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Nutritional and prebiotic efficacy of the microalga *Arthrospira platensis* (spirulina) in honey bees

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Abstract – We evaluated the microalga *Arthrospira platensis* (commonly called spirulina), as a pollen substitute for honey bees. Nutritional analyses indicated that spirulina is rich in essential amino acids and a wide variety of functional lipids (i.e., phospholipids, polyunsaturated fatty acids, and sterols) common in pollen. Feeding bioassays were used to compare dry and fresh laboratory-grown spirulina with bee-collected pollen and a commercial pollen substitute using sucrose syrup as a control. Diets were fed ad libitum as a paste to newly emerged bees in cages (10–13 cage replicates) and bees were sampled at days 5 and 10 for physiological and molecular measurements. Spirulina diets produced biomarker profiles (thorax weight, head protein content, and beneficial gut bacteria abundance) that were indicative of elevated nutritional states, meeting or exceeding the other diets in some metrics despite reduced consumption. Furthermore, spirulina diets led to significantly increased fat body lipid content and mRNA levels of the central storage lipoprotein vitellogenin. We conclude that spirulina has significant potential as a pollen substitute or prebiotic diet additive to improve honey bee health.

Apis mellifera / nutrition / microbiota / microalgae / pollen substitute

1. INTRODUCTION

To compensate for periods of forage scarcity or to bolster colony size prior to pollination services, beekeepers routinely feed pollen substitute (PS) diets to honey bees (Nabors 2000; Mattila and Otis 2006). PSs are generally considered a safe way to deliver protein to colonies since feeding bee-collected pollen is cost-prohibitive and difficult to standardize, can transmit disease, and can be contaminated by pesticides (Brodschneider and Crailsheim 2010). These artificial diets consist of a base protein derived from soy, yeast, egg, wheat,

or lentils. Early attempts at formulating PS diets failed to match the nutritional efficacy of pollen and had low palatability (Standifer et al. 1973). Soy products are common ingredients in PSs despite reports of potential anti-nutritional factors such as toxic sugars (Barker 1977) and protease inhibitors (Liener 1994; Sagili et al. 2005). Some more recently developed commercial PSs appear to match the nutritional value of pollen (DeGrandi-Hoffman et al. 2010; De Jong et al. 2009); however, these diets have not been robustly tested and their proprietary formulas confound efforts to investigate the effects of individual components. Feeding PSs has become a common management practice as landscape compositions shift to agriculturally intensive monocultures that may not meet the nutritional requirements of bees (Naug 2009). PSs are increasingly used by US beekeepers in the fall and winter months leading up to pollination of early crops such as almonds. Therefore, improving the effectiveness and

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sustainability of PS diets can be considered important to modern beekeeping.

While the nutritional value of pollen is largely attributed to its protein content and amino acid composition (Crailsheim 1990; Brodschneider and Crailsheim 2010), there has been comparatively less focus on lipids as they relate to supplemental nutrition for bees. Pollen contains a wide variety of lipids and is particularly rich in membrane-constituting phospholipids, fatty acids, and sterols (reviewed in Ischebeck 2016). Pollen consumption leads to global changes in honey bee tissue phospholipid composition. This pollen-influenced phospholipid spectra and the prevalence of esterified polyunsaturated fatty acid (PUFA) residues are strongly linked to abdominal *vitellogenin* (*vg*) expression (Wegener et al. 2018). *Vg* is the major lipoprotein produced by abdominal fat body cells and is central to brood production, life span regulation, oxidative stress response, and overwintering (Amdam et al. 2003; Amdam et al. 2005). *Vg* expression levels are also correlated with diet and landscape quality, making it a useful biomarker of individual (Alaux et al. 2011; Frias et al. 2015) and colony-level (Ricigliano et al. 2018; Ricigliano et al. 2019) nutritional status.

Pollen and nectar modulate bacterial communities (microbiota) in the honey bee gut that influence metabolism, immunity, and overall fitness (Engel et al. 2016; Kwong et al. 2017; Bonilla-Rosso and Engel 2018). Probiotic additives are used in some PS diets; however, there is little evidence to support their effectiveness or establishment when delivered as diet supplements (Stephan et al. 2019). An alternative approach takes into consideration the *pre*biotic (Samal and Behura 2015) efficacy of a diet to stimulate growth and metabolism of endogenous microbiota that promote bee health (e.g., *Lactobacillus*, *Bifidobacterium*, and *Snodgrassella*). Since bee-associated bacterial symbionts co-evolved with a natural pollen diet (Kešnerová et al. 2017), microbiota abundance could be an informative metric to evaluate extra-nutritive effects of artificial diets on bee physiology (Ricigliano et al. 2017; Zheng et al. 2018; Li et al. 2019).

Development of PSs for honey bees should aim to recapitulate the phytochemical profile,

palatability, and functional properties of pollen in a sustainable formulation. Microalgae, which are mostly photosynthetic, unicellular, or simple multicellular organisms, have recently gained traction as a feed source for aquaculture and terrestrial livestock (Roy and Pal 2014; Odjadjare et al. 2017; Lamminen et al. 2019), including honey bees (Jehlík et al. 2019; Ricigliano 2020). Extensive nutritional and toxicological evaluations have demonstrated the suitability of microalgal biomass as a feed additive or substitute for conventional protein sources (García et al. 2017; Caporgno and Mathys 2018). The rapid growth rates and biomass production of microalgae can enable them to outyield conventional protein feed resources on an area basis using non-arable land, mitigating some environmental burdens of intensive agriculture (Forján et al. 2014; Tallentire et al. 2018). The blue-green microalga *Arthrospira platensis* is grown on an industrial scale as a nutrition supplement for humans and livestock (Soni et al. 2017). This microalga, commonly called spirulina, is considered a complete nutrition source and is rich in essential amino acids, functional lipids, complex carbohydrates, vitamins, and minerals (Ciferri 1983). While the nutritional efficacy of spirulina has been well documented in other animals, their efficacy as a nutrition source for honey bees is largely unknown. The objective of this study was to assess the potential of spirulina as a nutrition supplement for honey bees during periods of pollen scarcity.

2. MATERIALS AND METHODS

2.1. Honey bees (*Apis mellifera* L.) and experimental design

Experiments were conducted in April–May 2019 at the USDA ARS honey bee lab in Baton Rouge, LA, USA. Newly emerged workers (< 24 h old) were obtained by incubating sealed brood combs sourced from multiple healthy colonies at 35 °C and 50% RH. Bees were collected into a single container, mixed thoroughly, then randomly assigned to diet treatment cages. Preliminary trials were conducted to optimize delivery and consumption of spirulina diets (Online

resource 1, Figure S1). Using the optimized conditions, two feeding tests were conducted using cage as the unit of replication (50 bees/cage) to compare the effects of different diets. Test 1 evaluated sucrose only, pollen, a commercial PS, and dry spirulina, while the objective of test 2 was to assess the nutritional value of fresh, laboratory-grown spirulina in parallel with the treatment groups from test 1. In test 1, 51 cages in total were established for the 4 diet treatments (12–13 cages per treatment). In test 2, 52 cages in total were established for the 5 diet treatments (10–12 cages per treatment). Physiological and molecular measures were made using pools of 10 bees collected separately from each cage at day 5 and day 10. Dead bees were counted and removed from the cages daily.

2.2. Diet preparation and consumption

Bees were fed ad libitum diets of pollen, commercial PS that lacks pollen, or spirulina (commercially sourced dry or fresh laboratory-grown). Diets were mixed into a paste with 50% sucrose (w/v) syrup containing 5% glycerol (v/v; to improve pliability) and loaded into small troughs then stored at -20°C before use. Control cages received sucrose syrup only. For the polyfloral pollen diet, mixed corbicular pollen pellets were collected using entrance-mounted pollen traps in a USDA ARS apiary in Baton Rouge and immediately frozen upon until ready for use (Mogren et al. 2018). For the PS diet, a commercial plant-based protein supplement was used. The dry spirulina diet consisted of commercially sourced dry spirulina powder. The fresh spirulina diet used in test 2 consisted of fresh harvested *A. platensis* UTEX LB 2340 biomass that was grown in our laboratory under standard conditions in Zarrouk medium (Vonshak et al. 1983), rinsed in dH_2O over a $30\text{ }\mu\text{m}$ filter, pressed to removed excess water, and mixed with syrup as above. The amount of diet consumed by each cage was recorded then the diet was refreshed with 3 small troughs ($\sim 700\text{ mg}$) of diet paste daily. The weight loss by diet samples maintained in a cage without bees was measured to determine the daily evaporation rate for each diet. Diet consumption in each cage was adjusted for daily moisture loss and

recalculated for the total diet consumed over 24 h per bee. Drip feeders of 50% (w/v) sucrose solution were provided to all cages.

2.3. Nutritional analysis

2.3.1. Amino acid content

Free amino acids in samples of pollen, PS, dry spirulina, and fresh spirulina were quantified by New England Peptide (Boston, MA, USA). Briefly, samples were subjected to vapor-phase acid hydrolysis, derivatization, and high-performance liquid chromatographic (HPLC) determination of 15 amino acids based on chromatographic peak identification and peak area quantification. This hydrolysis method destroys cysteine, methionine, and tryptophan residues and, therefore, these compounds are not included in the analysis.

2.3.2. Lipidomic analysis

Lipid analysis of dry spirulina was performed by Avanti Polar Lipids (Alabaster, AL, USA). Modified Bligh and Dyer extractions (Bligh and Dyer 1959) were performed with EquiSPLASH™ Lipidomix™, and Lipidizer™ standards (Avanti Polar Lipids), and different chain lengths of phosphatidylglycerol, phosphatidylserine, and phosphatidylinositol (Avanti Polar Lipids) were added as internal standards (IS). Total lipids were dried then resolvated in 1 mL of 1:1 methylene chloride:methanol. Samples were injected in triplicate without dilution for LC-MS analysis. The methodology used was hydrophobic interaction liquid chromatography (HILIC), which separates lipids into classes and subclasses that span a narrow retention time window (Hines et al. 2017). A standard of 18:1 cholesterol ester was used as an IS for stigmaterols and brassicasterols. Data are presented as ng/mg of sample. Values were calculated using a point calibration determined by multiplying area ratio averages for the analyte to IS and multiplying by the concentration of IS.

2.4. Nutritional assimilation measures

Bees were dissected into head, thorax (including legs and wings) and abdomen, and parts were

pooled into groups of 10. Average thorax weight per cage (test 2) was determined by drying to a constant weight (60 °C for ~48 h) and recording to the nearest 0.1 mg. Average head protein content per cage (test 2) was determined using spectrophotometric protein quantification (Sagili et al. 2005). Average fat body mass per cage (test 1) was determined using abdomens with guts removed and the ether extraction method described in (Wilson-Rich et al. 2008).

2.5. *Vitellogenin* (*vg*) expression and gut bacteria abundance

For gene expression and bacterial abundance measures (test 1), pools of 10 abdomens per cage with whole guts intact were homogenized in 2 mL lysis buffer (1.2 M guanidine thiocyanate, 0.6 M ammonium thiocyanate). Samples were centrifuged and total RNA was extracted from 300 μ L of supernatant using a GeneJet RNA Purification Kit (Thermo Fisher Scientific). cDNA was synthesized from 1 μ g of DNaseI-treated RNA using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). Quantitative PCR (qPCR), carried out in triplicate with previously published primer pairs and cycling conditions, was used to determine the relative expression levels of honey bee genes (*vg*, *vg-like-A*, *vg-like-B*) (Salmela et al. 2016) and species-specific bacterial 16S rRNA genes (*Lactobacillus Firm 5*, *Bifidobacterium*, *Snodgrassella*) (Kešnerová et al. 2017) across treatments using honey bee actin for normalization (Alaux et al. 2011).

2.6. Statistical analyses

The effects of diet treatments and bee age on food consumption, thorax weight, fat body mass, head protein, and *vg* expression were evaluated using two-way ANOVA. Kaplan-Meier survival estimates were used to determine the effect of diet on life span. The effects of diet treatments on gut bacteria abundance were evaluated using one-way ANOVA. Variables with deviations from normality were re-evaluated after log transformation. The Tukey HSD post hoc test was used to compare different treatment groups. Analyses were conducted in JMP v11 and Prism v7.

3. RESULTS

3.1. Nutritional analyses

We measured the abundance of 15 amino acids (AAs), including 8 essential AAs. The content of each essential AA detected in spirulina met or exceeded that of pollen with the exception of histidine and lysine (Figure 1), which were twice as high in pollen. Pollen substitute (PS) had the lowest essential AA content with the exception of histidine, which was the same as spirulina. The AA profiles of dry and fresh spirulina were highly similar.

Lipidomic analysis of spirulina identified and quantified 13 subclasses of phospholipids (309 species), 3 subclasses of neutral lipids (447 species), and two subclasses of sterols (37 species). The lipid categories were obtained by summing the levels of individual molecular species within each type (Table 1). The levels of each species are listed in detail in (Online resource 2). Phosphatidylglycerol, sphingomyelin, and lysophosphatidylglycerol were the major phospholipid subclasses, accounting for 62.4% of the total lipids. The sterol content (stigmasterol + brassicasterol) was 9.4%. The fatty acids 16:0, 18:2, 18:1, 18:0, 18:3, 20:0, 20:1, 20:3, 20:5, 22:2, 24:0, and 22:6 were predominant in the total esterified fatty acids (Online resource 2).

3.2. Diet consumption

Food consumption (corrected for evaporative loss) in test 1 was influenced by diet ($F_{2, 72} = 241.9$, $P < 0.001$) and age ($F_{1, 72} = 236.8$, $P < 0.001$), but not their interaction ($P = 0.69$). Cumulative consumption was highest for the pollen diet (65.37 mg/bee) and lowest for the dry spirulina diet (40.04 mg/bee) (Figure 2a). Test 2 consumption was influenced by diet ($F_{3, 76} = 36.92$, $P < 0.001$), age ($F_{1, 76} = 210.20$, $P < 0.001$), and their interaction ($F_{3, 76} = 9.88$, $P < 0.001$). Cumulative consumption was highest for the pollen diet (39.03 mg/bee) and lowest for the dry spirulina diet (28.41 mg/bee) (Figure 2b). Mortality was less than 3% overall for test 1 and less than 5% overall in test 2, so mortality was negligible for all treatments in the course of the study.

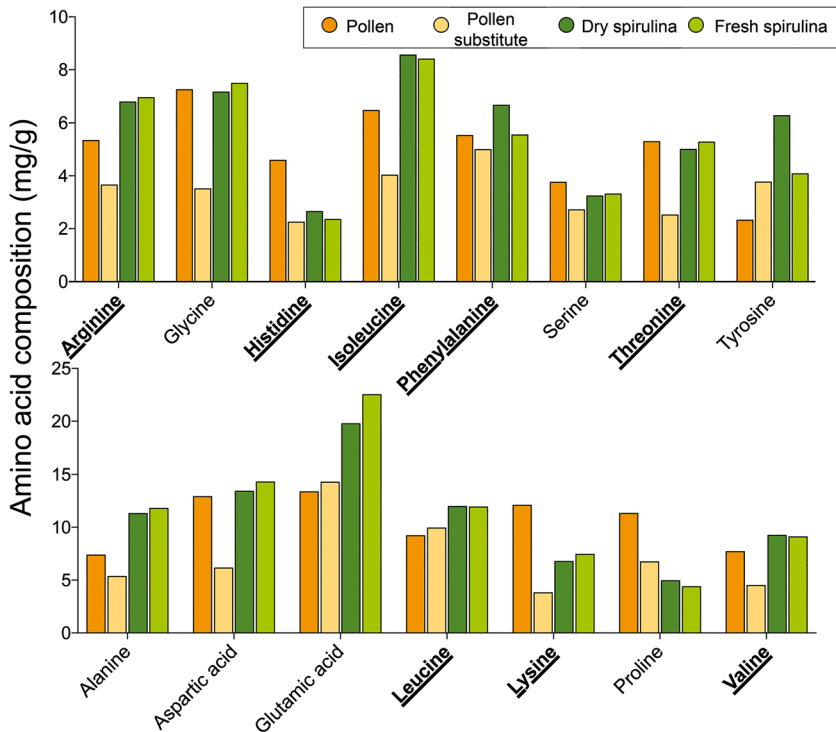


Figure 1. Amino acid content of different protein sources: bee-collected pollen, pollen substitute (PS), dry spirulina, and fresh spirulina. Essential amino acids are emphasized.

However, for the sucrose group, mortality was higher than the rest of the treatments in test 2 and no differences were noted in Trial 1 (Online resource 1, Figure S2). These results were consistent with preliminary tests to assess spirulina toxicity to bees, which revealed no significant difference in mortality compared with sucrose-fed control (Online resource 1, Figure S3).

3.3. Nutrient assimilation measures

Thorax weight was influenced by diet ($F_{4, 94} = 31.51$, $P < 0.001$) and age ($F_{1, 94} = 341.5$, $P < 0.001$), but not their interaction ($P = 0.200$). Thorax weight was highest in bees fed dry spirulina (Figure 3a). Similarly, preliminary feeding tests with a broader range of consumption values revealed a strong positive correlation between spirulina diet consumption and dried thorax weight ($F_{1, 58} = 69.7$, $P < 0.001$; $r^2 = 0.546$) (see Online resource 1 Figure S1c).

Soluble head protein content was influenced by diet ($F_{4, 94} = 111.2$, $P < 0.001$) and age ($F_{1, 94} = 21.14$, $P < 0.001$) but not their interaction ($P = 0.356$). Protein levels were highest in bees fed PS and lowest in bees fed only sucrose. There were no differences in head protein content among pollen and spirulina diets (Figure 3b).

Fat body mass was influenced by diet ($F_{3, 90} = 57.0$, $P < 0.001$), age ($F_{1, 90} = 41.98$, $P < 0.001$), and their interaction ($F_{3, 90} = 9.03$, $P < 0.001$). Fat body mass at day 10 was highest in bees fed pollen and dry spirulina (Figure 4).

3.4. Vitellogenin (vg) and vg-like expression

Vitellogenin (vg) expression was influenced by diet ($F_{3, 92} = 321.6$, $P < 0.001$) and age ($F_{1, 92} = 10.88$, $P = 0.001$) but not their interaction ($P = 0.950$). Vg expression was highest in bees fed PS and lowest in bees fed sucrose only. There was no difference between bees fed pollen or

Table I. Lipidomic analysis of spirulina. See Online resource 2 for fatty acid compositions of the molecular species in each lipid subclass

	Concentration (ng/mg)	Percent composition
Phospholipids		
Phosphatidylglycerol	946.16	43.03
Lysophosphatidylglycerol	222.83	10.14
Sphingomyelin	203.59	9.26
Phosphatidylcholine	133.95	6.09
Phosphatidylethanolamine	119.32	5.43
Phosphatidylinositol	37.66	1.71
Lysophosphatidylcholine	12.51	0.57
Phosphatidylserine	4.91	0.22
Lysophosphatidylinositol	2.01	0.09
Lysophosphatidylethanolamine	1.81	0.08
Ceramide	0.79	0.04
Dihydroceramide	0.62	0.03
Hexosylceramide	0.53	0.02
Neutral lipids		
Triacylglycerol	152.30	6.93
Diacylglycerol	124.95	5.68
Cholesterol ester	27.92	1.27
Sterols		
Stigmasterol	138.39	6.29
Brassicasterol	68.39	3.11

spirulina (Figure 5a). *Vg-like-A* expression was influenced by diet ($F_{3, 92} = 166.0$, $P < 0.001$), age ($F_{1, 92} = 66.36$, $P < 0.001$), and their interaction ($F_{3, 92} = 3.96$, $P = 0.010$). *Vg-like-A* expression was highest in bees fed PS at day 10 and lowest in bees fed sucrose only (Figure 5b). *Vg-like-B* expression was influenced by diet ($F_{3, 92} = 32.98$, $P < 0.001$), age ($F_{1, 92} = 39.49$, $P < 0.001$), and their interaction ($F_{3, 92} = 11.74$, $P < 0.001$). *Vg-like-B* expression was highest in bees fed spirulina at day 10 (Figure 5c).

3.5. Gut bacteria abundance

Lactobacillus Firm 5 abundance was highest in bees fed spirulina and lowest in bees fed pollen substitute or sucrose ($F_{3, 46} = 31.89$, $P < 0.001$; Figure 6a). *Bifidobacterium* abundance was highest in bees fed pollen and spirulina and lowest in bees fed pollen substitute and sucrose ($F_{3, 46} =$

25.48, $P < 0.001$; Figure 6b). *Snodgrassella* abundance was highest in bees fed spirulina and lowest in bees fed pollen substitute ($F_{3, 46} = 5.09$, $P < 0.001$; Figure 6c).

4. DISCUSSION

This study assessed the nutritional value and functional properties of spirulina in honey bees. We chose to evaluate spirulina due to its well-documented nutritional and non-toxic effects in a variety of animals including humans (Soni et al. 2017). The choice of microalgae species likely impacts animal health based on its chemical composition, including amino acid content, lipid content, and cell wall structure (Caporgno and Mathys 2018). Our results indicate that spirulina has significant potential as a feed additive or pollen replacement based on its nutritional content and effects on nurse-aged worker bee physiology.

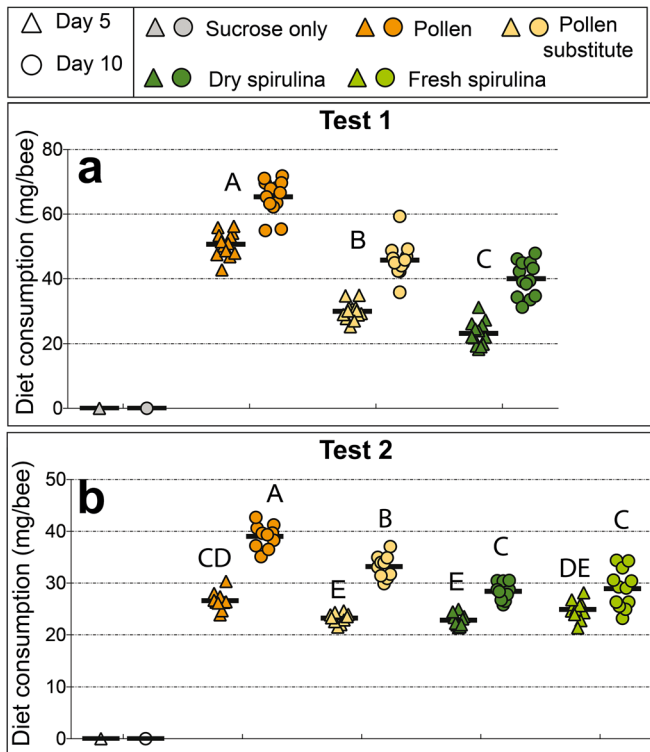


Figure 2. Diet consumption (milligrams per bee) by caged honey bees after 5 and 10 days. **a** Test 1, $n = 12\text{--}13$ cages. **b** Test 2, $n = 10\text{--}12$ cages. Each point represents an independent cage. Black horizontal lines indicate the mean. Different letters indicate Tukey HSD $P < 0.05$.

While this assessment is based on experiments with laboratory-reared bees and as such provides a snapshot into effects on workers, the findings warrant further study on the impact of spirulina-based diets at the colony level.

Since honey bees cannot synthesize arginine, histidine, lysine, tryptophan, phenylalanine, methionine, threonine, leucine, isoleucine, and valine, it is critical that these AAs are present in the diet (De Groot 1953). AAs are obtained from pollen and supplemented by feeding PS diets. The essential AAs detected in spirulina met or exceeded the other diets tested, with the exception of histidine and lysine, which were twice as high in pollen. PS had the lowest essential AA content but produced higher head protein levels than pollen or spirulina diets. The discrepancy between PS- and spirulina-fed bees could be attributed to higher consumption of PS. However, consumption of the pollen diet was higher than PS. The PS

contained a proprietary blend of plant-based proteins, which may have conferred increased bioavailability relative to the polyfloral pollen diet tested. The AA content of commercially sourced and lab-grown spirulina were highly similar, indicating that biomass grown under different conditions can reproducibly support the AA requirements of honey bees. This was confirmed by elevated thorax weights and head protein levels in spirulina-fed bees relative to sucrose control. Head protein levels are considered a biomarker of nutritional status as pollen consumption by workers leads to increased protein synthesis in head hypopharyngeal glands that feed the colony via proteinaceous secretions (Crailsheim 1990; DeGrandi-Hoffman et al. 2010). Taken together, the results suggest that spirulina could be incorporated as a sole protein source in future PS diets to support brood production in colonies.

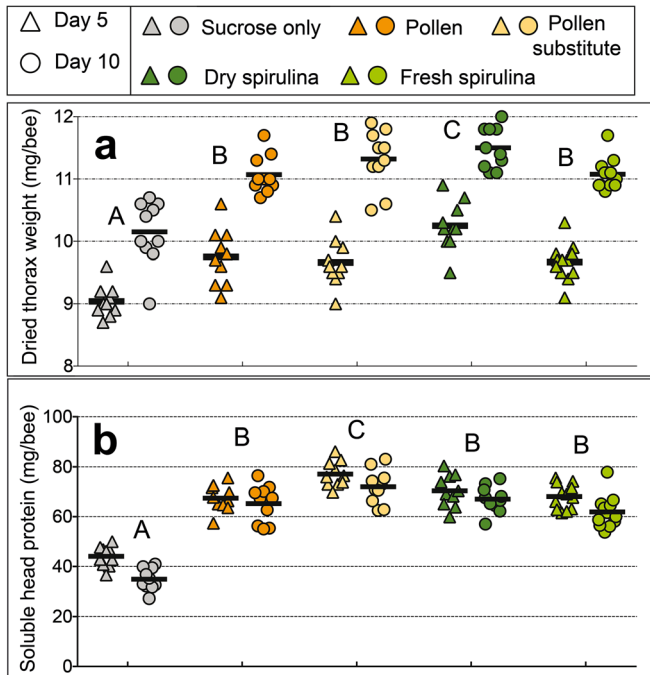


Figure 3. Protein assimilation in bees fed different diets after 5 and 10 days ($n = 10\text{--}12$ cages). **a** Thorax weight as a proxy for flight muscle development. **b** Soluble head protein as a proxy for hypopharyngeal gland development. Each point represents an independent cage. Black horizontal lines indicate the mean. Different letters indicate Tukey HSD $P < 0.05$.

Phospholipids are the major lipid components detected in honey bee tissues and pollen. Dietary phospholipids contain esterified fatty acid (FA) residues, which are hydrolyzed during digestion then reincorporated into cellular macromolecules such as

membrane lipids and lipoproteins. FAs are classified based on their degree of saturation (double versus single bonds), which influence the biochemical functions of lipids they comprise. Linoleic acid (LA, 18:2) and alpha-linoleic acid (ALA, 18:3) are

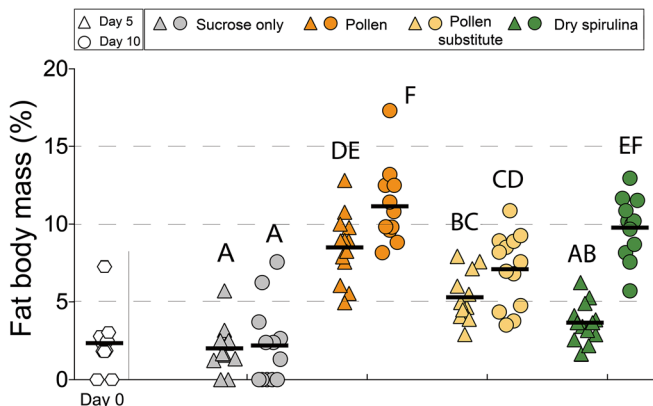


Figure 4. Fat body mass of bees fed different diets at 5 and 10 days ($n = 12\text{--}13$ cages). Each point represents an independent cage. Black horizontal lines indicate the mean. Different letters indicate Tukey HSD $P < 0.05$.

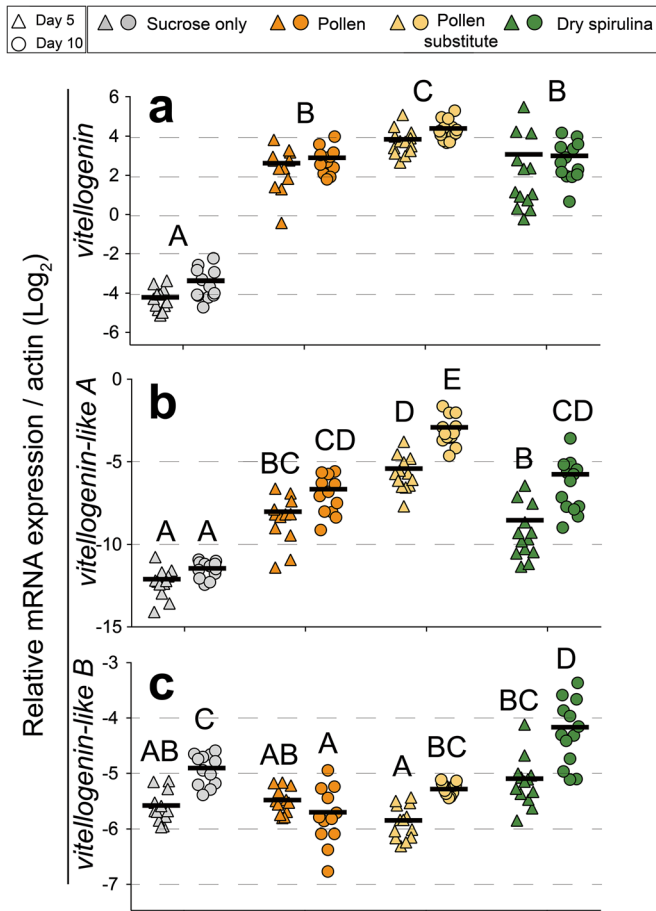


Figure 5. Relative mRNA expression levels of *vitellogenin* (*vg*) and *vg-like* gene homologs in bees fed different diets at day 5 and day 10 ($n = 12\text{--}13$ cages). Each point represents an independent cage. Black horizontal lines indicate the mean. Different letters indicate Tukey HSD $P < 0.05$.

two major polyunsaturated fatty acids (PUFAs) that are considered essential for higher animals (Hulbert et al. 1999), including bees (Avni et al. 2014; Arien et al. 2015). Spirulina has a broad diversity of phospholipid molecular species incorporating LA, ALA, and longer chain PUFAs such as eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6) (Online resource 2). Pollen consumption leads to global reconstruction of honey bee tissue phospholipid molecular species, and the abundance of esterified PUFAs is positively correlated with fat body expression of *vg* (Wegener et al. 2018). *Vg* is a highly abundant lipoprotein produced in fat body cells with central storage and regulatory functions (Amdam et al. 2003; Amdam et al. 2005). *Vg* itself is regulated at the mRNA and protein level by

pollen consumption, supporting its use as a biomarker of diet quality and nutritional status (Alaux et al. 2011; López-Uribe et al. 2020). Our results show that fat body mass and *Vg* mRNA levels in spirulina-fed bees matched that of pollen-fed bees. Pollen and spirulina diets led to higher fat body masses than PS, which produced the highest *Vg* mRNA levels. The PS diet also led to higher expression levels of *vg-like-A*, which likely plays similar roles to *vg* (Salmela et al. 2016). However, *vg* and *vg-like-A* proteins contain a large lipid-binding domain (Salmela et al. 2016) and it remains to be determined if dietary lipid composition impacts their function. Intriguingly, mRNA levels of *vg-like-B* were highest in 10-day-old spirulina-fed bees. Functional understanding of this *vg* homolog is limited

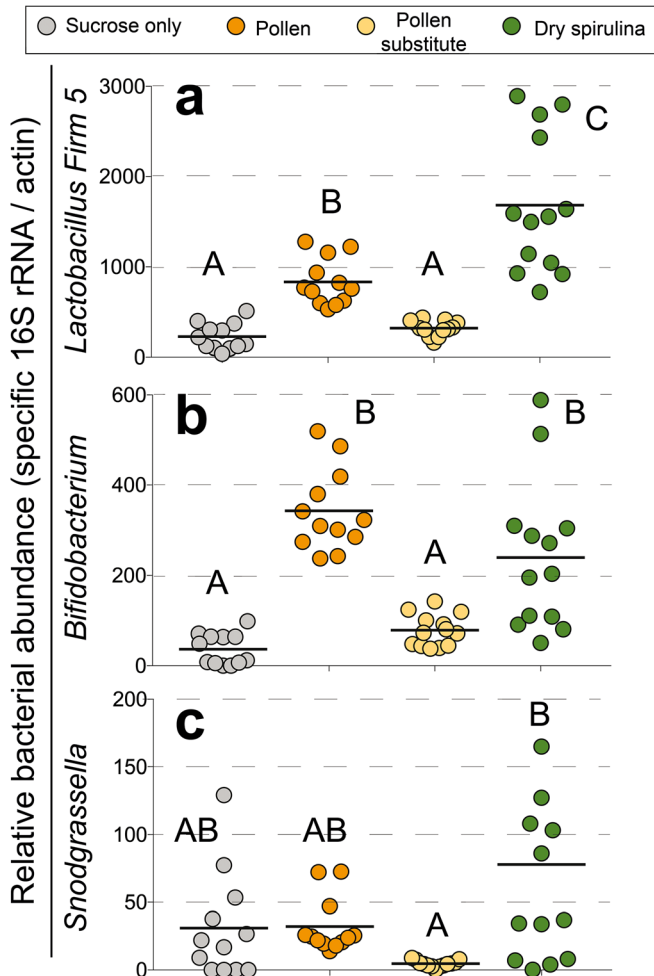


Figure 6. Relative core gut bacteria abundance in bees fed different diets at day 10 ($n = 12$ –13 cages). Each point represents an independent cage. Black horizontal lines indicate the mean. Different letters indicate Tukey HSD $P < 0.05$.

but it has been associated with the life span-regulating properties of vg (Salmela et al. 2016)

Bacterial abundance of core gut microbiota was significantly increased by a spirulina diet, which matched or exceeded the prebiotic effects of pollen and PS, respectively. These results are consistent with the prebiotic potential of microalgae as a nutrition supplement in other systems (de Jesus Raposo et al. 2016). *Lactobacillus spp* and *Bifidobacterium spp* are ubiquitous fermentative constituents of animal microbiota, including bees. The bee-specific gut symbiont *Snodgrassella alvi* is non-fermentative but participates in syntrophic (nutrient-sharing) interactions with fermentative community members (Kešnerová et al. 2017).

Gut bacteria fermentation products act as signaling molecules, influencing central physiological processes in honey bees (Zheng et al. 2017). Spirulina biomass stimulates the growth of *Lactobacillus* and other lactic acid bacteria (Parada et al. 1998), which can competitively exclude pathogenic bacteria via pH reduction (Douglas 2015). These results suggest that incorporating spirulina into PS diets as a prebiotic additive could promote bee health by stimulating the abundance and metabolism of beneficial gut bacteria.

Overall, the major findings that spirulina diets resulted in nutritional physiology measures that were nearly equal to a pollen-based diet show promise for the development of this microalga, and potentially

other microalgal species, as a nutrition supplement for honey bees. Spirulina consumption was less than that of pollen or PS but resulted in bees with equivalent fat body mass and increased thorax weight, respectively. This indicates that the nutritional value of spirulina is certainly at least sufficient for honey bees and its incorporation into future PS diets may be a sustainable solution to improve various aspects of honey bee health.

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AUTHORS' CONTRIBUTIONS

VAR conceived this research and designed the experiments. VAR and MSF performed the experiments and analyzed the data. VAR and MSF wrote the paper. Both authors read and approved the final manuscript.

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Efficacité nutritionnelle et prébiotique de la microalgue *Arthrospira platensis* (spiruline) chez l'abeille

***Apis mellifera* / nutrition / microbiote / microalgue / substitut de pollen**

Ernährungsphysiologische und präbiotische Effekte der Mikroalge *Arthrospira platensis* (spirulina) bei Honigbienen

***Apis mellifera* / Ernährung/ Mikrobiota/ Mikroalge/ Pollenersatz**

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