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Article

Efficacy of a Microalgal Feed Additive in Commercial Honey Bee Colonies Used for Crop Pollination

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Cite This: *ACS Agric. Sci. Technol.* 2023, 3, 748−759

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ABSTRACT: Honey bees are experiencing nutritional deficiencies related to intensive agriculture and climate change, posing major threats to the ecosystem services they provide. Colonies managed for crop pollination are fed artificial diets to offset reduced pollen in the environment and to stimulate population growth. Here, we evaluated the effects of a spirulina microalga feed additive on commercial honey bee colonies in an agriculturally intensive desert environment. One hundred honey bee colonies were used in a randomized block design that was replicated at two apiary sites over 4 months leading up to spring almond pollination. Colonies were fed a pollen-free artificial diet or the same diet containing 25% spirulina. Unfed colonies were randomly assigned within treatment blocks as controls. We measured colony population size, brood production, thermoregulation, and a panel of molecular biomarkers associated with nutritional status, stress responses, and gut microbiota. Hive brood imaging and continuous temperature data enabled sensitive detection of diet treatment effects. Spirulina feed significantly improved brood production and thermoregulation prior to almond pollination relative to unfed controls. Bees fed spirulina had distinct expression profiles of nutrition and stress response genes, but gene expression was primarily driven by the apiary site. We conclude that spirulina is a sustainable feed additive with potential to improve crop pollination efficiency by supporting larger, healthier honey bee colonies.

KEYWORDS: *pollinator health, Apis mellifera, sustainable diet, climate change, Arthrospira platensis, microalgae, pollen substitute*

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1. INTRODUCTION

A rapidly growing human population necessitates that modern agriculture sustainably produces more food while using less resources than ever before.[1−3] Over the last century, global biodiversity has plummeted largely because of anthropogenic climate change as well as land conversion for agricultural use and increased urbanization.[4−7] Whereas up to 90% of flowering plants require animal-mediated pollination—largely by insects[8]—approximately one-third of the human diet requires insect pollination.[9] Extirpations and range contractions of native insect pollinators have increased reliance on managed species for crop pollination.[4,10−13] Because of their large colony size and relative ease of husbandry, the Western honey bee (*Apis mellifera* spp.) comprises the bulk of the commercial pollination workforce in the US. However, colony health and the sustainability of pollination services provided by honey bees are threatened by a variety of interacting stressors. Honey bee colony losses are largely associated with pests, pathogens, and agrochemicals, but malnutrition is a growing threat that synergizes with other stressors.[14−21] Several compounding sublethal effects are linked to poor nutrition, including retarded immune function and increased susceptibility to diseases and pesticides.[22−27]

Diverse and abundant floral resources are crucial to honey bee colony growth, productivity, and resilience to biotic and abiotic stressors. Flower nectar serves as a carbohydrate source, whereas pollen provides essential proteins, lipids, and micronutrients. Both floral resources are also a source of secondary metabolites, which do not serve direct nutritional functions but exert a wide range of bee health-modulating effects.[28] Beekeepers have increased their reliance on supplemental feeding to offset nutritional deficiencies related to land use intensification and climate change, which have begun to drastically alter the landscape of floral resource availability.[13,29−31] Furthermore, monoculture cropping systems fail to provide colonies with adequate nutrition as honey bees require a varied diet to meet their nutritional needs.[22,25,32,33] Pollen nutrition is a major concern to beekeeping industries because it is not easily recapitulated by current dietary interventions. As such, there is room for improvement to develop efficacious feed that is tailored to seasonal and regional colony needs. Importantly, given the challenges of feeding a growing human population, sustainable ingredients that do not compete with human food production are ideal choices to address this critical need of modern beekeeping.

Microalgae are photosynthetic unicellular microorganisms that have become an attractive feed source for humans, livestock, and laboratory-reared model insects.[34−37] From a sustainability perspective, microalgal biomass production requires significantly less agricultural land use (ALU) when compared to common animal feed additives. For example, soymeal production requires about 3.11 m^2/kg, whereas microalgae have a measured ALU of approximately 0.034

Received: March 16, 2023
Revised: July 31, 2023
Accepted: August 1, 2023
Published: August 15, 2023

ACS Publications

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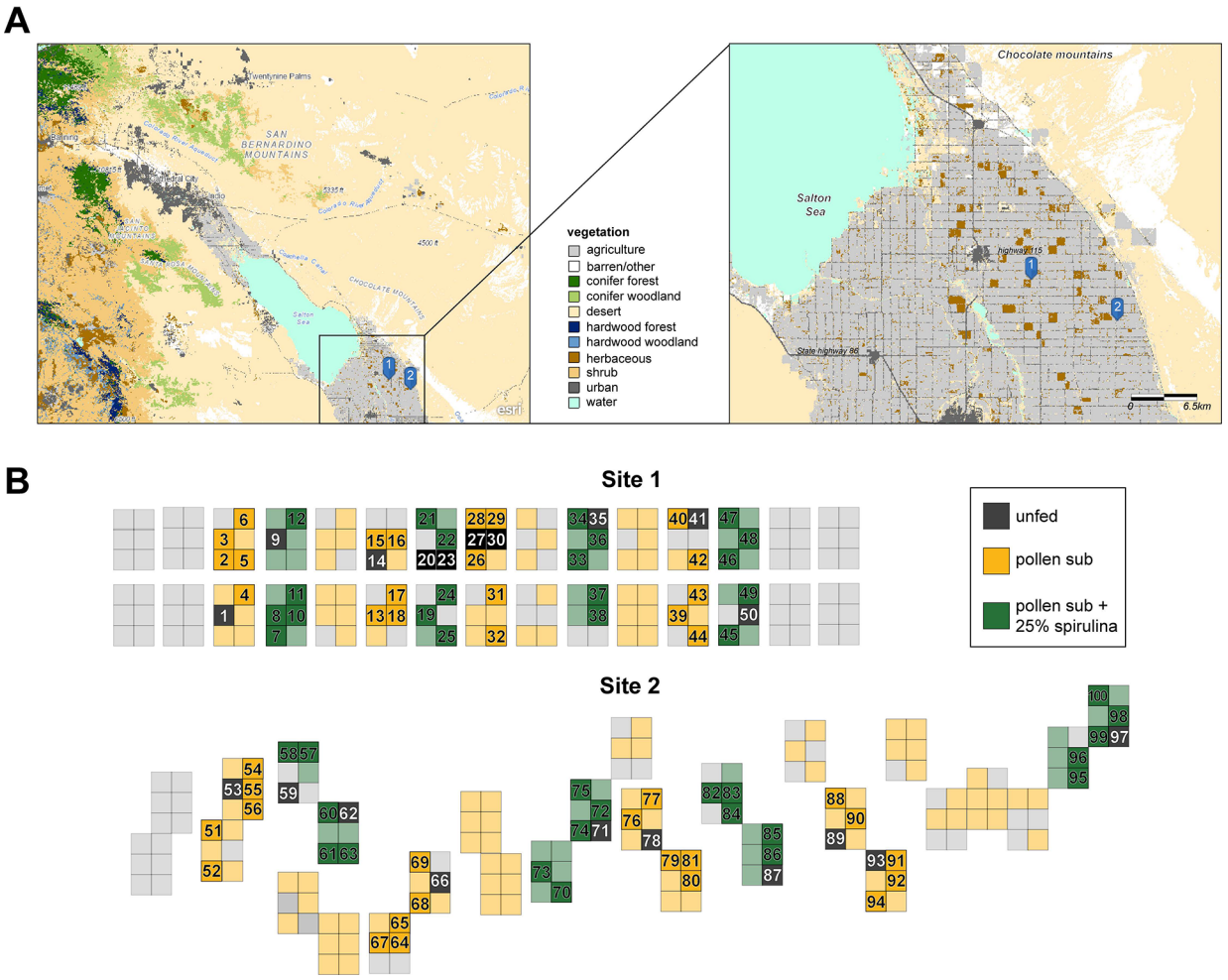

Figure 1. Study location and experimental site layouts. (A) The experiment was replicated in a randomized block design across two apiary sites denoted by blue flags as "1" and "2", respectively, on the maps. (B) Colonies were organized onto six-way pallets. Gold squares represent colonies that received a commercial artificial diet that did not contain pollen. Green squares represent colonies that received the same diet containing 25% spirulina. Black squares represent unfed controls. Gray squares indicate empty pallet positions. Only numbered colonies were included in the study. Panel A was generated using publicly available data from the State of California and the Department of Forestry and Fire Protection (URL: https://gis.data.ca.gov/maps/CALFIRE-Forestry::california-vegetation-whr13-types).

m^2/kg, a near 100-fold increase in land use efficiency.[38] Recently, microalgae have shown promise as feed additives for honey bees due to their essential nutrient density and biochemical overlap with pollen.[39] Laboratory experiments with individual bees indicate that diets containing the prokaryotic microalga *Arthrospira platensis* (commonly called spirulina) are nutritious pollen substitutes for bees.[40,41] From a practical standpoint, spirulina biomass could be formulated into patties for application to bee hives during routine colony maintenance. However, this requires validation in a field setting as honey bee nutrition is complex to evaluate because of emergent complexities of the colony and environmental stochasticity.

Pollen nutrition is especially important leading up to the use of bee colonies for crop pollination because larger, healthier colonies lead to improved pollination efficacy. In this study, we tested the effects of a microalgae diet additive on honey bee colonies managed using standard commercial apicultural practices in the Imperial Valley of southern California, United States, during a 4 month period leading up to their use in almond pollination. This location represents an agriculturally intensive environment with low rainfall and reduced natural forage that necessitates supplemental feeding. Replicated at two apiary sites, colonies were provisioned with a commercial artificial diet patty, a spirulina microalga-containing patty or were left unfed as controls. We monitored colony population size, brood production, thermoregulation, and a panel of molecular biomarkers associated with bee nutritional status, stress responses, and beneficial microbiota. We hypothesized that (i) colonies fed artificial diets would perform better than unfed controls, (ii) fine-scale brood frame imaging and continuous temperature monitoring will provide increased resolution on the impact of different feeding regimens, and (iii) a spirulina feed additive will positively influence colony performance and health biomarkers.

2. MATERIALS AND METHODS

2.1. Study Design and Honey Bee Colony Management. One hundred honey bee (*Apis mellifera*) colonies were established from splits of large, healthy parent colonies sourced from Ashurst Bee Co. Inc. (Westmoorland, CA), which managed all colonies in this study. Fifty colonies were maintained on six-way pallets at each of the two apiary sites: site 1 (33°06′12.7″N 115°27′32.4″W) and site 2 (33°04′04.5″N 115°22′09.8″W). At the start of the experiment in October 2019, feeding groups were designated as alternating rows of

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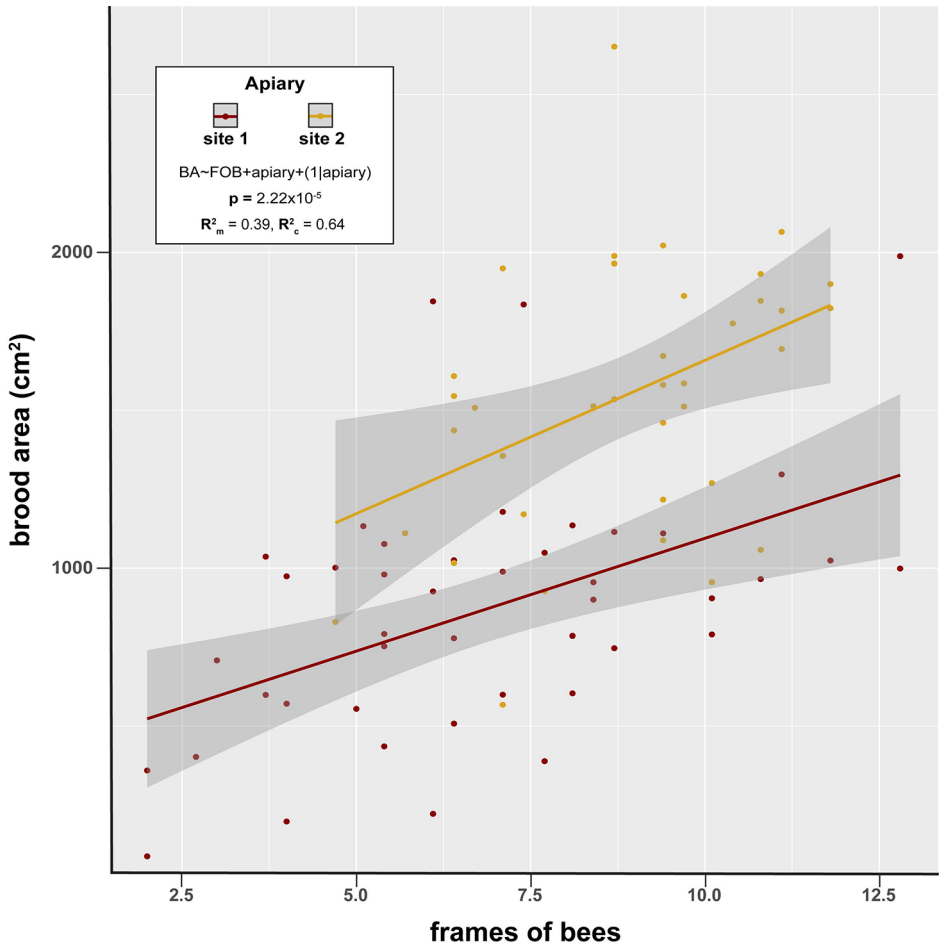

Figure 2. Brood area imaging is a quantitative and fine-scale measurement of colony performance. The strength of the linear relationship between brood area and frames of bees was tested by a mixed effects model with apiary as both a random effect and a fixed effect. Marginal R^2 (R^2_m) measures the variance explained by fixed effects alone, whereas conditional R^2 (R^2_c) measures the variance explained by the combined fixed and random effects. Asterisks denote significant covariates (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

pallets to control for positional effects within the sites (Figure 1). At each site, 40 colonies were fed an artificial diet containing a commercial pollen-free, terrestrial plant-based protein mixture (Ultra Bee, Mann Lake, the "pollen sub" colonies) or the same diet in which the protein source was substituted with 25% dried spirulina (*Arthrospira platensis* biomass; the "spirulina" colonies) (Earthrise Nutritionals, LLC, USA) (see Table S2 for full diet compositions). This ratio was determined after 100% spirulina patties proved to be unpalatable in an initial pilot experiment because of desiccation of the patties (Figure S3; Tables S3 and S4). Ten colonies at each site were randomly assigned as negative controls that were not fed and were dubbed the "unfed" colonies. Feeding treatments ("unfed", "pollen sub", or "spirulina") were applied to hives approximately every 21 days. Colonies were evaluated at the start of the experiment in October 2020 and again in February 2021 prior to their use in almond pollination services. Hive evaluations were carried out as follows: Frames of bees (FOB) were visually estimated in both top and bottom boxes by the number of between-frame spaces and half spaces covered by bees when observed from above. The same member of the team estimated all FOB values. Last, patty consumption was qualitatively assessed while replenishing patties to ensure that feed was consumed, although no quantitative measurements of consumption were taken.

All colonies were sampled for molecular analyses at the end of the study. A representative sample of brood-rearing nurse bees was collected from the central brood nest into 50 mL conical tubes, immediately frozen on dry ice, and stored at −80 °C for further processing. Molecular measures for three hives were excluded from the study because of insufficient nucleic acid yields.

2.2. Colony Brood Area Measurements via Hive Frame Imaging. For brood measurements, each brood frame was removed from the hive, gently shaken to dislodge adult bees, photographed on both sides using a 16.3 megapixel digital camera (Pentax K-01, Ricoh Imaging Co., Ltd.), and replaced in the hive. The area of sealed brood per frame was estimated from the photographs using CombCount, an open-source semiautomated Python program,[42] and ImageJ v 1.47 (W. Rasband, National Institutes of Health, USA).

2.3. Internal Hive Temperature Measurements and Analyses. Temperature sensors (iButton Thermochron, precision ±0.06 °C, accessed using 1-Wire Drivers x64, version 4.05) enclosed in plastic cassettes (Thermo Fisher Scientific, Waltham, MA) were stapled to the center of the top bar on the middle frame in the bottom hive box and set to record every 30 min. The data were then summarized for analysis as temperature amplitude, which is the daily change in brood nest temperature, by subtracting the lowest recorded temperature from the highest recorded temperature within a given day.

Three dates were chosen for *post hoc* analysis to test specific hypotheses related to hive temperature amplitude. We first tested whether treatment groups were identical at the beginning of the study as they were normalized by colony size. We then tested (2 February) if 4 months of continuous supplemental feeding would result in significantly improved colony thermoregulation. Finally (12 December), we tested if colonies experienced benefits from supplemental feed when temperature amplitude peaked, assuming that colonies

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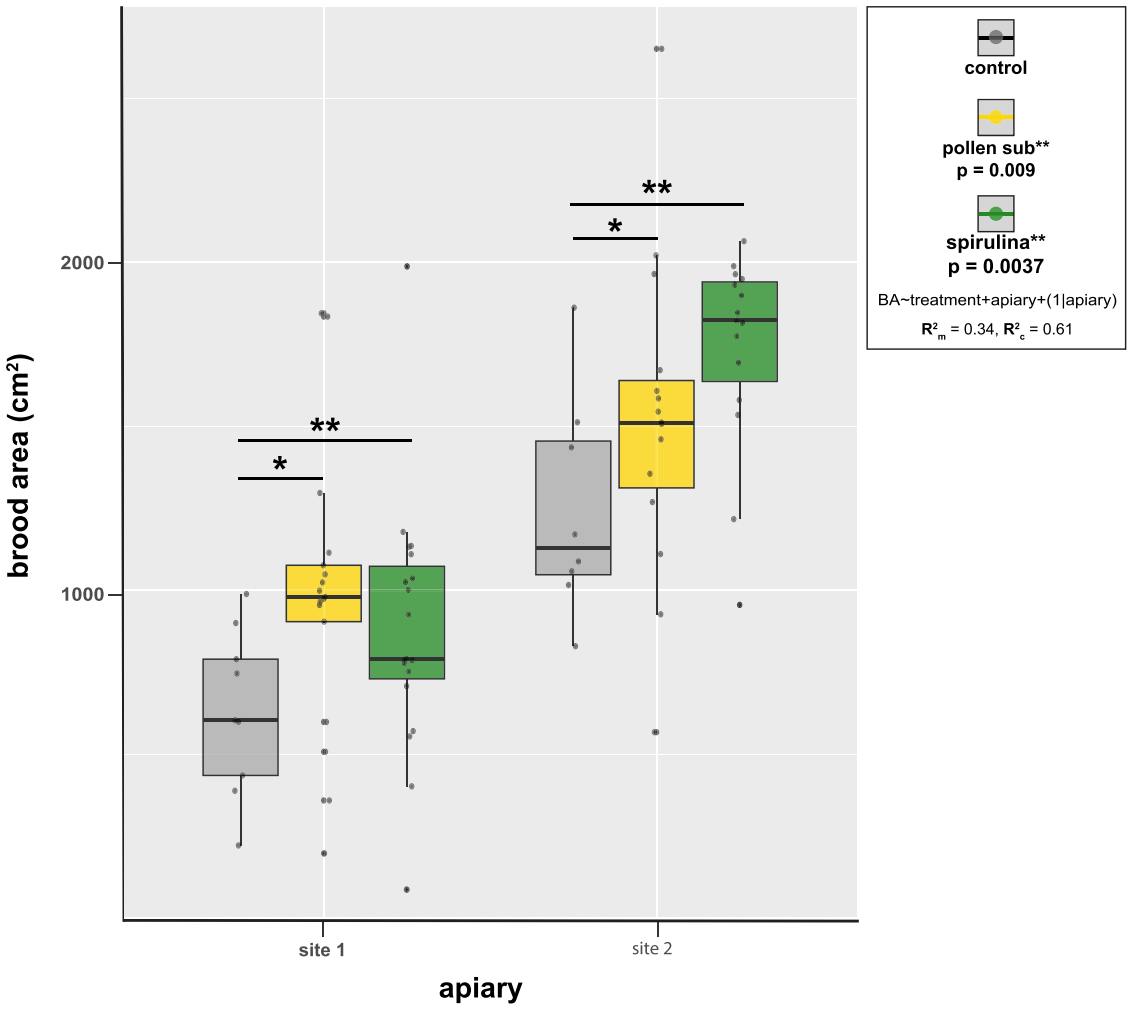

Figure 3. Artificial diets increase colony brood production relative to unfed controls. A mixed effects model was used to predict colony brood area as a function of treatment with apiary as both a random and fixed effect. Feed supplementation increased brood area overall. Marginal R^2 (R^2_m) measures the variance explained by fixed effects alone, whereas conditional R^2 (R^2_c) measures the variance explained by the combined fixed and random effects. Marginal means were extracted from the model to assess differences in brood area indicating that feed supplementation increased brood area within sites. Asterisks denote significant covariates (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

were most temperature stressed at this time. We also tested the date at which the difference in temperature amplitude between unfed and supplemented colonies peaked (15 November).

2.4. Molecular Biomarker Analyses via Quantitative Polymerase Chain Reaction (qPCR). Pools of 30 field-collected bee abdomens were homogenized in 2 mL of Maxwell simplyRNA homogenization solution (Promega, Madison, WI, USA) using a Bead Rupture Elite bead mill (OMNI International, Kennesaw, GA, USA). Samples were centrifuged, and 100 μL of the supernatant was removed for RNA extraction with a Monarch total RNA miniprep kit (New England BioLabs, Ipswich, MA, USA) according to the manufacturer's instructions. Complementary DNA (cDNA) template was generated using 1 μg of total RNA and a LunaScript RT SuperMix Kit (New England BioLabs) following manufacturer's instructions. Quantitative PCR was carried out using iTaq Universal SYBR Green Supermix (Biorad) in triplicate on a CFX96 Real-Time PCR Detection System (Biorad). See Table S1 for a full list of targets measured in this study. PCRs were performed in triplicate as follows: initial denaturation at 95 °C for 5 min; 40 cycles with denaturation at 95 °C for 15 s; and a primer-pair-specific annealing temperature for 30 s. All amplicons were verified by Sanger sequencing here or in a previous study (see the "reference" column on Table S1) to verify specificity. To confirm the absence of contaminating genomic DNA and primer dimers in the qPCR assay, we monitored amplification and melting curves in negative controls consisting of DNase-treated total RNA without reverse transcriptase. Relative gene expression was determined based on standardized Ct values (ΔCt) using the geometric mean of rp49 and β-actin Ct values as a composite reference value.

2.5. Statistical Analyses. Analyses were conducted and figures were generated in R version 4.1.2 "Bird Hippie".[43] Simple linear regression models were constructed using the base R *stats* package, and mixed effects models were fit using the *lme4* package.[44,45] All fixed effect structures for linear models were determined *a priori*, but random effect structures for random intercept models were determined using forward selection by REML criterion minimization. Hive ID, site, and date were assessed as potential random effects during model fitting to account for the autocorrelation of variables due to repeated measures of hives and the environmental effect on hive physiological parameters within a given date or site. Marginal means of brood area were calculated using the *marginaleffects* package.[46] Where the errors of evaluated models deviated from normality, the dependent variables were log transformed. A locally weighted running line smoother function was used to fit the polynomial function and corresponding 95% confidence interval of the true parameter shaded in gray in Figure 4A.

https://doi.org/10.1021/acsagscitech.3c00082
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3. RESULTS

3.1. Apiary Site Locations and Random Block Design. A randomized block design was employed across two apiaries in a setting with heavy agricultural land use surrounded by desert (Figure 1A). This environment is an ideal venue for field experiments involving nutritional manipulations because it features significantly reduced natural forage and is relatively homogeneous. Although site 2 was qualitatively in closer proximity to more herbaceous landcover (in brown) than site 1, both landscapes were dominated by agriculture. Given a typical foraging distance of under 5 km,[47] there was likely minimal overlap in the bee forage range between sites.

At the start of the experiment, 50 colonies at each site were chosen based on similar starting population sizes (hive frame coverage of bees) and then divided into pallet-level treatment blocks (pollen substitute or spirulina) with a randomized allocation of 10 unfed negative control colonies to treatment blocks at each site (Figure 1B). This design aimed to minimize random spatial effects within the apiaries that may confound field experiments.

3.2. Fine-Scale Imaging of Colony Brood Area Predicts a Beekeeping Industry Standard Performance Metric of Honey Bee Population Size. Colony size was assessed by "frame size" (i.e., number of frames more than two-thirds covered by adult bees), and brood production was quantitatively assessed from frame photographs by measuring brood area in square centimeters (cm^2). In this data set, frames of bees were strongly associated with brood area (Figure 2; estimate = 78.5, t = 4.5, $p = 2.22 \times 10^{-5}$, $R^2_{marginal}$ = 0.39, $R^2_{conditional}$ = 0.64 with apiary as a random effect). Therefore, we used brood area as a more quantitative and precise measure of colony performance for further analyses.

3.3. Spirulina Supplementation Increases Honey Bee Colony Brood Production. Brood area was measured at the beginning of the study (October 2020) and again at the end of the study (February 2021). By design, there was no difference between treatment groups in measured brood area or frames of bees in October 2020 (Figure S1). Feed supplementation resulted in significantly larger brood area than colonies that were unfed by February 2021 (Figure 3; pollen substitute-control: t = 2.8, p = 0.009; spirulina-control: t = 3.09, p = 0.0028; $R^2_{marginal}$ = 0.34, $R^2_{conditional}$ = 0.61 with apiary as a random effect). This effect was consistent within sites as well (site 1: pollen substitute-control: z = 2.68, p = 0.029; spirulina-control: z = 3.09, p = 0.0079; site 2: pollen substitute-control: z = 2.68, p = 0.029; spirulina-control: z = 3.09, p = 0.0079) Interestingly, in apiary site 2, spirulina-fed colonies had a mean brood area of 1736 ± 101 cm^2, whereas pollen-substitute-fed colonies had a mean brood area of 1517 ± 101 cm^2, although this difference was not statistically significant (simple linear regression with Bonferroni correction for multiple *post hoc* comparisons). In support of the use of this more quantitative colony performance metric, there was no treatment effect on frames of bees at the end of the study (Figure S2).

3.4. Spirulina Supplementation Improves Honey Bee Colony Thermoregulation. Internal hive temperature was recorded in 30 min increments in the brood chamber over the course of the study and summarized as temperature amplitude (i.e., the differences between maximum and the minimum temperature recorded in a day). Colonies had lower temperature amplitudes when they were provisioned with the pollen substitute (p = 0.001, linear mixed model with date and colony as a random effect) or the spirulina substitute (p = 0.0006), indicating that supplementation improved brood chamber thermoregulation (Figure 4A). Whereas there was no difference in baseline temperature amplitudes at the beginning of

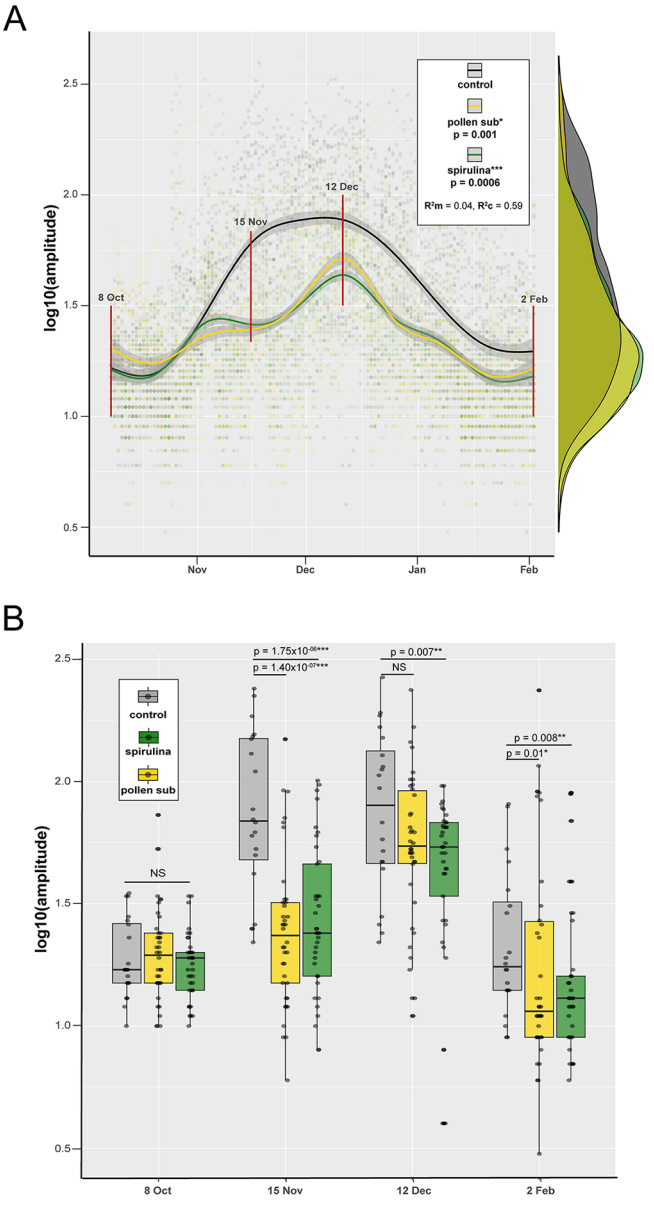

Figure 4. Artificial diets improve colony thermoregulation. (A) Temperature amplitude was predicted as a function of treatment. Pollen-substitute- and spirulina-containing diets both reduced temperature amplitude over the course of the study compared to the control, indicating improved thermoregulation. The density plot on the right margin shows the distribution of amplitude measurements by treatment group. (B) Temperature amplitude was extracted from four dates (8 Oct 2020, 15 Nov 2020, 12 Dec 2020, and 3 Feb 2021), and a mixed effects model was used to assess the treatment effect. There were no differences at the beginning of the study. Fed colonies had lower temperature amplitudes in November, December, and February relative to unfed controls. Marginal R^2 (R^2_m) measures the variance explained by fixed effects alone, whereas conditional R^2 (R^2_c) measures the variance explained by the combined fixed and random effects. Date and apiary were both included as random effects. Asterisks denote significant covariates (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

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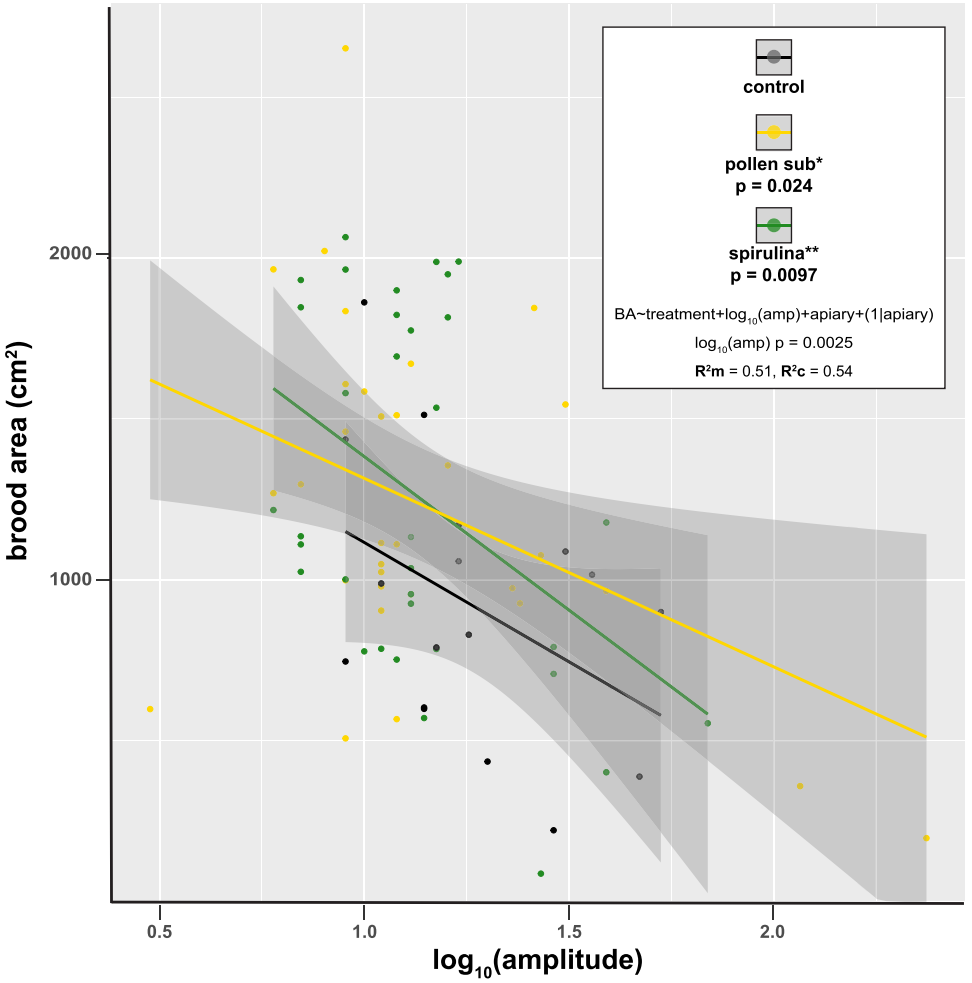

Figure 5. Improved colony thermoregulation is associated with larger brood areas. Brood area was predicted as a function of the log of the temperature amplitude using a mixed effects model. A higher amplitude was significantly associated with a smaller brood area. Treatment and apiary were included as covariates with apiary as a random effect. Marginal R^2 (R^2_m) measures the variance explained by fixed effects alone, whereas conditional R^2 (R^2_c) measures the variance explained by the combined fixed and random effects. Asterisks denote significant covariates (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

the study ($F = 0.712$, $p = 0.493$, simple linear regression), the difference in thermoregulation was the greatest around mid-November (pollen substitute-control: $t = -5.7$, $p = 1.4 \times 10^{-7}$; spirulina-control: $t = -5.11$, $p = 1.75 \times 10^{-6}$; simple linear regression; Figure 4B). However, when temperature amplitude peaked on December 12th, only the spirulina-fed colonies had lower amplitudes than unfed colonies ($t = -2.774$, $p = 0.0067$; simple linear regression).

At the end of the study, both pollen-substitute-fed and spirulina-fed colonies had lower temperature amplitude (pollen substitute-control: $t = -2.59$, $p = 0.011$; spirulina-control: $t = -2.72$, $p = 0.008$; multiple linear regression, Figure 4B). During the final inspection on February 3rd, the beekeeper made the decision to remove a medium hive body from several colonies due to reduced adult populations. This was a significant covariate as colonies that would have a medium removed on February 3rd had significantly higher temperature amplitude on February 2nd (estimate = 4.8 °C, $t = 6.7$, $p = 2.84 \times 10^{-9}$).

Furthermore, pollen-substitute- and spirulina-fed colonies had larger brood areas (pollen substitute-control: $p = 0.024$; spirulina-control: 0.0097, linear mixed model), and these lower temperature amplitudes significantly predicted larger brood areas ($p = 0.0025$, linear mixed model, $R^2_{marginal} = 0.513$, $R^2_{conditional} = 0.54$, Figure 5).

3.5. Honey Bee Colonies Fed Spirulina Had Distinct Gene Expression Profiles That Were Primarily Driven by Apiary Site. We measured the expression profile of putative molecular biomarkers (see Table S1 for all genes tested) in bee samples collected from each colony. Multidimensional analysis was applied to the relative expression of nine genes (including antiviral immune genes, heat shock proteins, and antimicrobial peptides), four core constituents of the honey bee gut microbiome, and the log of the temperature amplitude to test the hypothesis that feeding regimen would result in unique gene expression profiles. In fact, there was significant separation by treatment, but apiary site predominantly explains the majority of the variation in gene expression (FAMD, Figure 6). There was differentiation between bees from spirulina-fed and unfed control colonies along dimension 4 and differentiation between bees from spirulina-fed and pollen-substitute-fed colonies along dimension 5 (FAMD, Figure 7), although these dimensions only explained 7.3 and 5.2% of the variation in measured mRNA targets (12.5% of total variation). *S. alvi*, *Vg-like-A*, *VgMC*, and *hymenoptaecin* expressions are primarily loading onto dimension 4, suggesting that these are

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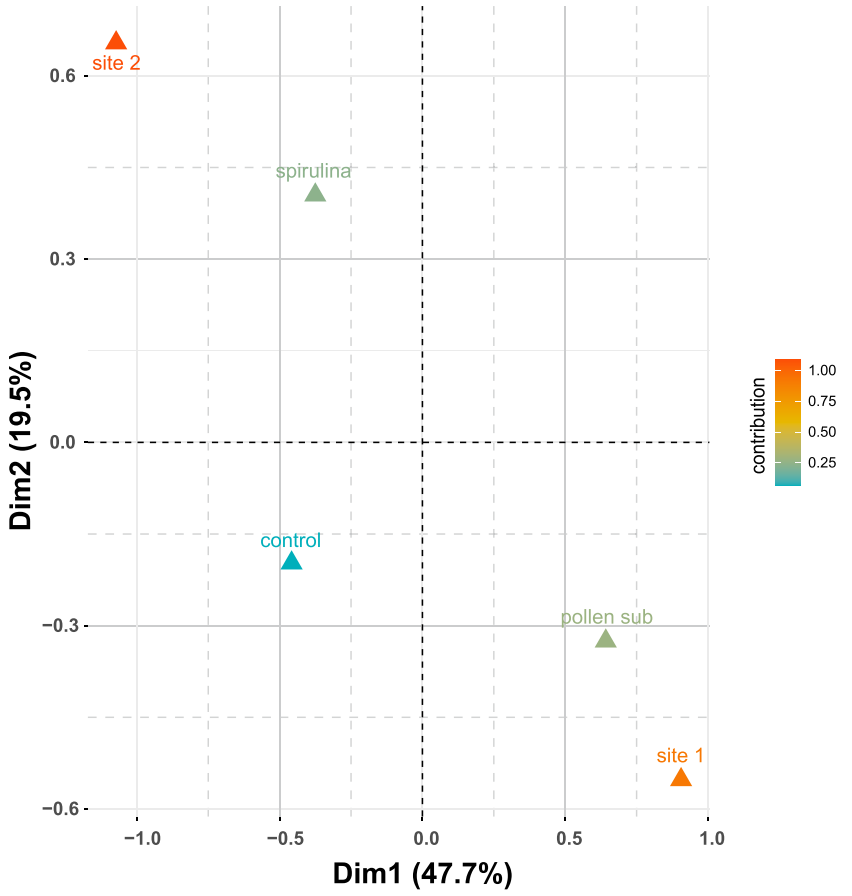

Figure 6. Expression profiles of putative molecular biomarkers are mainly driven by apiary site. Factor analysis of mixed data (FAMD) was used to test the hypothesis that gene expression profiles (see Table S1) were being driven by treatment (control, pollen substitute, or spirulina). Most of the variation was explained by site effects, indicating a landscape-level effect. "Contribution" is a measure of how much variance is explained by a given variable on a scale ranging from low contribution (teal) to high contribution (red).

driving most of the variation between treatment groups along that axis. It is less clear which variables are driving differentiation between pollen-substitute- and spirulina-fed bees along dimension 5, although the weak contributors are *Firmicutes 5*, *bee antiviral protein 1*, *heat shock protein 90*, and *heat shock protein 70 cognate 3* (FAMD, Figure S4).

Interestingly, site 2 had larger brood area than site 1 (F = 56.49, $p = 6.7 \times 10^{-11}$, ANOVA), and bees collected from colonies at site 2 had higher abundance of *S. alvi* (F = 144.2, $p = 2.2 \times 10^{-16}$, simple linear regression), lower *bap1* expression (F = 75.71, $p = 3.1 \times 10^{-13}$), and lower *hsc70-3* expression (F = 50.35, $p = 4.41 \times 10^{-10}$). We postulate that the design captured an environmental effect on gene expression differences associated with brood area. In support of this, a strong positive association between the expression of two putative molecular biomarkers (*bap1* and *hsc70-3*) was observed across and within apiaries (F = 289, $p < 2 \times 10^{-16}$, $R^2_{\text{marginal}} = 0.708$, $R^2_{\text{conditional}} = 0.908$, linear mixed model with apiary as a random effect, Figure S5) similar to what was seen in previous cage studies.[48]

4. DISCUSSION

Honey bees meet all of their nutritional needs from floral resources in the environment. Monoculture cropping systems are the dominant method of human food production, which have resulted in landscapes with reduced capacity to support plant-pollinator networks.[4,49−51] These depauperate floral landscapes may also fail to supply adequate nutrition to bees, especially when colonies are maintained at high densities leading up to and during pollination events.[32,33,52−54] Research aimed at feed development for honey bees is lagging behind other livestock and companion animals.[55] Therefore, as societal dependence on honey bee pollination services expands, it is imperative to develop sustainable and efficacious strategies to provision bee colonies on an agricultural scale. This study tested the effects of a spirulina feed additive on commercially managed honey bee colonies involved in almond pollination. The Imperial Valley of southern California represents an agriculturally intensive desert environment (Figure 1) with reduced natural forage that necessitates supplemental feeding. This location is ideal for testing novel feed formulations and provides an extreme example of the nutritional limitations that could be faced by modern beekeeping due to climate change and land-use conversion.

We used hive brood frame imaging and continuous temperature monitoring to evaluate honey bee colony performance in response to different supplemental feeding regimens. Although adult populations and brood production are traditionally estimated by visual inspection, more sensitive and accurate tools are necessary because bee nutritional responses are complex to evaluate, especially at the colony level. Advances in camera, software, and sensor technology have resulted in less expensive and more accurate methods that make using these approaches easier and more informative than

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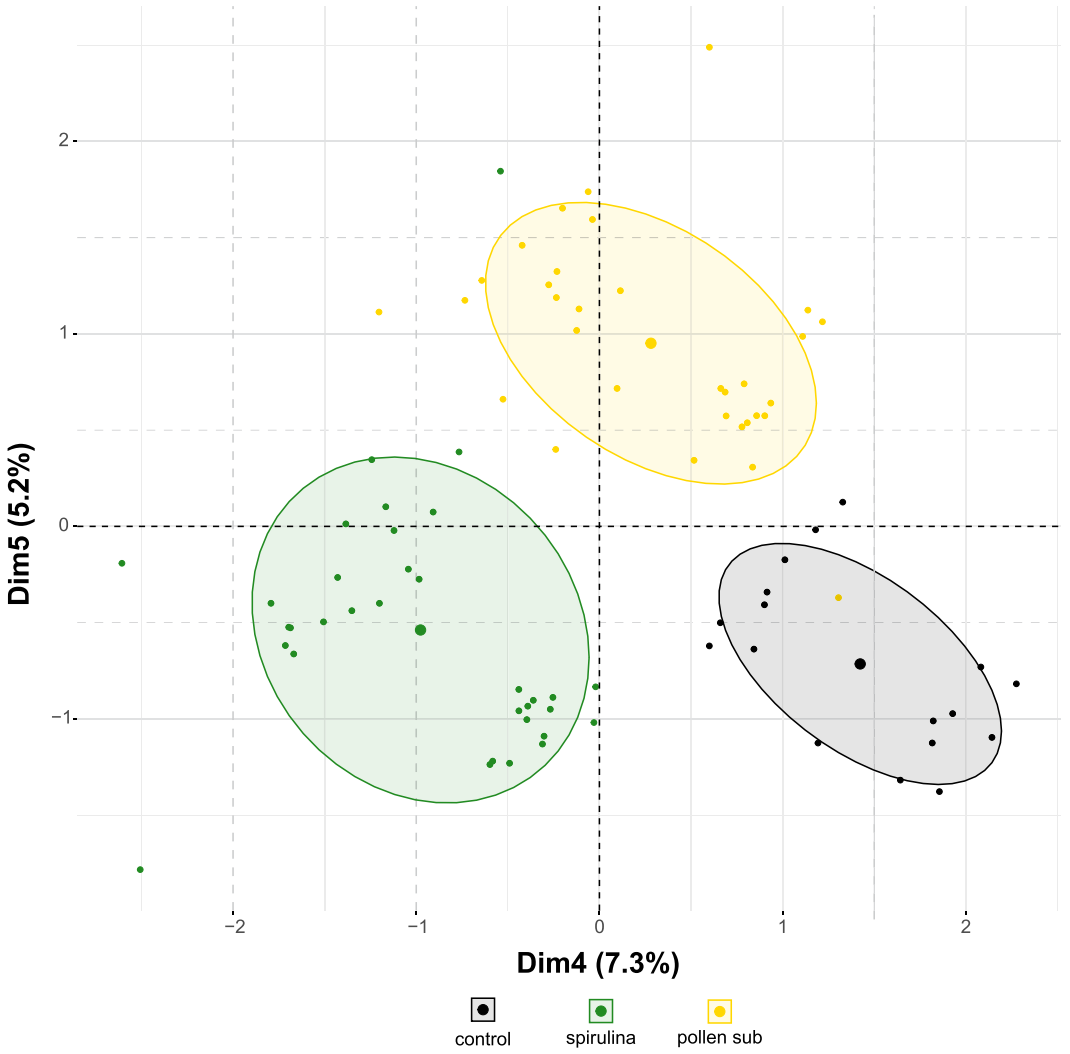

Figure 7. Spirulina-fed bees exhibit distinct gene expression profiles. FAMD analysis revealed that dimensions 4 and 5 explained cumulatively a small amount of variation (12.5%) in gene expression profiles and that (A) treatment groups were separating along these dimensions with (B) moderate loading of antimicrobial peptides *hymenoptaecin* and *apidaecin* onto dimension 4. Ellipses represent 90% confidence of treatment group membership.

visual inspection alone.[56] Spirulina supplementation led to increased colony brood production as measured by hive frame imaging. Our results agree with previous studies in which spirulina diets had positive impacts on individual bee nutritional physiology. In laboratory cages, bees fed spirulina had greater head weights, indicating nutrient assimilation into head glands that sustain brood via proteinaceous secretions.[57] Proteome analyses of the honey bee fat body, a tissue with central nutrient storage and metabolic functions, indicated that pollen and spirulina diets led to comparable protein assimilation and a marked overlap in proteome expression patterns.[58] Taking this into consideration along with the results of the current study, spirulina is bioavailable to honey bees and appears to support colony-level brood production under field conditions. A more granular approach in which the change in patty weight is measured would be useful in comparing actual consumption of patties with and without spirulina as well as any potential effects of the amount of the patty consumed on measured colony parameters.

It is intriguing that spirulina-fed colonies had marginally higher brood area than pollen-substitute-fed colonies at site 2, although the difference is not statistically significant (Figure 3). Although it is tempting to speculate about the complementary potential of provided supplements and environmentally available nutrients, it is equally likely that this observation was spurious. Commercially available diets for bees typically incorporate protein-rich ingredients such as soy, corn gluten, and yeast as a substitute for the essential amino acids provided by natural pollen. Honey bees cannot synthesize arginine, histidine, lysine, tryptophan, phenylalanine, methionine, threonine, leucine, isoleucine, and valine, and therefore, they must be obtained from the diet.[59] We previously compared essential amino acid compositions of bee diets formulated with mixed pollen, spirulina, and the same pollen substitute used in this study. Essential amino acid levels in spirulina matched or exceeded the other tested diets except for histidine and lysine, which were higher in pollen.[41] Although significant research effort has been focused on protein nutrition in bees, other macronutrients in spirulina could have contributed to the observed site-dependent increase in brood production.

Relative to what is known about bee protein requirements, the nutritional value and functions of dietary lipids are comparatively less understood. Linoleic acid and α-linolenic acid are two polyunsaturated fatty acids that are essential for

https://doi.org/10.1021/acsagscitech.3c00082
ACS Agric. Sci. Technol. 2023, 3, 748−759

bees.[60,61] Lipidomic analyses of spirulina bee diets indicated a broad diversity of lipid molecular species incorporating linoleic acid and α-linolenic acid residues.[41] Although spirulina can provide essential lipids, other microalga species may be a more promising source due to their increased lipid accumulation. For example, the lipid contents of edible green algae in the genus *Chlorella* range from 13 to 21%, whereas the lipid content of spirulina ranges from 2 to 7%.[36] Laboratory comparisons of bees fed *Chlorella vulgaris* had higher levels of linoleic acid and similar levels of α-linolenic acid relative to pollen-fed bees, whereas spirulina-fed bees had the lowest levels of both fatty acids.[40] Nevertheless, the protein content of spirulina ranges from 60 to 66%, and the protein content of *Chlorella* ranges from 38 to 48%,[36] indicating that both microalgae are promising feed additives to address different bee macronutrient requirements.

Colony brood nest temperatures were continuously monitored by sensors placed within the central brood nest of each hive. Thermoregulation is important to bee colony fitness because healthy brood development requires temperatures of 34−36 °C.[62] We observed that colonies with lower temperature amplitudes had larger brood areas and that both metrics were improved by supplemental feeding (Figures 4 and 5) due to improved overall colony physiology. It is also worth noting that, in this study, spirulina-fed colonies but not pollen-substitute-fed colonies had significantly improved thermoregulation compared to control colonies at the December midpoint of the study even though spirulina-fed colony temperature amplitude was not different from that of pollen-substitute-fed colonies (Figure 4B). Furthermore, although all colonies at had temperature amplitudes decrease back to near-starting levels, supplemented colonies still performed better at the end of the study (Figure 4B), an overwintering outcome that typically results in more robust spring population growth. Future studies should test whether winter spirulina supplementation can improve long-term colony survival.

Different landscape factors and pesticide exposure can influence colony thermoregulation.[63,64] In this study, we found that differences in hive temperature data corresponded to feed manipulations in a commercial beekeeping operation. This finding highlights the potential of sensor data to inform future studies and management practices related to supplemental feeding, especially in large-scale beekeeping operations. In fact, in-hive sensor data predicted a smaller adult population prior to the beekeeper making the management decision to downsize the hives to accommodate a smaller population. Taking this into consideration, remote data retrieval from in-hive sensors could have facilitated this management decision, thereby improving management efficiency.

Because honey bee colonies are adaptively organized groups of related individuals, we used a pooled sampling approach to overcome individual variation and to represent the average physiological status of adult bees localized to the central brood nest of each colony. Here we measured the expression of nine genes chosen for their roles in health (i.e., *vg*, *vg-like-A*) as well as stress resistance and immunity (i.e., *hymenoptaecin*, *apidaecin*, *ago2*, *hsp90*, *hsc70-3*, *pl2*, and *bap1*) as well as four core members of the honey bee microbiome. Some of these were previously used in laboratory studies of individual bees fed microalgae. For instance, both spirulina and *Chlorella* alga diets increased mRNA and protein levels of vitellogenin (Vg), a nutritional storage protein that is used as a biomarker of nutritional status and diet quality.[58,65,66] In this study, we did not observe a feeding treatment effect on *vg* levels. Instead, we found that *vg* expression was primarily driven by apiary site, which agrees with similar analyses of pooled, field-collected bee samples.[32,53,67] Previous studies indicate that mRNA and protein levels of *heat shock protein 90* (*hsp 90*) were upregulated in caged bees fed spirulina, suggesting improved stress resistance potential.[40] Like *vg* expression, *hsp90* levels were more strongly impacted by apiary site. Expressions of *hsc70-3* and *bap1* were both strongly negatively associated with brood area across sites but not within sites. The implication of this *post hoc* observation is not clear, but it is interesting to speculate that reduced expression of these genes indicates lower stress levels, which led to larger brood areas. However, we did not find a relationship between these physiological parameters within sites, indicating that another unmeasured difference between sites is driving greater *S. alvi* abundance, lower relative expression of *hsc70-3* and *bap1*, and higher brood area at site 2. Given the current state of knowledge of colony-level gene expression, it is difficult to form reasonable hypotheses about the direction and magnitude of expression of these particular molecular markers in response to feed supplementation. Therefore, we applied multidimensional analysis to test the hypothesis that different feeding regimens would result in unique broader gene expression profiles. Multidimensional factor analysis of colony gene expression data revealed clear differentiation by treatment along the fourth and fifth dimensions, which explained only 12.5% of the total variation. Clear separation of spirulina-fed colonies implies global gene expression differences, although the consequences of this remain largely unknown. Taken together with colony performance data, the result suggests that positive effects of spirulina supplementation may be associated with distinct impacts on bee physiology. More granular physiological studies and analyses of global gene expression by transcriptome sequencing are necessary to better understand how spirulina and other microalgae uniquely affect bee health.

Gut microbiota abundance was positively modulated in bees fed alga diets and supplements.[39] For instance, bees fed spirulina in laboratory cages had increased abundance of the gut symbiont *Snodgrassella alvi*, a gut symbiont associated with individual bee health.[41] In the present study, we found that site 2 had both higher relative abundance *S. alvi* and larger colony brood areas, although it was not impacted by feed type. As mentioned above, this relationship was confounded by apiary site, but this indicates a potential landscape scale effect of diet on gut microbe abundance. These data demonstrate the utility of highly quantitative, colony-level measurements to better understand the role of bee-associated microbes in a field setting.

Algae are attractive feed additives due to their nutrient density and sustainability of biomass production.[68−70] Adapting beekeeping management practices to incorporate microalga feed additives could help achieve objectives outlined in the United Nations Sustainable Development Goals (https://sdgs.un.org/goals) related to food security, sustainable water management, sustainable consumption, climate change, reversal of land degradation, and halting biodiversity loss. Industrial cultivation of microalgae has markedly accelerated over the last few decades.[71] Alga biomass is produced on a large scale and sold directly as food and diet supplements, whereas refined products or extracts are used by pharmaceutical and cosmetic industries.[72] In addition to its nutritional value, spirulina is cultivated for phycocyanin, a

https://doi.org/10.1021/acsagscitech.3c00082
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natural blue colorant extracted from biomass that still retains its protein content.[73] This spent biomass could prove useful as an essential amino acid source for bee feed development. Therefore, low-cost bee diets could theoretically be formulated with byproducts of industrial alga production.

There is significant potential for microalgae to be engineered for improved nutritional value and to serve as novel therapeutic production platforms.[74−76] The nutritional value of alga strains could conceivably be augmented via bioengineering to produce honey bee nutrient storage proteins, such as vitellogenin and royal jelly proteins. Such alga strains could more closely recapitulate the dietary amino acid requirements of bees.

Complex and difficult to synthesize biomolecules such as vaccines and monoclonal antibodies can be produced in algae.[74,77] For example, alga hosts could be used to produce nucleic-acid-based therapeutics against bee pests and pathogens. Bees depend on RNA interference (RNAi) as a post-transcriptional gene expression regulatory mechanism and core component of their antiviral immune system. Double-stranded RNA (dsRNA) molecules or hair-pinned microRNAs are processed and then used by the RNA-induced silencing complex (RISC), which triggers an immune cascade that inhibits translation of target RNA molecules in a sequence-specific manner.[78−81] Prokaryotic microalgae such as spirulina can produce large quantities of dsRNA using light and CO_2, making them a sustainable source of dsRNA, which is expensive to produce *in vitro*.[82] Recently, a honey bee gut symbiont was engineered to deliver sequence-specific dsRNAs, resulting in RNAi activation.[83] Similarly, engineered *E. coli* has been used to produce dsRNAs for bees, although dsRNA purification was necessary prior to administration via feeding.[84] These approaches present major obstacles for use in bee colonies, mainly due to their scalability to field applications. On the other hand, engineered algae could be formulated into nutritious patties and delivered to honey bees via feeding regimens that are already implemented by beekeepers.

■ ASSOCIATED CONTENT

Data Availability Statement

All raw data generated in this study are available in Table S3, S5, and S7.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsagscitech.3c00082.

Oligonucleotides used in this study (Table S1); compositions of diets used in this study (Table S2); raw temperature amplitude data used to generate Figure S3 (Table S3); output of the linear mixed model used to analyze temperature amplitude data in Figure 4 (Table S4); raw temperature amplitude data used to generate Figure 4 (Table S5); output of the linear mixed model used to analyze temperature amplitude data in Figure S3 (Table S6); and raw values for frames of bees, brood area, and qPCR targets collected from study colonies at the end of the study (Table S7) (XLSX)

Frames of bees and brood area in colonies at the start of the study (Figure S1); frames of bees at the end of the study (Figure S2); hive temperature amplitude in an initial field trial (Figure S3); dimensions 4 and 5 variable loadings corresponding to the factor analysis in Figure 7 (Figure S4); and correlation between the expression of *hsc70-3* and *bap1* in randomly sampled nurse bees (Figure S5) (PDF)

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Funding

This work was funded by Agriculture and Food Research Initiative grant no. 2021-67013-33556 from the USDA National Institute of Food and Agriculture. This research was supported in part by an appointment to the Agricultural Research Service (ARS) Research Participation Program administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy (DOE) and the U.S. Department of Agriculture (USDA). ORISE is managed by ORAU under DOE contract number DE-SC0014664. All opinions expressed in this paper are the authors' and do not necessarily reflect the policies and views of USDA, DOE, or ORAU/ORISE.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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■ REFERENCES

(1) Godfray, H. C. J.; Beddington, J. R.; Crute, I. R.; Haddad, L.; Lawrence, D.; Muir, J. F.; et al. Food Security: The Challenge of Feeding 9 Billion People. *Science* **2010**, *327*, 812−818.

(2) HLEF *Global agriculture towards 2050*; Division ADE; 2009 13 Oct 2009.

(3) FAO, IFAD, UNICEF, WFP, WHO *The State of Food Security and Nutrition in the World*; Rome, Italy; 2022.

(4) Goulson, D.; Nicholls, E.; Botías, C.; Rotheray, E. L. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science (New York, NY)* **2015**, *347*, No. 1255957.
(5) Raven, P. H.; Wagner, D. L. Agricultural intensification and climate change are rapidly decreasing insect biodiversity. *Proc. Natl. Acad. Sci.* **2021**, *118*, No. e2002548117.
(6) Wagner, D. L. Insect Declines in the Anthropocene. *Annu. Rev. Entomol.* **2020**, *65*, 457−480.
(7) Fenoglio, M. S.; Calviño, A.; González, E.; Salvo, A.; Videla, M. Urbanisation drivers and underlying mechanisms of terrestrial insect diversity loss in cities. *Ecol. Entomol.* **2021**, *46*, 757−771.
(8) Ollerton, J.; Winfree, R.; Tarrant, S. How many flowering plants are pollinated by animals? *Oikos* **2011**, *120*, 321−326.
(9) Calderone, N. W. Insect pollinated crops, insect pollinators and US agriculture: Trend analysis of aggregate data for the period 1992-2009. *PLoS One* **2012**, *7*, 24−28.
(10) Cameron, S. A.; Lozier, J. D.; Strange, J. P.; Koch, J. B.; Cordes, N.; Solter, L. F.; et al. Patterns of widespread decline in North American bumble bees. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 662−667.
(11) Gallai, N.; Salles, J.-M.; Settele, J.; Vaissière, B. E. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecol. Econ.* **2009**, *68*, 810−821.
(12) Aizen, M. A.; Harder, L. D. The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. *Curr. Biol.* **2009**, *19*, 915−918.
(13) Burkle, L. A.; Marlin, J. C.; Knight, T. M. Plant-Pollinator Interactions over 120 Years: Loss of Species, Co-Occurrence and Function. *Science (New York, NY)* **2013**, *339*, 1611−1615.
(14) Traynor, K. S.; Rennich, K.; Forsgren, E.; Rose, R.; Pettis, J.; Kunkel, G.; et al. Multiyear survey targeting disease incidence in US honey bees. *Apidologie* **2016**, *47*, 325−347.
(15) Faurot-Daniels, C.; Glenny, W.; Daughenbaugh, K. F.; McMenamin, A. J.; Burkle, L. A.; Flenniken, M. L. Longitudinal monitoring of honey bee colonies reveals dynamic nature of virus abundance and indicates a negative impact of Lake Sinai virus 2 on colony health. *PLoS One* **2020**, *15*, No. e0237544.
(16) Glenny, W.; Cavigli, I.; Daughenbaugh, K. F.; Radford, R.; Kegley, S. E.; Flenniken, M. L. Honey bee (Apis mellifera) colony health and pathogen composition in migratory beekeeping operations involved in California almond pollination. *PLoS One* **2017**, *12*, No. e0182814.
(17) Genersch, E.; Von Der Ohe, W.; Kaatz, H.; Schroeder, A.; Otten, C.; Büchler, R.; et al. The German bee monitoring project: A long term study to understand periodically high winter losses of honey bee colonies. *Apidologie* **2010**, *41*, 332−352.
(18) Budge, G. E.; Pietravalle, S.; Brown, M.; Laurenson, L.; Jones, B.; Tomkies, V.; et al. Pathogens as Predictors of Honey Bee Colony Strength in England and Wales. *PLoS One* **2015**, *10*, No. e0133228.
(19) Nazzi, F.; Brown, S. P.; Annoscia, D.; Del Piccolo, F.; Di Prisco, G.; Varricchio, P.; et al. Synergistic parasite-pathogen interactions mediated by host immunity can drive the collapse of honeybee colonies. *PLoS Pathog.* **2012**, *8*, No. e1002735.
(20) Dainat, B.; Evans, J. D.; Chen, Y. P.; Gauthier, L.; Neumann, P. Predictive markers of honey bee colony collapse. *PLoS One* **2012**, *7*, No. e32151.
(21) Dainat, B.; Neumann, P. Clinical signs of deformed wing virus infection are predictive markers for honey bee colony losses. *J. Invertebr. Pathol.* **2013**, *112*, 278−280.
(22) DeGrandi-Hoffman, G.; Chen, Y.; Huang, E.; Huang, M. H. The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (Apis mellifera L.). *J. Insect Physiol.* **2010**, *56*, 1184−1191.
(23) DeGrandi-Hoffman, G.; Chen, Y. Nutrition, immunity and viral infections in honey bees. *Curr. Opin. Insect Sci.* **2015**, *10*, 170−176.
(24) Tritschler, M.; Vollmann, J. J.; Yañez, O.; Chejanovsky, N.; Crailsheim, K.; Neumann, P. Protein nutrition governs within-host race of honey bee pathogens. *Sci. Rep.* **2017**, *7*, 14988.
(25) Di Pasquale, G.; Salignon, M.; Le Conte, Y.; Belzunces, L. P.; Decourtye, A.; Kretzschmar, A.; et al. Influence of Pollen Nutrition on Honey Bee Health: Do Pollen Quality and Diversity Matter? *PLoS One* **2013**, *8*, No. e72016.
(26) Castle, D.; Alkassab, A. T.; Bischoff, G.; Steffan-Dewenter, I.; Pistorius, J. High nutritional status promotes vitality of honey bees and mitigates negative effects of pesticides. *Sci. Total Environ.* **2022**, *806*, No. 151280.
(27) Alaux, C.; Ducloz, F.; Crauser, D.; Le Conte, Y. Diet effects on honeybee immunocompetence. *Biol. Lett.* **2010**, *6*, 562−565.
(28) Stevenson, P. C.; Nicolson, S. W.; Wright, G. A. Plant secondary metabolites in nectar: impacts on pollinators and ecological functions. *Funct. Ecol.* **2017**, *31*, 65−75.
(29) Bartomeus, I.; Ascher, J. S.; Wagner, D.; Danforth, B. N.; Colla, S.; Kornbluth, S.; et al. Climate-associated phenological advances in bee pollinators and bee-pollinated plants. *Proc. Natl. Acad. Sci.* **2011**, *108*, 20645−20649.
(30) Settele, J.; Bishop, J.; Potts, S. G. Climate change impacts on pollination. *Nat. Plants* **2016**, *2*, 16092.
(31) Biesmeijer, J. C.; Roberts, S. P. M.; Reemer, M.; Ohlemüller, R.; Edwards, M.; Peeters, T.; et al. Parallel Declines in Pollinators and Insect-Pollinated Plants in Britain and the Netherlands. *Science (New York, NY)* **2006**, *313*, 351−354.
(32) Ricigliano, V. A.; Mott, B. M.; Floyd, A. S.; Copeland, D. C.; Carroll, M. J.; Anderson, K. E. Honey bees overwintering in a southern climate: longitudinal effects of nutrition and queen age on colony-level molecular physiology and performance. *Sci. Rep.* **2018**, *8*, 10475.
(33) Donkersley, P.; Rhodes, G.; Pickup, R. W.; Jones, K. C.; Wilson, K. Honeybee nutrition is linked to landscape composition. *Ecol. Evol.* **2014**, *4*, 4195−4206.
(34) Qiu, S.; Wang, S.; Xiao, C.; Ge, S. Assessment of microalgae as a new feeding additive for fruit fly Drosophila melanogaster. *Sci. Total Environ.* **2019**, *667*, 455−463.
(35) Martins, C. F.; Ribeiro, D. M.; Costa, M.; Coelho, D.; Alfaia, C. M.; Lordelo, M.; et al. Using Microalgae as a Sustainable Feed Resource to Enhance Quality and Nutritional Value of Pork and Poultry Meat. *Foods* **2021**, *10*, 2933.
(36) Madeira, M. S.; Cardoso, C.; Lopes, P. A.; Coelho, D.; Afonso, C.; Bandarra, N. M.; et al. Microalgae as feed ingredients for livestock production and meat quality: A review. *Livest. Sci.* **2017**, *205*, 111−121.
(37) Caporgno, M. P.; Mathys, A. Trends in Microalgae Incorporation Into Innovative Food Products With Potential Health Benefits. *Front. Nutr.* **2018**, *5*, 58.
(38) Tallentire, C. W.; Mackenzie, S. G.; Kyriazakis, I. Can novel ingredients replace soybeans and reduce the environmental burdens of European livestock systems in the future? *J. Cleaner Prod.* **2018**, *187*, 338−347.
(39) Nichols, B. J.; Ricigliano, V. A. Uses and benefits of algae as a nutritional supplement for honey bees. *Front. Sustainable Food Syst.* **2022**, *6*, No. 1005058.
(40) Ricigliano, V. A.; Cank, K. B.; Todd, D. A.; Knowles, S. L.; Oberlies, N. H. Metabolomics-Guided Comparison of Pollen and Microalgae-Based Artificial Diets in Honey Bees. *J. Agric. Food Chem.* **2022**, *70*, 9790−9801.
(41) Ricigliano, V. A.; Simone-Finstrom, M. Nutritional and prebiotic efficacy of the microalga Arthrospira platensis (spirulina) in honey bees. *Apidologie* **2020**, *51*, 898−910.
(42) Colin, T.; Bruce, J.; Meikle, W. G.; Barron, A. B. The development of honey bee colonies assessed using a new semi-automated brood counting method: CombCount. *PLoS One* **2018**, *13*, No. e0205816.
(43) R Core Team *R: A language and environment for statistical computing*; 4.1.2 ed.; R Foundation for Statistical Computing: Vienna, Austria; 2021.
(44) R Core Team *R: A language and environment for statistical computing*: R Foundation for Statistical Computing; 2019.

(45) Bates, D.; Maechler, M.; Bolker, B.; Walker, S. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Software* **2015**, *67*, 1−48.
(46) Arel-Bundock, V. *marginaleffects: Predictions, Comparisons, Slopes, Marginal Means, and HypothesisTests*. R package version 0.12.0.9017 ed; 2023.
(47) Beekman, M.; Ratnieks, F. L. W. Long-range foraging by the honey-bee, Apis mellifera L. *Funct. Ecol.* **2000**, *14*, 490−496.
(48) McMenamin, A. J.; Brutscher, L. M.; Daughenbaugh, K. F.; Flenniken, M. L. The Honey Bee Gene Bee Antiviral Protein-1 Is a Taxonomically Restricted Antiviral Immune Gene. *Front. Insect Sci.* **2021**, *1*, No. 749781.
(49) Kareiva, P.; Wennergren, U. Connecting landscape patterns to ecosystem and population processes. *Nature* **1995**, *373*, 299−302.
(50) Holzschuh, A.; Steffan-Dewenter, I.; Tscharntke, T. Agricultural landscapes with organic crops support higher pollinator diversity. *Oikos* **2008**, *117*, 354−361.
(51) St Clair, A. L.; Zhang, G.; Dolezal, A. G.; O'Neal, M. E.; Toth, A. L. Agroecosystem landscape diversity shapes wild bee communities independent of managed honey bee presence. *Agric., Ecosyst. Environ.* **2022**, *327*, No. 107826.
(52) Ricigliano, V. A. Microalgae as a promising and sustainable nutrition source for managed honey bees. *Arch. Insect Biochem. Physiol.* **2020**, *104*, No. e21658.
(53) Ricigliano, V. A.; Mott, B. M.; Maes, P. W.; Floyd, A. S.; Fitz, W.; Copeland, D. C.; et al. Honey bee colony performance and health are enhanced by apiary proximity to US Conservation Reserve Program (CRP) lands. *Sci. Rep.* **2019**, *9*, 4894.
(54) Dolezal, A. G.; St Clair, A. L.; Zhang, G.; Toth, A. L.; O'Neal, M. E. Native habitat mitigates feast-famine conditions faced by honey bees in an agricultural landscape. *Proc. Natl. Acad. Sci.* **2019**, *116*, 25147−25155.
(55) Ricigliano, V. A.; Williams, S. T.; Oliver, R. Effects of different artificial diets on commercial honey bee colony performance, health biomarkers, and gut microbiota. *BMC Vet. Res.* **2022**, *18*, 52.
(56) Meikle, W. G.; Holst, N. Application of continuous monitoring of honeybee colonies. *Apidologie* **2015**, *46*, 10−22.
(57) Ricigliano, V. A.; Ihle, K. E.; Williams, S. T. Nutrigenetic comparison of two Varroa-resistant honey bee stocks fed pollen and spirulina microalgae. *Apidologie* **2021**, *52*, 873−886.
(58) Ricigliano, V. A.; Dong, C.; Richardson, L. T.; Donnarumma, F.; Williams, S. T.; Solouki, T.; et al. Honey Bee Proteome Responses to Plant and Cyanobacteria (blue-green algae) Diets. *ACS Food Sci. Technol.* **2021**, *1*, 17−26.
(59) de Groot, A. P. *Protein and Amino Acid Requirements of the Honeybee (Apis Mellifica L.)*; W. Junk; 1953.
(60) Avni, D.; Hendriksma, H. P.; Dag, A.; Uni, Z.; Shafir, S. Nutritional aspects of honey bee-collected pollen and constraints on colony development in the eastern Mediterranean. *J. Insect Physiol.* **2014**, *69*, 65−73.
(61) Arien, Y.; Dag, A.; Zarchin, S.; Masci, T.; Shafir, S. Omega-3 deficiency impairs honey bee learning. *Proc. Natl. Acad. Sci.* **2015**, *112*, 15761−15766.
(62) Stabentheiner, A.; Kovac, H.; Brodschneider, R. Honeybee Colony Thermoregulation − Regulatory Mechanisms and Contribution of Individuals in Dependence on Age, Location and Thermal Stress. *PLoS One* **2010**, *5*, No. e8967.
(63) Meikle, W. G.; Corby-Harris, V.; Carroll, M. J.; Weiss, M.; Snyder, L. A.; Meador, C. A. D.; et al. Exposure to sublethal concentrations of methoxyfenozide disrupts honey bee colony activity and thermoregulation. *PLoS One* **2019**, *14*, No. e0204635.
(64) Meikle, W. G.; Weiss, M.; Beren, E. Landscape factors influencing honey bee colony behavior in Southern California commercial apiaries. *Sci. Rep.* **2020**, *10*, 5013.
(65) Jehlík, T.; Kodrík, D.; Krištůfek, V.; Koubová, J.; Sábová, M.; Danihlík, J.; et al. Effects of Chlorella sp. on biological characteristics of the honey bee Apis mellifera. *Apidologie* **2019**, *50*, 564−577.
(66) Dostálková, S.; Kodrík, D.; Simone-Finstrom, M.; Petřivalský, M.; Danihlík, J. Fine-scale assessment of Chlorella syrup as a nutritional supplement for honey bee colonies. *Front. Ecol. Evol.* **2022**, *10*, No. 1028037.
(67) Alaux, C.; Allier, F.; Decourtye, A.; Odoux, J. F.; Tamic, T.; Chabirand, M.; et al. A 'Landscape physiology' approach for assessing bee health highlights the benefits of floral landscape enrichment and semi-natural habitats. *Sci. Rep.* **2017**, *7*, 40568.
(68) Santin, A.; Balzano, S.; Russo, M. T.; Palma Esposito, F.; Ferrante, M. I.; Blasio, M.; et al. Microalgae-Based PUFAs for Food and Feed: Current Applications, Future Possibilities, and Constraints. *J. Mar. Sci. Eng.* **2022**, *10*, 844.
(69) Gohara-Beirigo, A. K.; Matsudo, M. C.; Cezare-Gomes, E. A.; de Carvalho, J. C. M.; Danesi, E. D. G. Microalgae trends toward functional staple food incorporation: Sustainable alternative for human health improvement. *Trends Food Sci. Technol.* **2022**, *125*, 185−199.
(70) Fu, Y.; Chen, T.; Chen, S. H. Y.; Liu, B.; Sun, P.; Sun, H.; et al. The potentials and challenges of using microalgae as an ingredient to produce meat analogues. *Trends Food Sci. Technol.* **2021**, *112*, 188−200.
(71) Plaza, M.; Herrero, M.; Cifuentes, A.; Ibáñez, E. Innovative natural functional ingredients from microalgae. *J. Agric. Food Chem.* **2009**, *57*, 7159−7170.
(72) Borowitzka, M. A. High-value products from microalgae—their development and commercialisation. *J. Appl. Phycol.* **2013**, *25*, 743−756.
(73) Khandual, S.; Sanchez, E. O. L.; Andrews, H. E.; de la Rosa, J. D. P. Phycocyanin content and nutritional profile of Arthrospira platensis from Mexico: efficient extraction process and stability evaluation of phycocyanin. *BMC Chem.* **2021**, *15*, 24.
(74) Khavari, F.; Saidijam, M.; Taheri, M.; Nouri, F. Microalgae: therapeutic potentials and applications. *Mol. Biol. Rep.* **2021**, *48*, 4757−4765.
(75) Dehghani, J.; Movafeghi, A.; Mathieu-Rivet, E.; Mati-Baouche, N.; Calbo, S.; Lerouge, P.; et al. Microalgae as an Efficient Vehicle for the Production and Targeted Delivery of Therapeutic Glycoproteins against SARS-CoV-2 Variants. *Mar. Drugs* **2022**, *20*, 657.
(76) Banerjee, A.; Ward, V. Production of recombinant and therapeutic proteins in microalgae. *Curr. Opin. Biotechnol.* **2022**, *78*, No. 102784.
(77) Rizwan, M.; Mujtaba, G.; Memon, S. A.; Lee, K.; Rashid, N. Exploring the potential of microalgae for new biotechnology applications and beyond: A review. *Renewable Sustainable Energy Rev.* **2018**, *92*, 394−404.
(78) Kingsolver, M. B.; Huang, Z.; Hardy, R. W. Insect antiviral innate immunity: Pathways, effectors, and connections. *J. Mol. Biol.* **2013**, *425*, 4921−4936.
(79) McMenamin, A. J.; Daughenbaugh, K. F.; Parekh, F.; Pizzorno, M. C.; Flenniken, M. L. Honey Bee and Bumble Bee Antiviral Defense. *Viruses* **2018**, *10*, 395.
(80) Weaver, D. B.; Anzola, J. M.; Evans, J. D.; Reid, J. G.; Reese, J. T.; Childs, K. L.; et al. Computational and transcriptional evidence for microRNAs in the honey bee genome. *Genome Biol.* **2007**, *8*, R97.
(81) Toth, A. L.; Zayed, A. The honey bee genome– what has it been good for? *Apidologie* **2021**, *52*, 45−62.
(82) Wang, M.; Luan, G.; Lu, X. Systematic identification of a neutral site on chromosome of Synechococcus sp. PCC7002, a promising photosynthetic chassis strain. *J. Biotechnol.* **2019**, *295*, 37−40.
(83) Leonard, S. P.; Powell, J. E.; Perutka, J.; Geng, P.; Heckmann, L. C.; Horak, R. D.; et al. Engineered symbionts activate honey bee immunity and limit pathogens. *Science* **2020**, *367*, 573.
(84) Muntaabski, I.; Scannapieco, A. C.; Liendo, M. C.; Niz, J. M.; Russo, R.; Salvador, R. Bacterially expressed dsRNA induces Varroa destructor gene knockdown by honey bee-mediated oral administration. *J. Apic. Res.* **2022**, *61*, 511−518.

https://doi.org/10.1021/acsagscitech.3c00082
ACS Agric. Sci. Technol. 2023, 3, 748−759