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ORIGINAL ARTICLE

Honey bee immunity and physiology are enhanced by consuming high-fat diets

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Abstract

This study aimed to evaluate the nutritional behavior and some immunological criteria (encapsulation index and phenoloxidase – PO activity, the key enzyme for melanization) as well as to study the effect of protein to fat (P : F) diets on hypopharyngeal gland (HPG) protein content. Bees were restricted to consuming specific P : F diets varying in fat ratio under laboratory conditions. These diets included 25 : 1, 10 : 1, 5 : 1 (low-fat diet, LFD); 1 : 1 (equal-fat diet); 1 : 5, 1 : 10 (high-fat diet, HFD), and 1 : 0 (zero-fat diet) as a control. Bees preferred low-fat diets over high-fat diets, where it was 11.27 ± 0.68 µl · day^{-1} bee in 10 : 1 P : F, while it was 4.99 ± 0.67 µl · day^{-1} bee in 1 : 10 P : F. However, sucrose consumption was higher in high-fat diets where it was 25.83 ± 1.69 µl · day^{-1} bee in 10 : 1 P: F, while it was 30.66 ± 0.9 µl · day^{-1} bee in 1 : 10 P : F. The encapsulation index and phenoloxidase activity of bees were positively linked with the fat level they consumed during all 10 days. The maximum percentage of encapsulation index was 74.6 ± 7.2% in bees fed a high-fat diet, whereas the minimum percentage was 16.5 ± 3.6% in bees which consumed a low-fat diet. Similarly, phenoloxidase activity increased in the haemolymph with increasing fat consumed by bees (0.001 ± 0.0001 and 0.005 ± 0.0003 mM · min^{-1} · mg^{-1} at 25 : 1 and 1 : 10 P : F, respectively). The protein content of hypopharyngeal glands in bees which consumed HFD was double that of LFD. Overall results suggest a connection between a fat diet and bee health, indicating that colony losses in some cases can be reduced by providing a certain level of fat supplemental feeding along with sucrose and protein nutrition.

Keywords: fat, encapsulation, honey bee, immunity, phenoloxidase, protein

Introduction

Bee nutritional behavior

Numerous studies have reported that animals evolved their behavioral and physiological characteristics to reach the optimal diet, which is referred to as a "nutritional target"(Behmer 2009; Simpson and Raubenheimer 2012). For instance, a study revealed that bumblebees selectively gather pollen from flowers based on the protein-to-fat ratio (Vaudo *et al.* 2016b). It is generally agreed that animals have to be supplied with regular nutrition to meet their specific requirements, or intake target, and diversion away from their optima will have detrimental effects. For example, eating surplus protein increases the mortality risk in bees (Pirk *et al.* 2010; Paoli *et al.* 2014b; Stabler *et al.* 2015), ants, *Lasius niger* (Dussutour and Simpson 2012), and *Drosophila melanogaster* (Lee *et al.* 2008). However, consuming high protein diets improved the survival of African honey bees *Apis mellifera scutellate* (Archer *et al.* 2014). Also, bees have a very high requirement for carbohydrates and insufficiency leads to a high risk of mortality (Brodschneider and Crailsheim 2010). For example, 60–80% of the bees fed fructose, sucrose, melezitose, or sorbitol, survived for 10 days, whereas

those fed sorbose, lactose, xylose or mannose died within 3 days at the start of the experiment (Parkinson *et al.* 2022). Excess consumption of fat has also been demonstrated in increased mortality of bumblebees (Vaudo *et al.* 2016a). However, another study found that pollen with higher fat was preferable to low-fat pollen by bumblebees (Vaudo *et al.* 2016b).

Nutrition and immunity

In animals, the connection between nutrition and immunity has been previously studied by the effect of calorie restriction on immune function (Hultmark 1993; Cotter *et al.* 2011). Honey bees use essential amino acids for the synthesis of very important immunological compounds such as antimicrobial peptides (AMPs) needed in immune pathways by feeding on pollen protein (Grimble 2001; Schmid-Hempel 2005; Yi *et al.* 2014). Additionally, feeding on nectar or honey provides energy for metabolic processes required for innate humoral and cellular immune reactions. These carbohydrates can also have antimicrobial properties (Erler *et al.* 2014).

Regarding lipids, honey bees obtain them exclusively from pollen. Herbert *et al.* (1980) found that bees reared more broods when 2–4% lipid extracts from pollen were added to their diet. A study by Wright *et al.* (2018) showed that honey bees can regulate their consumption of specific proportions of macronutrients. For example, feeding honey bees with a high ratio of P : F caused an increase in HPGs (Stabler *et al.* 2021) and reduced deformed wing virus (DWV) levels in caged honey bees (Alshukri and Al-Esawy 2021). Furthermore, Paoli *et al.* (2014a) showed that worker honey bees preferred carbohydrates over proteins. Interestingly, honey bee survival decreased when eating low protein and high carbohydrate diets after exposure to low temperature and nicotine toxins (Archer *et al.* 2014).

To evaluate the efficiency of any diet for animal development and survival, it is important to consider several immune and physiological parameters. Immune parameters can involve either cellular responses, which include coagulation, phagocytosis, encapsulation, or humoral responses such as the prophenoloxidase activating system (Gillespie *et al.* 1997). This system is responsible for the production of phenoloxidases, which carry out: (a) melanin biosynthesis, (b) cuticle sclerotization, (c) wound healing, (d) nodule formation, (e) encapsulation, and (f) phagocytosis stimulation (Ratcliffe *et al.* 1984; Cerenius *et al.* 2008). Regarding melanin and encapsulation processes, PO converts phenols to quinones, which are eventually polymerized into melanin (Söderhäll and Cerenius 1998). Melanin is then deposited onto a foreign invader and, when further haemocytes participate, this can lead to the encapsulation of the attacker and protect the host body. Moreover, the number of hemocytes in the insect haemocoel can increase during some infections (Christensen *et al.* 1989; Coggins *et al.* 2012; King and Hillyer 2013).

Some factors can negatively affect the encapsulation response. For example, low nutritional quality of the insect's diet (Ojala *et al.* 2005; Klemola *et al.* 2007), and ingestion of some plant secondary metabolites (Haviola *et al.* 2007; Smilanich 2008). Briefly, melanization involves the following steps illustrated in Figure 1.

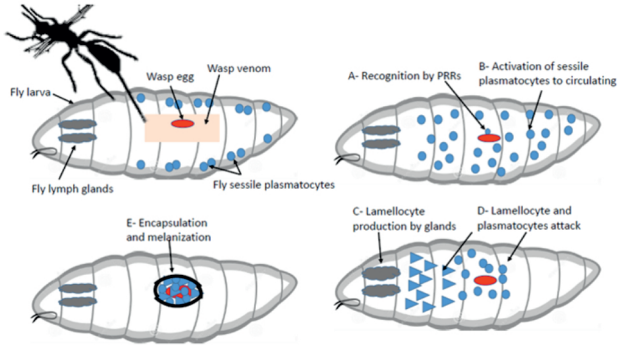

Fig. 1. Interactions between *Drosophila* larvae and endoparasitoid wasps. Wasps inject an egg into the body cavity of a fly larva, and the fly recognizes the egg as foreign and mounts a melanotic encapsulation response. A–C – the recognition of foreign intruders through plasmatocytes and lamellocytes; D – the recruitment of haemocytes to gather on the intruder; E – melanin release and encapsulating of the intruder (designed by the author)

Hypopharyngeal glands and nutrition

Honey bees have several exocrine glands such as mandibular, salivary, and HPGs (Fig. 2). The mandibular glands in young workers initially produce a fatty acid-rich secretion (Plettner *et al.* 1997). This is added to a protein-rich secretion from the HPGs. Together, these secretions are known as worker jelly, drone jelly or royal jelly (Crane 2009; Corby-Harris *et al.* 2019). The mandibular glands in field bees switch to the secretion of 'forage-marking' and alarm pheromones (Vallet *et al.* 1991). The rate of HPG protein synthesis is highest within the second week of honey bee nurse age (Knecht and Kaatz 1990). It is well known that pollen is the only source of protein and lipids for adult honey bees, and it is necessary for HPG protein production (Huang *et al.* 1989; Knecht and Kaatz 1990; Mohammedi *et al.* 1996; Feng *et al.* 2009; Renzi *et al.* 2016). Thus, summer bee nurses usually have HPGs with a higher level of protein content than winter bees (Brouwers 1982). The protein and lipid content of the

jelly produced from HPGs depends on the age of the brood or larval stage being fed. Brouwers *et al.* (1987) found that this content was high in the youngest larval jelly and decreased after age 3.5 days. However, the sugar content of the jelly increased simultaneously. The vast majority of literature focuses on the importance of pollen protein for honey bees (Schmidt and Buchmann 1985; Crailsheim 1986; Crailsheim 1990; Crailsheim 1992; Zheng *et al.* 2014). However, the literature is scarce regarding the importance of fat for HPG development and its protein content in young nurse bees.

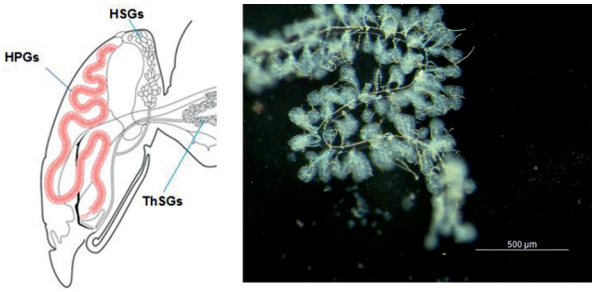

Fig. 2. Dissected HPGs, head salivary glands (HSGs), and thoracic salivary glands (ThSGs) of the honey bee worker – left (available from http://www.honey bee.drawwing.org). HPGs of honey bee *Apis mellifera* (photographed by the author) – right

Further studies on honey bees are needed to better understand how altered dietary lipid impacts the bee immune system, as well as the effects on development and performance.

This study aimed to evaluate the nutritional behavior and some immunological criteria such as encapsulation index and phenoloxidase (PO) activity, the key enzyme for melanization as well as to study the effect of protein to fat (P : F) diets on hypopharyngeal gland (HPG) protein content.

Materials and Methods

Nearly hatched frames of honey bee workers were collected from colonies of *A. mellifera* "Buckfast" hybrid strain kept on the roof of Ridley Building 2 / New castle University. Brood frames were placed in a wooden box inside a ventilated incubator (Sanyo MIR-553) set at 34°C in the dark to mimic natural field conditions (Winston 1991). Thirty newly emerged bees were taken each day for each cohort with 10 cohorts · treatment^{-1} . Bees were reared in a Perspex box (11 × 6 × 20 cm, Fig. 3) supplied with four, 2 ml Eppendorf tubes with four holes (3 mm diameter) for access as feeding tubes. A piece of paper was added to the hoarding box, covering the base.

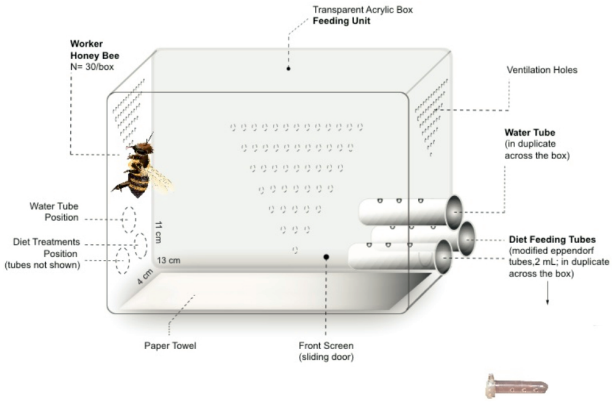

Fig. 3. The laboratory cage used for rearing honey bee *Apis mellifera*. under incubation conditions, 34°C and 66% R.H. (Wright lab: https://www.zoo.ox.ac.uk/wright-lab-oxford-bee-laboratory)

Experimental diets

Each protein part of the treatment was composed of a mixture of 10 essential amino acids (eAAs) required by honey bees (deGroot 1953): methionine, tryptophan, arginine, lysine, histidine, phenylalanine, isoleucine, threonine, leucine and valine (Tab. 1). This mixture was added to a 1.0 M sucrose solution, by adding 6.113 mg · ml^{-1} from eAAs mixture to 342.3 mg · ml^{-1} of sucrose to get 1 : 56 w/w protein to carbohydrate ratio (Vaudo *et al.* 2016b).

Table 1. Essential amino acids required by honey bees (deGroot 1953)

Amino acid	SLC*	g/16gN
Leucine	L	4.5
Isoleucine	I	4.0
Valine	V	4.0
Threonine	T	3.0
Lysine	K	3.0
Arginine	R	3.0
Phenylalanine	F	2.5
Methionone	M	1.5
Histidine	H	1.5
Tryptophan	W	1.0

*single-letter database codes

The fat source used in this study was lecithin (Optima® Bradford, UK). Lecithin was chosen as the fat source because it is an emulsifier and can be used for liquid diets. Ratios of eAAs/protein to fat (P : F) used in this study were calculated on a weight-to-weight (w/w) basis as the following: 25 : 1, 10 : 1 and 5 : 1 (low-fat

Table 2. Proportions of macronutrients in each dietary treatment

Treatment P : F	eAAs [mg · ml^{-1}]	Carbohydrate [mg · ml^{-1}]	Fat [mg · ml^{-1}]
25 : 1	6.113	342.3	0.24452
10 : 1	6.113	342.3	0.6113
5 : 1	6.113	342.3	1.2226
1 : 1	6.113	342.3	6.113
1 : 5	6.113	342.3	30.565
1 : 10	6.113	342.3	61.13
1 : 0	6.113	342.3	0

diet, LFD); 1 : 1; 1 : 5, 1 : 10 (high-fat diet, HFD) and 1 : 0 (zero-fat diet) as a control (Tab. 2).

Nutritional behavior and diet preference

Daily consumption (µl · bee^{-1}) was adjusted for the declining bee numbers in each cage during the experiment. Consumption of food was calculated by measuring the difference in the weight of feeding tubes before and after 24 h. To control the normal water evaporation from the feeding tubes, the same treatment tubes were put in hoarding cages but without bees. Each tube was replaced with a full one daily. Total daily consumption represented the sum of the adjusted weight of all four feeding tubes; the resulting number was then divided by the number of live bees remaining on that day. Experiments continued for 10 days with consumption recorded each day for each box (N = 10 per treatment). This study focused on strengthening the nurse bee's immunity, rather than older or foraging bees which have the most vigorous immune system (Wilson-Rich *et al.* 2008).

Fig. 4. A nylon thread implanted between the 2nd and 3rd abdominal segments of 10-day-old bees *Apis mellifera* fed different ratios of P : F diets (photographed by the author)

Encapsulation response

The encapsulation response was induced in the bee body by mimicking the *Varroa* mite behavior (Allander and Schmid-Hempel 2000; Sammataro *et al.* 2000; Wilson-Rich *et al.* 2008) by insertion of a nylon filament (0.08 mm diameter) as a 'pseudoparasite' (Cox-Foster and Stehr 1994; Di Prisco *et al.* 2016). Then, the filament was cut with a razor blade into approximately 5.0 mm long segments and sterilized in 95% ethanol. Bee nurses, 10-days-old, were first ice anaesthetized and then immobilized using a 'harnessing technique', exposing the dorsal side. Next, around 2.0 mm nylon cuts were implanted between the 2nd and 3rd tergites (Fig. 4).

After implantation, bees were released into the hoarding cages (Fig. 3) and incubated for 24 h (Brewer and Vinson 1971; Negri *et al.* 2014). Explanted threads were then observed at 80x magnification under a stereomicroscope (Leica M125 C, Leica®) and photographed with an attached digital camera (Sony DSC-H10). Images were analyzed using GIMP software (GIMP v.2.10.14). The encapsulation degrees were scored as the percentage of non-white pixels, which are covered by haemocytes and melanin (Di Prisco *et al.* 2016). The removed monofilament was photographed under a microscope from three different angles (Rantala *et al.* 2000; Wilson-Rich *et al.* 2008).

Phenoloxidase (PO) activity

A pulled 1.0 mm glass microcapillary tube was used to collect the bee haemolymph through the membrane between tergums 2 and 3. The haemolymph was added to phosphate-buffered saline (PBS, P4417- Sigma, pH 7.4 at 25°C) at a ratio of 1 : 25 (Adamo 2004; Alaux *et al.* 2010) and the tubes immediately were vortexed for 10s and kept at −80°C until use. As PO is highly immunologically active, causing a range of cytotoxic effects when worked, it is generally stored as its inactive precursor (prophenoloxidase, ProPO). Therefore, PO activity was measured after artificial activation of ProPO into PO with α-chymotrypsin, a common activator of ProPO (Kopacek *et al.* 1995). Levels of PO were calculated through its catalysis of the conversion of L-Dopa (3,4-dihydroxy-L-phenylalanine, colorless) to Dopachrome (red-brown), which can then be measured photometrically. Changes in the absorbance were measured for 30 min in 15s intervals at 475 nm (MRX Microplate Absorbance Reader, DynexTechnologies). The level of PO activity was measured by applying the Beer-Lambert Law (Oosterbroek and van den Berg 2003):

$$c = Abs/el,$$

where: *c* – concentration or activity, *Abs* – absorbance, *e* – molar absorption coefficient for the product dopachrome, *l* – length of the cuvette. Control tubes were composed of α-chymotrypsin without haemolymph.

Effect of fat diets on bee hypopharyngeal gland protein

Frozen bees (at age 10 days) were thawed and their HPGs (Fig. 2) were dissected under a stereoscope (Leica M125 C, Leica®) at 500x magnification. Two glands from two bees were mixed with 50µl PBS in a 1.5 ml Eppendorf tube. Subsequently, they were ground with a plastic homogenizer (Eppendorf® micro pestle, Z317314-Sigma) that was tightly fitted onto each tube. The homogenate was then vortexed briefly and then the sample was centrifuged at 336 g for 2 min (Suwannapong *et al.* 2010). To determine the soluble protein content, 10 µl of supernatant was used in a Bradford assay (Bradford 1976).

Statistical analysis

Analyses were conducted using Minitab (Minitab, State College, PA, USA, V. 19) with diet as the main effect. *Post hoc* comparisons were made using the Tukey analysis. GraphPad Prism 7 software was used to draw data figures. Data were analyzed using one-way ANOVA. We also reported an approximate F and its associated P-value. When the ANOVA was statistically significant ($p \leq 0.05$), H_0 was rejected.

Results

Nutritional behavior and diet preference

For 10 days, *A. mellifera* honey bee nurses were fed a sucrose-only diet and one of the P : F ratio diets (Tab. 2). The results indicated that bees differed in consuming PF diet [$F_{(6,63)} = 13.64$, $p < 0.001$; Fig. 5A]. Consumption of HFD was significantly less than all other treatments, where it was 4.99 ± 0.6707 µl · bee^{-1} · day^{-1} compared to 11.31 ± 0.9639 µl · bee^{-1} · day^{-1} in the control. However, in the HFD, bees ate more sucrose solution (although not significantly different) than LFD [$F_{(6, 63)} = 2.014$, $p = 0.0769$, Fig. 5B), where the highest rate of consumption was 30.66 ± 0.9076 µl · bee^{-1} · day^{-1} compared to 27.82 ± 1.299 µl · bee^{-1} · day^{-1} in the control.

Encapsulation

From the graph below (Fig. 6A), it can be seen that in general threads explanted from bee workers consumed HFD (1: 10 P : F) had a significantly greater degree of encapsulation than those fed LFD [$F_{(6,27)} = 6.7$, $p = 0.0002$]. The maximum percentage of encapsulation index was 74.6 ± 7.2% in 1 : 10 (P : F), whereas the minimum percentage was 16.5 ± 3.6% in 10 : 1 (P : F). Additionally, there was a clear difference between the encapsulated area on the threads taken from bees-fed LFD (Fig. 6C) and bees-fed HFD (Fig. 6D) compared to the normal nylon filament (Fig. 6B).

Phenoloxidase activity

The results obtained from the humoral immunity assay (PO) conducted in this study are presented in Figure 7A. Statistical analysis of PO results with multiple comparisons using the Tukey test revealed that there was a significant difference ($p < 0.05$) between HFD (1 : 5 and 1 : 10 P : F) and LFD (25 : 1 P : F). However, there was no significant difference in the variation of the activity of PO [$F_{(6,59)} = 1.426$, $p = 0.2199$] observed between other diets. Furthermore, PO activity in 10-day-old workers increased with an increase in fat consumed,

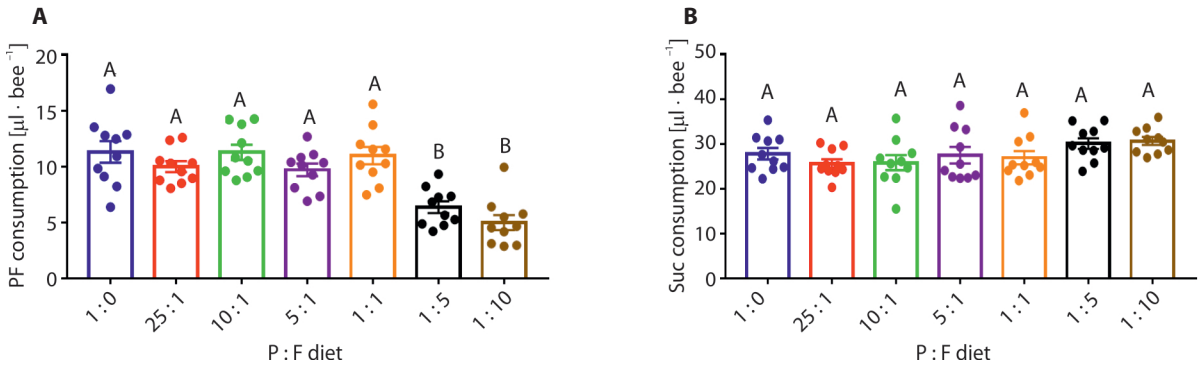

Fig. 5. Daily consumption of treatments – A and sucrose – B diets for *Apis mellifera* nurse bees in confined P : F diet assay. Treatments are represented by their protein : fat (P : F) diet ratio, including protein-only diets (1 : 0 ratio). Different letters indicate a significant difference between groups ($p < 0.05$). N (Replicates) = 10 cohorts per treatment with 30 bees in each cohort, data presented as means ±SEM

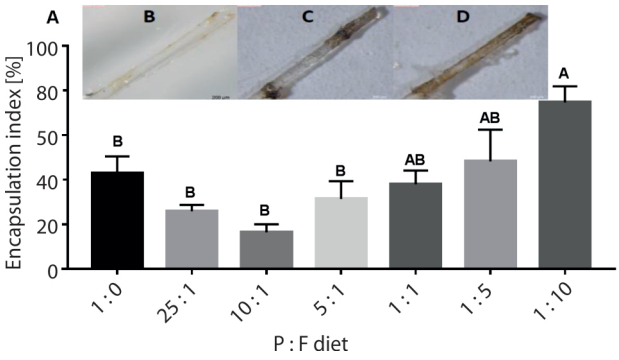

Fig. 6. A – immunocompetence of 10-day-old honey bee workers as affected by feeding on different ratios of P : F diets; B – normal nylon filament compared to C – partially encapsulated; D – completely encapsulated nylon thread at 24 h after implantation into the body of bees. Level of encapsulation of a nylon thread implant in honey bees with different levels of P : F ratio measured as a percentage of haemocytes/melanin area on the filament. Means labelled with the same letter do not differ significantly according to Post hoc test with the Tukey procedure

and it ranged in bee haemolymph from 0.001 ± 0.0001 to 0.005 ± 0.0003 mM · min^{-1} · mg^{-1} at 25 : 1 to 1 : 10 P : F diets (Fig. 7A). Furthermore, the current study revealed that there was a positive correlation, but not significant, between PO activity and encapsulation index (Pearson correlation, $r = 0.2$, $p = 0.07$) (Fig. 7B).

Hypopharyngeal (HPG) gland protein content related to fat consumption

The current study revealed that there was a significant difference in the protein content of HPGs in P : F treatments. Compared to LFDs, it was, 0.2 ± 0.05 mg · gland^{-1} · bee^{-1} at the highest fat diets (1 : 10 P : F), compared to 0.1 ± 0.006 mg · gland^{-1} · bee^{-1} in 25 : 1 P : F), while it was 0.12 ± 0.008 mg · gland^{-1} · bee^{-1} in the control [$F_{(6,28)} = 2.991$, $p = 0.021$; Fig. 8].

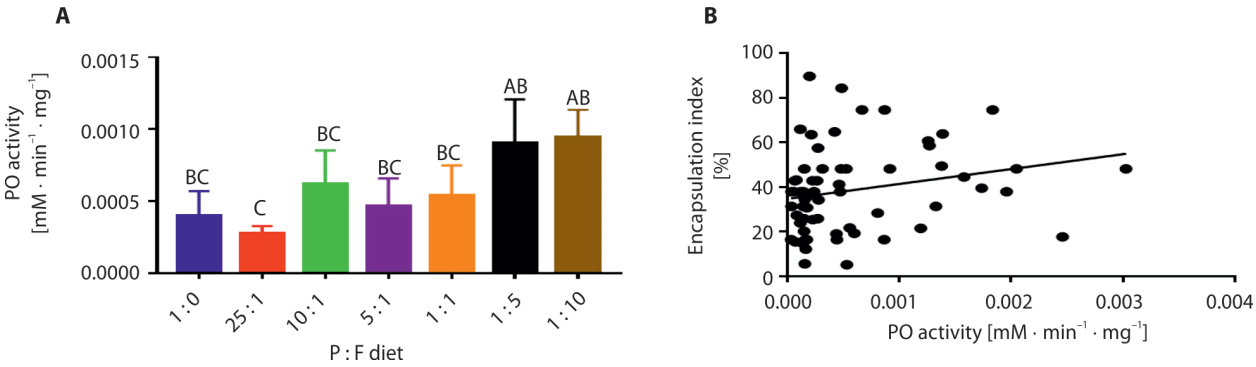

Fig. 7. A – effects of different P : F ratios on bee humoral immunity represented by phenoloxidase (PO) activity in the bee haemolymph of 10-day-old honey bee nurses *Apis mellifera*; B – correlation between PO activity and encapsulation. Means labelled with the same letter do not differ significantly according to Post hoc test with the Tukey procedure. Means ±SEM was calculated from 8–10 worker bees in each diet

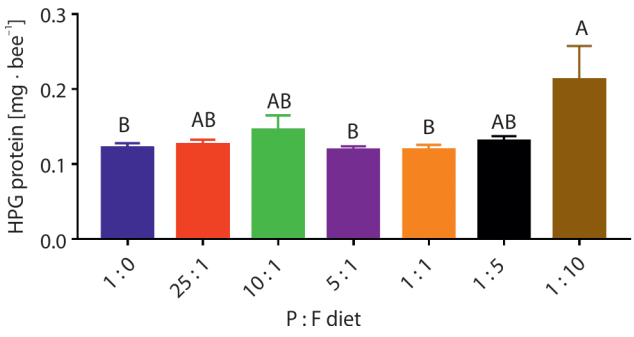

Fig. 8. Protein concentrations in the HPGs of 10-day-old worker bees *Apis mellifera* that were placed under broodless conditions. Means labelled with the same letter do not differ significantly according to Post hoc test with Tukey procedure. Means ±SEM were calculated from 4–5 worker bees in each diet

Discussion

Newly emerged honey bees were confined to diets in which the P : F ratio was manipulated. Lipid is one of the most important biochemical components of the animal diet, playing a key role in growth, survival and reproduction (Biebach 1996). Besides its primary function as an energy source, lipids have many additional functions such as preventing desiccation and participation in the chemical communication of insects, such as pheromones (Howard and Blomquist 2005).

This study demonstrated that bees on HFDs consumed significantly less than LFDs (Fig. 5). This can

be explained by the fact that bees tried to manage the toxicity resulting from high-fat diets by restricting food intake (Raubenheimer *et al.* 2005). Thus, bees on LFDs consumed twice as much as bees that were on HFDs. Although lipids naturally have a phagostimulatory effect, their high concentration caused decreased attraction and consumption (Fig. 5). However, hunger (or need for fat or carbohydrates) can activate lipolysis in the fat body to obtain energy. So, the concentration of fat in the fat body (in the form of anhydrous triglycerides) decreases, while the haemolymph fat (in the form of diglycerides) levels increases (Jimenez-Sanchez *et al.* 2012; Hossain *et al.* 2013).

This result is partially supported by Vaudo *et al.* (2016a) who found that the P : F ratio is the key stimulus of foraging in honey bees. However, in another study, Vaudo *et al.* (2016b) found that some of the most preferred plant species in the feeding of Bumblebee *Bumbus impatiens* were those with a high lipid content.

The encapsulation response is one of the most effective ways to assay the strength of immune defense in arthropods (König and Schmid-Hempel 1995; Rantala and Kortet 2003; Ahtiainen *et al.* 2004, 2005; Vainio *et al.* 2004). In this technique, nylon implants are considered immune challenges to activating encapsulation/wound healing in insect haemocytes (Wilson-Rich *et al.* 2008). Thus, in this study, broodless nurse-age honey bees were challenged with nylon thread implants to assess the impact of PF feeding on the bee immune system. The results provided in this study (Fig. 6) suggest that generally, feeding on high-fat diets plays a positive effect on the encapsulation response. The same trend was also found in the PO test (Fig. 7), where the results of the present study revealed that PO activity in HFDs was five times greater than the PO activity in LFDs.

Generally, the positive role of fat on the immune system may be attributed to two facts: first, fat is one of the nutrients which supports and stimulates the immune system as an "immuno-nutritional element" (Karacabey and Ozdemir 2012). In this regard, fats have a key role in some biological functions such as: the absorption of fat-soluble vitamins (A, D, E and K), a source of ω-3 and ω-6 oil acid, and providing permeability and stability for cell membranes (Simopoulos 2002; Jing *et al.* 2012; Ariena *et al.* 2015). Secondly, fat has more than twice as many calories as carbohydrates and proteins (Kritchevsky *et al.* 1986), and this can cover the high cost of energy required to activate and work the immune system (Moret and Schmid-Hempel 2000). The present findings support the study of Kritchevsky *et al.* (1986) who concluded that there was a positive correlation between dietary fat and encapsulation response in the male damselfly, *Calopteryx virgo* L. However, Adamo *et al.* (2007) found that force-feeding a high lipid diet reduced *Manduca sexta* caterpillar resistance to bacteria, *Serratia marcescens*. Moreover, some studies found that the genotype of honey bees and the location of the colonies influence the levels of endoparasite resistance such as tracheal mites. These genotypes included Buckfast, ARS-Y-C-1 (Yugoslavian) and Russian honey bees (Danka *et al.* 1995; Lin *et al.* 1996; de Guzman *et al.* 2002; 2005). It can be concluded from the current study that a low level of immunity was seen in bees eating low-fat diets, which could be partially explained by the low activity of PO enzymes or the encapsulation process that was shown in Figures 6 and 7. Many studies have revealed that PO activity can be influenced by diet quality (Lee *et al.* 2006; Klemola *et al.* 2007). For example, Brakefield (1987) mentioned that melanogenesis in the peppered moth (*Biston betularia*), which is controlled by PO might be costly in nitrogen. Although eating food with a high ratio of P : F had a negative impact on the health of many animals (Durand *et al.* 2005; Alzoubi *et al.* 2009; Moreira *et al.* 2012; Crean and Senior 2019), the results of the current study suggest the opposite, as bees eating HFDs had a better encapsulation index and high PO activity (Figs. 6, 7). Therefore, further research in this field would be of great help in better understanding bees' nutritional immunity.

HPGs are protein-producing glands situated in the head of worker honey bees (Klose *et al.* 2017). The current results showed clearly that feeding honey bees with diets high in fat (1 : 10 P : F), for the first 10 days of adult life, affected positively the protein content of HPGs, reaching 0.2 mg · gland^{-1} · bee^{-1} compared to 0.1 mg · gland^{-1} · bee^{-1} in the control and low-fat diets (Fig. 8). In fact, the exact reason for the positive relationship between HFDs and protein content of HPGs is unclear. However, the findings can provide insights into the potential of fat supplements to improve the effects of fat deprivation in workers and how this might translate into colony growth. The present result is supported by a study by Stabler *et al.* (2021) who revealed that providing HFDs to caged bees increased the HPG size. Moreover, DeGrandi-Hoffman *et al.* (2010) found that providing honey bees with protein supplements increased protein levels and the development of HPGs. Because the study was carried out using caged bees, the effects of fat diets on brood rearing and nestmate interactions at a colony level might have impacted the examined criteria. In beehives, fermentation and pre-digestion occur through the action of microbes (Gilliam *et al.* 1989; Zuluaga-Dominguez and Fuenmayor 2022). In the current study, the P : F diets were subjected solely to digestion in the gut, and this might limit the effectiveness of P : F supplements in colonies.

Conclusions

The current bioassays provided the first evidence that some cellular and humoral parameters in broodless nurse-age honey bees were affected by fat diets. Generally, there was a positive relationship between fat consumed and bee immunity. However, it is unknown whether honey bee nurses can adjust their fat nutritional preferences under the colony conditions, in the presence of broods, different ages and castes of bees, and natural resources. This study needs to be repeated in the field before the relationship between fat diets and changes in immune parameters can be fully confirmed. Further studies should be performed to identify the most efficient proportional fat diets enhancing honey bee life.

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