# Innate immunity mediated by nutrition during larval development

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## Introduction

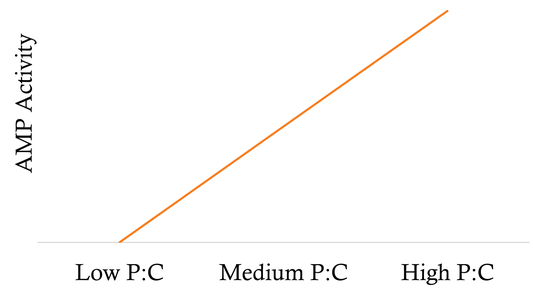
- Colony Collapse Disorder (CCD) has been associated with many pathogens and parasites, and recent changes in temporal and spatial foraging has been shown to leave the bees with a lower quality diet.
- Although changes in diet quality has shown to lower the immune defenses against pathogens in other insects, this has never been investigated in honeybees.
- Juvenile nutrition impacts both larval and adult immune defenses.
- Previous studies have found that insects fed a higher P:C ratio as juveniles have improved response to immune challenge. Yet the relationship between nutrition and immunity has not been investigated in honeybees specifically

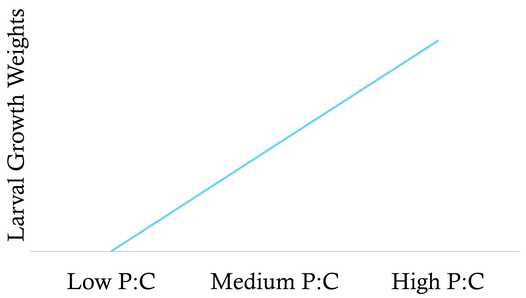
## Hypothesis

The higher protein:carbohydrate ratio diets will induce a more effective innate immune response



## Expected Outcomes




## Methods

**Rear honeybees in-vitro using 9 diet sampes**

| Diet | Fructose | Glucose | Yeast | Royal Jelly | Water | Royal Jelly to Carbohydrate ratio |
|---|---|---|---|---|---|---|
| 5 | 0.75g | 0.75g | 0.2g | 4.7g | 3.6mL | 1 : 3.13 |
| 6 | 0.6g | 0.6g | 0.2g | 5.0g | 3.6mL | 1 : 4.2 |
| 7 | 0.5g | 0.5g | 0.2g | 5.2g | 3.6mL | 1 : 5.2 |
| 8 | 0.35g | 0.35g | 0.2g | 5.5g | 3.6mL | 1 : 7.9 |
| 9 | 0.1g | 0.1g | 0.2g | 6.0g | 3.6mL | 1 : 30 |

**Figure 1:** This table displays the diets given to selected treatments and their protein to royal jelly ratio.

**On day 7, induce immune challenge using lipopolysaccharides (LPS)**

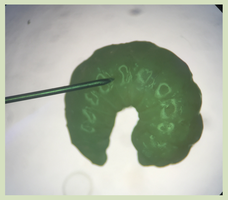


**Figure 2:** Image of larva being injected with lipopolysaccharides diluted in a .5mg/1ml ratio of Ringer's insect solution.

**24 hours after immune challenge, extract hemolymph**

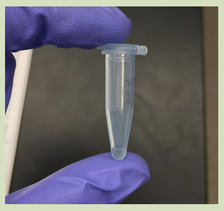


**Figure 3:** Image hemolymph post collection stored in an ependorph tube, ready to be centrifuged.

**Isolate AMP's from hemolymph**

**Figure 4:** Image of hemolymph stored in ependorph tubes and in centrifuge machine.

**Record zones of inhibition**

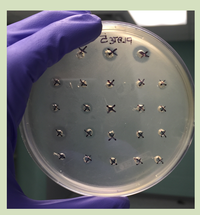
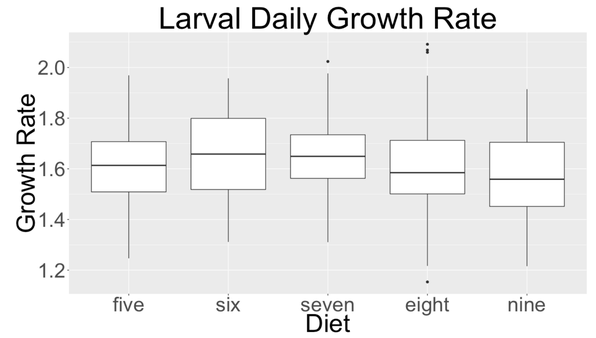
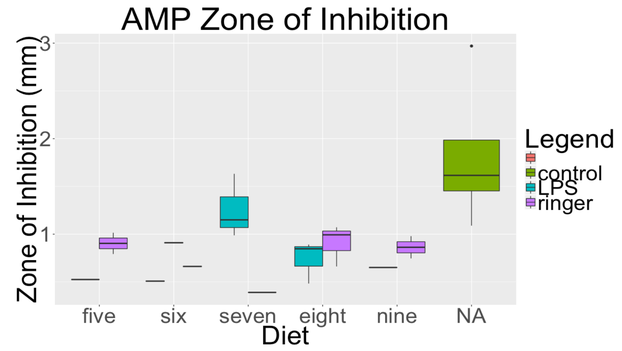


**Figure 5:** Image of zones of inhibition after being stored in an incubator for 24 hours at 25C.

## Results




**Graph 1:** Larval Daily Growth Weight: There was not a significant difference in larval weight at injection among diets (treatment groups). (F(4,208)=1.901, P=.112)




**Graph 2**: AMP Zone of Inhibition: There was a significant difference in larval weight at injection among diets (treatment groups). (F(5,17)=3.639, P=.0202). There was a significant difference between diet seven and eight(TukeyHSD, =.997, CI=.0979-1.897, P=0.025) and between diet six and seven(TukeyHSD, =1.129, CI=.0644-2.194, P=0.034)

## Future directions

- Observe the impact of the immune challenge in these treatments towards cellular immune response by testing the phenol oxidase activity
- Explore the relationship between nutrition during larval development and adult innate immunity
- Investigate the impact of starvation on larval and adult innate immunity
- Compare factors such as nutritional quality vs nutritional quantity towards innate immunity during larval development and in adults

## Acknowledgements

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