

# Influence of different pollen diets on the development of hypopharyngeal glands and size of acid gland sacs in caged honey bees (*Apis mellifera*)

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**Abstract** – We examined the consumption rate of protein diets in caged and free-flying honey bees, amino acid composition of diets, and diet effects on gland development. The effect of seven different diets (sugar solution only, Feedbee®, *Helianthus* pollen, *Sinapis* pollen, *Asparagus* pollen, *Castanea* pollen, and mixed pollen diet) on the development of the hypopharyngeal (HPG) and acid glands (AG) was tested in caged honey bees. Caged bees consumed the protein diet mainly at the age of 1–8 days, with the highest consumption rate on day 3. Different diets affected the development of both glands. The acini of HPG attained their maximum size in caged bees at an age of 5 days. Bees fed with *Castanea* sp., *Asparagus* sp., or mixed pollen had the largest glands among all test groups of this age. The AG sacs of caged bees grew in size between 5 and 12 days and were at day 18 less affected by different protein diets. *Castanea* sp. and mixed pollen diets were preferably consumed in free-flying colonies.

**hypopharyngeal glands / acid gland sacs / monofloral pollen / protein / nutrition**

## 1. INTRODUCTION

Pollen is the main source of protein for adult bees, and its protein content and quality influence the lifespan and performance of honey bee colonies (Brodschneider and Crailsheim 2010; Nicolson 2011). Pollen protein content can be used as an index of pollen nutritional value and quality (Nicolson 2011). Honey bees collect pollen grains from a great variety of plant species, and the nutritional composition of pollen varies greatly, according to the different plant species (Schmidt 1984; Roulston and Cane 2000). Schmidt (1982) reported

that the honey bee's preference for a type of pollen was not based on its protein content, but Cook et al. (2003) demonstrated that foraging preferences could reflect pollen quality. The nutritional value of pollen might be defined more accurately by its amino acid composition rather than its total protein content, because its nutritional value is reduced when inadequate amounts of essential amino acids are present (De Groot 1953; Cook et al. 2003).

Honey bee colonies obtain proteins, lipids, minerals, and vitamins required for brood rearing, maturation, and development from pollen (Schmidt and Buchmann 1985; Loidl and Crailsheim 2001). Poor-quality pollen leads to inferior weight gain and less protein or nitrogen content, reduced longevity, and an incomplete development of hypopharyngeal glands (HPG), which in turn reduces royal jelly production, that could impair growth of the larvae and queen (Kunert and Crailsheim 1988; Zaytoon

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et al. 1988; Schmidt et al. 1995; Brodschneider and Crailsheim 2010).

The HPG of nurse bees produce a protein-rich food called royal jelly. This food is fed to queen, larvae, drones, and other workers (Crailsheim 1991). For the optimal development of the HPG, newly emerged bees must feed on protein food (Kleinschmidt and Kondos 1978; Crailsheim 1990). Pollen consumption has been reported to positively correlate with gland development (Crailsheim and Stolberg 1989; Hrasnigg and Crailsheim 1998; Corby-Harris et al. 2015). Thus, the growth and development of the HPG can be considered as important criteria that can be used to evaluate the suitability of natural pollen diets or protein supplements fed to young bees (Maurizio 1954; Standifer et al. 1960). The diameter of the HPG acini has been used effectively to estimate the physiological status of worker honey bees (Moritz and Crailsheim 1987). DeGrandi-Hoffman et al. (2010) measured protein concentrations in the head and the development of HPG in worker honey bees that had been fed either patties of mixed pollen, a protein supplement, or food lacking protein (sugar solution). Workers consumed pollen more often than the protein supplement, but the amount and quality of the protein in these two diets did not seem to influence size of HPG acini. Bees that were fed only with a sugar solution had lower concentrations of protein and smaller HPG, as compared with bees fed with the other diets.

Honey bee venom is produced by two glands (the acid and alkaline glands) associated with the sting apparatus of the worker honey bee. In honey bee workers, the acid gland (AG) is a simple long structure split in two branches by its end, opening into an ovoid sac (Snodgrass 1984). Many factors have been demonstrated to affect honey bee AG including honey bee race, age of bees, and feeding supply (Owen and Bridges 1976; Nenchev 2003). Protein food is essential for venom production; bees fed only with sugar solution yielded only 23% of the venom produced by bees fed on a normal pollen diet (Lauter and Vrla 1939). However, the effects of different natural pollen diets on the development of the AG sac have not been studied before but might be a good criterion for evaluating nutritive value of different protein diets.

Honey bees foraging on monocultures such as sunflowers, a crop which has been demonstrated

to produce pollen lacking two essential amino acids (Nicolson and Human 2013), may hinder the internal gland development of honey bees. It is therefore important to identify the availability and nutritional value of the pollen from the most important and abundant local plant sources and consider the influence of this food on the physiological performances of honey bees of different ages. The purpose of the present study was to determine the effects of bee age and quality of selected protein diets on the development of the HPG acini and AG sacs. Additionally, the consumption rates of and preferences among several protein diets were examined in caged honey bees and free-flying honey bee colonies and amino acid analysis of the used pollen diets was made.

## 2. MATERIAL AND METHODS

### 2.1. Honey bees (*Apis mellifera* L.)

Newly emerged honey bee workers (0–24 h old) were obtained by incubating sealed brood combs from several unrelated honey bee colonies at 34.5 °C under standard conditions (Williams et al. 2013). Randomly chosen newly emerged bees from these brood combs were mixed and introduced into test cages. All experiments were conducted at the Institute of Zoology, University of Graz, in Graz, Austria, during the foraging season of honey bee colonies (June to September 2014).

### 2.2. Protein diets

Pollen loads gathered by bees from local plants were collected using entrance-mounted plastic pollen traps (Anel Standard) in the apiaries of the Institute of Zoology in Graz and the Department for Apiculture and Bee Protection in Vienna. Pollen traps were activated in the morning of days with good weather conditions when foraging activities of the bees were to be expected and emptied in the late afternoon. The harvested pollen pellets were immediately deep frozen (−18 °C) and stored until the grading by pellet color was carried out. Pellets of the same color were pooled. These pooled samples were checked for their botanical origin and purity by light microscopy from experienced staff of the Department for Apiculture and Bee Protection. A

homogenous suspension was prepared from each sample, and the percentage of main pollen types was evaluated by identifying and counting of 1000 pollen grains per sample. Results are given in Table I. All pollen diets were stored at  $-18^{\circ}\text{C}$  until the bioassays were conducted. Feedbee®, a commercial pollen substitute, was also tested as dough (diet 2, Table I). Diets 5, 6, and 7 were wetted with 10% ( $w/w$ ) water (Williams et al. 2013). The pollen loads of diets 3 and 4 were mixed with more water (30%) because the dough was still powdery with 10% water. All diets were kneaded until a homogeneous dough was formed. All prepared doughs were stored at  $-18^{\circ}\text{C}$  and thawed on the day of use.

### 2.3. Experimental cages

The experimental cages consisted of clear plastic cups with two holes, one in the base of the cup and the other on the side of the cup. The cages were also supplied with a wax comb to imitate natural conditions experienced by honey bees (Williams et al. 2013). The cage was closed from below by an iron grid that allowed air to pass through (Evans et al. 2009). Once bees had been placed into the cage, the hole in the base was covered by tape and the other hole was closed by a 1.5-mL vial in which three small holes had been punctured from which the bees could uptake 50% ( $w/v$ ) sucrose solution. As carbohydrate solutions containing  $\geq 50\%$  ( $w/v$ ) water are sufficient for hydration, no extra water was added (Williams et al. 2013). Protein diets were presented in one half of cylindrical 10-mL plastic tubes, and bees in cages were kept in the dark in an incubator at  $34.5^{\circ}\text{C}$  under standard conditions (Williams et al. 2013) for up to 18 days. During experimental period, dead bees were counted daily and removed from the cages. Daily consumption rates per bee were calculated on the basis of living bees during the observation period.

### 2.4. Experimental design

#### 2.4.1. Food consumption and gland development in caged bees

Experimental cages were provided with 1 mL sucrose solution and diet 6 as protein diet. We

started different cages containing 50 newly emerged bees each for each investigated age class. After 2, 5, 9, 12, 15, and 18 days, 20 bees each were taken for analysis and the remaining bees in this cage were not further used in experiments. This ensured a comparable number of bees in the cage for all age classes. During experimental period, the number of dead bees was counted daily and dead bees were removed from the cages.

The amount of consumed protein diet was recorded daily, and the diets were renewed. To measure the amount consumed, the difference between the weight before introduction and the weight after 24 h was measured on an analytical balance (Mettler Toledo XS205 dual range). Additionally, the weight lost by one sample of diet 6 in the incubator without bees was measured to determine the daily evaporation rate from the diet. For this calculation, the percentage of evaporation was subtracted from the recorded amount of the protein diet consumed (Pernal and Currie 2000; Williams et al. 2013). For food consumption calculation, dead bees were assumed not to have consumed any food since the prior food was changed. The time period during which the bees had access to the protein diet was measured accurately to the minute but was recalculated for the total consumption of protein diet over 24 h to standardize the values, and the consumption was also calculated per bee. The volumes of the HPG acini and AG sacs were measured in 15 honey bee workers from each cage after 2, 5, 9, 12, 15, and 18 days, respectively, to examine the development of the two glands.

#### 2.4.2. Comparison of the diets

Experimental cages received 2 mL of sucrose solution ad libitum and different protein diets (Table I). With sugar-only control (diet 1) and two replicates per cage, a total of 14 cages was used for this experiment. Cage design and incubation conditions were the same as described above, with the exception that each cage contained 100 bees. Samples of 10 bees per replicate were taken at age 5, 12, and 18 days for gland development analyses. The duration of this experiment was 18 days, and bee samples were taken with a minimum number of 70 bees present

**Table 1.** Diets used in first and second experiments and their constituents.

Diet number	Main component	Other species/details
Diet 1	No protein	50% sucrose solution
Diet 2	Feedbee®	41.72% Feedbee®, 42.39% icing sugar (sucrose), and 15.89% water
Diet 3	<i>Helianthus</i> sp. (94.8%)	Unknown (5.2%)
Diet 4	<i>Sinapis</i> sp. (91.2%)	Brassicaceae (5.2%), other
Diet 5	<i>Asparagus</i> sp. (70.6%)	<i>Silphium perfoliatum</i> (28%)
Diet 6	<i>Castanea sativa</i> (87.6%)	Unknown (3.8%), <i>Trifolium repens</i> (2.7%)
Diet 7	Pollen mix	Mix of 25% each of diets 3–6

in cages. As in the first experiment, evaporation rate was measured.

### 2.4.3. Diet preferences

This experiment was run during April 2015. Four free-flying colonies were chosen, all of approximately equal strength (2 supers with 20 frames in total) according to Bridgett et al. (2014). Diets 3, 4, 5, 6, and 7 were used in this experiment. Each diet was prepared as dough by mixing the pollen loads with water as described above before splitting them equally by weight ( $5.0 \pm 0.3$  g). Each pollen diet was placed into small plastic dishes (square weigh boats) and weighed before its introduction to the colonies, so that the consumption could be calculated by monitoring loss of mass over time. All diet dishes were placed simultaneously in the middle of the hive on top of the uppermost frames in each of the four colonies. Experiments were conducted over a period of 18 h, until the complete consumption of the first diet had been observed. All diets were removed from all four colonies and weighed again. The evaporation loss of the diets was measured by putting an extra diet dish for each diet with the same weight in one of the four colonies and covered those dishes with cages to prevent bees from accessing them. The experiment was made in two replicates.

### 2.4.4. Amino acid analysis

Content of amino acids of all essential and non-essential amino acids, except tryptophan, was measured by an amino acid analyzer in the

laboratory of the Austrian Agency for Health and Food Safety (AGES). For tryptophan determination, HPLC with fluorescence detection was used. All amino acid analyses were carried out according to the methodology of the European Commission (EC152/2009 2009). In addition to diet 6, a more purified sample of *Castanea sativa* pollen was available for amino acid analysis.

## 2.5. Physiological parameters

### 2.5.1. HPG development

After the sting apparatus of the living honey bee worker was dissected (see below), the heads of the bees were separated and stored at  $-18$  °C until further measurement of the HPG acini. To dissect the HPG, the head of the bee was opened dorsally with a razor blade. The paired glands were extracted carefully with forceps and put on a microscope slide in a droplet of 250 mM sodium chloride solution (Hrassnigg and Crailsheim 1998). To determine the developmental status of the HPG, the length and width of each oval acinus of 16 neighboring acini were measured using an ocular micrometer on Reichert Diavar microscope at hundredfold magnification. The volume of each acinus was calculated by applying the following equation to determine HPG development:

$$\text{Volume of an acinus} = \frac{4}{3} \pi \times \left( \frac{a+b}{4} \right)^3$$

where  $a$  is the maximum length and  $b$  is the maximum width of the acini or AG sac and  $\pi = 3.14$ .

### 2.5.2. AG development

The AGs were extracted of living bees and measured immediately thereafter. AG development was evaluated and expressed by measuring the volume of the AG sac, because the filling degree of the AG sac depends on the amount of venom secretion by the AG (Lauter and Vrla 1939; Nenchev 2003). The sting apparatus of honey bee workers was separated using forceps in physiological saline solution (250 mM sodium chloride solution) and examined under a binocular microscope. The dimensions of AG sac (length and width of the spherical sac) were measured using a binocular microscope (Olympus SZ 40). The volume of the AG sac was calculated by applying the same equation as above.

### 2.6. Statistics

Data on gland development and diet consumption are shown as box-whisker plots made in Statgraphics Centurion XVI (Statpoint Technologies), where the whiskers display minimum and maximum, the box the lower and upper quartile and median. Median notches show 95% confidence interval of the median.

The null hypothesis of equal means has been tested by a one-factorial analysis of variance (ANOVA). Since dispersions were not equal between the treatment groups (heteroscedasticity) in some variables, the choice of the Welch-type ANOVA was applied to account for heteroscedasticity. Multiple comparisons between treatment groups would inflate type-I error, which is compensated by Sidak's correction method. The latter method also allows separating the groups

into several subgroups, which are non-significant within the subgroup(s), but significant between subgroups. Sometimes, such different subgroups may overlap. Type-I error level was set to 0.05; testing procedures assumed two-sided testing. ANOVA with multiple comparisons were calculated with SAS 9.4 PROC GLM.

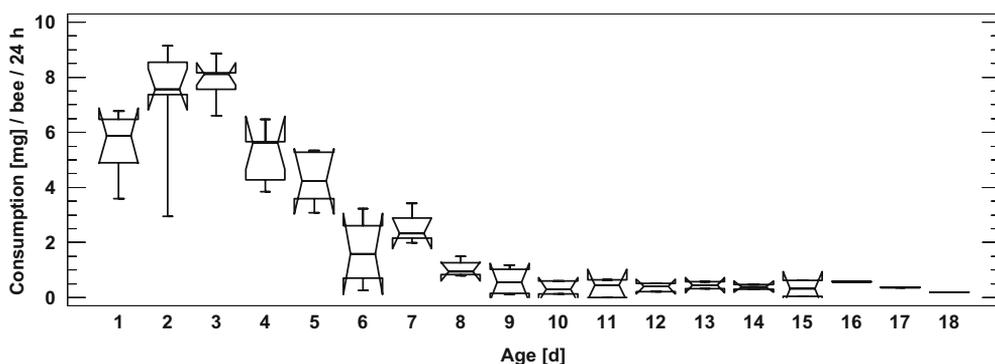
## 3. RESULTS

### 3.1. Food consumption and gland development of caged bees

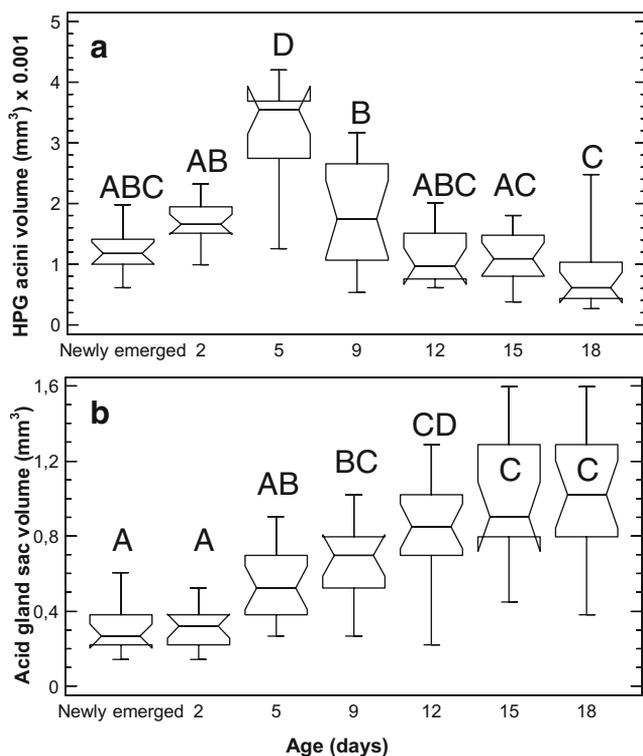
The protein diet (corrected for evaporative loss) was mainly consumed by 1–8-day-old bees, with the highest median consumption occurring at day 3 (Figure 1). From days 9 to 18, little consumption was observed. The acini of the HPG grew in size until the bees reached an age of 5 days, with the maximum developed HPGs, and then began to shrink afterwards (Figure 2a). The volume of the AG sac started to grow at age 5 and was significantly bigger after 9 days compared to younger bees (Figure 2b,  $P < 0.05$ , Welch ANOVA). The AG sacs reached their full size in bees at the age of 12 days and remain constant at least until day 18.

### 3.2. Comparison of diets

As in the first experiment, the main consumption of protein diets was between days 1 and 9, with the highest consumption on day 2 (data not shown). A difference between the six protein diets that were used to feed the caged honey bees in the



**Figure 1.** Daily consumption of diet 6 (milligrams per bee per 24 h) by caged honey bees over a period of 18 days ( $n = 6$  for age 1–2,  $n = 5$  for age 3–5,  $n = 4$  for age 6–9,  $n = 3$  for age 10–12,  $n = 2$  for age 13–15, and  $n = 1$  for age 16–18, where  $n$  is the number of cages). For ages with  $n = 1$ , the value is shown as a line.



**Figure 2.** **a** The development of the HPG acinus volumes of caged honey bees after emergence and at age of 2, 5, 9, 12, 15, and 18 days, which were fed with diet 6. **b** The development of the AG sac volume of caged honey bees fed with diet 6 after emergence and at age of 2, 5, 9, 12, 15, and 18 days.  $n = 14\text{--}16$  for each age. Groups with the same letter are not significantly different ( $P > 0.05$ , ANOVA with Sidak's multiple comparisons).

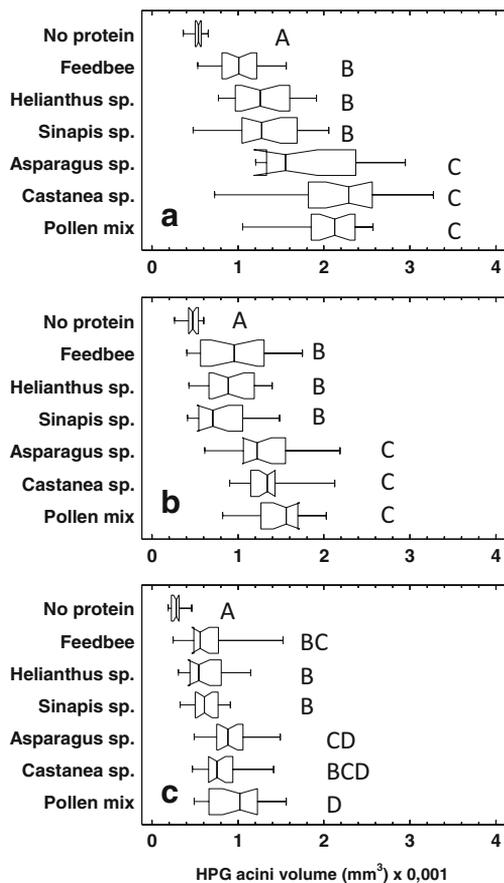
second experiment was observed with the lowest diet consumption over 18 days in the two replicates of diet 5 ( $R1 = 43.87$  mg/bee and  $R2 = 41.16$  mg/bee) and the highest consumption in the two replicates of diet 7 ( $R1 = 69.56$  mg/bee and  $R2 = 66.99$  mg/bee). More on the preference of diets in full colonies with higher number of samples can be found in "Section 3.3."

The volumes of the HPG acini in this experiment were largest at the age of 5 days and significantly different compared to the age of 12 days ( $P < 0.05$ , Welch ANOVA) in all diets except the no-protein diet and bees fed with Feedbee®. Bees from all groups had larger HPG acini on day 5 compared to day 18, and the acini on day 18 were also smaller than those on day 12 ( $P < 0.05$ , Welch ANOVA). The diets had different effects on the volumes of the HPG acini at the three ages investigated. At the age of 5 and 12 days, HPG acini were developed best in bees fed with *Asparagus* sp., *Castanea* sp., and mixed pollen

(Figure 3a, b). This trend was partly seen even in 18-day-old bees (Figure 3c). The protein-free diet always resulted in the lowest developed HPG glands ( $P < 0.05$ , Welch ANOVA).

The volume of the AG sacs of bees fed with all diets was significantly larger at day 12 compared to day 5 ( $P < 0.05$ , Welch ANOVA). No significant differences between day 12 and 18 were found ( $P > 0.05$ , Welch ANOVA). The diets influenced the volume of AG sacs in bees not as strong as the HPG acini. At the age of 5 days, *Asparagus* sp. pollen promoted AG sac growth significantly better than *Castanea* sp. pollen or the mixed pollen (Figure 4a). Bees aged 12 days fed on *Asparagus* sp., *Castanea* sp., and mixed pollen had significantly better developed AG sacs than bees deprived of protein (Figure 4b). At the age of 18 days, no more significant differences among different diets could be found (Figure 4c).

Different protein diets had no observable effect on the mortality of caged honey bees over a period of

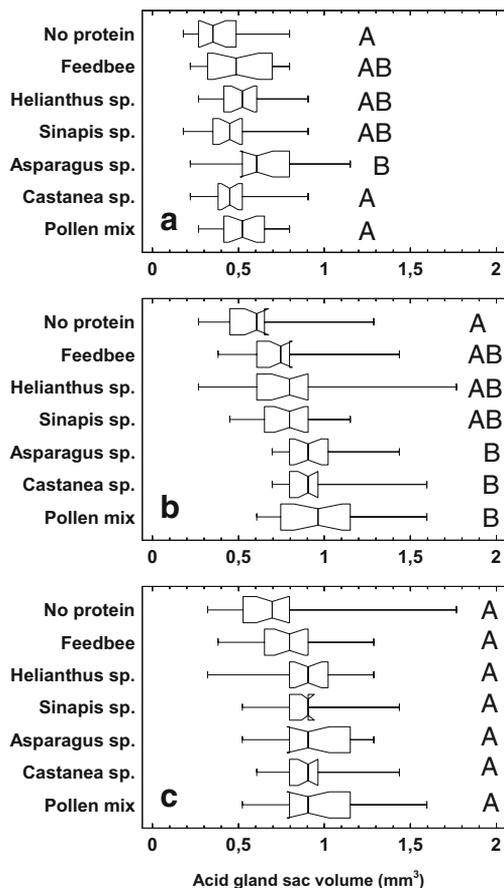


**Figure 3.** The HPG volumes (cubic millimeter) of caged honey bees fed with different diets, measured on **a** day 5, **b** day 12, and **c** day 18.  $n = 19-20$  for all age classes. Groups with the same letter are not significantly different ( $P > 0.05$ , ANOVA with Sidak's multiple comparisons).

18 days ( $P > 0.05$  Mantel-Cox log-rank test). Mortality was 9.0% (diets 3 and 5), 10.0% (diet 7), 12.5% (diet 6), and 15.0% (diets 2 and 4). Only protein-free diet resulted in a higher mortality of 21.0% ( $P < 0.05$  Mantel-Cox log-rank test). No other health parameters than mortality were evaluated.

### 3.3. Diet preference

A difference in consumption rate was observed among the five pollen diets that were presented simultaneously to test for dietary preferences in four honey bee colonies and two replicates. The lowest consumption was recorded for *Helianthus* sp. pollen with a median of 1.72 g/colony consumed in

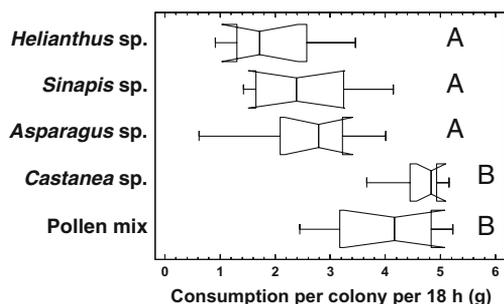


**Figure 4.** The AG sac volumes (cubic millimeter) of caged honey bees fed with different diets, measured on age of **a** day 5, **b** day 12, and **c** day 18.  $n = 20$  in all age classes. Groups with the same letter are not significantly different ( $P > 0.05$ , ANOVA with Sidak's multiple comparisons).

18 h (Figure 5). The highest consumption was recorded for *Castanea* sp. and mixed pollen with medians of 4.83 and 4.16 g/colony, respectively. There was a significant difference between consumption of *Castanea* sp. and mixed pollen compared to all other diets ( $P < 0.05$  Welch ANOVA).

### 3.4. Amino acid analysis

Amino acid composition of different protein diets is shown in Table II with the exception of diet 7, because it is a mix of the other analyzed pollen diets. Valine, isoleucine, and leucine are considered the three most required amino acids for young bees (De Groot 1953). According to



**Figure 5.** Consumption of pollen diets applied simultaneously to full free-flying honey bee colonies ( $n = 8$ , four colonies, two replicates). Groups with the same letter are not significantly different ( $P > 0.05$ , ANOVA with Sidak's multiple comparisons).

amino acid analysis in Table II, these are lowest in diet 3 compared to all the other diets.

#### 4. DISCUSSION

Protein diets were mainly consumed by caged honey bees from ages 1 to 8 days, which is the same age during which bees in a colony perform brood

care behavior (Schmidt 1984; Moritz and Crailsheim 1987; Crailsheim et al. 1992), with the highest consumption observed on day 3. The amount of diet consumed gradually decreased thereafter with no more noteworthy consumption after day 9. The size of the acini was larger in 5-day-old bees than in the newly emerged bees, because these bees feed on protein diets soon after emerging to get their HPG fully developed (Hagedorn and Moeller 1968). The acini reached their maximum size when the bees are 2 to 9 days old and then became smaller. This confirms previous findings in colonies or cages (Moritz and Crailsheim 1987; Altaye et al. 2010). The AG sac volumes continued to increase with age because bees prepare themselves for guarding tasks, during which they need venom for colony defense (Nenchev 2003).

Our results confirm those of Crailsheim and Stolberg (1989), conducted in colonies and caged honey bees, which showed that protein nutrition is important for the development of the HPG. Our results further indicate that different protein diets have various effects on the HPG development in bees

**Table II.** Amino acid composition of different protein diets as percentage. Refer to Table I for diet composition.

Amino acid	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Pure <i>Castanea sativa</i>
Tryptophan <sup>a</sup>	0.15	0.20	0.30	0.34	0.26	0.24
Cysteine	0.19	0.37	0.38	0.36	0.29	0.32
Aspartic acid	1.01	1.05	1.65	2.08	1.64	1.45
Methionine <sup>a</sup>	0.25	0.35	0.56	0.54	0.36	0.43
Threonine <sup>a</sup>	0.45	0.48	0.82	0.77	0.62	0.60
Serine	0.63	0.46	0.91	0.90	0.70	0.74
Glutamic acid	1.73	1.03	1.84	2.09	1.68	1.78
Proline	0.92	0.75	1.47	1.78	1.57	1.37
Glycine	0.43	0.56	0.89	0.94	0.71	0.72
Alanine	0.84	0.59	0.95	1.11	0.81	0.81
Valine <sup>a</sup>	0.62	0.56	0.95	0.90	0.83	0.76
Isoleucine <sup>a</sup>	0.56	0.51	0.83	0.83	0.70	0.67
Leucine <sup>a</sup>	1.34	0.77	1.30	1.54	1.15	1.15
Tyrosine	0.41	0.35	0.58	0.60	0.41	0.52
Phenylalanine <sup>a</sup>	0.72	0.50	0.83	0.85	0.70	0.69
Histidine <sup>a</sup>	0.38	0.60	0.55	0.56	0.47	0.50
Lysine <sup>a</sup>	0.54	0.81	1.50	1.49	1.10	1.11
Arginine <sup>a</sup>	0.59	0.49	0.86	0.89	0.74	0.76

<sup>a</sup> Essential amino acids for bees

during the first 18 days of their development, given the maximum consumption at the age of 3 days probably even earlier. The various effects of the different diets may be because of the quality of each diet which could be protein content, bioavailability of proteins, amino acid composition of proteins, or content of other important food sources as lipids or vitamins (Standifer et al. 1960; Brodschneider and Crailsheim 2010).

Standifer (1967) and Di Pasquale et al. (2013) found that certain aspects of nurse bee physiology, such as HPG development, were affected by pollen quality. Moreover, nutrition quality in combination with pesticide contamination has an effect on HPG development (Renzi et al. 2016). In our experiment, the pollen mixture was considered one of the best diets to support the development of HPG (Figure 3), but monofloral pollen diets comprised of *Asparagus* sp. or *Castanea* sp. pollen also supported HPG development same as mixed pollen. Bees fed only on a 50% sucrose solution had the poorest developed HPG, monofloral *Helianthus* sp. and *Sinapis* sp. pollen ranged between these two extremes. The strongest effects in HPG could be observed at the age of 5 days, although *Asparagus* sp., *Castanea* sp., and especially the mixed pollen also retarded the degeneration of HPG in 18-day-old bees. No significant differences in mortality between protein-fed bees were observed within the rather short period of 18 days, but bees without pollen diet had a significantly higher mortality, which supports the importance of protein diet for bee survival (Schmidt et al. 1987).

The AG sac volumes increased with the age of the bees and did not degenerate like the HPG. We detected fewer differences in bees fed with different protein diets compared to the HPG acini. While pollen of *Asparagus* sp. promoted AG sac development in bees aged 5 days, at day 12, the same diets identified as beneficial for HPG development (*Asparagus* sp., *Castanea* sp., and mixed pollen) resulted in significantly larger AG sacs compared to the protein-free control only. At the end of the experiment at day 18, all diets, surprisingly including the protein-free control, resulted in similar sized AG sacs, which could be due to loss of statistical power of multiple comparisons. We need to stress that we report morphological developments of the glands, and no direct proposition on relation of quality of venom production and AG sac volume can be made at this stage (Nenchev 2003).

Diets were not equally consumed, neither in cages nor in colonies. In our experiment in full colonies, following the protocol of Bridgett et al. (2014), the pollen mix (compare Schmidt 1984) and monofloral pollen of *Castanea* sp. have been eaten the most. This preference may have been due to the quality of the pollen, the composition and purity of each diet, or the presence of phagostimulants in the diet (Schmidt 1985).

Diets composed of mainly *Asparagus* sp. or *C. sativa* pollen performed as well as mixed pollen in our experiments. This is supporting findings of Tasei and Aupinel (2008) and Di Pasquale et al. (2013) who identified *Castanea* sp. pollen as high-quality pollen for bees. Also, *Asparagus* appeared to be highly nutritive but note that the *Asparagus* sp. pollen also contained 28% of *Silphium perfoliatum* pollen which could not be separated by color during grading of pollen pellets. Diets that contained more than 90% pure *Helianthus* sp. or *Sinapis* sp. pollen had the least positive effects on the development of the glands, which may indicate that they are low-quality pollens for the investigated parameters, HPG development in particular. This assumption is supported by the findings of Nicolson and Human (2013), who found that low-quality pollen such as from *Helianthus* sp. may hinder bee development, as well as by the results of Schmidt et al. (1995), who suggested that colonies located near fields of *Helianthus* will need to be provided with pollen supplements to prevent detrimental effects.

The amino acid composition of pollen of many important bee forages has not been investigated in detail; there is only one recent publication that discusses essential amino acid content of *Helianthus* sp. pollen on bee physiology (Nicolson and Human 2013). In contrast to this publication, which found methionine and tryptophan to be low in their investigated variety of sunflower, we found the lowest values of valine, isoleucine, and leucine, three important essential amino acids according to De Groot (1953). In our comparison of different protein diets, methionine and tryptophan were also lowest in sunflower pollen compared to other pollen diets but slightly higher than in Feedbee®, which had the lowest amounts of these two essential amino acids. The content of essential amino acids alone cannot explain the differential effects of pollen diets on

gland development. For example, pollen of *Sinapis* sp. has comparable high amounts of all essential amino acids but failed to promote HPG growth. Therefore, one or more limiting factors, amino acid, lipid, or vitamin, or their availability for the bees might exist. Amino acid composition of diet 6, which was not pure *Castanea* pollen, does not differ much from pure *Castanea* pollen which was later available for analysis (Table II).

The suitability of Feedbee® as pollen substitute is controversially discussed (De Jong et al. 2009; Altaye et al. 2010; Saffari et al. 2010; Archer et al. 2014). According to our experiments, it promotes HPG development better than a protein-free diet, similar to some naturally occurring monofloral pollen that poorly promotes development (*Helianthus* sp. and *Sinapis* sp.), but not as good as the pollen mixture or monofloral pollen from *Asparagus* sp. or *Castanea* sp. The latter diets emerged as the most beneficial for honey bee development in our experiments. Our findings therefore underline the importance of the natural availability of pollen with high nutritive value and diverse pollen sources for bee health.

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**Authors' contributions** Eslam Omar, Robert Brodschneider: lab experiments Rudolf Moosbeckhofer: provide some pollen samples Aly A. Abd-Ella, Mohammed M. Khodairy, Karl Crailsheim: supervisors.

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**Influence de différents régimes de pollen sur le développement des glandes hypopharyngiennes et la**

**taille de la poche à venin chez des abeilles élevées en cage (*Apis mellifera*)**

**pollen monofloral / protéine / nutrition / Apidae / poche à venin / glandes hypopharyngiennes**

**Einfluss unterschiedlicher Pollendiäten auf die Entwicklung der Brutfutterdrüsen und der Giftblase in gekäfigten Honigbienen (*Apis mellifera*)**

**Brutfutterdrüsen / Giftblase / monoflorale Pollendiät / Eiweiß / Ernährung**

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