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Orijinal araştırma (Original article)

Lipid, total sugar and glycogen composition in the parasitoid, *Bracon hebetor* Say, 1836 (Hymenoptera: Braconidae) during starvation

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Summary

The effect of starvation on lipid, total sugar and glycogen contents of braconid species, *Bracon hebetor* Say, 1836 (Hymenoptera: Braconidae) was examined under laboratory conditions. Adults of *B. hebetor* were starved for up to ten days. Lipid concentration in female and male parasitoids declined consistently throughout the starvation period when compared with the other nutrient reserves. In females a mild decline in total sugar levels followed emergence and continued through the fifth days of life. On subsequent days females had more or less a stable level of total sugar with slight oscillations. In males, total sugar concentration decreased at the beginning of starvation and remained constant over starvation period. The glycogen reserves of female and male parasitoids that had been starved for ten days reached a peak in 24 h and then declined slightly during experimental period.

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

Anahtar sözcükler: *Bracon hebetor,* açlık, lipit, toplam şeker, glikojen

Introduction

Insects have to expend energy constantly for survival and reproduction, and if they are not feeding, they must live on reserves accumulated in periods of food abundance (Visser & Ellers, 2008; Arrese & Soulages, 2010). There is remarkable similarity in the basic qualitative nutritional requirements and nutritional biochemistry of all insects (Thompson, 1999). Their nutritional behavior often reflects their physiological needs.

Adult parasitoids consume host materials (host-feeding) and/or other nutrient sources (*e.g*. nectar, pollen, plant exudates or honeydew) as food (Jervis & Kidd, 1986; Rivero & Casas, 1999). By feeding on these food sources, parasitoids can improve their longevity, fecundity and searching efficiency (Morales-Ramos et al., 1996; England & Evans 1997; Olson & Andow, 1998; Fadamiro & Heimpel, 2001; Lee et al., 2004; Hogervorst et al., 2007; Kapranas & Luck, 2008). In the food scarcity, they have to have some chemical changes in their metabolism. In that case, metabolic reserves which carried over from the larval stage serve as an insurance against the uncertainty of finding food sources during the first days of adult life of parasitoids.

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Many researchers have studied the starvation and the effects of this process on the metabolism of insects (Newton, 1954; Chaudhary et al., 1964; Auerswald & Gäde, 2000; Satake et al., 2000; Marron et al., 2003). In general, they suggested that stores of energy substrates in insect are depleted and can not be replenished during starvation.

Bracon hebetor Say 1836 (Hymenoptera: Braconidae) is a gregarious, larval ectoparasitoids of many lepidopteran species (Benson, 1973; Taylor, 1988; Brower & Press, 1990; Heimpel et al., 1997; Baker & Fabric, 2000; Darwish et al., 2003; Milonas, 2005; Magro et al., 2006). In our previous studies we reported that different abiotic and biotic factors change nutrient levels of *B. hebetor* adults (Gündüz et al., 2008; Gündüz & Gülel, 2010; Gündüz et al., 2010). However, none of these studies deal with the impact of starvation on lipid, total sugars and glycogen reserves of female and male *B. hebetor*. Therefore, the present study aims to determine the effect of starvation on nutrient levels of parasitoid *B. hebetor* during ten days of starvation period.

Material and Methods

Bracon hebetor Say, 1836 and late instar larvae of Mediterranean flour moth, *Ephestia kuehniella* Zeller 1879 (Lepidoptera: Pyralidae), were used throughout these experiments. The methods to establish and maintain stock cultures of both host and parasitoid species are described by Gündüz & Gülel (2004). Both the rearing of insects used in the experiments and the experiments themselves were done at 26 ± 2^{o}C, 60 ± 5% RH and 16L : 8D.

For investigating the effect of starvation, parasitoid adults were sexed at emergence and then they were placed individually into glass tubes (10 × 150 mm) and subjected to varying periods of starvation under the conditions described above. After the starvation period had elapsed, live adults were frozen at -20 ^{o}C for the biochemical analysis. We also used newly emerged wasps to determine initial amounts of lipid, total sugar and glycogen in adult females and males.

Lipid, glycogen and total sugar levels were determined using common biochemical analysis protocols (Olson et al., 2000; Fadamiro & Heimpel, 2001; Lee et al., 2004). Wasps were individually homogenized in 50 µl of 2% sodium sulfate and 450 µl chloroform-methanol (1: 2) in 1.5 ml microcentrifuge tubes. The mixture was centrifuged at 14 000 g for 2 min and 200 µl of the resulting supernatant was transferred to a glass test tube (10 × 50 mm) for the sugar assay and 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 ºC until all solution was evaporated from the lipid and glycogen tubes and approximately 50 µl of solution remained in the sugar tubes. For the lipid assay, 40 µl of sulfuric acid was added and heated at 90 ºC for 2 min. Then 960 vanillin-phosphoric acid reagent was added (Van Handel, 1985), mixed and kept at room temperature for 30 min. Absorbance at 525 nm was determined with a spectrophotometer and compared with lipid standards using corn oil. For sugar assay, hot anthrone test was used. We added 950 µl anthrone reagent to the sugar tube, heated at 90 ºC for 10 min and cooled on ice. Absorbance at 625 nm was read and compared with glucose standards. With the glycogen precipitate, 1 ml of anthrone reagent was added, mixed and heated at 90 ºC for 15 min. Absorbance at 625 nm was determined and compared with glucose standards.

Data Analysis

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

Results and Discussion

There is a strong relationship exists between the insect's nutritional state and their utilization of metabolic reserves. Under positive energetic balance, insects maintain their original levels, or accumulate reserves, whereas under negative energetic balance, they utilize their energetic reserves (Downer, 1981).

The effect of starvation on lipid, total sugar and glycogen composition of *B. hebetor* is illustrated in Figure 1 and Figure 2. The results of our analysis showed that adult parasitoids emerged with a high amount of lipid reserves when compared with the other nutrient reserves. Having large amount of lipids gets some advantages to insects. Because, lipids had a higher caloric content/unit weight of substrate than carbohydrates and can be stored in anhydrous form. In addition, they provide almost two times more metabolic water than carbohydrates. This water is especially important during non-feeding stages in the insect's life history (Downer & Mathews, 1976). In newly emerged wasps metabolic activity and energy requirements are high since they need to mate and to disperse to find new hosts in which to feed and lay eggs.

Pronounced declines occurred in lipid levels of female (Figure1) and male (Figure 2) parasitoids during starvation. Figure 1 shows that the lipid level of female parasitoids dropped consistently during starvation, from an initial 119.62 ± 5.44 µg of newly emerged females to 16.44±1.96 µg after 10 days of starvation (F = 53.713, df = 10,201, p = 0.000). The concentration of lipid in males declined significantly during the whole period of starvation. It declined from an initial value of 104.70 ± 4.19 to 10.35 ± 1.06 µg after 10 days of starvation (Figure 2) (F = 72.808, df = 10,175, p = 0.000). This would suggest that lipid probably seem to serve as a principal source of energy during the initial stages of starvation in this species. Similar results have also been recorded for different insect species such as *Lymantria dispar* Linnaeus 1758 (Stockhoff, 1991) and *Bombyx mori* Linnaeus 1758 (Satake et al., 2000). On the contrary, in fruit beetle, *Pachoda sinuata* Fabricius 1775, carbohydrate reserves used first, then switch to lipid and protein metabolism when carbohydrates are gone (Auerswald & Gäde, 2000). Similarly, Wheeler & Buck (1992) reported that starvation immediately prior to metamorphosis greatly decreased the carbohydrates in *Solenopsis xyloni* McCook 1879. However, lipid levels did not drop during starvation and even appeared to increase slightly in this species. On the other hand, there is an insect which use the glycogen and fat reserves concomitantly during starvation. This situation was observed in Japanese beetle larvae by Newton (1954). He observed that eighty percent of the glycogen and seventy-one per cent of the fat was utilized in 4 weeks of starvation in this insect. All of these studies show that the utilization of nutrient reserves varies greatly in different insects during starvation.

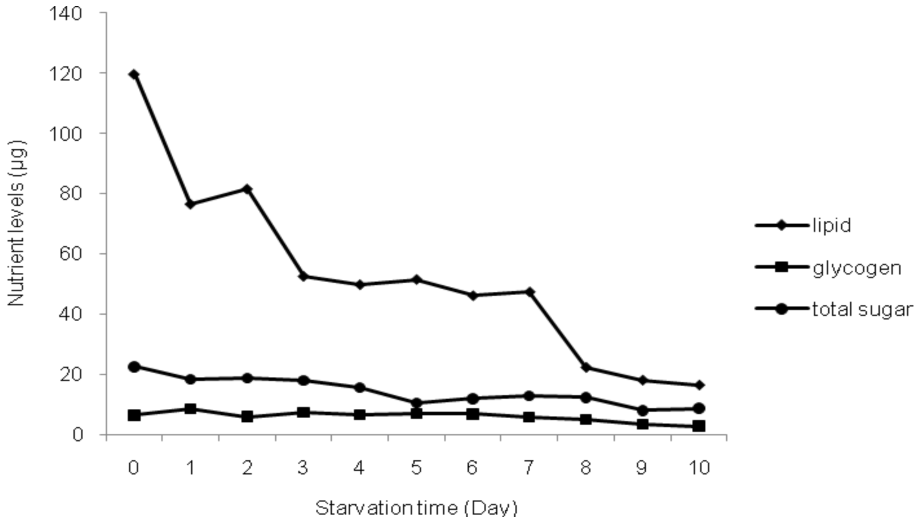

Figure 1. Effect of starvation on lipid, total sugar and glycogen composition of female *Bracon hebetor* Say (Hymenoptera: Braconidae).

In a previous study Gündüz & Gülel (2010) showed that lipid reserves is declined with time and cannot be replaced through feeding in *B. hebetor*. Interestingly, similar results have also been reported for different parasitoid species including *Macrocentrus grandii* Goidanich, 1937 (Hymenoptera: Braconidae) (Olson et al., 2000), *Nasonia vitripennis* Walker 1836 (Hymenoptera: Braconidae) (Rivero & West, 2002) and *Diadegma insulare* Cresson (Hymenoptera: Ichneumonidae (Lee et al., 2004). If the amount of lipids almost totally fixed at time of birth in these species, a prudent use of lipids is required to keep them as a spare fuel to survive periods of low sugar intake and for reproduction. Lipogenesis, however, is reported in a number of insect species including locusts, flies and some butterflies (Walker et al., 1970; Downer & Matthews, 1976; Warburg & Yuval, 1996).

In another study by Gündüz et al. (2010), lipid, glycogen, and total sugar levels of a female *B. hebetor*, were compared with those receiving different sugar-rich diet treatments. They reported that sucrose- or honey-fed females use the lipid reserves progressively with time, whereas completely starved and sugar-starved females use a disproportionate amount of their lipid reserves suggesting that access to carbohydrates slowed down the rate of lipid depletion. Similar results have been found for *N. vitripennis* (Rivero & West, 2002) and *Asobara tabida* Nees 1834 (Ellers, 1996).

Bracon hebetor females and males were emerged with lower amounts of total sugar and glycogen than lipid reserves. The concentration of total sugar was 22.46 ± 1.36 µg at the beginning of adult life. It declined slowly until about day 5 and then stabilized at this level during the entire period of starvation (Figure 1) (F = 13.404, df = 10,201, p = 0.000). The glycogen concentration of females raised from 6.32 ± 0.38 µg to 8.60 ± 0.35 µg after only one day of starvation and then declined to almost initial levels. This level showed some fluctuations until ninth days of adult life after which it significantly declined, reaching 2.75 ± 0.33 µg (Figure 1) (F = 14.365, df = 10,201, p = 0.000).

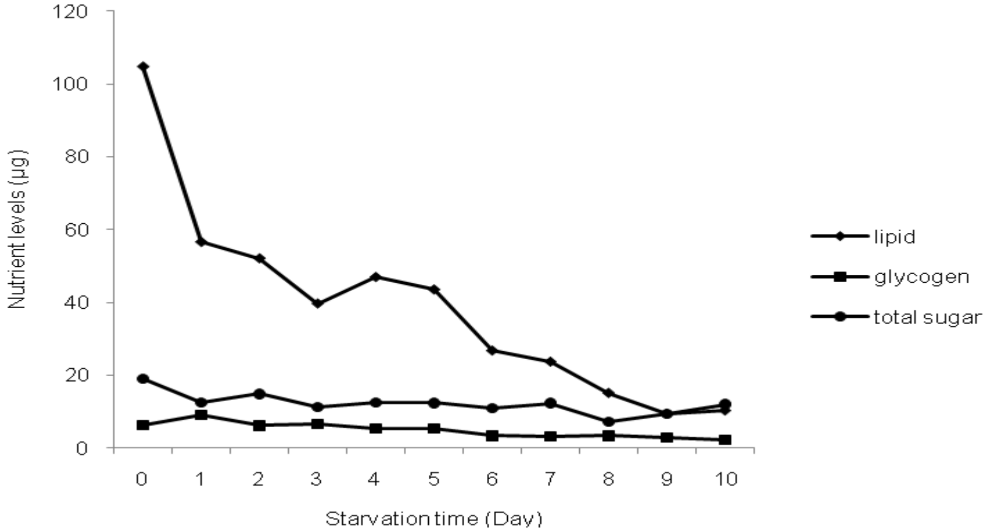

Figure 2. Effect of starvation on lipid, total sugar and glycogen composition of male *Bracon hebetor* Say (Hymenoptera: Braconidae).

In males, however, total sugar concentration decreased significantly at the beginning of starvation after which it remained at a stable level throughout the experiment (Figure 2) (F = 5.955, df = 10,175, p=0.000). Glycogen levels increased from the initial concentration of 6.41 ± 1.31 µg to 9.15 ± 0.66 after one day of starvation. This value was not significantly different from the initial value. It then declined to 2.29 ± 0.38 µg at the end of starvation period (Figure 2) (F = 25.067, df = 10,175, p = 0.000). In *M. grandii*, Olson et al. (2000) found that body sugars and glycogen reserves declined rapidly in starved individuals and were nearly depleted by the age of 4 days in both females and males. However, Giron & Casas (2003) described a different trend in body sugar and glycogen content of *Eupelmus vuiletti* CRW (Hymenoptera: Eupelmidae) during starvation. Body sugar levels of unfed *E. vuiletti* females increased weakly during the first 76 h following the emergence and this increase is associated with a concomitant decrease in glycogen reserves.

Overall, our experiment show that there is no possibility to refill stores during starvation and it results in a net loss of energy substrates. We assume, therefore, that all three main energetic resources were used to fulfill the various energetic requirements in *B. hebetor*, as demonstrated by statistically significant decreases at the end of starvation period. However, it should be emphasized that the laboratory results are only suggestive of what may occur under field conditions. Thereby, field studies are needed to determine the relationship between starvation and body composition in natural populations of this parasitoid species.

Özet

Açlık süresince parazitoit *Bracon hebetor* Say, 1836 (Hymenoptera: Braconidae)'un lipit, toplam şeker ve glikojen kompozisyonu

Açlığın, braconid türü, *Bracon hebetor* Say, 1836 (Hymenoptera: Braconidae)'un lipit, toplam şeker ve glikojen miktarına etkisi laboratuar koşullarında araştırılmıştır. *B. hebetor* erginleri 10 gün aç bırakılmıştır. Dişi ve erkek parazitoitlerdeki lipit konsantrasyonu açlık süresince diğer besin kaynaklarıyla karşılaştırıldığında, sürekli olarak azalma göstermiştir. Dişilerde, erginleşmeyi takiben toplam şeker seviyesi hafif bir şekilde azalmış ve azalma ergin hayatın ilk beş günü devam etmiştir. Sonraki günlerde dişiler, ufak dalgalanmalara rağmen az çok sabit şeker seviyesine sahip olmuştur. Erkeklerde, toplam şeker konsantrasyonu, açlığın başlangıcında azalmış, daha sonra sabit kalmıştır. On gün aç bırakılan dişi ve erkek parazitoitlerin glikojen kaynakları, ilk 24 saatte bir pik yaptıktan sonra deney periyodu süresince yavaş şekilde azalmıştır.

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