

Effect of age and host diet on protein content in parasitoid *Habrobracon hebetor* say (Hymenoptera: Braconidae), reared on *Corcyra cephalonica* stainton (Lepidoptera: Pyralidae)

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Abstract

Effect of host diet on the age-specific total protein content of *Habrobracon hebetor* Say, an idiobiont ectoparasitoid of the pyralid moths, was carried out. The rice moth, *Corcyra cephalonica*, a stored product pest causing severe losses to grains and products in storage, was used as host. The parasitoids were reared on 4th instar host larvae fed with different natural diets viz., wheat, maize, rice and sorghum. Total protein content of parasitoids was estimated for each group on first, fifth and tenth day of emergence. Age-specific total protein content of both male and female *H. hebetor* were affected significantly by the host diet. Protein content increased in both sexes from first to fifth day, however, towards the tenth day it had mostly decreased significantly in females but continued increasing in males. Females generally had more protein content than males. Parasitoids reared on host larvae fed on wheat showed the highest protein content initially in both males (6.10±0.04 mg/100mg ±SE) and females (7.86±0.15 mg/100mg), and reached the highest levels by day ten at 8.25±0.06 mg/100mg and 11.07±0.16 mg/100mg, respectively; also, in females the levels were found to increase from 5th to 10th day, unlike with other diets. Whereas, those reared on rice-fed host had the lowest protein content overall. It increased from 4.09±0.04 and 4.63±0.05 to 5.44±0.04 mg/100mg and 5.96±0.05 mg/100mg, in males and females respectively. Under similar conditions the differences between both sexes were statistically significant ($p < .05$), with the exception of sorghum on tenth day ($p = .079$). The protein level curves were also moderately correlated, with some even showing statistically significant association ($p < .01$). Since, *H. hebetor*, an idiobiont parasitoid, develop in closed systems without external nutritional supply, free amino acids and proteins, acquired via diet or through de novo synthesis, significantly influence the dietary and metabolic adaptations of insect parasitoids which may affect their performance. Therefore, it is critical to have in-depth knowledge of interactions between biological control agent and the target host insect to enhance the effectiveness of biological control.

Keywords: *Habrobracon hebetor*, *Corcyra cephalonica*, protein content, age, host diet, biological pest control

Introduction

Organisms engage in mutual interactions throughout the complex web of life on Earth, oftentimes called multitrophic interaction. The intricate relation between parasitoids and their hosts are among these interactions that offer insights into natural biological control. *Habrobracon hebetor* (Hymenoptera: Braconidae) is a parasitic wasp that parasitizes larval stage of several species of pyralid Lepidopteran moths which infests stored products (Nazarpour *et al.* 2015; Singh *et al.* 2009) [20, 26]. Employing *H. hebetor* to regulate the population of the stored product pest *Corcyra cephalonica* is an example of successful biological control. However, to gain a deeper understanding of this complex relation between parasitoid and host insect, further studies must be conducted. The biology of parasitoids, here *H. hebetor* is largely dependent on the protein content, which has profound effects on their performance as biocontrol agents.

All living organisms including insects need protein for proper growth and development (Kang *et al.* 2022; Liu *et al.* 2017) [13, 15]. Nutritional deficiency, in particular, long-term deficiency can affect biological function and even cause death in insects (Kirk, 2004) [14]. As biological control agents, the fitness and efficiency of parasitoid wasps such as *H. hebetor* are influenced by their protein content (Gündüz & Gülel, 2010) [8]. The age of parasitoid and host diet are two factors that can affect *H. hebetor*'s protein content. Understanding how these factors interact and affect protein content is crucial for optimizing the rearing and release of this parasitoid for pest management purposes.

When it comes to the physiology and behaviour of the parasitoid, age plays a critical role in the life cycle of *H. hebetor* (Mbata & Warsi, 2019) [18]. The requirement and protein metabolism of these parasitoids alter with age. The reproductive efficiency of *H. hebetor* is significantly affected by parasitoid age (Gündüz & Gülel, 2005) [7]. Another critical factor affecting the protein content of *H. hebetor* is the diet of the host insect, *Corcyra cephalonica* (Amadou *et al.* 2019) [1]. The protein content of the adult parasitoid largely depends on the quality and quantity of nutrients it obtains from its host during the larval stage. The host, in this case, serves as the primary source of nutrition for the developing parasitoid larvae (Hervet *et al.* 2023) [11]. *C. cephalonica* infests and feeds on a variety of stored grains and their products which have different nutritional composition (Vincent *et al.* 2021) [29]. Different grain-based diets have varying nutritional profiles, which may influence the quality and quantity of nutrients available to the developing parasitoid larvae (Thompson & Hagen, 1999) [28]. By examining how the diet of the host insect affects the protein content of *H. hebetor*, this research aims to determine the combined effect of age and host diet on protein content in the parasitoid, ultimately contributing to better pest control outcomes.

Materials and methods

All the insect cultures were kept (Singh, 2004) [27] and the experiments were conducted at $27 \pm 2^\circ\text{C}$, with a relative humidity of $70 \pm 10\%$, and 12:12 L:D photoperiod.

Rearing of the pest

C. cephalonica eggs were obtained from the Central Integrated Pest Management Centre (CIPMC), Gorakhpur and the culture was carried out based on Mathew (2019) [17]. Eggs obtained from the culture were also kept in different cereal diets, viz., rice (*Oryza sativa*. Linn.), jowar (*Sorghum vulgare*. Pers.), maize (*Zea mays*. Linn.) and wheat (*Triticum aestivum*. Linn.), in separate containers. Full-grown 4th instar larvae post 3-4 generation from these cultures were taken to be host for the parasitoid, *H. hebetor*.

Rearing of the parasitoid

For the culture of *Habrobracon hebetor*, adults were obtained from ICAR- National Rice Research Institute, Cuttack, Odisha. Culture method followed by Mathew (2019) [17] was used.

For the experiment, ten 4th instar larvae of *C. cephalonica* reared on fresh untreated individual grain diets were taken in 500 mL beakers and covered with muslin cloth. A gravid female *H. hebetor* was introduced in each beaker and after 24 hours of parasitization, adult female parasitoids were withdrawn from the beakers and parasitized host-larvae were kept for further development (Mathew, 2019) [17]. Male and female adult parasitoids emerging from the hosts on the same day, 5th day and 10th day were utilized for the estimation of protein content.

Estimation of Protein and Statistical analysis

Estimation of protein was carried out separately for males and females using the method described by Plummer (1971) [23]. For total protein content, adult parasitoids were anesthetized, weighed, and then homogenized in 500 μ L solution of 10% trichloroacetic acid (TCA) and then centrifuged at 3500 rpm for 15 minutes at room temperature. 500 μ L solution of 5% TCA was added to each tube after discarding the supernatant. The mixture was again centrifuged at 3500 rpm for 15 minutes. The pellet in each tube was then treated with 500 μ L of ethyl alcohol and centrifuged at 3500 rpm. Following centrifugation, the resultant pellet in each tube was dissolved in 1 mL of NaOH. The estimation of protein content was done using Folin-phenol reagent (Lowry *et al.* 1951), where Bovine serum albumin was used as the standard protein, and the absorbance was read at 660 nm with the help of a spectrophotometer. The absorption values were recorded and protein amounts were later calculated from resulting linear regression equation (Olson & Markwell, 2007) [22].

Protein content data was expressed as the mean quantity of protein (mg) per 100 mg of parasitoid mass. Means were subjected to analysis of variance (ANOVA), including both One Way and Two Way ANOVA, and when differences were significant, means were compared using Tukey test at a probability level of $P \leq 0.05$. Additionally, regression and correlation analyses were also conducted. SPSS Statistics version 20.0 (SPSS Inc., Chicago, IL, USA) Statistical Analysis Software was used for statistical analyses.

Results and discussion

Age-specific total protein content of male *H. hebetor* were significantly affected by the host diet. Protein content increased in both sexes from first to fifth day, however, towards the tenth day it had mostly decreased significantly in females but continued increasing in males (Fig 1.). Statistically significant increase ($p < 0.05$) was observed in

males from first through tenth day, however in females this trend was seen from first to fifth day only, except for the case of wheat: statistically significant fall ($p < 0.05$) were seen with the other three diets from fifth to tenth day. Females generally had more protein content than males (Fig 2.). Parasitoids reared on host larvae fed on wheat showed the highest protein content initially in both males (6.10 ± 0.04 mg/100mg \pm SE) and females (7.86 ± 0.15 mg/100mg), and reached the highest levels by day ten at 8.25 ± 0.06 mg/100mg and 11.07 ± 0.16 mg/100mg, respectively; also, in females the levels were found to increase from 5th to 10th day, unlike with other diets. Whereas, those reared on rice-fed host had the lowest protein content overall. It increased from 4.09 ± 0.04 and 4.63 ± 0.05 to 5.44 ± 0.04 mg/100mg and 5.96 ± 0.05 mg/100mg, in males and females respectively. Under similar conditions the differences between both sexes were statistically significant ($p < .05$), with the exception of sorghum on tenth day ($p = .079$) The protein level curves were also moderately correlated, with some even showing statistically significant association ($p < .01$). The effect of age on the combined means for all diets yielded interesting insights (Fig 3.). The protein content of females was statistically significantly higher ($p < .01$) in 0-day (6.03 ± 0.11 mg/100mg) and 5-day females (6.94 ± 0.13 mg/100mg) than that of the males (5.50 ± 0.14 mg/100mg, and 6.21 ± 0.14 mg/100mg, respectively). There was no such significant difference in 10-day parasitoids.

Age-specific total protein content of male *H. hebetor* were affected significantly by the host diet. The total protein content pattern of *H. hebetor* males emerging from host-larvae reared on wheat showed highly positive statistically significant correlation with that of individuals emerging from host-larvae reared on sorghum ($r = 1$, $p < .01$). Similarly, the total protein content pattern of *H. hebetor* males emerging from host-larvae reared on maize showed positive and statistically significant correlation with that of individuals emerging from host-larvae reared on rice ($r = .997$, $p < .05$).

Age-specific total protein content of female *H. hebetor* were affected by the host diet. The total protein content pattern of *H. hebetor* females emerging from host-larvae reared on wheat showed negative but not statistically significant correlation with that of individuals emerging from host-larvae reared on maize ($r = -.334$, $p > .05$). Similarly, the total protein content pattern of *H. hebetor* females emerging from host-larvae reared on wheat is correlated negatively but not significantly with that of individuals emerging from host-larvae reared on sorghum ($r = -.142$, $p > .05$). The total protein content pattern of *H. hebetor* females emerging from host-larvae reared on maize showed positive but not statistically significant correlation with that of individuals emerging from sorghum ($r = .980$, $p > .05$).

When comparing age-specific total protein content of male and female *H. hebetor* significant correlations were yielded. The total protein content pattern of *H. hebetor* males emerging from host-larvae reared on wheat showed positive and statistically significant correlation with that of female individuals emerging from host-larvae reared on wheat ($r = .999$, $p < .05$). Similarly, the total protein content pattern of *H. hebetor* males emerging from host-larvae reared on sorghum showed positive and statistically significant correlation with that of female individuals emerging from host-larvae reared on wheat ($r = .998$, $p < .05$). However, the total protein content pattern of *H. hebetor* males

emerging from host-larvae reared on wheat showed negative but not statistically significant correlation with that of female individuals emerging from host-larvae reared on maize ($r = -.381, p > .05$) and sorghum ($r = -.191, p > .05$). Similarly, the total protein content pattern of male *H. hebetor* emerging from host-larvae reared on sorghum was negatively but not significantly correlated with that of female individuals emerging from host-larvae reared on maize ($r = -.391, p > .05$) and sorghum ($r = -.202, p > .05$).

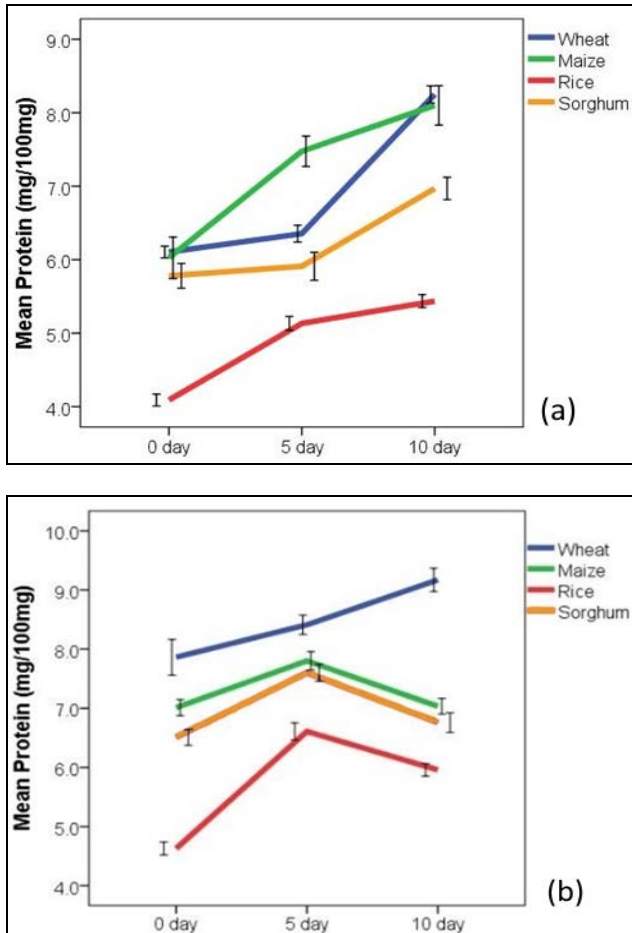


Fig 1: Mean amounts (mg/100 mg ± SE) of protein in male (a) and female (b) *Habrobracon hebetor* parasitizing *Corcyra cephalonica* host larvae reared on different diets

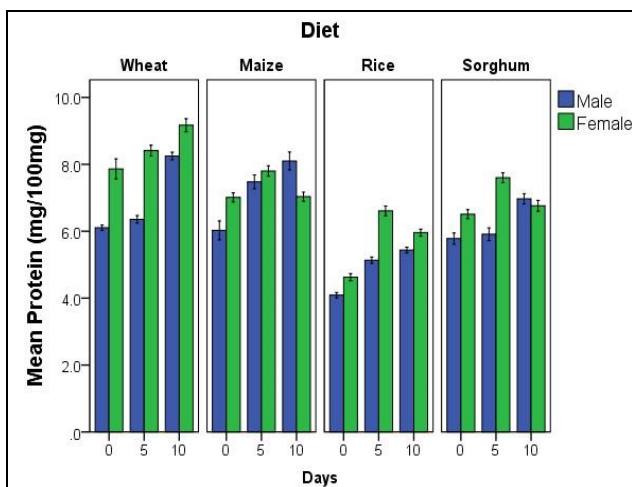


Fig 2: Male-female comparative mean amounts (mg/100 mg ± SE) of protein in *Habrobracon hebetor* parasitizing *Corcyra cephalonica* host larvae reared on different diets

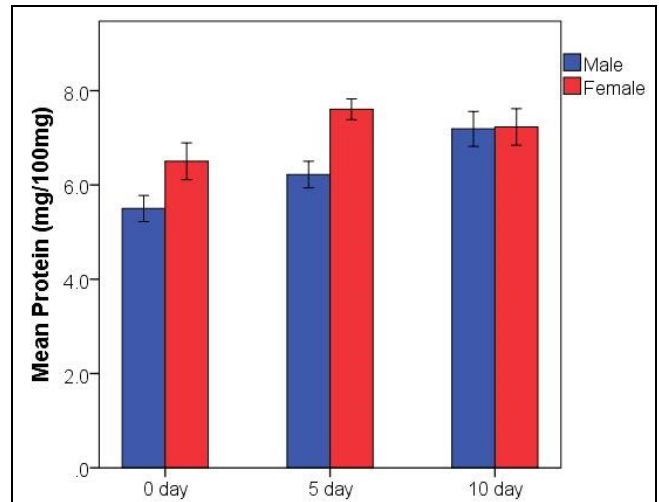


Fig 3: Combined age-specific mean amounts (mg/100 mg ± SE) of protein in males and female *Habrobracon hebetor* parasitizing *Corcyra cephalonica* host larvae reared on different diets

Conclusion

A successful use of bio-control agents for regulating pest populations is mostly dependent on their life-history parameters (Williams & Roane, 2007) [31]. The availability and quality of nutrients directly affects their several life-history parameters, and are therefore, of critical importance as their usefulness as bio-control agents. The importance of nutritional requirements and their availability; has long been recognized by investigators. Parasitoid nutrition is complicated because several factors (physiological, ecological, etc.) interact with one another in a tri-trophic context (Hare, 1992; Price *et al.*, 1980) [9,24]. Also, the feeding habits of adult and larval parasitoids differ where, the parasitic larval stages solely depend upon the host insect for nutrition, whereas, the adults generally depend upon non-host sources for energy. Thus, the host has a profound influence on growth, survival, and other life-history parameters of both larval and adult stages of parasitoids. The larval stages of parasitoids may exhibit effects of what they consume, as they spend the immature stages in/on one host with a confined food supply. Since, the quality of parasitoids' herbivorous hosts is influenced by the quality of host diet (Vinson & Barbosa 1987) [30], the effects may be reflected in the biochemical profile of the parasitoid. Thus, the quality and quantity of nutritional sources available to parasitoids affect several biological parameters. Proteins, in particular, play an important role in parasitoid reproduction and may have a direct influence on their efficacy as biocontrol agents.

Since, idiobiont parasitoids, like *H. hebetor*, develop without any external nutritional supply in closed systems (Mellini, 1986) [19] and also being a synovigenic species, i.e. emerging females have a limited quantity of mature eggs. The eggs are yolk-rich eggs, and they continue maturing throughout their life (Godfray, 1994) [4], so they need nutritionally very rich and concentrate food (Grenier, 2012) [6]. Protein and lipid are the two most important and essential components of any insect egg and their amounts in adults can have significant impact on the biology of insect (Nijhout, 1994) [21]. For normal development, nitrogen source, such as amino acids (Grenier, 2012) [6], and fatty acids (Yazgan, 1981) [33], etc., in the nutrition are required, and determines the quality of the adult parasitoid (Yazgan,

1972) [32]. Wheat and maize contain richer proportion of protein, than rice and jowar (Gopalan *et al.*, 2004) [5] which is reflected in the parasitoid protein levels as well. Benson (1973) [2] found that *B. hebetor* females emerge with nonmature eggs and the initial egg maturation sources its resources from fat body reserves. This explains the significantly higher levels of protein in 5-day-old females than 0-day ones (Isitan *et al.*, 2011) [12]. Host-feeding has been found to be essential for egg production and maturation in *B. hebetor* (Benson, 1973) [2] as it meets the high amino acid demand in the female during these processes (Giron *et al.* 2002, Heimpel *et al.* 1997) [3, 10]. In the absence of hosts, mature eggs are resorbed in females for maintenance and for sustaining oogenesis in adults. This may explain why the protein levels, in general, fall in 10-day females. However, if host is available then host feeding is essential means of obtaining the much-needed protein resources for the production of eggs (Rivero *et al.*, 2001) [25], which seem to happen with wheat diet. In males, however, the levels continue to rise which may be due to the continuous depletion of lipid resource. Information of host feeding in these conditions will allow us to get a better understanding in this matter, and will lead to a better and efficient biocontrol strategy.

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