Super DFM HoneyBee – Blind Study – 2014

**Goal:** investigate impact of direct-fed microbial product, Super DFM Honeybee, on honeybee gut microflora.

**Summary:** Supplementation with direct-fed microbial product, Super DFM Honeybee, significantly increases *Lactobacillus* and *Bacillus* populations in honeybee gut. Terramycin treatment decreases *Lactobacillus* populations in honeybee gut.

Objective 1. *Lactobacillus* are a natural constituent of commensal microflora of honeybees, *Apis mellifera*. However, pathological and drug-induced changes in microflora lead to a decrease in natural prevalence of the *Lactobacillus*. Supplementation with direct-fed microbial product, Super DFM Honeybee, is expected to result in significant increase in *Lactobacillus* population in honeybee gut. Gut *Lactobacillus* and other commensal bacterial play a role in inhibiting honeybee pathogens and therefore play a critical role in honeybee health.

**Result.** We are able to demonstrate a significant increase in *Lactobacillus* population in the guts of honeybees fed Super DFM Honeybee. Compared to untreated honeybee control group, honeybees fed SuperDFM showed 9-fold increase in *Lactobacillus* population in the gut (*p*-value = 0.03).
Objective 2. Antibiotic treatment (terramycin) negatively impacts commensal microflora of honeybees. Lactobacillus population in honeybee gut is expected to decrease after terramycin treatment.

Result. We were able to quantitate 10 fold decrease in Lactobacillus population after terramycin treatment.

![Graph showing LAB concentration in honeybee gut with negative control, SDFM before terramycin treatment, and SDFM after terramycin treatment.]

Objective 3. Optimal concentration of probiotic supplementation was tested.

Result. Our data indicate that increasing Lactobacillus concentration in probiotic supplementation 100 fold did not proportionately increase concentration of Lactobacillus in honeybee gut, suggesting that Lactobacillus in honeybee gut can reach a finite capacity. No adverse honeybee health effects were observed when probiotic supplementation was increased 100 fold.

![Graph showing LAB concentration in SDFM control and SDFM high dose.]
Objective 4. Analyze colonization of honeybee gut with beneficial *Bacillus* species.

Result. Besides *Lactobacillus*, we detected proprietary *Bacillus* species included in formulation of SuperDFM honeybee in the guts of all tested honeybee populations that were supplemented with SuperDFM.

Methods.

Honeybee maintenance.

Honeybee microbial analysis. Live honeybees were shipped to the laboratory (Figure 1) and euthanized by placing them into -20 degrees Celsius freezer. 1 gram of honeybees (approximately 3 honeybees) were homogenized in 1 milliliter of 1% peptone water, and 10 fold dilutions of gut contents in peptone were made (Figure 2). Dilutions were plated on MRS media for *Lactobacillus* quantification and TSA medium for *Bacillus* quantification.

Figure 1.
Figure 2.