AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
FUNDING CYCLE 2018 – 2020

TITLE: Understanding and improving honey bee nutrition to mitigate colony losses

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COOPERATORS: Oregon State Beekeepers' Association

EXECUTIVE SUMMARY: Sterols in insect diets play a physiologically vital role, as precursors of important molting hormones and building blocks of cellular membranes. However, sterol requirements and metabolism in honey bees is largely unknown. In honey bees, 24-methylenecholesterol is considered to be the most critical sterol for colony growth and worker longevity. Honey bees obtain this sterol from pollen. Honey bee colonies used for crop pollination often face nutritional stress as the quality or quantity of pollen forage available to them in such agricultural landscapes is inadequate. Beekeepers feed artificial protein supplements to their colonies during periods of nutritional dearth. However, current protein supplements available to beekeepers are deficient in 24-methylenecholesterol and therefore may be unable to sustain long-term brood rearing in honey bee colonies. Our goal in this proposal was to identify the importance of sterols in honey bee nutrition, bridge the gap in knowledge regarding sterol requirements and assess the nutritional composition of crop pollens, supplemental diets and natural forage surrounding agricultural farms. We expect our findings to provide practical recommendations for beekeepers to improve honey bee nutrition that will benefit both beekeepers and producers. Our best management recommendations related to nutrition based on our research findings will help mitigate risk to pollination services to all crops dependent on bee pollination.

OBJECTIVES:
1. Evaluate dietary sterol requirements of honey bees

2. Identify current dietary supplements used by commercial beekeepers

3. Examine the sterol composition of currently used honey bee protein diets and major forage (crop pollen and surrounding natural forage) available to honey bees

4. Disseminate relevant information to stakeholders

PROCEDURES:

1. Evaluate sterol requirements of honey bees

An artificial diet was supplemented with one optimal concentration of 24-methylenecholesterol based on our preliminary studies. Nucleus hives in flight cages were provided with the formulated artificial diets supplemented with sterol. There were control cages where honey bees

will were fed with diets containing no sterols. Parameters such as diet consumption by the honey bee nucleus colonies, colony population, brood production, larval growth etc. was measured. The dietary needs for sterols at different stages of honey bee development was also assessed indicating the prerequisites for suitable diets depending on the colony needs. Isotopically labelled 24-methylenecholesterol was used in the synthetic diets to specifically locate and quantify its presence across various honey bee tissues, brood food and different stages of bee life cycle. The impacts of a sterol deficit diet on honey bee physiology and health was measured by studying important proteins in the honey bee system.

2. Evaluate beekeeping practices and vegetation surrounding the target crops pollinated by honey bees

Beekeeping practices were surveyed to understand various types of dietary supplements provided to honey bees in between and during their movement across various cropping systems for pollination, constructing a timeline for provision of honey bee diets. The growers of selected crops were surveyed to obtain information regarding natural vegetation surrounding their fields where honey bees are employed for pollination. These data are essential for making management recommendations that are feasible for both beekeepers and growers.

3. Examine the sterol composition of bee diets and available forage (crop pollen and surrounding forage)

Pollen samples were collected from the various commercially important crops across Oregon and surrounding vegetation and pollen traps were placed to collect corbicular (honey bee gathered) pollen. The artificial dietary supplements provided to the honey bee by commercial beekeepers were also be sampled. The sterol composition of all the collected pollen samples and artificial diets were assessed. These data, coupled with our survey data, field observations and in-depth studies in the lab and flight cages will help us identify the dietary requirements of sterols in bees and inform beekeepers and growers of effective nutrition management strategies.

4. Disseminate relevant information to stakeholders

The findings and best management recommendations pertaining to optimal bee nutrition derived from our study will be disseminated to stakeholders at the regional and national meetings in the form of PowerPoint presentations and informational brochures. We will also publish our findings in peer reviewed scientific journals and stakeholder newsletters such as Beeline. Further, we will also work with stakeholders to compare the cost/benefit/feasibility of potential recommendations / interventions, enabling us to facilitate dialogue about the economic realities of each stakeholder group and conducting stakeholder advisory panel meetings to review scientific results and communication of best practices.

SIGNIFICANT ACCOMPLISHMENTS TO DATE:

We searched extensively for an appropriate source of synthetic 24-methylenecholesterol and procured a custom-synthesized 24-methylenecholesterol. We have established 20 flight cages to successfully perform controlled nutrition experiments with nucleus hives (Figure 1) and are currently analyzing the colony performance data. Further, we have established robust protocols for quantification of phytosterols and metabolites from pollen, honey bee tissues, vegetable oils and commercially available protein supplements. We have also established methods for conducting proteomics in honey bee tissues. We have also sought input and collaboration from stakeholders (beekeepers and farmers), which will be critical for successful execution of this proposed research.

Figure 1: Aerial image of the 20 flight cages – each having one experimental nucleus hive corresponding to a particular diet treatment group.

Liquid chromatography/mass spectrometry (LC/MS) methods have been established at the Oregon State University Mass Spectrometry Center (OSUMSC), in collaboration with the OSU Honey Bee Lab, to quantify the sterol profiles of different types of source samples – corbicular almond pollen, vegetable oil (borage oil), commercial diet and honey bee tissues. Figure 2 briefly shows the chromatographs obtained from phytosterol profiles of four representative sample types. Bar graphs indicating mean concentrations of different phytosterols obtained from two representative sample types are presented in Figure 3. In this study, 24-methylenecholesterol – our key phytosterol of interest – was found to be absent in the commercial diet sample that we tested; whereas the same phytosterol was abundantly present in almond pollen. The various other phytosterols were also found to be differentially available across the four types of samples. Proteomics methods (for identifying an extensive spectrum of proteins) have also been established which gives us knowledge about a large range of proteins in honey bee tissues. Honey bees fed on diets high in 24-methylenecholesterol had a number of protein expressions significantly enhanced – especially proteins which are directly or indirectly associated with nutritional physiology in honey bees. This method of extensive protein identification will help us strengthen our proposed research by analyzing a large number of sample types and being able to

identify protein markers. Protocols for metabolomics studies have also been established at the OSUMSC using a quadrupole-time-of-flight mass spectrometer (Triple TOF 5600, AB SCIEX) with MS/MS spectra recorded using Information Dependent Acquisition-Mass Spectrometry (IDA-MS). Metabolites are identified using the OSU-IROA in-house library covering 420+ measured standards based on accurate mass, fragmentation pattern, isotope distribution and retention time. Figure 4 represents an example total ion chromatographic run from the metabolomics studies. Honey Bee Lab personnel also actively participate in regional and national stakeholder and scientific meetings to disseminate the project results. This study has been published in the journal *Metabolomics*: Chakrabarti, P., Morre, J.T., Lucas, H.M., Maier, C.S. and Sagili, R.R. (2019) The Omics Approach to Bee Nutritional Landscape. Metabolomics 15: 127.

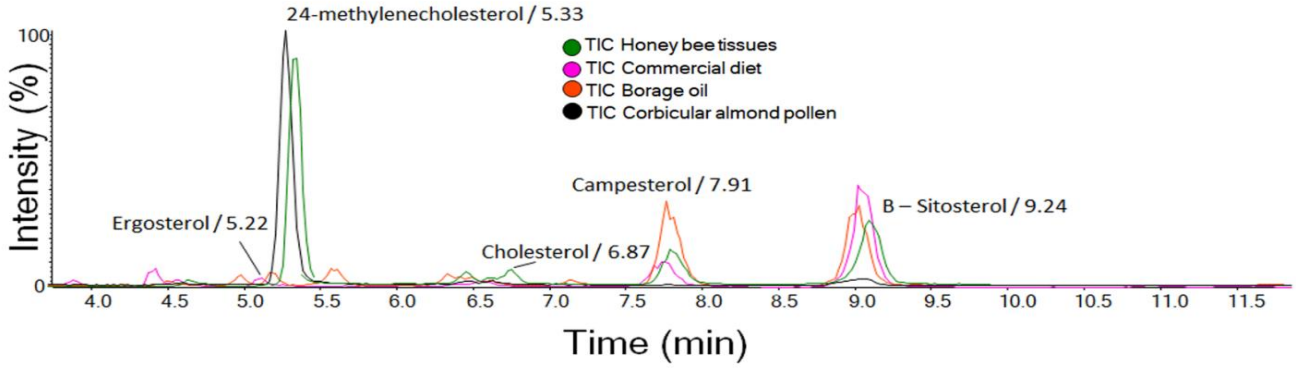

Figure 2: Liquid chromatography-mass spectrometric multiple reaction monitoring (LC-MS MRM) assay for quantifying plant sterols in honey bee tissues, pollen, borage oil and commercial diets. TIC indicates overlaid total ion chromatograms. Digits following the sterol standard name in the graph indicates its retention time during separation process in LC/MS/MS.

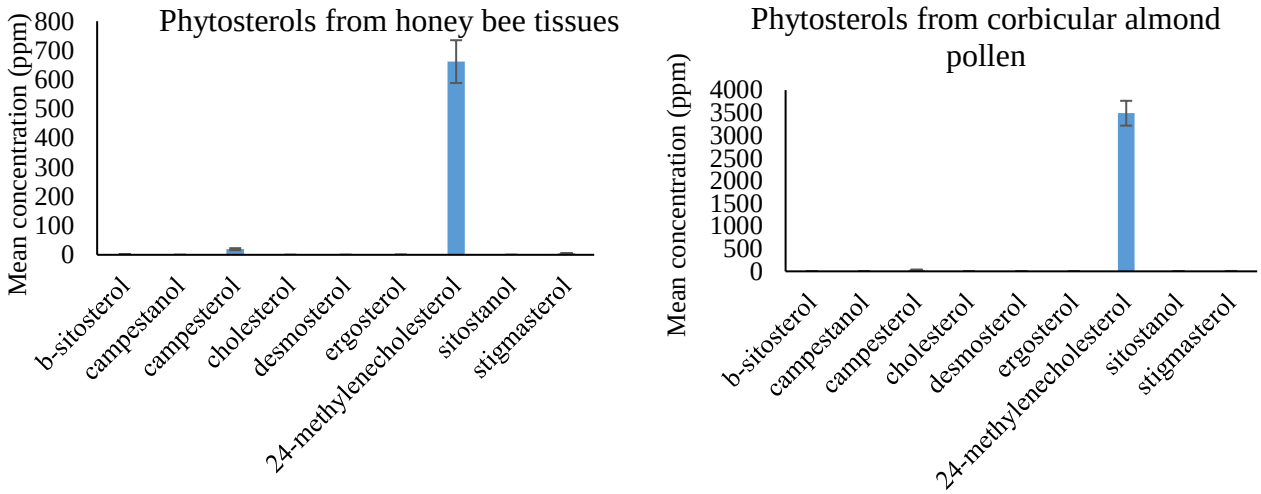

Figure 3: Graphs represent mean concentrations (ppm) of phytosterols analyzed from honey bee tissues and corbicular almond pollen samples. Error bars indicate ± standard errors of means.

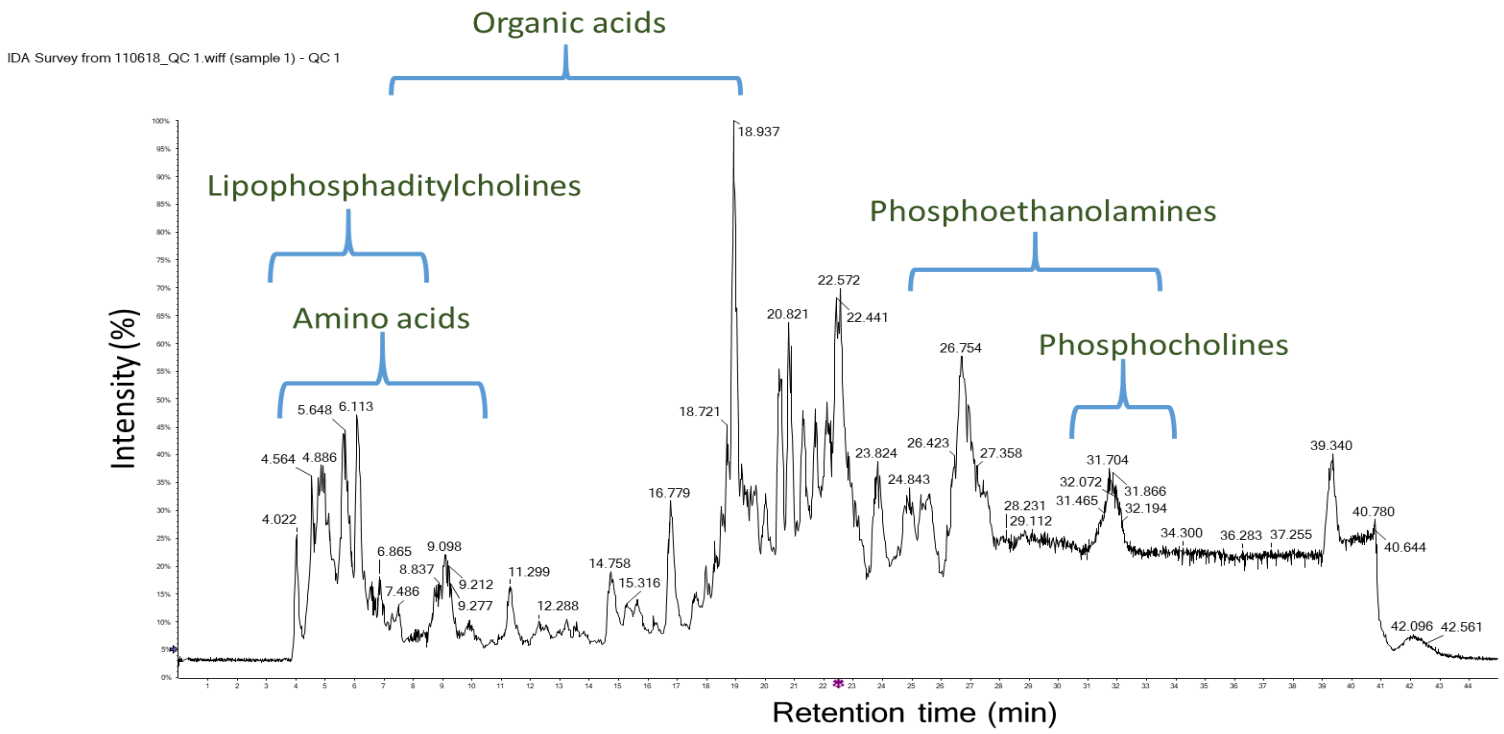

Figure 4: Total ion chromatogram of a representative metabolomics sample run using a quadrupole-time-of-flight mass spectrometer. The chromatogram represents samples separated in a positive ion separation mode.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:

We received the following two additional funding during this period:

1. Project Apis mellifera (National Honey Board) funding for $80,784
2. California State Beekeepers' Association grant $20,052

FUTURE FUNDING POSSIBILITIES:

With the data generated from this study, we aim to apply for grants from the United States Department of Agriculture and other relevant funding opportunities.