How Diet Affects the Production and Immunological Properties of Snail Slime

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Abstract

Snail slime is a key aspect of the snail innate immune system because it has anti-microbial properties. The ability for a snail to produce high volumes of chemically complex slime plays an integral role in fighting off various pathogens. However, we have a limited understanding of how the composition of snail slime may be altered. One possibility is that snail diet affects slime volume, composition, and its anti-microbial properties. To improve our understanding of how snail diet alters slime production and defensive properties, we collected slime samples from two groups of common land snails (*Cornu aspersum*) over a period of six weeks. Initially, we fed both groups a diet containing a 2:1 ratio of carrots and cuttlefish bones. After collecting baseline samples, we then fed one group (n=5) a high-protein diet containing a 2:2:1 ratio of cat food, oats, and eggshells. We collected additional slime samples from both groups at two subsequent time points. We tested all slime samples to determine effectiveness at inhibiting *Pseudomonas fluorescens*, a bacterium known to be pathogenic to the common land snail. Unexpectedly, we found that both groups exhibited decreases protein concentrations in the snail slime following the diet treatment. We also found that snail slime did not inhibit growth of *Pseudomonas fluorescens*. These results suggest that snail slime from *Cornu aspersum* may not have anti-microbial properties against *Pseudomonas fluorescens* as previously reported. Additional research on snail slime is warranted to further understand putative anti-microbial characteristics of the snail innate immune system.

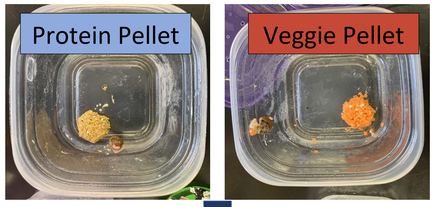
Introduction

- Snails have have multiple defense strategies against various pathogens.[1]
- The snail innate immune system uses a variety of strategies, including producing antimicrobial peptides (AMPs) in snail slime.[2]
- Diet may alter the volume, composition, and anti-microbial properties of snail slime.
- *Pseudomonas fluorescens*, a rod shaped, gram negative bacteria is one pathogen that is used to control snail populations in many southeast Asian countries.[3]
- *P. fluorescens* can infect and kill the common land snail, *Cornu asperum*.[3]
- We tested how two different diets could alter snail slime production and its ability to inhibit the growth of *P. fluorescens.*

Fig 1. The common land snail *Cornu asperum*

Fig 2. Petri dishes with *P. fluorescens* and a zone of Inhibition.

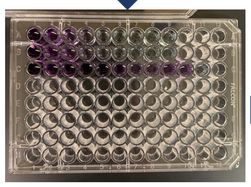
Methods



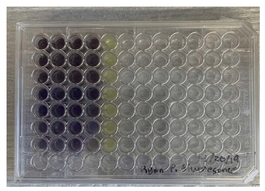
We created food pellets weighing three grams. The image on the left is of the high protein food mix, and the image on the right is the vegetable mix.

We placed the snails in 50 mL syringes and left them to roam for 40 minutes. We then flushed the produced slime with 500 µL of HPLC grade water and collected the mixture.

We placed the collected snail slime in aliquot tubes and froze the samples in a -20° C freezer.



We ran a BCA assay to determine slime protein concentration.



We ran an MTT assay to determine bacterial growth when snail slime is present.

Results- Snail Mass

- There were no differences in snail mass between time points
- Snails eating the high vegetable diet had slightly increased mass means; however, this finding can be explained by a higher initial mass.

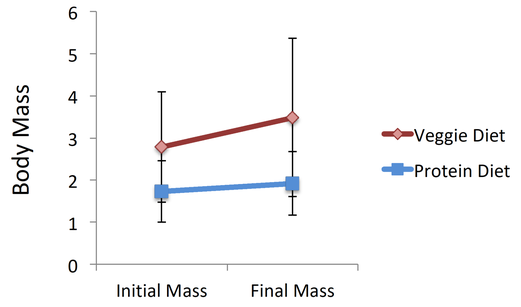


Fig 3. Average initial and final body mass for snails that ate different diets.

Results- *Inhibitory factors*

- *P. fluorescens* growth was not inhibited by our snail slime samples that were collected from snails that ate two different diets.
- The snail slime did not have an inhibitory effect on this pathogen from any time point.

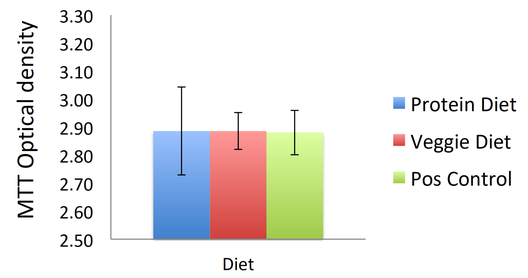


Fig 4. Optical density readings comparing *P. fluorescens* growth between diet groups to the positive control.

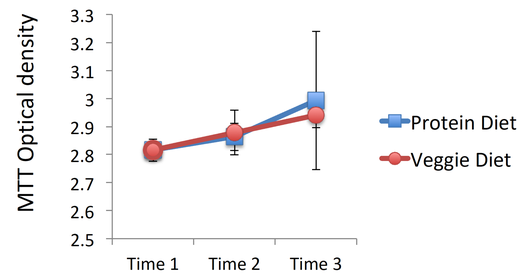


Fig 5. Optical density readings comparing P. fluorescens growth across three time points for both diets.

Conclusions

Our collected slime samples had no inhibitory effect on *P. fluorescens* growth. Additionally, we found no significant inhibitory difference between the veggie and protein diets, suggesting that a high protein diet may not affect the chemistry of slime for snails. Because several studies report inhibitory effects of snail slime, it may be that this species of snail has somewhat less inhibitory effects or that methods will need to be adjusted to concentrate the slime. Overall, our results underscore the need for additional research on snail slime and the invertebrate's immune system.

Acknowledgements/References

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[1] Bayne, Christopher & Hahn, Ulrike & Bender, R.C.. (2001). Mechanisms of molluscan host resistance and of parasite strategies for survival. Parasitology. 123 Suppl. S159-67. 10.1017/S0031182001008137.
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[3] Chobchuenchom, W. & Bhumiratana, A. World Journal of Microbiology and Biotechnology (2003) 19: 903.