Effect of Chitosan on the microbiome of *Musca domestica*

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Introduction

Musca domestica, commonly known as house flies, are the most common and widespread flies in the world. While they are often found near dumpsters, left-over food, manure, and barns, they are also found in our houses, as suggested by their name. In addition to being a nuisance, house flies also pose a significant public health risk. It has been shown that house flies are able to carry and spread more than 100 pathogens, some of which are even life-threatening like the E. coli, cholera, typhoid fever, and shigellosis. Flies tend to sit on all potential places where they might come in contact with bacteria, and thus aid in further transmission of bacteria to humans and animals by serving as a vector (Nayduch and Burrus, 2017). A recent study proposed the use of chitosan, a derivative of sugar, as a potential bioinsecticide agent to control house fly population in agricultural use. As seen in the figure below, a 2% diet of chitosan significantly reduced the longevity of house flies compared to a sucrose diet as a control.

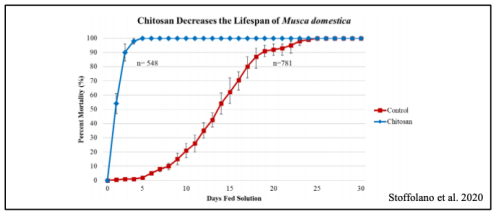


Figure 1: Chitosan decreases house fly longevity.
Control diet- 10% sucrose + 2% ascorbic acid;
Chitosan diet- 10% sucrose + 2% ascorbic acid + 2% chitosan

As seen in Figure 1, flies fed with a chitosan supplemented diet, shown in blue, reached 100% mortality within 5 days, while the control group reached 100% mortality in about 25 days, a normal life cycle of a house fly on a sucrose diet.

These findings suggest that chitosan can be used as a potential agent in the population control of house flies, however, there is little evidence to understand the mode of action of chitosan. Chitosan, a deacylated form of chitin, is currently used for various novel applications including wound healing, weight management, cosmetics, and food preservation. However, chitosan is also biodegradable, especially attracting interest for its sustainable use as an agriculture application in the growing times of climate change crisis.

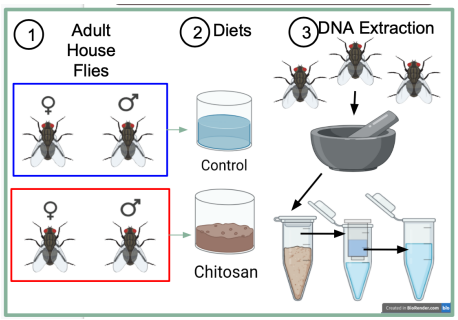
Meanwhile, the gut microbiome is known to change due to various factors such as age, diet, or health status. As microbiome ages, number of compositional changes occur that could negatively affect digestive health, nutrient absorption, and immune function. Therefore, as a part of the study, we are trying to understand the impact of chitosan on the gut microbiome of the house fly to better understand the cause of its reduced longevity after consuming chitosan.

Research Question - Does chitosan affect gut bacterial microbiota in house flies to affect their longevity?

Methods

Quantitative PCR (QPCR) was conducted using the fly DNA that was extracted during the chitosan fly mortality experiment. The purpose of doing the QPCR was to a) quantitatively analyze the effect of chitosan on the amount of gut bacteria in house flies, and b) to measure the number of bacteria per fly for control and chitosan amended diets. For QPCR, we specifically targeted the V3-V4 region of the 16S rRNA gene found in bacteria using the 515f and 806r primers to quantify the bacterial gene copy number in the flies with the control and chitosan amended diets.

The schematic below shows the set up for the DNA extraction process which was followed by QPCR.



Results

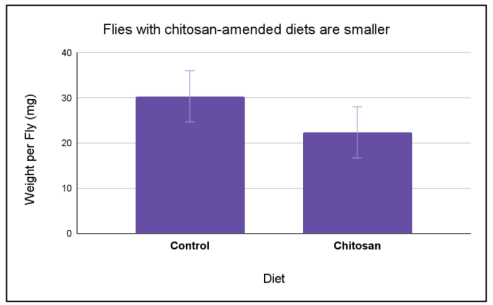


Figure 1- Comparison between fly weight and diet

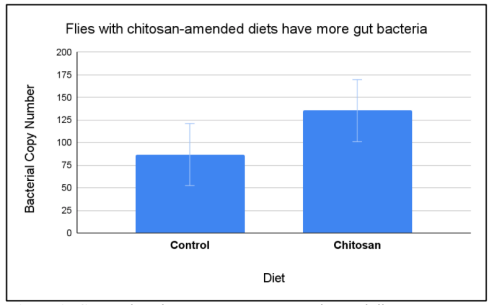


Figure 2- Comparison between gene copy number and diet

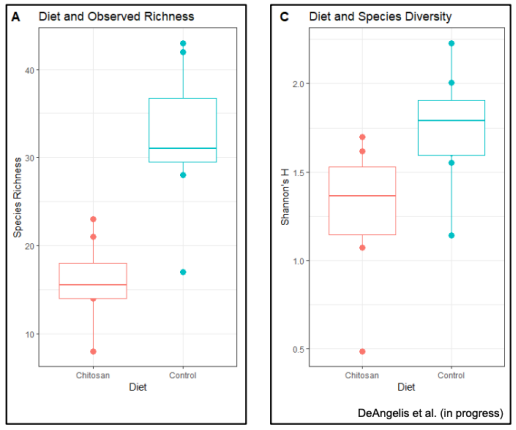


Figure 3- Comparison between diet and species richness and diversity

Conclusions

Synthesizing from the results, we can suggest the following -

1) Flies consuming the chitosan-amended diet weigh significantly lower and are physically smaller than flies consuming the control diet.

2) The quantitative results suggest that flies with chitosan-amended diets have a significantly more number of bacteria in their gut compared to the control diet.

3) The qualitative results suggest that chitosan fed flies have a lower bacterial species richness and diversity in their guts as compared to flies fed with the control diet.

Future Directions - Observing trends of the dominant genus that vary in diets and their intrinsic properties in correlation with fly immunity.

References

Stoffolano, J., Wong, R., Lo, T., Ford, B., & Geden, C. J. (2020). Effect of chitosan on adult longevity when fed, in no-choice experiments, to *Musca domestica L.*, *Tabanus nigrovittatus Macquart*, and *Phormia regina* (Meigen) adults and its consumption in adult *Musca domestica L. Pest Management Science*, *76*(12), 4293–4300. https://doi.org/10.1002/ps.5996

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