



Feeding with plant powders increases longevity and body weight of Western honeybee workers (*Apis mellifera*)

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Abstract – Beekeepers routinely substitute honey from managed Western honeybee, *Apis mellifera*, colonies with sugar water post-harvest, potentially leading to malnutrition. Although nutritional supplements have been created, a general consensus on proper colony nutrition for beekeeping has yet to be reached. Thus, finding easily obtainable fortified *A. mellifera* food alternatives is still of interest. Here, we test plant powder-enriched food supplements since evidence suggests plant extracts can enhance dry body weight and longevity of workers. Freshly emerged workers were kept in hoarding cages ($N=69$ days) and fed either with 50% (w/v) sucrose solution alone or additionally with one of 12 powders: *Laurus nobilis*, *Quercus* spp., *Curcuma longa*, *Hypericum* spp., *Spirulina platensis*, *Calendula officinalis*, *Chlorella vulgaris*, *Melissa officinalis*, *Moringa oleifera*, *Rosa canina*, *Trigonella foenum-graecum*, and *Urtica dioica* ($N=2028$ workers total). The dry body weight was significantly increased in *Quercus* spp., *Hypericum* spp., *Spirulina platensis*, *M. officinalis*, *M. oleifera*, and *T. foenum-graecum* treatments. Further, the longevity was significantly increased in *Quercus* spp., *C. longa*, *C. officinalis*, *C. vulgaris*, *M. officinalis*, *R. canina*, *T. foenum-graecum*, and *U. dioica* treatments. Given that plant extracts can enhance *A. mellifera* health (i.e., phenolics, flavonoids), plant powders possibly provide additional macro- (i.e., proteins, lipids, peptides) and micronutrients (minerals and vitamins) thereby enhancing nutrient availability. Further investigations into the mechanisms underlying these effects and field studies are recommended to validate these findings in real-hive scenarios.

Apis mellifera / nutrition / plant powder / bee health

1. INTRODUCTION

In light of ongoing global losses of managed Western honeybee, *Apis mellifera*, colonies (Neumann and Carreck 2010; Gray et al. 2020), finding viable and safe solutions for management is crucial. Among the identified key factors causally linked to *A. mellifera* colony failure (e.g., habitat loss (Naug 2009), disease (Mayack and Naug 2009; Neumann et al. 2012), climate change (Neumann and Straub 2023)), proper nutrition is

of foremost significance (van Engelsdorp et al. 2010). Indeed, malnutrition is linked to increased pathogen susceptibility (Alaux et al. 2010) and decreased resistance to xenobiotics (Wahl and Ulm 1983) and is an enduring reason, among many, that leads beekeepers to supplement foods to boost colony performance (Retschnig et al. 2021). Thus, finding low-risk, easily accessible fortified food alternatives in times of need seems reasonable to mitigate colony losses.

Indisputably, plant pollen benefits bee health (Haydak 1970; Brown et al. 2022b), yet pollen trade/supplementation can pose health-related-drawback, either from pesticide residues (Wahl and Ulm 1983) and/or disease (e.g., *Nosema* spp., Higes et al. 2008). Furthermore, due to agriculture-related

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landscape use, the floral foraging diversity of *A. mellifera* has significantly decreased (Naug 2009), resulting in heightened possibilities of protein deficiencies. Coincidentally, extensive research, spanning decades, has been devoted to understanding the nutritional needs of honeybees, focusing on macronutrients (Pudasaini et al. 2020) and, more recently, micronutrients (Jovanovic et al. 2021; Brown et al. 2022a), and products containing such nutrients are readily available to beekeepers. However, beyond macro- and micronutrients, researchers have also investigated fortifying *A. mellifera* supplements with plant secondary metabolites (Hýbl et al. 2021). Indeed, consuming phenolic and flavonoid compounds, both present in honey and pollen (Cianciosi et al. 2018; Hýbl et al. 2021), results in changes in detoxification gene expression (e.g., cytochrome P450 enzymes, Mao et al. 2009) critical for pesticide and pathogen immune defense (Mao et al. 2013), on top of increasing adult worker bee longevity (Hýbl et al. 2021). Furthermore, presence/absence assays of *p*-coumaric acid (a widespread phytochemical in honey and bee bread) has been causally linked as a key factor for determining the fate of female larval post-eclosion development (i.e., queen vs. sterile worker, Mao et al. 2015). Delving deeper, ample evidence, from contemporary to present, can be found of trials testing plant extracts on *A. mellifera* health parameters (Barker 1977; Pohorecka 2004; Roussel et al. 2015; Potrich et al. 2020), but to date, our literature review has not revealed any experiment that has yet simply tried to add whole plant powders to *A. mellifera* food supplements. If possible, this would indeed create an easily viable way of creating enriched nutrient supplements that may enhance bee health. Moreover, beyond aforementioned secondary metabolites from plant extracts, whole plant powders would arguably offer additional nutrients, such as a combination of macro- (i.e., proteins, lipids) and micronutrients (i.e., vitamins, minerals) generally recognized as beneficial to *A. mellifera* health (Pudasaini et al. 2020).

Myriads of *A. mellifera* studies have used longevity (Amdam and Omholt 2002; Zhang et al. 2019; Hýbl et al. 2021) and body weight (Maurizio 1954; Oskay 2021; Brown et al. 2022a) as validated indicators of health in *A. mellifera*

studies. To date, published literature persists to show that the nutritional status of bee colonies significantly correlates to changes in body mass at different developmental stages (e.g., larval body size (Tasei and Aupinel 2008)), adult fat body content (Alaux et al. 2010), and malnutrition (i.e., protein deficiencies) in nurse bees translates to decreased dry body weight and lifespan predictions (Brodschneider et al. 2022). Given that supplementation is common practice in beekeeping and that nutrition directly influences *A. mellifera* body mass and lifespan, a general consensus on proper nutrition for commercial beekeeping is in high demand (Paray et al. 2021). If properly established, this would indeed encourage managed *A. mellifera* colony success.

The goal of the present study was to investigate, in a hoarding cage trial, if 12 different plant powders (Table 1) would be readily consumed by adult *A. mellifera* workers and if so, whether these could influence body weight and longevity. The chosen plants studied here have either already been used in *A. mellifera* research (*Quercus* spp., *Laurus nobilis*, *Urtica dioica*, *Hypericum* spp., Supplement Table S.1) or as medicinal plants in mammalian research (*Calendula officinalis*, *Melissa officinalis*, *Curcuma longa*, *Rosa canina*, *Moringa oleifera*, *Chlorella vulgaris*, *Spirulina platensis*, and *Trigonella foenum-graecum*, Supplement Table S.1). Given the aforementioned roles plant secondary metabolites play in *A. mellifera* health, we would anticipate improvements in the measured parameters.

2. MATERIAL AND METHODS

2.1. Experimental set-up

Hoarding cage experiments were conducted at the Institute of Bee Health (Bern, Switzerland) from April 28th to July 6th ($N=69$ days) using standardized methods (Williams et al. 2013). Briefly, two worker brood frames, containing pre-emergent, late-stage pupae (i.e., dark-eyed, melanized) from three local unrelated queen-right *A. mellifera* colonies ($N=3$ colonies, $N=2$ frames/colony, $N=6$ frames total), were chosen

Table I Dietary treatment groups ($N=13$) administered to caged *Apis mellifera* workers. Concentrations (mg/mL) are shown, as well as number of replicates per treatment ($N=6$), bees/cage ($N=26$), and total number of experimental workers in the trial ($N=156/\text{treatment}$, $N=2028$ total)

Treatments	Concentration (mg/mL)	Replicates	Bees/cage	N workers
Sucrose (negative control)	Blank	6	26	156
<i>Calendula officinalis</i>	0.5	6	26	156
<i>Melissa officinalis</i>	0.3	6	26	156
<i>Quercus</i> spp.	0.3	6	26	156
<i>Laurus nobilis</i>	0.3	6	26	156
<i>Curcuma longa</i>	0.01	6	26	156
<i>Urtica dioica</i>	0.08	6	26	156
<i>Rosa canina</i>	0.3	6	26	156
<i>Chlorella vulgaris</i>	0.3	6	26	156
<i>Hypericum</i> spp.	0.04	6	26	156
<i>Spirulina platensis</i>	0.4	6	26	156
<i>Trigonella foenum-graecum</i>	0.08	6	26	156
<i>Moringa oleifera</i>	0.06	6	26	156
Total				2028

and incubated until adult emergence (48 h, 34.5 °C, > 60% relative humidity (RH); Williams et al. 2013). Freshly emerged workers were mixed in a single container (minimizing genetic influence) and randomly allocated to 100 cm³ polystyrol hoarding cages ($N=26$ workers/cage, $N=6$ replicates/treatment, $N=78$ total cages, $N=2028$, Williams et al. 2013). Post-cage assignment, all workers received their assigned dietary treatment ($N=13$ treatments, Table I) and were incubated at 30.4 °C and 60% RH (Williams et al. 2013).

2.2. Dietary treatments

All workers were fed sucrose solution (50% w/v) made with sterilized tap water and table sugar (i.e., sucrose), of which was either mixed with plant powders to desired concentrations or left blank (i.e., negative control, Table I). All treatments were provided ad libitum through vertically placed 5-mL syringes on each hoarding cage (Williams et al. 2013) and were freshly prepared and replaced on a bi-weekly basis. Given that no previous data exist, concentrations for the plant powders were

chosen based on recommended daily dosages for humans (~65 kg adult, <http://www.worlddata.info/>) and adapted to bees by adjusting for the average weight of an adult worker (~120 mg; Hrassnigg and Crailsheim 2005) and lastly by correcting for their mean daily sucrose consumption (~30 mg, Brown et al. 2022a). No data for humans were found for *Melissa officinalis* and *Quercus* spp., and as such, the concentration 0.3 mg/mL was arbitrarily chosen. Lastly, each individual plant powder was commercially purchased (Supplement Table S.1).

2.3. Sucrose consumption

Syringes were weighed once every 24 h on a Mettler Toledo ME103TE/00 scale (precision 10⁻³ g), and the resulting differences per day were divided by the number of workers present at the time in each cage respectively, yielding an average sucrose consumption/cage/day. All values were adjusted for evaporation and/or mechanical loss following standardized guidelines (OECD 2017).

2.4. Dry body weight

Here, we opted for dry weight given that it is a better approximation of true biomass (EFSA 2016; Brown et al. 2022a). On days 7 and 14, three randomly selected live workers were removed from each cage and weighed on a Mettler Toledo ME103TE/00 scale (precision 10^{-3} g) and subsequently frozen at -24°C . At the end of the cage trial, all workers selected for their weight were removed from the freezer and placed in a convection oven at 65°C for 96 h and reweighed on the same scale to obtain their dry weights (Brown et al. 2022a).

2.5. Mortality

Each treatment cage was monitored on a daily basis until the last remaining experimental worker bee died. All dead workers were removed and placed in labeled 1.5-mL Eppendorf tubes and frozen at -24°C .

3. STATISTICAL ANALYSES

Statistical analyses were performed using R version 4.3.2 (Team 2023), and for all linear models, data (i.e., error) distribution was visually confirmed with quantile–quantile (qq) plots and verified with Jarque–Bera normality tests (Jarque and Bera 1980). For the sucrose consumption, a least-squares regression model (lm) with raw (untransformed) data was used with both treatment group and time (modeled as a third-degree polynomial) as independent variables. Post hoc pairwise testing compared to the negative control “sucrose,” based on the linear model output, was done with the “multcomp” library (Hothorn et al. 2008) using the glth() command with the “Dunnnett” false discovery rate (FDR) option (Dunnnett 1955). For the dry body weight analysis, an lm() was also done with time as well as treatment as independent variables, and inspection of the data residuals in conjunction with a Jarque–Bera normality test with the variable

“treatment” suggested non-normality. Thus, a Kruskal–Wallis ANOVA, followed by pairwise–Wilcoxon tests, was done. All comparisons made with the negative control “sucrose” were extracted and adjusted for with the Benjamin–Hochberg FDR correction (Benjamini and Hochberg 1995) with the command p.adjust(). Lastly, for the survival analysis, the packages “survival” (Therneau 2021; Therneau and Grambsch 2000) and “surminer” (Kassambara et al. 2021) were used for calculating and plotting Kaplan–Meier survival curves. The survdiff function was used to calculate survival curves and log rank testing ($\rho=0$) as well as to perform a chi-square test. The pairwise survdiff function was used for multiple comparisons from the survival analysis between all treatment groups, and the resulting p -values compared to the control group “sucrose” were extracted and adjusted for with the Benjamin–Hochberg FDR correction (Benjamini and Hochberg 1995) with the command p.adjust().

4. RESULTS

4.1. Sucrose consumption

The sucrose consumption/day/treatment/cage was measured for 69 days (Figure 1) and ranged from 1.3 to 62.36 mg. All treatments displayed similar pattern of consumption through time (third-degree polynomial, Table II), and pairwise comparison to the control based on the linear model output revealed that no groups were statistically different to the negative control “sucrose” (multiple comparisons of means: Dunnnett contrasts, p -values > 0.05) with the exception of *C. officinalis* (multiple comparisons of means: Dunnnett contrasts, p -values = 0.05).

4.2. Body weight

Dry body weight ($N=468$ workers) was measured on days 7 and 14 and ranged from 16 to 59 mg. The first linear model revealed that dry weights

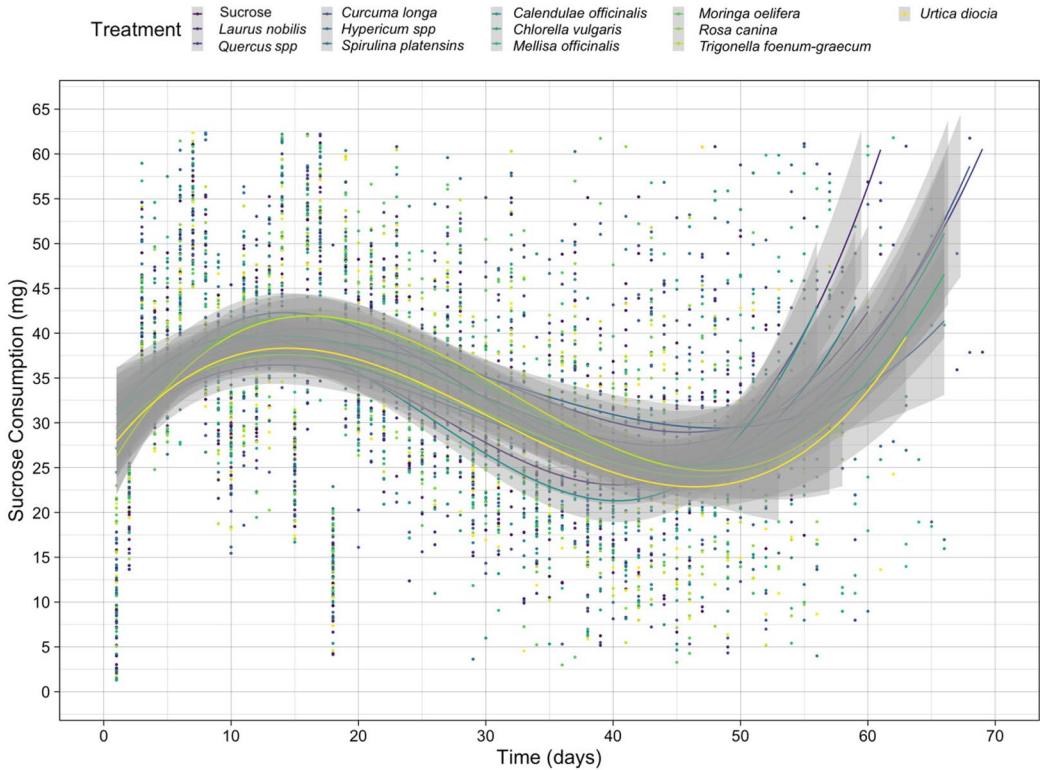


Figure 1. Sucrose consumption of adult *Apis mellifera* workers over time from 13 treatment groups: sucrose, *Laurus nobilis*, *Quercus* spp., *Curcuma longa*, *Hypericum* spp., *Spirulina platensis*, *Calendula officinalis*, *Chlorella vulgaris*, *Melissa officinalis*, *Moringa oleifera*, *Rosa canina*, *Trigonella foenum-graecum*, and *Urtica dioica* ($N=13$ treatments). Each point represents a data point taken at a specific time, with their corresponding least-squared lines and 95% confidence intervals shaded around the lines.

did not significantly change over time (lm, coefficient = 0.00008, t -value = 1.039, $p = 0.299$, AOV, $F = 1.039$, p -value = 0.2995), resulting in unchanged (i.e., flat) trends through time, justifying pooling the data for subsequent analysis (Figure 2). Significant variation between treatments was found (Kruskal–Wallis ANOVA, p -value < 0.001). The treatments *Quercus* spp., *Hypericum* spp., *Spirulina platensis*, *Melissa officinalis*, *Moringa oleifera*, and *Trigonella foenum-graecum* all resulted in significant increases in weight when compared to the “sucrose” negative control (pairwise Wilcoxon test, Benjamin-Hochberg FDR correction, p -values < 0.05, Table III).

5. MORTALITY

Mortality rate data of the 13 treatment groups were taken every day until the last worker has died ($N = 1$ –69 days, Table IV). Figure 3A shows the survival (%) of all treatment groups in a single plot, while Figure 3B shows all groups that were significantly different from the control “sucrose”: *Quercus* spp., *Curcuma longa*, *Calendula officinalis*, *Chlorella vulgaris*, *Melissa officinalis*, *Rosa canina*, *Trigonella foenum-graecum*, and *Urtica dioica* treatments (Kaplan Meier, log rank test, Benjamin-Hochberg FDR correction, all p 's < 0.05, Table IV).

Table II Linear model summary of sucrose consumption (mg) of adult *Apis mellifera* workers from 13 treatment groups: sucrose, *Laurus nobilis*, *Quercus* spp., *Curcuma longa*, *Hypericum* spp., *Spirulina platensis*, *Calendula officinalis*, *Chlorella vulgaris*, *Melissa officinalis*, *Moringa oleifera*, *Rosa canina*, *Trigonella foenum-graecum*, and *Urtica dioica*. Model estimates, standard error, *t*-values, and *p*-values are displayed

Treatments	Estimate	Std. error	<i>t</i> -value	<i>p</i> -value
(Intercept)	27.9800	0.7126	39.2670	< 0.001
<i>Laurus nobilis</i>	−0.7319	0.6657	−1.0990	0.27166
<i>Quercus</i> spp.	−0.3447	0.6663	−0.5170	0.60497
<i>Curcuma longa</i>	−0.3283	0.6640	−0.4940	0.62106
<i>Hypericum</i> spp.	−0.9200	0.6654	−1.3830	0.16687
<i>Spirulina platensis</i>	−1.7400	0.6586	−2.6420	0.00829
<i>Calendula officinalis</i>	1.0030	0.6691	1.4990	0.13401
<i>Chlorella vulgaris</i>	−0.9669	0.6609	−1.4630	0.14354
<i>Melissa officinalis</i>	−0.7652	0.6557	−1.1670	0.24333
<i>Moringa oleifera</i>	−3.6500	0.6621	−5.5120	< 0.001
<i>Rosa canina</i>	1.7510	0.6697	2.6150	0.00896
<i>Trigonella foenum-graecum</i>	−1.4300	0.6501	−2.1990	0.02794
<i>Urtica dioica</i>	−0.6241	0.6604	−0.9450	0.34473
Day ¹	1.7450	0.1006	17.3500	< 0.001
Day ²	−0.0807	0.0039	−20.9500	< 0.001
Day ³	0.0009	0.0000	21.4270	< 0.001

Significant values are indicated in bold, and superscripts for days 1, 2, and 3 refer to their respective polynomials

6. DISCUSSION

The data clearly show that plant powders can enhance both body weight and lifespan of adult honeybee, *A. mellifera*, workers, two common health proxies (Maurizio 1954; Retschnig et al. 2021). Since the powder solutions were readily consumed by the workers, these results offer a novel and promising avenue for supplementary feeding of managed colonies. In light of increasing climate change and pathogen challenges, such feeding may play an even more important role for future *A. mellifera* colony health.

The sucrose consumption data interestingly show that each and every treatment followed the same pattern: third-degree polynomial linear trends, highlighting that the feeding solutions very likely did not affect worker feeding behavior. Furthermore, this suggests that differences in longevity and weight cannot be attributed to sucrose consumption habits alone. On the other hand, unexpectedly, from the consumption

data, all treatment groups showed an observed increase in initial sucrose consumption, followed by a decrease, and once again, an increase until the end of the trial. Despite the highly artificial conditions of hoarding cages, which are arguably void of many social cues found in real-hive scenarios (e.g., absence of brood pheromone, Münch et al. 2013), these data could be hinting at age-related behavioral plasticity (i.e., temporal polyethism, Oster and Wilson 1978), where the last increase in sucrose consumption might be suggestive of transitional forager behavior (Corby-Harris et al. 2018). Indeed, nutrition-based social cues, especially sucrose response threshold (i.e., sucrose concentration that stimulates proboscis extension, Bitterman et al. 1983), are known to be drivers of social caste change in bees (Metz et al. 2018). Although the typical nurse-forager shift happens approximately ~3 weeks post-emergence (Winston 1991), if true, our data suggest in the cages that this might be happening around the ~fifth week. Furthermore, nectar sugar

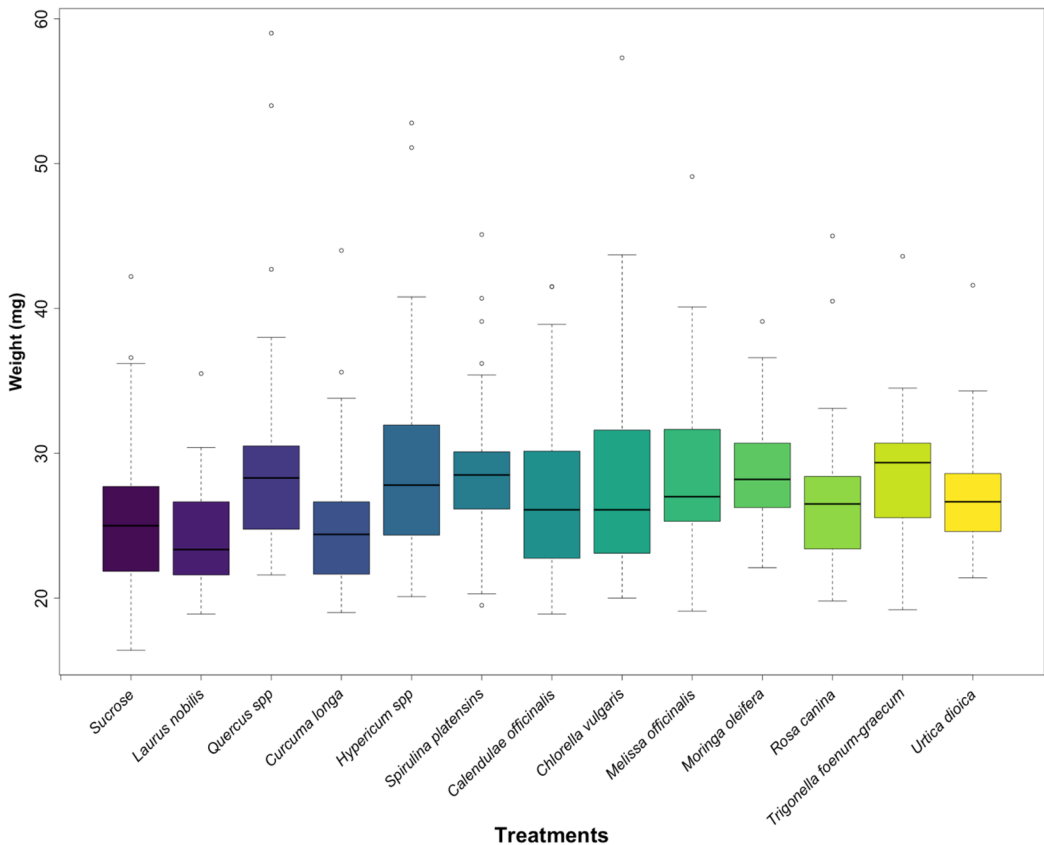


Figure 2. Boxplots of the dry body weights (mg) of adult *Apis mellifera* workers from 13 treatment groups: sucrose, *Laurus nobilis*, *Quercus* spp., *Curcuma longa*, *Hypericum* spp., *Spirulina platensis*, *Calendula officinalis*, *Chlorella vulgaris*, *Melissa officinalis*, *Moringa oleifera*, *Rosa canina*, *Trigonella foenum-graecum*, and *Urtica dioica* ($N=13$ treatments, $N=36$ workers/treatment, $N=468$ total workers). Minimum and maximum values, as well as lower and upper quartiles plus medians, are shown in each boxplot.

content is known to be highly variable ($2.5\% \geq 80\%$, Graham 1992; Wolff 2006), and higher sugar concentration has been shown to stagnate changes from nurse to foragers (Pankiw et al. 2001; Metz et al. 2018). Here, the bees were supplemented the upper end of this spectrum (50% sucrose (w/v)), offering a reasonable explanation for the last upward trend around the ~fifth week of our data. Indeed, our data align with other published literature, where increased caloric consumption in bees approaching the end of their lives was observed, regardless of dietary treatment (Bouchebti et al. 2022). Nonetheless, in order to truly come to such a conclusion of age-related polyethism, further look into physiological

and morphological cues, such as vitellogenin and/or juvenile hormone titers (Amdam and Omholt 2002) or hypopharyngeal gland development (Shakeel et al. 2020), would need to be done to help confirm if, indeed, caged bees undergo temporal polyethism. Undoubtedly, large gaps exist between extrapolating hoarding cage data to real-hive contexts, and knowing if caged bees underwent such above-mentioned physiological changes would help advance the ability of bee researchers to apply cage data to field-research scenarios.

Body weight is typically used as a token of bee health, since a larger body size is often associated to larger foraging areas and higher colony survival

Table III Summary results of pairwise testing of adult *Apis mellifera* dry weight from 13 treatment groups: sucrose, *Laurus nobilis*, *Quercus* spp., *Curcuma longa*, *Hypericum* spp., *Spirulina platensis*, *Calendula officinalis*, *Chlorella vulgaris*, *Melissa officinalis*, *Moringa oleifera*, *Rosa canina*, *Trigonella foenum-graecum*, and *Urtica dioica*. All treatments were compared to negative control “sucrose” and their median difference (mg) and corresponding *p*-values (FDR corrected) are shown. Treatment groups that were statistically significant compared to the negative control “sucrose” (*p* < 0.05) are highlighted in bold

Treatment	Median difference (mg)	<i>p</i> -value
Sucrose: <i>Laurus nobilis</i>	−1.65	0.28
Sucrose: <i>Quercus</i> spp.	3.3	0.01
Sucrose: <i>Curcuma longa</i>	−0.6	0.59
Sucrose: <i>Hypericum</i> spp.	2.8	0.04
Sucrose: <i>Spirulina platensis</i>	3.5	0.01
Sucrose: <i>Calendula officinalis</i>	1.1	0.34
Sucrose: <i>Chlorella vulgaris</i>	1.1	0.19
Sucrose: <i>Melissa officinalis</i>	2	0.04
Sucrose: <i>Moringa oleifera</i>	3.2	0.01
Sucrose: <i>Rosa canina</i>	1.5	0.21
Sucrose: <i>Trigonella foenum-graecum</i>	4.35	0.01
Sucrose: <i>Urtica dioica</i>	1.65	0.10

Table IV Summary statistics of survival analysis of adult *Apis mellifera* workers from 13 treatment groups: sucrose, *Laurus nobilis*, *Quercus* spp., *Curcuma longa*, *Hypericum* spp., *Spirulina platensis*, *Calendula officinalis*, *Chlorella vulgaris*, *Melissa officinalis*, *Moringa oleifera*, *Rosa canina*, *Trigonella foenum-graecum*, and *Urtica dioica*. Minimum, first quartile, median, third quartile, and maximum values (days) are shown. Treatment groups that were statistically significant compared to the negative control “sucrose” (*p* < 0.05) are highlighted in bold

Treatment	Minimum	1st quartile	Median	3rd quartile	Maximum
Sucrose	7	16	29	34	61
<i>Laurus nobilis</i>	1	14	31	40	61
<i>Quercus</i> spp.	3	14	33	42	69
<i>Curcuma longa</i>	2	16	31	39	68
<i>Hypericum</i> spp.	2	14	29	41	66
<i>Spirulina platensis</i>	3	14	28	39	59
<i>Calendula officinalis</i>	4	14	28	38	56
<i>Chlorella vulgaris</i>	1	14	33	41	66
<i>Melissa officinalis</i>	4	14	29	43	66
<i>Moringa oleifera</i>	3	14	27	38	53
<i>Rosa canina</i>	6	15	32	39	58
<i>Trigonella foenum-graecum</i>	4	17	33	41	57
<i>Urtica dioica</i>	3	14	35	46	63

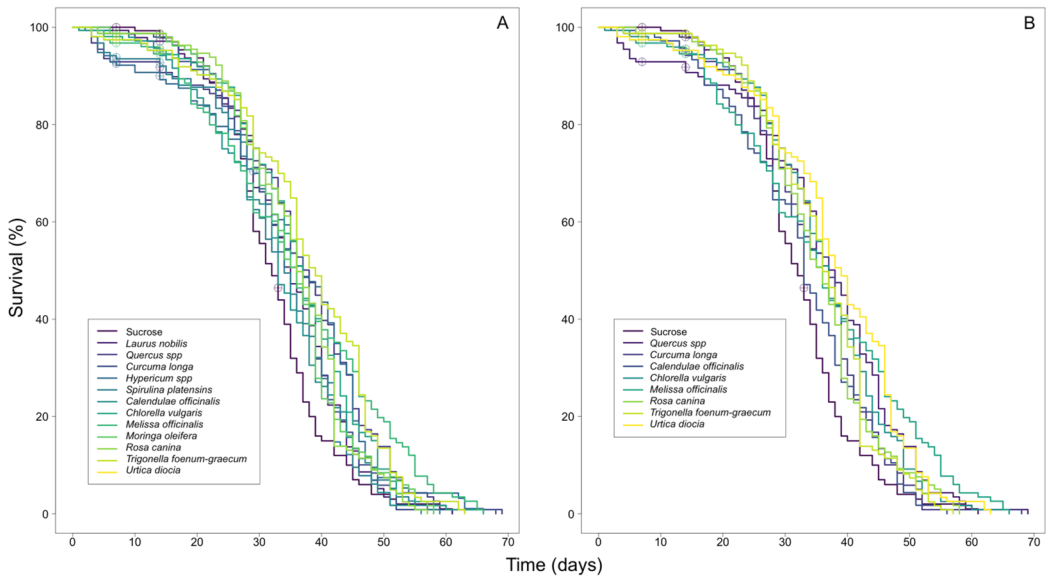


Figure 3. Kaplan–Meier survival curve (A, B) from mortality data of adult summer *Apis mellifera* workers from 13 treatments: sucrose, *Laurus nobilis*, *Quercus* spp., *Curcuma longa*, *Hypericum* spp., *Spirulina platensis*, *Calendula officinalis*, *Chlorella vulgaris*, *Melissa officinalis*, *Moringa oleifera*, *Rosa canina*, *Trigonella foenum-graecum*, and *Urtica dioica* ($N = 156$ workers per treatment group, $N = 6$ replicates/treatment, $N = 26$ bees/treatment, $N = 2028$ total workers). All groups are shown in A, and groups that were significantly different than the control group “sucrose” (log rank testing ($\rho = 0$), Benjamin-Hochberg FDR correction) are all shown in B.

rates (EFSA 2016; Beukeboom 2018; Vanderplanck et al. 2021), with the latter being particularly important in the context of colony losses (Neumann and Carreck 2010). Indeed, the goal of supplementing different regiments to experimental workers here was to improve their physical condition and survival predictions, and when considering both parameters together, *Quercus* spp, *Melissa officinalis*, and *Trigonella foenum-graecum* all statistically increase dry body weight (Figure 2), on top of increasing median and maximum longevity, or (in most cases) a combination of both (Table IV). *Quercus* sp is of particular interest here for several reasons. First, *Quercus* spp. are known to have high content in phytochemical compounds (e.g., antioxidant and anticarcinogenic) and as such have already been systematically investigated for disease treatment in humans (Vinha et al. 2016). Secondly, *Quercus* bark (used here, Supplement Table S.1) is naturally used by *A. mellifera* in the field to make propolis (Cheng and Wong 1996). Propolis has been long studied for its beneficial

properties (antiviral, antibiotic, etc.), namely, for its effectiveness against pathogenic fungi, viruses, and bacteria (Cheng and Wong 1996; Dolci and Ozino 2003; Muli and Maingi 2007). Although it does not appear that *A. mellifera* workers intentionally consume propolis for its nutrients, presence/absence assays exemplify propolis as promoting beneficial *A. mellifera* mouthpart microbiome (Dalenberg et al. 2020) with beneficial downstream consequences on gut microbiota (Saelao et al. 2020). Undoubtedly, favorable insect gut symbionts are key to many beneficial host-microbial interactions (e.g., detoxification and/or vitamin production, Kešnerová et al. 2017; Salem et al. 2014), and within the context of this paper, facilitating host-nutrient digestion and uptake (Brune 2014). Lastly, in addition to secondary metabolites, plant leaves generally contain varying quantities of macromolecules (e.g., carbohydrates, proteins, and lipids, Reiss and Ruse 2023), and both *M. officinalis* and *T. foenum-graecum* contain such macronutrients (Srinivasan 2006; Jovanović et al.

2022). As such, further investigation into their macronutrient profiles would be of interest, since the plant leaves are arguably adding other beneficial nutrients to the sucrose solutions used here.

Diverging from our findings, *Urtica dioica* has been shown to significantly increase body weight of adult *A. mellifera* worker bees (Pohorecka 2004). Although we did not detect this here (Figure 2), we did find that bees that received *Urtica dioica* leaf powder were predicted to have the longest median age among all treatment groups (6-day median life increase, Figure 3, Table IV). Similar to *Quercus* spp., *Urtica dioica* also contains many aforementioned polyphenols noted for their health benefits in humans (Esposito et al. 2019; Dhoubi et al. 2020), but more importantly, they are also rich in vitamins (e.g., C, B, and K, Joshi et al. 2014; Bhusal et al. 2022), essential nutrients for bees (Brodschneider and Crailsheim 2010) and of which are exclusively obtained from either plant material or gut microbial symbionts (Kwong and Moran 2016; Douglas 2017). B-vitamins in particular have already received attention in insect research, where biotin (B₇) is known to be needed for storing and metabolizing fatty acids, and folic acid (B₉) is necessary for de novo nucleic acid synthesis (Dadd 1973). With respect to *A. mellifera*, pyridoxine (B₆) is required for tasks such as brood rearing (Haydak and Dietz 1972; Anderson and Dietz 1976). It is clear, and contemporary evidence continues to show, that B-vitamins contribute to *A. mellifera* colony performance (Jovanovic et al. 2021). However, one cannot exclude that supplementing B-vitamins at improper dosages may result in costs (e.g., elevated mortality, (Brown et al. 2022a)). Therefore, having knowledge that organic material like *U. dioica* and *Quercus* spp. contains such essential micronutrients and antioxidants (Kuliev et al. 1997) coupled to the benefits associated with such compounds (e.g., anticancer/antioxidant properties (Zhang et al. 2015; Pérez et al. 2017; Morales 2021) and furthermore is shown to improve longevity here, replicating our results with long-lived winter bees would appear to be of considerable interest to future apiculture studies.

In conclusion, supplementary feeding with plant powders had striking positive effects on body weight and lifespan of honeybee workers, presumably due to their nutritional (i.e., vitamins, minerals, peptides) and phenolic contents. Further experiments with long-lived winter bees and individual compounds alone are required to identify the actual mechanisms explaining the data. Furthermore, given that laboratory findings may not necessarily reflect real-world scenarios (Retschnig et al. 2015), repeated experiments with field colonies would be of considerable interest. If our results hold true at the field colony level, these data highlight a very promising avenue at better understanding honeybee physiology and longevity as well as enhancing *A. mellifera* health via supplementary feeding.

SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at <https://doi.org/10.1007/s13592-024-01094-4>.

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AUTHOR CONTRIBUTION

AFB, GR, and PN designed the experiment, and AFB and SW conducted the trials. AFB and GR analyzed the data; and AFB, GR, SW, and PN wrote the manuscript; all authors revised and approved the final manuscript.

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DATA AVAILABILITY

The raw data of the study are on Dryad repository under <https://doi.org/10.5061/dryad.vmcvnd0w>.

CODE AVAILABILITY

See “readme” file available on the Dryad repository: <https://doi.org/10.5061/dryad.vmcvnd0w>.

DECLARATIONS

Ethics approval No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

Conflict of interest The authors declare no competing interests.

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