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PP-046 [Bee Health]

KENTUCKY STATE UNIVERSITY

Effects of Diet and Probiotics on the Health of Honey Bees

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Introduction

Honey bees {*Apis mellifera*} depend on pollen as their primary source of proteins, lipids, and micronutrients. Pollen consumption is critical for their physiological functions and influences multiple health parameters, including metabolic processes, immunocompetence, pathogen resistance, and detoxification capacity against pesticides. To offset pollen dearths, beekeepers often feed pollen substitutes to their colonies; however, the nutritional efficacy of these substitutes remains uncertain. In addition to these protein supplements, other nutritional supplements like probiotics have been introduced to the market to enhance colony health. Probiotics contain live microorganisms that are directly fed to bees to boost their gut microbiome. In this study, we conducted a comparative analysis of diets comprising natural pollen and a pollen substitute, both independently and in conjunction with a probiotic supplement. Controlled feeding trials were performed to assess the impacts of these dietary regimens, utilizing 16S rRNA amplicon sequencing for bacterial profiling and quantitative polymerase chain reaction (qPCR) to evaluate differential expression of honey bee immune, developmental, and metabolic genes. Our results indicate that diet has a significant effect on gene expression and bacterial diversity and abundance in the midgut, and probiotic supplementation may offer benefits when combined with a pollen diet.

Materials And Methods

Caged Trials

Emerging brood frames were collected from healthy honey bee colonies at the Harold R. Benson Research Farm (Franklin County, KY, USA) and incubated at 32.5°C for 24 hours. After 24 hours, newly emerged bees were caged in groups of 100 individuals. Cages were randomly assigned to treatment groups of diets with and without probiotic application, with 3 cages per treatment group. Diet was provided ad libitum, in addition to 50% sucrose syrup (w/w). Probiotic treatment was applied by dusting 1.0 gram of SuperDFM probiotic onto wax foundation suspended in each cage. Cages were kept in an incubator at 32.5°C for the 10-day duration of the experiment. On day 10, bees were flash frozen in liquid nitrogen and frozen at -80°C.

DNA Extraction, Library Preparation, and Sequencing

DNA was extracted from honey bee midguts using the Qiagen DNeasy blood and tissue mini kit. After extraction, all samples were quantified, standardized, and uniformly pooled by cage into groups of 8 individuals for 16s sequencing analysis. Pooled DNA was re-quantified with Equalbit 1x dsDNA HS Assay Kit. 50ng of DNA was amplified via PCR with 341F+805R primers covering hypervariable regions V3-V4. Libraires were quantified by Qubit 2.0 and quality was evaluated by TapeStation D1000 ScreenTape with Illumina 8-nt dual-indices. Sequencing was performed on an Illumina MiSeq with a read length configuration of 250 PE for 0.1 M PE reads per sample. Analysis was performed using the CosmosID-HUB.

RNA Extraction, cDNA synthesis, and qPCR

RNA was extracted from individual honey bees using the Qiagen RNeasy mini kit with on-column DNase treatment. Extracted RNA was quantified using a BMG Labtech Omega plate reader equipped with a LVis plate. 200 ng of RNA was used for cDNA synthesis using the Bioline Sensifast cDNA kit. qPCR was performed using an Applied Biosystems QuantStudio 3 machine. Data was analyzed using the delta cT method, using the geometric mean of GapDH and S8 as a reference gene.

Results

Taxa Diversity

Alpha diversity was analyzed using the CHOA1 index method (Fig. 1). Diet had a significant impact on bacteria taxa present in honey bee midguts ($p=<0.001$) but probiotic did not ($p=0.079$). Post-hoc diet comparisons indicate statistical differences between megabee-pollen ($p<0.001$) and pollen-sucrose ($p=0.005$). While there was an increase in taxa diversity with probiotics amongst the Megabee fed bees (9.67 vs 6.33), the result was not significant ($p = 0.130$).

Taxa Abundance

Taxa abundance was analyzed using two-way ANOVA . Certain bacteria taxa exhibited differences between treatment groups (Fig. 2). Apibacter was only found in pollen fed bees ($p<0.001$) and had a significant interaction effect with probiotic application ($p=0.007$). Apilactobacillus was significantly increased in pollen fed bees ($p<0.001$) with no impact of probiotics ($p=0.945$). Bartonella was present in all treatments and was highest in sucrose fed bees ($p=0.056$), with no significant difference in probiotic ($p=0.428$). Bifidobacterium was impacted by both diet ($p<0.001$) and probiotic ($p=0.001$) with a significant interaction effect ($p=0.011$).

Gene Expression

Normalized delta cT values were analyzed with two-way ANOVA. 6 target genes showed statistical differences between treatment groups (Fig. 3). Defensin, Catalase, ILP1, Hex70B, and Vitellogenin were only impacted by diet (all $p<0.001$), while InR1 showed differences based on diet ($p<0.001$) and probiotic ($p=0.006$), with a significant interaction effect ($p=0.041$).

Figure 1. Taxonomic Diversity

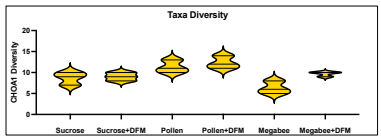


Figure 2. Taxa Abundance

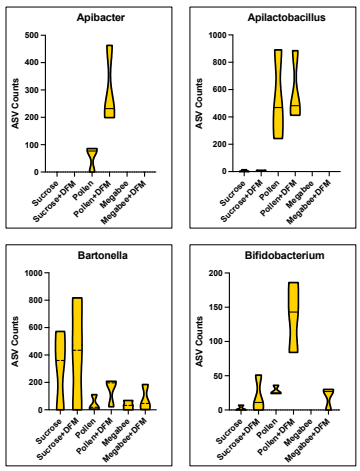
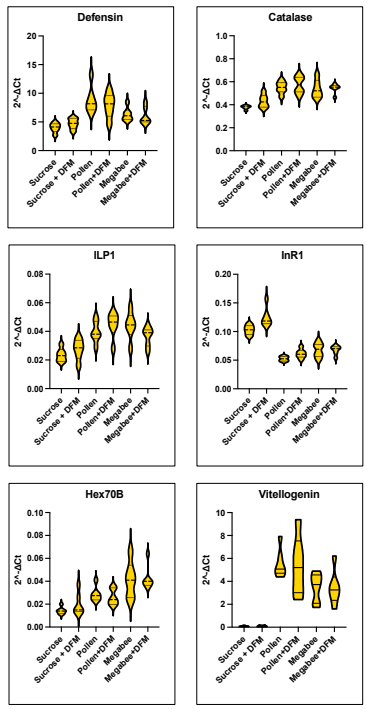


Figure 3. Gene Expression



Discussion

Pollen-fed bees exhibited significantly higher midgut bacterial diversity than those fed Megabee or sucrose, reflecting pollen's rich nutritional profile, which includes proteins, lipids, and micronutrients essential for microbial communities (Motta et al., 2018). Elevated Apibacter and Apilactobacillus in pollen-fed bees highlights their dependence on pollen-derived nutrients, while probiotic-enhanced Apibacter and Bifidobacterium suggest improved gut health under specific dietary conditions (Zheng et al., 2017). Conversely, Bartonella, which was most abundant in sucrose-fed bees, may indicate a less favorable microbial environment, as this genus is not typically associated with beneficial functions in honey bees. Gene expression analysis revealed protein-driven upregulation of Defensin, Catalase, ILP1, Hex70B, and Vitellogenin, underscoring protein's critical role in supporting immunity, detoxification, and development (Alaux et al., 2010). InR1's upregulation with a sucrose diet may be a compensatory response to ILP1 reduction, attempting to maximize nutrient utilization under poor dietary conditions. These findings affirm pollen's superiority, but suggest that pollen-substitutes are a valuable adjunct during pollen scarcity. Future research should be focused on improving pollen substitutes to match pollen's impact on midgut microbiota.

References

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Zheng, H., Powell, J. E., Steele, M. I., Dietrich, C., & Moran, N. A. (2017). Honeybee gut microbiota promotes host weight gain via nutrient digestion and hormonal signaling. *Proc. Natl. Acad. Sci. USA* , 114(28), 4775–4780.

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