frontiers | Frontiers in Sustainable Food Systems

ORIGINAL RESEARCH
published: 03 May 2022
doi: 10.3389/fsufs.2022.864741

Dream Team for Honey Bee Health: Pollen and Unmanipulated Gut Microbiota Promote Worker Longevity and Body Weight

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Gut microbiota are known to foster pollen digestion in honey bee workers, *Apis mellifera*, thereby enhancing longevity and body weight gain. However, it is currently not known how longevity and body weight gain are effected when gut microbiota are reduced in bees with or without access to pollen. Here, using a hoarding cage set-up with freshly emerged summer workers, we manipulated the gut microbiota of half the bees with the antibiotic tetracycline (ABX), and left the other half untreated on a sucrose solution diet. Afterwards, all bees were assigned to either sucrose diets or sucrose plus *ad libitum* access to pollen ($N = 4$ treatments, $N = 26$ bees/treatment, $N = 10$ replicates/treatment, $N = 1{,}040$ total workers). The data confirm that pollen has a positive effect on longevity and body weight in workers with an unmanipulated gut microbiota. Surprisingly, the antibiotics alone also improved the longevity and body weight of the workers fed a strictly sucrose diet, potentially explained by the reduction of harmful bacteria. However, this positive effect was reversed from an observed antagonistic interaction between pollen and antibiotics, underscoring the innate value of natural microbiota on pollen digestion. In conclusion, a combination of adequate pollen supply and an unmanipulated gut microbiota appears crucial to honey bee worker health, calling for respective efforts to ensure both in managed colonies.

Keywords: ***Apis mellifera*, honey bee, gut microbiota, nutrition, pollen**

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Agroecology and Ecosystem Services, a section of the journal Frontiers in Sustainable Food Systems

Received: *28 January 2022*
Accepted: *20 March 2022*
Published: *03 May 2022*

Citation:
Brown AF, Rodriguez V, Brzoska C, Pfister J, Neumann P and Retschnig G (2022) Dream Team for Honey Bee Health: Pollen and Unmanipulated Gut Microbiota Promote Worker Longevity and Body Weight. Front. Sustain. Food Syst. 6:864741. doi: 10.3389/fsufs.2022.864741

INTRODUCTION

The Western honey bee (*Apis mellifera*) is one of the most important insects for agriculture worldwide due to their pollination services (Garibaldi et al., 2013; Hung et al., 2018). In recent years, increasing numbers of honey bee colony losses throughout the northern hemisphere have been reported (Neumann and Carreck, 2010; Gray et al., 2020), likely as a result of the numerous stressors honey bee colonies are exposed to, including pests, parasites, depreciated food resources, and agrochemicals (e.g., Potts et al., 2010; Barron, 2015). Two specific aspects that have the potential to strengthen honey bees to cope with these challenges are honey bee nutrition (e.g., Dolezal and Toth, 2018; Stanimirović et al., 2019) and a functional worker gut microbiota (e.g., Alberoni et al., 2016; Kwong and Moran, 2016; Bonilla-Rosso and Engel, 2018), both of which have recently received increasing attention. Indeed, adequate nutritional supply plays a key role for *A. mellifera*

health (e.g., Haydak, 1970; Brodschneider and Crailsheim, 2010). Pollen collected by foragers in the environment constitutes the unique source of protein and is essential for the supply of macro- and micro-nutrients (i.e., proteins, lipids, vitamins, and minerals) (e.g., Haydak, 1970; Herbert et al., 1978; Wright et al., 2018) that are indispensable for development, tissue building, and growth of the individuals (Winston, 1991; Brodschneider and Crailsheim, 2010). Accordingly, pollen supply translates into a broad array of beneficial health effects including extended longevity (e.g., Wang et al., 2014) higher body weights (e.g., Retschnig et al., 2021) and immune competence (Alaux et al., 2010) as well as an enhanced ability to cope with pesticides (Barascou et al., 2021). On the other hand, pollen may be a source for pathogens (e.g., *Nosema ceranae*, viruses, Pereira et al., 2019) and has been shown to favor infection levels for certain pathogens, i.e., *Nosema ceranae* (Porrini et al., 2011).

Pollen digestion is complex (e.g., Nicolson et al., 2018), and honey bee gut bacteria have been reported to play a major role in the degradation of the complex cell wall polysaccharides including hemicellulose as well as pectin, which constitutes an obligatory requirement for the utilization of the nutrients contained in pollen (Lee et al., 2015, 2018; Zheng et al., 2019). Numerous reports assign beneficial health effects to the simple and distinctive microbial community of eusocial bees (Martinson et al., 2011; Kwong and Moran, 2016). Current evidence suggests that a functional gut microbiota contributes to a broad array of health aspects such as the efficient digestion of plant-based food, related body weight gain due to the availability of required nutrients, physiology, endocrine signaling, tolerance to parasites and pesticides, behaviors, and host immunity systems (Anderson et al., 2011; Koch and Schmid-Hempel, 2011; Vásquez et al., 2012; Engel and Moran, 2013; Schwarz et al., 2016; Ricigliano et al., 2017; Zheng et al., 2017; Dosch et al., 2021). Indeed, disturbance of the gut microbiota has been linked to diseases and malnutrition (e.g., Lozupone et al., 2012), including reduced protein digestive efficiency in honey bees (du Rand et al., 2020).

A wide range of xenobiotics can affect size, composition and functional properties of the honey bee gut microbiota (Daisley et al., 2020), as it has been demonstrated for pesticides (i.e., glyphosate, Motta et al., 2018), airborne particular matters (i.e., titanium dioxide, Papa et al., 2021) and antibiotics (e.g., tetracycline, Raymann et al., 2017). In certain countries, including the United States, antibiotics, mainly tetracycline derivates, are applied in apiculture as a preventive or control measure against two common larval diseases, American and European Foulbrood, caused by *Paenibacillus larvae* and *Melissococcus plutonius*, respectively (Genersch, 2010; Tian et al., 2012; Daisley et al., 2020). The accumulation and permanent exposure of honey bees to such broad-spectrum antibiotics affect the composition, diversity and functionality of the exposed honey bee's gut microbiota (e.g., Raymann et al., 2017). Indeed, several laboratory experiments have demonstrated that the antibiotic treatment disrupt the beneficial gut bacteria in the treated honey bees. For instance, a significant reduction of the gut bacteria has been reported when workers were treated with tetracycline (450 μg/ml of tetracycline) for 5 days (Raymann et al., 2017) or up to 9 days (Soares et al., 2021), with 500 μg/ml of the same substance for 3 days (Brown et al., 2022) or for the whole duration of the experiment (21 days, Retschnig et al., 2021). More specifically, antibiotic exposure can reduce the abundance of major gut bacteria such as *Lactobacillus* spp., *Bifidobacterium* spp., and *Snodgrassella alvi* (Raymann et al., 2017), and similarly, decrease genetic diversity on a strain level (i.e., *Gilliamella apicola*, Raymann et al., 2018). Accordingly, exposure of workers to antibiotics is a common method to inactivate gut microbiota (Zheng et al., 2018). Nevertheless, organismic studies addressing the mutualistic host-microbiota relationships in relation to honey bee nutrition remain scarce, even though the role of gut microbiota in protein digestion has been clarified (Lee et al., 2015; Zheng et al., 2019; du Rand et al., 2020). Potential parameters to evaluate the importance of the supply with certain nutrients on honey bee health include longevity and body weight (e.g., Retschnig et al., 2021). Nutritional reserves are associated with honey bee worker longevity (Mattila and Otis, 2006) and worker body weight has been identified as a predictive marker for longevity (Retschnig et al., 2021).

The goal of the present study was to investigate the potential effects of pollen supply on longevity and body weight of honey bee workers (*A. mellifera*) in the presence and absence of an unmanipulated gut microbiota. In a fully-crossed laboratory hoarding cage trial, workers were either treated with the antibiotic tetracycline (ABX) to inactivate the gut microbiota or allowed the establishment of a functional microbiota and were then assigned to a diet with or without pollen supply. Due to the known importance of protein supply for body weight and longevity (e.g., Haydak, 1970) and the importance of an unmanipulated gut microbiota (Kwong and Moran, 2016), we would expect an enhanced longevity and higher body weights in bees that had access to pollen and had an unmanipulated gut microbiota.

MATERIALS AND METHODS

Experimental Setup

The experiment was conducted from July 3rd to September 1st 2019 (local summer) at the Institute of Bee Health in Bern, Switzerland. To obtain defined age cohorts of newly emerged workers, capped worker brood frames pre-checked for final stage pupa (dark-eyed, pigmented cuticle pupae) were chosen from four local *A. mellifera* colonies ($N = 4$), brushed clean, and incubated following standardized conditions until adult emergence (48 h, 34.5°C, >60% RH; Williams et al., 2013). After 48 h, newly emerged workers from all colonies were randomly mixed to homogenize the impact of genetics, and placed in 100 ml (100 cm^3) clear polystyrol hoarding cages ($N = 40$, 26 bees/cage, $N =$ total workers 1,040; Williams et al., 2013). According to treatments, half of the workers were treated with the antibiotic (ABX) tetracycline for 72 h to reduce gut microbiota and the other half was fed sucrose solution *ad libitum* (Brown et al., 2022). Subsequently, cages were assigned to a diet with or without pollen (**Table 1**). All workers were maintained in an incubator at 30°C and >60% RH (Williams et al., 2013) until the last worker died.

TABLE 1 | Overview of the fully-crossed experimental set-up.

Treatment group	Tetracycline	Sucrose solution	Pollen paste	*N* total workers
1.ABX, Sucrose	+	+	−	260
2.ABX, Sucrose + Pollen	+	+	+	260
3.Sucrose	−	+	−	260
4.Sucrose + Pollen	−	+	+	260
				***N* = 1,040**

Treatment groups, applied treatments in each group, and the number of experimental adult Apis mellifera workers are shown. The Bold Value N = 1040 is to emphasize the total N value for the entire study. There were 4 groups, each with 260 individuals, meaning 4 × 260 = 1040 total.

TABLE 2 | Mean age (days) of each treatment group, ABX Sucrose, ABX Sucrose + Pollen, Sucrose, Sucrose + Pollen ($N = 4$).

Treatment	Mean age
ABX Sucrose	22.89
ABX Sucrose + Pollen	17.24
Sucrose	15.05
Sucrose + Pollen	19.9

Each treatment was replicated 10 times (N = 10), with 26 bees/cage (N = 40, 26 bees/cage, and N = total workers 1,040).

Antibiotic and Dietary Treatments

The antibiotic, tetracycline hydrochloride (©Sigma-Aldrich), was identified in a pre-trial screening to be the best suitable to inactivate the gut microbiota (i.e., significant reduction of colony forming units in the plated guts of antibiotic-treated vs. control workers) without significantly affecting longevity. Subsequently, a 50% (w/v) tetracycline-sucrose solution (500 µg/mL tetracycline hydrochloride) was prepared and fed *ad libitum* to the antibiotic groups (groups 1 and 2) for a time period of 72-h. Sucrose solution (50% w/v), made with sterilized tap water and stored at 4°C until use, was prepared freshly on a weekly basis and fed to all experimental workers *ad libitum*. Two treatment groups (2 and 4) were additionally fed *ad libitum* non-sterilized corbicula poly-floral pollen from honey bees harvest from an entire season and mixed (Swiss Pollen, Bienen Roth). The pollen was stored frozen at −24°C, and prior to feeding, was thawed, ground, and supplied to workers *via* 1.5 mL microcentrifuge tubes with a clipped tip. Syringes and pollen tubes were changed with fresh sterilized ones on a bi-weekly basis.

Parameters

Longevity

Worker mortality was recorded daily until the last worker has died. During the cage checks, dead workers were carefully removed and stored at −80°C.

Body Weight

Individual fresh body weights of three live workers per cage were measured on days 7 and 14 using a Mettler AT 400 scale, precise to 10^{-4} g ($N = 30$ per treatment and day, total $N = 240$).

Food Consumption

The workers from the groups 2 and 4 had permanent *ad libitum* access to pollen. Real pollen consumption was not quantifiable because the workers removed parts of the pollen paste from the tube and distributed it on the floor of the cage. To estimate consumption of sucrose solution per worker per day, the syringes were weighed daily on a Mettler AT 400 scale, and the differences in weight were divided by the number of live workers present in the cage at that time. To account for mechanic loss (i.e., evaporation from the incubator, dripping from syringes), control syringes were filled with sucrose solution and put in cages without workers, incubated, and measured daily, and the average evaporation rates were used to adjust sucrose-solution consumption across all treatments (OECD, 2017).

Pathogen Infection With *Nosema* spp.

In a subsample of individual experimental workers (1 worker per cage, 9–10 workers/treatment, N total = 39, workers that were collected on Day 14 for body weight measurements) *Nosema* spores were quantified visually using a standardized method (Cantwell, 1970). To do so, each worker was homogenized in a 2 mL Eppendorf tube using a bead mill homogeniser (MM300 Retsch), one glass bead and 1 mL of distilled water. Each homogenate was further diluted to 2 mL prior to spore quantification, which was done according to Cantwell (1970) using a haemocytometer (Thoma, L.O. Labor Optik) and a light microscope (Laborlux K, Leitz Wetzlar, Germany). Similarly, the pollen fed to the workers in the treatment groups 2 and 4 was checked for *Nosema* spp. spores using the same method.

Statistical Analyses

Statistical analyses were performed using the program, R, Version 4.1.2 (R Core Team, 2021). For the survival analysis, the packages "survival" (Therneau and Grambsch, 2000; Therneau, 2021) and "surminer" (Kassambara et al., 2021) were used for calculating and plotting Kaplan-Meier survival curves. The Surfdiff function was used to calculate survival curves and log rank testing (rho = 0) as well as to perform a chi squared test. The pairwise_survdiff function was used for multiple comparisons from the survival analysis between all treatment groups, and the resulting p-values were adjusted for multiple comparisons using a Bonferroni method (Bonferroni, 1936). Additionally, a simple linear regression model (lm) for the longevity data was performed, using the mean age per cage as a response variable, with the explanatory variables (i.e., treatments) expressed again as indicator variables (antibiotics = yes/no, pollen = yes/no) to estimate how the treatments, in days, influence life expectancy outcomes.

For the body weight analysis, raw untransformed data was used in a generalized linear mixed effect models (glmer), using the R package "lme4" (Bates et al., 2015) and fitted with a Gamma distribution, while defining "cage" and the time interval "day" as random factors. The model residuals were plotted with qqPlots from the R package "car" (Fox and Weisberg, 2019) to verify model assumptions (**Supplementary Figure 1**). The fitted (predicted) values were extracted from the model and used in boxplots. *Post-hoc* multiple comparison testing was done

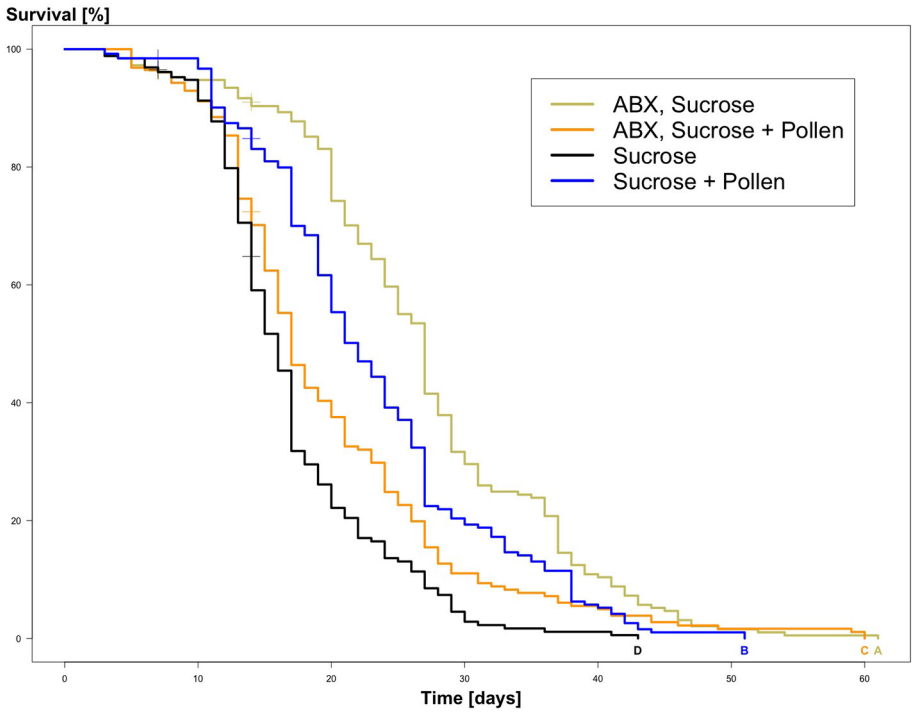

FIGURE 1 | Kaplan Meier Survival curves of experimental adult workers, *Apis mellifera*, from the four treatment groups: ABX + Sucrose, ABX + Sucrose + Pollen, Sucrose, Sucrose + Pollen ($N = 260$ workers per group). Significant differences between the survival of the experimental workers are indicated by compact letter display based on log rank tests and Bonferroni *p*-adjusted values ($Ps < 0.05$).

TABLE 3 | Bonferroni corrected pairwise comparisons of worker longevity using Log-Rank test.

Treatment group	ABX, Sucrose	ABX, Sucrose + Pollen	Sucrose
ABX, Sucrose			
ABX, Sucrose + Pollen	$3.76 \times 10^{-10***}$		
Sucrose	$3.95 \times 10^{-29***}$	0.0028**	
Sucrose + Pollen	$4.95 \times 10^{-4***}$	0.0103*	$1.62 \times 10^{-13***}$

Significant differences are marked in bold and with stars: $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

TABLE 4 | Simple linear regression model of average cage lifespan (in days) of adult *Apis mellifera* workers dependent on pollen and antibiotics summary output.

	Coefficient	Standard error	*t*-value	*p*-value
Intercept	15.05	1.129	13.329	**<0.0001*** **
Pollen	4.85	1.597	3.037	**0.004** **
Antibiotic	7.84	1.597	4.91	**<0.0001*** **
Antibiotic × pollen	−10.502	2.258	−4.65	**<0.0001*** **

Significant differences are marked in bold and with stars: $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

using the "multcomp" package, selecting "Tukey" comparison of means, with "Holm" correction (Holm, 1979). An additional linear mixed effect models (lmer) was carried out on the body weight data, with the explanatory variables (i.e., treatments) expressed as indicator variables (antibiotics = yes/no, pollen = yes/no), with "cage" and "day" defined as random factors, to estimate the effects of the explanatory variables as well as to test for any significant interactions. Sucrose consumption between the treatments was compared using a pairwise *t*-test with Bonferroni correction.

RESULTS

Longevity

The experimental workers lived between 3 and 61 days (global mean: 18.77 days, SD = 9.9; **Table 2**). From the Kaplan Meier survival analysis, all four groups showed significant differences in longevity (Kaplan Meier, log rank test, all $Ps < 0.05$, letters A, B, C, and D, **Figure 1**; **Table 3**). Complementing the survival analysis, a simple linear model regression analysis was ran on the longevity data, and the model summary revealed that the regression analysis was significant (Multiple R-squared = 0.45, F-statistic = 9.003, 3 and 36 degrees of freedom, $P < 0.0001$). In addition, it revealed that workers supplied sucrose combined with antibiotics are estimated to have the longest average lifespan (lm, coefficient 7.84, $P < 0.0001$; **Table 4**) followed by workers without antibiotic treatment supplied *ad libitum* access to pollen (lm, coefficient 4.85, $P < 0.004$; **Table 4**), and finally, in sharp contrast, there was a significant negative interaction between a disrupted gut microbiota and *ad libitum* access to pollen, leading to a significant decrease in average lifespan (lm, coefficient −10.502, $P < 0.0001$; **Table 4**).

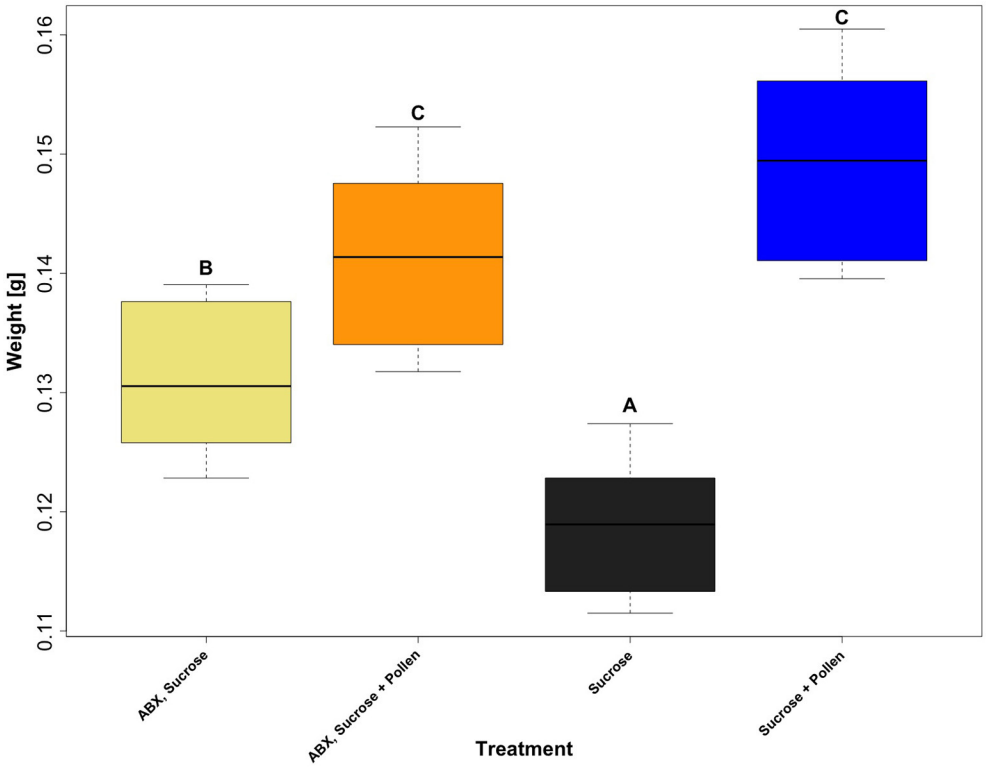

FIGURE 2 | Boxplots of the predicted body weight values of adult workers, *Apis mellifera*, from four treatment groups: ABX + Sucrose, ABX + Sucrose + Pollen, Sucrose, and Sucrose + Pollen, extracted from a generalized linear mixed effect model (glmer) which was calculated using the weight of the workers, dependent on diet (treatment), and "cage" and "day" as random variables ($N = 4$ treatments, $N = 60$ workers per treatment, and total $N = 240$ total workers). *Post-hoc* testing from the glmer, comparing group means (Tukey), with Holm correction was used to determine statistical significance. Compact letter display shows indicated groups who vary statistically ($P < 0.05$).

Body Weight

The fresh body weight of all experimental workers ranged from 0.0776 and 0.1886 g ($N = 60$ workers per treatment, total $N = 240$ total workers, mean = 0.13537 g, SD = 0.02235). A generalized linear mixed effect model, with "Gamma" as a defined distribution, proved to be the best fit for the model residuals (**Supplementary Figure 1**). *Post-hoc* testing on the fitted (i.e., predicted) values extracted from the glmer (**Figure 2**) revealed three distinct groups (glmer, Tukey multiple mean comparison, Holm adjusted *P*-values, all Ps < 0.05). Highest weights were observed in the two groups given *ad libitum* access to pollen (**Figure 2**, Letter C), and the lowest body weight was observed in the group with an unmanipulated gut microbiota and sucrose diet (**Figure 2**, Letter A). Pollen supply was shown to be the main driver for higher body weights in the experimental workers (lmer, coefficient 0.0307, $P < 0.0001$; **Table 5**). The antibiotic treatment also had a significantly positive impact when no pollen was supplied (lmer, coefficient 0.0126, $P < 0.0001$; **Table 5**) and a negative interaction in workers that had access to pollen (lmer, coefficient −0.0204, $P < 0.0001$; **Table 5**).

Food Consumption

The sucrose consumption values were pooled for the whole study duration ($N = 4$ treatments, total $N = 233$–393 observations/treatment, $N = 1{,}281$ total observations) and showed no significant differences between treatments (Pairwise *T*-test, Bonferroni correction, all *P*s > 0.05).

TABLE 5 | Summary output from a linear mixed effect model of weight (in grams) of adult *Apis mellifera* workers dependent on pollen and antibiotics, with cage and day defined as random factors.

	Coefficient	Standard error	*t*-value	*p*-value
Intercept	0.118	0.0076	15.544	**0.028***
Pollen	0.0307	0.0032	9.449	**<0.0001*****
Antibiotic	0.0126	0.0032	3.885	**<0.0001*****
Antibiotic × Pollen	−0.0204	0.0045	−4.435	**<0.0001*****

*Significant differences are marked in bold and with stars: *P < 0.05, **P < 0.01, and ***P < 0.001.*

Nosema spp. Infections

No *Nosema* spp. spores were detected in any of the experimental workers and in the supplied pollen.

DISCUSSION

Our data show that pollen supply had a positive effect on longevity in workers with an unmanipulated gut microbiota. In

sharp contrast, an adverse effect of pollen supply was observed in antibiotic-treated workers. Surprisingly, highest longevity was reported in antibiotic-treated workers supplied sucrose only. Pollen had a strong positive impact on worker body weight, and antibiotic treatment also showed a positive effect on body weight in workers without pollen supply. In contrast, the combination of antibiotic treatment and pollen supply showed a negative effect on body weight.

The observed mean longevity in the experimental summer bees was 18.77 days with a maximum lifespan of 61 days, and is in line with previous cage studies (e.g., Di Pasquale et al., 2016; Bernklau et al., 2019; Straub et al., 2019) or even exceeding observed longevities in other studies (e.g. Huang et al., 2014). The positive effect of pollen in workers without antibiotic treatment corresponds to the well-established beneficial impact of pollen supply on worker longevity (e.g., Schmidt et al., 1987; Malone et al., 1999; Di Pasquale et al., 2013; Tritschler et al., 2017; Retschnig et al., 2021). Pollen supplies the workers with the entire range of proteins, lipids and micronutrients (e.g., Haydak, 1970; Herbert et al., 1978) that are key for their development and growth (Winston, 1991; Brodschneider and Crailsheim, 2010). Additionally, pollen supply has been reported to be of importance for numerous further health parameters such as immune function (Alaux et al., 2010) or detoxification (Schmehl et al., 2014; Berenbaum and Johnson, 2015). However, the positive effect of pollen supply turned to the opposite in workers that were previously treated with antibiotics, which may be explained by the complex digestion of pollen (Nicolson et al., 2018) and the functional role of the gut microbiota in this digestion process (Vásquez and Olofsson, 2009; Lee et al., 2015). Pollen digestion is an arduous mechanical and enzymatic task for bees, given its multi-layered cell wall surrounding the nutrient dense center (Keller et al., 2005), and is therefore typically aided by symbiont bee-specific *Lactobacillus* microbiota (Vásquez and Olofsson, 2009). Further bacteria genera of the honey bee gut microbiota, *Bifidobacterium* and *Gilliamella*, have been linked to polysaccharide digestion and are the principal degraders of hemicellulose and pectin (Zheng et al., 2019). Indeed, a recent study showed that antibiotic treatment with oxytetracycline impaired protein digestion in honey bees (du Rand et al., 2020). If the worker gut microbiota loses its function following the exposure to antibiotics, the pollen may cause negative effects, potentially due to indigestible pollen components remaining in the gut or higher energy requirements for pollen digestion in absence of supportive gut bacteria (Klungness and Peng, 1984). Alternatively, pollen may be a source of pathogens or harming substances such as the pathogen *Nosema ceranae* (Higes et al., 2008), viruses (Singh et al., 2010) or pesticides (Chauzat et al., 2006) that might be harmful for workers, especially when they are weakened by a disrupted gut microbiota (Raymann et al., 2017). As no *Nosema* spp. spores were detected in the analyzed subsample of workers and the supplied pollen, a potential effect of this specific pathogen can be ruled out in the present study. The present study did not use sterilized pollen, leaving the door open for unknown opportunistic bacteria from the pollen to successfully populate ABX-treated workers. Therefore, food-borne disease resulting from pollen consumption and its potential to harm the health of honey bees with an unmanipulated vs. a compromised gut microbiota may be an interesting aspect to address in future research.

Antibiotic treatment has repeatedly been reported to affect honey bee worker longevity (Raymann et al., 2017; Li et al., 2019; Retschnig et al., 2021). In a recent study, Li et al. (2019) reported a significant decrease in the lifespan of workers when bees were treated with antibiotics. Further, they showed that pollen supply could partially counteract the negative effect of the antibiotic treatment, which is in line with previous reports (Retschnig et al., 2021), but differs considerably from the here obtained data. This dissenting outcome may be due to a different applied antibiotic substance (tetracycline vs. penicillin–streptomycin), dosage (Marceau et al., 2021), or the duration of antibiotic treatment (permanent vs. only 72 h). Further, the study duration may have an impact on the outcome. While Li et al. (2019) analyzed survival data for a 15-day time interval, in the present study, worker mortality was recorded until the last worker has died (61 days). This clearly shows that findings can vary considerably depending on applied methods. Finally, by removing the bees from their frames 48 h post-emergence, the experimental design (by default) disrupted the natural timeline and transmission pathways of microbiota acquisition from real-hive scenarios (Powell et al., 2014) before ABX treatment. Indeed, this entails an implicit issue when considering the non-ABX treated group (sucrose), indicating that they too may have a slightly "modified" microbiota. Nonetheless, we argue this is a necessary step to take in order to fully manipulate the nutritional diets of the bees, given that early-stage nutrition is critically tied to subsequent lifespan of the worker bees (Brodschneider and Crailsheim, 2010), the exact variables we wished to manipulate here.

Surprisingly and contradicting a large body of existing evidence where no negative (du Rand et al., 2020) or significant negative effects on survival (e.g., Raymann et al., 2017; Li et al., 2019; Retschnig et al., 2021) were detected, antibiotic treatment showed a positive effect on worker longevity when workers were fed with sucrose only. This may be explained by an antibiotic-induced reduced potential of pathogens in these workers (e.g., bacteria, viruses; Dutta and Basu, 2011), however, further investigations of possible mechanisms are needed. Additionally, the tetracycline screening pre-trial process was repeated in two separate cage trial experiments to confirm our results with a set 95% confidence level, yet here the ABX Sucrose treatment performed better in both weight and longevity to its direct counterpart "Sucrose only" treatment. Repeatability of experiments is paramount in science, and it is possible we achieved the 1/20th (5%) probability of obtaining a statistically different result than our ABX screening pre-trials. Further cage trials testing the present tetracycline dosing would clarify this important point. Finally, the highly artificial conditions associated with cage studies need to be taken into account for data interpretation (e.g., Retschnig et al., 2015); effects of treatments may be different under field colony conditions, where workers are involved with various in-hive and foraging activities (Winston, 1991).

Body weight is regularly measured as health parameter (e.g., Pettis et al., 2012; Retschnig et al., 2014; Straub et al., 2019) and has previously been identified as marker for longevity (Retschnig et al., 2021). The body weight results of this study indicate a significant positive signal from *ad libitum* access to pollen, likely leading to improved growth and body tissue development, which is supported by dated to contemporary publications (Haydak, 1937, 1970; Roulston and Cane, 2000; Tritschler et al., 2017; Retschnig et al., 2021). Previous reports have also shown that pollen can mitigate negative antibiotic-induced effects by increasing observed worker weights (Li et al., 2019), cohering with what was observed in this study. In contrast to pollen, evidence from previously mentioned studies underscore negative impacts of antibiotics on worker body weight in larvae (Duan et al., 2021) and adults (Retschnig et al., 2021) and demonstrate a positive effect of gut bacteria on weight gain in young adult workers (Zheng et al., 2017). Contrary to expectations, yet in line with the longevity data, the tetracycline treatment had a positive impact on worker body weight compared to their non-treated counterpart in the groups that were supplied with sucrose only. As sucrose consumption was consistent between treatment groups, the higher body weight cannot be attributed to altered sucrose intake habits and must be the result of another underlying mechanism. Tetracyclines, in general, are effective broad-spectrum antibiotics (Duggar, 1948), additionally acting as antiviral agents (Dutta and Basu, 2011; Mosquera-Sulbaran and Hernández-Fonseca, 2021). In case the experimental workers of this study harbored unnoticed pathogens and/or viruses, the latter may have been affected by the antibiotics, thereby leading to a beneficial effect on the workers. Investigating if tetracycline also has antiviral effects to common *A. mellifera* viruses would be of great interest, and if so, if this could further explain the current findings of this paper. Finally, in several areas around the world, tetracyclines are commonplace in agriculture, in part for the known ability to assist in increasing the weight of livestock (Cox, 2016). Although the underlying mechanisms are not fully understood, it is hypothesized that the ABX select for bacteria better at nutrient extraction, thus providing more calories to their host (Cox, 2016). Employing modern micro- and molecular biology techniques to see if this is the case in ABX treated bees would help shed light on accepting or rejecting notion of ABX positively selecting specialized microbes better at deriving nutrients, resulting in weight gain to their host.

In conclusion, this study adds to the evidence of beneficial effects of pollen supply on health parameters in honey bee workers in the presence of a functional gut microbiota. Beyond that, it also revealed a potential of harm of pollen access in antibiotic-treated workers with a compromised gut microbiota. This aspect and underlying mechanisms require further investigation and should be considered when honey bees and honey bee colonies are treated (preventively) with antibiotics against pathogens.

DATA AVAILABILITY STATEMENT

The datasets for this study can be found in the Dryad data repository: https://doi.org/10.5061/dryad.wstqjq2p2.

AUTHOR CONTRIBUTIONS

AB, VR, PN, and GR designed the experiment. AB, VR, CB, and JP conducted the experiment. AB, PN, and GR analyzed the data and wrote the manuscript. AB, VR, CB, JP, PN, and GR revised and approved the final manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

The financial support was granted by Ricola Foundation *Nature and Culture* (GR and PN) and the Vinetum Foundation (PN).

ACKNOWLEDGMENTS

The authors wish to thank the Ricola Foundation *Nature and Culture* (GR and PN) and the Vinetum Foundation (PN) for financial support.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2022.864741/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.