Effects of Natural Food Additives on The Protein Content of Adult and Immature Stages of The Wax Moth Endoparasitoid, *Apanteles galleriae*

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ABSTRACT

The endoparasitoid, *Apanteles galleriae*, is one of the effective parasitoids on the greater and lesser wax moths, *Galleria mellonella* and *Achroia greissella*. The parasitoid was kept in the laboratory on lesser wax moths, *Achroia greissella* as a host. Males and females were fed on pure honey, diluted honey (80%), diluted honey + royal jelly (20 mg/ml), diluted honey + Pollen (20 mg/ml) and diluted honey + royal jelly + pollen (same ratio). Food substitutes were offered to the parasitoid freely. The resulting generation was then used for the protein content studies. The protein contents of both males and females were monitored throughout their life span. In addition, the protein contents of parasitoid larvae and pupae were monitored as well. Protein electrophoresis for previous treatments has shown heterogeneous band distribution when compared with controls. Band distributions and densities were characteristically variable over time, food supplement and insect stages.

Key words: ***Achroia greissella*, *Apanteles galleriae*, protein content.**

INTRODUCTION

The braconid, *Apanteles galleriae* Wilk., is one of the major parasitoids of wax moths. It was studied and was identified to the species level by several authors (Ibrahim, 1980; El-Hemeasy, 1983; Ibrahim *et al.*, 1984; Al-Arnaooty, 1985 and Tawfik *et al.*, 1985). The parasitoid, *A. galleriae* is a koinobiont, solitary and early instar larval endoparasitoid of many lepidopterous wax moths like *Galleria mellonella, Achroia grisella, Achroia innotata* Walker and *Vitula edmandsae* Packard (Shimamori, 1987; Watanabe, 1987 and Whitfield *et al.*, 2001). Economic losses due to the infestation of wax moths could be of great concern since the damage caused could exceed several million dollars annually (Williams, 1976). In *Apis cerana* bee hives in China, Zhou *et al.* (1989) reported serious damages caused by *G. mellonella* reducing honey yields by more than 20%. Parasitoids of wax moths are among the foremost candidates in the reconstruction of ecological balance and biological control applications since they pose lower environmental risks, tend to be host specific and are considered as ecological life vests in this regard (Hokkanen and Lynch, 1995; Andow *et al.*, 1997 and Uçkan and Gülel, 2002). Earlier trials have been done through the use of egg parasitoids like *Trichogramma evanescens*, *Trichogramma cacoeciae* and *Trichogramma minutum* against species of wax moths (Boldt and Marston, 1974). Larval parasitoids have been known to be successful in the search for proper biological control agent. One of them is *Venturia (Nemeritis) canescens* (Grav.), an ichneumonid, which was found by Salt (1976) to parasitize the larval stage in the laboratory. While Al-Arnaooty (1985) reported its presence in wax moth infested hives in the area of Qualiobia governorate in 1983. Females of the ectoparasitoid, *Bracon hebetor* (Say), were found to oviposit on final instars of wax moth larvae (Awadallah *et al.*, 1985; Tawfik *et al.*, 1985 and Tharwat, 1991). The endoparasitoid, *Apanteles galleriae*, is one of the major parasitoids of wax moths. Both the greater and the lesser wax moth second and third instar larvae are liable to be parasitized by the *Apanteles galleriae* females.

Suitable food substituents and supplements have been reported to be important factors behind the success of any biological control program (Wolcott, 1942; Hocking, 1966). To raise the impact of parasitoids on their host populations, additional food source strategies could be implemented. To date, only circumstantial evidence is available to indicate that natural enemies benefit from the availability of food supplements and that supplemental feeding could actually be translated into better host regulation (Wäckers, 2002). In nature, adult parasitoids require sugar solutions, such as nectar or honeydew, as their main source of energy (Jervis and Kidd, 1986). Such feeding can increase parasitoid longevity (Wäckers, 1998, 2001; Siekmann *et al.*, 2001) as well as fecundity (Olson and Andow, 1998; Schmale *et al.*, 2001). Well-fed parasitoids are usually more active and focused in seeking out their hosts (Wäckers, 1994; Takasu and

Lewis, 1995). This concept of facultative phytophagy by entomophagous insects, may allow them to survive periods of low prey densities (Benton and Crump, 1981; Hemptinne and Desprets, 1986; Hagen, 1986; Hodek and Honek, 1996; Wiedenmann *et al.*, 1996), or provide them with critical or extra nutrients necessary for egg production or overwintering (Hagen, 1962; Schneider, 1969; Jervis and Kidd, 1986; Hodek and Honek, 1996). The general idea is that many arthropod predators and parasitoids exhibit stage-specific external feeding where they include plant-derived foods, such as pollen, nectar, extra-floral nectar or honeydew, in their immature and/or adult diet (Hagen, 1986).

It is well documented that insect natural enemies feed on pollen, but the nutritional suitability of pollen for these natural enemies is not well understood (Nordlund *et al.*, 2001; Lundgren and Wiedenmann, 2004). Studies have generally reported protein to be 23–27% of the dry weight of pollen grains (Goss, 1968; Roulston *et al.*, 2000). This was investigated by Lundgren and Wiedenmann (2004), who found that aphids + pollen grains were superior to corn pollen for the development of the predator, *Coleomegilla maculate* since there were significant increases in fecundity and weight as well as a significant decrease in larval durations. Another food supplement that could be used in feeding entomophagous insects is the honeybee royal jelly which is considered as a main source for proteins among other nutrients. Royal jelly contains various components: 60–70% water, 12–15% protein, and 10–16% total sugar, lipids, vitamin, salts and free amino acids (Chen & Chen, 1995; Howe, Dimick, & Benton, 1985; Simuth, 2001). Most of its soluble proteins are called major royal jelly proteins (MRJPs) (Schmitzova *et al.*, 1998; Simuth, 2001). MRJPs are thought to be the major factor responsible for the specific physiological role in queen honeybee development, as MRJPs include numerous essential amino acids, similar to known protein families like albumin and casein (Schmitzova *et al.*, 1998). MRJPs were found to be composed of five monomers that are bound by non-covalent bonds (Tamura *et al*, 2009). In the mean time, Abo Abdalla (2006) found that the supplemental feeding of the endoparasitoid, *A. galleriae* with pollen and royal jelly, have increased the female fecundity and decreased larval durations as well.

This project is aimed at addressing the question of how food supplements, namely pollen and royal jelly, may affect the parasitoid, *A. galleriae* protein content and protein profile.

MATERIALS AND METHODS

Insects

The lesser wax moth *Achroia grisella* (F.) was reared as the host insect. A laboratory culture of the insect was established after being collected from local infested apiaries. The culture was maintained for an entire year where the biology was monitored under laboratory conditions (21 – 28 °C and 60 – 85% RH). *A. grisella* larvae were reared on a semi-artificial diet that was developed by El-Kifl (1980) and modified by Abo Abdalla (2006).

A colony of the parasitoid, *Apanteles galleriae* Wilk. was kept in the laboratory on *A. grisella* larval stage. To maintain a good parasitoid laboratory population, parasitism was conducted on the third larval instars three times a week. Adults were kept on pure honey as a food source.

Food supplements

Four food supplements were used in these experiments, 80%-diluted honey (HW). Diluted honey + royal jelly (20 mg/ml) (HWRJ), diluted honey + Pollen (20 mg/ml) (HWP) and diluted honey + royal jelly + pollen (HWPRJ) (Abo Abdalla, 2006). The honey used was an alfalfa harvest and the pollen grains were bee-collected mostly from Faba bean flowers

Protein determination

An entire generation of *A. galleriae* was fed on the above food supplements before protein studies are commenced. Male and female adults were collected after 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 days of feeding. For the larval stage, first, second, early third and late third instar larvae in addition to the pupal stage were also collected. Protein content was estimated using the method of (Lowery *et al.*, 1951). Protein concentrations were measured using a double beam, Nicolete Evolution 100 UV-Visible spectrophotometer (Thermo Electron Corporation, UK)

Electrophoresis

Based on the data of the protein determination, adult males and females were chosen at 2 and 16 old-days of feeding in addition to late third larval instar and the pupal stage for the study of the protein electrophoresis. SDS-PAGE electrophoresis was conducted using the reported discontinuous buffer method by Laemmli (1970).

Sample preparation

Samples were mixed with a sample buffer (2% SDS, 10% glycerol, 0.002% bromophenol blue and 5% 2-mercaptoethanol). Afterwards, samples were submitted to heat treatment for 5 min. and were allowed to cool to room temperature prior to application.

Gel preparation

A 12.5 % resolving gel was prepared by mixing of acrylamid *bis*-acrylamid (10 mL), resolving buffer stock (3.0 M Tris-HCl, pH 8.8) (3.75 mL), SDS 10% (0.3 mL), freshly prepared Ammonium

persulfate 1.5% (1.5 mL), D-water (14.5 mL) and TEMED (N,N,N,N-tetramethylene diamide) (0.015 mL). The stacking gel was prepared similarly by the addition of acrylamid *bis*-acrylamid (2.5 mL), stacking gel buffer stock (0.5 M Tris HCl, pH 6.8) (5.0 mL), SDS 10% (0.2 mL), freshly prepared Ammonium persulfate 1.5% (1.0 mL), D-water (11.0 mL) and TEMED (0.015 mL). After polymerization, samples were loaded as 7.5 ul per well of each prepared sample. Electrophoresis was performed using Mini-Protean II (Bio-Rad, Richmond, CA) at 75v through the stacking gel followed by 125v through the resolving gel (2h). Reservoir buffer (0.25 M Tris, 1.9M glycerol, 1% SDS at pH 8.3), was used during the electrophoresis run. The gel was then stained with Comassie Blue R-250 for 2h, then the excess stain was removed from the gel by a de-staining solution (AcOH, MeOH, H_2O, 1:3:6).

Standared protein markers (Sigma, St. Louis, MO, USA)were myosin (200 kDa), B-Galactosidase (116 kDa), Phosphorylase b (97 kDa), Albumin (66 kDa), Glutamic Dehydrogenase (55 kDa), Ovalbumin(45 kDa), Glyceraldehyde-3-phosphate Dehydrogenase (36 kDa), Carbonic Anhydrase (29 kDa), Trypsinogen (24 kDa), Trypsin Inhibitor (20 kDs), a-Lactalbumin (14 kDa) and Aprotinin (6.5 kDa). Quantitative determination of the resolved protein bands were carried out using the TOTAL-LAB software v-2003 (Appligene).

Statistical Analysis

Experiments were replicated and statistical analysis was computed. Statistical analysis was done according to Gomez and Gomez (1984). Comparisons among means were carried out using the LSD at 0.01 probabilities.

RESULTS AND DISCUSSION

The endoparasitoid, *Apanteles galleriae* Wilk., was kept in the laboratory and was offered a supplemental feeding on 80%-diluted honey (HW), diluted honey + royal jelly (20 mg/ml) (HWRJ), diluted honey + Pollen (20 mg/ml) (HWP) and diluted honey + royal jelly + pollen (HWPRJ). The effects of such feeding on the parasitoid, *A. galleriae* protein content and protein profile are discussed.

Protein Content

The endoparasitoid, *A. galleriae*, males, females, larval instars and pupal stage that were allowed the four food supplements, were sampled over time and protein contents were determined. Male total proteins (Fig. 1) indicated that all treatments including the control started to increase from the 6th day while the pollen treatment (HWP) started earlier on the 4th day. The highest protein values were recorded on the 14th, 14th, 20th, 12th, 18th days for the control (honey), HW, HWP, HWRJ and HWPRJ, respectively. Female total proteins (Fig. 2) showed that all treatments started to increase from the 2nd day except for the water diluted honey and pollen which started from the 4th day. The highest value for the control, diluted honey, pollen, royal jelly and pollen & royal jelly were 12th, 16th, 14th, 20th, and 10th days, respectively. For the larval stage (Fig. 3) no difference in the protein content was recorded between all treatments in the early first, late first, second and early third instars. Differences were prominent at the late third instar where the pollen treatment exhibited the highest protein content followed by the royal jelly, the control, the water diluted honey and the least was in the case of pollen and royal jelly treatments. While, in the pupal stage (Fig. 4) protein content was at its highest value in the combined treatment and the least was for the royal jelly. Statistical analysis have shown that there was a high significant differences in the protein values between sex, time, stages and food supplements at p = 0.01 (Tables 1 & 2).

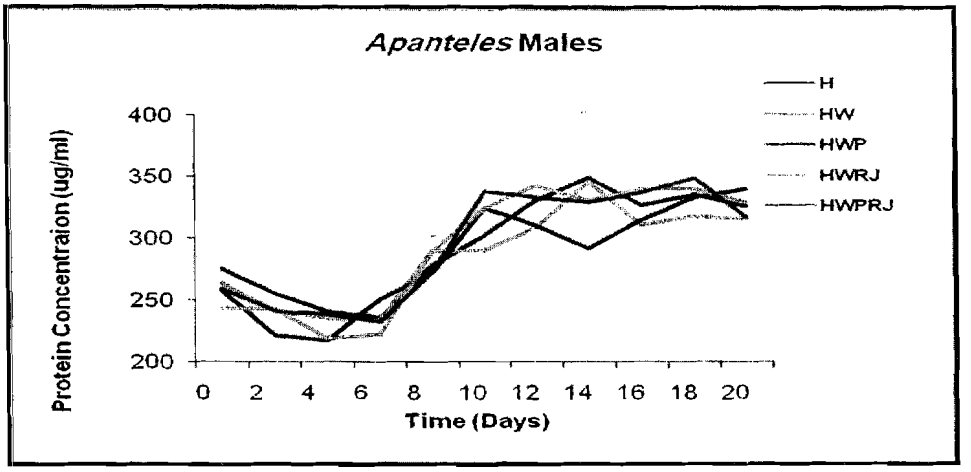

Figure 1: Protein concentration of adult males, *A. galleriae* fed on control honey (H), diluted honey (HW), pollen (HWP), royal jelly (HWRJ) and both pollen and royal jelly (HWPRJ) for a period of 20 days.

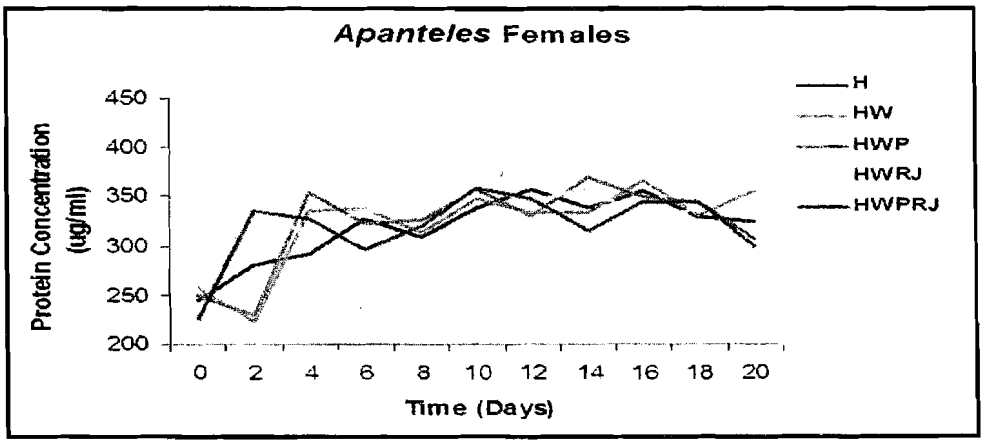

Figure 2: Protein concentration of adult females, *A. galleriae* fed on control honey (H), diluted honey (HW), pollen (HWP), royal jelly (HWRJ) and both pollen and royal jelly (HWPRJ) for a period of 20 days.

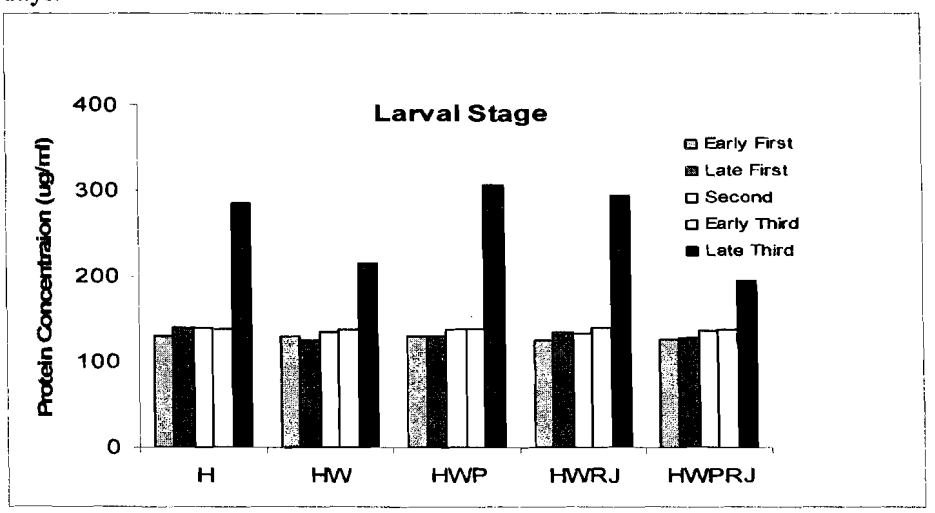

Figure 3: Protein concentration of the larval stage of *A. galleriae* fed (as adults) on control honey (H), diluted honey (HW), pollen (HWP), royal jelly (HWRJ) and both pollen and royal jelly (HWPRJ). Protein determinations were carried out on the early first, late first, second, early third and late third instars.

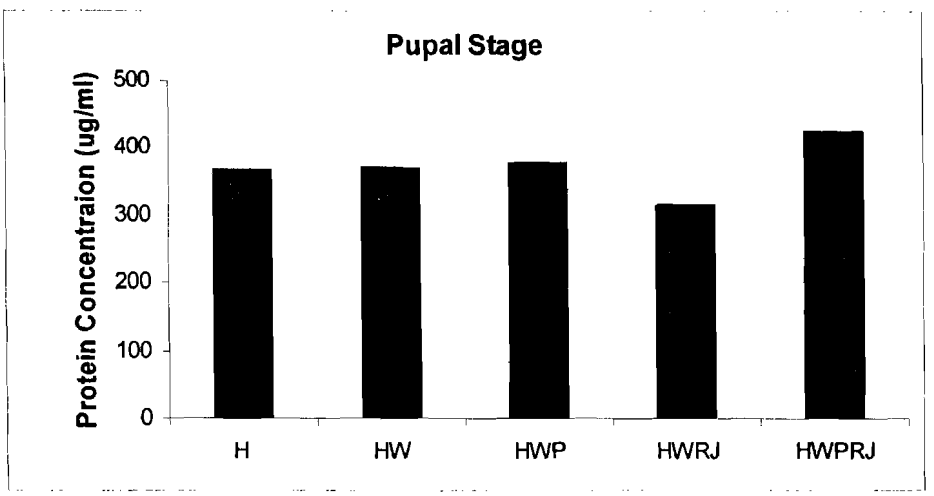

Figure 4: Protein concentration of the pupal stage of *A. galleriae* fed (as adults) on control honey (H), diluted honey (HW), pollen (HWP), royal jelly (HWRJ) and both pollen and royal jelly (HWPRJ).

Table 1: Differences in protein contents between the major stages of the endoparasitoid, *A. galleriae*.

Stage	Male	Female	Early 1st instar larvae	Late 1st instar larvae	2nd instar larvae	Early 3rd instar larvae	Late 3rd instar larvae	Pupal Stage
Protein Content (ug/ml)	278.6^{c}	310.8^{b}	132.6^{f}	128.5^{f}	139.6^{ef}	138.9^{e}	284.1^{d}	365.9^{a}

Table 2: Differences in protein contents between the food supplements given to adults of the endoparasitoid, *A. galleriae*.

	H	HW	HWP	HWRJ	HWPRJ	LSD
			Protein content (ug/ml)			
Males	278.6	279.6	283.2	285.3	286.7	14.8
Females	310.8^{b}	312.5^{b}	323.1^{ab}	330.4^{a}	327.9^{a}	11.5

Protein accumulation have started in the early third instar larvae and continued where it reached it's highest at the pupal stage (365.9 ug/ml). Females significantly contained higher protein concentrations than males being 310.8 and 278.6 ug/ml, respectively. Protein contents differed between treatments and sex as shown in Table(2). The highest protein value corresponded to the pollen treatment where it reached 228.4 ug/ml while the lowest was for the water diluted honey being 215.1 ug/ml for males. Wäckers(2002) argued in favor of using the additional food source strategies to raise the impact of parasitoids on their host populations. In addition, it does provide them with critical or extra nutrients necessary for higher egg production (Jervis and Kidd, 1986; Hodek and Honek,1996). Supplemental feeding on floral and extra-floral nectar and pollen is essential for them and are considered as excellent sources of carbohydrates and proteins for many entomophagous insects (Jervis & Kidd, 1996; Van Driesche & Bellows, 1996). Studies have shown that access to these floral resources increases survival and reproduction of these insects (Wäckers, van Rijn, & Bruin, 2005; Wade & Wratten, 2007; Kehrli & Bacher, 2008).

Protein bands of controls

The analysis of the protein profile for stages of *A. galleriae* was done using SDS-PAGE protein electrophoresis. Band patterns were checked and compared between controls and treatments. When controls (Honey fed only) were compared for males (2 and16 days, Fig. 5), females (2 and16 days, Fig. 6), larvae (Fig. 7) and pupae (Fig. 8), various band pattern differences appeared. Stage-specific distinctive bands distribution were noticed. Four of these bands appeared only in males age two-days at 50.5, 67.5, 84.9 and 119.8 kDa and their percentages were (5.5%), (8.6%), (3.9%) and (5.5%), respectively. After 16 days, males protein profile showed five characteristic bands at 36.5 (7.8%), 43.1 (8.9%), 69.7 (9.1%), 100.7 (5.1%) and 140.4 kDa (6.5%) (Fig. 5 and Table 3). Female band distribution (after 2 days) showed only 2 female-specific bands at 66.0 (5.2%) and 182.1 kDa (3.9%) that did not appear in other stages. After 16 days, four of these bands have appeared at 10.53 (6.6%), 58.2 (4.5%),80.2 (4.8%) and 136.7 kDa (4.4%). At the larval stage, four characteristic bands appeared at 32.8 (6.6%), 60.4 (7.6%), 83.4 (6.7%) and 172.6 kDa (5.8%). The pupal stage did not show any characteristic bands at any molecular weight. The above data would be useful when identifying bands that correspond to each stage of *A. galleriae* (Fig. 6 and Table 3).

Protein bands of treatments

The effect of the four treatments (food supplements) compared to the control were analyzed for both males and females of *A. galleriae*. For the two-days old males and against the 14 bands appeared in the control, only 12, 13, 10 and 12 bands appeared in the water diluted honey, pollen, royal jelly and the combined treatment, respectively. Out of these bands, four have appeared only in the control at 21.0(9.3%), 67.5(8.6%), 84.9(3.9%) and 119.8 kDa (5.5%) of the 2 days old males. In the same manner, the band at 28.0 kDa (5.7%) appeared only in the pollen & royal jelly treatment as well as the bands at 57.5(8.4%), 86.2(6.8%), and 172.7 kDa (9.0%) also. The royal jelly treatment showed only one band at 75.7 kDa (10.1%) that was not corresponded in the other treatments. Meanwhile, the pollen treatment has two distinctive bands at 62.7(10.9%) and 87.9 kDa (4.3%). In the mean time, the water diluted honey was characterized by 3 bands at 59.3 (7.0%), 90.0 (9.2%) and 136.3 kDa (7.4%). This led to speculate that the combined treatment caused a quick appearance of new proteins in 2 days old males.

The 16-days old males fed on the same supplements showed total number of bands being 12, 13, 10, 14 and 10 for the control, diluted honey, pollen, royal jelly and the combined treatment, respectively. Three bands in the control were not detected in all treatments at 28.4 (5.1%), 69.7 (9.1%) and 140.4 kDa (6.5%). The extensive effect shown by the combined treatment at the 2-days old treatment did not continue to the 16-days males. This treatment has given only two distinctive bands at 73.0(7.6%) and 200 kDa (7.1%). While the royal jelly treatment showed six bands at 25.1 (6.6%),

52.3 (9.8%), 62.9 (6.1%), 89.9 (6.3%), 116 (5.0%) and 180.2 kDa (6.5%). The pollen treatment gave two protein bands at 46.7 (10.4%) and 86.3 kDa (10.5%). The water-diluted honey gave three protein bands at 30.9 (3.8%), 50.3 (6.0%) and 81.9 kDa (13.4%). The effect of feeding on the above food supplements, for longer periods of time was obvious when compared to the 2-days old males especially at the royal jelly treatment where the number of protein characteristic bands was higher than all of other supplements (Fig. 5). When Males fed on the food supplements, most of the proteins appeared between 20 and 56 kDa. Some bands were present all the way up to the 200 kDa. At the 22 kDa protein, the concentration of this protein was always higher at the 2-days old males than the 16-days old ones in all treatments. In the same manner, the 38 and 40 kDa proteins showed high concentrations in all treatments including the control which is suspected to be one of the key proteins needed for male development. This specific protein was also found in higher concentrations in the 2-days old males than the 16-days old ones except in diluted honey supplement where it increased after 16-days of feeding. The same could be concluded for the 40 kDa protein where it kept a higher concentration in the 16-days old males for the control (22.42%) while the rest of the treatments either were less than the 2-days old males or disappeared totally. The pollen treatment was an exception since the 16-days old males had 17% of that protein and it was lacking in the 2-days old. At the 42 kDa, water diluted honey, pollen and the combined treatment showed an increase in the amount of this protein after 16-days. Meanwhile, at the 46kDa, all proteins disappeared after 16-days of feeding except for the pollen where it appeared only after 16-days.The 54 kDa protein band was at its highest (14.51%) at the combined treatment after 16-days compared to the other 16-days treatments. While the 56 kDa was at its highest with pollen after 16-days as well. The 70 kDa protein appeared only after 16-days in the control (9.11%) and the combined treatment (7.63%). The 96 kDa also appeared after 16-days in the royal jelly (4.89%) and the combined treatment (5.48%). Also, the 102 kDa protein was found only after 16-days of feeding in all treatments except the control (H) and the royal jelly. The same trend was found in the 122 and 174 kDa but only at the treatment of diluted honey & the pollen. The 180 kDa at the royal jelly (6.54%) and the 200 kDa at the combined treatment were very distinctive (7.1%). On the other hand, the 98 kDa protein band was only detected after 2-days in all food supplements in addition to the control. While the 112 kDa appeared after 2-days only in pollen (5.99%) and royal jelly (6.53%).

A parallel study on the effects of the above mentioned food supplements, was conducted on the females of *A. galleriae*. At the 2-days old females, total numbers of protein bands were 14, 16, 16, 13 and 15 for the control, diluted honey, pollen, royal jelly and the combined treatment , respectively (Fig. 6). The three bands which appeared in the control was not repeated in any of the treatments. The protein bands were at 16.2 (9.3%), 97.0 (5.5%) and 104.1 kDa (5.6 %). The water-diluted honey has also shown three bands at 44.3 (4.4%), 93.1 (3.4%) and 108.7 kDa (5.4%). Meanwhile, the pollen supplement was characterized by only two protein bands at 24.0 (11.8%) and 58.2 kDa (2.6%). The royal jelly treatment showed only one protein band at 90.5 kDa (5.3%). However, no characteristic protein bands were detected for the combined treatment.

As for the 16-days old females fed on the same set of food supplements, the total number of bands were 15, 11, 15, 13 and 12 for the control (honey fed only), diluted honey, pollen, royal jelly and the combined treatment, respectively. The control was characterized by the appearance of five bands not found in any of the other treatments. These bands were at 10.53 (6.6%), 16.2 (8.7%), 80.2 (4.8%), 99.7 (4.2%) and 136.7 kDa (4.4%). While the water-diluted honey had four distinctive bands at 40.1 (8.3%), 45.0 (3.8%), 75.8 (4.7%) and 116.0 kDa (11.6%). Pollen supplementation had four distinctive bands at 12.7 (6.8%), 44.3 (4.1%), 70.6 (4.1%) and 104.1 kDa (5.2%). No distinctive protein banding appeared at the royal jelly treatment. Meanwhile, the combined treatment showed two bands at 30.2 (6.4%) and 85.6 kDa (7.4%) (Fig. 6). Protein banding started to appear from the 10 kDa onward with most of the protein banding occurred between 18 till 58 kDa. Protein banding continued sporadically from 60 kDa till 182 kDa only. The band matrix showed that proteins at 20, 22, 24, 26, 38, and 56 kDa were found in all treatments as well as the control. Some of these bands may disappear in the 2 or 16-days old females but not in both ages. After 2-days, the 60 kDa protein appeared only in the royal jelly (3.7 %) and the combined treatment (8.4%). The 66 kDa appeared also after 2-days in the control (5.2 %) and the diluted honey (4.5 %) only. In the meantime, the 86 kDa protein appeared after 2-days in pollen and the combined treatment but continued in later to the 16 –days old females (7.4%). While the 100 kDa appeared after 2-days in pollen and the combined treatment except for the control where it appeared after 16-days of feeding. The 136 kDa protein appeared after 2-days in pollen (5.4 %), royal jelly (6.7 %) and the combined treatment (7.2%), while it was found after 16-days in the control (4.4 %). The highest molecular weight was at 182 kDa, appeared only in control (3.9 %) and in the diluted honey (3.6%) after 2-days.

Meanwhile, the 94 kDa appeared after 16-days in the pollen (4.5%) and the royal jelly (7.7 %) but appeared after 2-days in diluted honey (3.4%). The same trend was noticed for the 108 kDa protein where it appeared after 16-days in royal jelly (9.6%) and the combined treatment (13.2 %) but in the diluted honey (5.4 %) after two days of feeding. The effect of food supplementation on the females was affected by time. At the 2-days old females, the supplementation have shown higher molecular weight proteins reaching up to 182.0 kDa for the control and diluted honey. While, the 16-days old ones have only reached 136.7 kDa at the control.

The larvae of *A. galleriae* were dissected out of their hosts. These larvae were from eggs laid by females fed on the above mentioned food supplements. Only the late third instar larvae were analyzed for their protein patterns. The total number of protein bands for these larvae were 12, 11, 10, 10 and 10 bands for the control, diluted honey, pollen, royal jelly and the combined treatment. The protein bands characterizing the control and which were not found in the other treatments were three at 83.4 (6.7%), 103.3 (5.2%) and 172.6 kDa (5.8%). The water-diluted honey showed only one band at 52.2 kDa (8.2%). Meanwhile, the pollen supplement gave three distinctive bands at 36.8 (10.4%), 50.5 (2.9%) and 70.1 kDa (12.0%). Characteristic bands of the royal jelly treatment were two at 73.7 (2.3%) and 187.7 kDa (6.8%). The combined. Treatment was characterized by four protein bands at 23.1 (7.2%), 56.6 (11.9%), 78.6 (7.2%) and 200 kDa (7.8%) (Fig.7).

The protein distribution of the last larval instar was also studied to show the effects of food supplementation given to the adults. Hosts were dissected and the late 3rd larval instars of the endoparasitoid *A. galleriae* were collected for this experiment. The 22 kDa protein was specific for the larval stage eventually; it disappeared at the combined treatment. The same was noticed for the 36 kDa protein where it appeared under the effect of the pollen feeding (10.4%). The protein at 40 kDa, 44 kDa, 50 kDa and 52 kDa were also specific for the larval stage, although they differ between the treatments.

The pupal stage of *A. galleriae* of the corresponding females fed on the above mentioned food supplements was collected from cocoons that did not reach to the pharate adults for the protein profile determination. Total numbers of protein bands were 6, 5, 5, 5 and 4 bands for the control (H), water diluted honey, pollen, royal jelly and the combined treatment, respectively. Only one band characterizing the control was found at 37.9 kDa (9.6%). The same was noticed for the royal jelly treatment at 47.3 kDa (12.7%). No distinctive banding was detected for diluted honey, pollen and the combined treatment. All pupal protein bands were between 24 to 54 kDa with no high molecular weight proteins. Fewer number of protein band was also noticed (Fig. 8). Unlike the larval stage only one protein was specific for the pupal stage at 48 kDa (12.7%) with the royal jelly treatment. The 24 kDa protein also seemed to be specific for the pupal stage where it appeared in all treatments along with the control, eventually, it appeared at the larval stage at the combined treatment. This protein is also found at high concentration especially with the combined treatment being 40.2%.

Similar to the larval stage, the 54 kDa protein was important where it appeared in all treatments including the control. The highest concentration of this particular protein was 28.0% as an effect of the combined treatment supplement, while, the lowest was 15.1 % under the royal jelly supplement. No protein banding was detected higher than the 54 kDa. Except for the 54 kDa protein, all other proteins appeared commonly with both larvae and pupae. The 54 kDa protein seems to be an important protein which appears in all treatments including the control with its highest concentration at the royal jelly supplement being 31.5 %. All proteins above the 54 kDa were found in the larval stage only differing between treatments. High molecular weight proteins appeared with the royal jelly at 188 kDa (6.8 %) and with the combined treatment at 200 kDa (7.8 %).

In conclusion, protein accumulation in the female parasitic wasps started much earlier than the males, the third *A. galleriae* larval instar responded to almost all treatments while the pupal stage protein build-up was higher in the HWPRJ treatment, In general, the female protein contents were significantly higher in HWP, HWRJ and HWPRJ than other treatments and the males as well. The protein bands distributions and densities were characteristically variable over time, food supplement and insect stages. It remains a challenge to identify proteins and to relate them to their functions. The present study shows that non-host food supplements can have a profound effect on the protein profile of parasitoid system. To our knowledge, this study is considered one of the very few that deals with the protein profiling of the parasitic wasp, *Apanteles galleriae* (Hymenoptera: Braconidae).

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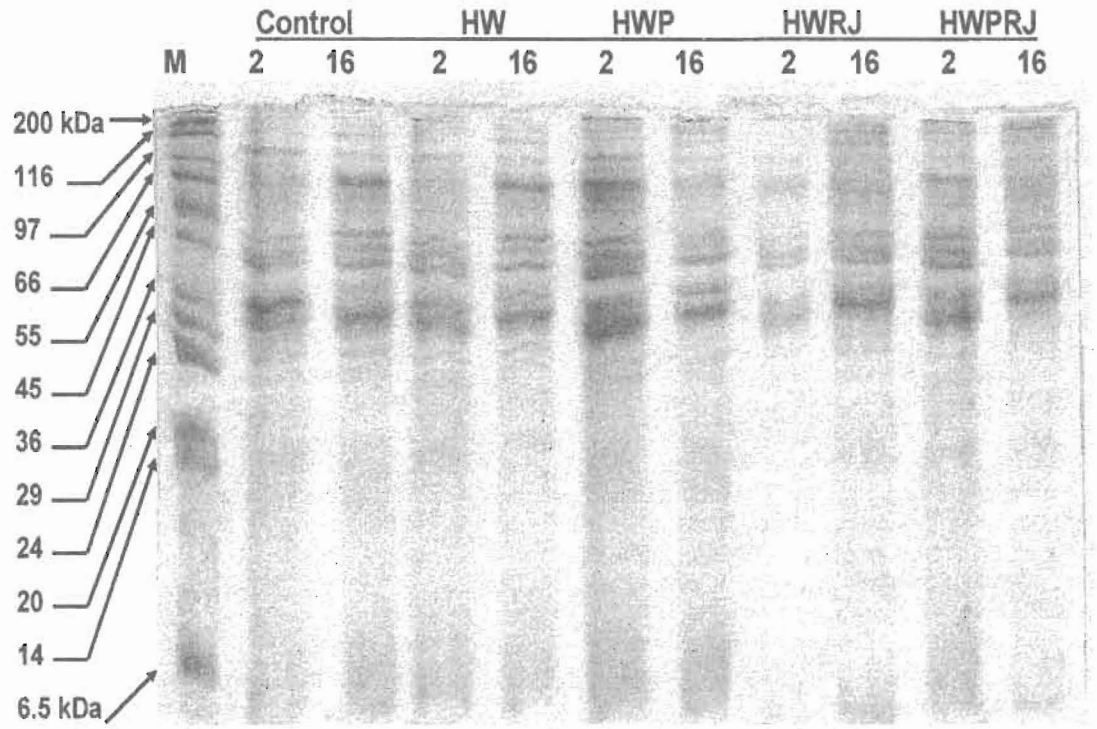

Figure 5: The SDS-PAGE of adult males, *A. galleriae* showing the protein profile when fed on control honey (H), diluted honey (HW), pollen (HWP), royal jelly (HWRJ) and both pollen and royal jelly (HWPRJ) for 2 and 16 days. The standard protein marker (M) is shown at the first lane.

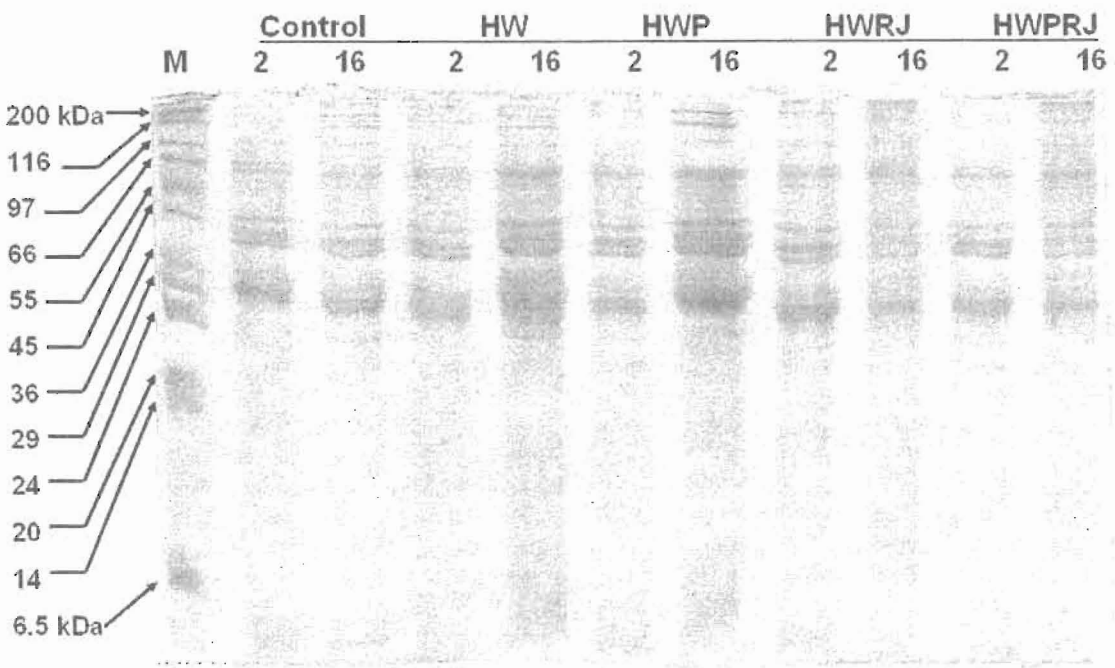

Figure 6: The SDS-PAGE of adult females, *A. galleriae* showing the protein profile when fed on control honey (H), diluted honey (HW), pollen (HWP), royal jelly (HWRJ) and both pollen and royal jelly (HWPRJ) for 2 and 16 days. The standard protein marker (M) is shown at the first lane.

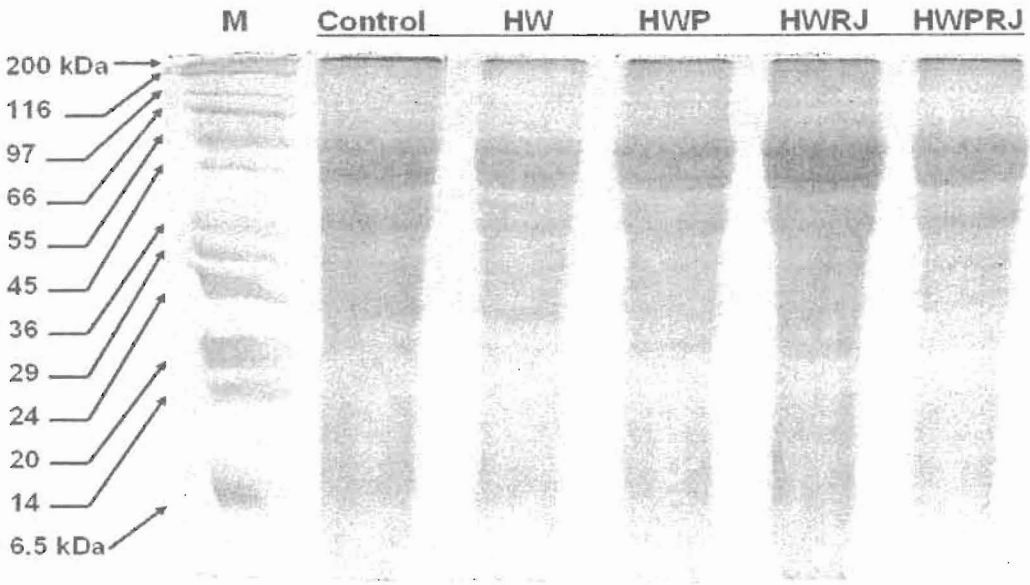

Figure 7: The SDS-PAGE of the larval stage of, *A. galleriae* showing their protein profile when fed (as adults) on control honey (H), diluted honey (HW), pollen (HWP), royal jelly (HWRJ) and both pollen and royal jelly (HWPRJ). The standard protein marker (M) is shown at the first lane.

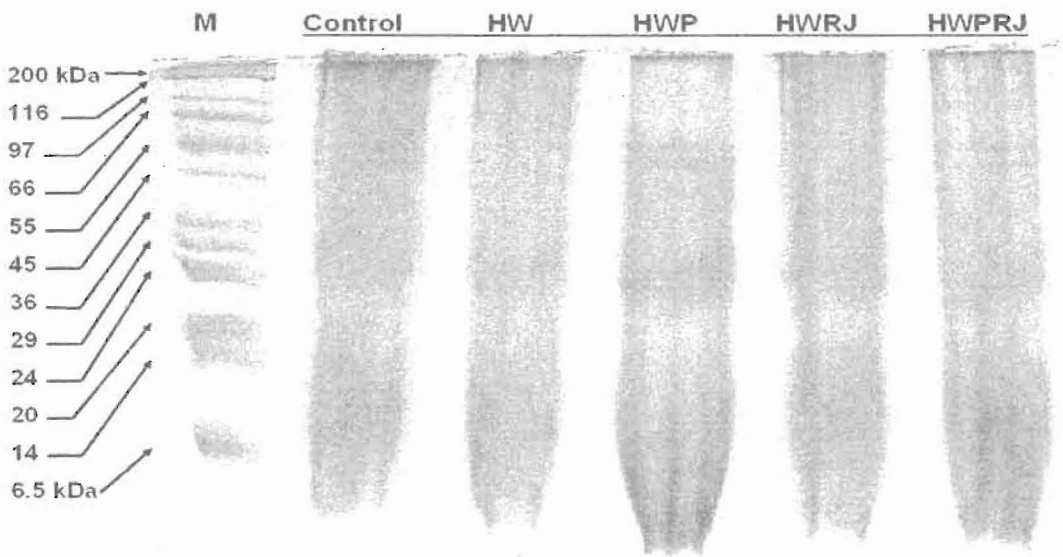

Figure 8: The SDS-PAGE of the pupal stage of, *A. galleriae* showing their protein profile when fed (as adults) on control honey (H), diluted honey (HW), pollen (HWP), royal jelly (HWRJ) and both pollen and royal jelly (HWPRJ). The standard protein marker (M) is shown at the first lane.

Table 3: Distinctive protein bands appeared in the controls of the males (2 and 16 days old), females (2 and 16 days old), larvae and pupae.

	Molecular Weight (kDa)					
	Males		Females			
Number	2 D	16 D	2 D	16 D	Larvae	Pupae
1	50.5	36.5	66	10.53	32.8	--
2	67.5	43.1	182.1	58.2	60.4	--
3	84.9	69.7	--	80.2	83.4	--
4	119.8	100.7	--	136.7	172.6	--
5	--	140.4	--	--	--	--

Table 4: Protein band distributions (%) of *A. galleriae* males fed on control honey (H), diluted honey (HW), pollen (HWP), royal jelly (HWRJ) and both pollen and royal jelly (HWPRJ) for 2 and 16 days.

MW (kDa)	Control		HW		HWP		HWRJ		HWPRJ	
	2 D	16 D	2 D	16 D	2 D	16 D	2 D	16 D	2 D	16 D
20	9.34	--	--	--	--	--	--	--	--	--
22	7.17	7.96	9.04	--	7.18	--	9.13	7.96	9.49	8.39
24	--	--	--	5.85	--	11.14	--	--	--	--
26	6.84	--	--	--	4.56	--	--	6.63	--	--
28	--	5.1	--	--	--	--	--	--	5.65	--
30	--	--	--	3.78	4.68	--	11.3	--	--	--
32	7.28	6.52	8.74	--	--	--	--	4.74	6.35	6.4
36	--	7.75	--	5.59	--	7.79	--	5.65	--	--
38	11.98	--	10.96	13.14	25.43	--	15.74	9.36	14.5	7.29
40	9.0	22.42	11.32	--	--	17	11.94	7.64	10.81	--
42	6.88	--	7.78	11.11	8.21	12.24	--	--	--	12.3
44	--	8.91	--	9.96	5.73	--	7.78	7.97	10.72	--
46	5.6	--	10.96	--	--	10.37	12.35	--	--	--
48	--	7.12	--	--	--	--	--	--	--	3.81
50	5.51	--	--	6.02	7.5	--	--	--	7.6	--
52	--	--	--	--	--	--	--	9.75	--	--
54	--	5.5	8.41	7.58	10.73	--	8.77	5.75	7.51	14.51
56	7.08	7.99	4.09	8.37	--	13.54	--	5.73	--	5.82
58	--	--	--	--	--	--	--	--	8.39	--
60	--	--	6.95	--	--	--	--	--	--	--
62	--	--	--	--	10.89	--	--	6.11	--	--
68	8.63	--	--	--	--	--	--	--	--	--
70	--	9.11	--	--	--	--	--	--	--	7.63
76	--	--	--	--	--	--	10.08	--	--	--
82	--	--	--	13.41	--	--	--	--	--	--
84	3.85	--	--	--	--	--	--	--	--	--
86	--	--	--	--	--	10.51	--	--	6.75	--
88	--	--	--	--	4.28	--	--	--	--	--
90	--	--	9.21	--	--	--	--	6.27	--	--
96	--	--	--	--	--	--	--	4.89	--	5.48
98	5.41	--	5.12	--	4.8	--	6.38	--	3.12	--
100	--	5.12	--	--	--	--	--	--	--	--
102	--	--	--	6.32	--	5.22	--	--	--	5.58
112	--	--	--	--	5.99	--	6.53	--	--	--
116	--	--	--	--	--	--	--	5.0	--	--
120	5.45	--	--	--	--	--	--	--	--	--
122	--	--	--	4.88	--	6.1	--	--	--	--
136	--	--	7.43	--	--	--	--	--	--	--
140	--	6.51	--	--	--	--	--	--	--	--
172	--	--	--	--	--	--	--	--	9.0	--
174	--	--	--	3.98	--	6.09	--	--	--	--
180	--	--	--	--	--	--	--	6.54	--	--
200	--	--	--	--	--	--	--	--	--	7.1

Molecular weight (MW, kDa) missing values showed no protein bands.

Table 5: Protein band distributions (%) of *A. galleriae* females fed on control honey (H), diluted honey (HW), pollen (HWP), royal jelly (HWRJ) and both pollen and royal jelly (HWPRJ) for 2 and 16 days

MW	Control		HW		HWP		HWRJ		HWPRJ	
(kDa)	2 D	16 D	2 D	16 D	2 D	16 D	2 D	16 D	2 D	16 D
10	--	6.64	8.4	--	8.38	--	--	--	--	--
12	--	--	--	--	--	6.79	--	--	--	--
14	--	--	5.97	--	4.92	--	6.8	--	6.27	--
16	9.32	8.67	--	--	--	--	--	--	--	--
18	--	--	5.87	--	7.63	5.39	7.06	5.97	7.06	--
20	8.35	5.28	6.22	8.78	5.03	5.83	6.06	6.72	5.68	7.5
22	5.51	4.82	8.33	3.57	--	6.95	12.2	9.99	14.54	7.05
24	--	13.41	--	14.67	11.8	--	--	9.14	--	14.69
26	9.86	6.64	8.34	--	1.73	17.43	12.03	6.08	1.7	2.52
28	9.05	--	4.07	10.69	5.26	--	--	6.21	5.87	--
30	--	--	--	--	--	--	--	--	--	6.39
32	5.9	5.45	--	--	--	5.61	--	--	--	--
34	--	--	6.1	9.66	8.05	--	5.64	9.29	6.05	8.95
38	6.26	8.4	6.77	8.21	7.45	6.77	7.38	5.6	8.09	8.68
40	--	--	--	8.25	5.35	--	6.66	--	--	--
42	--	5.94	6.45	--	--	7.25	--	8.35	6.12	8.22
44	--	--	4.41	--	--	4.05	--	--	--	--
46	11.96	--	--	3.83	--	--	5.47	--	--	--
48	--	6.69	--	6.11	4.36	--	--	--	5.32	--
50	--	--	5.59	--	--	6.68	4.98	--	--	--
52	--	--	--	--	--	--	--	2.76	--	3.65
54	5.48	--	--	--	--	--	--	--	8.84	--
56	8.18	10.17	6.55	9.97	11.23	9.8	10.04	--	--	11.79
58	--	4.48	--	--	2.6	3.7	--	12.6	--	--
60	--	--	--	--	--	--	3.69	--	8.41	--
66	5.19	--	4.48	--	--	--	--	--	--	--
70	--	--	--	--	--	4.07	--	--	--	--
76	--	--	--	4.68	--	--	--	--	--	--
80	--	4.83	--	--	--	--	--	--	--	--
86	--	--	--	--	5.67	--	--	--	4.67	7.4
90	--	--	--	--	--	--	5.32	--	--	--
94	--	--	3.43	--	--	4.5	--	7.65	--	--
98	5.52	--	--	--	--	--	--	--	--	--
100	--	4.18	--	--	5.08	--	--	--	4.16	--
104	5.56	--	--	--	--	5.19	--	--	--	--
108	--	--	5.39	--	--	--	--	9.62	--	13.15
116	--	--	--	11.58	--	--	--	--	--	--
136	--	4.39	--	--	5.44	--	6.67	--	7.22	--
182	3.85	--	3.61	--	--	--	--	--	--	--

Molecular weight (MW, kDa) missing values showed no protein bands.

Table 6: Protein band distributions (%) of *A. galleriae* larvae (L) and pupae (P) fed on control honey, diluted honey (HW), pollen (HWP), royal jelly (HWRJ) and both pollen and royal jelly (HWPRJ)

MW (kDa)	Control		HW		HWP		HWRJ		HWPRJ	
	L	P	L	P	L	P	L	P	L	P
22	7.21	--	4.65	--	9.89	--	7.12	--	--	--
24	--	22.69	--	16.42	--	20.33	--	29.26	7.18	40.24
26	8.74	--	11.66	--	9.41	19.89	8.74	--	10.32	13.08
28	7.21	15.6	8.14	22.75	7.75	--	--	22.31	--	--
30	6.86	--	5.77	--	--	19.04	8.95	20.64	--	--
32	6.62	17.24	7	21.96	12.12	--	6.74	--	8.38	--
36	--	--	--	--	10.42	--	--	--	--	--
38	--	9.56	12.3	--	--	--	10.29	--	--	--
40	9.29	--	--	--	--	--	--	--	12.94	--
44	--	--	8.61	--	9.98	--	--	--	--	--
46	6.69	18.46	--	15.97	--	22.7	9.92	--	8.39	18.67
48	--	--	--	--	--	--	--	12.7	--	--
50	--	--	--	--	2.88	--	--	--	--	--
52	--	--	8.18	--	--	--	--	--	--	--
54	20.06	16.46	18.85	22.9	16.07	18.04	31.45	15.09	16.97	28.01
56	--	--	--	--	--	--	--	--	11.85	--
60	7.59	--	7.99	--	--	--	--	--	--	--
70	--	--	--	--	12.03	--	--	--	--	--
74	--	--	--	--	--	--	2.26	--	--	--
78	--	--	--	--	--	--	--	--	7.2	--
84	6.69	--	--	--	--	--	--	--	--	--
100	--	--	6.84	--	--	--	--	--	9.01	--
102	--	--	--	--	9.45	--	7.38	--	--	--
104	5.2	--	--	--	--	--	--	--	--	--
172	5.83	--	--	--	--	--	--	--	--	--
188	--	--	--	--	--	--	6.75	--	--	--
200	--	--	--	--	--	--	--	--	7.75	--

Molecular weight (MW, kDa) missing values showed no protein bands.

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الملخص العربى

تأثير البدائل الغذائية الطبيعية على المحتوى البروتينى لكل من الاطوار الكاملة واليرقية للطفيل اليرقى الداخلى لدودة الشمع – ابنتيلس جاليرى

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يعتبر الطفيل الداخلى المسمى *Apanteles galleriae* من أهم الطفيليات على دودة الشمع الكبرى *Galleria mellonella* ودودة الشمع الصغرى *Achroia greissella*. تمت تربية هذا الطفيل معمليا ولعدة أجيال على يرقات دودة الشمع الصغرى. وكانت المعاملات المستخدمة فى تغذية الطفيل كالاتى: عسل نقى– عسل مخفف ٨٠% – عسل مخفف ٨٠% مضاف اليه غذاء ملكى(٢٠ ملجم)– عسل مخفف ٨٠% مضاف اليه حبوب لقاح(٢٠ ملجم)– عسل مخفف ٨٠% مضاف اليه غذاء ملكى وحبوب لقاح. وقد تم اتاحة البدائل السابقة للطفيل طوال الوقت. وقد استخدم الجيل الناتج من هذة التغذية لدراسة المحتوى البروتينى لكل من الذكور والاناث خلال فترة حياتها. بالاضافة الى دراسة المحتوى البروتينى ليرقات وعذارى الطفيل الناتجة عن نفس التغذية فى أعمارها المختلفة. وباستخدام الهجرة الكهربية SDS-PAGE لبروتين المعاملات السابقة تبين وجود اختلافات فى توزيع الحزم البيتيدية مقارنة بالكنترول. وبمقارنة كل من التوزيع والكثافة(التركيز) للحزم البيتيدية تبين وجود اختلافات تبعا لكل من الوقت وأطوار الطفيل المختلفة اضافة الى تأثير البدائل الغذائية.