0.75 \pm 0.07a$ (300)
10	$6.47 \pm 0.37a$ (200)	$7.97 \pm 0.40b$ (200)	$3.78 \pm 0.15c$ (100)	$2.91 \pm 0.27b$ (100)	$0.74 \pm 0.04c$ (300)	$0.77 \pm 0.05a$ (330)

Numbers in parentheses indicate number of adults used in the analysis. Means within columns followed by the same letter are not significantly different at $P \leq 0.05$ (Student-Newman Keuls test).

to emergence levels on the tenth day of adult life, whereas it remained virtually constant in males during the same time period. The observed increase in protein reserves in females during the initial days of adult life might have been related to the production of mature eggs. The material used for initial egg production comes from fat body reserves (Rivero and Casas, 1999). As female *B. hebetor* have very limited egg storage capacity, mature eggs removed by oviposition can provide space for others being formed. In the absence of hosts, mature eggs are resorbed by females. Thus, energy and materials contained in the eggs can be used both for maintenance and for sustaining future oogenesis (Jervis and Kidd, 1986). We conducted this study without providing hosts to the females. Although we did not investigate oosorption in this species, it is reasonable to suggest that the decrease in the level of protein in females after day 5 of adult life was related to oosorption.

Lipids are generally used by many parasitoid species in order to meet metabolic needs and to produce yolk-rich eggs (Ellers, 1996; Rivero and Casas, 1999). Lipids also produce acetyl groups that serve as the basis for the biosynthesis of nearly all other types of essential molecules, including amino acids (Nijhout, 1994). *B. hebetor* females and males emerge with a relatively high level of lipids. High lipid composition in newly emerged parasitoids is consistent with the importance of this component for both everyday activities and reproduction. Females utilized a significant amount of lipid reserves during the initial days of adult life. Similarly, males, although at a slower rate, catabolized their original lipid reserves. The present results suggest that lipids were synthesized only during the pre-adult stages and that *B. hebetor* adults had no lipogenic capability. Similar conclusions have been reported for *Anastrepha serpentina* (Wiedmann) (Diptera: Tephritidae), *Asobara tabida* (Nees) (Hymenoptera: Braconidae), *Macrocentrus grandii* (Goidanich) (Hymenoptera: Braconidae), *Nasonia vitripennis* (Walker) (Hymenoptera: Braconidae), and *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) (Jacome et al., 1995; Ellers, 1996; Olson et al., 2000; Rivero and

West, 2002; Lee et al., 2004). Nonetheless, lipogenesis has been reported in some other insect species, such as locusts and flies (Walker et al., 1970; Warburg and Yuval, 1996).

Adults of many parasitoid species obtain the materials required for adult maintenance and survival by feeding on many kinds of sugar sources (Jervis and Kidd, 1986; Heimpel et al., 1997; Fadamiro and Heimpel, 2001). Sugar feeding is especially important during the early phase of adult life in synovigenic parasitoids. This behavior probably prevents the consumption of fat body reserves for adult maintenance. Thus, fat body reserves can be used for egg production (Jervis and Kidd, 1986). Both *B. hebetor* females and males emerged with small glycogen reserves. Honey-fed female glycogen levels increased with age; however, males that were fed honey had a slight, but not significant, increase in glycogen levels with age. A possible explanation for this is that both *B. hebetor* adult males and females in the present study were able to synthesize glycogen from the sugars in honey; however, the rate of conversion of these sugars to glycogen reserves in females was higher than in males. Glycogen and total sugar levels increased with age in sucrose-fed *M. grandii* and nectar- or honeydew-fed *D. insulare* (Olson et al., 2000; Lee et al., 2004). In contrast, in *N. vitripennis* the glycogen content of honey-fed females did not increase significantly with time (Rivero and West, 2002).

In conclusion, the present results provide insight into the relationship between adult age and nutrient metabolism in the braconid *B. hebetor*. Further studies should continue to explore the accumulation and utilization of these nutritional reserves throughout *B. hebetor*'s lifespan.

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